PROTEIN FOLDING, STRUCTURE AND FUNCTION

Organizers: William DeGrado, Stephen Brenner and Dale Oxender

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Plenary Sessions Page

Poster Sessions

Structural Analysis of Proteins and Folding Intermediates
(joint with NMR meeting)

R 001 STRUCTURAL AND GENETIC ANALYSIS OF THE INTERACTIONS THAT STABILIZE PROTEINS. Sun Dao-pin, Hale Nicholson, Xue-Jun Zhang, Walt A. Baase, Michael Blaber, Dirk W. Heinz and Brian W. Matthews, Institute of Molecular Biology, Howard Hughes Medical Institute, University of Oregon, Eugene, OR 97403

The lysozyme from bacteriophage T4 is being used as a model system to determine the roles of individual amino acids in the folding and stability of a typical globular protein. Such studies can provide quantitative information on the contributions made by different types of interactions including H-bonds, hydrophobic interactions, salt bridges and disulfide bridges. The emphasis of the talk will be on two topics, first, the contributions of electrostatic interactions to protein stability, and, second, the influence of amino acid replacements within α-helices on protein stability.

Studies of mutant lysozymes suggest that engineered salt bridges between solvent-exposed residues on the surface of a protein contribute little to stability. In contrast, however, engineered electrostatic interactions with so-called "α-helix dipoles" consistently enhance the stability of the protein. Possible reasons for this difference will be discussed.

In order to determine the importance of "α-helix propensity" in protein stability, different replacements have been made within α-helical segments of the T4 lysozyme. Several such substitutions of the form Xaa → Ala increase the stability of the protein, supporting the idea that alanine is a strongly helix-favoring amino acid.

R 002 ELUCIDATION OF PROTEIN FOLDING PATHWAYS BY NMR AND AMIDE PROTON EXCHANGE. Heinrich Roder and Gülnur A. Elöve, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104-6059.

An important aspect of the protein folding problem concerns the structural characterization of intermediate states on the folding pathway. The fact that folding intermediates are generally unstable and short-lived limits the direct application of structural tools such as NMR. Nevertheless, it is possible to obtain detailed structural information on early events in folding by combining NMR spectroscopy with NH exchange pulse labeling and rapid mixing methods. The formation of hydrogen bonded structure during folding is probed by exposing the protein to a proton labeling pulse at different refolding times, followed by 2D NMR analysis of the patterns of amide protons trapped in the refolded protein. Earlier pulse labeling studies on cytochrome c [Roder, Elöve & Englander (1988) Nature 335, 700] revealed a 20 millisecond folding event in which amide sites in two α-helices near the chain termini become protected from exchange while amide sites in other helical segments and those involved in irregular H-bonds remain largely exposed out to about 100 ms. The fact that the N- and C-terminal helices form a tight contact in the native structure suggests that association of the two helices is an important early event in cytochrome c folding. Further evidence for such a helix pairing reaction was obtained by pulse labeling experiments involving systematic variation of the labeling conditions which show that amide protons on either helix exhibit the same degree of protection, indicating that the helices are stabilized by mutual contacts. The observation that the formation of stable helical structure is accompanied by docking of two helices illustrates the importance of key tertiary interactions in directing the pathway of folding. The pulse variation results also provide evidence for multiple parallel folding pathways. The role of the axial heme ligands in cytochrome c folding is addressed by stopped-flow and pulse labeling experiments under a variety of refolding conditions. The results indicate that His 18 remains ligated under most conditions, but the Met 80 ligand is replaced by His 26 or His 33 in a major population of unfolded molecules. This non-native ligand becomes trapped in the partially folded early intermediate and is replaced by the native methionine ligand in a subsequent folding phase on the 100 millisecond time scale.

R 003 NMR, PROTEIN STRUCTURES IN SOLUTION AND THE PROTEIN FOLDING PROBLEM. K. Wüthrich, Institut für Molekularbiologie und Biophysik, ETH-Hönggerberg, CH-8093 Zürich, Switzerland.

On a level of general availability and practicability, the NMR approach for the determination of three-dimensional protein structures in solution[1] has by now been in use for a period of about five years[2]. This lecture will emphasize features of NMR structures of proteins that have been shown during this period to be complementary to data that can be obtained from X-diffraction in protein crystals. A central theme will be the use of NMR for studies of protein hydration in solution[3].

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Folding in Vitro

R 004 CHARACTERIZATION OF MOLTEN GLOBULE-LIKE INTERMEDIATES IN PROTEIN FOLDING, Anthony L. Fink, Sally A. Swedberg and Takuzo Kurotsu, Department of Chemistry, University of California, Santa Cruz, California 95064

A number of proteins, on acid denaturation, adopt compact conformations with substantial secondary structure, as determined by circular dichroism. Kinetics experiments indicate that intermediates with similar properties are formed during refolding. Stopped-flow CD experiments show very rapid formation of secondary structure. The secondary structure content of the acid-induced molten globule conformation of several proteins was compared with that of the corresponding native state using FTIR spectroscopy. Quantitative analysis of the Amide I band revealed that for several proteins a substantial amount of secondary structure in the native state was replaced by different structure in the molten globule state. In most cases additional unordered structure was present in the molten globule state compared to the native. Analysis of the Amide III bands was consistent with the Amide I data. Thus, secondary structure is present in the molten globule state which is not found in the corresponding native state. The implications of this observation to protein folding will be discussed.

R 005 INTERMEDIATES AND THE TRANSITION STATE OF GLOBULAR-PROTEIN FOLDING

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Two important questions in protein folding are (1) what kind of structure is assumed early in refolding, and (2) what is the nature of the critical activated state ("transition state") that may exist between the early intermediate and the final native state.

To obtain a general picture of the early event in folding, refolding reactions from the fully unfolded (D) state have been investigated for various proteins including α-lactalbumin, lysozyme, parvalbumin, β-lactoglobulin, staphylococcal nuclease, and dihydrofolate reductase. The reactions were induced by concentration jump of a denaturant, urea or guanidine hydrochloride, and monitored by kinetic CD measurements including stopped-flow CD in the peptide region. In all proteins studied, a transient intermediate (I) with an appreciable amount of secondary structure accumulates rapidly, within the dead time of measurement (10~20 ms in the stopped-flow CD), although the stability of the I state strongly depends on protein species. Protein folding in general consists of at least two stages, as D→I→N. For α-lactalbumin, the I state has been shown to be identical with the so called "molten globule" state that is compact and partially unfolded. It is demonstrated that the first stage of refolding is brought about by long-range nonspecific hydrophobic interactions.

The kinetics of folding and unfolding have been investigated for α-lactalbumin, parvalbumin and staphylococcal nuclease for characterizing the transition state for folding. In all proteins, the activation free energy linearly increases for folding and decreases for unfolding with denaturant concentration. The transition state is partially organized in terms of the accessibility to denaturant molecules. Effects of specific ligands, Ca^{2+} for the three proteins and deoxythymidine 3',5'-diphosphate for the nuclease, on the folding kinetics have provided information about specific structure organized in the transition state. When the specific structure for ligand binding is organized in the transition state, the refolding rate is accelerated by ligand. The structure in the transition state is characterized by local, specific, tertiary structure. The secondary structure segments preformed in the I state may be organized as in the native structure but only in a localized region ("critical substructure"). This model of folding also implies that a negative heat capacity change observed in the temperature dependence of refolding kinetics must be ascribed in part to a change in the transition-state structure with temperature.

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Protein Folding in Vivo in Procaryotes

R 006 INTERACTION OF INTERMEDIATES OF PROTEIN FOLDING WITH CHAPERONINS

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The folding of many proteins from the unfolded state (U) to the native state (N) involves the transient formation of intermediates (I), variously described as 'molten globules' (1) or 'compact intermediates' (2). Generally the U->I transition is very rapid (msec or faster) whereas the I->N transition is slow with half times varying from seconds to hours. Rubisco-I, for example, is formed very rapidly and is only slowly converted to the N state . Its CD spectrum suggests much more secondary structure than the U-state and the trp fluorescence is much more N-like than U-like. However, ANS fluorescence measurements of Rubisco-I indicate a greater exposure of hydrophobic surfaces than in the N-state. In general, folding intermediates (I) tend to irreversibly aggregate. Rubisco-I, stable at 4°, rapidly aggregates upon warming to 25°. However, the I states of some proteins [e.g., rubisco (3,4), rhodanese (5), citrate synthase (6), dihydrofolate reductase (DHFR) (7), pre-β-lactamase (8)] can be stabilized by forming binary complexes with chaperonin 60 (cpn60). Formation of the cpn60·I complex prevents aggregation but also inhibits the transition to the native state. The binary cpn60·I complexes are sufficiently stable to withstand gel filtration. The formation of a stable binary cpn60·I complex is a common step in the interaction of the chaperonin proteins with all folding intermediates so far studied.

Subsequent steps leading to the formation of the N state lack mechanistic unity. [This may reflect thermodynamic differences between the I and N states of the various proteins and the strength of their interaction with cpn60.] For proteins such as rubisco (3,4) and rhodanese (5), significant formation of the N state from the cpn60·I complex requires the presence of chaperonin 10 (cpn10), MgATP and K^+. Hydrolysis of ATP appears necessary since non-hydrolysable analogs do not support the formation of active rubisco or rhodanese and the formation of the N state requires K^+. The I->N transition is presumed to occur on cpn60. On the other hand, for proteins such as citrate synthase (6), DHFR (7) and pre-β-lactamase(8), formation of the N state from the cpn60.I complex does not require cpn10. For DHFR, addition of ATP or of non-hydrolysable analogs induces the dissociation of the cpn60·I complex and permits DHFR-I to proceed to the N state; i.e. the I->N transition occurs off the chaperonin.

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R 007 KINETIC PARTITIONING BETWEEN POLYPEPTIDE FOLDING AND PROTEIN EXPORT, Linda L. Randall and Simon J. S. Hardy, Department of Biochemistry and Biophysics, Washington State University, Pullman, WA 99164-4660 and Department of Biology, University of York, York, England Y01 5DD.

SecB, an oligomeric molecular chaperone in *E. coli*, is involved in export of a subset of unrelated proteins (1-3). SecB not only enhances the efficiency of delivery of the precursors to the membrane but also is responsible for maintaining the proteins in a loosely folded state so that they remain competent for translocation from the cytoplasm across the cytoplasmic membrane to their final destination in either the periplasmic space or in the outer membrane (3,4). The interaction of cytoplasmic SecB with precursor maltose-binding protein (MBP), one of the proteins that depends on SecB for efficient export, occurs early in the export pathway *in vivo* but does not involve recognition of the amino-terminal leader sequence. SecB binds the precursor polypeptide at sites that are distinct from the leader sequence (5-7, see 8 for an opposing view). Investigations to be discussed indicate that precursor MBP contains no unique sequence of amino-acyl residues that serves as a hallmark for binding of SecB. SecB bound tightly to all nonnative polypeptides tested even though it did not interact with any native protein. If SecB recognizes all nonnative structures, how are proteins that are to be exported distinguished from those that are to remain in the cytoplasm? We propose that selectivity results from a kinetic partitioning. The pathway a polypeptide takes *in vivo* depends on the rate of its folding relative to the rate of its association with SecB.

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Protein Folding in Vivo in Eucaryotes

R 008 HSP70 MEMBERS AND PROTEIN FOLDING, Gregory Flynn and James E. Rothman, Department of Molecular Biology, Lewis Thomas Laboratory, Princeton University, Princeton, New Jersey 08544-1014.

Two members of the hsp70 family, termed hsc70 and Bip, have been implicated in promoting protein folding and assembly processes in the cytoplasm and the lumen of the endoplasmic reticulum, respectively. Short synthetic peptides were tested as possible mimics of polypeptide binding sites. Peptide binding elicits hydrolysis of ATP, with the subsequent release of bound peptide. To determine the specificity for peptide binding, we have synthesized random peptides of defined lengths. Both the binding of the peptide and the stimulation of the ATP hydrolysis of hsp70s increase in proportion to chain length, to seven-residues. With the random heptamer the specificity of binding can be determined.

Membrane Proteins

R 009 STABILITY STUDIES ON BACTERIORHODOPSIN C. G. Brouillette,[1] T. Marti,[2] L. Stern,[2,4] T. Mogi,[2,5] H. G. Khorana,[2] S. Mounter,[3] and C. K. Johnson[3]. [1]Univ. of Alabama at Birmingham, [2]Massachusetts Inst. of Tech, [3]Univ. of Kansas, [4]presently at Harvard Univ. [5]presently at Tokyo Univ.

Investigations of the thermodynamic stability of bacteriorhodopsin (bR) have been pursued to understand the forces that hold membrane proteins together. bR undergoes two thermally-induced conformational changes and each is the focus of a separate study.

A reversible conformational change preceeds thermal denaturation of bR. bR also undergoes at least two conformational changes during the course of its photocycle. The early stages in the photocycle (within the time frame of the first conformational change) are being studied by transient absorption laser spectroscopy as a function of temperature to determine the effect and possible relation of the thermal transition to photocycle conformational changes. The thermal transition decreases the rate of the K to L step of bR in lipid/detergent micelles. The implications these results have for the photocycle mechanism will be discussed.

The functional and structural roles for buried charges in bR are of considerable interest. Four buried charges, Arg82, Asp85, Asp212 and the protonated retinal Schiff base of Lys216 are positioned near each other in the structure and could form a quadrupolar interaction in which each charge interacts with two complementary charges on either side. The structure may, however, be better represented by a pair of discrete salt bridges. These residues are clearly important in the bR photocycle and how changes in their protonation state effect structural stability are relevant to the photocycle. The existence of stabilizing interactions was examined by thermal denaturation studies on micellar bR containing single "neutralizing" mutations (Arg→Gln and Asp→Asn) at Arg82, Asp85, Asp212, and double-site mutations of the complementary charge pairs Arg82Asp85 and Arg82Asp212. The stability changes are not additive in going from the single to either double mutation, and the mutant stabilities are similar. These results suggest the protein conformation is well-adapted to compensating for the loss of either a single charge or charge pair and that a quadrupolar interaction may exist among the four residues. Partial solvent accessibility from outside the proton channel and a more "open" structure of micellar bR may account for the modest changes in stability observed (1.5-3 kcal/mol). These and earlier studies on bR have led to the general conclusion that membrane and water-soluble proteins have more in common than, perhaps, was once thought. The hydrophobic effect is a factor in bR stability and ion pairs apparently do not play a critical role in stabilizing bR.

R 010 FUNCTIONAL AND BIOCHEMICAL STUDIES OF Shaker K^+ CHANNELS. Diane M. Papazian, Department of Physiology, UCLA, Los Angeles, CA 90024

Ion channels control the permeability of cells to specific ions. Because extremely sensitive measurements of ion channel function can be made electrophysiologically, these integral membrane proteins are interesting targets for structure/function studies. The Shaker gene of Drosophila encodes a K^+-selective, voltage-dependent channel which is opened by depolarization of the membrane. Shaker proteins contain a motif, the S4 sequence, which has basic amino acids at every third or fourth position. Since voltage-dependent Na^+ and Ca^{2+} channels also contain this motif, it has been widely proposed that the charged S4 sequence serves as the primary voltage sensor in a mechanism of voltage-dependent activation shared by these channels. This hypothesis was tested for the Shaker channel by site-directed mutagenesis of the S4 sequence and electrophysiological analysis.[1] The results indicate that the S4 sequence is important for activation. However, the sensitivity of the channel to voltage can be reduced without removing charges from the S4 motif. Therefore, activation of the K^+ channel cannot be explained completely by electrostatic interactions between the S4 basic amino acids and the membrane electric field.

Structure/function studies of K^+ channels have been hindered by a lack of structural information. Shaker channels have not been purified biochemically. In Drosophila, the channels are present in low abundance and may have a complex or heterogeneous subunit composition. To circumvent these problems, Shaker channels have been expressed in functional form in insect cells in culture using a recombinant baculovirus.[2] Biochemical experiments are underway to study the number of Shaker subunits in the K^+ channel, and their topology in the membrane. Analysis of the glycosylation of Shaker protein made in this system has provided some topological information.

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Antibodies

R 011 CATALYTIC ANTIBODIES: PERSPECTIVES AND PROSPECTS FOR THE FUTURE

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Recent attention has focused on the mammalian immune system as a source of highly specific, tailored catalysts. The construction of enzyme-like antibodies involves synthesizing compounds that mimic the transition state structure of a particular reaction, eliciting an immune response against such substances, and screening the resulting immunoglobulins for the desired activity. Because concerted chemical reactions are expected to be sensitive to induced strain and proximity considerations, and because they do not require the participation of specifically oriented catalytic groups within the binding pocket, they are especially suited for catalysis by antibodies. Such processes are, moreover, of enormous practical and theoretical interest. Our progress in generating immunoglobulins that catalyze concerted chemical reactions, particularly sigmatropic rearrangements, Diels-Alder cycloadditions and decarboxylations, will be outlined. In addition, the application of powerful genetic techniques for improving the chemical efficiency of first-generation antibody catalysts will be discussed. This work promises to contribute substantially to our general understanding of how natural enzymes work and evolve, how protein function is related to structure, and how entirely new enzymatic activities can be created on demand for use in research, industry and medicine.

R 012 ANTIBODY COMBINING SITES : PREDICTION AND DESIGN Anthony R.Rees, David Staunton, Jan Pedersen, Alison Jones, Kate Hilyard & Sally Roberts[1], Department of Biochemistry, University of Bath, Bath, England and [1] Department of Cancer Studies, CRC Laboratories, University of Birmingham, England. A knowledge of the rules involved in defining the three dimensional structure of antibody combining sites and in specifying those residues making specific interactions with a particular antigen, would allow the design and manipulation of antibodies by protein engineering techniques. Such "designer" antibodies might possess improved therapeutic properties such as higher affinity, higher specificity and greater accessibility to the desired tissue targets.

We have focussed on the study of antibody-antigen interactions using a variety of techniques. This multidisciplinary approach is essential if we are to understand the basis of antigen recognition at the molecular level. Modelling algorithms have been developed that are capable of predicting antibody combining site structures with high accuracy. Such models, however, are valuable only in the context of other techniques, such as x-ray crystallography, that seek to evaluate their accuracy. The modification and design of antibodies requires, in addition to the structural methods, the techniques of molecular biology, in particular protein engineering. We have developed protocols for engineering antibodies and are engaged in improving affinity and specificity as well as designing new properties into antibodies such as catalytic activity and metal binding. Such modifications may soon become a routine procedure in the rational design of antibodies for diagnostic and therapeutic applications.

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Protein Engineering

R 013 UNDERSTANDING AND ENGINEERING THE ION SPECIFICITY OF A CA^{2+} BINDING SITE, Joseph J. Falke, Brian W. Buoscio, Eric E. Snyder, Kay C. Thatcher, and C. Stefan Voertler. Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado, 80309-0215.

Protein Ca^{2+} binding sites selectively bind Ca^{2+} even in the presence of 10^3 to 10^5-fold higher concentrations of Na^+, K^+, and Mg^{2+}. This specificity is particularly important for regulatory sites in Ca^{2+} signaling proteins which must remain empty until a Ca^{2+} signal, and for Ca^{2+} channel sites which provide selective Ca^{2+} fluxes. The E. coli D-galactose and D-glucose receptor, an aqueous receptor which initiates bacterial sugar sensing and transport pathways, possesses a single Ca^{2+} binding site similar in structure and specificity to the EF-hand class of sites found in eukaryotic Ca^{2+} signaling proteins including calmodulin and its homologues. A universal feature of these sites is a pentagonal bipyramidal array of seven oxygens which coordinate bound Ca^{2+}. In order to probe the mechanisms of Ca^{2+} specificity we have quantitated the free energy of ion binding to the bacterial site as a function of ionic radius and charge, using the cations of Groups Ia, IIa, IIIa and the lanthanides as incremental substrates. The resulting free energy curves suggest that the coordinating array selectively binds Ca^{2+} by providing a substrate cavity highly optimized in both size and charge. To test this model we have engineered changes in cavity size and charge by replacing axial glutamine 142 with asparagine, glutamate, and aspartate. Dramatic changes in ion specificity result: sites containing an engineered smaller sidechain (asn or asp) bind large cations up to 50-fold more tightly than the native site. And sites containing an engineered negative sidechain (glu or asp) exhibit specificities for trivalent over divalent cations up to 1900-fold higher than the native site. The results demonstrate a) the control of ion specificity by the dimensions and electrostatics of the coordinating array, and b) the usefulness of incremental substrates in the design and evaluation of new substrate sites.

R 014 ENGINEERED VARIANTS TO STUDY DOMAIN FOLDING AND SUBUNIT INTERACTIONS OF DIMERIC ASPARTATE AMINOTRANSFERASE, Marzell Herold*, Bernd Leistler & Kasper Kirschner, *Hewlett-Packard GmbH, D-7517 Waldbronn 2, FRG; Biozentrum, Universität Basel, CH-4056 Basel, Switzerland.

The reversible equilibrium unfolding of the dimeric aspartate aminotransferase from E. coli (eAAT) consists of three steps with the dissociation/association partially uncoupled from the subsequent unfolding steps (1). Two monomeric intermediates were detected, one is stable at 1 M guanidine hydrochloride. It is inactive, but compactly folded and possesses properties of the "molten globule" state. Reactivation kinetics of eAAT occurred on two parallel pathways with the association of the folded but inactive monomers as the last step.
The native eAAT monomer consists of two domains. To test independent domain folding, the coenzyme binding domain (P-domain) was isolated. Since this domain is made up of an internal part of the polypeptide chain, the N- and C-terminal peptides that combine together the second domain were removed by recombinant DNA techniques. The isolated P-domain is inactive as expected and monomeric probably because of the deletion of the intersubunit contacts removed with the N-terminal peptide. But it folds autonomously in vivo and in vitro and binds the coenzyme pyridoxal-5'-phosphate specifically. The P-domain displayed a two-step unfolding and parallels the unfolding of the native monomer. It is somewhat less stable than the eAAT monomer.
Testing the N-terminus interaction a second monomeric mutant (ΔNTAAT) was obtained by deleting only the N-terminal peptide, i.e. one half of the second small domain. This variant is able to slowly catalyze the first half reaction with different substrates. Furthermore a full-length monomer was designed by selectively exchanging the residues at the N-terminus that interact with the second subunit.

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Enzymes

R 015 METALLOENZYME CATALYSIS, Joseph J. Villafranca, Department of Chemistry, The Pennsylvania State University, University Park, PA 16802

Metal ions can serve multiple roles in enzyme catalyzed reactions. Due to the electrostatic properties of cations, metal ions (e.g., divalent cations) can provide reversible binding sites for anionic substrates. In addition, cations can assist in bond polarization reactions acting as electrophilic centers. The enzyme glutamine synthetase has two divalent cations bound at the active site, one serving as the ATP binding site and the other binding the substrate glutamate. The enzymatic reaction has been studied by steady-state and rapid kinetic methods and two intermediates are proposed in the reaction mechanism. These intermediates were corroborated by biophysical investigations and a mechanism is proposed that involves each metal ion in a separate electrophilic event. Mutagenesis of the amino acids that are metal ion ligands has assisted in the interpretation of the overall catalytic reaction. Support in part by NIH Grant GM 23529.

R 016 MOLECULAR STUDIES ON THE CYCLOPHILIN IN CLASS OF PEPTIDYL PROLYL ISOMERASES: Christopher T. Walsh, Biological Chemistry and Molecular Pharmacology, Harvard Medical School

The immunosuppresant drug cyclophilin A (CSA) binds with high affinity to a protein termed cyclophilin in T cells, CSA binding to cyclophilin inhibits its catalytic activity as a cis-trans peptidyl isomerase (PPIase), an isomerization of possible relevance in late stages of folding of some proteins, Structure function studies on human, yeast and E. coli cyclophilins expressed in and purified from E. coli will be reported with regard both to PPIase mechanism, with regard to recognition of cyclosporin A, and with regard to possible physiologic role of this class of proteins.

R 017 MOLECULAR RECOGNITION AND THE ALPHA HELIX

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This lecture will review and illustrate the role of the alpha helix as a molecular surface that can recognise other components of proteins and nucleic acids. Within protein molecules there are well-known types of helix-helix and helix-sheet contacts that form the structural core. Active sites of enzymes include ligand-binding regions formed on or between helices. The architectural motifs of globular proteins range from simple four-helix bundles to more complex assemblies, such as the helix clusters in membrane proteins. Helices with specialised functions include parallel dimers in coiled coils, amphiphilic helices, membrane channel bundles,and helix elements that recognise DNA. We shall illustrate and discuss the characteristic types of molecular surface pattern that achieve these effects.

Theoretical Considerations

R 018 3-D PROFILES, David Eisenberg, James Bowie, and Roland Luthy, Molecular Biology Institute, University of California, Los Angeles, CA 90024-1570.

The PROFILE method, originally a method for detecting distantly related proteins by sequence comparison, has been extended to a method for detecting amino acid sequences compatible with a given 3-dimensional protein structure. The goal of the work is to be able to recognize 3-D structures from amino acid sequences.

In the original method, a sequence profile (also termed 1-D profile) is contructed from a family of aligned amino acid sequences and once made, this matrix can be used to search the sequence database for related sequences by the dynamic programming method. Tests with the globin and other families of sequences show that a 1-D profile can distinguish all members of the family from all other sequences in the database. Sequence profiles are not particularly effective, however, at recognizing more general protein folding classes, such as antiparallel beta barrels. Since being able to recognize these more general folding classes is the essence of the protein folding problem, we have extended the profile method so that a 3-D profile can be constructed from the coordinates of a protein structure. Results with globin and other 3-D profiles will be presented.

R 019 AN EMPIRICAL APPROACH TO FREE ENERGY AND SPECIFICITY OF MACROMOLECULAR INTERACTIONS. Jiri Novotny, Ronald Behling, Robert E. Bruccoleri and Stanley Krystek, Bristol-Myers-Squibb Research Institute, Princeton NJ 08543-4000. Using X-ray coordinates of macromolecular complexes such as antigen-antibody, enzyme-inhibitor, leucine zipper dimers and DNA-repressor/operator, we made semiquantitative estimates of Gibbs free energy changes (ΔG) accompanying noncovalent complex formation. Our empirical ΔG function, which implicitly incorporates solvent effects, has the following components: hydrophobic force, solvent-modified electrostatics, changes in side-chain conformational entropy, translational/rotational entropy changes, and the dilutional (cratic) entropy term. The calculated ΔG ranges often matched the experimentally determined values, thus supporting our empirical concept in principle. Future improvements to the empirical ΔG formula will concentrate on enumeration of conformational entropy using uniform sampling of conformational space (Bruccoleri & Karplus, Biopolymers (1987) 26, 137-168) and evaluation of electrostatic/desolvation binding energies via the linearized Poisson-Boltzmann electrostatic field algorithm (Sharp & Honig, Ann. Rev. Biochem. 1990). Results of our calculations obtained to date give us some insight into the attribution of ΔG and the thermodynamic origin of specificity in macromolecular interactions. In protein-protein complexes, usually only a subset of amino acids at the protein-protein contact surfaces contribute actively to binding energetics. For protein antigens, a concept of antigenicity emerges that involves attractive contributions mediated by "energetic" antigenic epitopes and a passive surface complementarity contributed by the surrounding contact area. In lecine zipper coiled-coil helical dimers the charged side chains abutting to the contact surface determine homo-heterodimer specificity while the hydrophobic contact area (leucines) contributes to dimer stability in a nonspecific manner. Rules such as these can be used to guide drug design, protein engineering, and site-specific protein mutagenesis.

R 020 COMPUTATIONAL APPROACHES TO BIOMOLECULAR DESIGN, **Zelda R. Wasserman, James D. Lear and William F. DeGrado, The Du Pont Merck Pharmaceutical Co., Wilmington DE 19880-0228.** **We have adopted a minimalist approach to protein design. Our de novo designed sequences, which feature simplicity and symmetry, are an attempt to extract from natural proteins' primary structure the information crucial for both folding and functionality. In our parallel computational efforts, using traditional force field methodology, we adopt a similar philosophy. We attempt to pinpoint structural features of the modeled designed systems which are common to a set of local minimum energy conformations, or which emerge during computer simulation of molecular motion. These structural hypotheses can be probed by suitably designed experiments, sometimes leading to completely unanticipated results. This interaction of experiment and theory will be discussed with respect to the structure, stability, and size and charge selectivity of ion channels formed from model peptides as a function of sequence variation.**

DNA Recognition

R 021 **ENGINEERING OF PROTEIN-DNA INTERACTION**, Richard H. Ebright, P. Shannon Pendergrast, Yon W. Ebright, Yan Chen, Xiaoping Zhang, and Angelo Gunasekera, Department of Chemistry and Waksman Institute, Rutgers University, New Brunswick, NJ 08855

A. PHOTOCROSSLINKING METHOD TO DETERMINE THE ORIENTATION OF A DNA BINDING PROTEIN IN THE PROTEIN-DNA COMPLEX

We have developed a simple, general approach to determine the orientation of a DNA binding protein relative to its DNA site in the protein-DNA complex. The approach involves covalently attaching a photoactivatible crosslinking agent--one capable of reacting with DNA nucleotides upon photochemical activation--to a defined site of the DNA binding protein. The approach has two steps:

(1) Two derivatives of the DNA binding protein are constructed: one derivative having a photoactivatible crosslinking agent covalently attached at amino acid *x*, the other derivative having a photoactivatible crosslinking agent covalently attached at amino acid *y*.

(2) For each derivative, the protein-DNA complex is formed. The photoactivatible crosslinking agent is photochemically activated *in situ*, resulting in crosslinking with nearby nucleotides. The locations of the crosslinked nucleotides are identified. The orientation of *x* and *y* relative to the DNA site in the protein-DNA complex is inferred from the locations of the crosslinked nucleotides in the case of the derivative modified at *x* and in the case of the derivative modified at *y*.

To test the approach, we have conducted experiments with the well-characterized sequence-specific DNA binding protein CAP. Two derivatives of CAP have been constructed and analyzed: [(N-acetyl-*p*-azidobenzene)-Cys170;Ser178]CAP and [(N-acetyl-*p*-azidobenzene)-Cys178]CAP.

B. CONVERSION OF HELIX-TURN-HELIX MOTIF SEQUENCE-SPECIFIC DNA BINDING PROTEINS INTO SITE-SPECIFIC DNA CLEAVAGE AGENTS

We have developed an approach to convert helix-turn-helix motif sequence-specific DNA binding proteins into site-specific DNA cleavage agents. The approach is to incorporate a chelator at amino acid 2, 6, or 10 of the helix-turn-helix motif.

We have constructed a prototype site-specific DNA cleavage agent in which the chelator 1,10-phenanthroline is incorporated at amino acid 10 of the helix-turn-helix motif of CAP: [(N-acetyl-5-amino-1,10-phenanthroline)-Cys178]CAP. [(N-acetyl-5-amino-1,10-phenanthroline)-Cys178]CAP binds to a 22 base pair DNA recognition site with $K_{obs} = 1 \times 10^8\ M^{-1}$. In the presence of Cu(II) and reducing agent, [(N-acetyl-5-amino-1,10-phenanthroline)-Cys178]CAP cleaves DNA at four adjacent nucleotides on each DNA strand within the DNA recognition site. The DNA cleavage reaction has been demonstrated using 40 base pair and 7164 base pair DNA substrates.

Such semi-synthetic site-specific DNA cleavage agents have potential applications in chromosome mapping, cloning, and sequencing.

R 022 ZINC FINGER-DNA INTERACTIONS: STRUCTURAL AND GENETIC STUDIES. Ross C. Hoffman, Sushil Thukral, Suzanna J. Horvath, E. T. Young, and Rachel E. Klevit, Department of Biochemistry, University of Washington, Seattle, WA 98195

ADR1, a transcription factor that binds to an upstream activator sequence of the Alcohol Dehydrogenase II (ADH2) gene of S. cerevisiae, contains two zinc-fingers of the TFIIIA class that are essential for its DNA-binding activity. Synthetic versions of the two zinc fingers are designated ADR1b (residues 102-130) and ADR1a (residues 130-159) and have been shown using 2DNMR to fold in a zinc-dependent manner into small, globular, heat-stable domains. A quantitative analysis of ADR1b has been performed, resulting in a family of distance geometry structures. ADR1b consists of a loop-like structure (residues 102-113), a "fingertip" (residues 114-115), and an α-helix (residues 116-126), similar to other NMR-derived structures of zinc finger peptides that have been reported.

A program of alanine mutagenesis, in which each residue in the two zinc fingers of ADR1 has been substituted with an alanine has identified point mutations that adversely affect DNA binding. In addition, chemical mutagenesis and site-directed mutagenesis at certain residues has been performed. Mutations that decrease DNA binding that occur in non-conserved residues are being investigated structurally. For example, a His118Tyr mutation completely abrogates DNA binding and a His118Ala mutation results in a 20-30 fold decrease in binding. Qualitative analysis of ADR1b peptides containing these mutations indicates they have the same zinc-dependent global fold as wildtype ADR1b. Thus, the structural changes that affect DNA binding are more subtle. Distance geometry structures of the mutant peptides have been generated and show some interesting differences when compared to wildtype. It is hoped that the combined approach of genetics and structure determination will yield insights into the details of the interaction between the zinc finger domains of ADR1 and its cognate DNA. (This work was supported by NIH PO1 GM32681.)

R 023 CRYSTAL STRUCTURE OF A ZINC FINGER - DNA COMPLEX AT 2.1 Å RESOLUTION, Nikola P. Pavletich[1] and Carl O. Pabo[1,2], [1]Department of Molecular Biology and Genetics, and [2]Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The crystal structure of a complex containing the three Zn fingers from the mouse immediate early protein zif268 and a consensus DNA binding site has been determined at 2.1 Å resolution. In this complex, the three Zn fingers (which are similar to the fingers of TFIIIA) bind in the major groove of B-form DNA. The relationship between each Zn finger and the DNA is essentially the same, and the fingers make their primary contacts with three contiguous 3 base pair "subsites". Each finger uses side chains from the N-terminal portion of its helix to contact the bases, and the majority of the contacts involve the purine rich strand of the DNA. The periodicity of the fingers in the complex, and the recurrence of certain base contacts in more than one finger, suggest useful rules and principles that would allow the design of novel DNA-binding proteins based on the Zn finger motif.

Protein Structure/Function; Structure Prediction

R 100 **A SYNTHETIC PEPTIDE BASED ON AN α-HELICAL SEGMENT OF THE COMPLEMENT PROTEIN Clq BINDS IMMUNE COMPLEXES,** **Byron Anderson and Michael Baumann, Department of Cell, Molecular and Structural Biology, Northwestern University Medical School, Chicago, IL 60611**

Clq is the 18 polypeptide complex of the first component of complement that shows selective binding to immune complexes (ICs) versus monomeric IgG. A peptide segment of the globular region of the B chain of the complement protein Clq was predicted to be an α-helical secondary structure and exhibited certain sequence and structural homologies to an α-helical portion of the S. aureus Protein A fragment B. The fifteen amino acid sequence was synthesized, purified and was shown to inhibit the binding of peroxidase-labeled IgG to both solid phase adsorbed Protein A and Clq. The peptide was covalently coupled by reductive amination to an LTQ (Chromochem) solid phase and column. Passage of peroxidase-anti-peroxidase ICs through the column resulted in a fraction of the ICs being bound whereas certain control columns bound little PAP. Using heat-aggregated human IgG (HAG) and biotinylated (B) HAG as IC standards, the peptide-LTQ column bound HAG, HAG in the presence of excess monomeric IgG, and HAG from diluted serum, greater than 90% of the HAG offered being bound to CBP column but not to control columns. These data suggest that the peptide mimics the IC binding activity and possible binding site of Clq.

R 101 BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF PEPTIDES COMPRISING THE HELIX-LOOP-HELIX MOTIF, Spencer J. Anthony-Cahill, Pamela A. Benfield, Robert Fairman, Zelda R. Wasserman, Stephen L. Brenner and William F. DeGrado, Biotechnology Department, Du Pont Merck Pharmaceuticals, P. O. Box 80328, Wilmington, DE 19880-0328

A number of recently discovered eukaryotic gene regulatory proteins contain a conserved amino acid sequence, the b-HLH motif, responsible for binding to DNA. This motif consists of an Arg- and Lys-rich basic region followed by two predicted α-helices, and mediates binding to DNA with approximate inversional symmetry (CANNTG). We have synthesized peptides spanning the b-HLH motif of E47 and myoD, two proteins that hetero-oligomerize and bind to muscle-specific promoters. These peptides form α-helical homo- and hetero-dimers, and the helical content of the dimers increases when they bind to DNA. Methylation interference experiments are consistent with interaction in the major groove, and show several distinct differences between the various homo- and hetero-dimeric complexes. We propose a structural model that accounts for the oligomerization and DNA-binding behavior of this motif.

R 102 Oct-1 BINDS TO DIVERSE TARGET SEQUENCES THROUGH INTERACTIONS WITH MULTIPLE REGIONS OF THE POU-DOMAIN, Rajeev Aurora and Winship Herr, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

The ability of site-specific transactivators to recognize a set of diverse DNA binding sites has important consequences on the flexibility of promoter structure. To understand the mechanism of flexible sequence recognition we have used the ubiquitously expressed transcription factor Oct-1. Oct-1 was discovered for its ability to bind to the octamer motif (ATGCAAAT), but it can also bind to the distantly related TAATGARAT motif. Although these two motifs share little sequence similarity, they can be related to each other, through an incremental progression of many Oct-1 binding sites.

The DNA binding domain of Oct-1 is a POU-domain which consists of two regions that are conserved among the POU-family; a C-terminal 60 aa POU-class homeodomain and a N-terminal 75 aa POU-specific region, separated by a 24 aa hyper-variable "linker". The POU-specific region itself has been divided into two sub-segments, called the A and B boxes, based on patterns of sequence similarity between the four founding members of the POU-family: Oct-1, the lymphoid transcription factor Oct-2, the pituitary transcription factor Pit-1, and the product of the nematode gene *unc-86*. The modular nature of the DNA binding domain may provide an explanation for the ability of Oct-1 to bind to such diverse sequences. However, the relative contribution of all the sub-segments to DNA binding specificity is not known.

We show here that Oct-1 binds to DNA as a monomer, and that the POU-domain is largely, if not entirely, responsible for DNA binding specificity. Oct-1 and Pit-1, which display different DNA sequence recognition, were used to create chimeras to evaluate the relative contribution of the different POU-domain segments. We find that the A and B boxes of the POU-specific region, the homeodomain and, surprisingly, the hyper-variable linker all contribute to DNA binding specificity. In addition, we find that the four segments cooperate with each other and contribute to DNA binding affinity in a sequence dependent manner. Oct-1 can thus bind to diverse DNA sequences because its DNA binding domain is composed of four flexible, modular and interdependent subsegments that together determine the DNA binding specificity of the entire POU-domain.

R 103 PEPTIDES CONTAINING THE PUTATIVE INTERNALIZATION SIGNAL FOR THE LDL RECEPTOR ADOPT A β-TURN CONFORMATION IN AQUEOUS SOLUTION, Anu Bansal[1] and Lila M. Gierasch[1,2], Departments of Biochemistry[1] and Pharmacology[2], UT Southwestern Medical Center at Dallas, 5323 Harry Hine Blvd., Dallas TX 75235.

We have used ^{1}H nuclear magnetic resonance (NMR) to analyze the conformations of nonapeptides corresponding to regions of the human LDL receptor. The first 22 amino acids of the 50-residue cytoplasmic domain of the LDL receptor are necessary and sufficient for the receptor internalization (Davis et al., JBC 262, 4076 [1987]). An aromatic residue must occur at position 807 for endocytosis of the receptor. Recent mutagenesis studies in which each position of the cytoplasmic tail was substituted by Ala indicated that the Asn, Pro and Tyr in the tetrapeptide sequence ^{804}NPXY were critical for rapid internalization of this receptor (Chen et al., JBC 265, 3116 [1990]). Since NPXY sequences are also present in several cell surface proteins, this sequence may represent a signal for receptor internalization in coated pits. We have synthesized nonapeptides from the LDL receptor comprising NPVY and variants in which alanine is substituted for individual amino acids in the NPVY sequence. NMR results on these peptides indicate that NPVY significantly favors a β-turn with N at the i position. Alanine substitutions affect the turn-forming tendency analogously to their effect on internalization. Other aromatic residues appear to be able to play the same role as Tyr, as in vivo, since the turn propensity of a peptide containing NPVF is essentially unaltered. While Asn and Pro are well-known to favor turn formation, the role of an aromatic residue in the fourth position of a turn has not previously been recognized. We will discuss both the conformational and functional significance of these results.

R 104 ANTIBODIES, Godfrey W. Caesar, 209 West, 137 th. Street, New York, 10030, N.Y.

Burnet and Fenner theorised in 1949 that if an animal is confronted with an antigen before it is capable of responding with the production of specific antibodies, then its capacity to do so in later life is inhibited or retarded. This theory was confirmed experimentally by Billingham, Brent, and Medawar in 1953. I, theorised and published in 1979, and 1980, that the converse of this idea should have the same effect. I confirmed this in a 1981 publication by conferring the tolerance of embryonic rabbits on genetically mature mice. Ready et al caused embryonic thymus lobes depleted of their endogenous lymphophaematopoietic cells by deoxyguanosine treatment in vitro to be successfully transplanted across major histocompatibility barriers, 1986, supports this hypothesis. McCune et al by creating in 1988 SCID-hu mice from SCID mice with human fetal liver, thymus, and lymph node cells further adds to this hypothesis. This concept is in consonance with current concepts in the field regarding the use of fetal cells as the constant in antibody antigen regulation.

R 105 SECONDARY STRUCTURE OF THE SCRAPIE ASSOCIATED FORM OF PRION PROTEIN BY INFRARED SPECTROSCOPY, Byron Caughey*, Aichun Dong** and W.S. Caughey**, *Laboratory of Persistent Viral Diseases, NIH/NIAID Rocky Mountain Laboratories, Hamilton MT 59840, **Department of Biochemistry, Colorado State University, Ft. Collins, CO 80523.

A common feature of scrapie, Creutzfeldt-Jakob Disease and related transmissible neurodegenerative diseases is the accumulation of a protease resistent form of prion protein (PrP-res) in tissues harboring substantial amounts of scrapie infectivity. Unlike the protease-sensitive, detergent soluble prion protein of normal tissues, PrP-res can aggregate into insoluble amyloid-like fibrils and plaques and has been identified as the major component of brain fractions enriched for scrapie infectivity. No scrapie-specific covalent modifications of PrP have been identified. Thus, it is possible that the unusual properties of PrP-res are due solely to conformational changes. Using a recently developed technique in infrared spectroscopy, we have initiated the conformational characterization of PrP-res as it exists in highly infectious amyloid fibril preparations. This approach has enabled us to quantitate the relative amounts of different types of secondary structure in PrP-res aggregates in aqueous media. As anticipated from its amyloid-like properties, the protease resistant core of PrP-res is predominantly composed of ß-sheet and turns with lesser amounts of α-helix and unordered structures.

R 106 DEHYDRINS: A CLASS OF ABUNDANT PROTEINS ASSOCIATED WITH DEHYDRATION OF PLANT CELLS, Timothy J. Close, Department of Botany and Plant Sciences, University of California, Riverside, CA, 92521

Dehydrins are a group of proteins of uncertain structure and function, which are characterized by high glycine content, the absence of tryptophan and cysteine residues, pronounced hydrophilicity, and several conserved and repeated amino acid sequence motifs that exist in regular arrays. The carboxyl terminus of virtually all known dehydrins includes the consensus sequence KIKEKLPG, a motif which occurs evenly spaced as few as two times and as many as nine times in barley dehydrins, whose sequences have been deduced from cDNAs and genomic clones. Dehydrins occur widely in plants as a consequence of any of several physiologically related stresses including desiccation, chilling, osmotic shock and exposure to the plant hormone abscisic acid (ABA). Through the use of a synthetic peptide, affinity purified polyclonal antibodies specific to the highly conserved carboxyl terminus of cereal dehydrins were produced and used to detect seven dehydrin proteins in dehydrating barley seedlings, ranging in size from 15 to 80 kDa. Various numbers and sizes of dehydrins were also observed in a broad range of additional plants within and outside of the Gramineae. The affinity purified anti-dehydrin antibodies were also used in sub-cellular localization studies, and to develop an immunoaffinity adsorbent useful for the purification and subsequent biochemical characterization of this set of stress-related proteins.

R 107 IDENTIFICATION OF THE IL-6 RESPONSIVE ELEMENT OF THE RAT T-KG GENE PROMOTER, Michelle L. Croyle, Elizabeth Mann, Jerry B Lingrel, Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati, Cincinnati, Ohio 45267-0524.

The 5'-flanking region of the Rat T-KG gene was linked to the reporter gene, bacterial chloramphenicol acetyltransferase (CAT) to identify the IL-6 responsive elements by transfection into HepG2 cells. Bal31 deletions localized the IL-6 response to -250 to +1 region. Site-specific mutational analysis identified two elements; a distal element at -250 and a proximal element at -184. Both elements are essential for the Il-6 response. Protein/DNA binding analysis using a fragment which contains both elements identified two specific complexes. Competition studies using fragments containing the individual sites indicates different affinities for binding. The two IL-6 responsive elements are homologous with the Type B elements identified in other acute phase proteins. The T-KG promoter did not contain any Type A elements.

R 108 A PROBABLE MECHANISM OF NEUROFILAMENT (NF-M) TANGLE FORMATION IN ALZHEIMER'S DISEASE, G. Fasman, M. Hollósi, L. Ürge, A. Perczel, J. Kajtár & L. Ötvös, Jr., Department of Biochemistry, Brandeis University, Waltham, MA, Institute of Organic Chemistry, Eötvös University, Budapest, Hungary, The Wistar Institute, Philadelphia, PA

Neurofibrillary tangles are a common feature of neurodegenerative diseases, and are organized in a cross-β-pleated sheet structure. Fragments of one of the three neurofilaments, the NF-M subunit, the 13mer sequence KSPVPKSPVEEKG (NF-M13), which is repeated contiguously 6 times, was synthesized [as well as the 17mer (EEKG-NF-M13) (NF-M17)]. The phosphorylated species, at Ser^2, Ser^7 and at $Ser^{2,7}$, were also produced. Circular dichroism (CD) studies and CD titrations with Al^{3+} and Ca^{2+} were performed. The unphosphorylated 17mer was random in H_2O and did not change conformation on the addition of metal ions. The three phosphorylated 17mer species all had an altered conformation in TFE. Upon titration with Al^{3+} or Ca^{2+}, monitored by CD, a conformational change occurred, which upon deconvolution of the CD spectra of the titrated phosphorylated fragments, suggested a high content of β-sheet structure. The original CD spectra of the phosphorylated species showed a predominantly random-like character, which on deconvolution yielded a high content of the Poly-L-Proline II structure. A mechanism of the metal ligand binding to the phospho-17mers is proposed which may be relevant to the formation of neurofilament tangles in Alzheimer's disease. Supported by NSF grants and an OTKA Hungarian grant.

R 109 THE ROLES OF ADP^{2-} AND Mg^{2+} IN CONTROL STEPS OF PHOSPHOGLYCERATE KINASE, Heidi C. Graham and Robert J.P. Williams, Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QR, England.
^{1}H-NMR measurements were made of solutions of yeast phosphoglycerate kinase containing the nucleotide, ADP, and Mg^{2+} in varying concentrations in order to investigate the affect that the metal ion has on the mode of ADP binding to the enzyme. A preliminary study of adenosine binding to PGK was made in order to be sure of the nature of the adenine site. From the change in chemical shifts of the 'basic patch' histidine resonances (H62, H167 and H170), the nucleotide C8-H, C2-H and C1'-H resonances and resonances 40 and 41 (assigned to Thr 373 and Thr 375 in the hydrophobic (catalytic) site) it is apparent that there are at least two ADP binding sites on the enzyme:- one at the hydrophobic (catalytic) site and one at the electrostatic site. A comparison of the results for ADP and ATP reveals differences due to the differential binding of the phosphate groups. The presence of Mg^{2+} results in further differences being observed. The data suggest that the primary binding site of ADP, in the absence of Mg^{2+}, involves electrostatic interactions between the diphosphate chain of the substrate and the 'basic patch' region of the N-terminal domain. In the presence of >1:1 ratio of Mg^{2+}:ADP, however, the primary binding site involves predominantly hydrophobic interactions between the adenosine moiety and the catalytic site, with the secondary binding occuring at the electrostatic site (presumably by competing for ADP). It is suggested that -helix XII moves differentially on binding ADP, Mg.ADP, ATP or Mg.ATP, consistent with Mg^{2+} assisting the transfer of the -phosphate of ATP to 3-PG during catalysis.

R 110 DISTANCES IN cAMP-RECEPTOR PROTEIN-DNA COMPLEX MEASURED BY FLUORESCENCE ENERGY TRANSFER, Tomasz Heyduk and James C. Lee, University of Texas Medical Branch, Department of Human Biological Chemistry and Genetics, Galveston, Tx 77550
Protein-induced DNA bending is one of the consequences and elements of recognition of specific binding site on DNA by cAMP receptor protein from E.coli. In this study the symmetry of CRP induced DNA bend was monitored by fluorescence resonance energy transfer (FRET) measurements. A 26 bp DNA fragment was labeled with fluorescent acceptor at the 5' end either upstream or downstream from the binding site. Distances between protein trp residues and the acceptors on these two DNA samples in the protein-DNA complex were compared at several salt concentrations. Under all conditions and within experimental error, the distances between the upstream or downstream end of DNA and CRP are the same. This distance is salt concentration dependent. The distance between the ends of a DNA molecule was estimated by measuring FRET between the fluorescent donor and acceptor attached to the ends of the same DNA molecule. A significant increase in FRET was observed when CRP was bound to DNA. This implies a shortening of the distance between these ends. The magnitude of the change indicates that the measured end to end distance changes from about 86 Å in free DNA to about 75 Å in CRP-DNA complex. Such a change corresponds to a CRP induced DNA bend of about 48 Å in radius.

R 111 LIMITED PROTEOLYSIS OF THE MEMBRANE-BOUND PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE (PAM) PRECURSOR RELEASES TWO CATALYTIC DOMAINS THAT CAN FUNCTION INDEPENDENTLY, E. Jean Husten and Betty A. Eipper, Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore MD 21205.
Amidation of glycine extended peptides involves the sequential action of two enzymes derived from the bifunctional PAM precursor. Peptidylglycine α-hydroxylating monooxygenase (PHM) catalyzes the copper, molecular oxygen and ascorbate dependent α-hydroxylation of the COOH-terminal glycine. At physiological pH this intermediate is subsequently converted to the α-amidated product by peptidyl-α-hydroxyglycine α-amidating lyase (PAL); at alkaline pH, this reaction proceeds spontaneously. The major forms of PAM mRNA in adult rat atrium encode two membrane associated PAM precursor proteins differing by the presence or absence of a 105 amino acid segment between the PHM and PAL domains. Rat atrial membranes were washed with 100 mM Na_2CO_3 (pH 11.5) to remove peripherally associated proteins. PAM proteins were visualized on Western blots using antibodies to peptides in the PHM, PAL, and cytoplasmic domains of the precursor. PAM proteins of molecular weights 105,000 and 120,000 were recognized by all three antibodies. The washed membranes were digested with various concentrations of trypsin for 5 minutes at 37°C and membranes were separated from solublized proteins by centrifugation. The activity of both PHM and PAL was retained over a broad range of trypsin concentrations (0.001 to 1 μg trypsin/μg atrial membrane protein). With trypsin concentrations in excess of 1% (w/w) of the concentration of atrial membrane protein, most of the PHM and PAL activity was recovered in the soluble fraction. When the atrial proteins released by trypsin were applied to a D-Tyr-Trp-Gly-Sepharose column, the PAL domain was recovered in the flowthrough, while the PHM domain bound to the resin. Western blot analysis demonstrated the separation of a 37 kDa PHM domain and a 43 kDa PAL domain. The release of similar sized, catalytically active PHM and PAL domains was obtained with several other endoproteases. The PHM activity of the intact PAM precursor exhibited a pH optimum of 4.5, with α-N-acetyl-Tyr-Val-Gly; upon trypsin treatment the pH optimum shifted to pH 6.0. The PAL pH optimum with α-N-acetyl-Tyr-Val-α-OH-Gly was pH 5.5 with or without digestion. Supported by DK-32949, DA-00098 and DK-08504.

R 112 IDENTIFICATION OF REGIONS WITHIN THE HUMAN FcERI ALPHA SUBUNIT WHICH PREVENT CELL-SURFACE EXPRESSION, Helen Kado-Fong and Jarema Kochan, Department of Molecular Genetics, Hoffmann La Roche, Nutley, NJ 07110

The human high affinity receptor for IgE (FcERI) is composed of at least two subunits, alpha and gamma. The alpha subunit, which contains the binding domain for IgE, is expressed on the surface of transfected COS cells only when co-expressed with the gamma subunit. We have used site directed mutagenesis to identify the regions of the FcERI alpha subunit which prevent cell surface expression and to characterize the interactions between the alpha and gamma subunits. The effects of these structural changes were analyzed in transfected COS cells by FACS analysis, IgE-binding, and immunoprecipitation experiments. We have identified at least three different regions of alpha which act to prevent cell surface expression. These regions act synergistically and/or independently, suggesting a novel mechanism of intracellular retention. Furthermore, we have identified a crucial residue in the FcERI alpha subunit which is involved in the interaction with the gamma subunit.

R 113 ANTIBODIES AND HETEROLOGOUS EXPRESSION AS PROBES OF CFTR STRUCTURE AND FUNCTION. N. Kartner, T.J. Jensen, O. Augustinas, Shizhang Sun, A.L. Naismith, L.-C. Tsui, J.M. Rommens, C.E. Bear and J.R. Riordan, The Hospital for Sick Childern and the University of Toronto, Toronto, Canada M5G 1X8.

The product of the gene which is defective in cystic fibrosis (CFTR) is thought to be a regulator of chloride conductance in epithelial cell membranes. To further our understanding of the structure and function of CFTR we have developed a series of polyclonal sera and monoclonal antibodies directed to a host of epitopes that include the termini and cytoplasmic domains of the protein. Western blotting of human cell membranes yields a 170 kd band that correlates with quantitative PCR data for CFTR expression. N-glycanase treatment increases the mobility of this band in SDS-PAGE to ca. 140 kd. Introduction of CFTR into Sf9 insect cells with recombinant baculovirus produced a 140 kd band in Western blots. Identification with all CFTR-specific antibodies suggests that the protein is intact, but unglycosylated. Moreover, iodide-125 transport studies indicate that expression of this protein correlates with a cAMP stimulated anion efflux which is not seen in control cells. Functional expression in this heterologous system suggests that CFTR itself may act as a regulated anion channel and that the carbohydrate moiety normally present in human epithelial cells is unnecessary for its function. Furthermore, it suggests that such heterologous systems will be useful for studying the structure, function and pharmacology of CFTR. Purification of CFTR from Sf9 cells and reconstitution expreriments are underway.

R 114 **PROCARYOTIC AND EUCARYOTIC EXPRESSION OF A MULTIPLE HYDROPHOBIC HUMAN CYTOMEGALOVIRUS MEMBRANEPROTEIN** **Robert Lehner and Michael Mach, Institut fuer Klinische und Molekulare Virologie, Loschgestr.7, 8520 Erlangen, FRG.**

As a member of the herpesvirus group, human cytomegalovirus (HCMV) is enveloped by a lipid bilayer which serves as a matrix for several membrane associated viral surface proteins. Most of these membrane proteins fit the type of glycoproteins with the bulk of the polypeptide at the outside of the virus particle and carrying the sugar residues, with a short transmembrane domain and an anchor sequence in the viral matrix. We have isolated a gene coding for a quite different type of membrane protein by immunological screening of a lambda gt11 cDNA library. The deduced amino acid sequence revealed eight markedly hydrophobic motifs which supposedly are membrane spanning domains. The aminoterminal part as well as the carboxyterminal tail are composed of hydrophilic amino acids. These features suggest a topology like the Epstein Barr virus latent membrane protein, which has been shown to serve as a target for cytotoxic T-lymphocytes. Parts of the open reading frame (ORF) have been expressed as fusion proteins in bacteria. The entire gene has been expressed in the eucaryotic baculovirus system. The different recombinant proteins are the prerequisite for further examination of biological and immunological functions of the protein.

R 115 ROLE OF N-LINKED CARBOHYDRATE CHAINS IN MEDIATING THE IMMUNOADHESION PROPERTIES OF THE HUMAN CD2 RECEPTOR. M. Luther, M. Knoppers, E. Neidhardt, S. Kandeckar, K. Gordon, M. Concino, P. Schimke, M. Francis, E. Rabin, E. Reinherz* and M. Recny. PROCEPT, Inc., 840 Memorial Drive, Cambridge, MA 02139, and the *Laboratory of Immunobiology, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Human CD2 (T11) is a T-lymphocyte glycoprotein receptor that facilitates adhesion between T-cells and antigen presenting cells by binding to the surface antigen LFA3. The extracellular region of CD2 consists of two polypeptide domains (residues 1-105, 106-182) containing three Asn-linked glycosylation sites, one within the amino-terminal domain (Asn^{65}) and two within the carboxy-terminal domain (Asn^{117}, Asn^{126}). All of the adhesion functions associated with LFA3 binding are associated with the first polypeptide domain (residues 1-105), based on structural and functional characterization of a recombinant soluble CD2 glycoprotein receptors expressed in eucaryotic cells. We have examined the role of the single Asn-linked glycan located within the immunoadhesion domain (residues 1-105) in CD2-LFA3 interactions. The native sCD2 glycoprotein receptor can be successfully renatured following denaturation in chaotropic agents without loss of functional properties. However, enzymatic removal of the glycan moiety at Asn^{65} completely disrupts native cell surface epitopes critical for CD2-LFA3 binding. Loss of this single carbohydrate moiety alters the conformational stability of sCD2 as measured by changes in the protein fluorescence spectrum for the deglycosylated receptor compared to the native receptor, and deglycosylated sCD2 cannot be functionally renatured. Transmembrane CD2 receptor containing a single Asn^{65}-Gln^{65} mutation can be expressed on the surface of COS cells but does not display functional immunoadhesion properties, does not bind to monoclonals that define critical activation/adhesion epitopes, nor does it bind to LFA3. sCD2 polypeptides can also be expressed in E. coli but cannot be functionally renatured, presumably due to the absence of the N-linked glycan at Asn^{65}. We propose that the carbohydrate chain at Asn^{65} plays a critical role in facilitating both proper folding of the CD2 immunoadhesion domain and maintaining the conformational structure required for LFA3 binding.

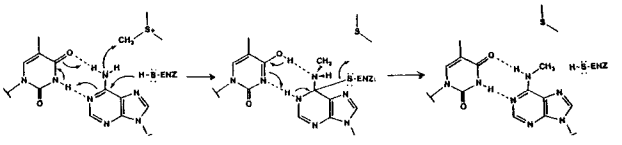
R 116 DIPHTHERIA TOXIN AS A VECTOR FOR INTRODUCING PEPTIDES INTO THE CYTOSOL. Inger H.Madshus, Harald A.Stenmark, Kirsten Sandvig and Sjur Olsnes. Department of Biochemistry, Institute for Cancer Research at the Norwegian Radium Hospital, Montebello 0310 Oslo 3, Norway.

Certain bacterial and plant toxins are the only established examples of external proteins that are able to enter the cytosol. We have investigated the possibility of utilizing diphtheria toxin as a vector for introducing oligopeptides into the cytosol of Vero cells. Peptides were fused by recombinant DNA technology with the N-terminal end of a low toxicity diphtheria toxin mutant. Genes encoding the heterologous proteins were transcribed and translated **in vitro**. The modified toxins were bound to cells, which were then exposed to low pH in order to induce translocation. Under these conditions all of the peptides tested were translocated to the cytosol along with the enzymatic part (A-fragment) of the toxin. We currently consider the possibility of using nontoxic variants of diphtheria toxin or related toxins to introduce peptides into the cytosol to elicit a HLA class I-restricted immune response.

R 117 COVALENT INTERMEDIATE IN *ECO* RI DNA METHYLTRANSFERASE INVESTIGATED BY SITE DIRECTED MUTAGENESIS.

K. Maegley, D. Shoemaker and N.O. Reich.
University of California Santa Barbara, CA 93106

Eco RI DNA Methyltransferase catalyses the methylation of the second adenine in the recognition sequence GAATTC, at the N6 position. Our protein modification studies have implicated cysteine 223 as an important residue in the chemical mechanism of the methyltransferase (JBC 265, 17713-17719). Cytosine N4 and adenine N6 methyltransferases are two unrelated classes of enzymes that catalyze similar reactions and our analyses show homology between both types of methyltransferases in the region of cysteine 223. Based on these results and the fact that the N6 of adenine is a poor nucleophile, we have proposed a chemical mechanism involving a covalent enzyme-DNA intermediate (see below). Three mutants have been constructed to test the proposed mechanism; ser223, ala223 and gly223. Characterization of these mutants will be described.



R 118 Electrostatic Field Around Cytochrome *c*::Theory and Energy Transfer Experiment. James B. Matthew, Theodore G. Wensel††, Claude F. Meares†, John J. Wendoloski% and Scott H. Northrup*, ICI Pharmaceuticals Group, Structural Chemistry, Wilmington, DE 19897, Chemistry Departments of *Tennessee Technological University, Cookeville, TN , †University of California Davis, CA ,% E. I. Dupont and ††Baylor College of Medicine ,Houston ,Texas.

Energy transfer in the "rapid diffusion" limit from electronically excited terbium(III) chelates in three different charge states to horse heart ferricytochrome *c* has been measured as a function of ionic strength. Theoretical rate constants calculated by numerical integration of the Forster integral (containing the Poisson-Boltzmann-generated protein electrostatic potential) have been compared with these experiments to evaluate the accuracy of protein electrostatic field calculations at the protein solvent interface. Two dielectric formalisms were used; a simple Coulombic/Debye-Huckel procedure and the finite difference method of Warwicker and Watson (1982, J. Mol.. Biol. 157,671-679) that accounts for the low dielectric protein interior and the irregular protein-solvent boundary. Good agreement with experiment is obtained and the ionic strength dependence of the reaction is successfully reproduced. The sensitivity of theoretical rate constants to the choices of effective donor sphere size and the energy transfer distance criterion has been analyzed. Electrostatic potential and rate constant calculations were carried out on sets of structures collected along two molecular dynamics trajectories of cytochrome *c*. Protein conformational fluctuations are shown to produce large variations in the calculated energy transfer rate constant. We conclude that protein fluctuations and the resulting transient structures can play significant roles in biological or catalytic activities that are not apparent from examination of a static structure. For calculating protein electrostatics large scale low frequency conformational fluctuations, such as charged sidechain reorientation, are established to be as important as the computational methodology for incorporating dielectric boundary effects.

R 119 MECHANISTIC STUDIES OF CYCLOPHILIN BY SITE DIRECTED MUTAGENESIS, H.E. McElroy, G.W. Sisson, D. DeLisle, S.A. Fuhrman and J.E. Villafranca, Protein Engineering Laboratory, Agouron Pharmaceutical, Inc., La Jolla, CA 92037

Cyclophilin has been implicated in the processing of nascent proteins by isomerization of normally slowly rotating prolyl-peptide bonds from the cis to the trans configuration which is more commonly required for native protein conformation. The exact nature of the prolyl-peptide isomerization mechanism is unknown. Recently, a non-covalent strain mechanism has gained favor over that which requires a covalent intermediate for catalysis. It has been shown by the activity vs. pH profile that activity is lost by titration of a residue with a pKa of 4.9, suggesting the requirement of an ionized acid residue for full activity. We propose a mechanism for stabilization of the isomerization transition state by electrostatic prohibition of peptide bond resonance by an ionized acid residue. In this way, facile rotation around the peptide bond can take place by interrupting this resonance without the requirement of a covalent tetrahedral intermediate. To investigate this proposal, conserved acid residues have been mutated to remove their charge and the effect on activity assessed. The effects of these mutations range from zero to 13-fold reduction in activity as measured by kcat/Km values.

R 120 THE EFFECT OF PHOSPHORYLATION AT SER-6 ON THE CONFORMATION OF ATRIAL NATRIURETIC PEPTIDE (ANP), Jennifer Reed, Claus W. von der Lieth, William E. Hull and Volker Kinzel, Institute of Experimental Pathology, German Cancer Research Center, Heidelberg, Germany

Atrial natriuretic peptide, a 28 residue peptide containing a single disulfide bridge, is a potent hormone regulating blood pressure. The peptide contains the sequence R-R-X-S distinguishing phosphorylation sites recognized by cAMP-dependent protein kinase, and can be phosphorylated at Ser^6 directly adjacent to the S-S bridge; such phosphorylation alters the biological activity of ANP. We have examined the phosphorylated and non-phosphorylated forms of ANP using circular dichroism and ^{31}P-NMR to determine whether phosphorylation is accompanied by a change in the solution conformation of the peptide. No change in the dichroism associated with either main chain conformation or with aromatic amino acids could be observed; ^{31}P-NMR spectra ruled out any type of permanent complex between the serine phosphate and the positively charged guanidino groups. The single detectable change is in the near UV CD, where signals arising from the disulfide are significantly altered in the phosphorylated peptide. The CD data are consistent with a change in the disulfide dihedral from at or near the degeneracy point in ANP (90-107^0) to around 45-70^0 in Ser^6-P-ANP. Molecular modelling has been used to assesss what effect such a rotation might have on the position of those side chains necessary for biological activity.

R 121 THE CYSTIC FIBROSIS GENE PRODUCT (CFTR) AS A METABOLICALLY REGULATED EPITHELIAL ANION CHANNEL? J.R. Riordan, Z. Grzelczak, T.J. Jensen and N. Kartner,
The Hospital for Sick Children and the University of Toronto, Toronto, Canada M5G 1X8
Electrophysiological and ion transport studies had shown a defective anion conductance in epithelial cells of the tissues affected in cystic fibrosis. This conductance which is normally activated by the action of protein kinase A (PKA) fails to respond to the action of this enzyme in CF. Hence, when the CF gene was identified and characterized by genetic means, the specificity of its expression in these same epithelial tissues together with the sequence prediction of a large membrane glycoprotein product seemed to at least superficially fit with the functional defect. However, on closer examination CFTR was seen to be a member of a super family (TM6-NBF) of membrane transporters. Its predicted structure suggested the presence of two membrane-associated domains (TMs) and two nucleotide binding folds (NBFs), characteristic of other members of this super family and an additional 'R-domain', rich in clusters of residues with charged side chains and putative PKA phosphorylation sites. Thus the genetic evidence of mutations in an apparent transporter and physiological evidence of a defective channel presented somewhat of an enigma. To obtain evidence that CFTR is indeed likely to be involved in epithelial chloride conductance, we have used antibodies to demonstrate its localization to the apical membranes of these cells and shown that a very highly conserved homologue is strongly expressed in a specialized salt secreting organ, the rectal gland of the shark, *Squalis acanthius*. Finally, we have shown that the heterologous expression of human CFTR in the baculovirus-insect cell system results in the appearance of a PKA activated anion conductance with properties very similar to that endogenously expressed in normal epithelial cells. We suggest that CFTR may be an actively regulated anion channel in which both nucleotide binding, and possibly hydrolysis, and the phosphorylation state of the R-domain play a role in the regulated translocation of anions across the plasma membrane. (Supported by the Canadian and U.S. CF Foundations and the NIDDK).

R 122 DIFFERENT FORMS OF HUMAN RECOMBINANT INTERLEUKIN-2 PRODUCED IN ESCHERICHIA coli, Frank Riske, Zafeer Ahmad, Joseph Revello, Lewis Apito, Yu-Ching Pan, Fazal R. Khan, Bioprocess Department, Hoffmann-La Roche, Inc., Nutley, NJ 07110
Human recombinant interleukin-2 (rIL-2), a 15 kd lymphokine and potent immunomodulator, is currently being evaluated as an anti-cancer agent in clinical trials. Two alternate forms of this protein present in the solubilized cell pellet were purified by reverse phase HPLC (RP-HPLC) and characterized. One alternate form exhibited a longer retention time on RP-HPLC then rIL-2, but had the same molecular weight (SDS-PAGE) and peptide map following trypsin digestion. Reduction of rIL-2 resulted in a protein with about the same retention time as this form indicating that this protein may be reduced rIL-2. A second alternate form of rIL-2 exhibited a shorter retention time on RP-HPLC lowered mobility in SDS-PAGE, and a pI which was approximately 1 unit less than rIL-2. This material had similar bio-activity as rIL-2, but showed a different peptide map upon trypsin digestion. Characterization of differences between rIL-2 and this protein (amino acid or other modifications) is underway.

R 123 EXPRESSION OF MITOCHONDRIAL P450 MONOOXYGENASE ENZYMES IN YEAST, TOSHIYUKI SAKAKI, MEGUMI AKIYOSHI-SHIBATA, YOSHIYASU YABUSAKI AND HIDEO OHKAWA, Biotechnology Laboratory, Takarazuka Research Center, Sumitomo Chemical Co., Ltd., Takarazuka, Hyogo 665, Japan.
Mammalian P450 monooxygenases consisting of NADPH-ferredoxin reductase, ferredoxin and cytochrome P450 localize in mitochondrial inner membrane. These enzymes are synthesized in cytoplasm as precursors, and then targeted into the mitochondria by their N-terminal signal sequences. We have constructed a number of expression plasmids for precursor, mature, and modified forms of bovine adrenal NADPH-adrenodoxin reductase (ADR), adrenodoxin (ADX) and rat hepatic $P450_{LMT25}$ for production of these enzymes in *Saccharomyces cerevisiae*. Substitution of the native signal sequences of these enzymes with that of yeast cytochrome *c* oxidase subunit IV (COXIV) succeeded in targeting of these enzymes into yeast mitochondria and processing to the mature forms. ADX with the COXIV signal was particularly overproduced and processed to the mature form, whereas the precursor of ADX was produced at an extremely low level. Thus, the use of signal sequences of yeast enzymes seemed to be important for heterologous expression of the mitochondrial enzymes. ADR, ADX and $P450_{LMT25}$ produced in the yeast constructed an electron-transport chain *in vitro* to exhibit both 5β-cholestane-3α,7α,12α-triol 27-hydroxylation and 1α-vitamin D_3 25-hydroxylation activities.

This work was performed as a part of the Research and Development Project of Basic Technologies for Future Industries supported by NEDO (New Energy and Industrial Technology Development Organization).

R 124 HUMAN ERYTHROCYTE SPECTRIN ELASTICITY INFERRED FROM CONFORMATIONAL ANALYSIS, Betty W. Shen, Fred Stevens, and Ursula Luthi, Argonne National Laboratory, Argonne, IL 60439

Spectrin, the major component of the membrane skeleton, can exist in extended and condensed configurations, both in the intact skeleton and as isolated molecules, suggesting that the transition between them could be the principal source of membrane elasticity. We analyzed the secondary structure predicted for the human alpha-spectrin from the sequence of its cDNA (Sahr, et al. JBC 265, 4434, 1990) using the alignment of homologous repeats proposed by Moon and McMahon (JBC 265, 4427, 1990) and a modified Chou and Fasman algorithm. The protein consists mainly of short amphiphilic helices of 10-30 residues connected by segments with high beta or beta-turn potential. The distribution of amino acids favors antiparallel association between consecutive helices, leading to stable domains of 3 or 4 helices. Most of these domains contain a small number of exposed hydrophobic side chains at their surface as well as charged residues. We, therefore, suggest that the spectrin molecule could be composed of domains of short amphiphilic helices stabilized by intra-domain hydrophobic interactions. Furthermore, both hydrophobic and electrostatic interactions between the surfaces of neighboring domains could stabilize the condensed configuration. Work supported in part by grant #HL 33254 from the National Institutes of Health (BWS) and by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract W-31-109-ENG-38.

R 125 STRUCTURE AND STABILITY OF THE THERMOSTABLE Sac7 DNA BINDING PROTEIN. John Shriver, Lingshi Qiu, James McAfee, and Steve Edmondson. Department of Medical Biochemistry, School of Medicine, Southern Illinois University, Carbondale, IL 62901.

We have initiated physical studies of the Sac7 DNA binding protein from the extreme thermophile *Sulfolobus acidocaldarius*. The protein has an unusually large number of attributes which make it suitable for physical and structural studies of thermostability and protein-DNA binding. The protein has a molecular weight of 7000, and contains no cysteine and a single tryptophan whose fluorescence is quenched by approximately 80% upon binding of the protein to poly(dGdC). Analysis of the fluorescence binding data indicates tight binding at low ionic strength with a K_a of 10^8 M^{-1} The protein preferentially binds non-specifically to double-stranded DNA and most likely serves to stabilize the duplex at the growth temperature of 85 °C. As might be expected, the protein is highly thermostable and at pH 7.0 it does not unfold below 100 °C. At lower pH the unfolding has been shown to be reversible and the energetics of unfolding have been studied by differential scanning calorimetry. At pH 2.7 the protein unfolds with a midpoint at 63 °C. The DSC endotherm can be fit with a van't Hoff enthalpy of 34.2 kcal/mole and a calorimetric enthalpy of 34.9 kcal/mole, indicating a non-aggregating, single domain protein. Collection and analysis of 2D NMR data is presently in progress and preliminary data will be presented. (Supported by the Biotechnology Research Development Corporation)

R 126 CORRELATION BETWEEN MOBILE ELEMENTS BY MOLECULAR DYNAMIC SIMULATION AND FRAGILE SITES IN IRRADIATED (Val^5)ANGIOTENSIN II. M. St-Louis, L. Thauvette, M. Bertrand* and M. Potier, Service de Génétique Médicale, Hôpital Ste-Justine, and *Département de chimie, Université de Montréal, Montréal, Québec, Canada H3T 1C5

Pure proteins irradiated with a ^{60}Co source produce defined radiolysis fragments. Such fragmentation patterns are consistent with a mechanism implicating random energy deposition along the peptide chain, translocation and final dispersion at defined "fragile" sites. In the catalytic subunit of aspartate transcarbamoylase, fragile sites were localized in mobile loops composed of more than 4 amino acid residues. Molecular dynamic simulation localizes mobile elements in protein structures. To test hypothesis correlating structural mobility and fragmentation sites in proteins, we choose (Val^5)angiotensin II as a model peptide. Molecular dynamic simulation using the Drieding II force field (BioDesign Inc.) on a model of the energy-minimized peptide identified a highly mobile N-terminal Asp residue and a fragile bond between residues Val^5 and His^6. Mass spectrometry of peptide (m/z 1032) and radiolysis fragments were consistent with breaks at these predicted sites: m/z 917 and 643. Structural mobility as predicted by molecular dynamic simulation correlates well with fragmentation sites under ionizing radiation exposure.

R 127 ACTIVITY AND STABILITY OF A PROTEOLYTICALLY TRUNCATED FORM OF GLUCOAMYLASE FROM *ASPERGILLUS NIGER*, Bjarne Stoffer, Peter K. Busk, and Birte Svensson, Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark.

Fungal glucoamylases possess a common structural organization consisting of a large catalytic domain linked at either the N- or C-terminus to a small granular-starch binding domain through a highly O-glycosylated segment. Glucoamylase G1 (aa 1-616) from A. niger consists of a catalytic domain (aa 1-440), a 72 residue long linker region and a granular-starch binding domain (aa 513-616) (1). Glucoamylase G2, a naturally truncated form of G1 (aa 1-512) is unable to adsorb onto and degrade granular-starch, but has the same activity as G1 towards soluble substrates (2). Furthermore, it has been shown that both the N- and C-terminal domains of A. niger glucoamylase can bind oligosaccharides specifically (3).

With the purpose to examine *i*) the architecture of glucoamylase, *ii*) the possible cooperation between the domains and *iii*) the structural and functional integrity of the individual domains, the G1 form was subjected to limited proteolysis. Conditions were established after screening for protease and incubation temperature under which subtilisin Novo produced a catalytically active fragment that extends into the O-glycosylated area. The thermostability was slightly reduced compared to G1 and G2 and the activity towards maltose was comparable to that of G1 and G2. The N-terminal sequence was identical to G1 and G2, amino acid composition of the entire fragment and of the relevant cyanogen bromide peptide suggested the C-terminus to be near aa 450-470. This is compatible with recent work by others where a granular-starch binding fragment of aa 471-616 have been isolated (4).

The present results indicate that the linker region and the C-terminal domain only have minor influence on the activity and stability of the N-terminal domain suggesting that the enzyme has an elongated structure.

References: (1) Svensson, et al. (1983), Carlsberg Res. Commun. **48**, 529. (2) Ueda (1981), TIBS **6**, 89. (3) Savel'ev et al. (1990), Biokhimiya **55**, 39. (4) Belshaw and Williamson (1990), FEBS Lett. **269**, 350.

R 128 CHOLERA TOXIN DETOXIFICATION . Shousun C. Szu and Rajesh Gupta LDMI, Natl. Institute of Child Health and Development, Natl. Inst. of Health, Bethesda, MD

Cholera toxin (CT) composed of five binding units (B-subunit) and one enzymatic unit (A-subunit) is too toxic for human vaccines. Formalin inactivated CT reverts after storage and in vivo. Other methods for inactivating bacteria toxin were evaluated: glutaraldehyde, hydrogen peroxide, carbodiimide and heating at 60° C to prepare procholeragenoid. CT treated by these methods were studied by circular dichroism for conformational alteration and by amino acid analysis. The biological activity of CT was assayed with Chinese hamester ovary cells. The antigenicity of CT after treatment was studied by reaction with hyperimmune anti-CT serum in immunodiffusion.

All preparations retain the antigenicity when reacted with anti-CT serum. CT treated with dilute glutaraldehyde was least toxic (~1/500 reduction).

R 129 FLEXIBILITY OF ENZYME ACTIVE SITES REQUIRED FOR FULL EXPRESSION OF ACTIVITY. Chen-Lu Tsou, National Laboratory of Biomacromolecules, Academia Sinica, Beijing, China.

Previous studies on the conformational and activity changes of a number of enzymes during guanidine denaturation showed that inactivation of these enzymes occurs before noticeable unfolding. It is proposed that the active sites of these enzymes are situated in limited and flexible regions of the molecules. That the initial phase of rapid inactivation is not due to an inhibition by the denaturating agent has recently been shown for D-glyceraldehyde-3-phosphate dehydrogenase by studying thermal denaturation. It has been reported that inactivation of lactate dehydrogenase paralleled its unfolding. This is now shown to be the case only during an initial phase and further unfolding occurs after complete inactivation. Lactate dehydrogenase can be stabilized in ammonium sulfate or by crosslinking of the subunits with glutaraldehyde and the stabilized enzyme has about 2/3 its original activity. With increasing concentrations of guanidineHCl the enzyme was first activated back to its original level, followed by complete inactivation and then conformational changes as detected by CD and fluorescence changes. It is suggested that the stabilized enzyme could have a more rigid structure at the active site which is responsible for the activity decrease and guanidine reactivates by restoring active site flexibility which may well be required for the full expression of the catalytic activity of this dehydrogenase.

R 130 SOLUTION STRUCTURES OF ALA-X PEPTIDES FROM VIBRATIONAL ANALYSIS

Robert W. Williams† and Alfred H. Lowrey‡. †Department of Biochemistry, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd, Bethesda, MD 20814-4799. ‡Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C., 20375-5000. Raman spectra of aqueous solutions of alanine-X peptides, where X represents an amino acid with a neutral sidechain, show that the amide III band shifts systematically (over 20 cm^{-1}) to lower frequencies as the sidechain of the X amino acid becomes larger. Our *Ab initio* and normal mode calculations reproduce these shifts, and show that they probably result from differences in the average conformational preferences of these peptides in water (Williams et al., Biopolymers 30, 599-608, 1990). We reject two other possible explainations by showing that this frequency shift is not due to differences in either sidechain masses or vibrational force constants. Our previous results have been obtained using only a single scale factor of 81% on *ab initio* force constants calculated using the 4-31G basis set. Many of the force constants calculated in this way are relatively inaccurate and may have given rise to an artifactual shift in the calculated amide III frequencies. To investigate this possibility, we have developed a set of differential scale factors for *ab initio* force constants that mimic the effects of hydration on peptides unfolded in water. These scale factors yield accurate force constants. Frequencies calculated with this force field are accurate, and reproduce the experimentally observed shifts. These results have the following implications: 1) Peptides with only two amino acids exhibit a conformational preference for extended structures in water. 2) Small variations in the side-chain dependent conformational preferences can be measured using Raman spectroscopy. 3) These results may provide information that can be used to refine empirical force fields. This work was supported by the NCI Supercomputer Facility and USUHS grant C07147.

R 131 A MOLECULAR DYNAMICS ANALYSIS OF BACTERIOPHAGE T4 LYSOZYME,

Gregory E. Arnold# and Rick L. Ornstein+, Molecular Science Research Center, Pacific Northwest Laboratory*, P.O. Box 999, Mail Stop K2-18, Richland, Washington 99352.

A 200 psec molecular dynamics simulation of bacteriophage T4 lysozyme, an endoacetylmuramidase with 164 amino acid residues and no disulfides, has been analyzed and the nature of the atomic fluctuations and average structures are described. The analysis was carried out at 300 K and all crystallographic waters were included in the simulation. Relationships between protein secondary structural units and internal motions were studied by examining the positional fluctuations of individual helix, sheet, and loop structures. Temporal analysis of the trajectory versus potential energy, root mean square deviation, hydrogen bond stability, and radius of gyration suggests that the simulation was both stable and representative of the average experimental structure.

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R 132 ISOLATION OF A STABLE PROTEIN FOLDING INTERMEDIATE,

David Baker, Julie Sohl, and David Agard, Dept. of Biochemistry, UCSF, San Francisco, CA 94143.

The high cooperativity of protein folding normally obscures analysis of folding intermediates and the energetics underlying folding partial reactions. An unusual feature of the folding of α-lytic protease allows an in depth analysis of a single step in a folding pathway. In vivo studies have shown that a 166 amino acid pro region is required either in cis or in trans for the proper folding of the protease domain. To allow detailed mechanistic study, we have reconstituted the pro region-dependent folding of the mature protease in vitro. Denatured α-lytic protease will refold in the presence but not in the absence of the pro region. Furthermore, we have been able to trap what appears to be a stable folding intermediate by omitting the pro region in a refolding reaction. The intermediate lacks enzymatic activity and is stable for months in low ionic strength buffers, but rapidly folds to the active, native state upon addition of the pro region. The intermediate chromatographs as a single species on a sizing column and has a CD spectrum distinct from both native and denatured states. Two very different conformations of the protease domain--the native state and the intermediate--are stable indefinitely under identical conditions, and, in the absence of the pro region, there is no detectable interconversion. The existence of kinetically trapped states with extended lifetimes is clearly at odds with the hypothesis that the stable conformations of polypeptide chains are at global energy minima.

We have also found that the pro region is an exceptionally high affinity inhibitor of the native protease (K_i ~10^{-10} M). Taken together, these results suggest that the pro region functions by stabilizing a late folding transition state having native-like structure. Protein folding involves kinetic competition between reactions on and off the pathway leading to the native state. In principle, the pro region could function by reducing the rate of off-pathway folding reactions (as suggested for the "molecular chaperonins") or by increasing the rate of an on-pathway reaction. As the pro region promotes folding in the absence of irreversible off-pathway reactions, the latter possibility is almost certainly correct. Furthermore, the observation that the pro region interacts strongly with the native enzyme suggests that the rate-limiting folding transition state has a native-like conformation.

R 133 HOMOLOGY BETWEEN VIRAL AND MAMMALIAN NEURAMINIDASES. M.-J. Champagne and M. Potier, Service de Génétique Médicale, Hôpital Sainte-Justine, Université de Montréal, Montréal, Québec, Canada H3T 1C5

The framework structure of influenza virus (FLU) neuraminidase (NA) is made of 6 ß-sheets disposed around a pseudo-six-fold axis of symmetry. Each ß-sheet is formed of 4 anti-parallel strands. Comparison of primary structures of FLU NA and mammalian NA suggests common active site residues but the overall similarity between the mammalian and viral enzymes is weak. Hydrophobic cluster analysis (HCA, Gaboriaud et al., FEBS Lett. 224, 149-155, 1987), a powerful tool for comparing proteins with weak sequence homology, revealed patterns of hydrophobic clusters which are associated to ß-strands and sheets in the NA structure. The comparison of FLU NA sequences between various A and B strains which can have as little as 12 % identical amino acid residues constitutes a good test of the method. Computation of HCA similarity scores in hydrophobic clusters between viral strains showed that strands $ß_1S_1$, $ß_3S_1$ and $ß_4S_3$ are the most conserved among the FLU NA sequences. These same strands were also the most conserved in a model of human and rat NA structure. The localization of these hydrophobic residues on the 3-D structural model of FLU NA indicates that these strands maintain hydrophobic cores for ß-sheet packaging in NA. Therefore, we conclude that the viral and mammalian NA probably have similar ß-sheet topology and overall framework structure.

R 134 NMR STUDIES ON THE PARTIALLY FOLDED STATE OF GUINEA PIG α-LACTALBUMIN, C.-L. Chyan, J. Baum, Chemistry Department, Rutgers University, Piscataway, NJ 08855, C. Hanley, C. Dobson, Oxford University, Oxford, England & P. Evans, Cambridge University, Cambridge, England

NMR is used to characterize a protein, α-lactalbumin, that exists in a state that is intermediate between a native and unfolded form. From bulk hydrogen exchange measurements and 2D pH-jump experiments, regions of secondary structure have been identified in the partially folded form of the protein. In particular, all but approximately 30 protons exchange at a rate expected for a fully unstructured protein. The slowly exchanging subset is attributed to two native-like helices. Limiting structures, for the partially folded form on molecular dynamics calculations are proposed.

R 135 PROBABLE LONG-RANGE INTERACTIONS IN SMALL GLOBULAR PROTEINS. David G. Covell and George N. McGregor ASCL/PRI/FCRDC, Frederick, MD 21701

The compact native state of small single domain globular proteins represents a balance between local and non-local interactions. Idealized lattice models of C^{α} backbones can be used to explore non-local interactions. Starting from the protein's amino acid sequence and a randomly generated non-compact C^{α} chain, a simplified Monte Carlo algorithm is used to determine important non-local ($|I-J|>3$) residue-residue contacts. For the eight proteins studied, crambin (46aa), rubredoxin (52aa), ferredoxin (54aa), trypsin inhibitor (58aa), neurotoxin (62aa) and amylase inhibitor (74aa), ubiquitin (76aa) and HIV-1 protease (99aa), between 49 to 74% of native non-local interactions were found. Distance plots of the final structures indicated similarities with those of the native folded structures. All model backbones were within 6-7.2Å RMS from the native structures. These models provide starting conformations for constructing models with more complete atomic details.

R 136 NMR Characterization of the Ethanol Induced Partially Folded Form of Monellin

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Monellin is a sweet tasting protein composed of two dissimilar chains (A and B chains). X-ray results indicate that the A-chain is predominantly composed of β-sheet while the B-chain contains both α-helix and β-sheet (Kim et. al., Protein Engineering, **2**, *571-575*, 1989).

When placed in ethanol/water mixtures, the protein undergoes a transition from the native state to a partially unfolded form. Using one and two dimensional NMR spectroscopy, we are characterizing the structure of this partially folded form. 1D NMR hydrogen exchange studies in 50% ethanol show that a small number of amide protons (8-10) are protected from exchange. We have mapped these slowly exchanging NH residues using 2D NMR and found that they are located in residues 9-25 of the A-chain, the most hydrophobic region of the protein.

The A and B-chains of monellin were separated. The isolated A-chain shows no evidence of secondary structure in aqueous solution. However, in 50% ethanol the A-chain contains a small number of slowly exchanging amides (~2-3). We hypothesize that the difference in the number of slowly exchanging amides observed in the ethanol induced form of intact monellin and the single A-chain arises from the interactions of the A and B-chains. We are determining by NMR whether the structured regions of the ethanol induced form are native-like, or whether they rearrange into a loosened α-helix, as indicated from CD measurements.

R 137 MOLECULAR DYNAMICS SIMULATIONS ON THE Ha-*ras* ONCOGENE PRODUCT p21, C.K. Foley[1], L.G. Pedersen[1,2], P.S. Charifson[3], T.A. Darden[1], A. Wittinghofer[4], E.F. Pai[4], and M.W. Anderson[1], [1]Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, [2]Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599, [3]Cray Research, Inc., Research Triangle Park, NC 27709, [4]Max Planck Institut für Medizinische Forshcung, Abteilung Biophysik, Jahnstrasse 29, 6900 Heidelberg, FRG

We have performed molecular dynamics simulations on the GTP-bound form of cellular Ha-*ras* p21, on the G12V mutant and on the GDP-bound form of the cellular protein. The X-ray crystal structure (E.F. Pai, et al., EMBO J. **9**, 2351-2359 (1990)) of cellular Ha-*ras* p21 was used to derive aqueous simulation structures for these three proteins. Thr-35, from the p21 effector region for GAP (GTP-ase Activating Protein), dissociates from the magnesium ion in all three structures. However, many of the GTP/Mg contacts identified in the crystal structure remain intact throughout the length of the simulations. Comparisons of the simulation structures after > 100ps will be given.

R 138 AMINO ACID COMPLEMENTARITY AND THE SEARCH FOR RECEPTOR-BINDING SITES: NEW DATA AND OLD HYPOTHESES, Andrew E. Gabrielian, Engelhardt Institute of Molecular Biology, USSR Academy of Sciences, 117984 Moscow GSP-1, V-334, Vavilov str., 32

Over the last ten years, a number of hypotheses have been proposed to clarify the question concerning the structural relationships of complementary protein sites, e.g. sites of interaction between ligand and their receptor. Now it became possible to compare the experimentally mapped sequences of such sites and by this means directly estimate the predictive abilities of various hypotheses (see (1) for refs.). We have done such comparison for the number of receptor-binding and other protein-protein interaction sites. Our results do not supported the hypotheses which stated the complementarity of protein sites is dependent on complementarity of corresponding DNA strands. The results of our investigations and possible combined algorithms of protein-protein interaction sites prediction will be discussed.

1. Gabrielian A., Heymann S. (1990) Biomed. Sci., 1, 311-313

R 139 UNDERSTANDING STEREOSPECIFICITY IN ENZYME SUBSTRATE INTERACTION - A MOLECULAR DYNAMICS APPROACH ,
Indira Ghosh Astra Research Centre India. 18th Cross Road, Malleswaram, Bangalore-560003, India.

Stereospecificity, one of the key features in enzyme specificity towards the substrate, has been studied using Thermolysin, a proteolytic enzyme as a model. It is well known that L- and D-hydrophobic aminoacids inhibit the enzyme with equal potency, but the substrate selectivity manifests at the level of di- ,tri- and higher number of peptides. Molecular dynamics simulations were carried out using free energy perturbation method , where L-Phe amino acid was converted to D-Phe in the active site in the presence of water as a solvent . It was found that due to the assymetric nature of the active site L- Phe and D- Phe bind differentially. L-Phe prefers to bind towards the catalytic cleft whereas D-Phe binds away from it. The results provide a guideline for altering the stereospecificity by suitably engineering the active site .

R 140 EFFECT OF PACKING DENSITY ON CHAIN CONFORMATION: WHERE DOES SECONDARY STRUCTURE COME FROM? Lydia M. Gregoret and Fred E. Cohen, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA, 94143-0446

Recent lattice polymer simulations by Chan and Dill (PNAS, 87:6388-6392, 1990) suggest that compactness may be a significant driving force in the formation of secondary structure. The origin of the compacting force is postulated to be the hydrophobic effect. We have addressed the robustness of this conclusion for non-lattice polymers using a rotational isomeric model of proteins. In our model, structures are generated via a self-avoiding walk inside a constraining ellipsoid. Virtual bond angles and dihedral angles between sequential alpha-carbons are chosen at random from the distribution of angles seen in proteins. Excluded volume effects are explicitly incorporated. As in the cubic lattice studies, compactness is seen to influence secondary structure content. This effect is modest for densities comparable to native proteins but dramatic for chains which are approximately 30% more dense than native proteins. α-helical structure is common but β-sheet structure is rare. It appears that lattices can impart an organizational bias to compact chains which favors β-sheet structure. We also report the effects of chirality and constraining ellipsoid shape on secondary structure formation.

R 141 STRAIN ANALYSIS OF CONFORMATION CHANGE, Robert W. Harrison and Lawrence C. Andrews, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702.

It is important to understand conformational changes in the structures of biological macromolecules because this is often related to control of the biological activity. In order to understand this effect we must be able to describe it. Classical elasticity theory can be used to describe the changes in terms of strain. Elasticity theory contains two related concepts, stress and strain. Stress is the force per unit area and is in general unmeasurable; strain is the fractional displacement caused by application of a force. The strains seen with conformational change are generally classifiable as elastic (volume conserving) and inelastic. Strain analysis of a number of cases of conformational change shows that both types of strain are important. The analysis shows in some cases such as Catabolite Gene Activator Protein (CAP) where there is a simple hinge connecting two domains, there is a simple inelastic strain. Complicated conformational changes show more complex strains.

This research was sponsored by the National Cancer Institute, DHHS, under contract NO. N01-C0-74101 with ABL-Basic Research Program.

R 142 IDENTIFYING FAVORED CONFORMATIONS FOR AMINO ACID SEQUENCE PATTERNS BY SEARCHING KNOWN PROTEIN STRUCTURES, Leslie A. Kuhn, Michael E. Pique, Michael A. Siani, Elizabeth D. Getzoff, and John A. Tainer, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037

A fast search algorithm allows identification in 2–5 seconds of all occurrences of either a specific amino acid sequence or a sequence pattern in the Protein Data Bank of protein structures. Gaps of specified or unspecified length are permitted at any position in the sequence pattern, and allowed residue substitutions in each position may be specified in one of three ways: 1) only an exact match is allowed (appropriate when a sequence position is absolutely conserved), 2) a specific set of residues is considered equivalent (useful when a set of permitted mutations is known), or 3) any residue having a value in an amino acid substitution matrix meeting a cut-off criterion is considered equivalent (appropriate when the variability of a sequence position is not known). Once all occurrences of the sequence pattern in the Protein Data Bank have been identified, structural conservation between the related sequences can be analyzed by superimposing their 3-dimensional coordinates. Applications of this algorithm for locating sequence patterns in structural databases include identifying favored conformations of recognition motifs (e.g., Collawn et al., 1990); designing fusion protein linkers by identifying structurally favorable, naturally occurring, linker sequences; and predicting the structural effects of residue mutations. The development and implementation of this algorithm were supported by N.S.F. grant 8822385 (M.E.P., E.D.G., and J.A.T.) and N.I.H. postdoctoral training grant DK07022 and fellowship CA08939 (L.A.K.).

J. F. Collawn, M. Stangel, L. A. Kuhn, V. Esekogwu, S. Jing, I. S. Trowbridge, and J. A. Tainer (1990) *Cell*, Vol. 63, No. 5.

R 143 CHORUS: A PROTEIN TERTIARY STRUCTURE PREDICTION PROGRAM, B. Lee and H. S. Kang, Division of Computer Research and Technology, National Institutes of Health, Bethesda, MD 20892

Chorus is a computer program for protein folding. It performs a Monte Carlo procedure, which involves biased random sampling of dihedral angles, partially localized correlated moves, real space main-chain and side-chain packing, and a Metropolis selection/rejection process based on simple energy criterion. The bias used in random sampling takes the form of a probability density function, which is set up initially for each dihedral angle in a sequence-dependent manner. If each residue is assumed to be independent, the folding entropy calculated from this probability density function gives approximately twice the total entropy change upon unfolding estimated from the calorimetric data by Privalov and his coworkers. Secondary structures can be predicted using this probability function with an average of 52% success rate for a three-way classification scheme. The energy terms used are the hydrogen bond energy, which is computed by a simple Coulomb sum, and the hydrophobic energy, which is set to be proportional to the accessible surface area. When tried on 20-residue homopolymers, polyalanine forms the alpha-helix most easily, polyglycine does not form a helix, and polyleucine forms the alpha-helix more easily than the polyisoleucine. A beta-turn can be made at the Pro-Asn junction of the sequence V_7PNV_7 if the hydrogen bond energy is computed only between specific pairs. The results of the program run on the 36-residue avian pancreatic polypeptide will be reported at the meeting.

R 144 MODELING THE STRUCTURE OF THE LIGAND-BINDING DOMAIN OF THE HUMAN INTERLEUKIN-4 RECEPTOR AS A TEMPLATE FOR PREDICTING THE FOLDING PATTERNS OF THE HEMATOPOIETIN RECEPTOR SUPERFAMILY, Carl J. March, Toohyon Cho, Theresa Farrah, Charles T. Rauch, Sucha Sudarsanam and Subhashini Srinivasan, Departments of Protein Chemistry, Gene Expression and Information Systems, Immunex Corporation, 51 University Street, Seattle, WA 98101

The extracellular, ligand binding domain of the human IL-4 receptor has been expressed in a Baculovirus system using Sf-9 cells. A 32 KD protein was expressed at high levels, purified to homogeneity on a recombinant human IL-4 affinity column and characterized by SDS PAGE and N-terminal sequencing. The soluble receptor inhibited IL-4 binding to its cognate, membrane-bound receptor. Using peptide mapping by HPLC, PDMS and sequencing, the disulfide bonding pattern was established. CD, UV and fluorescent spectroscopy were utilized to assess elements of secondary structure. Primary sequence dependent computer-based predictive algorithms were utilized to predict secondary structure and combinatoric methods were used to predict the folded tertiary structure for the IL-4 receptor extracellular domain. This information was extrapolated to draw global conclusions regarding the general attributes of the folding pattern for extracellular, ligand-binding domains for members of the hematopoietin receptor gene superfamily.

R 145 A FOLDING MODEL OF NEISSERIA MENINGITIDIS CLASS 1 PROTEIN. Kate Runeberg-Nyman, Sarah Butcher, Liisa Holm* and Matti Sarvas, Molecular Biology Unit, National Public Health Institute, SF-00300, Helsinki, Finland, *Biotechnical Laboratory, Technical Research Centre of Finland, SF-02151, Espoo, Finland

Neisseria meningitidis can cause meningitis, a potentially lifethreatening disease. All virulent meningococci are surrounded by a polysaccharide capsule, the composition of which is the basis of the serogrouping. Capsular polysaccharide vaccines have been developed against four out of five of the serogroups, however, the capsule of the fifth serogroup, B, is poorly immunogenic and therefore not a suitable vaccine component. Other components of the bacterial cell surface to which bactericidal antibodies are directed include the outer membrane proteins. Monoclonal antibodies to one of the major proteins, Class 1 (P1) have been shown to be bactericidal, and protective in an animal model.

In order to get a better understanding of the make up of the protective epitopes we have cloned and sequenced class 1 genes from several different subtypes. By comparing the sequences of the different subtypes, as well as by doing a profile alignment of the deduced amino acid sequence of one of the P1 proteins with porin proteins from other gram-negative, bacteria we arrived at a folding model. The model is similar to one described for OmpF and is based on identifying amphiphilic regions which would form membrane-spanning beta-strands. Antibody mapping support the model.

R 146 PREDICTION OF PROTEIN TERTIARY STRUCTURE BY USING A CONTACT MAP

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The backbone conformation of a protein is well characterized by a contact map which plots residue pairs in contact on the two dimensional plane. Once a contact map of the native structure is given, the corresponding tertiary structure can be reproduced by the distance geometry technique with an accuracy around 1.5Å rms. The contact map is directly related to the contact number (NXi), defined as the sum of contacting residues with a given residue i. Contact number N14i (defined with the cut-off distance of 14 Å) has a good correlation with the sequence information, and the experimental N14-profile plotted along a chain is best predicted from the amino acid sequence [J.Biochem (1986) 100,1043]. For a tertiary structure prediction,we assumed the experimental N14-profile instead of a predicted one as well as knowledge about disulfide bonding pairs and the total number of contacts. An initial guess of the contact map was made as follows : All the products of N14 values of two residues were sorted, and a residue pair with the largest value was taken one by one until the number reached to the fixed total number. Then a corresponding tertiary structure was generated by a distance geometry program. The generated structure was again converted to a contact map and embeded to the final structure so as to correct inappropriate bond lengths and angles. The prediction was good for small proteins ; the rms values in comparison with the crystal structure were 2.96 Å for crambin and 3.29 Å for BPTI. In general, predictions for larger proteins are getting worse.

R 147 RECURRENT αβ LOOP STRUCTURES IN TIM BARREL MOTIFS SHOW A DISTINCT PATTERN OF CONSERVED STRUCTURAL FEATURES.

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Eight stranded parallel α/β barrels also termed 'TIM barrel motifs' from seven enzymes have been analyzed for specific sequence and structure motifs in loop regions. It is found that a subset of the loops connecting α helices to β-strands (αβ loops) adopt characteristic conformations and sequence patterns previously identified *in other* α/β proteins as $\alpha\beta_1$ and $\alpha\beta_3$ loops by Edwards *et al.* (1987, Protein Engineering 1:173). Each of the analyzed α/β barrel domains contains one or more $\alpha\beta_3$ loops. At least one of these always occurs in the first half of the protein sequence. $\alpha\beta_1$ loops seem to be less common, with only one copy per barrel or none at all. It is found in addition, that $\alpha\beta_3$ loops invariably lead into even β-strands, whereas generally $\alpha\beta_1$ loops lead into odd β-strands. A detailed comparison of chain segments containing the two characteristic loop types reveals furthermore that they differ mainly by the orientation and position of their β-strand portion. The first strand residues in $\alpha\beta_1$ and $\alpha\beta_3$ loops have their side chains oriented in opposite directions relative to the β-sheet surface, and display a different hydrogen bonding pattern to neighbouring strands in the sheet. Despite of these differences however, the α-helix portions of both loop types are oriented similarly relative to the sheet. These findings are correlated with the analysis of Lesk *et al.* (1989, Proteins 5:139) on residue packing in the barrel interior, and their implication on the folding and evolution of TIM barrel motifs is discussed.

R 148 TOPOGENIC SEQUENCE MOTIFS - A MACHINE LEARNING APPROACH

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The chemical properties of eubacterial and metazoa signal peptides and of mitochondrial targeting peptides are examined by help of a machine learning program and statistical analyses. The experiments are based on a description of amino-acids according to their physico-chemical character. Rules describing the topogenic sequences are found and compared to the existing knowledge about topogenic sequences. The rules reveal a couple of known and hithero unknown features. We also investigate the known cleavage-sites of the precursor-protein processing proteases. Distinct sequence features for the different cleavage-sites can be found. We conclude that artificial intelligence is a powerful tool in investigating protein sequence information. The obtained results from these studies will be used on biotechnological important protein design.

R 149 SIMULATION OF HELIX ASSOCIATION IN MEMBRANES,

H. R. Treutlein, D. M. Engelman, A. T. Brünger, Department of Molecular Biophysics and Biochemistry, Yale University 260 Whitney Av., New Haven, CT 06511

We have developed a method for investigating energetic properties of helical protein structures associated inside a membrane. The method assumes a coiled-coil conformation of the helices. The helices are rotated against each other and their interaction energy is calculated as a function of the rotation angles around each of the two helix axes. The XPLOR program is used for rotating, equilibrating the structures and for energy calculations. Calculations have been performed in vacuo as well as inside a lipid bilayer. For vacuum simulations special restrains for backbone atoms were introduced to keep the helices in a coiled-coil conformation, however, no restraines on helix atoms were applied for simulations inside the lipid bilayer. Differences between vacuum and lipid simulations show the influence of the bilayer to the dimerization process. The results of our calculations for the unknown structure of the transmembrane portion of the Glycophorin A dimer [Welsh E., Thom D., *Bioploymers*, **24**(1985)2301] suggest the possibility for the prediction of helix orientation in membranes.

R 150 CONSERVED POSITIONING OF PROLINE IN AMPHIPATHIC HELICES OF GLOBULAR AND ION-CHANNEL PROTEINS

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Proline lacks an amide proton when found at any position other than the N-terminus of a protein. As a consequence the residue is expected to disrupt the hydrogen bonding pattern of an α-helix and to be restricted to the first four position of the structure. Those helices in which proline does occur after position four have a pronounced kink. We find that, in globular proteins, the proline residue almost always occurs on the solvent exposed face of the structure. This aids in the packing of long helices around the cores of globular proteins and allows hydrogen bonding between water and the carbonyl oxygen atom four residues down from the proline; this latter feature compensates for the interaction lost through proline being an imino acid. We also find considerable consistency in the positioning of proline in known and putative helices of protein and peptide ion-channels. The residue usually lies on the hydrophilic (interior) faces of the pore-forming helices. As a cosequence, kinking of the structures will produce funnel-shaped pores consistent with previous hypotheses. Such a structure can aid in the capture of ions by the channel (an entropic effect) and should help in the gating mechanism of the channel. This understanding of the conserved positioning of proline residues within the helices of known ion-channel structures will aid identification of putative transmembrane helices of ion-channel proteins and help direct mutagenesis studies.

Protein Folding, Structure and Function

Protein Folding

R 200 ^{1}H-NMR EVIDENCE FOR TERTIARY STRUCTURE IN ACID-DENATURED α- LACTALBUMIN, Andrei T. Alexandrescu,[¥] Yuet-Lan Ng,[†] Philip A. Evans,[§] Jean Baum,[‡] & Christopher M. Dobson[†], [†]Inorganic Chemistry Laboratory, University of Oxford; [‡]Department of Chemistry, Rutgers University; [§]Department of Biochemistry, University of Cambridge

The milk protein α-Lactalbumin (α-lac) exists in a partially folded "molten globule" (MG) state at pH* 2.0. [1,2] Circular dichroism (CD) studies of α-lac have indicated that the MG state retains much of the secondary structure of the native state but that tertiary structure is mostly lost.[1] NMR work in our lab has confirmed that regions of secondary structure, corresponding to secondary structure in the native protein, are stable in the MG state.[2]

The NMR spectrum of the MG state has less chemical shift dispersion than the native state, an attribute consistent with less extensive tertiary structure in the MG state. A number of residues in the MG state of α-lac, however, exhibit chemical shift values considerably different from those expected for a random coil. In addition, NOEs between side-chain protons on different residues indicate that some tertiary structure is retained in the MG state of α-lac. We have assigned the most strongly perturbed resonances in the ^{1}H-NMR spectrum of the MG state to side chain protons of Tyr^{103}, Trp^{104}, and His^{107}. Side-chain to side-chain NOEs between the three residues suggest that they form an aromatic residue cluster in the MG state. The NOE distance constraints are inconsistent with the structure of the native state and point to a structural rearrangement of the aromatic residues in the MG state. We are examining peptide fragments encompassing Tyr^{103}, Trp^{104}, and His^{107} in order to determine to what extent the observed tertiary structure is localized. The NOEs observed in the MG state are being used to monitor structural properties of acid denatured α-lac under a variety of solution conditions.

¥Work supported by NATO-NSF under a grant awarded to A.T.A. in 1989.

[1]Kuwajima, K. (1989) *PROTEINS: Structure Function and Genetics 6*, 87.

[2]Baum, J., Dobson, C. M., Evans, P. A., & Hanley, C. (1989) *Biochemistry 28*, 7.

R 201 PRODUCTION OF A RECOMBINANT HUMAN TISSUE PLASMINOGEN ACTIVATOR VARIANT (BM 06.022) FROM *ESCHERICHIA COLI* USING A NOVEL RENATURATION TECHNOLOGY,

D. Ambrosius, U. Kohnert, R. Rudolph, H. Prinz, U. Opitz, A. Stern, U. Martin*, S. Fischer, P. Buckel and G.-B. Kresse

Boehringer Mannheim GmbH, Biochemical Research Center, Penzberg, FRG; and Boehringer Mannheim GmbH, Department of Pharmacology*, Mannheim, FRG.

Human tissue-type plasminogen activator (tPA) is presently considered a promising fibrinolytic agent to be used in treatment of myocardial infarction. However, production of tPA in eucaryotic cells results in several drawbacks like high cost of production, variability in glycosylation, mixtures of single-chain (sc)- and two-chain (tc)-form and variability in the N-terminus. The same is true for the production of tPA-variants with improved pharmacological characteristics. Attempts to express recombinant tPA or tPA-variants in procaryotic hosts have as yet met with little success since the enzyme, as many other eucaryotic proteins expressed in procaryotes, is obtained as inactive and insoluble protein aggregates (inclusion bodies). The cDNA coding for the tPA-variant BM 06.022 was expressed at very high level in *E.coli*. After cell lysis and isolation of the inclusion bodies, the protein was solubilized under denaturing and reducing conditions. It was found advantageous to reversibly modify the thiol groups of the protein in order to increase solubility and to improve the yield renaturation. Subsequently, tPA was (re)natured under carefully controlled conditions with concomitant formation of the disulfide bonds. Purification by affinity- and ion-exchange-chromatography leads to a consistent final product which is non-glycosylated, completely in the sc-form and has a uniform N-terminus. Data will be presented that describe the renaturation conditions and the purification scheme, as well as detailed analysis of the purified product.

R 202 STABILITY OF MULTIMERIC PROTEINS: A STUDY OF FERRITIN MOLECULES AND MUTANTS. Paolo Arosio, Paolo Santambrogio, Sonia Levi, Anna Cozzi. Department of Biomedical Science and Technology, University of Milano, San Raffaele Institute, Milano, Italy

The subunits of the multimeric, 24-mer ferritin, fold in a 4-alpha-helix bundle. The high stability of this protein (e.g. up to 80^{o}C) may arise from both the compact subunit fold and the symmetrical an cooperative assembly. We analysed the stabilities of the two human ferritin types (named H and L, 174, 182 amino acids, 55% homology) which were over-expressed in *E. coli*. Guanidine-HCl and pH denaturation plots monitored by gel electrophoresis, circular dichroism and fluorescence showed the H to be less stable than the L, particularly when the end product of denaturation was a fully unfolded protein the stability difference was of 3-4 M Gua-HCl. We have also analysed 15 H-ferritin variants constructed by site directed mutagenesis. Six of them, with alterations in exposed areas, were as stable as the wild type. Alterations along the 3- or 4-fold axes, or the deletion of the N-terminal tail, (all sequences involved in inter-subunit interactions) decreased ferritin stability. By contrast three variant proteins, with the substitution of two amino acids in the bundle, exhibited increased stability (2-3 M Gua-HCl). Conclusions: i) both assembly and folding interactions contribute to ferritin high stability; ii) part of the stability difference between H and L ferritins is due to a salt bridge which stabilizes the folded L-monomer.

R 203 CHARACTERIZATION OF E. COLI PEPTIDYL-PROLYL CIS-TRANS ISOMERASES (PPIASE) AND THE INFLUENCE OF CYCLOSPORIN A ON THE IN VIVO FOLDING OF THE COLLAGEN TRIPLE HELIX, Hans Peter Bächinger and Larry A. Compton, Shriners Hospital and Department of Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR 97201

PPIase was recently shown to be identical to cyclophilin, a cyclosporin A binding protein. The enzyme is believed to be involved in the folding of proteins. We extracted and characterized two PPIases from E. coli. The two forms differ in their isoelectric point, (the major form has a pI of 9.0, the minor one of around 4.5), as well as in their antigenic properties (an antibody against an aminoterminal peptide of the major form does not crossreact with the minor form). Both forms are inhibited by cyclosporin A and the apparent K_i of the major form is 25-50 µM, a value about 1000 fold larger than that of eukaryotic PPIases. The specificity of the two E. coli forms towards synthetic peptide substrates is very similar, but distinct from the specificity of eukaryotic enzymes.

The folding of the triple helix of collagens is rate limited by cis-trans isomerization of peptide bonds. It has been shown that PPIases from pig and E. coli can catalyze the folding of type III and type IV collagen in vitro. In in vivo studies with fibroblasts from chick embryo tendons we show that folding of the triple helix of type I collagen is delayed in the presence of cyclosporin A, indicating a physiological role of PPIase in the folding process of type I collagen.

R 204 REDOX EFFECTS ON PROTEIN STABILITY AND FOLDING KINETICS OF HORSE CYTOCHROME C. Abani K. Bhuyan, Gülnur A. Elöve and Heinrich Roder, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104-6059.

Cytochrome c (cyt c) has been the subject of numerous equilibrium and kinetic protein folding studies, but this work has been largely limited to the oxidized form. We have found that under strictly anaerobic conditions, dithionite-reduced horse cyt c can be reversibly unfolded in guanidine hydrochloride (GuHCl) solution. The GuHCl-induced unfolding transition of oxidized and reduced cyt c was monitored by ^{1}H NMR, using the intensity of resolved resonances as a measure of the unfolding equilibrium constant. For reduced cyt c, identical transition curves, within errors, with a midpoint concentration of 5.35 M GuHCl were observed for different protons, indicating that the transition is highly cooperative. The transition curves measured for oxidized cyt c were centered around 2.8 M GuHCl, but significant dispersion was observed for different protons ($\pm$ 0.15 M). Linear extrapolation of ΔG to 0 M GuHCl, using $\Delta G=\Delta G(H_2O)-m[GuHCl]$, gave values of $\Delta G(H_2O)=6.5$ kcal/mol and $m=2.3$ kcal/mol/M for the oxidized form. For the reduced form, the slope was distinctly larger ($m=4.1$ kcal/mol/M), but $\Delta G(H_2O)$ is uncertain due to the length of extrapolation. The redox-dependent difference in free energy is about 7.5 kcal/mol at 4.5 M GuHCl. The dramatic increase in conformational stability upon reduction of cyt c is consistent with the high value for its redox potential, indicating that the reduced heme with zero net charge is strongly favored energetically compared to the positively charged oxidized heme. Stopped-flow measurements observed by Soret absorbance revealed large differences in the kinetics of refolding between the two oxidation states of cyt c. The reduced protein exhibits a dominant folding phase with a 15 ms time constant (95% amplitude), in contrast to oxidized cyt c which is characterized by three kinetic phases with time constants of 15 ms (20% amplitude), 500 ms (60%) and 20 s (20%) under the same refolding conditions (pH 6.5, 10°C).

R 205 EFFECT OF UNFOLDING ON THE TRYPTOPHANYL FLUORESCENCE LIFETIME DISTRIBUTION OF APO-MYOGLOBIN IN INVERTED MICELLES AT SUBZERO TEMPERATURE, E.Bismuto, I.Sirangelo, G. Irace, Dipartimento di Biochimica e Biofisica, Università di Napoli, Via Costantinopoli 16, 80138 Napoli, ITALY

It has been shown that the compact structure of some globular proteins unfolds upon cooling with heat release. This process has been found highly reversible; in fact, if heated, the protein refolds absorbing heat. In the case of apomyoglobin, this "cold denaturation" is expected to take place at temperature lower than 0°C and, therefore, in an experimentally unacesible thermal range in water. Reverse micelles of sodium ethylexylsulfosuccinate (AOT) in n-heptane provide a unique opportunity to study protein unfolding in aqueous solution at subzero temperatures. These systems are able to incorporate buffered solutions of proteins without loss of their native conformation.

In this comunication, we report the data relative to the fluorescence decay of tuna apomyoglobin, a single tryptophan containing protein, in reversed AOT micelles by frequency domain fluorometry. The investigated range of temperature was -20°÷20°C. The very heterogeneous emission was analyzed as continuous distribution of fluorescence lifetimes. The temperature dependence of the distribution parameters has been related to the conformation pattern experienced from the protein during denaturation. Moreover, the results have been compared to the previous apomyoglobin unfolding investigations on the "hot side".

R 206 ASSISTED PROTEIN FOLDING: A STUDY OF THE INTERACTIONS BETWEEN BIP AND TWO RENATURING PROTEINS: tPA AND INVERTASE, Sylvie Blond-Elguindi, *Mary-Jane Gething and Joseph F. Sambrook, Department of Biochemistry, University of Texas, Southwestern Medical Center, *Howard Hughes Institute, Dallas, TX 75235.

The endoplasmic reticulum protein BiP is believed to assist in the folding of secretory and transmembrane proteins via transient binding to nascent chains, and to bind more permanently to malfolded polypeptides that cannot be transported normally along the exocytotic pathway. To better understand how BiP recognizes and assists the folding of its substrates, we are studying in vitro its interactions with two purified secretory proteins, human tPA and yeast invertase. BiP has been purified either from canine pancreas or from *E. coli* cells following expression of a cDNA encoding the murine protein. In renaturation studies, canine BiP increased the yield of renaturation of invertase and tPA, probably by preventing irreversible aggregation. In the absence of substrate, BiP binds and exchanges ^{3}H-ATP at a slow rate. In the presence of renaturing tPA or invertase, the binding of nucleotide is delayed and the amount of bound ATP is significatively reduced. A model consistent with these data will be presented.

R 207 **pH Dependence of the Conformational Stability of Human Insulin and Asp(B10) Human Insulin.** Christopher Bryant, Alita A. Miller, and David N. Brems., Parenteral Research and Development, Lilly Corporate Center, Eli Lilly and Company, Indianapolis, IN 46285.
The substitution of an aspartic acid residue for histidine at position B10 of insulin has been described as the cause of a specific case of familial hyperproinsulinemia (1). A conformational change, possibly due to electrostatic interactions, has been postulated as the underlying basis for the abnormal processing of the Asp(B10) proinsulin (2). In an effort to gain a better understanding of the role that electrostatics play in determining conformational properties of human insulin and the Asp(B10) analogue, we have studied the effects of pH on the equilibrium denaturation characteristics of both molecules. The pH titration of the conformational stability of insulin is described by a single broad transition between pH 8 and 5.5 in which the Gibbs free energy of unfolding increases from 4.3 Kcal/mole to 6.0 Kcal/mole. In contrast, the Asp(B10) analogue undergoes two transitions, one transition occuring between pH 6.5 and 8.0 in which the conformational stability increases from 4.9 Kcal/mole to 6.2 Kcal/mole, and a second transition in which the conformational stability decreases to 5.0 Kcal/mole between pH 3 and 4. The role of specific electrostatic interactions in the observed effects of pH on the conformational stability insulin and Asp(B10) Insulin will be discussed.
1. Chan, S. J. et al. (1987) *Proc. Natl. Acad. Sci.* USA **84,** 2194-2197.
2. Schwartz, G. P. et al. (1989)*Proc. Natl. Acad. Sci.* USA **86,** 458-461.

R 208 CONFORMATIONAL MODIFICATION OF MUTANT YOLK PROTEINS IN *DROSOPHILA*. Frank Butterworth, Department of Biological Sciences, Oakland University, Rochester, MI 48309 and Mary Bownes, Institute of Cellular and Molecular Biology, University of Edinburgh, UK EH9 3JR
The relationship of amino acid sequence of a family of secretory proteins to their conformation is being studied at the cellular, genetic and molecular level. It is known that yolk proteins (YP1, 2 and 3) are coded for by three separate genes (*yp1, 2,* and *3*) which are expressed in the fat body and to a lesser extent in the follicle cells. Mutations in the coding sequences of *yp1* and *2* have been described earlier: *fs(1)1163*, where Ile^{92} becomes substituted by Asn^{92} in YP1; and *fs(1)K313*, where Met^{68} and Pro^{141} become substituted by Leu^{68} and Leu^{141}, respectively, in YP2. Another mutation *yp3*^{51} has been shown to have the amino acid Ala substituted by Asp in the leader sequence which in the mutant form is not cleaved off during processing. In all three cases molecular and cellular evidence shows that the proteins are secreted by the fat body, but trapped in the subbasement membrane space of the tissue, and as a result almost all the mutant protein and small amounts of the two normal proteins are retained by the tissue. Massive accumulations of electron-dense material seen in this region have a similar ultrastructure, and immunogold analysis indicates this material contains yolk. Normal animals transformed with the mutant *fs(1)1163* gene in a plasmid containing a fat body- and sex-specific promoter also express this electron-dense material, demonstrating the morphological effect is a direct result of the mutant gene. The possibility that these three mutations alter the normal folding pattern of the proteins will be discussed. Supported by Research Corporation (FB) and MRC (MB).

R 209 CLASSIFICATION OF ACID DENATURED STATES OF PROTEINS, Linda J. Calciano, Anthony L. Fink, Yuji Goto, Takuzo Kurotsu, and Daniel J. Palleros, Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064

Three major types of conformational behavior occur on acid denaturation, depending on the protein, the acid, the presence of salts or denaturant, and the temperature. The observed behavior ranges from no significant unfolding down to pH as low as 0.5, to unfolding in the vicinity of pH 3 or 4 followed by refolding to a compact, molten globule-like state at pH below 2, to direct transformation of the native state to the molten globule state. The particular behavior observed reflects the balance of attrative and repulsive forces, and can be converted from one type to another by the additon of anions or denaturants. Two types of intermediate states, both characterized by the presence of secondary structure and the absence of significant tertiary structure, but differing in their degree of compactness, are observed.

R 210 FOLDING IN ACTION: EARLY INTREMEDIATES IN THE FOLDING OF GLOBULAR PROTEINS, Henriette Cristensen, Paul G. Varley and Roger H. Pain, Dept. Biochemistry and Genetics, University of Newcastle upon Tyne, U.K., NE2 4HH.

The folding of different structural type proteins (β-lactamase, phosphoglycerate kinase and interleukin 1β) has been studied using ANS binding, intrinsic protein fluorescence. circular dichroism and stopped flow circular dichroism. Four main seperate stages were identified. While the kinetics of the formation of the first state are largely independent of temperature and pH , the later stages are not. When unfolding conitions are reintroduced after varying times of refolding i.e. unfolding from the different intermediate species formed in the early stages of folding, a mono exponential process is observed whose kinetics appear to be independent of temperature i.e. zero activation energy. The implications of these findings for the thermodynamics and the structure of the early intermediate species formed during folding are discussed.

R 211 MUTATIONS IN HUMAN INTERLEUKIN 1-β ALTER THE EXPRESSION OF THE PROTEIN IN INCLUSION BODIES, B. A. Chrunyk, J. Evans, J. Lilliquist, P. R. Young, and R. Wetzel, Macromolecular Sciences Department, SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd., King of Prussia, PA 19406.

The ever-growing use of recombinant DNA techniques to isolate and over-express eukaryotic and prokaryotic genes in bacterial hosts has allowed characterization of a wide array of molecules previously inaccessible. A drawback to this methodology has been that quite often the proteins of interest are expressed as insoluble cytoplasmic inclusion bodies (IBs). Active protein can be recovered but requires the use of chaotropic agents. Since there is no evidence for chemical modification, and since in many cases active protein can be recovered from the insoluble IBs, IB formation may involve incorrect folding. To investigate this possibility, we have begun a study looking at the effects of mutations in human Interleukin 1-β (hIL-1β) and formation of inclusion bodies. While about 8% of the wild type protein produced in *E. coli* is found in IBs, mutant hIL-1βs have been identified which form IBs from 1% to 83% of total hIL-1β produced. Mutations might influence IB formation by affecting the solubility of the unfolded or folded states, or by affecting the properties and/or kinetic lifetimes of folding intermediates (as suggested by King and coworkers for P22 tailspike protein (1)). The percentage of inclusion body formation in a series of mutant sequences referenced to the wild type will be presented. Preliminary results of in vitro studies to correlate the folding properties of the proteins with inclusion body formation will also be discussed.

(1) Mitraki, A. and King, J., *Bio/Technology* 7, 690 (1989).

R 212 **FEATURES OF UNFOLDED POLYPEPTIDES RECOGNIZED BY THE GROEL COMPLEX**, Deborah J. Clark*, Kerstin Braig*, John M. Flanagan+, F.-Ulrich Hartl§ and Arthur L. Horwich*, Departments of Human Genetics* and Molecular Biophysics and Biochemistry+, Yale School of Medicine, New Haven, CT, and Institut fur Physiologische Chemie, U. Munchen, Germany§

Folding of a guanidine-HCl-denatured monomeric polypeptide into its active conformation is catalyzed in vitro by purified groEL and groES components. The denatured protein is bound by groEL and does not reach an enzymatically active form until both groES and MgATP have been added. Using this reaction, we have begun to characterize the nature of binding of the unfolded protein to groEL. Specific peptides derived either from the protein itself or from other proteins were found to markedly inhibit the overall folding reaction. Peptides could compete only before the unfolded substrate became bound to groEL but not after, indicating a specific effect on recognition (as opposed to the subsequent steps of folding). Further studies should establish the character of domains within unfolded proteins that are recognized by groEL.

R 213 DEVELOPMENT OF RECOMBINANT STRAINS OF BORDETELLA PERTUSSIS PRODUCING INACTIVE PERTUSSIS TOXIN MUTANTS. S. Cockle, S. Loosmore, G. Zealey, R. Yacoob, R. Fahim, Y.-P. Yang, G. Jackson and M. Klein, Connaught Centre for Biotechnology Research, Willowdale, Ontario, Canada M2R 3T4.

Pertussis toxin (PT) is both an essential protective antigen in vaccines against whooping cough and a major virulence factor of the causative bacterium, *Bordetella pertussis*. In order to obtain inactive but immunogenic forms of PT for inclusion in a new pertussis vaccine, critical functional amino acids in the catalytic S1 subunit were identified and mutated. A technique was developed for replacement of the complete chromosomal toxin operon of a wild-type strain with reconstructed mutant alleles by double homologous recombination, via electroporetic transformation with linear DNA. Recombinant strains were therefore identical to the parent except for the specific point mutations introduced into the S1 gene, and displayed equivalent rates of bacterial growth and production of *B. pertussis*-specific antigens. Several fully-assembled PT analogues were generated that exhibited less than $1/10^5$ of the ADP-ribosyltransferase activity of wild-type PT, were non-toxic *in vivo*, yet were protective in a mouse pertussis model at lower doses than chemically detoxified PT. A prototype human vaccine comprising four purified antigens was prepared from a *B. pertussis* strain secreting a PT analogue with the S1 mutations $Arg_9 \rightarrow Lys$ and $Glu_{129} \rightarrow Gly$. This formulation elicited antibody responses in guinea pigs equivalent or superior to those induced by whole-cell and chemically detoxified non-recombinant component vaccines.

R 214 THE CONFORMATIONAL CHANGES OF APOCYTOCHROME C UPON BINDING TO PHOSPHOLIPID VESICLES AND MICELLES OF PHOSPHOLIPID BASED DETERGENTS : A CIRCULAIR DICHROISM STUDY, Harmen H.J. de Jongh and Ben de Kruijff, Centre for Biomembranes and Lipid Enzymology, State University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

The secondary structure of the mitochondrial precursor protein apocytochrome c at a water-lipid interface was investigated by CD techniques. Binding of the protein to phos-pholipid vesicles induced α-helical structures, which were more pronounced for negatively charged vesicles. The detergents decyl-, lauryl- and myristoyl-phosphoglycol or phosphocholine, were synthesized as micelle forming phospholipid analogs and are shown to mimic the phospholipids well in their ability to induce α-helices in the protein. A full assignment of the regions where the possible α-helices are formed is proposed by making use of derived fragments of apocytochrome c. Besides a helix at the N-terminus (res. 1-22) and at the C-terminal part (res. 80-101), two regions in the middle section (res. 49-54 and 59-70) are suggested to be helical. It is inferred that the two cysteines at the residues 14 and 17 at the N-terminal part are facing in the same direction, which could facilitate the covalent attachment of the heme group to the precursor in the translocation process. Future NMR-data are expected to give more detailed insight in the conformation and dynamics of the protein at a lipid-water interface. BBA 1029 ('90) 105-112

R 215 AN IMMUNOLOGICAL APPROACH TO DETERMINATION OF PROTEIN STRUCTURE: HUMAN ERYTHROPOIETIN, Steve Elliott, David Chang, Evelyne Delorme, Joan Egrie, James Grant, Lyndal Hesterberg, Anthony Lorenzini and Caren Talbot, Amgen, Amgen Center, Thousand Oaks, CA. 91320
Erythropoietin (EPO) is a 34 kDa glycoprotein hormone that regulates erythropoiesis in humans and other mammals. In an attempt to understand how this protein stimulates the formation of erythroid cells we have begun a program whose goal is to determine the tertiary structure of the protein as well as determine its active site(s). One method that can generate tertiary structural information is to identify conformational epitopes recognized by monoclonal antibodies. Three monoclonal antibodies have been studied; D11, F12 and 9G8A. In vitro bioassays indicate that D11 neutralizes activity 9G8A neutralizes partially and F12 does not neutralize. These results suggest that D11 binds at, or near to, the active site while F12 does not. D11 and F12 recognize folded EPO while 9G8A recognizes unfolded EPO. Therefore D11 and F12 recognize coformational epitopes and 9G8A recognizes linear epitope. 9G8A recognizes a peptide that includes aa 138-156. Other experiments indicate that the 9G8A epitope is partially buried. To determin the regions recognized by D11 and F12, a set of EPO analogs with point mutations spanning the length of the molecule were constructed by in vitro mutagenesis. The ability of each antibody to recognize each analog was tested. The results will be presented.

R 216 ^{1}H-NMR STUDIES OF INTERMEDIATES IN THE FOLDING OF LYSOZYME

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The nature of protein folding intermediates remains largely unknown. ^{1}H NMR has been applied to the study of intermediates in the folding and unfolding of hen lysozyme. A partially folded species of hen lysozyme, containing three of the four native disulphide bridges has been trapped during reductive denaturation of the protein. This species is believed to correspond to an intermediate formed late in the folding pathway. It has also proved possible to trap intermediates during the oxidative refolding of reduced hen lysozyme *in vitro*. The characterisation by NMR of these species, which contain partially formed disulphide bonds is in progress.

R 217 **CONFORMATIONAL HETEROGENEITY OF A WOUND HEALING FACTOR IS RELATED TO ITS STRUCTURAL INSTABILITY**, Kenneth A. Field, Nicholas W. Warne, and Benjamin S. Isaacs, Pharmaceutical R&D, Genetics Institute, Andover, MA 01810.

Our heparin-binding wound healing factor (GNI-600), which affects cells of mesodermal and neural origins, may be useful as a pharmaceutical agent in the acceleration or induction of wound repair. As produced by *E. coli* and purified at Genetics Institute, the 17.5 kD protein has a single tryptophan and two cysteine residues. Others have demonstrated that the two cysteines, which are not believed to be essential for activity, are fully reduced. Using the collisional quenchers I^-, Cs^+, and acrylamide, we have detected varying degrees of solvent accessibility to the single tryptophan within this protein. Furthermore, we have shown that approximately one fifth of all cysteine residues are accessible to sulfhydryl-specific probes. Thermal denaturation experiments have demonstrated that a fraction of the molecules show a greater tendency to aggregate when stressed. This heat induced precipitation of GNI-600 results in both disulfide and non-disulfide bonded aggregates. This process may be enhanced by the presence of the minor species which has greater sulfhydryl exposure. From the above studies, it appears that approximately 20% of the protein is conformationally distinct. The identification and characterization of this species provides a model by which an apparently subtle folding difference can have a profound effect on protein drug stability.

R 218 HEAT SHOCK COGNATE-70 PROTEINS MAY HAVE A PEPTIDE BINDING SITE SIMILAR TO THAT OF MAJOR HISTOCOMPATIBILITY COMPLEX-ENCODED CLASS I MOLECULES, Martin Flajnik, Department of Microbiology and Immunology, P.O. Box 016960 (R-138), University of Miami, Miami, FL 33101.

The polymorphic class I MHC molecule of Xenopus was cloned from an expression library prepared from liver mRNA of an MHC-homozygous frog. The peptide-binding domains (alpha-1 and alpha-2 domains) of the MHC molecule have low but significant homology to a C-terminal region of the heat shock cognate-70 (HSC70) proteins of human, rat, and human. Although the amino acid similarity is low, mapping of the HSC70 sequence onto the known crystal structure of MHC class I strengthens the notion that the peptide-binding regions could adopt a similar folding pattern. The following features are of note: shared prolines occurring at bends of loops or as helix breakers; shared potential glycosylation sites on loops; very similar hydropathy profiles. Further, the proteolytic site of HSC70, known to separate the ATP and peptide-binding regions, occurs on an exposed loop. Lastly, secondary structure predictions of this C-terminal region of HSC70 (amino acids 384-565) predict the crystal of MHC class I better than class I molecules themselves. If HSC70 and MHC class I truly have similar peptide-binding sites, very simple models can be envisaged concerning the evolution of class I and class II MHC proteins.

R 219 ***IN VITRO*** **FOLDING OF PRO-α-LYTIC PROTEASE IS TEMPERATURE SENSITIVE**, Amy Fujishige* and David A. Agard‡*, *Depts. of *Pharmaceutical Chemistry and ‡Biochemistry and Biophysics , U.C.S.F., San Francisco, CA, 94143-0446*

The serine proteases comprise two distinct evolutionary families distinguishable by sequence and structural homology to either trypsin or subtilisin. The extracellular bacterial serine proteases share the common feature of a large pro-region; the role of the pro-region in folding has been previously demonstrated for a bacterial representative of each evolutionary family. Alpha-lytic protease, a member of the trypsin family, originates as a preproenzyme before being secreted extracellularly by the Gram-negative soil bacterium, *Lysobacter enzymogenes*. The protease exhibits temperature sensitivity for expression in *E. coli* that parallels the viable growth temperature profile of the native *L. enzymogenes* host. At permissive temperatures in *E. coli*, the mature (proteolytically processed and active) protease is secreted into the media, and at nonpermissive temperatures the inactive proenzyme accumulates in the outer membrane. We have now isolated and refolded the proenzyme from *E.coli*, and demonstrate that the folding process itself is temperature sensitive *in vitro*.

R 220 IDENTIFICATION OF FUNCTIONALLY IMPORTANT RESIDUES FOR BINDING OF SecB TO PRECURSOR PROTEINS, Pamela M. Gannon*, Linda L. Randall** and Carol A. Kumamoto*, *Department of Physiology, Tufts University School of Medicine, Boston, MA 02111 and **Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164

SecB is an *E. coli* chaperone protein which interacts with a subset of exported proteins to maintain them in a conformation competent for translocation. The binding of SecB to precursor molecules, such as maltose-binding protein (MBP), is an important determinant for protein export. We have isolated a collection of 26 independent SecB point mutants which are defective for protein export *in vivo*. *In vitro* fluorescence experiments with purified mutant SecB indicate that the mutant proteins block the folding of MBP more effectively at lower molar ratios compared to wild-type SecB, suggesting that the mutant SecB may bind more tightly or with higher affinity to MBP. Sequence analysis of mutants which produce full length SecB at steady state indicate that 10/13 mutants contain an amino acid substitution at residues Leu^{75}-Cys^{76}-Glu^{77}. Based on the *in vivo* and *in vitro* data, we propose that these residues are functionally important for binding of SecB to precursor molecules.

R 221 *Abstract Withdrawn*

R 222 IMPROPER FOLDING AND THE FORMATION OF PROTEIN AGGREGATES IN*ESCHERICHIA COLI* AND *INVITRO*, George Georgiou, Department of Chemical Engineering, University of Texas, Austin, Texas 78712-1062

Proteins that cannot fold correctly within the cell are either subjected to rapid degradation or self-associate forming aggregates that are resistant to proteolysis. High level expression of the secreted protein TEM β-lactamase in *Escherichia coli* results in the formation of aggregates within the periplasmic space. The ratio of correctly folded to aggregated β-lactamase depends on: (i) the rate of protein synthesis, (ii) the mode of secretion from the cytoplasm and specifically the nature of the leader peptide and (iii) the growth conditions [Bowden G.A. and G. Georgiou, *J. Biol. Chem.* **265**:16760 (1990)]. Addition the appropriate concentrations of (non-metabolizable) protein stabilizing agents such as sugars, polyhydroxylated alcohols or D_2O abolishes protein aggregation essentially completely without affecting cell growth or protein synthesis. The physiologically active concentrations of additives are also effective in preventing the aggregation of β-lactamase *in-vitro* during refolding from guanidium-HCl solutions. Equilibrium experiments indicated that aggregation arises from the association of an intermediate which is populated at increased temperatures and intermediate concentrations of denaturant.

In parallel with the above studies we have investigated the effect of secretion on the folding of β-lactamase. Removal of the signal sequence resulted in extensive aggregation of the mature protein within the cytoplasm. It was shown that the lack of proper folding was not due to the inability to form the single disulfide bond within the reducing environment of the cytoplasm. Protein aggregates formed by the expression of mature β-lactamase in the cytoplasm or following secretion within the periplasmic space, were isolated to apparent homogeneity. Electron microscopy revealed significant differences were observed in the size, morphology and surface structure of the protein particles. Regardless of the mode of expression, more than 95% of the aggregated protein corresponded to β-lactamase or degradation products. However, the addition of denaturants solubilized the aggregates to varying degrees suggesting differences in the interaction of the associated polypeptide chains.

R 223 LEU 91→PRO: A "TIGHT" TEMPERATURE SENSITIVE MUTANT OF T4 LYSOZYME. Terry M. Gray, Eric Arnoys, Steve Blankespoor, Darla Plowman, Department of Chemistry, Calvin College, Grand Rapids, MI 49546.

We have selected a class of temperature sensitive (ts) mutants of T4 lysozyme with reduced activity at 30°C and no activity at 43°C. "Tight" ts mutants are of special interest because the extreme phenotype is likely the result of major conformational changes in the protein. These mutants differ from most other T4 lysozyme mutants which are active at 43°C, but only manifest their ts lesion by a reduced halo size around phage plaques after exposure of the growth plates to chloroform vapors. We have identified two mutants with this phenotype by DNA sequencing: L91P and L66P. Both of these mutants insert proline residues into α-helical regions of the wild type (WT) protein structure. We have cloned the L91P gene into a high expression vector, expressed, and purified mutant protein. We have compared the enzymatic activity and stability of the L91P and WT protein. The activity of the mutant protein at 25°C is 29% of WT by a turbidometric assay and 27% of WT by a lysoplate assay. The mutant protein is inactivated to 25% activity after 1.3 min of incubation at 65°C, compared to 18 min for WT. We have also examined stability by urea-induced denaturation monitored by UV difference spectroscopy. Many other T4 lysozyme mutants have crystallized under conditions that produced crystals for WT protein. L91P does not crystallize under those conditions. We have also obtained a revertant (same site or second site) of L91P with a non-WT phenotype.

R 224 EFFECT OF UNFOLDING ON THE TRYPTOPHANYL FLUORESCENCE LIFETIME DISTRIBUTION OF APOMYOGLOBIN IN INVERTED MICELLES AT SUBZERO TEMPERATURE, E.Bismuto, I.Sirangelo, G. Irace, Dipartimento di Biochimica e Biofisica, Università di Napoli, Via Costantinopoli 16, 80138 Napoli, ITALY

It has been shown that the compact structure of some globular proteins unfolds upon cooling with heat release. This process has been found highly reversible; in fact, if heated, the protein refolds absorbing heat. In the case of apomyoglobin, this "cold denaturation" is expected to take place at temperature lower than 0°C and, therefore, in an experimentally unaccesible thermal range in water. Reverse micelles of sodium ethylexylsulfosuccinate (AOT) in n-heptane provide a unique opportunity to study protein unfolding in aqueous solution at sub zero temperatures. These systems are able to incorporate buffered solutions of proteins without loss of their native conformation.

In this comunication, we report the data relative to the fluorescence decay of tuna apomyoglobin, a single tryptophan containing protein, in reversed AOT micelles by frequency domain fluorometry. The investigated range of temperature was -20°÷20°C. The very heterogeneous emission was analyzed as continuous distribution of fluorescence lifetimes. The temperature dependence of the distribution parameters has been related to the conformation pattern experienced from the protein during denaturation. Moreover, the results have been compared to the previous apomyoglobin unfolding investigations on the "hot side".

R 225 FOLDING AND UNFOLDING OF A MITOCHONDRIAL PRECURSOR PROTEIN, Ana Iriarte, Joseph Mattingly, Ting-huai Wu and Marino Martinez-Carrion, School of Basic Life Sciences, University of Missouri, Kansas City, MO 64110

The structure of the precursor to a mitochondrial matrix targeted protein can now be studied since the precursor to aspartate aminotransferase (pmAAT) has been isolated in a stable conformation (Altieri et al., J. Biol. Chem. (1989) 264, 4782). This 94,000 dalton dimeric protein contains a chromophore at the active site and shows catalytic competence. The role of the signal peptide in lending stability to the final precursor conformation or in affecting the folding process of the two identical polypeptide chains can be compared since similar enquiries can be made in parallel experiments with the mature (mAAT) enzyme. We have carried out a study of the thermal unfolding process of pmAAt and as a consequence of the effect of denaturants such as urea and guanidine hydrochloride. For these studies we use the techniques of differential scanning calorimetry, UV absorbance and circular dichroism as well as monitor catalysis as an index of final integrity and formation of the dimeric state since the active site contains elements of the two protein subunits. Since the folded protein, except for a hinge region of the signal peptide in pmAAT is extremely resistant to proteolysis, this resistance is used to study folding in freshly synthesized pmAAT prepared by in vitro translation procedures using reticulocyte lysate. Folding in the in vitro system appears to be affected by proteins in the lysate; furthermore, the isolated precursor protein shows some differences in folding characteristics with mAAT. The folding state appears relevant to the efficiency of import into the mitochondrial matrix. (Supported by NIH grants GM-38341 and GM-38184.)

R 226 EVIDENCE FOR DISULFIDE INTERCHANGE IN THE FOLDING OF HUMAN LYSOZYME IN YEAST, Masakazu Kikuchi, Yoshio Taniyama and Chisako Seko, Protein Engineering Research Institute, 6 - 2 - 3 Furuedai, Suita, Osaka 565 Japan

Human lysozyme (hLZM) is a lytic enzyme with 4 disulfide bonds: Cys6 - Cys128, Cys30 - Cys116, Cys65 - Cys81 and Cys77 - Cys95. A mutant hLZM C77A, in which Cys77 is replaced with Ala, was secreted by Saccharomyces cerevisiae as two proteins (C77A - a and C77A - b) with different specific activities. Mutant C77A - a was found to have mixed disulfide linkage with GSH at the Cys95 residue. Among eight mutants lacking one cysteine, four mutants (C30A, C77A, C81A and C95A) were secreted by yeast. Among the four mutants, only C77A could be modified by GSH at Cys95, suggesting that the attachment site of GSH is specific for the Cys95 residue. This specific attachment of GSH at Cys95 may be a mimic of an intermediate in the formation of a disulfide linkage in the folding process of secreted hLZM.

R 227 RANDOM MUTAGENESIS OF THE LamB SECRETION SIGNAL, Barbara K. Klein and Joseph O. Polazzi, Monsanto Corporate Research, Monsanto Company, St. Louis, MO 63198

bST is secreted from *E. coli* at moderate levels of 1-2 μg/ml/OD using expression vectors in which the bST gene is fused to the LamB secretion signal. To improve the secretion of properties of LamB-bST precursor, libraries of random mutants were created in the secretion signal and the ribosome binding site (RBS) and the levels of secreted bST determined. A total of 248 independent clones were screened for the amount of bST released by osmotic shock. Mutagenesis was performed using synthetic oligonucleotides, which contained a low percentage of each other base at each location. To screen for secreted bST, individual clones were grown in duplicate in microtiter wells, induced with IPTG and the periplasmic proteins were released by osmotic shock. The level of bST in the periplasmic fraction was determined using a particle concentration fluorescent immunoassay (PCFIA). Western blot analysis was used to confirm the screening technique. None of the variants tested released significantly more bST than the parental plasmid. Eighty-five random clones were sequenced to estimate the extent and distribution of the base changes. The library of mutants in the ribosome binding site showed approximately the expected frequency of changes evenly distributed throughout the insert. Sequence analysis of these clones did reveal a high fraction a incorrect ligation events especially at the blunt *EcoRV* restriction site. The library of mutants constructed at the C-terminal half of the secretion signal showed fewer mutants that expected.

R 228 STRUCTURAL CONSEQUENCES OF POINT MUTATIONS IN THE DNA-BINDING DOMAIN OF THE GAL4 "ZINC FINGER PROTEIN, Thomas Kodadek, Andrew Hansen and Michael VanHoy, Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712. The yeast GAL4 protein stimulates the transcription of several genes involved in galactose metabolism. It binds as a dimer to several related 17bp sequences by virtue of a novel zinc finger domain, composed of six cysteine side chains that complex two zinc or cadmium atoms in a binuclear, bridged fashion. While the presence of the metal is clearly required for the sequence-specific recognition of DNA, the precise role it plays in organizing the DNA-binding domain is unclear. This point was probed by examining purified fragments of the protein containing the entire DNA-binding and dimerization domains, particularly GAL4(1-140)P26L, a point mutant that requires unusually high concentration of zinc to bind DNA sequence-specifically. The mutated proline is located in the loop between the third and fourth cysteines in the binuclear complex. The results of trypsin cleavage, CD and UV-Vis experiments suggest that the proline is required for proper folding of the zinc finger, perhaps by helping to preorganize the metal-binding site.

R 229 PROCESSING AND FOLDING OF HUMAN LYSOZYMES WITH ALTERED N-TERMINAL REGION, Atuko KOHARA, Yoshio YAMAMOTO and Masakazu KIKUCHI, Protein Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565 Japan

Signal sequences play a central role in the initial membrane translocation of secretory proteins. However, some characteristics of mature proteins, especially those of the N-terminal region, might also affect the function of the signal sequences. To examine this possibility, several mutants of human lysozyme modified in the N-terminal region of the mature protein were constructed, and their folding and secretion in yeast as well as in vitro translocation into canine pancreatic microsomes were analyzed using an idealized signal sequence L8=MR(L)$_8$PLAALG. Our results show that N-terminal region of mature protein is concerned in various events through secretory pathway as follows:

(1) Efficient cleavage of the signal sequence (2) Translocation of the precursor protein into endoplasmic reticulum (3) Correct folding of mature protein.

R 230 MEMBRANE INSERTION-INCOMPETENT M13 PROCOAT PROTEIN CAN BE POST-TRANSLATIONALLY CONVERTED INTO AN INSERTION-COMPETENT FORM, Andreas Gallusser and Andreas Kuhn, Dept. of Applied Microbiology, University of Karlsruhe, Kaiserstr. 12, D-7500 Karlsruhe

Since M13 procoat protein inserts into the membrane independent of the Sec proteins, it requires certain features for a direct protein-membrane interaction. Previously we have shown (1) that the initial membrane binding involves both positively charged regions, at the N-terminus and at the C-terminus. A number of procoat mutants show that membrane insertion is retarded if positively charged residues are changed on one end and is totally prevented when both regions lack positive charges or have negatively charged residues. Extensive analysis showed that these mutants fail to bind to the membrane surface in vivo and in vitro to artificial liposomes.

Replacing the seven lysyl residues of M13 procoat protein by histidines (procoat NH/CH7) offered the possibility to test whether the protonation of the residues is important for membrane binding. Since the intracellular pH of E.coli is neutral (2), less than 10% of the histidines are positively charged and the procoat NH/CH7 accumulated in the cytoplasm, incompetent for membrane insertion. The intracellular pH of E.coli can be lowered in the presence of the K^+/H^+ antiporter nigericin in a K^+-free medium. Indeed, the in nigericin-treated cells NH/CH7 was efficiently cleaved to coat protein, suggesting that the now more protonated precursor protein can electrostatically interact with the membrane surface. (1) Gallusser and Kuhn (1990) EMBO J. 9, 2723-2729; (2) Zilberstein et al. (1984) J. Bacteriol. 158, 246-252

R 231 Membrane-Protein Interaction and Molten Globule State,
Anil K. Lala & Poonam Kaul, Department of Chemistry, IIT Bombay, Powai, Bombay 400076, INDIA.

Soluble proteins can insert into membranes under certain environmental conditions and either reside in the membrane or go across it. This dual property of these proteins has led to several investigations involving different approaches. We have studied the interaction of bovine α-LA (α-LA) with membranes. The α-LA could be incorporated in single bilayer vesicles prepared from egg phosphatidylcholine at low pH. A new method involving pH shock was developed so that the protein stays inserted into the vesicle even on raising the pH to 7.4, a pH value at which α-LA poorly interacts with vesicles. The α-LA inserted vesicles were identified and studied by fluorescence quenching. These studies indicate that the membrane-bound form of α-LA is quite different from the conformation of the protein in solution. The fluorescence quenching studies involving both extrinsic and intrinsic quenchers indicated that the membrane-bound state of α-LA is similar to that in the molten globule state. The current study indicates that while the soluble proteins cannot easily penetrate the membrane, the same proteins in the molten globule state readily insert into membranes. Similar results were obtained using diazofluorene as a hydrophobic photolabeling reagent.

R 232 A SYNTHETIC PEPTIDE DERIVED FROM RHODANESE FORMS A HELIX IN ASSOCIATION WITH THE CHAPERONIN, GROEL. Samuel J. Landry*, Jose Mendoza+, Paul M. Horowitz+, and Lila M. Gierasch*, *Dept. of Pharmacology, UT Southwestern Medical Center, Dallas, TX 75235-9041 and +Dept of Biochemistry, Univ. of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

The E. coli chaperonin, GroEL and its eukaryotic homologues in mitochondria and chloroplasts interact with proteins in intermediate stages of folding, assembly, or translocation across membranes. Chaperonins bind proteins in a non-native conformation and thus may serve to prevent formation of unproductive aggregates by folding intermediates. Prior to or during membrane translocation, the chaperonin may stabilize the protein in a partially folded (translocation-competent) conformation. We have begun to examine the conformational behavior of synthetic peptides derived from two proteins known to interact with GroEL, RuBisCO small subunit [Landry and Bartlett, JBC 264, 9090 (1989)] and rhodanese [Mendoza and Horowitz, unpublished results]. Various experiments have shown that some of the peptides bind to GroEL and inhibit the GroEL-assisted folding of rhodanese. One peptide corresponding to an N-terminal alpha-helix in rhodanese appears to be unstructured in aqueous solution by CD and 2D-NMR spectra. However, the peptide forms an alpha-helix on binding to GroEL as indicated by transferred NOEs. It is tempting to speculate that the GroEL polypeptide binding site accommodates protein segments as helices.

R 233 IN VIVO MICROENVIRONMENTAL MIXING OF NASCENT ALDOLASE SUBUNITS.
Lemire, I., Sygusch, J., Dept. de biochimie, Faculté de Médecine, Université de Sherbrooke, QC, Canada J1H 5N4

Biogenesis of recombinant maize aldolase, a tetrameric glycolytic enzyme of identical subunits was utilized to study in vivo self-assembly of nascent subunits in E.coli. Heterotetramer formation among recombinant aldolase mutants was used to assay for microenvironmental mixing of nascent subunits prior to self-assembly. Recombinant aldolase mutants having differential electrophoretic mobility were constructed by site-directed mutagenesis modifying amino acid residues of the COOH terminal region, distant from subunit interfaces. Mutants corresponding to different electrophoretic mobility were cloned into expression plasmids having identical promotor regions and transcription terminators and distinguishable on basis of conferred ampicillin or tetracycline resistance upon E.coli transfection. Electrophoretic migration of mutant aldolases purified from $Amp^{+}Tet^{+}$ E.coli hosts is consistent with predominant heterotetramer formation. Controls suggest no evidence for subunit exchange among pre-existing aldolase tetramers. These results are indicative of diffusion by nascent subunits from their site of synthesis. Pattern of hybrid tetramer formation is consistent with random mixing of nascent subunits prior to oligomerization. Subunit self-assembly is consequently distinct from subunit polypeptide chain folding. Supported by Medical Research Council (Canada) Grant MT-8088 to J. Sygusch and FCAR studentship to I. Lemire.

R 234 Structural Consequences of Disruptive Mutations in the Core of λ Repressor,
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We have constructed a set of mutations in the N-terminal domain of λ repressor which are expected to severely disrupt interactions in the protein's hydrophobic core. Two types of core mutants have been studied: 1) packing mutations, which maintain hydrophobicity but dramatically alter the total core residue volume, and 2) mutations that have polar residues inserted at core positions. All of the proteins in this set are perturbed enough in structure and/or stability to show no detectable activity. However, using CD, NMR, and antibody reactivity as low resolution structural probes, we find that many of these mutants are able to adopt helical structures with properties similar to the wild-type protein. Many of the packing mutants, including one with an increase in calculated core volume of six methylene groups, are nearly as thermally stable as the wild-type protein. These results suggest that the protein can adjust its packing relatively easily in order to achieve most of the interactions required for stable folding. Polar mutants which show evidence of structure, however, have non-cooperative thermal unfolding transitions, indicative of a molten globule-like state.

R 235 ARGININE RESIDUES AS STABILIZING ELEMENTS IN PROTEINS
N. Mrabet[1], P. Stanssens[1], J. Jenkins[2], M. Chiadmi[2], H. van Tilbeurgh[1,2], J. Janin[2], I. Lasters[3], M. De Maeyer[3], Y. Laroche[1], A.M. Lambeir[1], G. Matthyssens[1] and S.J. Wodak[4].

[1]Plant Genetic Systems, Plateaustraat 22, 9000-Gent Belgium, [2]Laboratoire de Biologie Physicochimique, Université Paris Sud, 91405-Orsay Cedex, France, [3]Plant Genetic Systems, [4]Unité de Conformation de Macromolécules Biologiques, CP160 P2 Université Libre de Bruxelles, av. Héger, 1050-Brussels, Belgium.

Enhancing protein stability by rational design has been one of the great challenges of protein engineering. Site-directed mutagenesis experiments combined with X-ray diffraction studies as well as denaturation studies have already been very useful in determining the contributions of specific residues to protein stability. Here we describe a strategy which involves site-specific substitutions of arginine for lysine residues in D-xylose isomerase from *A. Missouriensis*, a large tetrameric (M_r 172000) enzyme that undergoes irreversible thermal denaturation and is moreover thermostable. These substitutions enhance the stability of the enzyme in the presence of sugar substrates. It is moreover shown that they are also able to improve the thermostability of the protein in absence of any sugar derivatives, a behavior which is postulated to result from additional beneficial effects, particularly on the reversible phase of the denaturation process. Rationalizations of these observations are proposed on the basis of a detailed analysis of the crystal structures of the wild-type xylose isomerase enzyme and of two engineered mutants, the single mutant K253R and the triple mutant K309R/K319R/K323R. We show furthermore that specific substitutions of lysine by arginine result in a significant stability enhancement in two other unrelated proteins, human cupro-zinc superoxide dismutase and glyceraldehyde-3-phosphate dehydrogenase from *B. Subtilis*. These results, together with the observations that Lys -> Arg substitutions are among the most commonly observed in sequences from evolutionarily related proteins, suggest that arginine residues may be important stabilizing elements in proteins.

R 236 PROTEIN FOLDING AND SECRETION IN *BACILLUS SUBTILIS*, Mark S. Payne and Ethel N. Jackson, CR&D Dept., E. I. Du Pont de Nemours and Co., Wilmington, DE 19880

An increasing body of evidence is implicating the importance of protein folding in the process of protein secretion. We are using a model protein and a secretion reporter vector to investigate the relationship between protein folding and secretion in *Bacillus subtilis*. The model protein, alpha-helicin, was designed *de novo* and synthesized by W. DeGrado (L. Regan and W. F. DeGrado, *Science* 241, 976 [1988]). Alpha-helicin is composed of four identical 16-amino acid alpha helices connected by three identical loop regions, and was designed to assume a four-helix bundle conformation. The secretion reporter vector is based on a fusion of the *B. amyloliquefaciens* alkaline protease signal sequence (*apr* ss) to the mature *E. coli* alkaline phosphatase gene (*phoA*) in a manner which allows heterologous coding sequences to be inserted between *apr* ss and *phoA*. Since alkaline phosphatase (AP) activity is dependent on secretion, it is used to monitor secretion of various *phoA* fusions. When the alpha-helicin coding sequence is subcloned between *apr* ss and *phoA*, a fusion protein is synthesized in *B. subtilis* as determined by incorporation of labelled amino acids; but it is not secreted since no AP activity appears in the supernatant. However, when a normally secreted protein fragment was inserted between *apr* ss and alpha-helicin, AP activity was detected in the supernatant, demonstrating that alpha-helicin can be translocated across the cell membrane. To test the hypothesis that the folding rate of alpha-helicin influences its ability to be secreted, alterations within alpha-helicin which result in secretion of the alpha-helicin/*phoA* fusion, and their impact on folding rate, will be investigated.

R 237 PROTEIN FOLDING IN THE PATENT LITERATURE

Reinhard R.W. Hermann, European Patent Office, Munich, Germany

An inquiry into disclosures concerning protein folding techniques has been performed in the EP, WO, and US patent literature[1)].

Although the basic methods for protein folding stem from the 60ies and 70ies, the first patent applications dealing with protein folding techniques appear only in the context of gene technology. The majority of these applications apply standard techniques to activate recombinant proteins [i.e. (1) unfolding in a denaturant, and (2) removal of the denaturant by dialysis or dilution], and introduce at best only subtle, protein-specific modifications. Only few patent disclosures suggest substantial modifications, e.g. ***EP248362:** removal of the denaturant in a desalting centrifuge. ***EP123928 & WO86/05809:** refolding of non-covalently immobilised proteins. ***EP325691:** folding by incubation together with an IEC-resin.

[1)] Hermann, R. in Protein Folding, Monograph, to be published in 1991

R 238 DEFECT IN ASSEMBLY OF CARBOXYL-TERMINAL ALTERED B SUBUNITS OF HEAT-LABILE ENTEROTOXIN IS SUPPRESSED AT LOWER TEMPERATURES AND BY THE PRESENCE OF A SUBUNITS. Maria Sandkvist[1], Timothy R. Hirst[2] and Michael Bagdasarian[1]. [1]Department of Microbiology, Michigan State University and Michigan Biotechnology Institute, Lansing, MI 48909 and [2]Biological Laboratory, Canterbury, Kent CT2 7NJ, Great Britain.

Heat-labile enterotoxin from *Escherichia coli* is a diarrhea causing toxin composed of one A subunit (EtxA) and five identical B subunits (EtxB). The EtxA and EtxB polypeptides are translocated independently across the cytoplasmic membrane and are assembled into a hexameric protein complex which resides within the periplasmic space.

Nine mutant B subunits with deletions and substitutions of carboxyl-terminal amino acids were obtained by Bal31 nuclease digestion and oligonucleotide-directed mutagenesis of the *etxB* gene. It was found that minor alterations to the carboxyl-terminus of the B subunit caused a defect in its assembly into stable pentamers. The extent of inhibition of assembly correlated with the magnitude of the alteration that had been introduced. Removal of the C-terminal asparagine had no effect, whereas deletion of the last three amino acids completely inhibited subunit assembly. Mutant monomers were found to be associated with the periplasmic face of the cytoplasmic membrane whereas the pentamers were located in the periplasm.

The assembly defect of mutant EtxB polypeptides could be suppressed by growth at lower temperatures. Expression at 30° rather than 37° C increased the yield of assembled pentamers in the periplasm five fold, and decreased the amount of membrane bound monomers. When the mutant B subunits were produced in the presence of A subunits the concentration of assembled mutant EtxB pentamers in the periplasm increased up to 30 fold.

These results indicate that a correct structure of the carboxyl terminus is essential for the subunit assembly. The A subunit may act as specific folding and/or assembly factor in the normal pathway of holotoxin formation.

R 239 ***Ligand-linked Conformational Changes of Calmodulin***

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Calmodulin is the primary eukaryotic intracellular calcium receptor. Cooperative binding of divalent cations to calmodulin causes a large increase in helical content; these conformational changes permit it to activate a wide range of target enzymes. The position as well as number of ions bound may control the sequence of populated conformations. To determine the nature of these ligand-induced structural transitions, we are monitoring individual sites and individual species of calmodulin to identify and quantitate intermediate states in the pathway.

Ligand-linked conformational changes of calmodulin were probed by investigating changes in backbone susceptibility to limited exposure to chemical reagents and specific and non-specific proteases. Products were screened electrophoretically and quantitated using reverse phase HPLC; amino acid analysis was used to identify the resulting peptide fragments. From these data, primitive isotherms or reactivity profiles were determined. It was found that divalent ion binding to calmodulin protected it from cleavage at most positions; however, there were notable exceptions. Non-monotonic reactivity of some residues suggests the existence of intermediates (i.e., more than 2 global conformations populated). Calmodulin fragments manifested a wide range of electrophoretic mobility shifts as a function of ligand levels, pH and buffer composition. Conformations of peptides containing site 3 were most sensitive to [Ca^{2+}]; peptide length and composition beyond the site also influenced folding properties. Distinct ligation species of whole calmodulin were trapped and separated on the basis of differential electrophoretic transport properties at -45°C; again, more than 2 states were evident.

R 240 DETECTION AND CHARACTERIZATION OF INTERMEDIATES IN THE FOLDING OF LARGE PROTEINS BY THE USE OF GENETICALLY INSERTED TRYPTOPHAN PROBES.

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L-Lactate dehydrogenase (LDH) from B. stearothermophilus was rebuilt using site-directed mutagenesis to produce an enzymically active, tryptophanless enzyme by replacing all the wild-type tryptophans (80, 150 and 203) by tyrosines. Nine single tryptophan-containing active enzymes were constructed from this enzyme by genetically replacing one of nine tyrosines by tryptophan. The equilibrium and the time-resolved tryptophan fluorescence intensity and anisotropy were used to report unfolding events in guanidine-HCl monitored from these 9 defined positions. Three transitions defined four folding intermediates I (native), II (expanded monomer-1), III (expanded monomer-2) and and IV (random coil). Intermediate II is a globular monomer. All the probed α-helices and most of the β-structure was intact. There was an increase in the rate but not the extent of the mobilities of six of the probed-tryptophan side-chains indicating loss of tertiary structure. Circular dichroism (CD) showed all the secondary structure to be intact. Intermediate III is monomeric and still globular, but the tryptophan anisotropy indicated an increase mobility at positions 36, 85, 190, 203, 279 and 285. Helix α-B is further disrupted but helices α-1F, α-2G and α-3G were still rigid. CD showed half the secondary structure to be still intact. Intermediate IV is random coil in which all tryptophans have complete rotational freedom and the helix CD signal is lost. Intermediates II and III both have characteristics ascribed to a molten globule state. Probes close together in the primary sequence (279 and 285) reported identical unfolding events. The 9 probes suggest partial structures for the two monomeric intermediates in folding of B. stearothermophilus LDH which can be compared to two kinetic intermediates proposed by Jaenicke (1987).

R 241 THE PRO-REGION OF CARBOXYPEPTIDASE Y IS A CO-TRANSLATIONAL CHAPERONE,

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We have analyzed the *in vitro* folding of carboxypeptidase Y (CPY) and its precursor from *Saccharomyces cerevisiae* and find that the pro-region is nescessary for the refolding of denatured CPY. Our results show that the pro-region of CPY is a "co-translational chaperone", like reported recently for two bacterial proteases (Ellis,R.J., Semin. Cell Biol. 1,1 (1990)). ProCPY was denatured in 6 M guanidinium hydrochloride and renaturation was initiated by dilution into a refolding buffer. As judged by the recovered activity, proCPY could be refolded to 85 % of a non-denatured control. In contrast, mature CPY could not be renatured under these conditions. Reducing the three disulphide bridges in the denatured precursor did not prohibit renaturation although the level of recovered activity was lower. Intrinsic fluorescence measurements show that proCPY has a lower stability under denaturing conditions than mature CPY, suggesting that a metastable state is nescessary for the correct folding.

R 242 SOME MUTANTS OF STAPHYLOCOCCAL NUCLEASE DISPLAY A THREE STATE UNFOLDING EQUILIBRIUM, W. E. Stites and D. Shortle, Department of Biological Chemistry, School of Medicine, Johns Hopkins University, Baltimore, MD 21205

The guanidine hydrochloride induced equilibrium unfolding behaviors of wild type and most mutant staphylococcal nucleases fit well to a two-state approximation where protein is either in the native or denatured state. A number of mutant nucleases with tryptophan substituted for other residues were made. These mutants exhibit characteristics of three-state unfolding behavior where an additional non-native state is present. The guanidine hydrochloride induced cooperative breakdown of this apparent non-native folding intermediate can be monitored by fluorescence and circular dichroism. The apparent stability of the intermediate has been determined in these mutants. Experimental evidence in at least one case indicates that mutation can also stabilize this putative intermediate in a fragment model of the denatured state of staphylococcal nuclease. The possibility that three-state behavior may be present in wild-type nuclease and other mutants that apparently fit the two-state model can be demonstrated from the experimental parameters obtained for the mutants studied in this work. The existence of this intermediate can explain some of the previously observed changes in the slope of free energy against denaturant concentration ($d\Delta G/ d[GuHCl]$) supporting arguments that changes in this parameter reflect changes in the denatured state.

R 243 COMPLEX INTERACTIONS BETWEEN CHAPERONIN 60 AND DIHYDROFOLATE REDUCTASE, Paul V. Viitanen, Gail K. Donaldson, Thomas H. Lubben, George H. Lorimer and Anthony A. Gatenby, Central Research and Development Department, E. I. DuPont de Nemours & Co., Wilmington, DE 19880-0402.

The spontaneous refolding of chemically denatured mouse dihydrofolate reductase (DHFR) is completely arrested in the presence of the bacterial chaperonin cpn60 (GroEL). Inhibition results from the formation of a complex between cpn60 and the unfolded or partially folded DHFR. This complex is sufficiently stable to withstand gel filtration and exhibits no detectable DHFR activity. Binding studies with ^{35}S-labelled DHFR suggest that each cpn60 14-mer can bind up to 2 to 3 molecules of DHFR. Furthermore, it appears that ribulosebisphosphate carboxylase (Rubisco) and DHFR compete for a common saturable site on cpn60. While sequestered on the chaperonin, DHFR is extremely sensitive to proteolysis in comparison to the unassociated native enzyme. Enzymatically active DHFR can be discharged from the cpn60·DHFR complex by the addition of ATP. Discharge of DHFR does not require the participation of the co-chaperonin cpn10, although the latter potentiates the effect of ATP. Surprisingly, ATP hydrolysis is also not required for the release of DHFR, since certain non-hydrolysable analogs of ATP are capable of discharge. Under appropriate conditions 'native' DHFR can also form a stable complex with cpn60. However, in this case, complex formation is not instantaneous and can be prevented by the presence of the enzyme's natural substrates (NADPH or dihydrofolate). This observation suggests, that native DHFR is in equilibrium with at least one conformational substate which can efficiently interact with cpn60.

R 244 GENERAL IMMUNOCHEMICAL SCREENS DETECT MUTATIONS WHICH INFLUENCE THE DEPOSITION OF HUMAN INTERFERON GAMMA INTO INCLUSION BODIES WHEN PRODUCED IN *ESCHERICHIA COLI*, Ronald Wetzel*, L. Jeanne Perry** and Constance Veilleux, Biomolecular Chemistry Department, Genentech, Inc., 460 Point San Bruno Blvd., So. San Francisco, CA 94080 (*present address: Macromolecular Sciences Dept., SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd., King of Prussia, PA 19406; **present address: Molecular Biology Institute, UCLA, Los Angeles, CA 90024).

Wild type human interferon-gamma (HuIFN-γ) produced at high levels in the cytoplasm of *E. coli* is localized predominantly in dense, poorly soluble inclusion bodies. We have prepared collections of clones expressing randomly mutagenized cDNAs for HuIFN-γ. In order to analyze these clones we have developed a series of microtiter plate immunochemical screens and assays. Using these screens we have identified a series of HuIFN-γ mutants which exhibit altered distributions of the protein between soluble and inclusion body forms, in some cases yielding more than 90% of the IFN in the soluble fraction. The sequences of these mutants will be presented and discussed. Biophysical characterization of these molecules should help to define the mechanism of inclusion body formation. Conversely, mutants in inclusion body distribution may provide molecules whose *in vitro* analysis will help elucidate *in vitro* folding characteristics. A major point is that these screens should be quite general and thus, in principle, can be applied to any protein, even those, like IFN-γ, which have no metabolic or regulatory interaction with a heterologous host cell. In addition to its use in facilitating folding studies, immunochemical screening for soluble expression - coupled with random mutagenesis - may provide useful sequence variants for maximizing soluble expression of otherwise recalcitrant proteins.

R 245 DENATURATION OF RECOMBINANT HUMAN STEFIN B AS REVEALED BY SPECTROSCOPY AND SIZE - EXCLUSION CHROMATOGRAPY, Eva Žerovnik, Roman Jerala and Vito Turk Department of Biochemistry, J.Stefan Institute, 61000 Ljubljana, Yugoslavia

Stefin B, a cysteine proteinase inhibitor (3-dimensional structure in the complex with papain has been recently determined by Bode W. et.al., (1990) EMBO J. 9, 1939-1947) is a small globular protein (M_r 11000) without disulphide bridges. Urea, GuHCl, heat and extreme pH denaturation was followed by circular dichroism in the near and far UV. The size-exclusion chromatography (FPLC system) was used in addition, to get some information on the compactness of conformational states. Some stable intermediate states appear to exist during denaturation.

Protein Engineering

R 300 SINGLE PROLINE SUBSTITUTIONS IN PREDICTED ALPHA HELICES OF MURINE GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR RESULT IN A LOSS IN BIOACTIVITY AND ALTERED GLYCOSYLATION, Scott W. Altmann, G. Douglas Johnson, and Michael B. Prystowsky, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

Contributions of alpha helices to biological acitivity in murine granulocyte-macrophage colony stimulating factor (mGM-CSF) were analyzed using site directed mutagenesis and protein expression in COS-1 cells. A series of single proline substitutions were made for residues within the four predicted alpha helices as a means of disrupting local helical secondary structure. Mutations in three of the four helices resulted in marked reductions in bioactivity. Five mutants E21P, L56P, E60P, L63P, and L107P showed 10^2- to 10^4-fold reduction in bioactivity as well as hyperglycosylation. The same Pro substitutions made on non-N-glycosylated molecules had a similar loss in bioactivity implying that a Pro induced structural change and not hyperglycosylation was responsible for the major decrease in bioactivity. Additional amino acid substitutions at these residues which conserve charge or hydrophobicity, or replace the original residue with an Ala verified that conformational changes in the protein structure were specifically due to steric constraints imposed by the Pro residue rather than loss of important side chain functions.

R 301 SYNTHETIC METAL-BINDING SITES IN CYTOCHROME C AND BOVINE SOMATOTROPIN: HIS-X_3-HIS IN AN α-HELIX, Frances H. Arnold, Sung-Sup Suh, and Robert Todd. Division of Chemistry and Chemical Engineering 210-41, California Institute of Technology, Pasadena, CA 91125.

Metal-binding sites consisting of two histidines positioned His-X_3-His in an α-helix have been engineered into the surfaces of *S. cerevisiae* cytochrome c and bovine somatotropin. Association constants for formation of a complex between this site and Cu(II) chelated by iminodiacetic acid-derivatized poly(ethylene) glycol (IDA-PEG) have been measured by partitioning in aqueous two-phase polymer systems. The association constants for four different sites range from 2×10^4 to 1.6×10^6 M^{-1}, which correspond to free energies of chelation ($\Delta\Delta G^o_{chelate}$) of 0.9 to 3.5 kcal mol^{-1}. These $\Delta\Delta G^o$ values are equivalent to the free energy that metal binding contributes to the stability of the folded protein. The metal affinity of a particular site depends on the structure and rigidity of the helix into which the site is engineered. Incorporation of the His-X_3-His site into an unconstrained helix at the N-terminus of cytochrome c yields a synthetic metal-binding protein whose metal affinity is sensitive to environmental conditions which alter helix stability.

R 302 **GENETIC ANALYSIS OF E. coli TRP APOREPRESSOR RESIDUE ALA77,** **Dennis N. Arvidson James Pfau, Janet K. Hatt, Francis S. Pecoraro and Philip Youderian, California Institute of Biological Research, 11099 N. Torrey Pines Rd., La Jolla, CA 92037**

The side chain of residue alanine 77 of the Trp repressor is located at the turn between α-helices D and E, the flexible DNA recognition helices. Codon-directed mutagenesis was used to make mutant Trp aporepressors with each of the 19 possible amino acid changes of residue 77. To exclude possible second site mutations introduced during mutagenesis, DNA sequences of mutant plasmids were determined between the endpoints of a small fragment of each mutant trpR gene, which was subcloned into an otherwise wild-type backbone of the parental plasmid. Each mutant plasmid was transformed into Salmonella strain MS1868/F'lacI^Q and tested for the ability to be lysogenized by a P22 challenge phage with a consensus, symmetric trp operator, as a function of L-tryptophan concentration. Mutant proteins with substitutions of Cys, Val, Ile, Leu, Thr, Lys or Ser for Ala77 require less L-tryptophan for activation in vivo than wild-type aporepressor and are thus superrepressors. Replacment with Phe, Tyr, Trp, Asp or Glu caused loss of activity in this assay. Each mutant plasmid was also transformed into otherwise isogenic E. coli strains bearing trpE-lacZ gene fusions with wild-type or mutant (trm) operators. Comparisons of the abilities of mutant aporepressors to repress these operators suggest that no changes of Ala77 give rise to repressors with altered abilities to bind in tandem. Tryptophan dependance data from ß-galactosidase assays and challenge phage assays were similar.

R 303 SWITCHING SUBSTRATE SPECIFICITY OF A THERMOPHILIC XYLOSE ISOMERASE BY REDESIGNING THE SUBSTRATE BINDING POCKET. M. Bagdasarian[1,2], M. Meng[1,2], C. Lee[4], and J. G. Zeikus[1,2,3]. [1]Michigan Biotechnology Institute, Lansing, MI 48909, [2]Department of Microbiology, [3]Department of Biochemistry, Michigan State University, East Lansing, MI 48824, [4]Department of Pharmaceutical Chemistry University of California, San Francisco, CA 94143.

The substrate specificity of thermophilic xylose isomerase from *Clostridium thermosulfurogenes* was examined by using predictions from amino acid sequence homologies to other xylose isomerases, the crystal structure of the *Arthrobacter* enzyme and site-directed mutagenesis of the thermophile *xyl*A gene. The results are consistent with the orientation of the substrate molecule in the active site with the C^5-OH toward the His_{101} residue in the substrate binding pocket. The location of Met_{87}, Thr_{89}, Val_{134} and Glu_{180}, which contact the C^6-OH of the substrate analog, sorbitol, in the *Arthrobacter* enzyme are equivalent to those of Trp_{139}, Thr_{141}, Val_{186}, and Glu_{232} in the thermophilic enzyme. Substitution of Trp_{139} by Phe or Tyr reduced the K_m and enhanced the k_{cat} of mutant thermophilic enzymes towards glucose whereas the K_m for xylose was increased and the k_{cat} decreased. Replacement of Val_{186} with Thr also enhanced the catalytic efficiency of the enzyme towards glucose. A designed Trp_{139}-Phe/Val_{186}-Ser mutant enzyme displayed a 12-fold higher k_{cat}/K_m on glucose than that of wild-type enzyme and, a substrate specificity preference for glucose. These results provide evidence that alteration in substrate specificity of factitious thermophilic xylose isomerases can be achieved by designing reduced steric constraints and enhanced H-bonding capacity for glucose in the substrate binding pocket of the active site.

R 304 CORRELATION OF STRUCTURE OF OMEGA LOOPS FROM iso-1-CYTOCHROME C WITH PROTEIN ACTIVITY, Judy K. Barnett and Martha S. Briggs, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332

We have synthesized omega loops[1] A and C from yeast iso-1-cytochrome c and are characterizing their structures in the absence of the native protein. Deletion of these loops affects the biological activity of the protein differently[2]. In the absence of loop A [residues 23-37] *in vivo* there is no activity and the protein is not detected by low temperature spectroscopic examination of intact cells. In the absence of loop C [residues 45-59] the protein is present, but has reduced activity. To correlate structure with function we are characterizing the conformations of peptides with sequences corresponding to loops A and C using NMR. The peptides have been synthesized with the residues Gly-Cys added to each terminus. The added cysteines allow us to determine the propensity for the formation of a closed loop (oxidized form) versus an open chain (reduced form). Preliminary 1D and 2D NMR data indicate that loop A may take on structure while loop C appears to be unstructured in aqueous solution.

[1]Leszczynski and Rose, *Science* **234**(4778):789-912
[2]Fetrow et al., *Proteins* **6**(4):372-381

R 305 IDENTIFICATION OF RESIDUES IMPORTANT FOR FUNCTIONALITY OF Tn*10* TET REPRESSOR BY SITE-SPECIFIC DELETION AND MISSENSE MUTAGENESIS Ch. Berens, K. Pfleiderer and W. Hillen, Institut für Mikrobiologie und Biochemie, Friedrich-Alexander Universität, D-8520 Erlangen, FRG

The tetracycline resistance determinant from Tn*10* is regulated at the level of transcription by the Tet repressor protein. Tet repressor has 207 amino acids, forms a dimer that recognizes *tet* operator, and is induced by tetracycline. DNA-binding is mediated by a helix-turn-helix motif. Since λ repressor uses its NH_2-terminal arm to contact DNA, a site-specific deletion analysis of the Tet repressor amino-terminus was carried out. Deletion of amino acids 2–23 yields a protein that is rapidly degraded. Deletion of amino acids 3–6 or 6–8 leads to 3-fold, deletion of amino acids 3–8 to a 1500-fold reduction in repression while retaining full inducibility. Individual mutagenesis of amino acids Arg3, Asp5, Lys6 and Lys8 to Ala leads to fully inducible repressor proteins with decreased DNA-binding. This shows that the N-terminus of Tet repressor is important for DNA-binding, either by providing additional contacts to *tet* operator, or by positioning the DNA-binding motif. Consecutive deletions of amino acids 9 to 9–13 result for Δ9 in 400-fold, for Δ9–12 in 1900-fold reduction of repression. Deletions Δ9–10, Δ9–11 and Δ9–13 show a transdominant phenotype with a complete loss of DNA-binding. We interprete these mutations as internal deletions in an α-helix leading to the "helical phasing" observed for DNA-binding. The function of carboxy-terminal residues was examined by constructing truncated proteins. Termination at residues 138, 151, 159 and 174 yields mutants that are degraded proteolytically *in vivo*, and at amino acid 202 a protein retaining near wildtype activity and stability. This result suggests that Tet repressor is not composed of two or more isolated structural domains, as many other gene-regulatory proteins. Internal deletion of amino acids 151–166 or 164–166 renders the mutants non-inducible by tetracycline, assigning a functional role to this region of the protein for the first time.

R 306 ALANINE→SERINE SUBSTITUTIONS WITHIN α-HELICAL SECONDARY STRUCTURE: EFFECTS UPON PROTEIN STABILITY, Michael Blaber and Brian Matthews, Institute of Molecular Biology, University Of Oregon, Eugene OR 97403

Alanine to serine substitutions represent one of the largest changes in side chain α-helical propensity for the substitution of a single functional group. A set of mutations for this type of substitution at positions 41, 73, 82, 93, 130 and 134 of T4 lysozyme comprise a wide variety of solvent accessibilities and positions within an α-helical secondary structure. Analysis of this set of mutations will allow the understanding of both positional and solvent accessible contexts on the α-helical secondary structure stability for this particular substitution. These mutant lysozymes have been constructed and expressed and thermodynamic and structural analyses are in progress. The structure and thermodynamic constants for the Ala49→Ser mutant are presented and the effects upon the stability of the protein are discussed.

Residue Positions In T4 Lysozyme For α-Helical Stability Analysis Of Ala→Ser Substitutions

Wild Type Residue	Side Chain Accessibility	Position From Helix N- Terminal	Position From Helix C- Terminal
Ala 41	0.47	N +2	C -8
Ala 49	0.28	N +10	C -1
Ala 73	0.73	N +13	C -6
Ala 82	1.34	N	C -8
Ala 93	1.16	N	C -12
Ala 130	0.15	N +4	C -4
Ala 134	0.39	N +8	C

R 307 SWAPPING DIMERIZATION DOMAINS: THE JUN LEUCINE ZIPPER MOTIF CAN FUNCTIONALLY RE PLACE THE LEXÅ DIMERIZATION REGION, Laurent Bracco, Christine Pernelle, Cristina Giuliacci, Christine Dureuil, Manfred Schnarr*, Michele Schnarr*, Pascalle Oertel-Buchheit*, Thomas Schmidt#, IBV, Rhone-Poulenc Rorer, 13 Quai Jules Guesde, BP 14, 94403 Vitry-sur-Seine, France, *IBMC, CNRS, 15 rue Rene Descartes 67084 Strasbourg, France and #Alexander von Humboldt Stiftung, Jean-Paul Str. 12, D-5300 Bonn 2, Germany. The jun leucine zipper sequence has been substituted for the carboxy-terminal domain of the lexÅ repressor which is responsible for its dimerization. This hybrid repressor is fully functional in binding to several *lexÅ* operator sites *in vivo* and *in vitro*. Extensive site-directed mutagenesis has been used to estimate the importance of some residues in the specificity of the recognition process between two leucine zipper domains. The homodimerization of lexÅ /jun mutants have been studied *in vivo* thanks to a beta-galactosidase assay and *in vitro* through a gel retardation assay. Competition experiments with several synthetic peptides derived from the jun and fos leucine zipper sequences have enabled us to obtain additional information about this recognition process.

R 308 ENZYMES AT AN INTERFACE: SITE-SPECIFIC MUTANTS OF SUBTILISIN BPN' WITH ENHANCED SURFACE STABILITY, Philip F. Brode, III, Christopher R. Erwin, Deborah S. Rauch, James M. Armpriester and Donn N. Rubingh, The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH 45239-8707

Enzyme adsorption and inactivation at the solid/liquid interface for subtilisin BPN' show a strong dependence on the surface energy of the solid surface. The half-life of the wild-type BPN' is approximately 6 times greater when equilibrated with a model hydrophilic surface than with a hydrophobic surface. Similarly, adsorption of BPN' at the solid/liquid interface is greater for a hydrophobic surface than for a hydrophilic one.

Several site-specific variants of subtilisin BPN' have been made in an attempt to alter the surface-inactivation of the wild-type enzyme. Although the basic kinetic parameters of the variant enzymes, which include the turnover number (k_{cat}), the binding constant (K_M) and the product inhibition constant (K_i), are not significantly different on a soluble substrate, the surface autolytic stability is found to vary significantly from one variant to another. A model will be presented which can explain these observations.

R 309 LOOP MUTATIONS CAN CAUSE A SUBSTANTIAL CONFORMATIONAL CHANGE IN THE CARBOXY-TERMINUS OF THE FERRITIN PROTEIN. Gianni Cesareni, Roberto Iappelli, Alessandra Luzzago and Paola Tataseo.
Department of Biology, II University of Rome "Tor Vergata", Via Carnevale, 00173 Rome Italy.
We have previously shown that assembled ferritin can exist in two substantially different conformations (EMBO J. 8, 569-576). In the wild type protein 24 monomers assemble to form a hollow shell with the carboxy-terminal α-helix pointing inside (Flip conformation). Amino acid substitutions of some of the last 20 aminoacids, however, can cause the α-helix to flip of approximately 180°C and to point toward the outside of the protein shell (Flop conformation). In some mutants, Flip and Flop conformations coexist in the same molecule. We have recently asked whether the aminoacid sequence of the loop connecting the carboxy-terminal E-helix to the core of the molecule (D-E loop) can influence the relative proportions of the two conformations. By exploiting a colony colour test that discriminates between mutants able to fold into the native conformation and those presenting "folding assembly problems" we have characterized a large number of site directed and random loop mutants. We have been able to show that, although the side chains of the loop residues are not involved in intramolecular contacts, the loop primary sequence can dramatically influence the conformation of the carboxy-terminus of the molecule. In the sequence of the wild type loop (GAPESG), none of the four central residues plays a key role and the conformation of loops having most of the possible aminoacid combinations is indistinguishable from wild type. Some loop sequence however cause either the protein to accumulate in an insoluble aggregate or to assemble into Flop conformation. We have observed that insolubility correlates with the presence of positively charged residues in position 1, 2 and 3, while Flop mutants are characterized by the absence of Gly residues at position 4 and 6. We have also shown that the D-E loop is rather tolerant to insertions and that oligopeptides up to 7 aminoacids in length can be accommodated without disturbing the conformation of the rest of the protein shell.

R 310 *Abstract Withdrawn*

R 311 *IN VIVO* SYNTHESIS OF REPETITIVE POLYPEPTIDES: DEFINING REQUIREMENTS FOR β-HAIRPIN FORMATION, Dougherty, M. J.[1], Tirrell, D. A.[2], Mason, T. L.[1], and Fournier, M. J.[1], Program in Molecular and Cellular Biology, [1]Department of Biochemistry and [2]Department of Polymer Science and Engineering, University of Massachusetts, Amherst, MA 01003

Beta (β) hairpin turns positioned periodically in repetitive polypeptides should favor a tight reversal of chain direction and thus aid the formation of lamellar crystals. The structural requirements for forming tight hairpins are not known, but among naturally occurring two-amino-acid β-hairpins a high percentage can be categorized by their dihedral angles as being type I' or type II' β-turns. In general, types I' and II' turns are quite rare in nature, comprising only 3% and 5% of all β-hairpins, respectively, but they are highly favored in the two-residue connections joining two hydrogen-bonded antiparallel β-strands. The bias in turn composition of the two-amino-acid hairpins, first noticed by Sibanda and Thornton (Nature 316, 1985), has led them to designate certain amino acid pairs as consensus sequences for type I' turns (Asp-Gly, Asn-Gly and Gly-Gly) and type II' turns (Gly-Ser and Gly-Thr). We are undertaking a systematic investigation of these pairs to determine if the consensus turn combinations can generate hairpins in *de novo* designed proteins. The goals are to define the side chain parameters that govern protein folding of the two-residue β-hairpins and to apply these design principles to the synthesis of chain-folded polypeptides of defined thickness and surface functionality. The sequences of interest are described by the general formula $\{(GlyAla)_3XY\}_n$ where the repeated glycine-alanine dyads form an extended β-strand and X and Y represent the turn positions. A strategy of mixed site DNA synthesis has been used to generate artificial genes for all five of the pertinent consensus sequences and a number of variations. The genetic monomers have been ligated and cloned to produce multimeric genes which are stable *in vivo*. We have expressed several of these genes in *E. coli* using T7 and tac based promoter systems. The properties of these proteins and the basis for β-hairpin formation will be described.

R 312 CONSTRUCTION, SYNTHESIS AND INVESTIGATION OF THE DE NOVO POLYPEPTIDE WITH PREDICTED THREE-DIMENSIONAL STRUCTURE, A.N.Fedorov[1], D.A.Dolgikh[1], V.V.Chemeris[1], B.K.Chernov[1], A.V.Finkelstein[1], A.A.Schulga[2], Yu.B.Alakhov[1], M.P.Kirpichnikov[2] and O.B.Ptitsyn[1]. [1] Institute of Protein Research, [2]Academy of Sciences of the USSR, 142292 Pushchino, Moscow Region, USSR and [2] Institute of Molecular Biology, Academy of Sciences of the USSR, 117984 Moscow, USSR

An artificial polypeptide was designed in order to obtain three-dimensional structure consisting of four antiparallel β-strands covered from one side by two α-helices. The gene coding for this polypeptide was chemically synthesized and cloned. The polypeptide was synthesized in the mRNA-dependent cell-free wheat germ translation system. The approach has been developed to study the synthesized polypeptide with only nanogram amouts of a radiolabelled sample. Rapid cooperative transition between compact and unfolded forms was observed by urea gradient electrophoresis with transition midpoint at 4.5 M urea. Limited proteolysis has shown that polypeptide has a different sensitivity for trypsin in a buffer than in 4 M urea. These data suggest that the polypeptide has a stable compact structure.

R 313 TYPE II REVERSE TURN CONFORMATIONAL STUDIES IN SOLUTION UTILIZING SHORT PEPTIDES, Stewart L. Fisher, Rex A. Moats and Barbara Imperiali, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.

Efficient *de novo* protein design is reliant upon establishing stable secondary and super-secondary structure in solution. Our goal has been to design a stable type II reverse turn utilizing 4-6 amino acid residues, which can then be incorporated in longer polypeptides to create stable super-secondary structures. To this end, we have studied the conformational properties of peptides with the basic sequence **L**aa-**Pro**-**D**aa-**L**aa. All the peptides studied were capped at both the N- and C-termini to avoid strong electrostatic interactions (between these groups), and to best emulate a sequence within a longer polypeptide chains. Specific nOe connectivities from 2D NMR ROESY experiments and low temperature coefficients from 1D NMR variable temperature experiments in d6-DMSO were used as a basis for assessing conformational stability. It was found that steric crowding at the n+2 position destabilizes a type II turn, while branching at the β-carbon in the n position appears to stabilize the turn. Also, in all the peptides studied to date, it appears that the N-terminal cap has a tendency to hydrogen bond with the n+2 residue amide proton. This poster will aim to define the peptide secondary structure which is consistent with our experimental results.

R 314 SATURATION MUTAGENESIS OF HUMAN INTERLEUKIN-1α, Richard B. Gayle III, Kurt Poindexter, Rita Jerzy, Steve Dower, Subhashini Srinivasan and Carl March, Immunex Corporation, Seattle, WA 98101

Human interleukin-1α (IL-1α) is a potent cytokine, involved in inflammatory responses, as well as being an important molecule in regulation of the immune system. It shares approximately 22% homology with another protein, interleukin-1β, which binds with similar affinity to the same receptors as IL-1α. IL-1α and IL-1β have been intensely studied by biophysical means such as NMR and X-ray crystallography. Although the structures for both molecules have been determined, and some site-directed mutants have been examined, no coherent model has been produced which identifies the residues important for activity. Using a new protocol for contaminating the phosphoramidites, saturation mutagenesis utilizing cassettes of the human IL-1α gene was performed, resulting in cloning efficiencies in *Escherichia coli* greater than 95%. This protocol resulted in approximately equal frequencies of 1-5 nucleotide changes per oligonucleotide, with very few wild type sequences. A relatively small number of clones can then produce nucleotide changes at every possible residue. Protein production was initiated with a novel induction scheme utilizing a pH shift of the growth medium. This allowed over 200 colonies to be grown and induced in 1 day by a single person. Both biological activity and binding activity were examined. Mutants which altered one of these activities to a larger extent than the other are easily identified. Mutants with altered activities will have a ratio of biological to binding activity significantly different from the ratio of wild type IL-1α. Mutants which had no effect on the ratio, as well as some which had significant effects, were identified.

R 315 **THE FUNCTIONAL CONSEQUENCES OF INTRODUCING A POSITIVE CHARGE AT CLOSE PROXIMITY TO THE CATALYTIC SITE OF SUBTILISIN BPN'**, Thomas P. Graycar, I. Helena Sagar, and David A. Estell, Department of Research, Genencor International Inc., 180 Kimball Way, South San Francisco CA 94080.

It has recently been stated that the "electrostatic complementarity" of the active site of serine proteases to changes in the charge distribution that occur during the hydrolysis reaction is the source of their catalytic activity (1). To examine the functional consequences of introducing a positive charge at less than 10 Å from the site of peptide bond hydrolysis in the Bacillus serine protease subtilisin BPN', a site-specific mutant having the methionine at position 222 replaced with lysine was kinetically characterized using a variety of synthetic substrates.

(1) A. Warshel, et. al., Biochemistry 28, 3629 (1989).

R 316 **De Novo Design and Structure of Metal-Binding Four-Helix Bundle Proteins.** Tracy M. Handel and William F. DeGrado. Central Research and Development Department; E. I. DuPont de Nemours; Wilmington, DE, 19880-0328.

In previous studies, NMR spectroscopy has been used to probe the conformation and dynamics of a 16-residue α-helical peptide, α_1B (Ac-GELEELLKKLKELLKG-CONH$_2$), that self-associates into a four-helix bundle. In particular, it was possible to to show that the peptide is helical between residues 2 and 15 by 2-D NOESY experiments (W. DeGrado, T. Handel, J. Hoch, D. Live, J. Osterhout, D. Weaver). However, because of the symmetry of the bundle and the degeneracy of the amino acid sequence, it was not possible to establish the orientation of the helices with respect to each other based on cross peaks observed in NOESY spectra. To circumvent this difficulty we have recently carried out both 2-dimensional NOESY and 3-dimensional NOESY-TOCSY experiments of analogues of the original peptide which have Val and Phe at positions 3 and 13 and which are selectively deuterated in the Leu residues.
Building on the framework of the four-helix bundle model, we have also introduced metal binding sites containing 3-His residues into a helix-loop-helix peptide which dimerizes to give a 4-helix bundle protein (α_2) and a full-length 4-helix bundle (α_4). In one instance, a zinc coordination site modelled after carbonic anhydrase was designed on the exterior of these peptides. This site, which consists of 3 His residues, was constructed to probe the folding of the bundles and as a first step in the design of a bundle with catalytic activity. A second design modelled after blue copper proteins has a Cu^{+2} site (His-Cys-His) on the interior of the bundle. Characterization of these proteins by NMR and CD will be described.

R 317 **TOLERANCE OF MULTIPLE XAA→ALA SUBSTITUTIONS IN AN α-HELIX OF T4 LYSOZYME.** Dirk W. Heinz, Walt A. Baase and Brian W. Matthews, Institute of Molecular Biology and Howard Hughes Medical Institute, University of Oregon, Eugene, OR 97403

Single and multiple Xaa → Ala substitutions were introduced into an α-helix comprising residues 40-49 in T4 lysozyme. Single substitutions located on the solvent exposed side of the helix (e.g. N40A, S44A, E45A) slightly enhanced the thermostability of the protein, whereas substitutions of amino acids involved in interactions with the hydrophobic core (e.g. K43A, D47A and especially L46A) showed substantial decreases in stability. The results support the idea that alanine is a helix-favoring residue and that replacements of solvent-exposed residues in α-helices with alanines can increase protein stability. The mutant with alanines in position 40 through 49 folded correctly and was active, but was substantially less stable than wild type T4 lysozyme. The thermostability of the wild-type enzyme, however, could be restored by keeping both K43 and L46 unmutated. Crystal structures and thermodynamic data for the mutant lysozymes will be presented.

R 318 DESIGN AND CONSTRUCTION OF NOVEL LIGAND BINDING SITES IN PROTEINS.

H.W. Hellinga, F.M.Richards. Department of Biochemistry and Biophysics, Yale University, New Haven CT06511

We have devised a molecular model building computer program *(DEZYMER)* which builds new ligand binding sites into proteins of known three-dimensional structure. It alters only the sequence and the side-chain structure of the protein, leaving the protein backbone intact. The program searches for a constellation of backbone positions arranged such that if appropriate side-chains were placed there, they would bind the ligand according to a pre-defined geometry of interaction specified by the experimentalist. These binding sites are introduced by the program taking into account rules known to maintain the integrity of a protein structure. It is hoped that this program provides a very general method for the design of ligand binding sites and enzyme active sites which can then be tested experimentally.

A test case is presented where the copper binding site found in blue-copper proteins such as plastocyanin and azurin is introduced into *E. coli* thioredoxin. This mutant thioredoxin, which we have named "chelatin", was constructed by site-directed mutagenesis. A variety of techniques have been applied to show that chelatin is properly folded, retains thioredoxin-like properties, and has obtained an extra copper binding site with a binding constant of $3x10^{-7}$ M. We are in the process of applying spectroscopic techniques to investigate the nature of the coordination chemistry of this new copper site.

R 319 SEQUENCE REQUIREMENTS FOR COILED-COILS: ANALYSIS WITH λ REPRESSOR-GCN4 LEUCINE ZIPPER FUSIONS, James C. Hu[1], Erin K. O'Shea[2], Peter S. Kim[2] and Robert T. Sauer[1], [1]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139 and [2]Whitehead Institute for Biomedical Research, Cambridge, MA 02142

The leucine zipper is a structural motif that consists of two α-helices arranged in a short parallel coiled-coil. Leucine zippers are the archetype of an emerging family of dimerization motifs that play an important role in the control of gene expression by mediating the assembly of homodimeric and heterodimeric transcription factors. We have developed a genetic approach to study protein dimerization structures, using protein fusions to the N-terminal DNA binding domain of bacteriophage λ repressor. Repression of λ gene expression acts as a phenotypic reporter for assembly of dimers in *E. coli*. This system was used to analyze the importance of amino acid side chains in the dimer interface of the leucine zipper from the yeast transcriptional activator GCN4. We find that, as in the cores of globular proteins, residues buried in a functional dimer interface tend to be hydrophobic. Furthermore, although many substitutions that replace one buried hydrophobic amino acid with another are tolerated, leucine is preferred at the positions that it normally occupies. We also examined the importance of side chains that participate in interchain salt bridges. Although these interactions appear to determine the specificity of dimerization, they are not required for formation of stable dimers.

R 320 DESIGN OF IMPROVED HYDROPHOBIC CORE PACKING ARRANGEMENTS IN T4 LYSOZYME. James H. Hurley and Brian W. Matthews, Institute of Molecular Biology and Howard Hughes Medical Institute, University of Oregon, Eugene, Or. 97403.

A procedure for the design of protein hydrophobic cores with maximal hydrophobic stabilization and minimal strain has been developed and applied to the design of mutant T4 lysozymes. Each combination of the residues Ala, Ile, Leu, Phe, and Val at the seven solvent-inaccessible sites 91, 99, 102, 111, 121, 133, 153 in the α-helical C-terminal core of T4 lysozyme was tested for acceptable packing. 56 core sequences had a net hydrophobicity greater than or equal to the wild-type core sequence, no violations of packing more severe than wild-type, and no net reduction in helical propensity. Model structures for each allowed combination of side-chain conformers for these sequences were subjected to constrained energy minimization. A correction based on the estimated interaction of each hydrophobic residue with solvent in the unfolded state was applied to the calculated energy. Mutant sequences with the lowest calculated energies, differing at 2 or 3 positions from wild-type, were constructed by site-directed mutagenesis. The crystal structures and thermal stabilities of these mutants are compared with prediction.

R 321 CRYSTAL STRUCTURE OF THE APPI/TRYPSIN COMPLEX & CONTRIBUTION OF INDIVIDUAL SIDECHAINS TO SPECIFICITY USING SITE-DIRECTED MUTAGENESIS.
Thomas Hynes, Laura Kennedy, Anthony Kossiakoff, Genentech, South San Francisco, CA 94080
Alzheimer's amyloid β-protein precursor contains the Kunitz protease inhibitor domain APPI. To aid the identification of physiological targets of the inhibitor, the crystal structure of the complex of APPI with trypsin has been determined and refined to 1.8 Å resolution. The backbone conformation of APPI was found to be very similar to the homologous inhibitor BPTI, indicating that altered specificity of APPI results from the five residues in the inhibitor-protease interface which differ between APPI and BPTI. Arg-15 of APPI occupies the P1 binding site of trypsin and forms a direct salt bridge with Asp-189 of trypsin as compared to the water bridged interaction of Lys-15 in BPTI. APPI side chains Met-17 and Phe-34 are involved in hydrophobic interactions with Tyr-151 of trypsin in contrast to the hydrogen bond between Arg-17 of BPTI and the His-40 backbone oxygen of trypsin. This suggests that protease targets will have hydrophobic residues facing this region of the inhibitor. To map the contributions of individual inhibitor residues to protease specificity, sidechains of APPI and BPTI in the inhibitor-protease interface have been mutated to alanine and assayed for binding affinity to a number of serine proteases.

R 322 PROTEIN STABILIZATION BY ENGINEERED PAIRS OF HISTIDINE RESIDUES WHICH CAN CHELATE METAL IONS.
James T. Kellis, Jr. and Frances H. Arnold, Division of Chemistry and Chemical Engineering, 210-41, California Institute of Technology, Pasadena, CA 91125.

We have inserted histidine residues into *S. cerevisiae* iso-1-cytochrome *c* by site-specific mutagenesis in order to form synthetic metal-binding sites. The sites were chosen so the imidazole nitrogens of the histidine pairs would be positioned to favor metal chelation; for example, His-X_3-His in an α-helix brings the side chains close to one another in space and provides the proper orientation for bidentate binding to Cu(II) and other transition metals. This strategy was originally conceived to allow the purification of the recombinant protein by metal-affinity chromatography and aqueous two-phase extraction using metal-chelating polymers. We predicted that metal ions would shift the thermodynamic folding/unfolding equilibrium by binding preferentially to the folded state of the protein, where the preorganization of the histidine ligands results in a low entropic cost for forming both bonds to the metal simultaneously. Equilibrium unfolding measurements of the proteins reveal that their conformational stability is increased by metal binding. Incorporating synthetic metal-binding sites into recombinant proteins thus provides a useful strategy for their purification and stabilization.

R 323 NMR STUDIES OF A CONSENSUS ZINC FINGER PEPTIDE AND AMINO ACID VARIANTS
Valda J. Kilfoil, Barbara T. Amann, Beth A. Krizek, Scott F. Michael and Jeremy M. Berg
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In order to investigate the structural features of TFIIIA like zinc fingers, a prototypical single zinc finger peptide was designed using a data base of 131 sequences. This consensus peptide (CP1), which has the sequence P-Y-K-C-P-E-C-G-K-S-F-S-Q-K-S-D-L-V-K-H-Q-R-T-H-T-G has been synthesized and the structure of the zinc bound form has been determined by two dimensional nuclear magnetic resonance (nmr) techniques. General features are that residues 1-11 adopt an antiparallel β hairpin structure and residues 15-25 form a helix. These results are consistent with a predicted structure and with structures determined for other zinc finger binding domains. CP1 forms a very stable zinc bound structure as evidenced by its binding constant and pH stability. An nmr monitored pH titration has shown that the second histidine can be protonated and dissociated from the metal center with only loss of local structure. Further investigation of structure has involved the synthesis of amino acid variants. For example nmr studies of a variant with the phenylalanine switched from position 11 to 9 have shown a flip in orientation of the aromatic ring. To investigate minimum requirements for formation of a TFIIIA like zinc bound structure a peptide has been synthesized in which most residues apart from the three conserved hydrophobics and the four metal binding ligands (underlined above), have been replaced by alanine. This peptide has been found to bind zinc with a unique structure in which the hydrophobic residues have similar chemical shifts to those in CP1.

R 324 THERMAL STABILITY OF A MUTANT HUMAN LYSOZYME HAVING AN ENGINEERED CALCIUM BINDING SITE, R. Kuroki, K. Yutani*, M. Kikuchi. Protein Engineering Research Institute, 6-2-3 Furuedai, Suita, OSAKA 565, JAPAN, *Institute for Protein Research, 3-2 Yamadaoka, Suita, OSAKA 565, JAPAN.

We constructed a mutant human lysozyme (D86/92) having a calcium binding ability(1). In order to investigate the stabilization mechanism of D86/92 induced by the calcium binding, the thermal stability of D86/92 was measured by using differential scanning calorimeter (Privalov type, DACM-4). The denatured temperature(Td) of D86/92 was 85.0°C at the presence of 1mM $CaCl_2$ and pH 5.5, and the Td was 6.5°C higher than that of wild type (Td; 78.5°C). Moreover, the Td of D86/92 increased with the increase of Ca^{2+} concentration. The phenomenon suggests that a ligand binding in the folded state shifts the unfolding equilibrium to the folded state. The change of enthalpy(dH) in the unfolding of holo-D86/92 was measured to be 126.5 kcal/mol at 78.5°C, and almost equal to the values of the wild type and apo-D86/92 at the same temperature. We concluded that the stabilization of the holo mutant was mainly caused by the change of unfolding entropy. It is a result of the release of Ca^{2+} in the unfolding process.

REFERENCE; (1)R.Kuroki et al. (1989) Proc. Natl. Acad. Soc. USA 86,6903-6907.

R 325 ROLE OF DISULFIDE BONDS IN ACTIVITY OF INTERLEUKIN-6

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Interleukin-6 is a cytokine with various regulatory functions, such as induction of the acute phase response and involvement in differentiation of B-cells and multipotential hemopoietic progenitor cells. The tertiary structure of IL-6 is unknown, but it has been shown that the four cysteines in IL-6 form two disulfide bonds between Cys45-Cys51 and Cys74-Cys84. We studied the role of these disulfides on the function of IL-6 by replacing one or both pairs of cysteines with other amino acids.

Replacement of all cysteines by serines caused a significant loss of activity of IL-6 in assays using human B-cells or hepatocytes (<0.1 % activity of native rIL-6). This loss of activity was greater in human cell lines than in rodent cell lines (activity 2-20% of native rIL-6). Replacement of the cysteines with alanines gave similar results. Replacement of individual disulfide bonds showed that the bond between Cys74-Cys84 is far more important for biological activity than the Cys45-Cys51 bond. The loss of activity can be attributed to decreased binding of IL-6 to its receptor, as was demonstrated by binding studies, measuring the inhibition of binding of radiolabeled rIL-6 by unlabeled mutant IL-6. In attempting to stabilize the IL-6 molecule in the absence of disulfide bonds, we have replaced the cysteines by residues having the potential to form salt bridges. Replacement of all cysteines resulted in a similar loss of activity as found for the other residues mentioned above. The mutant with Cys45-Cys51 replaced and Cys74-Cys84 intact had a slightly lower activity than native rIL-6.

We suggest that the disulfide bond between Cys74-Cys84 is essential for normal conformation of IL-6. Replacement of this disulfide structure in IL-6 results in loss of the ability to bind to the IL-6 receptor and consequently loss of its activity.

R 326 Conformation and folding studies of a designed collagen model peptide. Ming-Hua Li, Barbara Brodsky, and Jean Baum. Dept. of Chemistry, Rutgers University and Dept. of Biochemistry, Robert Wood Johnson Medical School, Piscataway, N.J.

The triple helix is well suited for defining amino acid sequence features which direct protein folding and stability. The presence of glycine as every third residue and a high imino acid content are required for triple helix stability, and this conformation is adopted by small peptides, such as $(Pro\text{-}Hyp\text{-}Gly)_{10}$. NMR was used to characterize the conformation, stability and folding of $(Pro\text{-}Hyp\text{-}Gly)_{10}$. Several peaks in the ^1H-NMR spectra shift in going from the unfolded to the triple-helical state, and 2-dimensional NMR was used to assign these helix sensitive resonances to the GlyαCH_2 and ProδCH_2. The intensity of the helical peaks was used as a measure of helicity. Thermal equilibrium measurements show a sharp melting transition at 62°C, and the folding follows third order kinetics. CD folding and equilibrium curves differ from NMR data, and the differences suggest a extended single chain folding intermediate.

The ten tripeptide units in $(Pro\text{-}Hyp\text{-}Gly)_{10}$ could not be distinguished by NMR, so a more complex peptide, DP2, was designed to allow specific assignment of residues and the determination of 3-dimensional structure. The DP2 peptide, $(Pro\text{-}Hyp\text{-}Gly)_3$-Lys-Asp-Gly-Val-Arg-Gly-Leu-Thr-Gly-$(Pro\text{-}Hyp\text{-}Gly)_4$, contains 9 residues from the collagen αI(I) chain with Pro-Hyp-Gly caps at each end, and is found to form a stable triple helix. Two-dimensional ^1H-NMR spectra of DP2 show a reasonable chemical shift dispersion, and the spin system of the 9 central residues can be assigned. However, the three chains in this peptide cannot be differentiated. A peptide with ^{13}C and ^{15}N on the central 9 residues will be used to solve the structure by 3-D analogy NMR experiments. This would be the first characterization of a triple helix structure at the molecular level, and would provide a basis for detailed studies of folding and site directed substitutions.

R 327 MUTATIONAL ANALYSIS OF THE RAT CATECHOL-O-METHYLTRANSFERASE GENE, Kenneth Lundström, Hannele Ahti and Ismo Ulmanen, Orion Corp., Orion Pharmaceutica, Laboratory of Molecular Genetics, Helsinki, Finland. Catechol-O-methyltransferase (COMT) catalyses the inactivation of catechol hormones, catecholamine neurotransmitters and many neuroactive drugs. For increased efficiency of these drugs, like L-dopa in Parkinson's disease, specific inhibitors against COMT have been designed. To study the interaction between the COMT enzyme and inhibitors and for molecular analysis, we have cloned the rat liver COMT gene from a cDNA library. The COMT gene with an ORF of 663 nts encodes for a 221 aa long polypeptide.The COMT coding region was introduced into an E. coli expression vector and soluble active recombinant COMT was produced in bacteria. For studies of the COMT structure, series of 5' and 3' end deletions and site-specific mutations of the COMT gene were made applying the PCR technique. The expression of the mutant COMT enzymes in E. coli was characterized in SDS-PAGE, Western blotting and a COMT activity assay using dihydroxy-benzylamine as a substrate. All mutants showed high expression levels of COMT immunoreactive recombinant products. However, loss of COMT activity was seen for all 5' end deletions while a 5 aa deletion at the 3' end still resulted in fully active enzyme. Interestingly, aa-substitutions at positions 33 (Cys to Ala) and 38 (Trp to Leu) led to complete loss of enzyme activity, while Cys to Ala mutations at positions 69, 157 and 191 retained full activity.

R 328 THE STRUCTURE AND PROPERTIES OF A SURFACE-LOOP DELETION VARIANT OF SUBTILISIN BPN' . Colin Mitchinson and Richard Bott , Genencor International ,South San Francisico ,CA94019 .

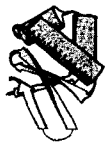
The bacillus subtilisins are a family of serine-proteases with high sequence and structural homology. There has been some success in recruiting properties of one subtilisin into another by making limited homologous substitutions (see Wells,J.A. and Estell,D.A. ,1988 , Trends Biochem. Sci. 13 ,291-297 , for a review). One marked variation among these proteins is that a surface loop present in BPN' (amongst others) is shortened in some other subtilisins (including that from *Bacillus lentus*). This difference interested us because ; 1) Surface loops are difficult to model and predict accurately. 2) Surface loops are commonly sites of proteolytic attack in proteins and autolytic stability is important in subtilisins . 3) The subtilisins from *B.amyloliquefaciens* and *B.lentus* have quite different enzymatic properties .

We modeled a deletion in BPN' based mainly on that found in the *B.lentus* subtilisin (Patent WO 89/06279.NOVO ,Hastrup et al.) . The variant was constructed ,expressed in B.subtilis ,purified ,crystallised and a high-resolution structure determined . We will discuss the variant's structure ,stability ,and enzymatic properties with reference to the above three points .

R 329 STRUCTURE AND STABILITY OF MONOMERIC VARIANTS OF λ CRO REPRESSOR CONTAINING DIFFERENT β HAIRPIN TURNS, Michael C. Mossing, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556.

A series of proteins have been constructed in which the dimer interface of λ Cro (an antiparallel β ribbon made up of a chain from each subunit) has been replaced by a β hairpin. Monomers have been characterized which are more stable than the wild type dimer and retain weak DNA binding activity. The structures (by NMR) and stabilities (by CD and calorimetry) of these proteins are being correlated with the sequence of amino acids which make up the turn. A crude screen for stability against proteolysis in *E. coli* indicates the following ranking of turn propensities of sequences making up the L1 and L2 positions of the hairpins: GG, NG, DG, GS > DA, GA, GT, NS > DT, ET, KT, NT, RT, ST, KS, SS. Among the most stable proteins are those with the consensus sequences identified by Sibanda et al. (J. Mol. Biol. **206** 759 (1989)) for the common type I' (underlined above) and Type II' (double underlined) turns.

Cro Dimer



Engineered Cro Monomer

R 330 **DEVELOPMENT OF A 3-D MODEL OF HIV REVERSE TRANSCRIPTASE,** Lakshmi S. Narasimhan and Gerald M. Maggiora, Computational Chemistry, Upjohn Laboratories, Kalamazoo, MI 49001

Development of a three dimensional model of HIV reverse transcriptase (HIV RT), a key enzyme in the pathogenesis of the human immunodeficiency virus, has presented a challenge. Structural information is not available for any close homologue, and 3-D structural understanding of polymerases is limited to the structure of the Klenow fragment of E. coli DNA Polymerase I, for which a C-alpha trace is available in public domain. The sequence homology between E. coli DNA Pol I and several other polymerases is rather weak, and the homology between HIV RT and E. coli DNA Pol I is even more tenuous.

A study of the sequences of several polymerases has led to a somewhat speculative hypothesis that the various DNA/RNA polymerases including Klenow fragment and HIV RT share a common structural motif around the polymerase active site. However, HIV RT and Klenow fragment seem to be distantly related in that there is very little sequence homology between the two enzymes, except for the few residues that make up the functionally critical motifs. In addition, in order to be consistent with results from affinity-based chemical modification experiments on the two enzymes, an exon-shuffling type of rearrangement of the HIV RT sequence with respect to the Klenow fragment has to be assumed. Starting with the assumptions of structural homology in the active site of these two enzymes, and a shuffled sequence, the sequence of HIV RT has been mapped on to that of Klenow fragment, using predicted secondary structure (mostly pattern-based methods) for guidance. This has led to a preliminary model for the tertiary fold of HIV RT. The features of this model and supporting rationale will be presented.

R 331 DESTABLIZATION OF THE E. COLI recA PROTEIN BY MUTATION OF ARGININE-243, Tien T. Nguyen and F. R. Bryant, Department of Biochemistry, Johns Hopkins University, Baltimore, MD 21205

The recA protein promotes the ATP-dependent pairing of DNA strands during homologous recombination in E. coli. It has recently been proposed that the region of the recA polypeptide from glutamic acid-241 to glutamic acid-259 forms an antiparallel ß-loop structure that may be involved in DNA binding (deJong et al., J. Mol. Biol. 206, 133 (1989)); this proposed structure is stablized, in part, by a salt bridge between arginine-243 and glutamic acid-259 at the base of the loop. To test this structural model, we have used site-directed mutagenesis to replace arginine-243 of the recA polypeptide with an alanine residue, a mutation that should disrupt the putative salt bridge. The [R243A]recA protein has been purified to homogeneity and is active in the standard recA protein-promoted strand pairing reactions. The binding of [R243A]recA protein to ssDNA, however, is more sensitive to inhibition by salt than is the binding of wild-type recA protein. The [R243A]recA protein is also less stable than the wild-type recA protein, with a half-life of 6 min at 37°C (based on loss of ssDNA-dependent ATPase activity), compared to 2.5 hours for the wild-type recA protein. Thus, the properties of the mutant [R243A]recA protein are consistent with the ß-loop DNA binding domain model proposed by deJong et al.

R 332 THE REDESIGN OF D-RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE-OXYGENASE, L. Jeanne Perry and David Eisenberg, Molecular Biology Institute, University of California, Los Angeles, CA 90024-1570

In addition to the pivotal importance of RuBisCO (D-ribulose 1,5-bisphosphate carboxylase-oxygenase) in photosynthesis and photorespiration, RuBisCO is the world's most abundant protein. The reason for this abundance is, at least in part, its catalytic inefficiency. Although structures are known for RuBisCO, its mechanism and regulation remain obscure. Conceivably, RuBisCO's catalytic efficiency can be improved, thereby increasing photosynthesis, enhancing agricultural yields. We propose to study RuBisCO's mechanism by designing a simpler version of the molecule. RuBisCO from tobacco leaves is a hexadecameric structure of eight large subunits and eight small subunits (L8S8). It has been shown that the catalytic active site is found at the interface of a $\beta\alpha$-barrel of one large subunit with another. The function of the small subunit is is unclear. We are using computer modeling and mutagenesis to engineer an enzyme composed of a single $\beta\alpha$-barrel (BAB RuBisCO). The purpose is to define the essential features of RuBisCO structure and function.

R 333 SITE SPECIFIC MUTAGENENSIS OF *BACILLUS STEARTHERMOPHILUS* AND *BACILLUS LICHENIFORMIS* ALPHA AMYLASE HYBRIDS, Carol Requadt, Stan Mainzer, Alisha Stephens-Jarnagin and Scott D. Power, Protein Chemistry, Genencor International, South San Francisco, CA 94080.

Procaryotic alpha amylases fall into several categories based on their relative thermostability. Among the more thermostable and industrially useful are the enzymes from *Bacillus stearthermophilus* and *Bacillus licheniformis*, both of which are used in starch liquefaction processes at temperatures greater than 95C. Despite their 60% homology and similar thermostability however, these enzymes differ in specific activity and action pattern. It would be useful from a processing point of view, to be able to recruit either the high specific activity of the *stearthermophilus* enzyme into the *licheniformis* amylase or the action pattern of the *licheniformis* enzyme into the *stearthermophilus* product. One process to accomplish this is to use recombination *in vivo* to generate new hybrid enzymes of the two. The synthesis of these hybrids has led to the determination of areas key to thermostability, activity and calcium binding. This work extends these initial observations, focussing on the use of site specific mutagenesis at positions 204, 206 and 210 of the *Bacillus stearthermophilus* enzyme (and its *B.licheniformis* hybrids), to probe structure/activity relationships in a region of the enzyme predicted to be involved in Calcium binding.

R 334 TRANSPLANTING ENZYME CATALYTIC MOTIFS INTO ANTIBODIES, Victoria A. Roberts, Elizabeth D. Getzoff, Stephen J. Benkovic*, Richard A. Lerner, and John A. Tainer, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037 and *Department of Chemistry, Pennsylvania State University, University Park, PA 16802.

To complement more conventional studies of enzyme active sites, which employ site-directed mutagenesis to probe the chemical function of amino acid residues, we have developed an approach involving the transplantation of catalytic motifs into antibody combining sites. To identify structurally conserved regions within the sequence-variable complementarity determining regions (CDRs) of antibodies, we superposed the framework regions of ten sets of antibody light and heavy chain variable domains (V_L and V_H). These conserved regions were then compared with catalytic motifs. One example of a catalytic motif is the three-dimensional pattern of catalytic zinc sites, which includes a main-chain hydrogen bond joining two of the zinc ligands. In carbonic anhydrase, the three zinc ligands lie on two hydrogen-bonded antiparallel β-strands. This motif was found to be accommodated within five structurally conserved sites general to all known antibody structures. For one such antibody light chain general site, the replacement of CDR1 position 34 and CDR3 positions 89 and 91 with histidine residues formed a zinc-binding site with an open coordination position at the bottom of the antigen-binding pocket. Creation of this site in the anti-fluorescein antibody 4-4-20 provided a mutant antibody that bound copper, zinc, and cadmium with the same preferences as carbonic anhydrase. This template-based multi-site design proved successful for remodeling an antibody to contain a cofactor-binding site, without requiring further mutagenesis and screening. This technique should not only improve the design of catalytic antibodies by allowing the addition of appropriate residues and metal cofactors, but also help evaluate the function of residues in enzyme catalytic motifs independently of the other features of the evolved enzyme active site.

R 335 BINDING CHARACTERISTICS OF A HIV-1 SPECIFIC SINGLE CHAIN FV FRAGMENT, Florian Rüker, Johann Kohl, Marlies Skias, Franz Steindl, Gottfried Himmler and Hermann Katinger, Institute for Applied Microbiology, University of Agriculture, Peter Jordanstraße 82, A-1190 Vienna, Austria

A single chain Fv fragment was constructed from the variable regions of the human monoclonal antibody, 3D6, and expressed in Escherichia coli. After refolding and purification, the binding characteristics of the recombinant protein to the native antigen, HIV-1 gp41, as well as to synthetic peptides representing the antigen were analysed and found to be similar to those of the native antibody produced by conventional cell culture. Use of the single chain Fv fragment for the affinity purification of HIV-1 envelope protein will be shown. Application of a fusion protein consisting of the single chain Fv fragment fused to E. coli Alkaline Phosphatase for the detection of HIV-1 gp41 in different samples will also be discussed.

R 336 STUDIES ON THERMOSTABILITY OF ENGINEERED SUBTILISIN MUTANTS BY TEMPERATURE-GRADIENT GEL ELECTROPHORESIS, Andrea Sättler and Detlev Riesner, Institut für Physikalische Biologie, Heinrich-Heine-Universität Düsseldorf, 4000 Düsseldorf, F.R.G.

The thermal unfolding of microbial serine proteases was studied by temperature-gradient gel electrophoresis (TGGE). In this method a temperature gradient is applied to the gel perpendicular to the direction of electrophoretic migration (Rosenbaum und Riesner (1987), *Biophys. Chem.* 26. 235-246). Mobility changes of the molecules in the temperature gradient are indicative for thermally induced conformational changes. Transition temperature can be determined with good accuracy. TGGE is used routinely for structure analysis of nucleic acids (Riesner et al. (1989), *Electrophoresis* 10, 377-389) and proteins (Birmes et al. (1990), *Electrophoresis* 10, 795-801). TGGE of proteins is carried out under the conditions of a native PAGE. The study of subtilisin with TGGE is rather difficult as compared to non-proteolytic enzymes: due to autoproteolysis the protein in its native conformation is digested at elevated temperature. Above the midpoint of transition, the protein could not be detected any longer in the temperature-gradient gel. After inhibition by PMSF, the complete structural transition became detectable. The transition is 'discontinous', i.e. the thermal unfolding of the protein is either very slow or it is irreversible as it is known for a subtilisin from calorimetric data. The catalytic activity of the native and non-inhibited protease conformation was shown by an esterolytic assay in the gel. The loss of activity is correlated exactly with the structural transition of the molecule. With a TGGE, where the temperature-gradient is parallel to the direction of sample separation, we are able to investigate the thermostability of a series of protein samples in one and the same temperature-gradient gel. Random Mutagenesis was directed to the weak Calcium-binding site of subtilisin Carlsberg. Three amino acid positions were varied leading to a set of 30 000 mutants. Currently, these mutants are tested for improved thermostability by parallel TGGE.

R 337 THE ROLE OF CHARGE ON THE ION CHANNEL FORMING PROPERTIES OF PARDAXIN Yechiel Shai, Diana Bach & Orelia Finkels.
Department of Membranes and Molecular Recognition. Weizmann Institute of Science, REHOVOT, 76100,ISRAEL

Pardaxin is a shark repellent peptide toxin (33 residues), isolated from the secretion of the Red Sea Moses Sole fish. At concentrations below 10^{-7}M, pardaxin was shown to form single channels in lipid bilayers, while at higher concentrations (10^{-7}M -10^{-4}M) cytolysis is induced. We have synthesized six analogues of pardaxin, by the solid phase method; [Glu^{8},Glu^{16}] pardaxin, [N^{1}-succinamido,Glu^{8},Glu^{16}] pardaxin, [N^{1},Lys^{8},Lys^{16}-trisuccinamido] pardaxin, [N^{1},Lys^{8},Lys^{16}-triacetyl] pardaxin, [des 1—>9]pardaxin, and [des 1—>9][Glu^{16}]pardaxin. The spectroscopic and functional characterization of the analogues is described. The peptides were characterized by; (1) circular dichroism (CD) before and after their binding to vesicles, (2) the potential to dissipate diffusion potential, (3) the ability to induce release of calcein and ANT/DPX from sonicated unilamellar liposomes, (4) by their ability to create single channels in planar bilayers, and (5) by measuring their cytolytic activity on human erythrocytes. Their activities were obtained at three pH values; 7.4, 5 and 4.5. All the analogues were found to produce single channels however of different properties. Cytolytic activity was found only for pardaxin. The release of calcein and ANTS/DPX from few of the analogues are pH dependent.

R 338 ADENYLATE KINASE: SITE-DIRECTED MUTAGENESIS VERSUS X-RAY AND NMR. Zhengtao Shi, Honggao Yan, Terri Dahnke, and Ming-Daw Tsai, Department of Chemistry and Ohio State Biochemistry Program, The Ohio State University, Columbus, OH 43210.

Prior to 1987 the X-ray studies by G. E. Schulz et al. and the NMR studies by A. S. Mildvan et al. led to two dramatically different sets of substrate binding sites for adenylate kinase. We then undertook site-directed mutagenesis studies to test the contradicting results. For each mutant enzyme the kinetic data was not interpreted until the structures of the free enzyme and its binary and ternary complexes had been examined by proton NMR to ensure no global conformational perturbations relative to the wild-type enzyme. Once the kinetic data had been interpreted and a particular residue had been suggested to be important to catalysis, further NMR and/or kinetic studies were then performed to identify the specific function of the residue. Such studies led to the following conclusions: Asp-93 is involved in the binding of Mg^{2+}, Arg-44 and Arg-97 are involved in the binding of AMP, Arg-138 and Arg-149 stabilize the ternary complex modestly and the transition state greatly, and Arg-132 prevents AMP from binding to the MgATP site. Three residues, Lys-27, His-36, and Thr-39, which have been suggested to be interacting with substrates by previous NMR or X-ray studies, appear to be nonessential. These results taken together suggest that the binding sites deduced by earlier NMR and X-ray studies both require serious revisions.

R 339 **COMBINATORIAL MUTAGENESIS OF NON-CONTIGUOUS PROTEIN SEGMENTS; STUDIES ON THE ACTIVE SITES OF ENDONUCLEASE *Eco*RI AND BETA-LACTAMASE.** Xavier Soberón, Joel Osuna, Humberto Flores, Héctor Vladiu and Ernesto Cota.
Dep. de Biología Molecular, CIIGB, National University of México, Apdo. Postal 510-3, 62271, Cuernavaca, Mor. México.

We focus our studies on clusters of residues, in the vicinity of active sites, which have been implicated directly in the catalytic activity or the determination of specificity. We apply mutagenesis schemes, using degenerate oligonucleotides and polymerase chain reaction, to generate collections of fragments that comprise a number of variants commensurate with the type of assay that we have available. We do this over windows of one to six or more residues that may be distant in the primary structure, in a combinatorial fashion; we also adjust the mutagenesis rate (at the oligonucleotide synthesis step), so as to get the desired distribution of amino acid replacements, from single to multiple.
Using the above approach, we have generated collections of mutants for the enzymes *Eco*RI endonuclease and beta-lactamase. Through suitable genetic screenings like phenotype of colonies or selection for growth, we have isolated mutants that reveal the involvement of some residues in the activities of the enzymes. Details on the mutagenesis methods, as well as the properties of some mutant proteins will be presented.

R 340 C-TERMINAL PROCESSING, FOLDING AND ACTIVE SITE MUTAGENESIS OF BARLEY α-AMYLASE, M. Søgaard*, F.L. Olsen #,‡ and B. Svensson*, Departments of Chemistry* and Physiology#, Carlsberg Laboratory and Carlsberg Research Laboratory‡, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark.

A barley α-amylase 1 cDNA was expressed in yeast leading to secretion of four different forms of the corresponding enzyme as directed by the endogenous signal peptide (Søgaard, M. and Svensson, B. (1990), Gene, 94, 173-179). The purified forms differed in pI between 4.7 and 5.1 and had very similar M_r. Two lacked Arg-Ser at the C-terminus and were also generated by treatment of the two other forms with carboxypeptidase Y from yeast or malt carboxypeptidase II leading to a decrease in pI. One such set of recombinant, truncated/full-length α-amylase 1 was fully active, while the other showed a 20-fold reduced k_{cat} towards p-nitrophenylmaltoheptaoside as well as disulfide mispairing. Otherwise, the 4 recombinant products were indistinguishable from α-amylase 1 isolated from malt with respect to substrate affinity, β-cyclodextrin-binding at a surface site, reaction with 3 monoclonal antibodies, Ca^{2+}-binding and limited proteolysis by proteinase K.

The catalytic mechanism of α-amylase is not clear. Barley α-amylase 1 derivatives mutated at each of three carboxyl (Asp180, Glu205 and Asp291) or two histidyl (His93 and His290) residues conserved at the catalytic site of all α-amylases were produced in yeast. The specific activity of the carboxyl group mutants was reduced 2-3 orders of magnitude and indicated a role in catalysis for all three residues.

Transient precursors of α-amylase 1 are found in aleurone protoplasts. Their conversion to mature α-amylase 1 is accompanied by a decrease in pI and was inhibited by added serine carboxypeptidase inhibitors. Furthermore, since precursors purified from protoplasts could be converted into the mature forms with malt carboxypeptidase II *in vitro* and α-amylase 1 purified from malt lacks the C-terminal 7 aa residues, it is strongly indicated that *in vivo* processing during seed germination involves a serine carboxypeptidase.

R 341 RESIDUE HELICAL PREFERENCE VALUES OBTAINED FROM CIRCULAR DICHROIC MEASUREMENTS OF MONOMERIC PEPTIDES, Earle Stellwagen, Soon-Ho Park, Gene Merutka, Wolfgang Schaller and John Rovang, Department of Biochemistry, University of Iowa, Iowa City, IA 52242

Each of the 20 peptides in the homologous series having the sequence acetylY(EAAAK)(EAXAK)(EAAAK)Aamide exhibits circular dichroic properties characteristic for a monomeric two-state helix/coil equilibrium. This equilibrium is markedly dependent upon the temperature, pH and ionic strength of the solvent and upon the identity of the residue X at the central position in the peptide sequence. Systematic analysis of these variables suggests that electrostatic interactions of the charged residues with each other and with the helical macrodipole significantly perturb the helix/coil equilibria in solvents of low ionic strength at neutral pH. These electrostatic interactions appear to be substantially diminished at pH 2 thereby illuminating the inherent helical preference of the individual residues. The monomeric helix/coil equilibria of the 20 peptides observed in solutions of low ionic strength at pH 2 and 0° compare very favorably with the dimeric helix/monomeric coil peptide equilibria recently reported for the 20 residues by O'Neil and DeGrado, Science 250 646-651 (1990).

R 342 EXPRESSION OF ANTIBODY Fv FRAGMENTS SPECIFIC FOR A HEAVY METAL CHELATE (INDIUM-EDTA) IN E. COLI, Willem P. Stemmer, Suzanne K. Morris, Curtis R. Kautzer and Barry S. Wilson, Therapeutics Division, Hybritech Inc. San Diego CA 92126-9006.

The expression of monoclonal antibody binding fragments (Fv) in E. coli was approached using the pIN-III plasmid controlled by the lpp-lac promotor-operator. For initial studies, a single gene containing only a light chain sequence (mouse lamda V region and human kappa C region) was used to evaluate the impact of leader peptide type, drug marker choice and bacterial strain on secretion of light chains into the periplasmic space and the media. Based on these data, we chose to use the ompA leader peptide, the chloramphenicol resistance gene and RV308 as the host strain. The variable regions of the murine monoclonal antibody CHA255, specific for Indium-EDTA, were used to code for an Fv binding fragment. This antibody has been used as part of a bifunctional antibody delivery system to image colon carcinoma through capture of a ^{111}Indium-EDTA benzyl derivative. The structural genes were inserted into the vector as a bi-cistronic message with the light chain followed by the heavy chain, both with their own leader peptides. Antibody activity assays based on an ELISA inhibition format indicated that active antibody molecules were secreted to the periplasmic space and were also released to the media. Binding studies performed at 37°C versus room temperature indicated instability of the two chain molecule at the elevated temperature. These data suggest that some form of interchain stabilization will be needed to utililze this antibody in an in vivo setting.

R 343 PRODUCTION AND CHARACTERIZATION OF RECOMBINANT KRINGLE 1 DOMAIN OF HUMAN PLASMINOGEN, Seiji Sugimoto, Tatsunari Nishi, Seiga Itoh, and Yoshiharu Yokoo, Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Machida, Tokyo, 194 Japan

Kringle 1 (K1) is a functional domain for fibrin affinity of plasminogen and plasmin. Therefore, fusion proteins of K1 and plasminogen activators have been investigated to increase fibrin clot affinity of the plasminogen activators. In order to investigate the fibrinolytic therapy by a chemical conjugate of K1 and other agent, we established a novel procedure for production of K1 with recombinant technique. A fusion protein of B domain of protein A and K1 was expressed in Escherichia coli cells. The fusion protein was extracted, fractionated by ammonium sulfate precipitation, refolded, purified on IgG column utilizing the specific affinity between protein A and IgG, and digested by trypsin. Released K1 was purified on lysine column and reversed-phase high performance liquid chromatography. Total yield was 40 %. We found that trypsin selectively digests K1 that was refolded incorrectly. Schachard analysis of K1 with Lysine-Sephrose revealed that purified K1 had a single dissociation constant. Therefore, purified K1 has natural structure. We also produced a derivative of the fusion protein with a single point mutation in the B domain of protein A to introduce a zinc binding site. The fusion protein was purified on zinc chelate column instead of IgG column.

R 344 STRATEGIES FOR SECRETION OF ANTIBODY FRAGMENTS AND SINGLE CHAIN ANTIBODIES IN *ESCHERICHIA COLI*, Tuula T. Teeri, Kristiina Takkinen, Dorothea Sizmann, Marja-Leena Laukkanen, Ari Hemminki, Liisa Vanne, Kaija Alfthan, VTT Biotechnical Laboratory, P.O. Box 202, SF-02151 Espoo, Finland

The cDNAs coding for the Fab fragments of different antibodies have been cloned and expressed in *E. coli* under the tac promoter. Expression, activity and stability of Fab, Fv, V_H and V_L fragments as well as secretable single chain antibodies in *E. coli* culture supernatants have been compared. Results concerning the effects of different signal sequences and different linker peptides on the secretion and stability of single chain antibodies will be discussed.

R 345 DESIGN OF DNA BINDING DOMAINS WITH DESIRED DNA TARGET SPECIFICITIES USING THE ZINC-FINGER SPECIFIC RECOGNITION CODE DETERMINED BY THE TARGET DETECTION ASSAY (TDA). Hans-Jürgen Thiesen and Christian Bach, Basel Institute for Immunology CH-4005 Basel, Tel.: CH-61 6051256; Fax: CH- 61 681 13 04

Recently, thirty nonoverlapping cDNAs (cKox1-cKox30) were isolated from human T cells encoding zinc finger structures. Comparisons of individual zinc fingers of cKox1 with finger domains of cKox2 to cKox30 showed that some Cys_2/His_2 zinc fingers are highly conserved in their putative α-helical DNA binding region, supporting the notion of a zinc finger-specific DNA recognition code (New Biol. 2, 363-367). A collection of recombinant SP1 proteins mutated in their zinc finger domains were subjected to the target detection assay (TDA) (NAR 18, 3203-3209). Using randomized double-stranded oligonucleotides (DNA and Protein Engineering Techniques 2, 92-95) target sites were selected by these mutant proteins. Specific amino acid positions were identified as being involved in sequence-specific DNA recognition. By mutating amino acids at these positions the target specificity of SP1 switched from one oligonucleotide to another. Different specific mutations displayed different strength of DNA binding affinity for different DNA target sequences which allowed us to generate a table describing which amino acid combinations recognize which nucleotides with what affinity. This zinc finger specific recognition code can be used to predict target sites for zinc finger proteins. In particular, this binding code is applied for designing zinc finger domains for desired DNA target sites. The analysis of protein mutations in combination with the target detection assay (TDA) turns out to be a very successful approach for developing therapeutical proteins with desired target specificities for RNA and DNA recognition.

R 346 ENGINEERING OF A LOW MOLECULAR WEIGHT GLUTENIN SUBUNIT OF WHEAT EXPRESSED IN INSECT CELLS, Stephanie A. Thompson, Peter R. Shewry* and David H.L. Bishop, NERC Institute of Virology and Enviromental Microbiology, Oxford, UK and Department of Agricultural Sciences, Long Ashton Research Station, University of Bristol, UK*.
Wheat seeds contain 50 or more proteins which make up a cohesive mass called gluten, the visco-elastic properties of which are important for food production such as breadmaking. Gluten proteins are divided into two groups, the gliadins which are monomeric, and glutenins which are polymeric consisting of individual subunits held by interchain disulphide bonds. Gliadins and glutenins also interact by non-covalent forces to produce gluten. Studies of gluten protein structure and functionality has been hampered by difficulty in the purification of individual subunits from such a heterogeneous mass of protein. A Low Molecular Weight Glutenin gene has been expressed in insect cells to high levels using a baculovirus vector. Protein has been purified and was found to be incorrectly folded inside the insect cells. A system has been developed to refold purified expressed protein, and mutants produced in which cysteine residues involved in intermolecular disulphide bond formation have been changed to serine in order to explore the molecular basis for polymer formation.

R 347 DESIGN AND SYNTHESIS OF LYTIC PEPTIDES, William J. Todd[4,5], William G. Henk[1], Kenneth L. White[2], and Jesse M. Jaynes[3], Departments of Anatomy[1], Animal Science[2], Biochemistry[3], Veterinary Science[4], and Veterinary Microbiology and Parasitology[5], Louisiana State University, Baton Rouge, LA 70803.
A variety of lytic peptides exist in nature primarily as components of venoms and as defense mechanisms against microbial pathogens. The lytic peptides usually consist of only 20 to 40 amino acids arranged in tertiary structure as an amphiphathic α-helix. Cecropin B, a principle component of the humoral defense system of the giant silk moth, Hyalophora cecropia, is composed of 35 amino acids. This peptide is lytic for bacteria. We have designed and synthesized functional analoques of cecropin B. By conserving hydrophobicity and charge on amino acid substitutions, we were able to alter about 60% of the sequence while retaining lytic function. The analogs were synthesized by Fmoc chemistry, purified by sephadex column chromatography followed by reverse phase HPLC. The synthetic analog with 60% amino acid substitution was as bacterialcidal as native cecropin B. Ultrastructural analysis revealed that the cecropin B analog killed bacteria by cell lysis, and that pores were formed in the outer membrane of the treated bacteria. The lytic peptides melittin, from bee venom, and Magainin 2, from the frog Xenopus laevis, were also used as models for the synthesis of functional analogs of lytic peptides.

R 348 PHOSPHOLIPASE A_2 ENGINEERING: STRUCTURAL AND FUNCTIONAL ROLES OF ACTIVE SITE AND SURFACE RESIDUES. M.-D. Tsai, C. A. Bingman, T. Deng, C. M. Dupureur, K. J. Hamilton, J.-G. Kwak, J. P. Noel, C. Sekharudu, and M. Sundaralingam, Department of Chemistry, The Ohio State University, Columbus, OH 43210.

The structural and functional roles of some active site and surface residues of bovine pancreatic phospholipase A_2, overproduced in *E. coli* and refolded *in vitro*, have been investigated with site-directed mutagenesis in conjunction with structural analysis by X-ray crystallography. The results to be presented are summarized as follows: (a) Substitution of Lys-53 and/or Lys-56 with neutral and/or hydrophobic residues resulted in the increase of activity toward phosphatidylcholine substrates but not toward negatively charge substrates. These surface residues appear to be interacting with micellar substrates. (b) Substitution of the active site Asp-99 by Asn resulted in a 1000-fold decrease in the activity of the enzyme. (c) Replacement of Tyr-52 and/or Tyr-73 (which form H-bonds with the carboxylate of Asp-99) with Phe caused little change in the activity. However, substitution with non-aromatic amino acids resulted in 10^2- to 10^3-fold decreases in activity. Thus these two tyrosine residues are more important for structure than for function. (d) Replacement of Phe-106 (which is involved in an aromatic-aromatic interaction with Phe-22) with Ile or Ala prohibited the enzyme from folding, which implies that this residue could be involved in the folding of phospholipase A_2. (e) Structural and functional roles of other residues still under investigation will also be presented.

R 349 MULTIPLE ALTERATIONS AT THE TWO DOMAIN INTERFACE OF PAPAIN BASED ON THE IDENTIFICATION OF COORDINATED MUTATIONS, Thierry Vernet[1] and Danièle Altschuh[2], [1]Biotechnology Research Institute, National Research Council of Canada, Montréal, Québec, H4P 2R2, Canada; [2]Institut de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, 64084 Strasbourg Cedex, France.

Redesigning the packing of amino acids in a given region of a protein is a challenging problem since alternative packing solutions must respect complex combinations of compositional, volumetric and steric constraints. Homologous proteins provide examples of alternative sequences leading to similar functional structures. An analysis of the pattern of amino acid substitutions in families of homologous proteins has shown that some amino acids close together in the crystallographic structure are substituted together. We have previously suggested that such positions with coordinated changes may represent alternative packing solutions (Altschuh, et al., Protein Engineering, **2**, 193, 1988). To test this hypothesis we have transferred into the precursor of papain such a coordinated contact, whose side chains interact across the interface of the two domains of papain. Alterations of the contact affect characteristics of the precursor but not the enzymatic activity of the mature form of the enzyme. The various mutants never perfectly mimic the properties of the wild type precursor. However, detrimental effects of the single mutations can be partly suppressed by a coordinated mutation that reproduces naturally occurring contacts.

R 350 SUBSTRATE SPECIFICITY OF HIV PROTEASE ANALYZED BY MOLECULAR MODELING BASED ON CRYSTAL STRUCTURES, Irene T. Weber, Clare Sansom, and Alla Gustchina, Crystallography Laboratory, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702.

The human immunodeficiency virus (HIV) protease (PR) is a target for antiviral drugs to treat AIDS, since it is essential for viral replication and infectivity. Several co-crystal structures of HIV-1 PR with different inhibitors have been determined. We have used these as a basis for molecular modeling of the related (about 40% identical residues) HIV-2 PR. Conserved residues predominate in the structural core and around the substrate binding region, while variable residues occur on the surface loops of the HIV-1 PR dimer structure. Several inhibitors and substrates have been modeled in HIV-1 and HIV-2 PRs and the structures compared with kinetic measurements made in other laboratories. Both qualitative and quantitative analysis are being developed to correlate the experimental results with the model structures. Four residues differ in the substrate binding regions of HIV-1 and HIV-2 PRs. Changes in the relative Ki values can be explained by differences in some of these residues.

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R 351 COMPUTER-BASED DESIGN OF A MUTANT ALPHA-LYTIC PROTEASE WITH ALTERED SUBSTRATE SPECIFICITY. Charles Wilson, James E. Mace, and David A. Agard. Department of Biochemistry and Biophysics, University of California, San Francisco, CA, 94143-0448.

We have developed an accurate and extremely rapid method for predicting the effects of mutagenesis on enzyme substrate specificity. Our algorithm makes use of an approximate free energy force field to evaluate the energy of a given conformation, and a library of side chain rotamers to sample conformation space. By estimating the energetic effect of a mutation on an enzyme-substrate complex, and then the effect on each of the components of the complex, it is possible to identify mutations which will increase or decrease activity of an enzyme for a given substrate. This algorithm accurately reproduces experimental data for 42 enzyme-substrate kinetics measurements for alpha-lytic protease. To test the ability of the algorithm to design enzymes with desired specificity, we evaluated all single-site mutations in the alpha-lytic binding pocket for the ability to cleave leucine substrates and to discriminate against isoleucine substrates. Replacement of Met192 by valine was predicted to create a protease with this specificity. The mutant protein was made and isolated and enzyme kinetics were determined. The wild-type enzyme was found to show little discrimination between the Leu and Ile substrates (k_{cat}/K_M = 4 and 2 $M^{-1}s^{-1}$ respectively). The M192V mutant is significantly more active for Leu (k_{cat}/K_M = 5300 $M^{-1}s^{-1}$), >200-fold more active than for the the Ile substrate (24 $M^{-1}s^{-1}$). Future work will attempt to increase the accuracy of the algorithm and the design of other proteases with significantly altered specificity.

R 352 STRUCTURE-FUNCTION STUDIES ON MYELIN-ASSOCIATED GLYCOPROTEIN (MAG) USING CONFORMATIONALLY SENSITIVE FUNCTION-BLOCKING MONOCLONAL ANTIBODIES (mAbs). Michael B. Tropak, John C. Roder, Division of Molecular Immunology and Neurobiology, Samuel Lunenfeld Research Institute at Mount Sinai Hospital, Toronto, Ontario, CANADA M5G-1X5

MAG is a cell adhesion molecule which mediates the interaction between myelinating glia and neurons via a neuronal receptor. It is a member of the immunoglobulin (Ig) superfamily and consists of five Ig-like domains. We have used insertion, deletion and substitution mutagenesis to map the epitopes of conformationally sensitive function-blocking mAbs which may recognize the receptor binding site on MAG. Because lack of binding may be attributed to improper folding and/or loss of epitope we have designed our constructs to determine the minimum size of protein which can bind. We have used surface expression, ER retention and mAb binding to assess the native state of MAG. The conformational epitopes of two function blocking mAbs, 513 (M. Schachner) and 15 (A.Meyer, M.Schachner) require domains 1,2 and 3 and domains 3 and 4, respectively. To localize the epitopes more precisely we are constructing chimeras between MAG and homologous proteins, CD33 and CD22, with the intention of reconstituting mAb and/or receptor binding. The linear epitopes of two other mAbs, GenS3 (N. Latov) and B11F7 (R. Quarles) have been localized to residues 633-642 and 1200-1227, respectively. The behavior of several insertion mutants suggests that the folding of domains 1,2 and 3 appear to be interdependent. This further suggests that domains 1, 2 and 3 may be conformationally linked. We have created contraction mutants by substituting portions of domain 3 with domain 4. The fact that we have obtained native-like mutants argues that domains 3 and 4 are structurally similar.

Late Abstracts

MOLECULAR CHARACTERIZATION OF BGH(96-133) AGGREGATION, S. Russ Lehrman, Jody L. Tuls, and Marilyn Lund,
Control Biotechnology, The Upjohn Company, Kalamazoo, MI 49001

Bovine growth hormone (bGH) is an antiparallel α-helix bundle protein that aggregates when partially denatured at high protein concentrations. The aggregated species has been identified using a number of biophysical methods including size exclusion chromatography, near-UV circular dichroism and dynamic light scattering.[1] This species appears to be stabilized by specific intermolecular interactions that involve an amphiphilic α-helix spanning residues 106 to 127.[2] We are interested in defining the specific intermolecular interactions that stabilize bGH aggregation. Our approach is to model the aggregation process by using bGH(96-133) and related peptides. The fragment derived from native bGH, which includes the amphiphilic α-helical protein region noted above, selectively inhibits protein aggregation, and itself forms α-helical aggregates in aqueous solution.[3] We find that the analog peptides have differing propensities for the formation of α-helical aggregates. Only those peptides that have strong potential for α-helix formation inhibit bGH aggregation. Of these, the inhibition of bGH aggregation is positively correlated with increasing peptide hydrophobicity. A model of peptide aggregation will be presented and its relevance to bGH aggregation will be discussed.

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3. Brems, D.N., Plaisted, S.M., Kauffman, E.W., Lund, M., and Lehrman, S.R. (1987) Biochemistry 26, 7774-7778.

ELECTRON MICROSCOPIC 3D-STUDY OF ISOLATED AND 2D-CRYSTALLIZED YEAST RNA POLYMERASE A (I). Schultz P., Célia H., Riva M°., Huet J°., Sentenac A°., and Oudet P. LGME-CNRS/U184-INSERM, 11 rue Humann, 67085 Strasbourg Cédex; (°) Biochimie Bat. 142, CEA 91191 Gif sur Yvette (F).

Isolated monomers and dimers of the yeast RNA polymerase A adsorbed on positively charged lipid layers were characterized by electron microscopy and a 3D-reconstitution was obtained from sets from tilted views. Processing of electron microscopic images allowed us to demonstrate that the yeast enzyme both as monomers or dimers are adsorbed on essentially one orientation on positively charged lipid films. The dimers, spontaneously formed in solution in low ionic strength can from organized linear arrays at the water liquid interface. In paralell 2D-crystals of the same material were obtained [P. Schultz et al., J. Mol. Biol. (1990) 26n 353-362). Using tilt series, the different projections were merged into a 3D model of the enzyme. The resolution obtained was better than 3nm. ALready some important features are visualized. The macromolecule shows an elongated shape and overall dimensions of the 15 subunits enzyme are 15-11-11nm. The enzyme is composed of two curved and twisted arms of different shapes delineating a large stain accessible groove.

The localisation of the biggest subunits is presently under way using immunological probes as well as the pathway of the DNA using synthesized fragments. Our approach demonstrates that the present electron microscopy and image processing techniques can provide strucutral informations of biological interest on large and complex macromolecular assemblies.