PROTEIN AND PHARMACEUTICAL ENGINEERING

Organizers: Charles Craik, Robert Fletterick, C. Robert Matthews and James Wells

January 17-22, 1989

Plenary Sessions Page

Poster Sessions

Theoretical Analysis of Proteins (joint)

A 001 CHARGE-CHARGE AND CHARGE-SOLVENT EFFECTS IN PROTEINS AND NUCLEIC ACIDS, Barry Honig, Kim A. Sharp and Michael K. Gilson, Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168 St., New York, NY 10032.

In the past few years it has been demonstrated in a number of laboratories that the Finite Difference Poisson-Boltzmann (FDPB) method provides a reliable means of treating electrostatic interactions in proteins and nucleic acids. This method makes it possible to account for solvent screening of electrostatic interactions, solvation energies and salt effects while maintaining a detailed atomic level description of the macromolecule[1]. In the treatment of phenomena including the electrostatically enhanced diffusion of the superoxide anion to superoxide dismutase[2], pK shifts induced by site-directed mutagenesis of charged residues in subtilisin[3] and the solvation energies of charged molecules[4], the method has yielded good agreement with experimental observations. One of the more striking findings that has emerged in a number of cases is that the shape of the boundary between the low dielectric macromolecule and high dielectric solvent plays an important role in determining the sign and magnitude of the electrical potential. In this talk the basis of the method will be outlined and a number of new applications will be discussed. These include: the effect of surface charge on the active site potential of enzymes; the destabilizing effect of the helix dipole, due to the loss of solvation energy, in the assembly of 4-helix bundle proteins; the electrical potential and ion atmosphere around DNA[5] and t-RNA; the magnitude of solvation terms in the conformational analysis of proteins and solvent effects on forces used in molecular dynamics simulations.

1. K. Sharp, M. Gilson, R. Fine and B. Honig, Protein Structure, Function and Design 2, Alan R. Liss Inc., p. 235 (1987).
2. K. Sharp, R. Fine and B. Honig, Science, 236, 1460 (1987).
3. M. Gilson and B. Honig, Nature, 330, 84 (1987).
4. M. Gilson and B. Honig, Proteins, 4, 7 (1988).
5. B. Jayaram, K. Sharp and B. Honig, Biopolymers, (in press).

A 002 EXPLORING ENZYME CATALYTIC MECHANISM BY CAD APPROACHES, Arieh Warshel, Department of Chemistry, University of Southern California, University Park, Los Angeles, CA 90089-1062

The origin of enzyme catalysis is explored by Computer Simulation approaches and the feasibility of Computer Aided Design of enzymes is examined. The CAD program used (referred to as ENZYMIX) combines the EVB method and a free energy perturbation method(1) in evaluating the free energy of enzymatic reactions. The simulations explore both the effect of the mutations and the more challenging aspect of the overall storage of catalytic free energies. Several test cases are presented and discussed. It is found in agreement with our early studies(2) that the electrostatic free energies of the reacting system is the key factor in structure-function correlation of enzymatic reactions.

(1) A. Warshel, F. Sussman and J-K. Hwang, J. Mol. Biol., 201, 139 (1988).
(2) A. Warshel, Act. Chem. Res., 14, 284 (1981).

Structural Analysis of Proteins (joint)

A 003 **HUMAN *ras* ONCOGENE PROTEIN: STRUCTURAL DIFFERENCES BETWEEN NORMAL AND TRANSFORMING FORMS,** Sung-Hou Kim, Liang Tong, Abraham M. de Vos, Michael V. Milburn, Pedro M. Matias and Jarmila Jancarik, Department of Chemisry, University of California, Berkeley, CA 94720.

Earlier studies of the *ras* oncogene at the genetic and molecular biological levels revealed that a point mutation in one of a few key regions of normal *ras* gene results in the production of a *ras* oncoprotein with single amino acid substitution, which is capable of transforming mammalian cells. One of the most commonly found transforming *ras* oncogenes in human tumors has a valine codon replacing the glycine codon at position 12. The crystal structure of the transforming protein at 2.2 Å resolution reveals that the mutant protein has an enlarged loop that binds the ß phosphate of the guanine nucleotide. Such a change in the "catalytic site" conformation could explain reduced GTPase activity of the protein, thus, keeping the protein in GTP bound state, which is thought to signal cell proliferation. Structural comparison of normal and transforming *ras* oncoproteins provides a basis for eventual understanding of cell transformation at the structural level.

A 004 PROTEIN STRUCTURES IN SOLUTION: COMPARISON WITH CORRESPONDING CRYSTAL STRUCTURES, Kurt Wüthrich, Eidgenössische Technische Hochschule-Hönggerberg, CH-8093 Zürich, Switzerland.

Compared to the data available on amino acid sequences, relatively few three-dimensional protein structures have been determined. Furthermore, until recently three-dimensional protein structure determination was limited to the crystalline state. This scarcity of experimental data on protein conformations is one of the principal bottle-necks in protein design and protein engineering. The introduction of nuclear magnetic resonance spectroscopy as a second technique for protein structure determination besides X-ray diffraction in single crystals (K. Wüthrich, NMR of Proteins and Nucleic Acids, Wiley, New York, 1986) promises to improve the situation both by providing complementary information to that contained in crystal structures and by the addition of new structures. New structures will cover proteins which could either not be crystallized, or for which the molecular architecture in noncrystalline states is different from that in single crystals. A complementation of the crystal structure may be obtained when the same molecular architectures are preserved in the different states, but the structures vary by subtle, local differences. With regard to all these different possible situations detailed comparisons of protein structures in solution and in single crystals are of fundamental interest. Such comparisons will be presented, with special emphasis on the variability of conformational ordering along the amino acid sequence in the crystal structures and in solution.

Protein Folding and Stability

A 005 BIOPHYSICAL GENETICS OF THE β-TURN, Robert O. Fox, John F. Gill, Margaret A. Goodman, Thomas R. Hynes, and Roger Kautz, Department of Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale University, New Haven, CT 06511 The role of the amino acid sequence in determining β-turn type, and protein structure has been investigated by combining a genetic approach with the biophysical methods of NMR spectroscopy and x-ray crystallography. Analysis of a library of random sequences at a β-turn site in staphylococcal nuclease suggests that constraints on amino acid usage are moderately strong. NMR and crystallographic analyses of nuclease variants demonstrate that amino acid substitutions in β-turn elements can result in an alteration of β-turn type while not destabilizing or greatly altering the protein structure.

A 006 STABILITY OF PROTEIN STRUCTURE AND HYDROPHOBIC INTERACTIONS, Peter L. Privalov, Institute of Protein Research, Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR

The hydrophobic interactions are usually considered as a main factor stabilizing the compact native state of protein molecule, as hydrogen bondings and van der Waals interactions are supposed to be almost identical between the groups of protein and that with water molecules. According to the conventional concept, the hydrophobic effect results from water which expels the non-polar groups as hydration of these groups is a thermodynamically unfavorable process. However, the recent thermodynamic analysis of the calorimetric data on dissolution of non-polar solutes in water and denaturation of protein shows that hydration of non-polar groups is a thermodynamically favorable process and the hydrophobicity of these groups results from van der Waals interaction between these groups. Since van der Waals interaction is a short range one and the hydration effect is a long range one and these two effects are of opposite signs, the integral complex effect, which is just what is assumed under the "hydrophobic interaction", should be attractive at a short distance and repulsive at a long distance, exceeding the size of a water molecule. This repulsive action of water hydration increases in magnitude as the temperature decreases and at sufficiently low temperature causes cooperative unfolding of the protein compact structure, i.e. its cold denaturation.

Enzyme Specificity and Catalysis

A 007 DETERMINANTS OF SUBSTRATE SPECIFICITY: STRUCTURE AND FUNCTION OF α-LYTIC PROTEASE VARIANTS. Roger F. Bone, Joy Silen, David A. Agard, Dept. of Biochemistry and Biophysics, University of California, San Franciso, CA 94143-0448. The structures of the complexes formed between a-lytic protease, a serine protease secreted by *Lysobacter enzymogenes*, and a series of inhibitory peptide boronic acids have been studied by X-ray crystallography. The boronic acid group of the most tightly bound inhibitors forms a covalent tetrahedral adduct with the active-site serine. Weakly bound inhibitors form unusual covalent adducts with the active-site serine and/or histidine residues. The primary specificity of the enzyme is for small nonpolar peptides with Ala in the P_1 position. Mutants were constructed in which Met residues(192, 213) lining the primary specificity pocket were converted to Ala. Conversion of Met 192 to Ala resulted in a dramatic shift in the specificity of the enzyme towards larger hydrophobic residues. Activity towards substrates with Phe in the P_1 position was increased by nearly 6 orders of magnitude and improved the overall catalytic efficiency of the enzyme by a factor of 15. Conversion of Met 213 to Ala also increased activity of the enzyme towards large hydrophobic residues in the P_1 position, but eliminated the selectivity of the enzyme. The structures of these two mutants in both the absence and presence of inhibitors have been determined and reveal that flexibility and structural heterogeneity are key factors in generating broad substrate specificity.

A 008 THE EFFECTS OF ACTIVE SITE MUTATIONS ON THE STRUCTURE AND ACTIVITY OF STAPHYLOCOCCAL NUCLEASE, John A. Gerlt,[1] David W. Hibler,[1] Tayebeh Pourmotabbed,[1] Mark Dell'Acqua,[1] Susan M. Stanczyk,[2] Philip H. Bolton,[2] Pat Loll,[3] and Eaton Lattman[3], Department of Chemistry and Biochemistry,[1] University of Maryland, College Park, MD 20742; Department of Chemistry,[2] Wesleyan University, Middletown, CT 06457, and Department of Biophysics,[3] Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Using site-directed mutagenesis we have generated a number of mutations for Glu 43, Arg 35, and Arg 87 in the active site of Staphylococcal nuclease. High resolution x-ray studies suggest that Glu 43 acts as a general base catalyst and both Arg 35 and Arg 87 act as electrophilic catalysts to assist the attack of water on the phosphodiester bond of a substrate. All of the substitutions made for these residues significantly decrease the catalytic efficiency but do not totally inactivate the enzyme. High resolution NMR studies suggest that all of the substitutions we have made produce long range conformational changes in the protein molecule. High resolution x-ray studies of the E43D mutant reveal the presence of conformational changes both within and remote from the active site. The presence of these conformational changes preclude detailed, quantitative interpretation of the roles of these active site residues in catalysis. Also, the substitutions made in the active site produce changes in the ^{1}H NMR spectral properties of the four histidine residues in the nuclease which suggests that conformational heterogeneity which has been attributed to *cis-trans* isomerism of Pro 117 may actually be the result of other structural effects.

A 009 ENHANCED SUBSTRATE BINDING AND EXTERNAL CATALYSIS EXHIBITED BY A CRIPPLED ACTIVE SITE MUTANT OF ASPARTATE AMINOTRANSFERASE, Michael D. Toney, Wayne L. Finlayson, and Jack F. Kirsch, Department of Biochemistry, University of California, Berkeley, CA 94720 Conversion of Lys-258, the active site residue in aspartate aminotransferase to alanine (mutant K258A) results in a 10^7-fold reduction in k_{cat} for the transamination of L-aspartate. This finding supports the earlier postulated role of Lys-258 in catalyzing the critical 1,3 prototropic shift. The mutant does bind pyridoxal phosphate, and the bound cofactor reacts with aspartate and other amino acids to form external aldimines, but at rates which are 50,000-fold less than the corresponding reactions catalyzed by wild-type enzyme. Interestingly, K258A binds amino acid substrates at least 10^4-fold *more* tightly than does wild type enzyme. The pyridoxamine phosphate form of the enzyme correspondingly will condense with α-ketoacids to form ketimines. The ketimine formed from oxalacetate decarboxylates in an enzyme catalyzed reaction to form the enzyme-bound ketimine of pyruvate which disproportionates in approximately equal measure to pyruvate and to the enzyme-bound aldimine of alanine. Overall transamination activity is restored to K258A by chemical rescue through the addition of exogenous amines. Eleven amines generated a Brønsted correlation with β of 0.4 for the transamination of cysteine sulfinate, when steric effects were included in the regression analysis. Localized mutagenesis thus allows the classical Brønsted analysis of transition state structure to be applied to enzyme catalyzed reactions.

A 010 TRYPANOTHIONE REDUCTASE, A PARASITE SPECIFIC ENZYME IN OXIDANT STRESS METABOLISM, Christopher T. Walsh, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115
Trypanosomatid parasites have much of their glutathione present as $N^1$18 bis-spermidinyl glutathione, known as trypanothione. Trypanothione is not reduced by glutathione reductase but instead by a parasite-specific trypanothione reductase and these two flavoprotein reductases show mutually exclusive substrate specifity. Studies on enzyme characterization, gene cloning, sequencing and heterologous expression of trypanothione reductase will be discussed.

Drug and Peptide Design

A 011 ENZYME MIMICS, Ronald Breslow, Department of Chemistry, Columbia University, New York, NY 10027

Synthetic enzyme models have a role to play in helping us understand catalysis by enzymes themselves, but enzymes also have a role to play in inspiring us to develop novel catalysts that can imitate their remarkable accelerations and specificities. In synthetic chemistry, the specificity of enzymes is of most interest: if we can imitate it, we can develop much better methods to prepare important materials, such as pharmaceuticals. We shall describe enzyme mimics that use various binding forces to orient substrates with respect to reagents or catalysts, including hydrophobic binding, metal coordination, and ion pairing. The functional groups involved in catalyzing the reactions include coenzymes, proton transfer catalysts, and templates for radical processes. With these mimics, catalyzed reactions have been developed that show the four types of selectivity typical of enzymatic processes: **substrate** selectivity, **reaction** selectivity, **regioselectivity**, and **stereoselectivity**. Future prospects for such mimics, and their potential relative to catalytic macromolecules such as enzymes, RNA, and antibodies, will also be described.

A 012 A PEPTIDE MODEL OF A PROTEIN FOLDING INTERMEDIATE

Terrence G. Oas and Peter S. Kim

Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02139; and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142.

One of the basic goals of protein folding studies is to determine the structures of folding intermediates. It is difficult to characterize the structures of intermediates in the folding process because folding is a highly cooperative process; a high resolution structure has not yet been determined for any intermediate of a single-domain protein.

We have designed and synthesized a small (30 residues) synthetic analogue of the first crucial intermediate in the oxidative folding of bovine pancreatic trypsin inhibitor (BPTI). The analogue is a disulfide-bonded peptide pair in which two short peptides are connected via a disulfide bond corresponding to the 30-51 disulfide of BPTI. As judged by CD and NMR, the peptide model is over 90% folded in aqueous solutions at low temperatures. 2D-NMR (NOESY) data indicate that the peptide model contains much of the secondary and tertiary structure present in the corresponding region of native BPTI.

These results demonstrate that native-like structure can form early in protein folding, and they provide a structural explanation for why the [30-51] intermediate is populated at high levels in early folding intermediates of BPTI. Peptide models of folding intermediates can circumvent the cooperativity of folding and thereby allow the structures of folding intermediates to be characterized.

A 013 DESIGN OF ENZYME INHIBITORS[1]. Daniel H. Rich, School of Pharmacy, University of Wisconsin-Madison, 425 N. Charter ST. Madison, WI 53706

De novo design of enzyme inhibitors has not yet been achieved. Today, attempts to develop enzyme inhibitors usually begin by rationally modifying naturally-occurring inhibitors which were discovered by screening methods, or by rationally modifying an enzyme substrate (itself a natural product) to resemble a postulated transition state for the reaction catalyzed by the target enzyme.

These methods can lead to effective enzyme inhibitors. Very potent inhibitors of pepsin and penicillopepsin will be described that have been designed by applying molecular modeling techniques to the crystal structures of inhibitors bound to *R.chinensis* pepsin. However, some attempts to design inhibitors by application of transition-state analog inhibitor concepts have failed, in part, because of an overly simplified picture of catalysis. A multistep catalytic mechanism for aspartic proteinases is proposed and the free energy profiles for this mechanism predict a biphasic relationship between V_{max} and substrate binding affinity. Derivation of the reciprocal relationship between V_{max}/K_m and K_i shows that good correlations are possible only for limited cases. Both good and bad correlations will be illustrated. The study of the naturally-occurring inhibitor, bestatin, illustrates an unanticipated binding mode to aminopeptidases. The phenomenon of "slow binding inhibition" that is often associated with tight-binding enzyme inhibitors, is shown for several systems to be an artifact of multiple enzyme:inhibitor complexes and is not evidence that the inhibitor resembles the transition state for catalysis.

1. Abstracted in part from "The Dependence of Enzyme Inhibition on the Distribution of Enzyme Forms. Implications for Substrate and Inhibitor Structure-Activity Correlations." by D. H. Rich and D. B. Northrop. In "Computer-Aided Drug Design" ,edited by T. Perun and C. Probst, Marcel-Dekker (1989) in press.

Hormones and Binding Proteins

A 014 STRUCTURE-FUNCTION ANALYSIS OF HUMAN INTERLEUKIN-2 BY SITE-SPECIFIC MUTAGENESIS, Grace Ju[1], Lisa Collins[1], W.-H. Tsien[2], John Hakimi[2], Pascal Bailon[3], and Warner Greene[4], Departments of [1]Molecular Genetics, [2]Immunopharmacology, and [3]Protein Biochemistry, Roche Research Center, Hoffmann-La Roche, Inc., Nutley, NJ 07110 and [4]Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC 27710.

Human interleukin-2 (IL-2) is a polypeptide hormone of 133 amino acids. We have created a series of analogs of IL-2 that contain defined amino acid substitutions and deletions. These analogs were assayed for bioactivity and for competitive binding to the high-affinity human IL-2 receptor and its two component subunits, the low-affinity p55 receptor and the intermediate-affinity p70 receptor. Substitution or deletion of specific residues resulted in biologically inactive analog proteins that were unable to interact with the p55/p70 complex or the p70 subunit. These analogs, however, retained the capacity to compete for binding to the p55 subunit. In contrast, substitutions of other residues created analogs that were inactive in the bioassay and in all three binding assays. Circular dichroism was used to assess the effects of these mutations on overall protein conformation, and to confirm that specific substitutions caused a drastic alteration in structure. These results, which demonstrate that specific residues in the NH_2 and COOH termini of IL-2 are crucial for its structure and activity, can be related to the three-dimensional structure of this protein.

A 015 ATOMIC STRUCTURES OF BINDING PROTEINS AND THEIR COMPLEXES WITH CARBOHYDRATES, OXYACIDS, AND AMINO ACIDS, F. A. Quiocho, N. K. Vyas, J. S. Sack, M. N. Vyas, and J. S. Spurlino, Howard Hughes Medical Institute, Departments of Biochemistry and Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030

Periplasmic binding proteins, which serve as initial receptors for active transport and chemotaxis in Gram-negative bacteria, have as many as three functional sites that can be studied in detail by X-ray crystallography: (1) a tight binding site (K_d=0.5 μM) for substrates such as saccharides, oxyacid anions, and amino acids, (2) a set of sites for interacting with other transport protein components lodged in the cytoplasmic membrane, and (3) a set of sites for interacting with the transmembrane signal transducer proteins. We have determined and extensively refined the X-ray crystal structures of the L-arabinose-binding protein at 1.7 Å resolution, the sulfate-binding protein at 1.7 Å, the D-galactose-binding protein at 1.9 Å, the Leu/Ile/Val-binding protein at 2.4 Å and the maltose-binding protein at 2.3 Å. All these structures are very similar despite the lack of significant sequence homology; each protein is ellipsoid and is composed of two similar globular domains with a deep cleft between the two domains where substrate is bound and sequestered. Perhaps the most unusual common feature of these proteins is that the diverse substrates (e.g., uncharged saccharides, sulfate dianion, and leucine zwitterion), are bound primarily by several strong hydrogen bonds. These high resolution studies have further revealed the atomic features of the following biologically important *interactions*: *Protein-sugar interactions* — Saccharide binding stereospecificity and affinity are conferred primarily by hydrogen bonds involving polar residues with planar side chains (i.e., Asn, Asp, Glu, Arg, His) and secondarily by stacking or non-polar interactions of aromatic residues with the sugar ring. Hydrogen bonds take on three forms: cooperative, bidentate, and networked hydrogen bonds. *Electrostatic interactions in charged substrate binding and in protein structure* — Three common key features characterize the interactions of the sulfate dianion with the sulfate-binding protein, the leucine zwitterion with the leu/ile/val-binding protein, and the positively charged Arg 151 residue in the binding site of the arabinose-binding protein: (i) The interactions are mediated primarily via hydrogen bonds; there are no counter-charged residues or counterions. (ii) The hydrogen bonds are formed chiefly with main-chain peptide units — the NH groups with the sulfate, both the NH and CO groups with the leucine, and the CO group with Arg 151. (iii) The peptide units associated with the charged moieties are in turn coupled to a variety of hydrogen bond arrays. These features constitute the basis of our conclusion that the isolated charges on the various buried groups are stabilized by dipoles of hydrogen bonds, especially those involving main chain peptide units. The hydrogen bond arrays could further disperse the charges. *Protein-membrane protein interactions* — We have located sites on the galactose- and maltose-binding proteins which are involved in interacting with respective transmembrane signal transducer proteins. The sites, which are quite polar, are located on the surface of both domains. A site on the galactose chemoreceptor contains an extensive ordered water structure. The features and important implications of the various interactions and the functions of binding proteins will be further discussed in light of the atomic structures.

A 016 DECODING THE STRUCTURAL INFORMATION IN PROTEIN SEQUENCES, Robert T. Sauer, James U. Bowie, Wendell Lim, and John Reidhaar-Olson, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Although the amino acid sequence of a protein determines its three-dimensional structure, it is widely recognized that a particular structure can be determined by many different, albeit related sequences. This raises several interesting questions: (*i*) How many total sequences are compatible with a given tertiary fold? (*ii*) Where in the sequence does the critical information for folding reside? (*iii*) Can significant information about the secondary or tertiary structure be gleaned from the list of compatible primary structures?

We have been using methods of combinatorial cassette mutagenesis to generate lists of mutations that are structurally and/or functionally silent. These studies have been applied to the N-terminal domain of λ repressor, a protein of known structure, and the P22 Arc protein, a protein of unknown structure. In λ repressor, we find that there is significant variation in the spectrum of allowed substitutions from position to position. Residues that are part of the hydrophobic core are generally the most restricted, although significant repacking of the core is allowed as long as each of the substitutions is hydrophobic and the overall volume is kept within 10-15% of the wild type value. By contrast, most surface positions in the N-terminal domain are extremely tolerant of substitutions. At present, the only surface position that plays a significant structural role is a proline at the N-terminus of an α-helix. In Arc, we find that only about 40% of the residues are key determinants of structure. Many of these have properties expected of hydrophobic core residues. From the patterns of allowed substitutions in Arc, it is possible to make strong predictions of secondary structure. These predictions are currently being tested.

Antibody Structure and Engineering

A 017 CATALYTIC ANTIBODIES, S.J. Benkovic, A.D. Napper, K. Janda, R.A. Lerner, Dept. of Chemistry, The Pennsylvania State Univ., University Park, PA 16802 and Scripps Institute, LaJolla, CA 92037.

Monoclonal antibodies have been elicited to transition state analogs that are capable of catalyzing: i) a stereospecific 6-membered ring cyclization; ii) a stereospecific bimolecular aminolysis reaction and iii) a hydrolysis of an aromatic amide. Examination of the kinetics of these reactions reveals that these reactions have much in common with enzyme catalyzed processes in that they follow Lineweaver Burke kinetics, show high enantioselectivity, and in the presence of the eliciting hapten show tight binding competitive inhibition. The rate acceleration observed in the presence of antibody relative to the spontaneous reaction may be predicted from the values of the binding constants for the substrate versus that of the eliciting hapten. Ratios that lie above this range suggest the presence of catalytic functionality in the antibody binding site. Given the behavior of the presently known systems some predictions can be made about the nature of reactions that would be particularly sensitive to antibody catalysis.

A 018 3-D STRUCTURES OF INFLUENZA VIRUS NEURAMINIDASE-ANTIBODY COMPLEXES P.M. Colman, W.R. Tulip, J.N. Varghese, W.G. Laver (1) and R.G. Webster (2), CSIRO, Division of Biotechnology, 343 Royal Parade, Parkville 3052, Australia, (1) John Curtin School of Medical Research, Australian National University, Canberra, Australia, (2) St. Jude's Children's Research Hospital, Memphis, Tennessee, U.S.A.

Data on the three-dimensional structures of antigen-antibody complexes are now emerging from studies of two different systems, one using the influenza virus antigen neuraminidase (1), and the other using hen egg-white lysozyme as antigen (2,3). A number of reviews have already addressed the central findings of these studies (4,5,6) although the database of structures remains small and may not be truly representative of the vast number of possible antigen-antibody interactions. It appears these interactions have much in common with other protein-protein interactions, including the detailed nature of structural and chemical complementarity, the size of the interacting interfaces, and the role of structural change and/or flexibility in complex formation (5).

1. Colman, P.M., Laver, W.G., Varghese, J.N., Baker, A.T., Tulloch, P.A., Air, G.M. and Webster, R.G. (1987) Nature (London) 326, 358-363.
2. Amit, A.G., Mariuzza, R.A., Phillips, S.E.V. and Poljak, R.J. (1986) Science 233, 747-753.
3. Sheriff, S., Silverton, E.W., Padlan, E.A., Cohen, G.H., Smith-Gill, S.J., Finzel, B.C. and Davies, D.R. (1987) Proc. Nat. Acad. Sci. USA 84, 8075-8079.
4. Mariuzza, R.A., Phillips, S.E.V. and Poljak, R.J. (1987) Annu. Rev. Biophys. Chem. 16, 139-159.
5. Colman, P.M. (1988) Advances in Immunology 43, 99-132.
6. Davies, D.R., Sheriff, S. and Padlan, E.A. (1988) J. Biol. Chem. 263, 10541-10544.

Receptors and Membrane Proteins

A 019 THE PHOTOSYNTHETIC REACTION CENTER FROM RHODOPSEUDOMONAS VIRIDIS, J. Deisenhofer[1] and H. Michel[2], [1]Howard Hughes Medical Institute / University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Texas 75235-9050, [2]Max-Planck-Institut fuer Biophysik, Heinrich-Hoffmann-Str. 7, D-6700 Frankfurt 71, Federal Republic of Germany

Photosynthetic reaction centers (RCs) are membrane-bound complexes of proteins and pigments. They catalyze the primary reaction in photosynthesis: light driven charge separation across a membrane.

The RC from the purple bacterium Rhodopseudomonas viridis was one of the first membrane proteins for which well ordered 3-D crystals were obtained (1). The X-ray structure analysis at 3.0Å resolution of these crystals (2,3,4) allowed the construction of an atomic model including the RC's four protein subunits, and the major pigment cofactors. Crystallographic refinement at 2.3Å resolution (5) further improved the model, and led to the discovery of additional cofactors, and of localized solvent molecules.

The RC is an elongated complex whose surface is hydrophobic in the center, and polar at both ends. The central membrane spanning polypeptide chains are folded into 11 helices, connected by chains which contain shorter helices or peptides without regular secondary structure. The transmembrane helices vary in length between 21 and 28 amino acid residues.

The cofactors located in the membrane spanning region of the RC form two approximately symmetric pathways for electron transfer across the membrane. However, under most circumstances, only one of these pathways is actually used. This functional asymmetry in the presence of structural symmetry is one of the most surprising properties of the RC complex.

(1) Michel, H. (1982) J. Mol. Biol. 158, 567-572
(2) Deisenhofer, J., Epp, O., Miki, K., Huber, R. , Michel, H. (1984) J. Mol. Biol. 180, 385-398
(3) Deisenhofer, J., Epp, O., Miki, K., Huber, R. , Michel, H. (1985) Nature 318, 618-624
(4) Michel, H., Epp, O., Deisenhofer, J. (1986) EMBO J. 5, 2445-2451
(5) Deisenhofer, J., Epp, O., Michel, H. manuscripts in preparation

A 020 MUTATIONAL ANALYSIS OF THE LIGAND BINDING DOMAIN OF THE LOW DENSITY LIPOPROTEIN RECEPTOR, David W. Russell, Michael S. Brown, and Joseph L. Goldstein, Department of Molecular Genetics, The University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, Texas 75235.

The ligand binding domain of the low density lipoprotein (LDL) receptor contains seven imperfect repeats of a 40-amino acid cysteine-rich sequence. Each repeat contains clustered negative charges that have been postulated as ligand-binding sites. The adjacent region of the protein, the growth factor homology region, contains three cysteine-rich repeats (A-C) whose sequence differs from those in the ligand binding domain. To dissect the contribution of these different cysteine-rich repeats to ligand binding, we used oligonucleotide-directed mutagenesis to alter expressible cDNAs for the human LDL receptor which were then introduced into monkey COS cells by transfection. We measured the ability of the mutant receptors to bind LDL, which contains a single protein ligand for the receptor (apoB-100) and β-migrating very low density lipoprotein (β-VLDL), which contains apoB-100 plus multiple copies of another ligand (apoE). The results show that repeat 1 is not required for binding of either ligand. Repeats 2 plus 3 and repeats 6 plus 7 are required for maximal binding of LDL, but not β-VLDL. Repeat 5 is required for binding of both ligands. Repeat A in the growth factor homology region is required for binding of LDL, but not β-VLDL. Repeat B is not required for ligand binding. These results support a model for the LDL receptor in which various repeats play additive roles in ligand binding, each repeat making a separate contribution to the binding event.

Theoretical and Structural Analyses

A 100 SPIN LABELED BACTERIORHODOPSIN MUTANTS PROVIDE INFORMATION ON TERTIARY STRUCTURE, Christian Altenbach[1], Sabine Flitsch[2], Gobind Khorana[2], Wayne L. Hubbell[1]. [1]Jules Stein Eye institute and Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024-1771; [2]M.I.T., Cambridge, MA 02139. Site directed mutagenesis was used to make mutants of bacteriorhodopsin where either glycine-72, threonine-90, leucine-92 or serine-169 was replaced by a cysteine. A very short and compact spin label (methane thiosulfonate spin label) or one with a longer more flexible chain (pyridine disulfide spin label) was then covalently attached to these sites. The selection of attachment sites covered two postulated loops (72, 169) and a membrane spanning segment (90, 92). It was not possible to properly refold the protein labeled at position 90, presumably due to steric problems, but the EPR spectra of the other mutants reconstituted in phospholipid vesicles provided information on the local motional state. EPR power saturation curves reflect with very high sensitivity the spin relaxation times, which in turn can be influenced by collisions with paramagnetic species. The differential effect of oxygen, a water-soluble chromium complex, and a lipid-soluble copper complex on the power saturation behavior of the spin labeled mutants could be used to obtain topographical information and localization of the sites in the membrane bound protein.

A 101 VIRAL BRANCHES OF THE TRYPSIN-LIKE PROTEASE FAMILY, J. Fernando Bazan and R. J. Fletterick, Dept. of Biochemistry and Biophysics, University of California, San Francisco, CA 94143. Crystallographic analysis of a number of trypsin homologs reveals a specificity pocket and a conserved catalytic triad of His, Asp and Ser residues nested in a distinctive bilobal β-barrel structure. We have identified virally-encoded proteases homologous to trypsin in a set of related positive-strand RNA viruses. Strikingly, the group of trypsin-like viral proteases includes both Ser and Cys active-center enzymes. The singular replacement of the trypsin Ser-195 nucleophile for a Cys is observed in picorna-, como-, nepo- and potyvirus proteases. Contrastingly, sobemo-, flavi- and pestivirus proteases are predicted Ser enzymes. This variation of the catalytic triad and novel changes in the substrate-binding pockets of viral proteases test our understanding of trypsin catalytic function. In addition, we examine the implications for the structural evolution of the trypsin fold as the viral proteases may represent structurally primitive molecular fossils that are not well classified by the two extant trypsin structural types. Approximate tertiary structures for representative viral proteases have been constructed (based on the known protein frameworks of trypsin-like molecules) as a prelude to model-assisted refinement of crystallographically determined structures.

A 102 HUMAN MUSCLE GLYCOGEN SYNTHASE: WHY SO NEGATIVE? Michelle F. Browner, Kenichi Nakano, Anne Bang and Robert J. Fletterick, Department of Biochemistry, University of California, San Francisco, CA 94143. Phosphorylation of proteins is an important control of enzyme activity. Glycogen synthase is inactivated by progressive phosphorylation events involving at least seven different kinases and is activated by dephosphorylation of the phosphorylated serine residues. The conformational changes associated with inactivation of a protein by multiple phosphorylation have not yet been elucidated. Amino acid sequence analysis of the cDNA for human muscle glycogen synthase revealed that the enzyme has an unusual asymmetric negative charge distribution. The negatively charged regions are at the N- and C-termini of the protein and correspond to the sites of phosphorylation control. The C-terminal region, which extends for 117 amino acids, is also unusual in that there are only 9 hydrophobic amino acids. Given the extreme hydrophilic and charged nature of the C-terminus this region is unlikely to adopt a typical secondary structure. The C-terminal region is either involved in intermolecular quaternary interactions or in intramolecular interactions with a molecule such as glycogen. Unlike the ordering of the N-terminal region observed when glycogen phosphorylase is activated by a single phosphorylation event, inactivation of glycogen synthase by multiple phosphorylation likely involves novel structural changes.

A 103 ANALYSIS OF THE CATALYTIC IMPORTANCE OF A HYDROGEN BONDING NETWORK IN SUBTILISIN USING SITE-DIRECTED MUTAGENESIS. Robert Caldwell*, Thomas Graycar*, Mark Ulltsch+, Frank Graham#, David Estell* and Richard Bott*, *Research Dept., Genencor, Inc., +Dept. of Medicinal and Biomolecular Chemistry, Genentech, Inc., So. San Francisco, CA., 94080 and #McMaster University, Hamilton, Ontario, Canada.

The three-dimensional structures of related subtilisins show a number of highly conserved structural features. The catalytic serine, S221, is found at the beginning of a helix extending through the molecule, the sequence of which is conserved in evolutionarily related subtilisin-type serine proteases. This helix contains a discontinuity that has been noted in a number of subtilisin x-ray structures; the first turn is in fact more like a 3_{10}-helix, with main chain residues forming hydrogen bonds with side chains rather than main chain atoms. These hydrogen bonds are in turn part of extensive hydrogen bonding networks. Also, it has been proposed that the dipole of this helix may have a functional role in catalysis (1).

To determine the significance of these features we have prepared subtilisin variants replacing amino acids in this central helix and are now analyzing the structural and functional consequences of these substitutions.

1. Hol,W.G.J. Prog. Biophys. Mol. Biol. 45:149-195 (1985).

A 104 STRUCTURAL AND THEORETICAL ANALYSES OF THE CRYSTALLINE SURFACE LAYER PROTEIN ANTIGEN OF TYPHUS GROUP RICKETTSIAE. W-M. Ching, M. E. Dobson, M. Falk, J. Weaver, G. A. Dasch, M. Carl, and R. Williams, Naval Medical Research Institute and Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

The immunodominant surface protein antigen (SPA) of typhus rickettsiae belongs to the class of S layer proteins which form two-dimensional regular arrays on several pathogenic bacteria. Since SPA is efficacious as a subunit vaccine against typhus rickettsiae, structural and theoretical analyses of the SPA were done to define the T and B cell epitopes essential for SPA immunogenicity. SPA contains 27% Asx+Gsx, 3% Lys+Arg, 1% His, 4% Tyr+Phe, 17% Ser+Thr and has an isoelectric point of 4.1. Molecular weights by SDS-PAGE varied (90-135 KDa) depending on gel percentage. Zone sedimentation and Hedrick-Smith plots both indicated monomer molecular weights of 87 KDa. Circular dichroism spectra indicated 50% beta and <10% alpha helix. Raman amide I and III spectra indicated 62% beta, 21% turn, and 2-6% alpha helix. DNA sequence of 30% of the SPA was obtained from pUC subclones of lambda gtll expression clones. The amino acid sequence deduced from DNA data was analyzed for theoretical T cell epitopes by the amphipathic helix model of Margalit et al. and for B cell epitopes by the Pustell hydropathy plot. Although twice as large as alpha helix rich myoglobin and highly hydrophobic, this region of the beta rich SPA had about as many amphipathic helices (9 vs. 7, amphipathic score >8) as myoglobin. The hydropathy plot suggested 6 regions that might comprise B cell epitopes. Three of the proposed B and T cell epitopes overlapped. The validity of the predicted T and B cell SPA epitopes is being determined with synthetic peptides using monoclonal antibodies and human T cell clones.

A 105 STRUCTURE AND FUNCTION RELATIONSHIPS OF THE HEPARIN BINDING SITES OF ANTITHROMBIN III, HEPARIN COFACTOR II AND PROTEIN C INHIBITOR. Frank C. Church, Herbert C. Whinna, Rebecca L. Brown, Mark R. Harris and Charlotte W. Pratt. The Center for Thrombosis and Hemostasis, The University of North Carolina School of Medicine, Chapel Hill, NC 27599-7035.

Antithrombin III **(AT)**, heparin cofactor II **(HC)** and protein C inhibitor **(PCI)** are distinct plasma proteinase inhibitors yet their inhibitory activity is greatly stimulated by the highly negatively-charged glycosaminoglycan, heparin. We are characterizing the structural similarities of these heparin-dependent inhibitors. Because of their high degree of sequence homologies, the three-dimensional structures of AT, HC and PCI were modeled after that of α_1-proteinase inhibitor [Loebermann, H. et al. (1984) J. Mol. Biol. 177: 531-556] using an Evans & Sutherland PS 340 with MENDYL graphics. An area rich in basic amino acids in HC corresponded to an similar region in AT. In contrast, the areas richest in basic amino acids in PCI were near but did not correspond with the same area as that found in AT and HC. As a further probe of possible heparin binding site similarities, these regions in AT (Ala 124 to Val 141 has been previously found to be critical for heparin binding), HC (Lys 173 to Leu 190 and Phe 183 to Arg 200) and PCI (Gln 81 to Phe 92 and Ser 264 to Glu 281), were diagrammed as Edmundson wheel plots (assuming 3.6 amino acids/turn of the helix). All of these possible heparin binding sites in AT, HC and PCI yielded amphipathic α-alpha helices. Finally, a PCI synthetic peptide consisting of the sequence S_{264}-E-K-T-L-R-K-W-L-K-M-F-K-K-R-Q-L-E-L-Y_{283}, and a "random sequence" peptide containing these same amino acids were eluted from a heparin-agarose column at ionic strengths of 0.8 and 0.4 M, respectively. The results imply that specific secondary and tertiary protein structures may contribute to heparin binding regions in these proteinase inhibitors.

A 106 PROBING THE STRUCTURE OF AN ENZYME: USE OF AMBER SUPPRESSION TO REPLACE AMINO ACIDS IN CHLORAMPHENICOL ACETYLTRANSFERASE, Robert M. Crowl and Daniel K. Burns, Department of Molecular Genetics, Roche Research Center, Hoffmann-La Roche, Inc., Nutley, NJ 07110

To extend our understanding of the structure/function relationships of the bacterial enzyme chloramphenicol acetyltransferase (CAT), we have undertaken an extensive genetic analysis by introducing amber mutations within the CAT coding sequence, and then making amino acid substitutions at those sites by amber suppression. We currently have a set of suppressors that allow for the substitution of up to 11 different amino acids at any site within the protein, when expressed in E. coli. Over 150 mutations in CAT have been examined at 14 sites. Most, but not all, of the changes at residues located within the chloramphenicol-binding pocket result in severely diminished or completely abolished activity. Amino acid replacements at residues outside the active site have only minor or no effect on activity, even though some of these residues are conserved among the known variants of CAT. Our genetic analysis has yielded some surprising results that would not have been predicted from existing information obtained from the recently published crystal structure of the CAT (type III) enzyme.

A 107 **TAB LINKER AND SITE-SPECIFIC MUTAGENESIS OF THE HUMAN DIHYDROFOLATE REDUCTASE GENE.** Adam P. Dicker, Barry Schweitzer, Robert Sheridan, Babu Venkataragha, Francis Barany and Joseph R. Bertino, Lederle Laboratories, Cornell University Medical College, Department of Molecular Pharmacology, Sloan-Kettering Institute. New York, New York 10021.

The widespread clinical use of methotrexate (MTX) as a antineoplastic and anti-inflammatory agent has focused the attention of our laboratory on the interaction between MTX and the human dihydrofolate reductase (DHFR). We have been interested in developing a dominant selectable marker using the human DHFR gene. The atomic crystal coordinates of the avian liver DHFR were used to study hydrophobic interactions between MTX and DHFR. Using the computer programs Macromodel and Mogli on a Evans and Sutherland PS 390 terminal, we have examined the effects of TAB Linker (Two Amino acid Barany insertional mutagenesis) and site specific mutations that will preferentially affect the binding of MTX to DHFR without greatly altering the catalytic activity. We have been able to examine the contributions of protein domains to methotrexate binding with the use of TAB Linker mutagenesis and in certain cases focused on individual residues with site-directed mutagenesis. After verification of mutations by restriction analysis and DNA sequencing, the altered enzymes were expressed in a prokaryotic T7 based promoter expression vector. Characterization of these mutants by enzymatic and pharmacologic means has shown that one can create altered enzymes with different binding affinities without greatly altering catalytic function.

A 108 SITE-DIRECTED MUTAGENESIS OF THE REGULATORY SUBUNIT OF cAMP-DEPENDENT PROTEIN KINASE: CHARACTYERIZATION OF cAMP BINDING SITE B, W. Dostmann, D. Øgreid, G. E. Ringheim, and S.S. Taylor, *Chemistry Department, University of California, San Diego, La Jolla, CA 92093*

The regulatory (R) subunit of cAMP-dependent protein kinase has two tandem high affinity cAMP binding sites at the carboxy-terminus. The most amino-terminal binding site (A) shows a rapid exchange of bound cAMP under high salt conditions [$t_{1/2}$ (30 ^{0}C) = 90 sec.] and preferentially binds cyclic nucleotides modified at N-6 of the adenine base. In contrast, site B is characterized by a slower exchange [$t_{1/2}$ (30 ^{0}C) = 90 min.] and shows preference for some cyclic nucleotides modified at C-2 and C-8 of the adenine ring. A CPK-model has been constructed for site B based on sequence homologies with the cAMP binding domain of the *E. coli* catabolite gene activator protein (CAP). The model showed that a) Tyr 371 could form a hydrophobic, stacking interaction with the adenine base of cAMP and b) that Arg 331 and Arg 333 could possibly form a Coulomb-interaction with the equatorial and axial oxygens of the phosphorous in cAMP. The CPK model also is being used to explain the analog specificities of each site. Based on this model, recombinant techniques have been used to selectively change several residues. Tyr 371 has been substituted by Phe, Ser, Thr and Trp, and Arg 331 has been changed to Lys. The recombinant proteins for each mutation were expressed in *E. coli* 222 and purified. Holoenzyme was prepared from each recombinant protein and a) stoichiometry of cAMP binding, b) K_D (cAMP), c) K_a (cAMP) and d) k_d (off-rate, cAMP) were determined. All of these mutant proteins showed altered exchange rates compared to the exchange from site B of the wild-type protein but relatively small changes in K_D. All mutations at position 371 showed very rapid exchange rates. Several mutants showed a monophasic off-rate, so that the exchange from site A and could no longer be distinguished. The Arg 331/Lys mutation showed a normal exchange from site-A but the exchange from site B was twice as fast as the wild-type protein. We conclude that Tyr 371 and Arg 331 are both important for the slow exchange between cAMP and binding domain B of the R I Subunit. We further conclude that the CPK model, coupled with site-directed mutagenesis, provides a powerful tool for predicting critical interactions between cAMP, cAMP-analogs and the R-subunit.

A 109 Computer Modeled Salt Bridge Variants and the Thermal Stability of Subtilisin BPN'

C.R. Erwin, B.L. Barnett and J.F. Sullivan, Biotechnology Department, Corporate Research Division, The Procter & Gamble Company, Miami Valley Laboratories, P.O. Box 398707 Cincinnati Ohio.
The geometric strategies implemented in the computer program, PROTEUS, were used to model salt bridge variants in subtilisin BPN' with the aim of increasing enzyme thermostability. The mutant proteins made from these models are for the most part secreted and differ little in K_m and k_{cat} from wildtype enzyme. Several of the mutant proteins have significant changes in their pI compared to wildtype subtilisin. The observed differences in pI indicated that they were caused by the amino acid substitutions made to the mutant proteins. In thermal inactivation studies where the enzymes are exposed to a hydrophobic surface, one mutant protein had a ten fold higher half life at elevated temperature than wildtype subtilisin. None of the mutant proteins showed a substantial increase in T_m as seen by differential scanning calorimetry.

A 110 A METHOD FOR GENERATING AND SCREENING MUTANT TRYPSINS, Luke B. Evnin (1) and Charles S. Craik (2), Department of Biochemistry and Biophysics (1,2) and Department of Pharmaceutical Chemistry (2), University of California- San Francisco, San Francisco, CA 94143.
A method for efficiently generating and screening variants of the serine protease trypsin has been developed. The mutagenesis is performed by extension of mismatched oligonucleotide primers annealed to trypsin DNA. Sequence analysis of the transformants derived from the mutagenesis reaction identifies the mutants. As the expression vector contains an M13 origin, it can be isolated in single-stranded form. Consequently, the mutagenesis is performed on the expression plasmid and no subcloning of the mutagenized DNA is necessary. Trypsin and trypsin variants are expressed in *E. coli*. In this system, the enzyme is secreted into the periplasmic space in the mature (active) form with the signal peptide acting both as a propeptide and as a prepeptide. The activity of the trypsins is assayed by two distinct methods. In one method, the crude periplasmic extract containing the trypsin is assayed by measuring cleavage of a p-nitroanilide substrate. The reactions take place in a 96-well microtiter dish and are monitored by a microplate reader. In the other method, the crude extract is fractionated by SDS-PAGE and the activity subsequently detected by overlaying the separating gel with a gel containing an ester substrate. Cleavage of the ester bond is detected indirectly by a color change in the overlay gel. Both assays are extremely rapid and easily can accomodate a large number of samples. We have explored the substrate specificity of trypsin and 19 trypsin variants each containing a different amino acid at postion 189- a critical position in detemining the primary substrate specificity. This work on position 189 demonstrates the ability of the system to rapidly generate and screen mutants. Other mutagenesis stratagies aimed at elucidating the interactions that are criticial in the productive binding of substrate will be presented.

A 111 COOPERATIVITY OF CARBOHYDRATE MOEITY ORIENTATION AND β-TURN STABILITY IS DETERMINED BY GLYCOPEPTIDE INTRAMOLECULAR H-BONDS, G.D. Fasman, M. Hollósi*, and A. Perzcel*, Dept. of Biochemistry, Brandeis University, Waltham, MA 02254-9110, and *Institute of Organic Chemistry, L. Eötvös University, Budapest, Hungary. Anomeric mixtures of 2,3,4,6-tetra-O-acetyl-D-gluco-, -D-galacto-, and -D-mannopyranosides of Boc-X-Y-$NHCH_3$ dipeptides (X-Y=Pro-Ser, Pro-D-Ser, Val-Ser, Val-D-Ser and Gly-Ser) have been synthesized. Circular dichroism and infrared spectroscopic studies were performed to characterize the peptide backbone conformation and examine the possible formation of intrapeptide and glyco-peptide intramolecular H-bonds. It was found that O-glycosylated peptides containing a D-serine residue are likely to adopt a type II β-turn while those with the Pro-Ser or Val-Ser sequence feature a type I(III) β-turn in solution. Glycosylation also increases the magnitude of the CD bands, characteristic of the given type of β-turns, which can be interpreted as an indication of the stabilization of the folded backbone conformation. IR data showed that in non-polar solutions, the peracetyl glycopeptides adopt both single- and double H-bonded conformations whose ratio, in some cases, depends on the position at C-2′ of the H-bond acceptor acetoxy group. These data suggest that five-, seven-, or ten-membered glyco-β-turns, may play an important role in fixing the steric orientation of the carbohydrate antennae systems in glycoproteins. Supported by NSF Grant # DMB-8512570.

A 112 FORCE FIELD PARAMETERIZATION FOR THE 4-FLUOROPHENYL GROUP, D.H. Gregory and J.T. Gerig, Department of Chemistry, University of California, Santa Barbara, Santa Barbara, CA 93106

Empirical intermolecular force field parameters for the fluorine atom of the 4-fluorophenyl group have been developed for use with the AMBER software Partial charges on the ring system were obtained by Mulliken population analysis of wave functions generated by molecular orbital calculations using an STO-3G basis set and Gaussian-86. Fluorine van der Waals parameters were developed by non-linear least-squares fitting to crystallographic data using the procedure described by Hagler and Lifson. The van der Waals non-bonded terms R_o and ϵ for fluorine, optimized with respect to the AMBER force field, were found to be 0.348 nm. and 0.644 kJ/mol. These values are compared to similar fluorine parameters reported previously. Also described are preliminary minimization and molecular dynamics calculations using these fluorine parameters within a version of AMBER installed on the Cray X-MP at the San Diego supercomputing center. These calculations will be directed toward developing and analyzing molecular dynamics trajectories for proteins modified with 4-fluorophenylalanine.

A 113 A COVALENT ANGIOGENIN-RIBONUCLEASE HYBRID CONTAINING FOUR DISULFIDE BONDS GENERATED BY REGIONAL MUTAGENESIS, J. Wade Harper and Bert L. Vallee, Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, Boston, MA 02115. Angiogenin is a blood vessel inducing protein whose primary structure displays 33% identity to that of bovine ribonuclease A (RNase A). While angiogenin catalyzes limited cleavage of ribosomal RNA, it is 5-6 orders of magnitude less potent than RNase A in standard assays. A striking difference between angiogenin and RNase is the virtual absence of sequence similarity within the region of RNase that contains the Cys-65-Cys-72 disulfide bond. Indeed, angiogenin lacks this disulfide linkage. Regional mutagenesis was used to generate an angiogenin-RNase hybrid protein (ARH-I) where residues 58-70 of angiogenin have been replaced by the corresponding region of RNase A (residues 59-73). The protein expressed in E. coli readily folds to form the four expected disulfide bonds. While the angiogenic potency of ARH-I is markedly diminished, its enzymatic activity toward standard RNase substrates (e.g. wheat germ RNA, poly(C), CpA, and UpA) is increased dramatically (200-660 - fold). Moreover, the specificity of ARH-I toward dinucleotide substrates is qualitatively similar to RNase A; angiogenin prefers CpG to UpA while both ARH-I and RNase A prefer UpA to CpG. ARH-I also displays >10-fold enhanced activity toward rRNA in intact ribosomes, while abolishing the capacity of the ribosome to support cell-free protein sunthesis. The enhanced enzymatic properties of ARH-I parallel a 2-fold increase in chemical reactivity of active site histidine and lysine residues based on chemical modification. The data indicate that introduction of a region of RNase A containing the Cys-65 - Cys-72 linkage into angiogenin dramatically increases RNase-like enzymatic activity while reducing its angiogenicity.

A 114 UV RESONANCE RAMAN AND FLUORESCENCE STUDIES OF ACID-INDUCED STRUCTURAL ALTERATIONS IN PORCINE, BOVINE AND HUMAN GROWTH HORMONE. Henry A. Havel, E. Wayne Kauffman and Thomas J. Thamann, The Upjohn Company, Kalamazoo, MI 49001

UV resonance Raman and fluorescence spectroscopy have been used to study acid-induced structural alterations in growth hormones from three species. Resonance Raman data for porcine (pGH), bovine (bGH) and human (hGH) growth hormones using 222 nm laser excitation show strong enhancement of aromatic residues (Phe, Trp and Tyr). Pro vibrations are also enhanced. Protein amide backbone and non-aromatic amino acid vibrations are only enhanced slightly, thus they contribute little to electronic absorption at 222 nm. Resonance Raman data indicate that Pro is present in both cis and trans configurations over the pH 8 to 2 range. Vibrational intensities due to the single Trp (observed at 756 and 1557 cm^{-1}) increase dramatically when bGH or pGH is partially unfolded in acid. Trp vibrational intensities for hGH change little at acidic pH, and are comparable to the intensities for partially unfolded bGH and pGH. Fluorescence quantum yields from the single Trp in bGH and pGH increase at low pH in a similar manner to the Trp vibrational intensity increase, while the hGH quantum yield does not change at low pH. Since fluorescence intensity changes for bGH as a function of pH appear indistinguishable from the resonance Raman intensity changes, it appears that Trp Raman and fluorescence properties are influenced by the same molecular interactions. A pK_a of 3.7 for the resonance Raman and fluorescence spectral changes indicates that acidic groups (aspartic or glutamic acids) are involved in the structural alterations. The possible roles of disulfide bridges, lysines, and histidines in decreasing Trp vibrational and fluorescence intensities in native bGH and pGH are examined.

A 115 IS THE TROPONIN C CENTRAL HELIX ESSENTIAL? Sarah E. Hitchcock-DeGregori, Zbigniew Dobrowolski, and Gong-Qiao Xu, Department of Anatomy, UMDNJ-Robert Wood Johnson Medical School, Piscataway, N.J. 08854.
A central helix connecting the two Ca binding domains is conserved in the X-ray structures of four-site Ca binding regulatory proteins. In avian troponin C (TnC) the sequence of the helical linker (res. 87-97) is KEDAKGKSEEE. To learn the functional significance of this structure we used oligonucleotide-directed mutagenesis to change its length (dEDA, dKGK, dKG, dSEEE, dKEDAKGK, and inEELAKSE at res. 95; d=deletion, in=insertion). The mutants proteins were expressed in E. coli, and purified for functional analysis (Xu and Hitchcock-DeGregori, 1988, J. Biol. Chem. 263, 13962-13969). All mutants show Ca dependent changes in electrophoretic mobility, though certain mutants differ from wildtype in the extent. The Ca dependence of the fluorescence intensity of TnC modified at Cys 101 with eosin-5-iodoacetamide (IAE) showed two transitions with midpoints ~$6x10^{-6}$M and ~$2x10^{-5}$M Ca^{2+} reflecting Ca binding to the high and low affinity sites of the protein. The fluorescence response of all the mutants was generally the same as wildtype with the exception of the insertion mutant in which the second transition was small relative to the wildtype. All mutants show Ca dependent binding to TnI as measured in urea gels and a Ca dependent increase in the monomer/excimer fluorescence ratio of pyrene-TnI as a function of Ca^{2+}. The midpoint of the transition is ~$1x10^{-6}$M Ca^{2+} and reflects Ca^{2+} binding to the Ca specific sites. The ratios in the absence of calcium of dKGK and dKEDAKGK are greater than the wildtype. The total increase in the ratios of dSEEE and insertion mutants are greater than wildtype. All mutants relieve inhibition of the actomyosin ATPase by TnI in the absence of calcium. dKGK and dKEDAKGK are more effective than wildtype, consistent with the fluorescence results. All mutants can form functional complexes with TnI and TnT though all are not equally effective. We conclude that TnC mutants with large changes in the length of the central helix (shorter or longer by two turns) or changes in the orientation of the two halves of the molecule with respect to each other retain the fundamental properties of TnC. Supported by NIH-GM36326, MDA, AHA NJ Affiliate.

A 116 SIMULATION OF THE CRYSTAL OF *STREPTOMYCES GRISEUS* PROTEASE A. David H. Kitson[1], Franc Avbelj[1,2], John Moult[2], Michael N. G. James[3] and Arnold T. Hagler[1,4], [1]The Agouron Institute, La Jolla, CA 92037; [2]Center for Advanced Research in Biotechnology, Gaithersburg, MD 20899; [3]The University of Alberta, Edmonton, Alberta T6G 2H7; [4]Biosym Technologies Inc., San Diego, CA 92121. The solvent system which makes up the environment of biological macromolecules plays a central role in both structure and function. An understanding of the nature of the interactions between these molecules and water is therefore important in developing a full picture of the behavior of macromolecules in biological systems. Simulation techniques provide a powerful tool for investigating the behavior of solvent in such systems, which defies description at the molecular level by conventional experimental techniques due to its disordered or semi-ordered nature. We have run a 10,000,000 configuration Monte Carlo simulation and a 60 ps molecular dynamics simulation to study the energetics and dynamics of the water and counter ions in the crystal of the protein *Streptomyces griseus* Protease A (SGPA) (whose structure has been solved to 1.5 Å resolution with an R-factor of 12.1%). An analysis of the ability of the methods and force field which we use to reproduce the experimentally observed structural and dynamic properties of the system is now being carried out. The time-averaged structures for the 2 simulated proteins over the time period 16-60 ps have RMS deviations from the experimental structure of 1.67 Å and 1.25 Å when atoms with experimental temperature factors greater than 20 $Å^2$ are omitted from the comparison. The second major focus of this study has been on the behavior of the ions. Preliminary results indicate that ion translation occurs through a jump diffusion mechanism, where jumps in the ion position can be correlated with decreases in its electrostatic energy. We have also observed unexpected clusters of ions of like charge in the simulated structure, which can be accounted for by examining the electrostatic environment of the ions and the ion-water interactions. This effect has led to a reinterpretation of some of the original X-ray data for this structure.

A 117 STRUCTURE-FUNCTION STUDIES OF THE *lac* REPRESSOR OF *E. coli* K12, Lynn G. Kleina and Jeffrey H Miller, Department of Biology, University of California, Los Angeles, CA 90024. We have used a set of synthetic suppressor tRNAs (Normanly et al. 1986, PNAS. 83:6548-6552) to insert an amino acid in response to an amber (UAG) codon in the lac repressor. We have 142 amber sites in the repressor, of which 54 were made by oligonucleotide site-directed mutagenesis. Eleven to twelve amino acid substitutions have been made by nonsense suppression at each of these sites generating approximately 1500 mutant repressor proteins. The mutants have been analyzed, and their significance to structure-function relationships are discussed.

A 118 THE USE OF A NOVEL *IN VITRO* COMPLETE MUTAGENESIS METHOD FOR THE STUDY OF *BACILLUS STEAROTHERMOPHILUS* α-AMYLASE

Jonathan Knowles, Anu Koivula, Päivi Lehtovaara and Liisa Holm

Technical Research Centre of Finland (VTT), Biotechnical Laboratory, Tietotie 2, SF-02150 Espoo, Finland.

A novel *in vitro* complete mutagenesis method based on a controlled use of polymerases has been developed[1]. With this method it is possible to generate mutant libraries containing all possible single base substitutions within a cloned gene. A partial mutant library of *B. stearothermophilus* α-amylase gene has been generated. Over 100 different amino acid changes have been so far localized along the 515 amino acids long protein sequence. In addition, a structural model of *B. stearothermophilus* α-amylase has been constructed by computer aided molecular modelling using the known three dimensional structure of a fungal α-amylase as a starting point. Different mutants can be used to verify the model and the model can be used to explain the effects of different mutants.

[1]Lehtovaara, *et al.* Protein Engineering *2* (1988) 63-68.

A 119 A NOVEL CONFORMATIONAL SEARCHING ALGORITHM: "ANTI-TEMPLATE FORCING"

Steven C. Koerber[1], Genzo Tanaka[1], R. Scott Struthers[1] & Arnold T. Hagler[1,2], The Agouron Institute[1], La Jolla, CA 92037 and Biosym Technologies[2], San Diego, CA 92121. A powerful feature of classical molecular mechanics and dynamics approachs to describing a molecule's potential energy is that the energy expression can be modulated towards specific ends (DHJ McKay, AJ Cross & AT Hagler, *Prediction of Protein Structure and the Principles of Protein Conformation*, G Fasman ed., in press.) Restraining equations can be used to apply torques about specific bonds, impose interatomic distances, or force the adoption of a reference conformation. The latter "template forcing" technique (RS Struthers, J Rivier & AT Hagler, *Proc. ACS Symp. on Drug Design Based on Peptide and Nucleic Acid Conf. and Struc.*, JA Vida & M Gordon eds., 1984) imposes an energy penalty on the target function the more the test and reference molecules *differ*. By analogy, we have developed "anti-template" forcing whereby the target function is penalized the more the molecules are *alike*. The net result after dynamics or minimization is a low energy structure that differs maximally from the reference. This structure can then be added to the reference database and the process repeated. Ideally, the end result after multiple iterations is a set of structures that spans conformational hyperspace as an orthogonal set of basis vectors spans a mathematical hyperspace. As an initial investigation we have subjected cyclohexaglycyl to anti-template forcing. Starting with the lowest energy structure (65.0 Kcal/mol) minimized from the X-ray structure (Karle & Karle, *Acta Cryst. 16*, 969, 1963,) 19 rounds of anti-template forcing resulted in 19 unique minimized structures in the energy range 66.0-81.1 Kcal/mol. The RMS deviations of all 20 structures spanned the range 0.96-2.77 Å. As judged by these initial results, anti-template forcing is an efficient algorithm for exploring conformational space.

A 120 ANALYSIS OF PROTEIN STRUCTURE USING FT-IR SPECTROSCOPY AND LINEAR DISTANCE PLOTS,

Michael N. Liebman, AMOCO Technology Co., Naperville, IL 60566 and Steven J. Prestrelski, Mount Sinai School of Medicine, New York, N.Y. 10029.

Resolution enhanced Fourier-transform infrared spectroscopy holds great promise as a method for observing protein secondary structure because it exhibits bands associated with the amide I, II and III transitions which tend to result from the protein backbone. The resolving capability reveals bands beyond those assigned to the traditional conformations, e.g. helices, beta-sheets and turns, etc. To expand our ability to interpret these spectra, we have generated a substructure library, using a dynamic programming algorithm based on the Linear Distance Plot representation (Liebman; Williams and Liebman), which more completely spans those proteins whose three-dimensional structure has been determined by x-ray crystallographic methods. To aid in the assignment of these bands to substructures, we have further developed an analysis based on conformational perturbation, using the serine proteases as an example, through analysis of the linear distance plot of zymogenic, native enzyme and enzyme-inhibitor complexes. We have used this database of localized structural changes to effect band assignments and to evaluate these assignments for application to other proteins through the substructure library. We have further studied the process of autolysis and its associated conformational and chemical changes using this approach.

FT-IR spectra were collected at the National Resource for FT-IR, Batelle Memorial Laboratories Columbus, Ohio.

A 121 SECONDARY STRUCTURAL ANALYSIS OF RETROVIRUS INTEGRASE PROTEINS: CHARACTERIZATION BY CIRCULAR DICHROISM AND EMPIRICAL PREDICTION METHODS, Thy-Hou Lin and Duane Grandgenett, Institute for Molecular Virology, St. Louis University, St. Louis, MO 63110
The retrovirus integrase (IN) protein is essential for integration of viral DNA into host DNA. The secondary structure of the purified IN protein from an avian retrovirus was investigated by both circular dichroism (CD) spectroscopy and five empirical prediciton methods. The CD analysis demonstrated that the avian IN contained 17±2% α-helix, 32±0.5% β-sheet, 18±2% β-turn and 33±5% random coil. The secondary structures determined from the resolving of CD spectra through a least-square curve fitting procedure were compared with those predicted from four statistical methods, e.g. the Chou-Fasman, Garnier-Robson, Nishikawa-Ooi (NO), and a joint prediction scheme which combined all three of these methods, plus a pure a priori one, the Ptitsyn-Finkelstein method. Among all the methods used, the NO prediction gave the closest match in the composition of secondary structure to the CD result although the other methods each correctly predicted one or more secondary structural group. Most of the α-helix and β-sheet states predicted by the Ptitsyn-Finkelstein method were in accord with the NO method. Secondary structural predictions by the NO method were extended further to include IN proteins from five phylogenetic distinct retroviruses, as well as HIV-1, HIV-2, SIVagm, SIVmac, HTLV-1 and HTLV-2. The structural relationships between the four most conserved amino acid blocks of these IN proteins were compared using sequence homology and secondary structure predictions. Secondary structural predictions of avian retrovirus mutant IN proteins containing single amino acid changes and correlation of these predicted structural changes to biological activity will be presented.

A 122 MODEL-BUILDING OF PROTEASE DOMAINS OF PLASMINOGEN ACTIVATORS, Parthasarathy Manavalan, David J. Livingston, David Haney* and Roger Fontes*, Protein Engineering Laboratory, Integrated Genetics, Framinham, MA 01701 and *Biosym Technologies, San Diego, CA 92121
Tissue plasminogen activator (tPA) and urokinase (UK) are serine proteases which catalyze the conversion of plasminogen to plasmin. The rate of plasminogen cleavage in the absence of fibrin is more than 20-fold higher for UK than tPA. We are studying differences in active site residues between the proteins which may be responsible for the higher catalytic efficiency of UK. The high degree of homology of these domains with several proteases with solved high-resolution X-ray crystal structures has enabled us to predict the geometry and examine differences in the active site catalytic and binding pocket residues in tPA and UK. Interesting differences between tPA and UK are seen near the catalytic triad (Asp^{365} and Ala^{473} of tPA) and in the side-chain binding pocket (Lys^{277} and Arg^{462} of tPA). We have used energy calculations to predict the effect of mutating these residues in tPA to the corresponding residues of UK on tPA tertiary structure and substrate-binding. These predictions are being tested by site-directed mutagenesis and enzyme kinetic studies.

A 123 A COMBINED APPROACH TO THE MOLECULAR MODELLING OF ANTIBODY COMBINING SITES, Andrew C.R. Martin, Janet C. Cheetham and Anthony R. Rees, Laboratory of Molecular Biophysics, The Rex Richards Building, University of Oxford, South Parks Road, Oxford, OX1 3QU, U.K.
The overall structure of an antibody is highly conserved, consisting of two heavy and two light chains of high overall sequence homology. Their exquisite specificity is dictated by six 'hypervariable' loops or 'complementarity determining regions' (CDR's). Modelling of antibodies concentrates on attempting to describe the backbone conformation and the sidechain orientations of the CDR's to an accuracy approaching that obtained by X-ray crystallography.
In Oxford, our approach to modelling has been to combine both knowledge-based and purely computational methods. Database searching looks at loops in all proteins in the protein databank rather than just antibody loops as previous methods have done. Loops extracted from the database are subjected to further analysis by conformational searching. This allows non-conservative differences between loops observed in the database and the loop being modelled to be accounted for. Each conformation is subjected to 10 cycles of energy minimisation and models for the CDR's are then selected from the conformations produced on the basis of energy calculations. The assembled antibody is then subjected to exhaustive energy minimisation.

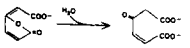
A 124 ELECTROSTATIC CALCULATIONS: THE STATIC STRUCTURE PROBLEM, James B. Matthew and J. J. Wendoloski, E. I. du Pont de Nemours & Co., Inc., Experimental Station, Central Res. & Dev. Dept., Wilmington, DE 19898.
Electrostatic calculations on structural conformers of Tuna cytochrome c generated by Molecular Dynamic simulation were done to evaluate the validity of assuming a static structure in comparing calculated electrostatic effects with experimental observations. Molecular dynamic simulations were carried out with four macroscopic dielectric formalisms to simulate the dielectric effects of water, and one computation that explicitly includes solvent water molecules. Individual pK values, overall titration curves and electrostatic potentials surfaces were calculated for each static structure along each trajectory using the solvent accessibility modified Tanford-Kirkwood algorithm (Matthew and Gurd, Methods in Enzymol. 130, 413). Differences between the proteins' structural conformers within each trajectory give rise to substantial changes in calculated local electrostatic interactions. Our findings show the importance of proper dielectric formalism in Molecular Dynamic simulations and the potential problem of using a single static structure, like a crystal structure, to differentiate between dielectric formalisms. The implications for protein-protein electrostatically mediated association will be discussed.

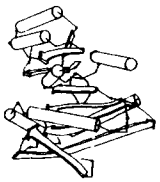
A 125 CONSERVED PROTEOGLYCAN DOMAIN STRUCTURES, Peter J. Neame, Shriners Hospital for Crippled Children, 12502 N. Pine Drive, Tampa, FL 33612. The re-assembly of domains in proteins into new structures is exemplified by proteoglycans (PGs). These large extracellular matrix macromolecules (sizes from 50 kDa to 5000 kDa) consist of a protein core to which are attached one or more glycosaminoglycan (GAG) chains. A combination of protein chemistry and molecular biology has recently resulted in a variety of structures being described for these species of macromolecules. The protein cores clearly divide into a variety of domain types, some of which contain GAGs and some of which mediate other functions of the PGs. Other domains have no known function as yet. The structures of two proteoglycans will be described. One, a chondroitin sulfate PG from cartilage, has been completely sequenced by Doege et al (1987, JBC, 262, 17757-67) and the structure of a globular domain which binds to hyaluronic acid has been described by Neame et al (1987, JBC, 262, 17768-78). The domain structure of this globular region will be described and arguments put forward for function, based on theoretical considerations of available sequences and the properties of synthetic peptides. Another proteoglycan, dermatan sulfate, consists of two sub-types, one of which (DS-PG II) has been described by Krusius and Ruoslahti ((1986) PNAS 83, 7683-7) and the other (DS-PG I) by Neame et al (1988, submitted JBC). Again, this PG has a number of domains: a GAG-containing domain, a metallothionein-like domain, a leucine-rich domain and a fourth domain with no outstanding features and which is distinct from other known proteins. These defined domains have clearly derived from genetic rearrangement and will be amenable to functional and 3-dimensional structural analysis if synthesized de novo or dissected out from their parent proteins. This work was supported by grant AM 35322 and a grant from The Shriners Hospitals for Crippled Children.

A 126 STRUCTURE AND ASSEMBLY OF PROTOCATECHUATE 3, 4-DIOXYGENASE PSEUDOMONAS AERUGINOSA, D.H. Ohlendorf[1], J.D. Lipscomb[2], and P.C. Weber[1], [1]Central Research and Development
[2]Department, E.I. du Pont de Nemours & Co., Inc., Wilmington, Delaware 19880-0228 and [2]Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55392

The structure of protocatechuate 3,4-dioxygenase (3,4-PCD) from *Pseudomonas aeruginosa* has been determined at 2.8 Å resolution. 3,4-PCD catalyzes the intradiol cleavage of protocatechuic acid by molecular oxygen to produce β-carboxy-*cis*, *cis*-muconic acid. The reaction is accomplished using a non-heme ferric ion. 3,4-PCD holoenzyme is composed of twelve protomers each of which contains a single active site located between two similar polypeptide subunits. All four ligands of the catalytic irons are provided by the larger subunit. The subunits contain a novel β barrel composed of both parallel and antiparallel strands. The 3,4-PCD protomers are organized to form a hollow, truncated tetrahedron with local symmetry *23(T)*. Interactions between the protomers are primarily mediated by the amino termini and some interstrand loops of the larger subunit.

A 127 X-RAY CRYSTALLOGRAPHIC STRUCTURE OF A HYDROLASE: ACTIVE SITE SIMILARITY WITH SERINE/THIOL PROTEASES, Dushyant Pathak and David Ollis, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208 The structure of dienelactone hydrolase (DLH), an enzyme of the β-ketoadipate pathway has been determined at 2.8Å (schematic below) and a tentative identification of its active site made. DLH catalyzes the hydrolysis of dienelactone to maleylacetate (see below). Inspection of the crystal structure of native and product-bound DLH, combined with inhibition and site specific mutagenesis studies, lead us to believe that the mechanism of DLH may be similar to that of the serine and thiol proteases. This example of conservation of, or convergence to, an active site efficient for ester hydrolysis provides insights into how enzyme specificities are altered, and in turn may be engineered for the processing of related but different substrates.





A 128 THE USE OF TERTIARY TEMPLATES IN THE ANALYSIS OF FAVORABLE HELIX-HELIX PACKING SITES, J. W. Ponder and F. M. Richards, Department of Molecular Biophysics and Biochemistry, Yale Univeristy, New Haven, CT 06511. The ability of two helical amino acid sequences to pack together has been investigated using our recently developed protein tertiary template algorithm. A helix interaction site is defined as a central residue and four of its nearest neighbors from the same face of a helix (residues i, i±3 and i±4). Given two such interaction sites, we have performed packing computations to determine the allowed sequences consistent with the usual helix-helix geometries. When, starting from a known sequence, these methods can be used to find pairs of interaction sites which mesh to form favorable packing arrangements. All ten residues in the pair of sites are subjected to a tree search to identify allowed side chain rotamer combinations consistent with the known sequence. At present, we have results on some simple model systems and are starting to apply the techniques to members of the globin family. In the latter systems, lists of favorable pairs of sites can be combined with other geometrical constraints to provide a low resolution tertiary structure. In this sense the current work is an extension of earlier helix packing algorithms of Richmond and Richards. Implications of these modeling techniques for analysis of helix-helix packing in membrane proteins is discussed.

A 129 STRUCTURAL AND FUNCTIONAL ANALYSIS OF YEAST GLYCOGEN PHOSPHORYLASE, Virginia L. Rath, Peter K. Hwang, and Robert J. Fletterick, Dept. of Biochemistry and Biophysics, University of California, San Francisco, CA 94143. We wish to elucidate the structural details of allosteric regulation in yeast phosphorylase. Allosteric activation by covalent phosphorylation is a common feature of both yeast and mammalian phosphorylases but involves different mechanisms stemming from nonhomologous N-termini which contain dissimilar phosphorylation sites and intersubunit contacts. Thus, rabbit muscle phosphorylase and the less studied yeast phosphorylase represent alternative structural designs for achieving functionally equivalent results upon phosphorylation. We have now purified yeast phosphorylase to near homogeneity from overexpressing yeast strains. The purified enzyme migrates as a single band on SDS gradient gels but as many as four bands on isoelectric focusing gels with isoelectric points in the range of 5.4-5.8. Judging by the specific activity of the enzyme, it is primarily phosphorylase *b*, the unphosphorylated form. Protein sequencing indicates that the enzyme N-terminus is heterogeneous. Several crystal forms have been grown under conditions which are expected to promote either R or T states of the enzyme. Further progress in crystallizations and crystallographic analysis of yeast phosphorylase will be reported.

A 130 Isolation and Sequencing of Disulfide Bridged Peptides from Bovine Adrenal Dopamine ß-Hydroxylase James G. Robertson and Joseph J. Villafranca, Department of Chemistry, The Pennsylvania State University, University Park, PA, 16802
Bovine dopamine ß-hydroxylase is a tetrameric glycoprotein of M.W. 290,000 that catalyzes the physiologically important conversion of dopamine to norepinephrine. Two identical monomers form a disulfide bridged dimer, and two dimers noncovalently associate to form a tetramer. The enzyme contains 13-14 cysteines per monomer and all the cysteines are involved in disulfide bridges. However, the number and location of intrachain and interchain disulfides has not been determined. In order to identify cysteine containing sequences and to assign the disulfide linkages in DßH, several approaches were followed. First, in order to identify cysteine containing sequences, enzyme was unfolded in 6 M guanidine-HCl, reduced with dithiothreitol, and derivatized with 5,5'-dithio-bis-(2-nitrobenzoate). Labelled enzyme then was digested with trypsin and the resulting peptides were separated by HPLC. Cysteine containing peptides were identified by absorbance at 330 nm, were repurified by HPLC, and were sequenced by Edmond degradation on gas phase and liquid phase automated sequencers. Second, tryptic peptides from oxidized DßH were prepared and separated by HPLC. Disulfide containing peptide fractions were identified chemically, then reduced, repurified, and finally sequenced. Alternatively, disulfide containing peptide fractions were analyzed by FABMS, then reduced and reanalyzed by FABMS. The identity of the paired peptides was determined from the unique masses of the reduced peptides and the known masses of peptides previously sequenced. These analyses have yielded the sequences of eight peptides containing nine cysteines. Preliminary analysis by FABMS indicates that one peptide contains a Cys-Thr-Cys sequence apparently doubly disulfide bridged to the identical Cys-Thr-Cys sequence on a second copy of the same peptide. These peptides appear to be candidates for an interchain disulfide linkage.

A 131 IDENTIFICATION OF CRITICAL REGIONS IN MOUSE GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR USING SCANNING DELETION ANALYSIS, Armen B. Shanafelt and Robert A. Kastelein, DNAX Research Institute of Molecular and Cellular Biology, 901 California Street, Palo Alto, CA 94304. The structure-function relationships in mouse Granulocyte-Macrophage Colony Stimulating Factor (mGM-CSF) were examined using a series of deletion mutants which scanned the entire length of the molecule. Deletions of three amino acids were introduced every five amino acids by site-directed mutagenesis on the mature mGM-CSF gene. The mutant proteins were expressed in E. Coli, and unpurified cell extracts, adjusted to give comparable amounts of the deleted mGM-CSF molecules, were assayed for biological activity. Four regions were identified that are critical to the activity of the protein, spanning approximately amino acids 19-21, 34-41, 54-60, and 94-116. Implications with respect to protein folding and receptor binding interactions are discussed, as well as comparisons to human GM-CSF.

A 132 MECHANISMS OF MOLECULAR RECOGNITION IN HEME PROTEINS, Stephen G. Sligar, Departments of Chemistry and Biochemistry, University of Illinois, Urbana IL 61801. Equilibrium and dynamic structures of heme proteins dictate the mechanisms and specificity of polypeptide chain folding, macromolecular association, electron transfer physics, recognition of substrates, and the chemistry of catalysis. In order to probe the structure-function correlations in heme proteins we have utilized site-directed mutagenesis of cloned or totally synthetic genes of sperm whale myoglobin, Aplysia myoglobin, rat liver cytochrome b_5, putidaredoxin, cytochrome $P\text{-}450_{cam}$ and the four-helical bundle cytochrome b_{562}. In order to precisely understand the physics and chemistry of the fundamental processes described above, concerted structural, catalytic, dynamic, spectroscopic and biochemical information must be obtained. In the case of sperm whale myoglobin, for instance, we have obtained gram quantities of the following mutants. For examining distal pocket effects, Histidine E7 (H64) was replaced by Gly, Cys, Lys, Tyr, Val, Met, Arg, Phe, Asp, and Thr. Valine E11 (V68) has been replaced with Ile, Phe, Ala, and Glu. E7/E11 double mutants Gly/Ile, Gly/Phe, and Gly/Ala have been constructed and over-expressed. The contribution of Arg 45 to the salt linkage at one pocket access channel has been examined by replacing this residue with Gly, Asp, Ser, and Asn. The role of axial ligands in the bio-inorganic chemistry of myoglobin has been realized by removing the normal histidine at F8 (H93) in favor of Cys and Tyr. Determining the precise effects of our sperm whale myoglobin mutations on protein function requires extensive biophysical investigations. In my presentation, I will describe the basis for molecular recognition in the myoglobin system as elucidated through work in our own laboratory and that of our collaborators, Drs. Phillips (X-ray structure), Olson and Gibson (ligand binding and geminate recombination), LaMar and Wright (NMR), Frauenfelder (ligand binding and infrared spectroscopy), Champion (Raman), Debrunner (EPR), and Peters (photoacoustic spectroscopy).

A 133 The Thermodynamic Components of Protein Secondary Structure Formation, S.F. Sneddon, D.J.Tobias and C.L. Brooks III, Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA 15213

Thermodynamic perturbation calculations, along with a newly developed constraint method, have been employed to obtain thermodynamic profiles for the formation of an amide hydrogen bond (C=O···H-N) in gas phase, carbon tetrachloride and water. Our results provide insight into the relative stabilities of secondary structure, and the thermodynamic driving force for their formation (i.e. energetic versus entropic contributions to the free energy). By calculating these properties in the three solvents we obtain the relative solvation free energy of the amide hydrogen bond (C=O···H-N). We draw relations to experimental results (M.A. Roseman, J. Mol. Biol., 201, p621, (1988)), and consider implications for protein folding pathways.

A 134 PROBING ACTIVE SITE AND PROTEIN-PROTEIN MOLECULAR RECOGNTION IN THE CYTOCHROME P-450 ENZYME SYSTEM, Patrick S. Stayton, William M. Atkins, and Stephen G. Sligar, Depts. Biochemistry and Chemistry, University of Illinois, Urbana, Il 61801. Cytochrome $P\text{-}450_{cam}$ interacts highly specifically with both a small molecule substrate and protein redox partners. The structural features responsible for molecular recognition have been probed with a concerted series of surface and active site mutants of cytochrome $P\text{-}450_{cam}$, cytochrome b_5, and putidaredoxin. Cytochrome b_5 has been genetically engineered to afford a surface cysteine that has been labeled with acrylodan. Fluorescence binding titrations with $P\text{-}450_{cam}$ yield a K_d of 1 uM and further demonstrate that putidaredoxin competitively inhibits $b_5\text{-}P\text{-}450_{cam}$ association. The b_5-P-450 complex has been computer modeled and features electrostatic binding contacts between the conserved b_5 anionic surface and P-450 basic amino acids on the surface of nearest proximal approach to the buried heme. The model features conserved Phe 350 of P-450 on the path between the heme centers and may represent the physiological binding site of putidaredoxin. Lys 344 and Arg 72 of P-450 provide model binding contacts and have been mutated to neutral amides as a test of the model. Putidaredoxin carboxylates have also been mutated to Asn or Gln and the binding effects investigated. The molecular active site features responsible for the observed regio and stereoselectivity of camphor hydroxylation have been probed with Y96F, V247A, and V295I mutants and substrate analogs. These mutants provide molecular rationale for differential substrate affinity, substrate dependent spin state equilibria, and the regiospecificity of hydroxylation with camphor and several analogs.

A 135 CONTRIBUTIONS OF ELECTROSTATIC AND HYDROPHOBIC INTERACTIONS TO THE FORMATION OF THE THROMBIN-HIRUDIN COMPLEX. Stuart R. Stone, Andrew Wallace, Stanley Dennis, and Jan Hofsteenge, Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.

Hirudin is a polypeptide that inhibits thrombin through the formation of a tight complex ($K_d = 10^{-14}$ M). Our earlier studies had suggested that ionic interactions involving the C-terminal region of hirudin were important for complex formation. Consequently, the contribution of acidic residues in this region has been evaluated by site-directed mutagenesis. By examining the effect of ionic strength on the kinetics of inhibition, it was possible to separate the electrostatic and nonelectrostatic contributions to the binding energy. The results indicated that, whereas the nonelectrostatic fraction of the binding energy remained constant for each mutant, the electrostatic fraction was proportional to the number of acidic residues. Each of the acidic residues contributed about 4 kJ mol^{-1} to the binding energy. The total contribution of electrostatic interactions was about one third of the overall binding energy. The importance of the hydrophobic N-terminus was also examined and it was found that a correlation could be made between the hydrophobicity of the two N-terminal amino acids and the binding energy. The effect of ionic strength on the kinetics indicated that only the nonelectrostatic fraction of the binding energy was significantly affected by the N-terminal mutations.

A 136 CYSTEINE ANALOGS OF BASIC FIBROBLAST GROWTH FACTOR
Stewart A. Thompson, Jack Rose, Joshua Hatch, Thomas Palisi and Andy Protter. California Biotechnology, Mt. View, Cailfornia 94943 Human basic fibroblast growth has been cloned and expressed in E. coli. The recombinant protein consists of 155 amino acids with four cysteines located at positions 34, 78, 96 and 101. In the absence of reducing agents, the protein appears heterogenous when chromatographed on heparin affinity and reverse phase columns. Through site directed mutagenesis of the cysteine residues, we have established that the heterogeneity is due to oxidation of cysteines 78 and 96. Intermolecular disulfides are indicated by an increase in molecular weight on size exclusion chromatography and non-reducing SDS-PAGE after carboxymethylation. Construction of the C78/96S analogs of bFGF results in a protein that is resistant to multimerization without a loss of biological activity. The physical and chemical differences between the wild type protein and the C78/96S analog will be discussed.

A 137 PROTEIN MOTIFS AND A DATABASE OF THREE DIMENSIONAL STRUCTURE
Janet M. Thornton, Steven Gardner, Suhail Islam, Michael J.E. Sternberg, Carrie M. Wilmot; Crystallography Department, Birkbeck College, Malet Street, LONDON WC1E 7HX

The explosion in the number of protein sequences, with more than 10,000 already known, compared to less than 400 independent protein structures, has fuelled the need for accurate structure prediction from sequence. Recently there has been a shift in emphasis from 'ab initio' prediction to the more pragmatic approach of recognition of structures from sequence patterns. The basis for prediction by recognition must be an integrated database of protein structures and sequences. At Birkbeck College and Leeds University we have established such a database of protein structures and sequences supported by the U.K. Protein Engineering Club. The first release of this database is now generally available and will be described.

We are particularly interested in storing the common structural motifs in the database for easy access. Progress towards this goal will be described.

A 138 1.6Å STRUCTURE OF AN ENGINEERED CYSTEINE PROTEASE
Marjorie E. Wilke, M. M. McGrath, T. N. Earnest, J. Higaki, C. S. Craik, R. J. Fletterick Dept. of Biochemistry and Biophysics, San Francisco, CA 94143-0448 We have determined the structure of a cysteine protease made by mutating the active site serine of rat cationic trypsin. S195C was solved to 1.6Å resolution using data measured at -120°C to reduce the effects of radiation damage and thermal motion. The k_{cat}/K_M of S195C is down 10% from papain. The structure shows that the sulfur is in the correct conformation and is the same distance from the active site His as it is in papain, a naturally occurring Cys protease. However, the sulfur to substrate distance is altered. This difference in the distance may be the sole cause of the reduced catalytic rate. But, it is also possible that the altered chemical environment of the active site will not stabilize the transition state as well as trypsin. Currently we are determining the structure of S195C with a covalent tetrahedral intermediate analog to address the question of geometric factors in the course of the reaction. To study the effects of the chemical environment we have begun constructing second site variants designed to test the role of charge in the region around the active site.

A 139 CRYSTAL STRUCTURE OF RIBONUCLEASE WITH DOXYNUCLEOTIDES COVALENTLY BOUND TO THE HISTIDINE RESIDUES IN THE ACTIVE SITE, Alexander Wlodawer, Joseph Nachman, Maria Miller, NCI-Frederick Cancer Research Facility, BRI-Basic Research Program, Frederick, MD 21701, and Robert Carty, State University of New York, Health Science Center at Brooklyn, Brooklyn, NY 11203-2098.

RNase-T-(N3)His12, ribonuclease with a deoxythymidine covalently bound to N^{ϵ} of His12, one of the residues which, together with Lys41 and His119 forms the active site of ribonuclease, crystallizes in space group $P2_12_12_1$ with unit cell dimensions a = 53.14Å, b = 64.61Å, c = 73.64Å. There are two molecules in the asymmetric unit related by a non-crystallographic pseudo-twofold rotation axis. The structure was solved by molecular replacement, using the native monoclinic ribonuclease as a starting model. It was refined to 1.7Å resolution with an R of 0.16 and the orientation of the deoxythymidine within the active site is clearly indicated by the residual positive density from the F_o-F_c map. Structural comparison of both crystallographically independent molecules, as well as of the modified enzyme with the starting model, have been performed.

Crystals of RNase-U-(N2)His119, ribonuclease with a deoxyuridine bound to N^{δ} of His119 are isomorphous with those of RNase-T-(N3)His12 and refinement of the structure to 1.8Å resolution is in progress (currently R =0.20).

Research sponsored in part by the National Cancer Institute, DHHS, under contract NO. NO1-CO-74101.

A 140 **FUNCTIONAL AND IMMUNOLOGICAL PROPERTIES OF HUMAN FERRITIN H-CHAIN MUTANTS.** P. Arosio, S. Levi, A. Luzzago, A. Cozzi, S. Ingrassia, F. Franceschinelli and G. Cesareni. Department of Biomedical Science and Technology, University of Milano, San Raffaele Hospital, Milano, Italy, and EMBL, 6900 Heidelberg, FRG.

Ferritin H-chain mutants were obtained by performing site directed mutagenesis of the plasmid pEMBLex2HFT. Some of the mutants whiche are expressed in high yield by E. coli and that assemble in stable 24-mer ferritin-like molecules were purified and analyzed for functional and immunological properties. They included deletions of the first 13 and of the last 22 aminoacids, duplication of a 18 aminoacid sequence, and various point mutations along the two, three and four fold simmetry axes sequences of the molecule. None of the mutageneses eliminated the ferro-oxidase activity of ferritin H chain, which could not be precisely mapped. Two mutants with extensive alteration at the C terminal sequence could not form a stable iron core. In order to map ferritin epitopes, 12 anti-H ferritin monoclonal antibodies were reacted with the mutants. They could be subdivided into 5 grups with distinct, but overlapping binding site, the affinity of all of them largely decreased or disappeared after deletion of ferritin N-terminal and duplication of the loop sequence. It is concluded that the C terminal sequence is important for iron core formation, and the N terminal and Loop sequences are involved in most/all ferritin epitopes.

A 141 **THE COMPLETE AMINO ACID SEQUENCE OF BOVINE MILK ANGIOGENIN**

P. Maes[+], D. Damart[*], C. Rommens[+], J. Montreuil[*], G. Spik[*], and A. Tartar[+], [+]Service de Chimie des Biomolécules, (Unité Associée au CNRS D-1000), Institut Pasteur de Lille, 1 rue Calmette 59109 Lille, France ; [*]Laboratoire de Chimie Biologique, (Unité Associée au CNRS n° 217), Université des Sciences et Techniques de Lille Flandres-Artois, 59655 Villeneuve d'Ascq Cedex, France.

Angiogenin isolated initially from HT-29 tumor conditionned media and later from human plasma by the group of Vallee is able to induce the neoformation of blood vessels. Fully active angiogenin was shown to be present in large quantities (500-800 µg per liter) in bovine milk. The amino acid sequence of bovine angiogenin was deduced by gas-phase sequencing of the protein and its fragments. The protein contains 125 residues and has a calculated Mr of 14577 daltons. The sequence is highly homologous (65% identity) to the sequence of human angiogenin, most of the differences being the result of conservative replacements. Like human angiogenin, the bovine protein is also homologous to bovine pancreatic RNase A (34% identity) and the three major active site residues known to be involved in the catalytic process His-14, Lys-41 and His-115 are conserved. When tested against conventional substrates for RNase A activity, bovine angiogenin displays the same selective ribonucleolytic activity as human angiogenin. The sequence of bovine angiogenin contains the cell recognition tripeptide Arg-Gly-Asp which is not present in the human protein.

A 142 DOMAIN STRUCTURE OF HIV-1 REVERSE TRANSCRIPTASE ANALYSED BY LIMITED PROTEOLYSIS, Denise M.Lowe, Alastair Aitken+, Graham K.Darby, Brendan A.Larder, Kenneth L.Powell, Dorothy J.M.Purifoy, Margaret Tisdale and David K.Stammers, Department of Molecular Sciences, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, U.K. and +National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.
Bacterially expressed recombinant HIV-1 reverse transcriptase is active as both a homodimer of Mr 66,000 subunits and a heterodimer of Mr 66,000 and 51,000 subunits. The heterodimer is formed by cleavage of a C-terminal fragment from one 66,000 Mr polypeptide.
Limited proteolysis of the homodimer with α-chymotrypsin resulted in cleavage to a stable 66,000/51,000 Mr mixture, and proteolysis with trypsin resulted in the transient formation of some 51,000 Mr polypeptide, which was then cleaved after residue 223 to yield peptides of apparent Mr 29,000 and 30,000 which remained in complex with the original 66,000 Mr polypeptide. The resistance of one subunit of the homodimer to proteolysis indicates that the subunits are not symmetrically related.
During both chymotrypsin and trypsin digestion there was an increase in the reverse transcriptase activity caused by a doubling of Vmax with little change in Km for dTTP.
These results are consistent with the reverse transcriptase molecule having a protease-sensitive linker region following a structured domain of Mr 51,000. Mutant proteins with deletions in this region are currently being characterized.

Protein Folding and Enzyme Catalysis

A 200 SITE-DIRECTED MUTAGENESIS STUDIES ON THE NUCLEOTIDE-METAL BINDING SITE OF *E. COLI* GLUTAMINE SYNTHETASE, Lynn M. Abell+, Pamela J. Keck and Joseph J. Villafranca, Department of Chemistry, The Pennsylvania State University, University Park, PA 16802. Glutamine synthetase from *E. coli* requires two divalent metal ions per active site to catalyze the ATP dependent synthesis of glutamine from glutamate and ammonia. The two metal binding sites display markedly different affinities for metal ions, and possible protein ligands at each site have recently been identified from x-ray crystallographic data (Almassy et. al. (1986) *Nature 323*, 304). Binding of a metal ion at the high affinity (n_1) site induces a conformational change converting the inactive apoenzyme to the catalytically active form. Three glutamic acid residues have been proposed as metal ligands at this site. The protein ligands to the lower affinity nucleotide-metal binding site (n_2) are thought to include not only oxygen ligands from glutamates 129 and 357 but also one nitrogen ligand from histidine 269. A series of mutants has been constructed which contain potential oxygen and nitrogen ligands at position 269. The effects of these mutations on the steady-state kinetic parameters and the metal ion binding properties of the enzyme have been investigated. (+Supported by NIH fellowship GM 11994-02. This work is supported by NIH grant GM 23529.)

A 201 T4 LYSOZYME: SPECIFIC INTERACTIONS OF IONIZABLE GROUPS PLAY THE DOMINANT ROLE IN DETERMINING THE pH DEPENDENT STABILITY OF THE FOLDED STATE, D. Eric Anderson, W.J. Becktel and F.W. Dahlquist, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403
The pH of the maximum stability of T4 lysozyme is approximately 5.8 despite the rather basic isoelectric point (~10) of the protein. This suggests that specific interactions of ionizable groups rather than simply the net charge of the protein play a role in determination of the stability of the folded state. We have identified one such interaction between HIS31 and ASP70 that contributes 5 kcal to the stability of the folded state at neutral pH. Using nuclear magnetic resonance techniques, combined with site directed mutagenesis the pKa values of HIS31 and ASP70 have been determined. The pKa of HIS31 is shifted by 2.3 units to a value of 9.1 as a result of the interaction with the ionized form of ASP70. Remarkably, ASP70 is found to be a strong acid in the native structure with no indication of protonation even at pH 1.4. The implications of this finding on the role and strength of salt bridges in protein folding will be discussed.

A 202 FORMATION OF ISOASPARTATE IN CALMODULIN AND HUMAN GROWTH HORMONE, Dana W. Aswad, Brett A. Johnson and Steven M. Potter. School of Biological Sciences, University of California, Irvine, CA 92717. Isoaspartate has been found to arise in flexible peptides and in structured proteins, primarily via deamidation of succinimide-prone Asn-X bonds. Protein carboxyl methyltransferase (PCMT) catalyzes a selective methylation at the α-carboxyl of these atypical, isoaspartyl sites, using S-adenosyl-L-methionine (SAM) as the methyl donor. We have used this selective methylation reaction to estimate the kinetics and location of isoaspartate formation in bovine brain calmodulin and a recombinant form of human growth hormone. In incubations carried out at 37 °C, pH 7.4, the rates of isoaspartate formation were estimated at 4.8 residues per day per 100 molecules of calmodulin and 1.8 residues per day per 100 molecules of growth hormone. To locate the sites of formation, the incubated proteins were digested with trypsin and then enzymatically methylated with [methyl-^{3}H]SAM. The peptides were separated by reversed-phase HPLC and assayed for ^{3}H-methyl incorporation. In growth hormone, methylation occurred in peptide 146-158, correlating with deamidation of Asn-149, and in peptide 128-134, correlating with isomerization of Asp-130. For calmodulin, methylation occurred mainly in peptides 91-106, 127-145, and 38-71. The exact sites of methylation in these peptides is currently under investigation. These studies indicate that isoaspartate can arise at an appreciable rate in structured proteins at physiological pH and temperature. Both asparagine and aspartate can make significant contributions as progenitors of isoaspartate. PCMT offers a powerful analytical tool for assessing the extent and location of these modifications.

A 203 HIV-PROTEASE: PURIFICATION AND CHARACTERIZATION, Lilia M. Babe, Sergio Pichuantes and Charles S. Craik, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143

The human immunodeficiency virus (HIV-1) is the etiologic agent of AIDS. As other retroviruses, it encodes a protease activity responsible for the processing of viral polyprotein precursors. This protease has been expressed in *E. coli* and shown to autocatalyze its maturation from a larger fusion protein precursor. The 99 residue mature protein has been purified to near homogeneity and its sequence confirmed by amino and carboxy-terminal sequence analysis. Incubation with p53 gag polyprotein precursor results in the generation of the expected products as confirmed by immunological analysis and amino terminal sequence analysis of the 24Kd cleavage product (p24). Other assays are currently being developed to detect enzymatic activity on short synthetic peptide substrates containing the consensus cleavage sites for this protease. Such assays will enable us to assess the properties of this enzyme and test its sensitivity towards potential inactivators.

A 204 PROBING ISOENZYME CATALYSIS AND SUBSTRATE SPECIFICITY, Luc Berthiaume, Sylvie Berardi, Danièle Beaudry, Dean R. Tolan and Jurgen Sygusch, Département de biochimie, Université de Sherbrooke, Sherbrooke, Québec, Canada, J1H 5N4 and Biological Science Center, Boston University, 2 Cummington Street, Boston, MA. 02215. Sequence comparison of class I fructose-1, 6-diphosphate aldolases, found in vertebrates and invertebrates as well as higher plants, have shown the active site residues as determined from crystallographic studies of the rabbit muscle enzyme to be conserved throughout evolution. Crystallographic evidence suggest that the active site located in the center of the aldolase β barrel is covered by a conformationally mobile COOH terminal region which possesses minimal homology among aldolases. Sequence alignment and subtyping of the aldolase's isoenzymes are consistent with conserved COOH termini among members of the same subtype. Mutagenesis experiments were designed to specifically modify the COOH terminal region of the maize aldolase to include relevant sequence information of the rabbit muscle and liver COOH terminal regions. Discussion of the kinetic properties of the various mutant aldolases will be presented.

A 205 INACTIVATION OF HUMAN ARGININE-143, LYSINE-143, AND ISOLEUCINE-143 Cu,Zn SUPEROXIDE DISMUTASES BY H_2O_2: MULTIPLE MECHANISMS FOR INACTIVATION, C. L. Borders, Jr. and Pamela J. Horton, Department of Chemistry, The College of Wooster, Wooster, OH 44691 Human Cu,Zn superoxide dismutases (SODs) containing either arginine (Arg-143), lysine (Lys-143), or isoleucine (Ile-143) at position 143 are inactivated by H_2O_2 (I). At low concentrations, I is an affinity reagent for the inactivation of Arg-143. At pH 9.0 and 25°C, the process is characterized by a half-saturation constant, K(50) of 5.1 mM and a maximum pseudo-first-order rate constant for inactivation, *k*(max), of 0.53 per min. At pH 11.5, the corresponding values are 0.63 mM and 1.23 per min. The active species in the inactivation is likely HO_2^- (II), as previously found with other Cu,ZnSODs. Lys-143 is also inactivated by I by an affinity mechanism. At pH values of 9.0 and 11.5, the *k*(max) values are 0.92 and 1.08 per min, respectively; however, the corresponding K(50) values increase to 42.5 and 15.8 mM. Ile-143 is also inactivated by I, but by a non-affinity mechanism. Inactivation of all three enzymes parallels the loss of one histidine/subunit. At high [II], a second non-affinity mechanism of inactivation of both Arg-143 and Lys-143 was found, in which a second equivalent of H_2O_2 reacts with the Cu,ZnSOD II complex to give a competing second-order inactivation. The positive charge of Arg at position 143 in "wild type" human Cu,ZnSOD likely plays a role in the binding of II during inactivation; replacement by lysine gives an enzyme with a similar mechanism of inactivation, but with a reduced affinity for II. However, replacement with isoleucine results in a different inactivation mechanism; this suggests that the mechanism of catalysis of superoxide dismutation by Ile-143 is also be different.

A 206 ENGINEERING LARGE INCREASES IN THE STABILITY OF SUBTILISIN BPN' THROUGH INCREMENTAL INCREASES IN THE FREE ENERGY OF UNFOLDING, *Philip Bryan, Michele Rollence, Jay Wood, Steven Dodd, Mark Whitlow, Karl Hardman, and Michael Pantoliano,* **Genex Corporation, 16020 Industrial Drive,Gaithersburg, MD 20877 USA**

We have used several approaches to engineer large increases in the stability of the Bacillus serine protease, subtilisin. These include introducing disulfide cross-links, improving electrostatic interactions at calcium ion binding sites, and the use of in vitro random mutagenesis coupled with a phenotypic screen to identify stabilizing mutational events. More than twenty individual stabilizing mutations of subtilisin BPN' have been identified. Thermodynamic analysis has shown that individually these modifications contribute between 0.3-1.5 Kcal/mol to the free energy of stabilization. We have further found that combining individual stabilizing mutations results in cumulative increases in stability. Calorimetric and crystallographic data demonstrate that increases in the free energy of stabilization are often independent and additive. We therefore have been able to create extremely stable versions of subtilisins in a step by step manner. Thermodynamic stability of subtilisin was also shown to be related to resistance to irreversible inactivation at high temperature and high pH. The most stable versions have half-lives at high pH or high temperature approaching 1000-times longer than the wild type subtilisin BPN'.

A 207 cAMP-DEPENDENT PROTEIN KINASE: IDENTIFICATION OF ESSENTIAL CARBOXYL GROUPS, J.A. Buechler, S.S. Taylor, and W.M. Yonemoto, *Chemistry Department, University of California, San Diego, La Jolla, CA 92093.*

The catalytic (C) subunit of cAMP-dependent protein kinase phosphorylates Ser or Thr residues flanked by a common recognition sequence, Arg-Arg-X-Ser/Thr. The two arginines are essential features of this recognition site. Several carboxylic acid residues have been identified by chemical modifications of the C-subunit that may be essential for function. In order to identify amino acids that contribute to peptide recognition and, in particular, to recognition of the two arginines, the C-subunit was modified with a water soluble carbodiimide, 1-ethyl 3(3-dimethyl amino-propyl)-carbodiimide (EDAC) in the presence of [^{14}C] glycine ethyl ester. Due to its hydrophilic properties, this carbodiimide should react only with carboxylic acids that are exposed to the aqueous solvent. EDAC irreversibly inhibited the C-subunit, and MgATP afforded only partial protection against inactivation. Of the six carboxyl groups that were modified by EDAC in the apoenzyme, only Glu 170 and Asp 328 were totally protected when MgATP and a 20 residue inhibitor peptide were bound to the C-subunit. We concluded that these two residues, Glu 170 and Asp 328, may contribute to recognition of the arginine residues in the protein substrates. Site-directed mutagenesis is being used to determine whether those residues contribute to peptide recognition. The C-subunit also was chemically modified with dicyclohexylcarbodiimide (DCCD). DCCD is a hydrophobic carbodiimide that partitions into hydrophobic regions of the protein, such as the ATP binding site, and consequently reacts with different carboxylic acids than EDAC. The C-subunit was irreversibly inhibited by DCCD and MgATP protected against inactivation. Two carboxyl groups, Glu 91 and Asp 184, were modified by DCCD in the apoenzyme, and MgATP protected both residues from reacting with DCCD. Each of those residues are invariant in all protein kinases. Treatment of the apoenzyme with DCCD also lead to covalent crosslinking between Asp 184 and Lys 72. Lys 72 was shown previously, by affinity labeling, to be associated with the ATP binding site. Site-directed mutagenesis also is being used to help elucidate the function of these residues.

A 208 ENGINEERING SUBTILISIN FOR SITE-SPECIFIC PROTEOLYSIS, Paul Carter, Bjorn Nilsson, John P. Burnier, Daniel Burdick & James A. Wells, Department of Biomolecular Chemistry, Genentech Inc, 460 Point San Bruno Blvd, S. San Francisco, CA 94080
The serine protease, subtilisin BPN', has been engineered by rational design to be exquisitely specific for a target site in a fusion protein. A mutant in which the catalytic histidine was replaced by alanine (H64A) is exceedingly specific for histidine containing substrates because the substrate histidine can substitute to some extent for the missing catalytic histidine (substrate-assisted catalysis). The catalytic efficiency of the H64A mutant has been increased 20-fold (K_{cat}/K_M = 1.0 x 10^3 s^{-1} M^{-1}) by introducing further site-directed mutations and by judicious choice of substrate. This penta-mutant subtilisin efficiently and quantitatively cleaves a model fusion protein containing a synthetic IgG binding domain of Staphylococcus aureus protein A linked by an accessible target site to Escherichia coli alkaline phosphatase, with no detectable cleavage occurring at additional sites. This penta-mutant subtilisin has many properties which we consider to be desirable for an ideal enzyme for site-specific proteolysis: it can be recovered in high yield (>30 mg/l) free of contaminating proteases, it is active in the presence of denaturants (modest concentrations), detergents, reductants and a cocktail of protease inhibitors or after immobilization on a solid support.

A 209 THE EFFECTS OF MULTIPLE REPLACEMENTS ON THE FOLDING OF THE ALPHA SUBUNIT OF TRYPTOPHAN SYNTHASE FROM ESCHERICHIA COLI, Boris A. Chrunyk, Neil B. Tweedy, Mark R. Hurle, and C. Robert Matthews, Department of Chemistry, The Pennsylvania State University, University Park, PA 16802
The alpha subunit of tryptophan synthase from E. coli has been found to be an excellent system for investigating the molecular process by which the amino acid sequence directs the folding of the protein. The protein has been shown to unfold through a stable intermediate, consisting of a folded amino terminal portion (residues 1-188) and an unfolded carboxyl region (residues 189-268). The association reaction between these two domains is the rate-limiting step in the folding process. The study of the effects of single amino acid replacements on the stability and folding of the protein has been used to identify the residues which play key roles in the process. A number of point mutations at position 211 in the alpha subunit, which recent crystallographic results indicate is near the interface of the two domains, have been isolated by Yanofsky and co-workers. The folding characteristics of six replacements, G211E,R,D,S,V,W, were examined. The results show that electrostatics play a significant role in the rate-limiting association step while the effects of residue volume are of lesser importance. This data, coupled with previous studies, suggests that the docking of the two domains may involve a smaller area of the interface region rather than the entire interface.

A 210 INACTIVATION OF PERTUSSIS TOXIN BY SITE-DIRECTED MUTAGENESIS. S. Cockle, S. Loosmore, K. Radika, G. Zealey, H. Boux and M. Klein, Connaught Research Institute, Willowdale, Ontario, Canada M2R 3T4.
Pertussis toxin (PT) is a multimeric protein comprising an A subunit (S1) with ADP-ribosyltransferase activity and a B oligomer that interacts with target cell receptors. Site-directed mutagenesis was performed on the S1 gene to produce inactive but still immunogenic forms of PT for inclusion in a new pertussis vaccine. Mutated S1 genes were recombined with the native B oligomer genes and the complete polycistron introduced into a PT-negative Bordetella host, so that fully assembled mutant toxins were secreted. Certain sites for mutagenesis were chosen by sequence comparison with other ADP-ribosylating bacterial toxins. The two Cys residues were selected because S1 is activated by reduction. Most importantly, an active-site Glu was identified by photocrosslinking of native S1 to the substrate [14-C]NAD. Several amino acid substitutions were made at each site, and replacements were also introduced simultaneously at more than one site. Expression levels in culture supernatants were determined by ELISA, and residual biological activity was estimated by a CHO cell clustering assay, an indirect measure of ADP-ribosylation. Mutants were then purified for further work by affinity chromatography. Some 50 mutants were obtained exhibiting greatly decreased activity (0.1-1% relative to native PT), of which 40% also retained an important protective S1 epitope. Selected mutants were checked for toxicity and immunogenicity in vivo in a mouse model. The contribution of different S1 residues to catalysis and binding was judged by enzyme kinetic studies of NAD hydrolysis and of ADP-ribosylation of bovine transducin.

A 211 PROGRESS IN DEFINING THE EcoRI ENDONUCLEASE CATALYTIC MECHANISM, J.P. Day, N.O. Reich, J.M. Rosenberg, P. Greene, H.W. Boyer

We are investigating the mechanism of phophodiester cleavage by the EcoRI endonuclease. The x-ray structure of the protein-DNA complex has successfully predicted which amino acids form hydrogen bonds with the bases of DNA which comprise the canonical recognition sequence (3'-GAATTC-5'). There appear to be only two amino acids in the EcoRI endonuclease which could effectively function in hypothetical catalytic mechanisms as general acids or general bases. These are E143 and K138 (single letter amino acid code).

The ES143 mutant enzyme has the same k_{cat} and K_m as the wildtype so this residue is probably not catalytically significant. The wildtype enzyme has a rate of first strand cleavage vs. pH which increases from pH 6 to 9.5 and then is constant to pH 12. The shoulder of this curve at 9.5 is consistent with K138 as a catalytic residue. The increase of the rate with pH indicates that the neutral side chain is the active form and is probably acting as a general base. A water molecule is deprotonated which subsequently attacks the phosphorous atom. Mg^{2+} seems not to shift the pK_a of K138, but it may be involved in stabilizing the leaving group. This proposed role of K138 is consistent with inversion of configuration of the phosphate (F. Eckstein). Also, K138 has a pK_a not optimal for cleavage at pH 7.5. EcoRI optimizes specificity and not catalysis. Lengthening the time between binding and catalysis may function as an editing mechanism. Preliminary analysis of mutants at position 138 support K138 as an important catalytic residue.

Another possible contributor to catalysis is the phosphate (ApA) next to the phosphate (GpA) which is cleaved. Unlike B-DNA, the DNA of the complex is kinked in such a way that an oxygen atom of the ApA phosphate is shifted into a position nearly in line with the bond that is broken. This phosphate may act as a general base, or to coordinate the attacking water. The kink is likely sequence specific, thus this phosphate may contribute to the specificity of the enzyme.

A 212 COMPARATIVE HIGH LEVEL EXPRESSION OF CHICKEN AND HUMAN LYSOZYMES IN THE YEAST SACCHAROMYCES CEREVISIAE, A. de Baetselier, M. Heusterpreute, M. de Beukelaere, V. Ha Thi, M. Brauer*, A. Vasavada*, and S. Rosenberg*, UCL-ICP, UCL7539, Avenue Hippocrate 75, B-1200 Brussels, Belgium and *Chiron Research Labs, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94602

Human and chicken lysozymes have been expressed at high levels in the yeast, Saccharomyces cerevisiae utilizing the chicken lysozyme signal sequence for secretion and a hybrid ADH2-GAFDH promoter for transcriptional regulation. Plasmid transformants were shown to produce up to 20 mg/L of either lysozyme when grown in high glucose medium in which yeast growth and lysozyme production were cleanly separated. Production of chicken lysozyme could also be achieved with ethanol as carbon source where growth and production occurred concurrently, but little or no expression of human lysozyme could be obtained under the same conditions. Measurements of plasmid stability showed that with ethanol as carbon source the human but not the chicken plasmid are rapidly lost from the yeast. This is not due to the higher specific activity of human lysozyme against Micrococcus luteus, since a mutant of human lysozyme with the same specific activity as chicken lysozyme also shows plasmid instability.

A 213 THE ROLE OF HYDROPHOBIC INTERACTIONS IN THE FORMATION OF MOLTEN-GLOBULE-LIKE CONFORMATIONAL STATES AT LOW pH, A.L. Fink, L.J. Calciano and Y. Goto, Department of Chemistry, University of California, Santa Cruz, CA 95064.

Under conditions of low ionic strength, many proteins become denatured and substantially unfolded in the vicinity of pH 2 to 3. We have found that the addition of anions, either as salts or in the form of increased acid concentration, cause the protein to refold to a compact molten-globule-like conformation with substantial secondary structure, but disordered tertiary structure. To understand the mechanism of this anion-induced structure formation we examined the effect of different salts and strong acids on the low-pH unfolded form of several proteins. The effectiveness of different anions in inducing structure was consistent with their position in the Hofmeister series. These results demonstrate the importance of hydrophobic interactions in the formation of the molten-globule state. The criteria used to characterize the partially folded state included circular dichroism, Trp fluorescence and volume measurements from light scattering or molecular exclusion chromatography.

A 214 EQUILIBRIUM AND KINETIC FOLDING OF trp APOREPRESSOR FROM ESCHERICHIA COLI, Mitchell S. Gittelman and C. Robert Matthews, Department of Chemistry, The Pennsylvania State University, University Park, PA 16802
The urea induced folding of trp aporepressor from Escherichia coli was examined using optical spectroscopy to study the folding mechanism of this interdigitated dimeric protein. The equilibrium unfolding transitions, as monitored by difference ultraviolet, fluorescence and circular dichroism spectroscopy are nearly coincident; the midpoints are 5.47, 5.55 and 5.47 M urea, respectively. This result is well described by a two-state model containing both native dimer and unfolded monomers. The unfolding transition is protein concentration dependent and the free energy of folding under standard state conditions is approximately 25.4 kcal mol^{-1}. Kinetic studies of the unfolding reaction reveal a single kinetic phase which accounts for the total amplitude change expected from equilibrium studies. Three kinetic phases were detected in refolding which depend in complex ways on both final urea and protein concentration. Protein folding and/or association are rate-limiting at intermediate urea concentrations while isomerization reactions appear to predominate at low urea concentrations. These results are consistent with a folding model in which folding proceeds by an association reaction followed by a conformational change to yield the native conformation.

A 215 CLONING AND CHARACTERIZATION OF ACTIVE SITE MUTANTS OF E. COLI THIOREDOXIN, F.K. Gleason, C-J. Lim, M. Gerami-Nejad and J.A. Fuchs, Departments of Plant Biology and Biochemistry, University of Minnesota, St. Paul, MN 55108.
Thioredoxin is a small redox protein with an active disulfide/dithiol, having the active site sequence, Cys-Gly-Pro-Cys. It functions both as a reducing agent and a protein disulfide reductase. Although studies with model peptides indicate considerable strain in a 14-membered disulfide ring, it is an invariant feature of all naturally occurring thioredoxins and related proteins such as thioredoxin reductase, glutaredoxins, and protein disulfide isomerases. Mutants of E. coli thioredoxin with either a larger or smaller disulfide ring were produced by digesting the gene with AvaII. One AvaII restriction site is found at the Gly-Pro codons of the gene. One mutant thioredoxin has a 17-membered ring with the active site sequence, Cys-Gly-Arg-Pro-Cys; the other protein has a 11-membered ring with the sequence, Cys-Ala-Cys. Both proteins were isolated and characterized. Molecular modeling indicates little change in the structure of the thioredoxins except for the larger ring where several amino acids must shift to accomodate the extra residue. Both mutant proteins interact with E. coli thioredoxin reductase and are reduced by NADPH. Km's are higher, but turnover rates are comparable to wild type thioredoxin. The mutant protein with the Arg inserted into the active site can also function as a reducing agent and protein disulfide reductase. In contrast, the Cys-Ala-Cys active site does not reduce other proteins, as the dithiol form of the mutant thioredoxin does not readily reoxidize due to steric strain.

A 216 A GENETIC SCREEN FOR MUTANTS OF BPTI WITH ALTERED FOLDING ENERGETICS, D.P. Goldenberg, L.J. Coplen and R.W. Frieden, Department of Biology, University of Utah, Salt Lake City, UT 84112

We have developed a novel genetic screen for identifying variants of bovine pancreatic trypsin inhibitor (BPTI) with altered unfolding kinetics. The method utilizes an expression plasmid that directs the production of native active BPTI in *Escherichia coli*. Small cultures of bacteria containing mutagenized plasmids are grown in 96-well microtiter plates. The cultures are lysed and treated for 60 min at room temperature with dithiothreitol (DTT), conditions where the native conformation of the wild-type protein is thermodynamically unstable but unfolding is very slow, with a half-time of about 20 h. Under these conditions, one of the three disulfides of the native wild-type protein is selectively reduced, but the protein retains its native conformation and activity. Trypsin is then added to the extracts, followed by a chromogenic substrate. The active protein produced by the wild-type gene inhibits trypsin while mutant proteins that fail to fold or are inactivated by the treatment with DTT allow hydrolysis of the substrate. Approximately 10,000 mutagenized clones have been screened in this way. About one percent of the clones produce proteins that can fold but are inactivated by DTT. The mutant proteins may be inactivated by DTT either because they are fully unfolded much more rapidly than the wild-type protein, or are selectively reduced to yield inactive forms. The genes coding for over 50 "DTT-sensitive" mutant proteins have been sequenced, and over 20 different amino acid substitutions at 14 of the 58 residues of the protein have been identified. These mutations identify residues that establish a "kinetic lock" on the native conformation or are important for inhibitor activity. The amino acid substitutions appear to fall into two clusters in the native conformation, suggesting that even a protein as small as BPTI may be genetically dissected into subdomains. Further studies of the disulfide-coupled folding and unfolding of these mutant proteins will enable us to determine the roles of the altered residues throughout the folding process.

A 217 EXPLORING AMINO ACID SEQUENCE PREFERENCES AT A TYPE I' β-HAIRPIN SITE IN STAPHYLOCOCCAL NUCLEASE, Margaret Ann Goodman and Robert O. Fox, Department of Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale University, New Haven, CT 06511 We have developed a genetic system to explore sequence preferences at a type I' β-hairpin in staphyloccal nuclease (residues 27-31). An M13 based cassette vector has been constructed which allows an oligonucleotide to be cloned into the region encoding this β-turn. An oligonucleotide mixture was synthesized with random base incorporation in the codons for residues 27-31, thus encoding all pentapeptide sequences in the turn region. This mixture of oligonucleotides was cloned into the vector, producing a library of nuclease mutants, each with a unique sequence at the hairpin site. Mutants were selected on the basis of enzymatic activity on assay plates. Only 5-10% of the sequences tested resulted in detectable activity. A library of 130 β-hairpin sequences from active enzyme variants has been sequenced. Each position in the β-turn region is characterized by a different distribution of amino acid preferences.

A 218 *Eco*RI AND THE CLEAVAGE OF NONCANONICAL DNA SEQUENCES. Paul W. Hager, Patricia J. Greene, Herbert W. Boyer, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143, and John M. Rosenberg, Department of Biological Sciences, University of Pittsburg, Pittsburg, PA 15260. In order to understand the specificity of the *Eco*RI endonuclease we have investigated the first and second strand cleavage of noncanonical DNA sequences by *Eco*RI under standard conditions. The comparison of the second strand cleavage reaction indicates that *Eco*RI is 4500 times slower on noncanonical sequences. The striking feature of noncanonical cleavage is that the reaction mechanism is altered, enzyme releases from the nicked intermediate. Since the nonspecific binding of DNA is of the same order of magnitude as the binding of the nicked intermediate, at low enzyme/DNA ratios the bulk DNA acts to reduce the velocity of the reaction on the nicked intermediate. Even this low rate of cleavage is potentially lethal to the cell. We suggest that ligase acts in an editing role to repair the nicked intermediate before the lethal double strand cleavage can occur.

A 219 THE ROLE OF SURFACE LOOPS IN TRYPSIN STRUCTURE AND FUNCTION Jeffrey N. Higaki, Guo Hong and Charles S. Craik, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143 Surface loops, though variable in length and amino acid sequence, are presumed to be necessary for the proper folding and/or the structural integrity of a protein molecule. Using site-directed mutagenesis, we have deleted portions of two surface loops of trypsin in order to probe the role and requirement of these loops in maintaining trypsin structure and function. The deleted sequences were chosen based on a computer modeling of the enzyme, focusing on those residues that showed sequence variability within the serine protease family of enzymes to which trypsin belongs. The deletion of residues 145-149 (located in the autolysis loop) and 70-79 (located in the calcium binding loop) does not affect the level of expression in E. coli; however, both deletions render trypsin more susceptible to proteolysis by pepsin and chymotrypsin These loop deleted variants have peptidase activities that are 30 to 400 fold lower than wild type typsin although their Km values are normal. In contrast, the deletion of the last three residues (243-245) from the C-terminus dramatically reduces the expression level of the enzyme presumably by destabilizing the molecule in the periplasmic space of the host. These results show that two complete surface loops of trypsin are not absolutely required for either its proper folding or function although the overall catalytic efficiency of the enzyme is reduced. The major effect is not in substrate recognition but in substrate turnover, thus illustrating the sensitivity of the active site to minor structural alterations.

A 220 SITE-DIRECTED MUTAGENESIS OF HUMAN $\beta\beta$ ALCOHOL DEHYDROGENASE: THE ROLE OF AMINO ACID 47 IN COENZYME BINDING, Thomas D. Hurley, Howard J. Edenberg, and William F. Bosron, Indiana University School of Medicine, Indianapolis, IN 46223.

Alcohol dehydrogenase (ADH) catalyzes the NAD^+ dependent oxidation of ethanol to acetaldehyde. Arg-47 has been implicated in the tight binding of NAD^+ by β_1 ADH. Using site-directed mutagenesis, we have substituted His(H), Lys(K), and Gln(Q) for Arg(R) at position 47 of β_1 ADH. The wild-type and mutant cDNA's were expressed in E. coli. The V_{max} (U/mg) and the kinetic constants (mM) of these ADH's were determined at pH 7.5 and 25°C.

ENZYME	K_M(NAD)	K_i(NAD)	K_iAMP	K_iADP-R	V_{max}	V_{max} + Cl^-
β47R(β_1)	0.020	0.050	0.33	0.12	0.097	0.26
β47K	0.10	0.12	1.7	0.11	0.25	0.32
β47H	0.21	0.33	0.45	0.072	8.9	7.7
β47Q	0.48	0.49	1.3	0.31	21	20

β47Q has high and β47R has low K_M and K_i values for NAD and K_i values for the competitive inhibitors AMP and ADP-ribose. β47K and β47H exhibit intermediate values for K_M, K_i, and V_{max}. NADH dissociation is rate limiting for β_1-ADH; therefore, a low K_M for NAD correlates with a low V_{max}. The anomalously high K_i of β47K for AMP may be due to ion-pairing of K47 with D50 instead of the AMP phosphate. Cl^- activates β47R 2.5 fold, but has only minor effects on the other three enzymes. We conclude that changing the strong base, Arg-47, in the anion binding site of β_1 decreases coenzyme binding and increases the V_{max} of these enzymes, by accelerating the rate-limiting dissociation of NADH. (AA07117, AA07962)

A 221 TRANSFER OF A β-TURN STRUCTURE TO A NEW PROTEIN CONTEXT, Thomas R. Hynes, Roger A. Kautz, Margaret A. Goodman, John F. Gill & Robert O. Fox, Department of Molecular Biophysics and Biochemistry, Howard Hughes Medical Institute, Yale University, 260 Whitney Ave., New Haven, CT 06511 Turns and loops are an attractive starting point to develop protein design principles, as they involve a small number of consecutive residues, and are largely unconstrained by packing interaction due to their surface location. Correlations between the sequence, and conformation of β-turns and loops will permit the rational engineering of the backbone conformation of surface regions to create novel functions.

We have inserted a type I β-turn sequence from concanavalin A, (residues 160-165) into staphylococcal nuclease A, replacing a type I' β-turn (residues 27-31). The resulting hybrid protein folds to yield an active enzyme with a significant decrease in the overall stability. A crystal structure of the hybrid protein refined to 1.8 Å reveals that the guest turn sequence retains the type I β-turn conformation found in concanavalin A. This result suggests that β-turn elements may be used as structural cassettes in protein design.

A 222 PROBING PROLINE ISOMERISM IN STAPHYLOCOCCAL NUCLEASE USING SITE-DIRECTED MUTAGENESIS AND NMR SPECTROSCOPY. Roger A. Kautz, Thomas R. Hynes and Robert O. Fox, Department of Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale University, New Haven, CT 06511

NMR investigations of staphylococcal nuclease have revealed two native conformations in slow exchange[1]. The multiple His $H^{\epsilon 1}$ proton resonances indicative of the two states result from a mixture of *cis* and *trans* isomers of the Lys116-Pro117 peptide bond.[2] Nuclease variants generated by site-directed mutagenesis of this type VI β-turn region (115-118) have been investigated by NMR spectroscopy and X-ray crystallography. Substitutions at Pro117 (P117G and P117T) abolish the observed *cis/trans* equilibrium. A substitution at Lys116 (K116G) shifts the equilibrium from this *cis* form (~85%) to the *trans* form (~85%). This system provides an opportunity to use the type VI β-turn cis-trans equilibrium as a probe of the forces which contribute to the stabilization of globular protein molecules.

[1] Fox, R.O., Evans, P.A., Dobson, C.M. *Nature* **320**:192-194 (1986)
[2] Evans, P.A. *et al.* *Nature* **329**:266-268 (1987)

A 223 REPLACEMENT OF HIS 64 WITH TYR RESULTS IN INCREASED THERMAL STABILITY OF t-PA KRINGLE-2 DOMAIN WITHOUT LOSS OF LYSINE BINDING, Robert F. Kelley & Scott Cleary, Biomolecular Chemistry Dept., Genentech, South San Francisco, CA 94080. Tulinsky et al., [PROTEINS: Structure, Function & Genetics (1988) 3: 85-96.] have proposed that histidine 64 of the kringle-2 domain of tissue plasminogen activator (t-PA) provides the cationic site for binding the carboxylate of the ligand l-lysine. We have used site-directed mutagenesis to replace this histidine with a tyrosine, the residue found at this position in t-PA kringle-1 domain, using a plasmid vector constructed for secretion of the isolated kringle-2 domain in *E. coli*. The mutant and wild type domains were purified using affinity chromatography on lysine-Sepharose. Far ultraviolet circular dichroic spectra suggest that the mutant and wild type domains have the same overall folding. Equilibrium dialysis experiments indicate that the mutant and wild type proteins have an identical affinity for l-lysine and have a similar pH dependence of lysine affinity. Thermal denaturation of wild type kringle-2 at pH 4.5 has a Tm of 65 °C while the Tm for the H64Y mutant is 79 °C. The difference in melting temperature between the two proteins is slightly pH dependent, increasing to 17 °C at pH 3 and decreasing to 10 °C at pH 7.5.

A 224 EVIDENCE FOR DIFFERENT FUNCTION OF CYSTEINE RESIDUES IN FOLDING OF HUMAN LYSOZYME IN YEAST

M. Kikuchi, Y. Taniyama, Y. Yamamoto, K. Inaka, K. Morikawa and M. Ikehara, Protein Engineering Research Institute, 6-2-3, Furuedai, Suita, Osaka 565 Japan.

Human lysozyme (hLZM) has four disulfide bonds; Cys6/Cys128, Cys30/Cys116, Cys65/Cys81, and Cys77/Cys95. C81A(Ala-substitution at Cys81) and C77/95A gave one molecule with the same specific activity as that of native hLZM. And crystals of C77/95A were isomorphous with those of native hLZM. C77A and C95A were purified as two molecules with different specific activities. C30A, C116A, C30/116A and C65/81A reduced their secretion and specific activities. C6A, C65A, C128A and C6/128A were insoluble and inactive. These indicate that Cys6, Cys65 and Cys128 are indispensable to correct folding, but that Cys81 or Cys77/Cys95 are unnecessary. Cys65 and Cys81, which form a disulfide bond in native hLZM, were found to function differently in folding of hLZM in yeast.

A 225 THE USE OF *E. COLI* NONSENSE SUPPRESSORS TO RAPIDLY INTRODUCE AMINO ACID SUBSTITUTIONS IN THYMIDYLATE SYNTHETASE

Choll W. Kim, Mark L. Michaels, Peter G. Markiewicz, and Jeffrey H. Miller, Molecular Biology Institute and the Department of Biology, University of California, Los Angeles, CA 90024. We have used nonsense suppressors of *E. coli* to rapidly introduce amino acid substitutions in thymidylate synthetase (5,10-methylene tetrahydrofolate:dUMP C-methyl transferase), a highly conserved enzyme required in the sole *de novo* pathway for thymidine synthesis. A combination of naturally occurring nonsense suppressor tRNAs and nonsense suppressor tRNAs constructed *in vitro* has been used to efficiently insert lysine, serine, glutamine, tyrosine, leucine, phenylalanine, cysteine, histidine, alanine, glycine, glutamic acid, or proline at specific 5'UAG3' amber termination codons. Using this method, we have introduced 11 or 12 amino acid substitutions at over 25 sites to generate over 300 variants of the *E. coli* thymidylate synthetase. Correlating the effect of each of these mutations on enzymatic activity with the three-dimensional structure of the bacterial thymidylate synthetase (Dave Matthews and co-workers, Agouron Pharmaceuticals Inc., unpublished results), we have examined the structure-function relationship of this enzyme.

A 226 TEMPERATURE SENSITIVE AMINO ACID SUBSTITUTIONS IN PHAGE T4 LYSOZYME INCREASE THE RATE OF PROTEIN UNFOLDING, Juli Klemm*, Joan Wozniak^, Tom Alber* and David Goldenberg*, *Departments of Biology and Biochemistry, University of Utah, Salt Lake City, UT 84112, ^Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

To determine the relative effects of amino acid substitutions on the native state and the transition state for denaturation of phage T4 lysozyme, we have examined the kinetics of unfolding of 28 lysozyme variants by urea gradient electrophoresis.

The wild type protein reversibly unfolds in about 7 M urea at pH 7.0, 22° C. The denaturation transition is discontinuous on the gels, indicating that unfolding and refolding are slow on the time scale of electrophoresis (20 min.). In contrast, all 26 temperature sensitive (ts) lysozymes examined unfold at lower urea concentrations and show continuous patterns in the transition zone characteristic of rapid unfolding and refolding behavior. Two mutant proteins with thermodynamic stabilities equal to or greater than the wild type protein display slow unfolding transitions.

In summary, all of the ts mutations increase the rate of unfolding. This observation indicates that the ts substitutions destabilize the native protein with respect to both the unfolded state and the transition state for folding and unfolding. None of the 26 ts substitutions exclusively stabilize the unfolded state. This pattern of effects on unfolding kinetics, in conjunction with the observation that the sites of destabilizing mutations frequently have low solvent accessibility and low thermal mobility, is consistent with the view that the ts mutations disrupt stabilizing interactions that are strongest in the native state.

A 227 SITE DIRECTED MUTAGENESIS OF THE POLIOVIRUS PROTEINASE 2A ACTIVE CENTER AND A CLEAVAGE SITE, Hans-Georg Kräusslich, Michael Faecke, Chong-Kyo Lee, Christopher Hellen, and Eckard Wimmer, State University of New York, Stony Brook, N.Y. 11794.

All picornaviral proteins are expressed from the genomic RNA as a single polyprotein. This polyprotein is proteolytically processed to yield mature viral proteins. In poliovirus all but one of these processing steps are catalyzed by two virus-encoded proteinases, 3C and 2A. The initial cleavage of the polyprotein, which separates capsid- and non-structural proteins occurs co-translationally and is facilitated by proteinase 2A which cleaves a Tyr-Gly dipeptide at its own amino-terminus. This proteolytic enzyme is believed to have a Cys residue as nucleophile but to be otherwise closely related to the serine proteinases of the trypsin family. Accordingly, we changed the conserved Cys residue by site directed mutagenesis to a serine. This serine-2A gave weak but detectable activity in autocatalytic processing of an *in vitro* synthesized precursor protein. This residual activity was, however, not sufficient to yield viable virus when engineered into a full-length cDNA of poliovirus. In additional experiments we studied complementation of this mutant and the activity of serine-2A when overexpressed in *Escherichia coli*. We also performed site directed mutagenesis of the 2A cleavage site at its own amino-terminus and thereby analyzed substrate requirements for this viral proteinase.

A 228 THE SUBUNIT COMPOSITION OF REFOLDED GIZZARD TROPOMYOSIN.

S.S.Lehrer & Y.Qian, Boston Biomedical Research Institute, Boston, MA 02114.

Native chicken gizzard tropomyosin (GTm) is an α-helical coiled-coil, containing 2 similar subunits existing predominently as a heterodimer, $\beta\alpha$ (Sanders et. al., J.B.C., 261, 1274, 1986), where α is the slower moving chain on SDS-PAGE. GTm undergoes a single thermal helix-coil transition (Woods, Aust.J.Biol.Sci. 29,405, 1976; Lehrer et.al., Biochem., 23, 1592, 1984). Refolding of GTm from GdmCl into 1M NaCl predominently produces $\alpha\alpha$ and $\beta\beta$ homodimers exhibiting two thermal unfolding transitions (Graceffa, P., to be published). We have obtained additional thermal and denaturant-induced unfolding data with CD and have identified the first transition as originating from the unfolding of the $\alpha\alpha$ species, consistent with previous data (Sanders et.al., 1986), using a sample enriched in $\alpha\alpha$ ($0.8\alpha\alpha$:$0.2\beta\beta$). We also refolded the dissociated subunits by dialysis from denaturant into 0.05M NaAcetate, 5 mM $MgCl_2$, 5 mM Na phosphate buffer, pH 7.0 at 36° and 5°. CD studies on the sample refolded at 36° showed one thermal transition similar to native GTm ($\alpha\beta$); whereas the sample refolded at 5° showed 2 transitions, similar to renaturation from GdmCl, suggesting at least 2 species present. Thus, refolding close to physiological temperature *in vitro* results in preferential formation of native GTm ($\alpha\beta$) whereas refolding at lower temperatures produces homodimers. Supported by NIH HL22463.

A 229 SITE-DIRECTED MUTAGENESIS AS A PROBE OF THE THERMAL INACTIVATION OF SUBTILISIN BPN'.Colin Mitchinson and James A. Wells,Genencor,Inc. and Genentech,Inc.,South San Francisco,CA 94080. Studies of the thermal inactivation of wild-type and site-directed mutants of subtilisin suggest that the inactivation is fundamentally irreversible,in that the denatured form is incapable of refolding to the native state.This could explain the results seen when disulphides were engineered into subtilisin,as disulphides are thought to exert a stabilising effect primarily by increasing the rate of refolding of an unfolded protein: A total of seven disulphide bonds have been introduced,seperately,into the normally cysteine-free subtilisin.All the bonds formed fully and spontaneously in the secreted enzymes without major effects on their activities or expression levels.The measured redox-strengths of the engineered bonds were within the range seen for natural bonds.However,when first assayed,none of these disulphides resulted in a mutant subtilisin with greater than wild-type stability to thermal inactivation. Recent mechanistic studies suggest that there are two processes contributing to the thermal inactivation;**unfolding** of the native enzyme and **autolysis** of the active species. When re-assayed under conditions where the inactivation seems to proceed mainly by the autolytic path one of the disulphide-mutants is now stabilised and inactivates at only half the rate of wild-type subtilisin.

A 230 PAPAIN ASSISTED RESOLUTION OF α-AMINO ACIDS AND NUCLEOPHILES, Jean-L. Morinière, Jean-Y. Lacolle, Laboratoires Debat, 153 Route de Buzenval, 92380 Garches, France.

The production of α-amino acids, a present-day problem closely linked to the development of peptides, can be partially solved either by resolution or by asymetrical induction during synthesis by chemical chiral catalysts, or by catalysis using an isolated enzyme or a complex biological system (yeast, fungi, bacteria). We would like to describe the experimental results obtained using papain, an enzyme extracted from plants, wich allows resolution of natural and xenobiotic α-amino acids in favorable conditions.

$$(DL)\ X - AA - OH + (DL)\ NuH \overset{E}{\rightleftharpoons} (L)\ X - AA - (L) - Nu + (D)\ X - AA - OH + H_2O$$

AA - OH = natural and xenobiotic amino acids NuH = ROH, RNH_2, RSH

X = Z, Boc, Fmoc, Ac, Ar - SO_2, Bz, Ar - Co E = crude papain

The mild experimental pathway and the low cost of crude enzyme permit the production on preparative scale of optically active acyl donnors (AA-OH) and nucleophilic acyl acceptors (NuH).

A 231 DETERMINANTS OF *ECO*RI ENDONUCLEASE SEQUENCE DISCRIMINATION, Michael C. Needels, Sharon R. Fried, Herbert W. Boyer and Patricia J. Greene, Department of Biochemistry and Biophysics, University of California at San Francisco, San Francisco, CA 94143. Interactions of proteins with specific sequences of DNA are of fundamental importance for biochemical processes. In order to elucidate the molecular basis of one example of DNA sequence discrimination, we are investigating the DNA binding and cleavage mechanisms of the *Eco*RI endonuclease. This enzyme binds tightly to the unique DNA sequence GAATTC in the absence of Mg^{2+}. In the presence of Mg^{2+}, it hydrolyzes the phosphodiester bond between the guanylic and adenylic acid residues. Hydrogen bonding between three key amino acids and the purine bases in the major groove is thought to be the primary determinant of sequence specificity. One of these amino acids, the arginine at position 200, forms two hydrogen bonds to the guanine of the sequence GAATTC. In order to determine the role of hydrogen bonds in sequence discrimination, we have substituted this residue with each of the other 19 naturally occuring amino acids using cassette mutagenesis. The 19 mutant enzymes, which are each harbored on inducible plasmids in *E. coli*, were characterized with respect to *in vivo* activity, as judged by relative growth on plates and relative viability in liquid culture. Crude cell extracts were used to assay the relative activities of the mutant enzymes *in vitro*. Certain mutants which exhibit residual activity provide evidence that hydrogen bonding may not be the sole determinant of sequence discrimination.

A 232 THE DEPENDENCE OF ENZYME INHIBITION ON THE DISTRIBUTION OF ENZYME FORMS: IMPLICATIONS FOR SUBSTRATE AND INHIBITOR STRUCTURE-ACTIVITY CORRELATIONS. Daniel H. Rich and Dexter B. Northrop, School of Pharmacy, University of Wisconsin, Madison, WI 53706
Evidence for multiple forms of enzyme-substrate and enzyme-inhibitor complexes raise serious concerns about how these multiple forms can complicate our attempts to interpret structure-activity data and how to design novel enzyme inhibitors. Of particular importance is the necessity to abandon the common assumptions that a single catalytic step is rate-limiting, that rapid equilibrium conditions apply to binding of substrates and inhibitors, and that the Michaelis constant is a simple binding constant. Partial rate limitation by multiple steps during catalysis and slow product release are incorporated in a 5-step general steady-state kinetic mechanism, in which structure-activity relationships are alternatively evaluated in terms of V and V/K (or k_{cat} and k_{cat}/K_m). The postulate that values of V/K correlate with values of K_i for transition-state analog inhibitors will be challenged with the aid of computer simulations of free energy profiles of the 5-step mechanism. Similarly, the notion that slow-binding kinetics are diagnostic of transition-state analog inhibitors will be challenged as inconsistent with the Hammond postulate. Consideration of the steady-state mechanism of proteases provides an alternative explanation for pH-dependent kinetics which would otherwise be interpreted as implicating ionizations at remote binding sites. Considerations of isozymes and negative cooperativity raise concerns about the significance of kinetic data acquired with alternative or artificial substrates, often chosen for the convenience of spectrophotometric assays.

A 233 CALCULATION OF AMINO ACID CONTRIBUTIONS TO THE STABILITY OF AN α-HELIX. Karyn T. O'Neil, James W. Bryson, and William F. DeGrado. Central Research and Development Department; E. I. DuPont de Nemours; Wilmington, DE, 19880-0328. To assess the individual contributions of each amino acid toward the overall stability of an α helix, we have designed a synthetic peptide predicted to form a two stranded α-helical coiled coil. Substitution of each of the twenty naturally occurring amino acids into the same position in the peptide will allow us to determine the contributions from each amino acid to the relative stability of the coiled coil. We have determined that the peptide sequence chosen (AcELKAWEEKLAAL EXKLAALEEKLEALEG-NH2) with guest position X=phenylalanine adopts a dimeric α-helical conformation in concentrated solution. The guest amino acid is located on the face of the helix opposite the aligned hydrophobic residues that make up the proposed site of interaction between the two helices. In addition, the site is surrounded by small alanine residues to minimize steric effects. By spectroscopically monitoring the two state equilibirum between monomeric random coil and dimeric α-helical coiled coil we can quantify, energetically, the side chain contributions from each amino acid towards the stabilization of the coiled coil.

A 234 A NEW METHOD FOR DETERMINING THE HEAT CAPACITY CHANGE FOR PROTEIN FOLDING, C. Nick Pace and Douglas V. Laurents, Dept. of Biochemistry, Texas A&M University, College Station, TX 77843-2128.
There is currently considerable interest in measuring the conformational stability of globular proteins, i.e., how much more stable the folded biologically active conformation is than unfolded conformations under physiological conditions. With thermal denaturation curves or calorimetry, this requires a knowledge of the heat capacity change for unfolding, ΔC_p, in order to extrapolate measurements made in the narrow temperature range where unfolding occurs to an ambient temperature, such as 25°C. This is generally done with an equation such as: $\Delta G(T) = \Delta H_m(1-T/T_m) - \Delta C_p[(T_m-T) + T\ln(T/T_m)]$, where $\Delta G(T)$ is ΔG at temperature T, T_m is the midpoint of the thermal unfolding curve, and ΔH_m is the enthalpy change for unfolding measured at T_m. T_m and ΔH_m can be readily determined from a thermal denaturation curve, but ΔC_p is difficult to determine accurately and the best method available requires a calorimeter. We describe a new method of estimating ΔC_p which is based on results from urea and thermal denaturation curves, but does not require a calorimeter. We use the method to show that ΔC_p = 1650 ± 200 cal/deg mole for the unfolding of ribonuclease T_1, and ΔC_p = 2200 ± 300 cal/deg mole for the unfolding of ribonuclease A. (Supported by grants from NIH (GM37039) and the Welch Foundation (A1060).)

A 235 AMINO ACID REPLACEMENTS ALTERING THE ALLOSTERIC PROPERTIES OF THE ESCHERICHIA COLI ADPGLUCOSE PYROPHOSPHORYLASE, Jack Preiss, Paritosh Ghosh, Anil Kumar, Toshio Tanaka, and Young Moo Lee, Department of Biochemistry Michigan State University, East Lansing, MI 48824

The ADPglucose pyrophosphorylase of E. coli K12 mutant 618 has a higher apparent affinity for the activator fructose 1,6-P_2 and lower apparent affinity for the inhibitor, 5' AMP than the parent strain enzyme. The structural gene, glgC of the mutant enzyme has been cloned and sequenced. From the deduced amino acid sequence it has been shown that the amino acids changed in the mutant enzyme that altered the allosteric properties were Lys^{296} to Glu and Gly^{336} to Asp. Single mutant enzymes, Glu^{296} and Asp^{336}, were constructed using oligonucleotide directed mutagenesis. The Glu^{296} enzyme had the same kinetic constants as the wild type enzyme with respect to substrates and allosteric effectors. The Asp^{336} enzyme showed only 3-5% of the activity seen with the wild type enzyme. Thus the mutations at 296 and at 336 alone could not affect the allosteric alteration seen with mutant 618 enzyme. Alteration of the normal allosteric properties thus were due to changes of both Lys^{296} to Glu and Gly^{336} to Asp.

A 236 USE OF PROTEIN ENGINEERING TO IMPROVE THE IMMOBILIZATION PROPERTIES OF SUBTILISIN BPN'

Tapani Reinikainen, Tarja Nevanen and Jonathan Knowles

Technical Research Centre of Finland (VTT), Biotechnical Laboratory, Tietotie 2, SF-02150 Espoo, Finland.

In an attempt to improve the immobilization properties of the protease subtilisin BPN' we have generated a number of single and multiple Lys-Ala mutants. Glutaraldehyde, often used for the chemical immobilization of proteins reacts principally with the aminogroups of lysines and thus by changing the number of distribution of lysines it should be possible to modify the characteristics of the immobilized enzyme. In addition changes in the surface charge distribution of the enzyme could affect the catalytic properties. Eight different mutants were constructed as follows K136A, K170A, K213A, K213AK170A, K213AK136A, K136AK170A and K136AK170AK213A. A number of parameters of both free and immobilized mutant enzymes were studied. More importantly, when immobilized under appropriate conditions, certain mutants showed clearly higher activity than immobilized wild type enzyme. This work suggests that it may be possible to make significant improvements in the properties of immobilized enzymes by rational design.

A 237 PROBING THE MECHANISM OF H^+/LACTOSE SYMPORT BY SITE-DIRECTED MUTAGENESIS. Paul D. Roepe and H. Ronald Kaback, Dept. of Biochemistry, Roche Institute of Molecular Biology, Nutley, NJ 07110.

The *lac* permease of *E. coli* catalyzes the coupled translocation of a single β-galactoside molecule with a single H^+ across the plasma membrane, and experiments utilizing site-directed mutagenesis demonstrate that Arg302 (putative helix IX), His322 (putative helix X) and Glu325 (putative helix X) play a critical role in the mechanism, possibly as components of a H^+ relay [Kaback, H. R. (1987) *Biochemistry 26*, 2071]. Recent experiments show that the "apparent pK" for lactose efflux is downshifted ca. 0.5 pH units by replacing Glu325 with Asp, thereby providing a strong indication that deprotonation of Glu325 in the wild-type permease is rate-limiting for lactose efflux. In addition, 13 of the Tyr residues in the permease have been replaced with Phe, and four of the mutations have been found to have a significant effect on permease activity. Two of the mutations (Y26F and Y336F) completely abolish all modes of activity and significantly raise the K_D for ligand, one (Y236F) appears to partially uncouple H^+ from lactose translocation, and the fourth mutation (Y350F) decreases turnover of the permease by 60% without uncoupling. Finally, three different five-residue sequences that exhibit homology to sugar binding sites in the crystal structures of periplasmic sugar-binding proteins [Quiocho, F.A. (1986) *Ann. Rev. Biochem. 55*, 287] have been extensively mutagenized. Binding and transport experiments are consistent with the notion that two of these regions (R135-E139 and K335-S339) may be involved in substrate binding in the permease.

A 238 SEQUENCE, MUTAGENESIS, AND HIGH LEVEL SECRETION OF VARIABLY GLYCOSYLATED ASPERGILLUS NIGER GLUCOSE OXIDASE, S. Rosenberg, K. Frederick, M. Brauer, W. Tung, S. Chamberlain, and F. Masiarz, Departments of Biochemistry and Protein Chemistry, Chiron Research Labs, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608.

We have obtained cDNA and genomic clones of A. niger glucose oxidase. The enzyme consists of 583 amino acids as predicted by the cDNA sequence; this is preceded by a 22 amino acid N-terminal extension, which must be finally processed at single basic cleavage site (R - S) to yield the mature N-terminus. The N-terminus of the enzyme shows homology with other AMP binding proteins, and the entire protein is homologous with alcohol oxidase of H. polymorpha. We engineered a full length GO cDNA for expression in S. cerevisiae using either the GO or S. cerevisiae alpha-factor pre-prosequences for secretion. Transformants of yeast containing high copy number plasmids secreted more than 100 ug/ml of active GO into the yeast media with either signal. Analysis of the proteins produced showed more extensive N-linked glycosylation of the yeast derived enzymes than that from A. niger. Site-directed mutagenesis has been used to ascertain which of the 8 potential glycosylation sites are utilized in yeast, as well what role the 3 cysteines play in enzyme structure and function.

A 239 A TRUNCATED CHORISMATE MUTASE INSENSITIVE TO FEEDBACK INHIBITION BY PHENYLALANINE

R. Balakrishnan, K. C. Backman, D. L. McKay and E. A. Rudd
BioTechnica International, Inc., Cambridge, MA 02140.

The first enzyme in the phenylalanine pathway of *E. coli* is a bifunctional protein having both chorismate mutase (E.C. 5.4.99.5) and prephenate dehydratase (E.C. 4.2.1.51) activity. Both of these activities are subject to feedback inhibition by phenylalanine. A truncated enzyme was made by genetic engineering that was insensitive to feedback inhibition by phenylalanine. The truncated enzyme contains the N-terminal 337 amino acids of the native enzyme. Both of the enzyme activities were resistant to inhibition by phenylalanine at concentrations of 1.2 mM. The only significant negative effect of truncating the enzyme is a 10-fold increase in the K_m for chorismate.

A 240 PROTEIN ENGINEERING IN ORGANIC SOLVENTS.

A.J. Russell and A.M. Klibanov. Department of Chemistry, MIT, Cambridge, MA 02139.

The ability of an enzyme to memorize active conformations in anhydrous organic solvents has been investigated. By lyophilizing subtilisin Carlsberg, a serine protease, in the presence of active site directed inhibitors it has been possible utilize the remarkable rigidity of proteins in non-aqueous media to lock the enzyme into an active conformation.

Subtilisin was lyophilized from aqueous solution in the presence or absence of N-acetyl-L-tyrosine amide. The dried enzyme powder was then washed exhaustively with acetonitrile to remove the ligand from the enzyme. Subtilisin prepared in the presence of inhibitor was up to 100 times as active as that prepared in the absence of inhibitor. The two preparations have identical activity in aqueous solution. Thus, the activating phenomenon is dependent on the properties of the solvent. Molecular imprinting was successful with a number of ligands, all of which induce increased activity of subtilisin with a variety of substrates. Furthermore, the induction of enzyme memory led to significant changes in the stability and specificity of subtilisin.

A 241 PROBING THE ROLE OF TWO HYDROPHOBIC ACTIVE-SITE RESIDUES IN THE HUMAN DIHYDROFOLATE REDUCTASE BY SITE-DIRECTED MUTAGENESIS, Barry I. Schweitzer, Adam Dicker, Srinivasan Srimatkandada, and Joseph R. Bertino, Yale University School of Medicine, New Haven, CT 06510 and Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

The phenylalanines at residues 31 and 34 in the human dihydrofolate reductase (hDHFR) have been shown by X-ray crystallography to be involved in the binding of the substrate dihydrofolate (DHF) and the inhibitor methotrexate (MTX). Using oligonucleotide-directed mutagenesis and a bacterial expression system producing the wild-type and mutant hDHFRs at levels of 10% of the bacterial protein, we have constructed and purified a Serine 31 (P-31-S) mutant and a Serine 34 (P-34-S) mutant. Fluorescence titration experiments indicated that P-31-S bound DHF 5 times tighter and NADPH 2 times tighter than the wild-type hDHFR. Furthermore, this mutation had little effect on the enzyme's steady-state kinetics but produced a 6-fold increase in the MTX Kd. This was in agreement with our results for a MTX-resistant human cell line in which we found a phenylalanine to serine mutation in DHFR at position 31. The P-34-S mutant had much greater alterations in its properties than P-31-S; specifically, it had a 25-fold higher DHF K_M, a 25-fold lower NADPH K_M, a 6-fold decrease in kcat, and a 2400-fold increase in Kd for MTX. Clearly, phenylalanines 31 and 34 contribute very differently to hDHFR's ligand binding and catalysis.

A 242 EFFICIENT COMBINATORIAL MUTAGENESIS OF PROTEIN SEGMENTS: APPLICATION TO EPITOPE - - DISPLAY AND ACTIVE SITE MODIFICATION. Xavier Soberón, Joel Osuna and Yolanda Fuchs. Dep. de Biología Molecular, Centro de Investigación sobre Ingeniería Genética y Biotecnología/UNAM, Cuernavaca, Mor. México.

Our approach to the study of protein structure-function relationship is based on the generation of large numbers of mutant genes coding for proteins that difier from the wild in one or a few amino acid residues. We have used chemically synthesized DNA and trinucleotide - - building bloks to seek the introduction of a controlled distribution of side chain replacements in the targeted area. Theoretical analysis has shown that the use of trinucleotides -- as mutagenic units is a significant advantage in studies requiring the simultaneous appearance of more than one amino acid replacement. The actual performance of the method will be discused in light of our results with model proteins in which such combinatorial mutagenesis has been applied: conformationally restrained grafting of protein epitopes and the active - sites of restriction endonuclease Eco RI and beta-lactamase.

A 243 ACTIVATION ENERGY AND pH DEPENDENCE OF SYNOVIAL COLLAGENASE AND GELATINASE, M. S. Stack and R. D. Gray, Dept. of Biochem., Univ. of Louisville, Lou., KY 40292.

Several cell types secrete neutral metalloproteinases that degrade collagen, gelatin and proteoglycan. Collagenase hydrolyzes each chain of collagen at -Gly_{775}-$Leu(Ile)_{776}$-, after which gelatinase can make additional cleavages. We have shown that collagenase and gelatinase can be copurified from conditioned medium of pig synovial tissue using a peptide inhibitor affinity column. Both enzymes cleave the fluorogenic substrate Dnp-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH_2 at the Gly-Leu bond. The activation energies of collagenase and gelatinase on the synthetic substrate were determined (9180 cal and 11030 cal, respectively) and compared to those for the natural substrates (collagen 81060 cal and gelatin 26360 cal). These data indicate an increase in the energy dependence of catalysis as the structure of the substrate becomes more ordered. The fluorogenic substrate was also used to determine the effect of pH on the stability, substrate binding and catalytic activity of collagenase and gelatinase. Both enzymes were stable over a broad pH range with maximum stability between pH 6-8 for collagenase and 7-9 for gelatinase. Preliminary experiments of the pH dependence of kinetic constants indicate that different ionizable groups are involved in substrate binding and catalysis for both enzymes. Supported by NIH AR-39573 and the Graduate School, University of Louisville.

A 244 STUDIES OF THE MECHANISM AND SUBSTRATE SPECIFICITY OF PAPAIN USING SITE DIRECTED MUTAGENESIS. Andrew C. Storer, Henry Khouri, Robert Ménard, Daniel C.Tessier, David Y. Thomas and Thierry Vernet. National Research Council of Canada, Biotechnology Research Institute, 6100 Royalmount Ave, Montréal, Québec, Canada. A synthetic gene coding for papain has been expressed in a Baculovirus -insect cell system (see accompanying poster; T. Vernet et al.). Through site directed mutagenesis, several papain mutants have been produced and expressed in this same system. These mutants were designed to investigate both the specificity and mechanism of papain. For example, several mutants at position 158 have been used to study the role of aspartic acid at this position in the native enzyme. The carboxyl acid group of Asp 158 is close to the active site thiolate-imidazolium ion pair and through electrostatic interactions is thought to influence the enzymes pH-activity profile. The role of an oxyanion hole in papain's mechanism has been questioned and we have therefore produced mutants aimed at modifying the H-bond donors thought to be involved in this oxyanion hole (glutamine 19, serine 176). The enzyme's catalytic triad (cysteine-histidine-asparagine) has also been modified (cys25→ser; asn175→asp) in an attempt to obtain mechanistic information. In addition, preliminary results indicate that modifications designed into papain's S_2 subsite have resulted in dramatic changes in substrate specificity.

A 245 THE INTRACELLULAR ROLE OF THE DISULFIDE BONDS OF HUMAN CHORIONIC GONADOTROPIN β SUBUNIT, Nobuhiko Suganuma, Martin M. Matzuk and Irving Boime, Departments of Pharmacology and Obstetrics & Gynecology, Washington University School of Medicine, St. Louis, Missouri 63110.
Human chorionic gonadotropin (hCG) consists of two noncovalently joined α and β subunits similar to the other glycoprotein hormones. Site-directed mutagenesis of the 12 cysteine (cys) residues of hCGβ was used to study the function of the disulfide bonds of hCGβ in secretion, subunit assembly, and post-translational processing. These mutant hCGβ genes (cys to alanine conversion) were transfected alone or together with the wild-type α gene into Chinese hamster ovary cells. Only 5-10% assembly could be achieved with derivatives containing cys mutations at positions 26, 72, 90 or 110, whereas the other 8 mutants failed to assemble. Thus, alterations at any cys in hCGβ affected dimer formation. However, the secretion rate of the individual mutants varied greatly. While the cys 23 mutant was secreted normally ($t_{\frac{1}{2}}$ ≈2.3 hr) and cys 26 mutant was secreted faster ($t_{\frac{1}{2}}$ ≈1.1 hr); the other 10 mutants showed slower secretion ($t_{\frac{1}{2}}$ ≈3.1-7.3 hr). Furthermore, these mutants were quantitatively recovered in the medium except for mutant 88 (≈40% secreted). Thus, interruption of any disulfide bonds in hCGβ changes the structure, possibly due to altered folding, that blocks assembly without greatly affecting the stability and secretion of the mutant hCGβ subunits. To examine if carbohydrate processing is altered by disrupting particular disulfide bonds, sensitivity to endoglycosidase H (Endo H) was examined. Absence of cys at proposed pairs 9-90, 38-57, 34-88 resulted in mutants bearing oligosaccharides that were partially sensitive to Endo H whereas the other mutants and native hCGβ were Endo H resistant. Thus, alteration of specific disulfide bonds modifies oligosaccharide processing of hCGβ. These data suggest that regions bounded by particular disulfide bonds are associated with key events in the intracellular behavior of the hCGβ subunit.

A 246 ENZYMATIC PEPTIDYL α-AMIDATION PROCEEDS THROUGH FORMATION OF AN α-HYDROXYLGLYCINE INTERMEDIATE, Paul P. Tamburini, Angelo P. Consalvo and Stanley D. Young, Unigene Laboratories, Inc., Fairfield, NJ 07006. The peptidyl α-amidation enzyme catalyzes the conversion of C-terminal glycine-extended peptides to desglycine α-amidated peptides in an oxidative reaction dependent both on L-ascorbate and on enzyme bound copper ion(s). Derivatives of the model substrate N-dansyl-Tyr-Val-Gly containing either a C-terminal N-hydroxylglycyl residue or an α-hydroxylglycyl residue were synthesized. The capacity of these peptides to undergo α-amidation enzyme catalyzed conversion to N-dansyl-Tyr-Val-NH_2 was evaluated. Neither the N-hydroxylpeptide nor one of the α-hydroxylglycine stereoisomers were converted to N-dansyl-Tyr-Val-NH_2, whereas both N-dansyl-Tyr-Val-Gly and the other α-hydroxylglycine stereoisomer were efficiently α-amidated with similar K_m values. In contrast to α-amidation of N-dansyl-Tyr-Val-Gly, amidation of this α-hydroxylglycine stereoisomer was neither affected by metal chelators nor dependent on L-ascorbate, and occurred with a V_{max} ten times greater than that observed with the model glycine-containing substrate. The pH optima for amidation of N-dansyl-Tyr-Val-Gly and this α-hydroxylglycine containing peptide were at pH 5.0 and pH 6.0-6.5 respectively. Collectively, these data strongly implicate a mechanism for α-amidation involving the rate limiting and stereoselective formation of a C-terminal α-hydroxylglycine intermediate.

A 247 SYNTHESIS, EXPRESSION, AND SITE SPECIFIC MUTAGENESIS OF A GENE FOR BOVINE (PRO) PHOSPHOLIPASE A2, Ming-Daw Tsai, Joseph P. Noel, Tiliang Deng, and Jian Tan, Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

A gene coding for the (pro)phospholipase A2 (PLA2) from bovine pancreas has been designed, synthesized, and expressed in *E. coli.* The total gene spans 404 base pairs, and was divided into 33 oligonucleotides. The gene was constructed in two halves of 224 and 180 base pairs from the oligonucleotides by the shotgun ligation technique using pBSM13- as the cloning vehicle. The two fragments were then ligated and cloned into pBSM13- to complete the gene. The gene was expressed to high levels (50-100 mg/liter) from a high copy number vector, designated as pJPN, derived from the *E. coli* secretion vector, pIN-III-ompA3. Although the protein failed to be secreted and was in the form of insoluble inclusion body, active PLA2 could be obtained by renaturation of the inclusion body pellet followed by tryptic activation which removes the signal sequence and the pro-peptide of (pro)PLA2. The PLA2 thus obtained reacted with the antisera raised against the natural PLA2 purified from bovine pancreas, and the specific activity of the expressed PLA2 was identical to that of the natural PLA2. Several site specific mutants (substitutions of the active site residues His-48, Asp-99, Tyr-69, Tyr-73, Phe-22) of bovine PLA2 have been constructed and. The characterization and properties of these PLA2 mutants will be presented.

A 248 STUDIES ON THE FUNCTION OF ASP-102 IN THE CATALYTIC TRIAD OF SERINE PROTEASES John R. Vasquez[1] and Charles S. Craik,[1,2] Departments of Pharmaceutical Chemistry[1] and Biochemistry and Biophysics[2], University of California, San Francisco, San Francisco, CA 94143 We have been studying the function of active site residues in rat anionic trypsin through the analysis of mutant trypsins. Mutants are generated using oligonucleotide-directed mutagenesis and are expressed in *E. coli* as signal peptide-trypsin fusion products which are processed to yield trypsin in the periplasm. The periplasm is analyzed for trypsin content by immunological analysis (Western) and for ester hydrolysis activity by activity stain. Using this system, we have screened several mutants including D102G, D102A, D102S, and D102N. No trypsin activity is detected in the periplasm of *E. coli* expressing these mutants. Western analysis reveals that the mutations D102G, D102A, and D102S destabilize trypsin to proteolysis by endogenous proteases while D102N stabilizes the protein. These results suggest that Asp-102, in addition to the known catalytic role, plays an important structural role in trypsin. Our previous enzymatic and structural studies of D102N have shown that Asp-102 stabilizes the correct tautomeric form of His-57. To further test this role of the negative charge and hydrogen bonding of Asp-102, the carboxylate of Asp-102 was replaced by a carboxylate from S214E. Computer modelling indicated that the mutation D102G was necessary to make room for the side chain of S214E since Ser-214 hydrogen bonds to Asp-102 in the wild-type enzyme. No activity was detected in the D102G, S214E double mutant because the D102G mutation destabilizes the protein. Routine screening, however, indicated that the single mutant S214E was not only stable, but also had significant ester hydrolysis activity relative to trypsin. The S214E protein has been purified to homogeneity using bovine pancreatic trypsin inhibitor (BPTI) affinity chromatography according to the same protocol used for the purification of wild-type trypsin. Preliminary kinetic analysis of the amidolysis activity of S214E trypsin indicates that K_M has increased while k_{cat} is virtually unaffected. Future kinetic and structural studies will focus on the effect of the S214E side chain on the catalytic mechanism of trypsin. Further mutation studies of Ser-214 are underway to elucidate the role of this evolutionarily conserved residue.

A 249 PRODUCTION OF PRECURSOR OF PAPAIN PROTEIN AND ITS MUTANTS IN INSECT CELLS, Thierry Vernet, Daniel C. Tessier, Christopher D. Richardson, Henry Khouri, Andrew Storer and David Y. Thomas, National Research Council of Canada, Biotechnology Research Institute, Montréal, Québec, Canada. The enzymatic properties and the 3-D structure of papain are well-defined and therefore make this cysteine protease an attractive candidate for protein engineering. A synthetic gene coding for the precursor of papain cloned into the modified insect virus vector pAC373/f1 was recombined in vivo with baculovirus DNA. The recombinant virus was used to infect insect cells. Immunoreactive and enzymatically active papain was obtain upon treatment of insect cell extracts with a reducing agent. The inhibitors of cysteine proteases mercury or E64 prevented this enzyme activation and a higher molecular weight precursor was detected. These preliminary results indicate that the processing of the papain precursor requires access to the active site of the enzyme. Localization of the protein was assessed by immunofluorescence. In the presence of the putative signal sequence (26 N-terminal amino acids) the precursor of papain was found in the membranes of the insect cells, whereas deletion of the signal sequence lead to a cytoplasmic localization of the protein. A series of mutants at position Gln19 of the oxyanion hole and at position Asp158 are now under investigation. Purification of the inactive Cys25Ser mutant will be used to determine the structure of the papain precursor enzyme and to elucidate the mechanism of processing.

A 250 **DISSECTING THE CATALYTIC APPARATUS OF SUBTILISIN**

James A. Wells and Paul Carter, Department of Biomolecular Chemistry, Genentech Inc, 460 Point San Bruno Boulevard, South San Francisco, Ca 94080.

Subtilisin BPN' is a serine protease and has the characteristic catalytic machinery of this class of enzymes comprising the Ser-His-Asp triad, an oxyanion binding site and possibly additional binding determinants. For subtilisin BPN' these elements together impart a total rate enhancement of at least 10^9-10^{10} fold over the non-enzymatic hydrolysis of amide bonds. The interplay between the catalytic triad residues was examined by replacing them singly or in various combinations by site-directed mutagenesis. Alanine substitutions were chosen to avoid introducing new charge interactions or hydrogen bonds from the substituted side chains and to minimize unfavourable steric contacts. Single mutations of the triad residues: Ser221, His64 and Asp32 lower k_{cat} by factors of 2×10^6, 2×10^6 and 3×10^4 fold respectively, with very minor changes of the Michaelis constant , K_M (increased up to 2-fold). The residual activity of the Ala221 single mutant ($k_{cat} / k_{uncat} = 3000$) is NOT reduced further by removing one or both of the remaining members of the triad. The side chain of Asn155 is believed to hydrogen bond to the oxyanion developed on the substrate carbonyl oxygen in the tetrahedral intermediate[2]. It has previously been shown by us[3] and by others [4] that mutating Asn155 to a variety of residues lowers k_{cat} by 10^2-10^3 fold. The mutation Asn155->Gly has been introduced into the context of various catalytic triad mutants to investigate whether the oxyanion hole has any relevance to their residual catalytic activity.

A 251 MECHANISM OF ADENYLATE KINASE. TEST OF THE NMR, X-RAY, AND MOLECULAR MECHANICS MODELS BY SITE-SPECIFIC MUTAGENESIS. Honggao Yan, Gaochao Tian, Binbing Zhou, and Ming-Daw Tsai, Department of Chemistry, The Ohio State University, Columbus, OH 43210.

Using site-specific mutagenesis, we have continued to define the adenosine 5'-triphosphate (ATP) site of adenylate kinase (AK) from chicken muscle expressed in *Escherichia coli*. The sites of mutations were chosen on the basis of the NMR model [Mildvan, A. S., and Fry, D. C. (1987) *Adv. Enzymol. 58*, 241-313], the X-ray model [Pai, E. F., Sachsenheimer, W., and Schirmer, R. H (1977) J. Mol. Biol. 114, 37-45], and the model derived from molecular mechanics calculations [Caldwell, J. W., and Kollman, P. A. (1988) *Enzyme 39*, 61-77]. The specific mutants were characterized for their kinetics, proton NMR, and conformational stability. The results suggest that the molecular mechanics model can best explain the binding of MgATP. Some residues involved in the transition state stabilization, and in substrate-induced conformational changes have also been identified. Details of these results will be presented.

Late Additions

A 252 A MAMMALIAN MITOCHONDRIAL PRECURSOR PROTEIN CAN BE ISOLATED AS STABLE SOLUBLE ENTITY, M. Martinez-Carrion, F. Altieri, J. Mattingly and A. Iriarte, School of Basic Life Sciences, University of Missouri, Kansas City, MO 64110

E. coli containing the cDNA encoding for rat liver mitochondrial precursor for aspartate aminotransferase expresses this protein. This precursor has been isolated as a stable, soluble, dimeric protein from E. coli extracts. Through protein characterization procedures, including amino acid analysis and sequencing of the N-terminal region, we find the isolated precursor contains the pertinent amino acid sequence including the presequence peptide. This protein has a higher MW than that of the mature enzyme and, unlike the in vitro expressed precursor material using rabbit reticulocyte lysates expression systems, is resistant to proteases. Instead, trypsin converts it only to a mature-like protein. The isolated precursor is also unusual in two other aspects; it contains bound coenzyme (pyridoxal phosphate) at each active site of the dimeric protein and is capable of full enzymatic catalytic activity. On the other hand, as a protein, the precursor shows different physical properties from that of the mature enzyme, whose tertiary structure is known in detail. Specifics of the properties of this precursor and of some of the events in the mechanism of processing by mitochondria will be presented.

A 253 ISOLATION AND CHARACTERIZATION OF TAQI RESTRICTION ENDONUCLEASE MUTANTS, Francis Barany, Department of Microbiology, Cornell University Medical College, New York, NY 10021.

The gene encoding TaqI restriction endonuclease (recognition sequence T↓CGA) has been subcloned downstream of an inducible phoA promoter (1), and overproduced to 30% of cellular proteins (2). E. coli cells remain viable at 37°C when endonuclease is expressed, even in the absence of (protective) methylation. Analysis of wild type TaqI endonuclease 'star' activity (cleavage at closely related sequences) revealed that for a particular star site, one strand of the DNA duplex was preferentially cleaved. Based on these preferences, a model is proposed that sequence specific discrimination is mediated by 8 hydrogen bonds formed by TaqI and specific base groups in the major groove (3). Using a strain that produces β-galactosidase upon DNA damage (4), and a temperature dependent in vivo plate assay as screens, over two dozen two-codon insertion mutants have been isolated (5). These are being characterized with respect to in vitro canonical site activity, DNA binding using gel mobility shifts, and 'star' site activity.

1. Barany, F. (1987) Gene 56: 13-27.
2. Barany, F. (1988) Gene 65: 167-177.
3. Barany, F. (1988) Gene 65: 149-165.
4. Heitman, J. & Model, P. (1987) J. Bacteriol. 169: 3243-3250.
5. Barany, F. (1988) DNA and Protein Engineering Techniques 1: 29-35.

Peptide Design, Hormones, Antibodies and Receptors

A 300 TESTING PREDICTIONS OF T CELL STIMULATING PEPTIDE SECONDARY STRUCTURE. D.C. Anderson, W.C.A. van Schooten, M. Barry and R.R.P. deVries. Dept. of Pathobiology, University of Washington, Seattle, WA 98195, and Dept. of Immunohematology and Blood Bank, University Hospital, Leiden, The Netherlands. Leprosy is a disease abetted by an M. leprae-specific defect in the cellular immune response to Mycobacterium leprae. A synthetic vaccine, containing immunodominant T cell epitopes from this bacillus for different HLA types may thus possibly help control this disease. The 65 kD protein, analogous in sequence to human and bacterial heat shock proteins, is prominent in the immune response. We have previously mapped helper T cell stimulating peptides in the sequence of this protein, and defined the minimal size of an HLA-DR2 restricted, M. leprae-specific, potent stimulatory peptide. To explore hypotheses that these peptides may act to cause T cell proliferation as amphipathic alpha helices, we have designed and tested analogs of the minimal peptide, LQAAPALDKL, containing four identical amino acids added at both ends of the sequence. Residues such as ala, lys or glu were added to enhance helicity; pro was added to destabilize a potential helix. The helical tendencies of the peptides in aqueous trifluoroethanol solutions do not correlate with the rank order of helper T cell stimulation for either fixed or unfixed T cells. Peptide analogs designed to manipulate amphipathicity, assuming a helical structure, were also tested. Although again no correlation was seen, an analog was found which half-maximally stimulated T cell proliferation in the picomolar range. Implications for vaccine design are discussed.

A 301 MANIPULATION OF PROTEIN-IMMOBILIZED METAL INTERACTIONS BY ALTERING SURFACE HISTIDINE CONTENT, Robert Todd and Frances H. Arnold, Division of Chemistry and Chemical Engineering 206-41, California Institute of Technology, Pasadena, CA 91125.

Variants of yeast iso-1 cytochrome c containing single histidine mutations have been constructed and their intreactions with immobilized Cu^{2+} cations studied. Quantitative chromatographic analysis of natural and genetically engineered cytochromes has been used to demonstrate that interaction with immobilized metals is largely determined by the number and orientation of surface histidine side chains. Single histidine mutations significantly alter affinity to immobilized metals. High-affinity metal binding sites consisting of two or more histidines can be engineered into the cytochrome surface without disrupting the biological activity. Relatively simple surface modifications such as these may lead to much-improved purifications of recombinant proteins using immobilized metal affinity separations.

A 302 BINDING DOMAINS OF HUMAN IGF-I, Marvin L. Bayne, Joy Applebaum, Dennis Underwood, Gary G. Chicchi, Barbara G. Green, Nancy S. Hayes, and Margaret A. Cascieri, Merck Sharp & Dohme Research Laboratories, Rahway, N.J. 07065

Human insulin-like growth factor I (IGF-I) is a 70 aa protein that is structurally similar and functionally related to insulin. We have characterized the binding properties of 3 structural analogs of IGF-I. These analogs are [1-62]IGF-I, an analog lacking the carboxy terminal 8 aa D region of IGF-I; [1-27,Gly_4,38-70]IGF-I, an analog in which residues 28-37 of the C region of IGF-I are replaced by a 4 aa glycine bridge; and [1-27,Gly_4,38-62]IGF-I, an analog with the C region glycine replacement and the D region deletion. The removal of the D region of IGF-I has little effect on binding to the types 1 and 2 IGF receptors. [1-62]IGF-I has 2-fold higher affinity for the insulin receptor and 4-fold higher affinity for IGF binding proteins. The replacement of the C region of IGF-I with 4 glycines results in a 30-fold loss of affinity for the type 1 IGF receptor. However, this analog has near normal affinity for the type 2 receptor, the insulin receptor and IGF binding proteins. Incorporating the C region glycine replacement and the D region deletion into 1 analog does not affect binding to either the type 2 receptor or to IGF binding proteins. As predicted from the single deletion analogs [1-27,Gly_4,38-62] IGF-1 has reduced affinity for the type 1 receptor (40-fold) and increased affinity for the insulin receptor (5-fold). These data indicate that determinants in the C region are involved in maintaining high affinity binding to the type 1 IGF receptor and that neither the C region nor the D region are required for high affinity binding to the type 2 IGF receptor or to binding proteins.

A 303 MOLECULAR MODELING AND FREE ENERGY CALCULATIONS ON ANTIBODY VARIABLE REGIONS, Michael B. Bolger, Jenn-Kang Hwang, Steve Creighton, Arieh Warshel, Departments of Biomedicinal Chemistry and Chemistry, University of Southern California, Los Angeles, CA 90033. Microscopic computer simulations have been applied to the X-ray structure of the myeloma protein MCPC603 with phosphorylcholine in the combining site. Free-energy perturbation methods, as implemented in the program ENZYMIX, were used to explore the changes in binding free energy of phosphorylcholine and its analogs. Particular emphasis has been placed on the role of electrostatic energy in the association reaction. The feasibility of using these calculations for predicting the results of antibody engineering in diagnostic monoclonal antibodies and abzymes will be discussed.

A 304 THE MOLECULAR BASIS OF THE INTERACTION OF AN ANTIBODY (IgG) WITH A HUMAN Fc RECEPTOR (FcRI), Dennis Burton, Jenny Woof, Lynda Partridge, Roy Jefferis+, Alex Duncan* and Greg Winter*, Department of Biochemistry, University of Sheffield, Sheffield S10 2TN, +Department of Immunology, University of Birmingham, Birmingham B152TJ, *MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

Having earlier confirmed the role of the C_H2 domain of IgG in the human monocyte Fc receptor I-IgG interaction, we have further assayed several IgGs from different species for their abilities to inhibit the binding of radiolabelled human IgG to FcRI. Categorisation of the IgGs into three groups, exhibiting either tight, intermediate or weak binding to FcRI, followed by amino-acid sequence comparisons between the groups has allowed postulation of a possible monocyte FcRI binding site on human IgG. This comprises residues Leu_{234} - Ser_{239} in the lower hinge region with possible involvement of the nearby folded C_H2 domain.

Recent protein engineering work has provided strong evidence for the importance of the lower hinge region in the FcRI interaction. Site-directed mutagenesis of a single amino-acid within this region has enabled us to convert a non-binding IgG to one with high affinity for FcRI. Further engineering experiments support and extend this site identification.

A 305 STRUCTURE-FUNCTION STUDIES OF INTERLEUKIN-3. **Ian Clark-Lewis.** The Biomedical Research Centre, University of British Columbia, Vancouver, Canada.

Automated solid phase peptide synthesis of mouse, rat and human interleukin-3 (IL-3) analogs has been used to investigate the relationship between its polypeptide structure and its biological functions. Three general questions have been addressed. (1) The minimal structure required for IL-3 activity has been approached by synthesis of fragments shortened at both the C-terminus and N-terminus. The results indicate that residues 109 to 118 are critical for a fully active murine molecule. (2) The question of the amino acids/regions of the molecule that are required for activity has been addressed in two ways. Firstly, a novel amino-acid substitution strategy which has identified critical regions/residues will be discussed. Secondly, interspecies human hybrid molecules have been designed with the aim of transferring the species specificity of the molecule by interchanging structural and functional domains. (3) The identification of regions of IL-3 that can be modified without affecting its activity suggests that IL-3 can be modified at defined amino-acid residues without substantial loss in activity. Several experiments indicate that this is the case and that IL-3 can be modified in biologically useful ways. Some such Analogs designed to probe the structure of the receptor will be discussed.

A 306 A MODEL FOR THE SOLUTION CONFORMATION OF HUMAN IgE, K.G. Davis, (Biochemistry Department, Sheffield University, S10 2TN, England), M.R. Walker (Clinical Chemistry Department, Birmingham Medical School, B15 2TN, England), S.E. Harding (Applied Biochemistry Department, Nottingham University, LE12 5RD, England), D.R. Burton (Biochemistry Department, Sheffield University, S10 2TN, England). In the absence of a crystallographic structure of an intact immunologically active antibody, we have tried to assess the average solution conformation of human IgE. The strategy adopted involved the collection of macroscopic biophysical data such as sedimentation coefficient and radius of gyration and then attempting to reproduce the experimental data by calculation. The calculations were performed on models constructed to include as many of the known properties of IgE as possible. In essence the model arrived at suggests that IgE does not adopt an extended conformation but is compacted in a way that is consistent with recent proposals on the location of the mast cell binding site at the $(C\ 2)_2/(C\ 3)_2$ interface.

A 307 AMINO ACID-BASE PAIR CONTACTS INVOLVED IN SEQUENCE-SPECIFIC PROTEIN-DNA INTERACTION PROBED BY SITE-DIRECTED MUTAGENESIS: CONTACTS BY ARG180 AND GLU181 OF E. COLI CATABOLITE GENE ACTIVATOR PROTEIN (CAP), R.H. Ebright,[1] X. Zhang,[1] Y. Ebright,[1] and T. Kunkel.[2] [1]Rutgers University, New Brunswick, NJ 08855, [2]NIH-NIEHS, Research Triangle Park, NC 27709.

The consensus DNA half site for CAP is 5'-AAATGTGATCT-3'. One contact between an amino acid of CAP and a base pair of the DNA site has been identified: i.e., Glu181 of CAP contacts G:C base pair 7 (Nature 311:232, 1984; PNAS 84:6083, 1987). Here, we use site-directed mutagenesis (1) to further investigate this contact, and (2) to identify a second contact.

1. Glu181: We have constructed and analyzed all 19 possible amino acid substitutions at position 181 of CAP. We find that thirteen substitutions eliminate specificity at base pair 7 of the DNA site (Ala181, Arg181, Asp181, Cys181, Gly181, Ile181, Leu181, Lys181, Met181, Pro181, Ser181, Thr181, Val181). The six other substitutions result in strong functional specificity at base pair 7 of the DNA site; three result in specificity for G:C, the base pair recognized by wild-type CAP (Gln181, Phe181, Tyr181); three result in new, different specificities (Asn181, His181, Trp181).

2. Arg180: The present model for the structure of the CAP-DNA complex (PNAS 81:3973, 1984; PNAS 81:7274, 1984) predicts that Arg180 of CAP contacts G:C base pair 5 of the DNA site. To test this prediction, we have substituted Arg180 by Gly and Ala. We find that the Gly180 and Ala180 substitutions eliminate specificity at position 5 of the DNA site. In contrast, the Gly180 and Ala180 substitutions do not eliminate specificity at other positions of the DNA site. We conclude that, as predicted by the model, Arg180 makes a direct contact with G:C base pair 5.

A 308 ABERRANT 5'-CAPS AND CAP ANALOGUES AND THEIR EFFECT ON mRNA TRANSLATION EFFICIENCY. +I. Ekiel, *E. Darzynkiewicz, *J. Stepinski, #Y. Jin, and #S.M. Tahara, +Biotechnology Research Institute, NRCC, Montreal, Que., H4P 2R2, Canada, *University of Warsaw, 02-089 Warsaw, Poland, #Dept. of Microbiol., USC Sch. of Med., Los Angeles, CA, 90033-1054.

A characteristic cap structure, $m^7G(5')ppp(5')N$, present at the 5'-end of cellular messengers and most viral templates, plays an important role in the initiation of protein translation and in splicing. Several key interactions between the 5'-cap of mRNA and cap binding proteins are required for optimum binding. As a part of a systematic examination of the structural features of the cap which are recognized by cap binding proteins, we tested for the translation inhibitory activity various 7- and 2-substituted cap analogues. Some of these analogues were incorporated into mRNA, which was then tested for the translational efficiency in reticulocyte lysates. Particularly interesting was increased activity (as compared to m^7G) of the 7-benzyl G, possibly caused by the enhanced binding to the 24K cap binding protein via interaction with Trp residue(s). Similarly, increased activity was observed for the $m^{2,7}2G$ cap, but on contrary, $m^{2,2,7}3G$ analogue was inactive. The two last caps are particularly interesting as they are produced by togaviruses. Potential for the use of the aberrant caps analogues as antiviral and anticancer drugs as well as to enhance translation will be discussed. [S.M.T was supported by Amer. Cancer Soc (MV-257) and NHI (GM38512), ED by CPBR-3.13 from Polish Acad. Sci].

A 309 MOLECULAR MECHANISM OF PARADAXIN TOXICITY: DESIGN AND SYNTHESIS OF CYTOTOXIC ANALOGS, Jay W. Fox, Susan Hefler, Daniel E. Hershey, and David C. Benjamin, Department of Microbiology, University of Virginia Medical School, Charlottesville, VA 22908.

Paradaxin is a toxic, cytolytic pore-forming peptide isolated from *Pardachirus marmoratus*. We have proposed a model for the biologically active peptide which states that the peptide in the presence of lipid bilayer forms an amphipathic helix in the amino terminal region. These monomers aggregate to form a pore with the monomers interacting in a side by side, head to tail arrangement. The carboxyl terminus is projecting outside of the bilayer. The highly charged, polar carboxyl terminus is in part responsible for the observed biological activity. The exact function of the tail is as yet unclear, however, the analogs synthesized for these studies yield new information on the structural/functional mechanism of this family of pore-forming toxins.

A 310 **X-RAY CRYSTALLOGRAPHIC STUDIES OF A FAMILY OF ANTIBODIES RAISED AGAINST A PEPTIDE.** R.E. Griest, P.D. Jefferey, G.L. Taylor and A.R. Rees, Laboratory of Molecular Biophysics, Rex Richards Building, University of Oxford, South Parks Rd., Oxford OX1 3QU, United Kingdom

Fab fragments of four monoclonal antibodies (Gloop1, 2, 4 and 5) which bind the 'loop' peptide fragment (residues 57 through 84) of hen egg white lysozyme (HEWL) and cross react with the native protein have been crystallized using polyethylene glycol as the precipitant. Native data sets of all four crystal forms have been collected and the space group and cell dimensions determined using a Xentronics area detector and the Xengen suite of programs. The structures of two distinct crystal forms of Gloop2 (space groups P1 and $P2_1$) have been solved at a resolution of 3.3 Å by the method of molecular replacement. Higher resolution data is being collected. Gloop2 and Gloop5 Fab fragments have also been crystallized in the presents of excess 'loop' peptide. These crystals are morphologically distinct from any native ones grown under identical conditions except for the absence of peptide. The crystals are fragile and difficult to work with but preliminary crystallographic data indicates that they are completely new crystal forms. Comparison of the native Fab structures with those of Fab:peptide complexes plus the known structure of HEWL would help answer some of the questions concerning antibody:antigen recognition, the role of conformational change in antigen binding and the selection and design of peptides for vaccines.

A 311 DESIGN, EXPRESSION, AND PRELIMINARY CHARACTERIZATION OF FELIX: A MODEL PROTEIN, Michael H. Hecht, David C. Richardson and Jane S. Richardson (Dept. of Biochemistry, Duke University Medical Center, Durham NC 27710) and Richard Ogden (The Agouron Institute, La Jolla CA 92037).
We have designed a sequence for FELIX (Four hELIX), a model protein that is intended to fold into a four helix bundle. The non-repetitive sequence contains 79 amino acids and shows no homology to natural proteins:

```
M  P  E  V  A  E  N  F  Q  Q  C  L  E  R  W  A  K  L
                                                  S
                                                  V
                                                  G
      K  L  I  A  E  A  A  Q  N  A  M  H  A  L  E  G
   G
   G
    N  E  A  Q  L  K  N  A  Q  A  L  M  H  E  A  M  K  T  R
                                                           K
                                                           Y
    Q  S  A  R  A  K  Y  A  C  H  A  F  E  Q  A  L  Q  E  S
```

Felix has been expressed in E. coli both by itself and as a part of a cleavable fusion protein. Purification of Felix has enabled us to initiate structural studies of the designed protein.

A 312 PEPTIDES BINDING TO MURINE AND HUMAN MHC CLASS II PROTEINS
C.M.HILL R.BUSCH J.B.ROTHBARD Laboratory of Molecular Immunology,Imperial Cancer Research Fund, London

The ability of MHC proteins to bind fragments of immunogens is a necessary requirement for immunological responsiveness. Unlike other known ligand receptor interactions which have exquisite specificity, MHC proteins appear to have evolved to allow them to bind a wide range of peptide ligands.

To determine the molecular details of the MHC-peptide interactions an assay has been developed that can detect binding of biotinylated peptides to MHC class II proteins on cell surfaces by subsequent incubation with fluorescent streptavidin and analysis by fluorescent activated cell sorting. Interestingly, identical peptides bind not only many human class II alleles, but also murine class II proteins.By locating the lysine biotinyl group at different positions in the peptide the steric availability of each residue can be determined which provides information on the conformation of the bound peptide . Using the orientation of the peptide relative to the T-cell receptor and the MHC protein as a guide, a model of the location of the peptide in the MHC binding site can be constructed based on the presence of complementary residues in the peptide and restriction element. The model currently is being tested by mutating both the restriction element and the peptide sequence.

A 313 PROTEIN ENGINEERING OF ANTIBODY COMBINING SITES: INTRODUCTION OF CATALYTIC ACTIVITY,Katherine L. Hilyard,Sally Roberts,Janet C. Cheetham, and Anthony R. Rees, Laboratory of Molecular Biophysics, South Parks Road, Oxford, OX1 3QU, England.

An anti-lysozyme mAb, Gloop2, has been used as the basis for protein engineering studies to explore the possibility of introducing catalytic activity into an antibody combining site. The naturally ocurring aspartic proteases have been surveyed and essential features of their active sites used in the design of a catalytic site in Gloop2. Tyr32 in CDR1 of the light chain was identified as a potential site for the introduction of catalytic residues. In all antibody structures there is a prevalence of tyrosine residues within the combining site, but their contribution to antigen binding is unknown.The substitutions Tyr32 to Phe, Ser and Asp have been made by site-directed mutagenesis, the mutant antibodies expressed, and their binding profiles characterised. Initial results show that the Tyr to Phe substitution has no effect on antigen binding. In contrast, the Tyr to Ser mutant showed a reduction in binding .The effect of the Asp substitution has still to be determined. The structural implications of the mutations are discussed. Two other antibodies, HyHel-5 and HyHel-10,are also being considered as possible "abzyme" templates.

A 314 STRUCTURE-FUNCTION ANALYSIS OF THE VITAMIN B12 RECEPTOR OF ESCHERICHIA COLI. Simon E. Hufton, Anthony J. P. Fletcher, Nigel A. C. Bunce, Geoffrey C. Rowland & Robert E. Glass. Biochemistry Department, Nottingham University Medical School, Clifton Boulevard, Nottingham, NG7 2UH, England. The vitamin B12 receptor in E.coli is coded for by the btuB gene and serves to translocate B12 from the external medium into the periplasmic space. This integral outer membrane protein is also utilised by certain colicins and bacteriophage BF23. This multivalent nature of BtuB makes it particularly ameneable to study.

The initial step in the generation of a fine structure function map of BtuB was to isolate forty-eight chromosomal btuB nonsense mutations. These mutations give some four hundred protein variants by means of informational suppression. The nonsense sites are being located by a combination of marker rescue, restriction enzyme analysis and immuno-blotting, the positions being confirmed by DNA sequencing. This information, in conjunction with the phenotypes of the suppressed derivatives and structural predictions, will allow the generation of a detailed structure-function map of BtuB.

A 315 **BIOLOGICAL AND MOLECULAR MODELING STUDIES COMPARING MURINE MONOCLONAL ANTIBODIES WITH THEIR ENGINEERED CHIMERIC AND HUMANIZED COUNTERPARTS,** **M. Jacqueline Johnson, Michael Sierzega, Jose Cardenas, William Butler, Department of Immunobiology, Therapeutics Division, Hybritech, Inc., San Diego, CA. 92121**

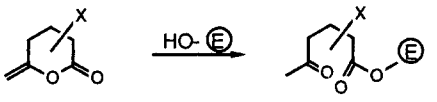
A murine monoclonal antibody (CEM231) having specificity for human carcinoembryonic antigen was engineered to create a chimeric immunoglobulin having murine VJ_L, VDJ_H sequences fused to human constant region sequences (Ck and G1 respectively). The chimeric heavy chain of CEM231 was further engineered to replace the murine framework regions with human sequences derived from the KOL antibody. All three immunoglobulins were expressed, allowing for comparison of specificity, affinity and immune reactivity between the murine, chimeric and humanized versions of the antibody. The grafting of murine CDR loops onto human framework regions was modeled using Biosym's Insight and Discover software. Data from both the modeling/energy minimization and biological studies will be presented.

A 316 SINGLE-CHAIN ANTIGEN-BINDING PROTEINS. *Johnson, S., Hardman, K., Huyhn-Pham, K., Jacobson, J., Kaufman, B., Pope, S., Riordan, G., Small, S., Whitlow, M., Wood, J., & Bird, R. Genex Corp., Gaithersburg, MD 20877.*

We have produced single-chain antigen-binding proteins which consist of the V_L and V_H of an antibody tethered together by a short polypeptide linker. The designed linker connects the carboxyl terminus of the V_L to the Amino terminus of the V_H such that the protein can be synthesized as a single polypeptide chain which folds to form the correct antigen binding site. We have designed and produced single-chain proteins from the sequences of one anti-bovine growth hormone and two anti-fluorescein monoclonal antibodies. One of the single-chain proteins to fluorescein, 4-4-20/202', has been expressed in *Escherichia coli*, refolded from insoluble protein, purified, and characterized. Quantitative assays of the binding to fluorescein show that the K_a of 4-4-20/202' for fluorescein is nearly equal to that of F_{ab} made from the 4-4-20 monoclonal antibody and that fluorescein is bound in the same manner. Single-chain antigen-binding proteins are expected to have significant advantages over monoclonal antibodies in a number of applications.

A 317 ENOL LACTONE INHIBITORS OF SERINE PROTEASES, John A. Katzenellenbogen, Du-Jong Baek, Guy W. Bemis, and Peter E. Reed, Department of Chemistry, University of Illinois, 1209 West California Street, Urbana, IL 61801

Enol lactones can inhibit serine proteases: Halo enol lactones form acyl enzymes in which a halomethyl ketone is revealed; the acyl enzyme can then partition between deacylation (turnover) and alkylation (inactivation), and in certain cases, highly efficient inactivation of chymotrypsin has been demonstrated. Certain protio enol lactones can also act as alternate substrate inhibitors, giving transient inhibition of chymotrypsin as a result of the formation of stable acyl enzymes. In some cases, deacylation rates (k_d) for enantiomers differ by two orders of magnitude. Molecular mechanics computer modeling has been used to investigate the structural determinants of this selectivity, and it appears that in the more stable acyl enzyme, the ketone carbonyl oxygen can either block access of water to the acyl serine linkage or replace this group in the oxyanion binding hole. This phenomenon is of use in designing small molecule inhibitors of serine proteases.



X	k_d (min^{-1})	
	R	S
β-Ph	0.0029	0.17
α-(1-Np)	0.0009	0.067

A 318 STRUCTURAL CONTROL OF PROTEIN DEGRADATION. PEPTIDE MODELS OF DEAMIDATION SITES IN PROTEINS. Kamlesh Patel, Cecilia Oliyai, Ronald T. Borchardt, and Mark C. Manning, Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66045.

Deamidation of amino acid side chains, particularly of asparagine, is one possible decomposition pathway which proteins may undergo. It is still unclear whether this reaction is controlled by sequence considerations (via possible side chain participation) or by secondary structure. The hexapeptide, Val-Tyr-Pro-Asn-Gly-Ala, corresponding to the deamidation site in adrenocorticotropin hormone, ACTH, has been synthesized and the kinetics of the deamidation reaction have been followed by HPLC. Marked dependence on pH, temperature, and buffer composition have been demonstrated. Reaction proceeds through a cyclic imide intermediate with formation of both normal aspartate and the β-carboxyl-linked isoaspartate products, with the isoaspartate predominating at basic pH. This peptide has been structurally characterized by ^1H NMR spectroscopy. In aqueous solution, there is significant isomerization of the Tyr-Pro amide bond (~ 30% at pH 7). Isomerization is slow enough to allow detection and characterization of both forms. Indication of β turn formation, particularly in the trans isomer, is observed. The effect of conformation on deamidation rates and products will be discussed.

A 319 MAPPING OF IgG SUBCLASS- AND T-CELL EPITOPES ON HIV-PROTEINS BY SYNTHETIC PEPTIDES. Tiit Mathiesen, Per Anders Broliden, Jonathan Rosen and Britta Wahren. Department o Virology, National Bacteriological Laboratory, 105 21 Stockholm, Departments of Neurosurgery, Virology and Immunology, Karolinska Institute, 104 01 Stockholm, Johnson & Johnson Biomedical Research, La Jolla, California,

Pentadecapeptides, sequentially overlapping by 10 a.a., were syntesized based on the HTLV-III sequences of gag and env proteins and used as antigens in IgG-subclass ELISAs and T-cel stimulation assays. Sera and cells were obtained from 30 asymptomatic, HIV-infected homosexuals. Reactivity in all subclasses could be found to parts of the gag-protein. Extensive areas of IgG1 and 3 reactivity were indentified mainly in the p17 and N-terminal half of p24 with an additional region in the N-terminal end of p15. The highest IgG1 and 3 reactivities were detected in a.a. 8-33, rich in glycine, arginine and lysine and thereby hydrophilic with a high segmental flexibility; IgG2 and 4 epitopes were found in the hydrophilic COOH-terminal of p17; the second highest IgG1 and 3 reactivities were found in the hydrophilic N-terminal of p15. For gp120, the IgG1 epitopes identified were in the putative loop-region (a.a. 296-331) and in the hydrophilic COOH-terminal end of gp120 (a.a.489-503). The immunodominant region of gp41 (a.a. 582-617) showed some IgG2, 3 and 4 responses in addition to IgG1. T-cell proliferative responses were detected against many peptides usually in areas not binding IgG. Still, simultaneously T- and B-cell reactive peptides could be detected, and the showed a distinctly preference for IgG1. The variation between different patients was considerable regarding both T-cell reactivity to peptides and the subclasses of reactive IgG. A mapping of subclass restricted epitopes allows an elucidation of anti-viral immunological mechanisms.

A 320 SITE-DIRECTED MUTAGENESIS, OVEREXPRESSION, AND CRYSTALLIZATION OF THE *E. coli* BIOTIN OPERON REPRESSOR, Junichi Matsuzaki, Sanjay Vasu, and Anthony Otsuka, Department of Genetics, University of California at Berkeley, Berkeley, CA 94720
The *bio* operon of *E. coli* is negatively regulated by a 321-amino-acid repressor protein (BirA) which also acts as the acetyl-CoA carboxylase biotin holoenzyme synthetase (BHS). To study interaction of repressor with operator DNA, Ser32, Arg33, andAla34 in the DNA-binding domein were altered by site-directed mutagenesis. Although most mutants lacked repression activity, the Cys32 mutant was found to require high levels of biotin (1 mM) while the wild type was repressed at lower levels (41 nM). A new expression plasmid, pJM1, produces BirA as 5-10% of total cellular protein. The Cys32 mutant protein was also overexpressed by a similar plasmid, pJM2. The results of site-directed mutagenesis and methylation protection experiments support our computer generated model of the protein-DNA interaction. The detailed structure of the BirA DNA-binding region may soon become known because the protein has been crystallized.

A 321 MONOCLONAL ANTIBODIES AGAINST VERO CELLS WHICH PROTECT FROM DIPHTHERIA TOXIN, John L. Middlebrook, Bengt J. Rönnberg and Burt C. Lidgerding, U. S. Army Medical Research Institute of Infectious Diseases, Frederick, MD 21701, U.S.A.
Mice were immunized with a cell line (Vero) that possesses a high number of membrane receptors for diphtheria toxin (DT). Spleen cells from these mice were fused with SP2/0-Ag14 cells and 2 cell lines (1A2 and 2D2) isolated by screening for their secreted antibody's ability to inhibit binding of radiolabeled DT to Vero cells. These antibodies protected Vero cells from the inhibition of protein synthesis mediated by diphtheria toxin. The antibodies were purified, iodinated, and their binding characteristics investigated. At 4°C, the association of 1A2 and 2D2 with Vero cells was saturable (KD ~ 10^{-8} M) and indicated about 2 x 10^6 binding sites/cell. DT did not inhibit the binding of either radiolabeled antibody. 1A2 completely inhibited ^{125}I-2D2 binding and vice-versa. Trypsin or phospholipase C treatment of Vero cells, which ablates DT binding capacity, had no effect on the ability of the monoclonal antibodies to bind to the cells. These findings suggest that 1) the two monoclonal antibodies recognize the same or closely related epitopes and 2) the antibodies bind a domain distinct from the toxin binding site or to a subcomponent of the DT receptor, which is present at many other cell surface sites. Nevertheless, these antibodies are powerful tools to study the structure, processing, and mode of action of DT receptors.

A 322 HIV BINDING REGION AND EPITOPES FOR BLOCKING MONOCLONAL ANTIBODIES OF THE CD4 MOLECULE DEFINED BY SITE-DIRECTED MUTAGENESIS, Tamio Mizukami, Thomas R. Fuerst, Edward A. Berger, and Bernard Moss, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892
The binding region for human immunodeficiency virus (HIV) and epitopes for a panel of HIV-blocking anti-CD4 monoclonal antibodies of the CD4 molecule were defined using *in vitro* site-directed mutagenesis. Codons for two amino acid (serine-arginine) residues were inserted at selected positions within the region encoding the first and second immunoglobulin-like domains of CD4. A vaccinia virus-based expression system was used to produce soluble full-length extracellular CD4 fragments containing the insertions. The mutant proteins were tested for direct binding to soluble gp120 (the CD4-binding subunit of the viral envelope glycoprotein) and to a series of HIV-blocking anti-CD4 monoclonal antibodies. Impaired gp120-binding activity resulted from insertions after amino-acid residues 31, 44, 48, 52, 55, and 57 in the first immunoglobulin-like domain. The epitopes for two HIV-blocking monoclonal antibodies, OKT4A and OKT4D, were also mapped in the gp120-binding region in the first domain. Insertions after amino-acid residues 21 and 91 in the first domain had no effect on gp120 binding, but impaired the binding of OKT4E, suggesting that this antibody recognizes a discontinuous epitope not directly involved in gp120 binding. Moderate impairment of gp120 binding resulted from the insertion after amino-acid residue 164 in the second immunoglobulin-like domain, where the epitopes for monoclonal antibodies MT151 and OKT4B were also mapped.

A 323 **SOLUTION STRUCTURES OF MURINE EPIDERMAL AND HUMAN TYPE-α TRANSFORMING GROWTH FACTORS DETERMINED BY NMR AND DISTANCE GEOMETRY**, G.T. Montelione[1], M.E. Winkler[2], A.W. Burgess[3], K. Wüthrich[4], H.A. Scheraga[5], & G. Wagner[1], [1]Institute of Science and Technology, Biophysics Research Division, Univ. of Michigan, Ann Arbor, MI 48109; [2]Dept. of Medicinal and Biomolecular Chemistry, Genentech, Inc., S. San Francisco, CA 94080; [3]Ludwig Institute, Melbourne Tumour Biology Branch, Victoria 3050, Australia; [4]ETH-Hönggerberg, CH-8093 Zürich, Switzerland; [5]Baker Laboratory of Chemistry, Cornell Univ., Ithaca, NY 14850.

Three dimensional structures for natural murine epidermal (mEGF) and recombinant human type-α transforming (hTGFα) growth factors in aqueous solution have been determined by ^{1}H-NMR spectroscopy. Both mEGF and hTGFα have two small β-sheets, which are structurally homologous. For mEGF, the ^{1}H-NMR data were used as input for DISMAN distance geometry calculations to determine the polypeptide chain fold. This solution structure was subsequently refined by incorperating many more NOE-derived distance constraints (particularly between the two β-sheet domains) and spin-spin vicinal coupling constants. These additional data determine more precisely the relative orientations of the two domains and the side-chain conformations. Distance geometry calculations have also been used to determine the polypeptide chain fold of hTGFα. Despite their overall three-dimensional structural homology, amide proton exchange studies reveal differences in the structural dynamics of mEGF and hTGFα at pH 3.5 and 28° C. Implications of these results for design of growth factor analogs will also be described.

A 324 SPECTROSCOPIC CHARACTERIZATION OF THE INTERACTION OF KRINGLE 2 OF t-PA WITH ω-AMINO CARBOXYLIC ACIDS. Michael G. Mulkerrin, and Robert F. Kelley, Department of Biomolecular Chemistry, Genentech, Inc., 460 Point San Bruno Blvd., So. San Francisco, CA 94080. The kringle-2 domain of t-PA is shown to bind ω-amino carboxylic acids (Cleary, S., Mulkerrin, M. G., Kelley, R. F., Biochemistry submitted). Absorption spectra of this kringle show the tryptophan residues are in a very hydrophobic environment. These spectra show there is a possibility for hydrogen bonding between either a carboxylic acid or a histidine and one or more of the three trytophan residues in the molecule. Upon binding ligand the hydrogen bonding interaction is not significantly perturbed. However, upon binding ligand, there is a decrease in the fluorescence quantum yield. This decrease is employed to titrate the binding of ligands to the kringle. Stern-Volmer experiments show that the tryptophans are about 20% accessible to quencher in the absence of ligand but in the presence of an ω-amino carboxylic acid the tryptophans are completely shielded from solvent, consistent with the modeled structure of kringle-4 of plasminogen by Tulinsky et al.(Proteins: Structure Function and Genetics, 3, 85-96, 1988).

A 325 CLONING OF cDNA CORRESPONDING TO HEAVY AND LIGHT CHAIN IMMUNOGLOBULIN VARIABLE DOMAINS. R. Orlandi*, P.T. Jones°, D. Güssow°, G. Winter°. *Experimental Oncology E., Istituto Nazionale Tumori, Milan, Italy; ° MRC Laboratory of Molecular Biology, Cambridge, U.K.

Numerous murine monoclonal antibodies (MAbs) with restricted reactivity against human tumors have been used for therapy, but the development of an anti-antibody (HAMA) immune response has frequently been observed in patients. To circumvent this problem, recombinant DNA technology has been applied to 'humanise' murine MAbs. The cloning of the immunoglobulin genes was one of the most critical steps in the production of recombinant antibodies. In the attempt to obtain a general methodology, a new strategy in the cDNA production and cloning of light and heavy variable domains has been developed. As a prototype, the murine MAb MBr1 (IgMk) directed against human carcinomas was used. The cDNA corresponding to the variable domains was prepared using synthetic primers in which suitable restriction sites were introduced to facilitate the cloning into either a sequencing vector or an expression vector. The applied strategy also proved to be useful for the cloning of other MAbs.

Partially supported by grants from the Associazione Italiana per la Ricerca sul Cancro.

A 326 **ALTERING THE SPECIFICITY OF THE LEUCINE BINDING PROTEINS OF *E. COLI*.**
Dale. L. Oxender, David J. Maguire, and Mark D. Adams, Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109-0606.

High affinity branched-chain amino acid transport in *E. coli* involves two periplasmic binding proteins and three membrane components. The branched-chain amino acids diffuse through the outer membrane of *E. coli* and form a complex with the soluble binding proteins. These complexes interact with the inner membrane components to produce the transport of the ligand. Two periplasmic binding proteins have been isolated, purified and crystallized. The three dimensional structures have been determined to high resolution by Quiocho and coworkers (personal communication). The genes for these proteins livJ and livK have been cloned and sequenced. They encode the leucine-isoleucine-valine (LIV) and leucine-specific (LS) binding proteins, respectively. The amino acid sequences of LIV-BP and LS-BP are approximately 75% homologous, suggesting they have derived from a gene duplication event. The two binding proteins show different specificities. LIV-BP shows a high affinity for all three branched-chain amino acids while LS-BP is specific for leucine. The x-ray structural analysis by Quiocho suggests that six amino acid residues line the leucine binding pocket of LIV-BP. Three of these residues — leu_{77}, cys_{78}, and ala_{101} are conserved in the two proteins. The three amino residues that are not conserved are tyr_{18}->trp, ala_{100}->gly, and phe_{276}->trp. We have prepared hybrid proteins by gene splicing techniques and specific mutants using site-directed mutagenesis in an attempt to understand the molecular basis of the difference in specificities of the LIV- and LS- binding proteins. Supported by NIH grant GM 11024.

A 327 The Characterization of Single Chain Antibodies Synthesized in *Bacillus Subtilis*. *Pantoliano, M., Alexander, P., Dodd, S., Bryan, P., Rollence, M., Wood, J., & Fahnestock, S. Genex Corp., Gaithersburg, MD 20877.*
An anti-fluorescein antibody ("Fluorescein Hapten: An Immunological Probe" ed. Voss, E.W., 1984, CRC Press) was chosen as a model to explore the feasibility of engineering antibodies for enhanced therapeutic and industrial applications. A single chain antigen-binding protein was designed by connecting the carboxyl terminus of the V_L domain to the amino terminus of the V_H domain with a short 15 amino acid linker. This single chain antibody was expressed in *B. subtilis* using alpha-amylase, neutral protease, and alkaline protease promoter and signal sequences derived from *B. amyloliquefaciens*. Binding assays demonstrated that the protein, as synthesized, was able to bind fluorescein with high affinity. The K_a and mechanism of binding of fluorescein to the redesigned antibody was found to be similar to that for the comparable F_{ab}.

A 328 **CONFORMATION OF PEPTIDES CONTAINING DEHYDRO-Phe RESIDUES AT (i+1)TH POSITIONS.** H.C. Patel, P. Narula, T.P. Singh and V.S. Chauhan, Department of Biophysics, All India Institute of Medical Sciences, New Delhi-110029, India. We have been designing the peptide structures by introducing dehydro-Phe at various positions in the amino acid sequences. We found that the single dehydro-Phe residue has values of ϕ and ψ either in the vicinity of -60° and 120° respectively or these values are close to 80° and 0° respectively. The values of ϕ and ψ i.e. ϕ_1 = -60°, ψ_1 = 120°, ϕ_2 = 80° and ψ_2 = 0° correspond to β -turn II conformation. It suggests that the dehydro-Phe tends to promote β-turn conformation. We further examined by placing dehydro-Phe at (i+1)th position. We repeatedly found that the values of ϕ and ψ fall in the vicinity of -60° and 120° respectively. Then we synthesized and determined the structures of peptides with the dehydro-Phe at (i+2)th position. In this case, we obtained the ϕ and ψ values close to 80° and 0° respectively. It, therefore, suggests that the substitution of dehydro-Phe residue in a peptide sequence results in the formation of β-turn II conformation. Thus, it provides an exact tool to design β-turn II structures of peptides by using α,β-dehydro-Phe-residue.

A 329 BACTERIAL EXPRESSION AND CHARACTERIZATION OF ANTIGEN BINDING FRAGMENTS OF THE ANTIBODY McPC603, A. Plückthun, J. Stadlmüller, A. Skerra, R. Glockshuber, and B. Steipe, Max-Planck-Inst. f. Biochemie, Genzentrum, D-8033 Martinsried, FRG.

The phosphorylcholine binding antibody McPC603 is particularly well studied and the crystal structure of its Fab fragment with hapten bound is known. Thus, the Fv and Fab fragment of this antibody are convenient model systems for quantitatively investigating binding interactions and transition state stabilization (i.e. catalysis) by systematic modification of the antigen binding site and the hapten. We have developed an expression system with which fully functional Fv or Fab fragments can be expressed in *E. coli*. Both chains are co-expressed and co-secreted into the periplasm of E. coli, with correct signal-processing, disulfide formation, and chain association. The Fv and Fab fragment can be purified to homogeneity in a single step by hapten affinity chromatography. The binding constant of the hapten to the Fv fragment was found to be identical to that of the whole antibody. Also, the variable domains were expressed as fusion proteins with ß-galactosidase, precisely cleaved with the protease factor Xa, and refolded *in vitro* to give a functional Fv fragment. The association constant between the VH and VL domains was determined by crosslinking and fluorescence experiments. Furthermore, we could show that VL dimerizes with itself with an association constant similar to that of the heterodimer, but VH does not. The binding of the hapten stabilizes the Fv fragment considerably. We also showed that the recombinant Fv fragment of McPC603 possesses catalytic activity toward the hydrolysis of suitable carboxylic acid derivatives. This constitutes the first catalytic antibody for which a three-dimensional structure and a convenient expression and mutagenesis system is available.

A 330 THE DE NOVO DESIGN OF HELICAL PROTEINS, Lynne J. Regan and William F. DeGrado, C.R.&D., The Experimental Station, E. I. du Pont de Nemours & Co., Wilmington, DE 19898

The question of how the primary amino acid sequence of a protein determines its 3-dimensional structure is still unanswered. Our approach to this problem involves the *de novo* design of model peptides and proteins that should adopt desired 3-dimensional structures. Characterization of these novel proteins (produced by solid-phase peptide synthesis or by expression in *E. coli*) allows us to assess our design successes and failures.

Our model proteins are idealized versions of the naturally occuring 4-helix-bundle motif found in proteins such as myohaemerythrin. A step-wise approach allowed peptides corresponding to the helices to be optimised. Connecting loops were added subsequently and finally the full-length model protein was designed. Structural features of the model protein were probed by circular dichroism, guanidine hydrochloride denaturation and the introduction of disulfide bridges to link segments of the protein modelled to be appropriately positioned. Further NMR and x-ray crystallographic analyses are in progress.

The model 4-helix bundle protein is extremely stable, with a free energy of folding of approximately -20 kCal mol^{-1}. Thus a stable framework is provided onto which we are adding functional groups. The first designs are for ligand binding sites, specifically for metals. Subsequently we hope to add to these designs to include catalytic activity.

A 331 DNA BINDING STUDIES OF ACRIDINE AND ANTHRACYCLINE DRUGS CONJUGATED TO PEPTIDES AND PSEUDOPEPTIDES, Robert T.C. Brownlee, Anastasis Eliadis, Robert J. Hook, Don R. Phillips and James A. Reiss, Departments of Biochemistry and Chemistry, La Trobe University, Bundoora, Victoria 3083, Australia.

Synthetic drug conjugates derived from adriamycin and acridine have been prepared by condensing short polyamide or polymethylene linkers with 14-bromodaunomycin by means of an ester group. The precursor linkers and acridine derivatives were characterised by H-1 and C-13 NMR spectra, mass spectra and elemental analysis. The derivatives have been purified by HPLC and characterised by FAB mass spectrometry. The biophysical analysis of these new compounds together with a previously reported series of acridines conjugated to the netropsin/distamycin class of peptide analogues are reported. Viscometric analysis of solutions of calf-thymus DNA in the presence of drugs, dissociation rates of the drug/DNA complexes using a SDS drug sequestration technique, and DNA-footprinting and DNA transcription experiments were carried out. Footprinting studies of the acridine linked netropsin and distamycin ligands were determined on a 180-mer restriction fragment by inhibition of DNase I, and transcription inhibition experiments were carried out on an *E. coli* RNA polymerase system using a 497 base-pair DNA template. In general, bifunctional binding of these conjugates to DNA were observed with enhanced binding (100 to 1,000-fold) and increased base-pair specificity associated with recognition by the intercalating chromophore. In particular, the acridine-linked netropsin/distamycin conjugates behaved as true bifunctional mixed ligands for DNA showing enhanced preference for A-T rich sites in which co-operativity between the peptide residue and the intercalator occurred in the recognition of DNA. The acridine-anthracycline conjugates show promise as bifunctional ligands for DNA, and the presence of the peptide-like linkage should provide for increased specificity of recognition.

A 332 PROBING THE INTERACTIONS BETWEEN AN ANTIBODY AND ITS PROTEIN DETERMINANT BY SITE-DIRECTED MUTAGENESIS(SDM), Sally Roberts, Janet C. Cheetham, Phil D. Jeffrey and Tony R. Rees, Laboratory of Molecular Biophysics, South Parks Road, Oxford, OX1 3QU, England.

The interactions between the mAb Gloop2 and the 'loop' determinant of hen-egg lysozyme (HEL) are under investigation using SDM of the cloned heavy and light chain cDNAs. A computer-derived model of the Gloop2 ACS docked to the epitope on HEL was used to identify residues important for Ag binding. More recently the structure of the Fab of Gloop2 has been determined and has aided the design of mutant Abs. Mutagenesis of charged residues which lie at the periphery of the ACS did not destroy Ag binding, and in some cases affinity was improved. In contrast, mutation of glutamate, identified as possibly important in maintaining the structure of the ACS, results in the abolition of binding. The role of other charged residues are under investigation.Mutations in CDR3 of the light chain result in reduced Ag affinity. This CDR forms the central region of the ACS where there is good complementarity between the two proteins and thus interactions in this region may be critical for complex formation. Attempts to improve affinity further by multiple mutations will be discussed.

A 333 **HUMAN I_gG_3: STRUCTURE-FUNCTION ANALYSIS OF HINGE REGION MUTANTS.** **Inger Sandlie and Terje Michaelsen. Dept. of Biology, University of Oslo and Dept. of Immunol. National Inst. Public Health, OSLO, NORWAY.**

Four subclasses of human I_gG exist. They have 95% homology in their aminoacid sequence except in the middle part of the heavy chains, the hinge. It has been suggested that the disparity in the hinge sequences may be partly responsible for the observed differences in effector functions seen amongst the I_gG subclasses. The hinge region of I_gG_3 is four times as large as that of the other subclasses, and is made of four distinct exons separated by short introns.

To investigate the biological significance of this extended hinge, we have made hinge region deletion mutants having none, one, two and three hinge exons and compared the structure and effector funtions of the mutants. All the mutants having at least one hinge exon reacted with antibodies against the hinge region. Thus, the basic antigenic structure is preserved in the shortened mutants. The difference in effector functions will be presented.

A 334 THE SUBSTITUTION OF A HYDROGEN BOND WITH A COVALENT MIMIC IN AN ALPHA HELICAL PEPTIDE, Satterthwait, A., Arrhenius, T., Lerner, R.A., Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037. A major goal of peptide chemistry is to develop methods for the synthesis of three-dimensional mimics of protein surfaces. Our approach is to replace hydrogen bonds with covalent mimics. To the extent that hydrogen bonding patterns define protein structure this might serve as a general method for shaping inactive, disordered peptides into biologically active conformers. A hydrazone-ethane link (N-N=CH-CH_2-CH_2-) has now been substituted for the (i + 4) → i amide-amide hydrogen bond (N-H···O=C(R)-NH) in a series of pentapeptides with alpha-helical potential. The modified cyclic peptides have been extended from the carboxyl terminus with short peptides. Nuclear magnetic resonance experiments indicate that the cyclic pentapeptide serves as a nucleation site for the extended peptide folding it into an alpha helical structure.

A 335 MODIFICATION OF ANTIBODY BINDING SITE THROUGH ALTERED V-V DOMAIN INTERACTIONS,* M. Schiffer, C.-H. Chang, Z.-B. Xu, and F. J. Stevens, Biological, Environmental, and Medical Research Division, Argonne National Laboratory, Argonne, IL 60439-4833

Quaternary interactions expressed by the immunoglobulin light-chain dimer Loc depend on the solvent of crystallization. Previously, we had determined the structure of light-chain dimer Loc in a crystal form obtained from ammonium sulfate (Chang et al., 1985, Biochemistry 24:4890). The two variable domains form a very unusual antigen binding site consisting of a protrusion with a groove on each side.

We have now determined the crystal structure of Loc crystallized from distilled water. At present, the R = 22% for 2.8 Å data. In this structure, the association of the two variable domains forms a cavity that resembles the "conventional" antigen binding site observed in the Mcg protein and in Fabs.

Though the association constant of the Loc VL domains is > 10^6 M^{-1} (the same order of magnitude of the VL-VH interaction), this protein can have at least two different quaternary structures. The observation with Loc suggests that V-V interactions are less restricted than was previously apparent. Therefore, it can be anticipated that the antigen binding site conformation in an Fab can be changed by altering the residues in the interface that affect the association properties of the domains comprising an Fab.

*This work supported by the U.S. Department of Energy, Office of Health and Environmental Research under contract No. W-31-109-ENG-38.

A 336 **DESIGNING OF PEPTIDE STRUCTURES USING α, β-DEHYDRO-AMINO ACIDS.** T.P.Singh, P. Narula, H.C. Patel and V.S. Chauhan, Department of Biophysics, All India Institute of Medical Sciences, New Delhi-110029, India. α,β -dehydro-amino-acids have been found to occur naturally in peptides of microbial origin and in some proteins. We have used α,β -dehydro-residues to design a large number of specific structures of peptides. We have synthesized these peptides and determined their conformations using NMR and X-ray diffraction. We have observed that the β -turn type II conformation is invariably found in these peptides whether the dehydro-residue is placed at (i+1)th position or (i+2)th position. If the sequence contains alternate dehydro-residues or if the dehydro-residue in a sequence follows two saturated residues, it results in the helical structures. If all the residues in the sequence are dehydro-residues, the structure resembles with the extended β-chain conformation. We have also synthesized specific structures of inhibitors of Proteinase K and Trypsin with the sequences: H3N-Ala-Ala-Gly-dehydro-Phe-Lys-OCH3 and N-Ac-Ala-Ala-Lys-dehydro-Ile-Tyr-OCH3 respectively. We have observed 100% inhibitions of Prot K and Trypsin with these peptides. Both the structures have β-turns at the dehydro-residues. We have found that the judicious placements of dehydro-residues in peptide sequences can give rise to highly predictable secondary strucutres.

A 337 SYNTHESIS & REFOLDING OF THE FIRST REPEAT IN THE LIGAND-BINDING REGION OF HUMAN LOW DENSITY LIPOPROTEIN RECEPTOR, Stephen R. Sprang, H. Thomas Steely, Jr. and Clive Slaughter, Howard Hughes Medical Institute, The University of Texas Southwestern Medical Center, Dallas, Texas 75235. We have used automated solid-phase synthesis methods to generate the first of seven heterologous repeats near the amino terminus of human LDL receptor (Sudhof *et al.* Science **228** 815, [1985]). The seven repeated sequences are each composed of approximately forty amino acids and have six conserved cysteine residues which form three internal disulfide bridges. The sequence of the first repeat is VGDRCERNEFQCQDGKCISYKWVCDGSAECQDGSDESQETC. Treating the two peptides with oxidized glutathione and the disulfide isomerase thioredoxin yields the same result: generation of folded, monomeric and dimeric molecules which bind to a monoclonal antibody directed specifically against this portion of the LDL receptor [Russell *et al.* (1984) *Cell* **37**, 577-585]. At least 35% of the monomeric material which recognizes the antibody is stable (*i.e.* does not chain to multimers *via* disulfide bonds). Furthermore, these stable monomers can repeatedly bind to immobilized MAb columns. Based on its homology, with respect to the spacing of cysteine residues, with constituent domains of wheat germ agglutinin (J. Finer-Moore, personal commun.), we have constructed a model for the three dimensional structure of the peptide.

A 338 STRUCTURE AND ACTIVITY OF MUTANT α_2-PLASMIN INHIBITOR,

Sumi Y.(1)Ichikawa Y.(1)Nakamura Y.(2)Miura O.(2) and Aoki N.(2)
Biotechnology Research Laboratory, Tokyo Research Center, Teijin Limited(1) and the 1st Department of Medicine, Tokyo Medical/Dental University (2), Tokyo, Japan.

α_2-Plasmin Inhibitor (α_2PI), one of the serine protease inhibitors in plasma, was expressed in baby hamster kidney (BHK) cell line. The expression vector was constructed with its genomic DNA and cDNA, and was transfected into BHK cell by the calcium phosphate method. The recombinant α_2PI, 70k dalton on SDS-PAGE, was purified by using monoclonal antibody affinity column. The structure was analyzed, and 12 amino acids of the leader peptide were found at the amino terminus of recombinant α_2PI. This finding suggests that α_2PI has pre-pro type processing and that the pro-peptide is not removed in BHK cell. This mutant α_2PI shows the same plasmin inhibitory activity and the same affinity for plasmin as those of normal α_2PI. However, the ability of closs-linking to fibrin is reduced to less than 25%. The closs-linking site is the glutamine located in the second position from the amino terminus of normal α_2PI. The conformational change of this region caused by the addition of the pro-peptide may have affected the closs-linking capacity of the inhibitor.

A 339 CONFORMATION INDUCTION AT INTERFACES IN RELATION TO THE DESIGN OF BIOACTIVE PEPTIDES,

John W. Taylor, Laboratory of Bioorganic Chemistry and Biochemistry, The Rockefeller University, New York, NY 10021

Peptides which are designed to have the appropriate periodicity of hydrophobic amino-acid residues in their linear sequence for formation of either amphiphilic α helices or amphiphilic β strands form stable monolayers at the air-water interface which have very distinctive compression isotherms. When these peptide monolayers are transferred to siliconized quartz glass slides, their CD spectra may be measured and are indicative of helical structure and β-sheet structure, respectively, in the plane of the interface. Furthermore, similar spectra corresponding to the amphiphilic helix or sheet conformations are obtained when these model peptides are adsorbed from aqueous solutions onto planar phospholipid monolayers coating the siliconized glass slides. Peptide hormones which have the potential to form amphiphilic α helices, including β-endorphin, calcitonin and neuropeptide Y, appear to do so both at the air-water interface and on the phospholipid surfaces. The study of peptide models of β-endorphin indicates that the formation of this structure at interfaces correlates with the opioid activities of that hormone. Compression isotherms are, however, shown to be unreliable as an indicator of helix formation at the air-water interface, although they do appear to be a reliable indicator of β-sheet structure. In addition, an example where ionic interactions dominate over a potential amphiphilic conformation in determining the type of secondary structure formed at these interfaces is also described.

A 340 STUDY OF THE PUTATIVE HAPTEN BINDING SITE OF OX1-IgG BY COMPUTER AIDED MOLECULAR MODELLING

Tuula T. Teeri, Liisa Holm, Leif Laaksonen, Matti Kaartinen* and Jonathan K.C. Knowles
Technical Research Centre of Finland (VTT), Biotechnical Laboratory, Tietotie 2, SF-02150 Espoo, Finland.
*Dept. of Serobacteriology, University of Helsinki, Haartmaninkatu 3B, SF-00290 Helsinki, Finland.

A number of natural variants of the anti-2-phenyloxazolone IgG (OX1-IgG) have been sequenced and the dissociation constants for hapten binding have been determined previously[1,2]. A structural model of the OX1-Fab' fragment was constructed by computer-aided model building with FRODO using as templates homologous structures from the Brookhaven Protein Data Bank. A possible binding site for the hapten was initially localized by comparison with phosphocholine binding to MCP 603. The hapten was docked to the putative binding site interactively by HYDRA and AMPAC was used to calculate the charges of the hapten. CHARMM was used both for energy minimization to remove any close contacts and for molecular dynamics simulations to scan molecular conformations. The known properties of the antibody variants are being used to test and evaluate the model. REFERENCES. 1. Kaartinen, M., Griffiths, G.M., Markham, A.F. and Milstein, C. (1983) Nature 304: 320-324. 2. Griffiths, G.M., Berek, C., Kaartinen, M. and Milstein, C. (1984) Nature 312: 271-275.

A 341 DESIGN OF IONOPHORE-CONTAINING PEPTIDES. Normand Voyer and William F. DeGrado, Central Research and Development Dept.; E.I. du Pont de Nemours and Co.; Wimington, DE 19898-B328. Recently, crown ether-bearing amino acids based on 3,4-dihydroxyphenylalanine (Dopa-crowns) have been synthesized (Biopolymers, 25, 189 (1986)). These ionophores are capable of forming 2:1 complexes with certain cations in which a single metal is sandwiched between the crown ether rings. Thus, two sites that are distant in sequence may be bridged in a ligand-responsive manner. Several peptides will be described that have been designed to test the possibility of using this system to introduce ligand-gated conformational changes into peptides.

A 342 CONSTRUCTION AND EXPRESSION OF A CDR-GRAFTED ANTI-TNF ANTIBODY. Nigel Whittle, Beth McIntosh, John Adair, Mark Bodmer. Dept. of Molecular Immunology, Celltech plc, 216, Bath Road, Slough, UK.

Gram negative septicaemia has a high mortality rate due to the induction of endotoxic shock, following over-stimulation of the host response by bacterial LPS. Many of the effects observed in shock are believed to be mediated by the cytokine TNF, released by activated macrophages. It is likely that antagonists capable of blocking TNF activity will have potential for treating patients in shock. 61E71 is a mouse mab raised against recombinant hTNF, which is capable of blocking the activity of TNF *in vitro*, and neutralising the endotoxic effects of TNF *in vivo*. The clinical use of this antibody will be limited by the possible generation of an immune response by the recipient against mouse-specific epitopes. Accordingly, a humanised version of the mouse mab was constructed by 'grafting' antigen-binding loops (CDR's) from the mouse mab into a human framework structure. These loops were tentatively identified by computer-modelling and by comparison with the Kabat hypervariable sequences. A series of genes for each of the antibody chains was constructed with slightly varying lengths of mouse sequence at each of the CDRs in order to define precisely the regions involved in antigen-binding. These genes were analysed by expression in COS cells together with the corresponding chimaeric chain gene. The secreted antibodies were screened for their assembly and antigen-binding ability by ELISA. Antibodies which were shown to bind antigen were then investigated for their ability to inhibit cell-killing in an L929 cytotoxicity assay. A fully CDR-grafted mab was constructed by combining optimally grafted chains. Permanent cell lines expressing the humanised antibody have been established, and animal model studies are underway.

A 343 MAGAININ PEPTIDE ANALOGS SHOW STRUCTURE–ACTIVITY CORRELATIONS Robert W. Williams*, David Covel§, and Hao-Chia Chen‡, *Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda MD, 20814. §Department of Mathematical Biology, and ‡Endocrinology and Reproduction Branch, National Institute of Child Health and Human Developement, National Institutes of Health, Bethesda, MD 20892. Magainins are 23-residue peptides isolated from the skin of the african clawed frog. They protect the frog from infection under adverse conditions and exhibit a broad-spectrum antimicrobial activity in vitro [Zasloff (1987) PNAS 84, 5449]. This activity appears to involve the interaction of magainin with microbial membranes. Synthetic analogs of the magainins show antimicrobial activities that are correlated with the secondary structures of the peptides in solution and bound to membranes. The activity of some synthetic analogs are higher than those of native magainins, but some of these are also hemolytic. Hemolytic activity also appears to be correlated with structure. Raman measurements show that magainin 2 amide is 95% unfolded in physiological saline, 71% helix upon forming an electrostatic interaction with the negatively charged surface of DPPG vesicles, and only 50% helix when it interacts with DPPC vesicles and SDS. Magainin 2 amide has about twice the antimicrobial activity of magainin 2, but is not hemolytic. When glycines 13 and 18, and serine 8 are replaced with L-alanine, the structure is 78% helix in saline, 70% helix bound to DPPG, and 72% helix bound to DPPC. This analog, magainin B, is much more active than magainin 2, but is also hemolytic. When the same substitutions are made with D-alanine, the structure is 73% unfolded in saline, and 36% helix bound to DPPG. This analog, magainin H, is neither antimicrobial or hemolytic.

A 344 BIOLOGICALLY ACTIVE PEPTIDE ANALOGS OF VASOACTIVE INTESTINAL POLYPEPTIDE, T. Ryskamp, G. Musso, S. Patthi, S. Provow and G. Velicelebi, SIBIA, P.O. Box 85200, San Diego, CA 92138

In earlier work, we introduced a group of VIP analogs, which were designed based on the hypothesis that the region from residue 6 to 28 of VIP adopts an amphipathic π or twisted α helical structure when bound to its receptor. In particular, one peptide, Model 5, in which the hydrophobic surface was identical to that of VIP but the hydrophilic surface was idealized, behaved as a full VIP agonist. In this work, we describe the design, synthesis, and characterization of three additional VIP analogs based on the structure of Model 5. The principal focus in the design of these peptides was the hydrophobic surface of the peptide, with particular emphasis on the role of Arg14 in maintenance of function. The peptides were tested for binding to rat lung VIP receptors as well as for stimulation of secretion of α-amylase in guinea pig pancreatic acini. The results indicated that all the peptides bound to lung receptors with affinity equal to that of mammalian VIP. However, in the amylase assay, the analog whose hydrophobic surface differed most significantly from that of VIP, displayed one-tenth the biological potency of VIP, suggesting some degree of tissue selectivity.

Late Additions

A 400 CHARACTERIZATION OF DNA- AND PROTEIN-BINDING DOMAINS OF THE PRODUCTS OF THE *fos* AND *jun* PROTO-ONCOGENES. Cory Abate, Reiner Gentz and Tom Curran, Department of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, NJ 07110

The proto-oncogenes *fos* and *jun* encode nuclear proteins (Fos and Jun respectively) that have been implicated in transcriptional regulation. We have demonstrated that Fos and Jun form a protein complex that interacts specifically with the regulatory element known as the AP-1 binding site. Although Jun can bind to DNA in the absence of Fos, Fos increases the affinity of binding by stabilizing the DNA-protein complex. To investigate the exact nature of the protein-protein and protein-DNA interactions, we have over-expressed certain domains of these proteins in *E. coli* and the recombinant proteins have been purified to apparent homogeneity. Additionally, site-directed mutagenesis studies have been carried out using c-*fos* and c-*jun* cDNA clones. Taken together the data indicate that two separate domains are involved in protein-protein and protein-DNA associations. The regions of Fos and Jun containing heptad repeats of leucine residues (the "leucine zipper") are responsible for protein complex formation.

A 401 DESIGN OF A MARKER PEPTIDE SEGMENT FOR RECOMBINANT PROTEIN IDENTIFICATION AND PURIFICATION, Thomas P. Hopp, and Kathryn S. Prickett, Departments of Protein Chemistry and Transgenics, Immunex Corp., 51 University Street, Seattle WA 98101

A short peptide segment, AspTyrLysAspAspAspAspLys, has been developed for use as a marker segment attached to recombinant proteins for their detection and purification. This segment was designed to incorporate a number of desirable properties: it possesses an antibody binding site and a cleavage site for removal by enterokinase digestion, it can be encoded in a short, synthetic oligonucleotide, and its overall strong hydrophilicity was intended to lead to a high degree of solvent exposure. That this latter quality was achieved, is supported by our observations that a number of recombinant proteins can be made in soluble, active form with the marker sequence attached, that they can be immunoaffinity purified using a monoclonal antibody, and that enterokinase efficiently removes the marker sequence without detectable degradation of the desired product. Binding to the antibody is calcium-dependent, so that a one step purification of products to homogeneity can be accomplished under mild conditions by eluting with EDTA. This process represents a successful practical application of *de novo* polypeptide design.

A 402 STRUCTURAL INVESTIGATIONS OF CYCLIC GMP PHOSPHODIESTERASE FROM CATTLE RETINA. N. V. Khramtsov, I. A. Vasilevskaya, N. V. Atabekova, K. G. Muradov, T. M . Shuvaeva, V. V. Gubanov and V. M. Lipkin. Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, ul. Miklukho-Maklaya, 16/10 117871 GSP Moscow V-437, USSR.
Cyclic GMP phosphodiesterase (PDE) participates in transduction and amplification of the visual signal. PDE from bovine retina consists of three subunits: α, β and γ. The enzyme catalytical subunits are α and β, the γ-subunit being internal inhibitor of the protein enzymatic activity. We utilised both the protein and nucleotide techniques to study subunits primary structure. The complete primary structure of the enzyme was established. Close homology between the segments of peptide chains of α- and β-subunits adjoining the C-terminal and analogous segments of other phosphodiesterases being revealed. So the catalytical center in α- and β-subunits is presumed to be located in the C-terminus of the molecule, while regulatory regions interacting with the γ-subunit and transducin are likely to be in the N-terminal part.

A 403 A MICROBIOLOGICAL ASSAY FOR THE DETECTION OF LAC PERMEASE MUTATIONS AFFECTING SUBSTRATE BINDING AND TRANSPORT, Jonathan A. Lee, Irene B. Puttner, and H. Ronald Kaback, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110
The lac permease of Escherichia coli is a transmembrane protein which couples the movement of H+ down its electrochemical gradient to the transport of galactosides against their concentration gradient. Recent studies utilizing site-directed mutagenesis has implicated three residues, Arg302, His322, and Glu325, as possible components of a proton relay system which is postulated to function as a chemical pathway for H+ translocation through lac permease (Menick et al 1987 Biochemistry 26, 6638). As an alternative to site-directed mutagenesis, classical mutagenesis techniques can be utilized to generate random mutations in lac permease providing that appropriate screening assays are available to differentiate between wild-type and mutant permease molecules. Towards this goal a simple plate screening assay was developed to detect bacterial colonies expressing permease forms that are defective in transport or binding of non-metabolizable lactose analogs. Utilizing this assay in conjunction with random mutagenesis of the wild-type gene by E. coli mutator strain mut D5, 35 lac permease mutants were isolated that are defective in either transport or the binding of galactosides. One of these mutants (Asn 245 to Asp) catalyzes the active transport of lactose to only 20 percent of the control steady-state level. Further biochemical and genetic characterization of these mutants will define specific residues in lac permease important to substrate translocation and binding.

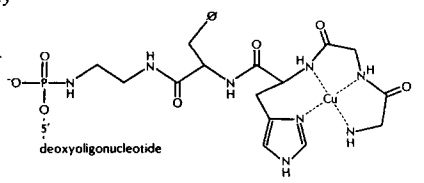
A 404 USE OF SELECTIVE GLYCOSYLATION TO ENGINEER ANALOGS OF HUMAN INTERFERON-γ WITH ALTERED BIOLOGICAL ACTIVITY. Wai-Choi Leung, Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205-7199.
Selective addition and/or deletion of N-linked carbohydrate at predetermined sites was employed to study the structure & function relationship of human interferon-γ(HuIFNγ). The mature polypeptide consists of 143 amino acids with two potential N-linked glycosylation sites at Asn-25 & Asn-97. Recently, we were able to express and secrete HuIFNγ in filamentous fungus Achlya ambisexualis. The fungal-derived HuIFNγ was found to be efficiently glycosylated at either Asn-25 or -97 sites, therefore providing a system for examination of the role of individual carbohydrate on the biological activity of HuIFNγ. Site specific mutagenesis was then performed to generate a number of mutants with altered recognition sites at either Asn-25 or Asn-97. These mutants were first expressed in E. coli to allow the synthesis of nonglycosylated proteins in order to examine the effect of amino acid substitution per se on the biological activity. Only those mutants with antiviral activity comparable to controls were retained for expression in fungus for examination of the role of selective glycosylation on biological activity. Mutant with selective glycosylation at Asn-97, but not Asn-25, exhibited a 8 to 10 fold increase in antiviral activity, with comparable increase in induction of 2,5A synthetase activity. Removal of carbohydrate with N-glycanase but not O-glycanase, was able to convert the antiviral activity of this mutant back to control level, indicating that N-linked, but not O-linked, glycosylation, was responsible. This study provides an avenue in utilization of selective glycosylation in generating useful protein analogs.

A 405 REDUCTION OF THE HETEROGENEITY OF MURINE MONOCLONAL IgG1 BY C-TERMINAL PROCESSING *IN VITRO*. James P. McDonough[1], Rodney A. Jue[2], Thomas C. Furman[1], Richard M. Bartholomew[2], Chris J. Vlahos[1], and Susan M. Hochschwender[2], [1]Eli Lilly & Co., Indianapolis, IN 46285 and [2]Hybritech, Inc., San Diego, CA 92121.

Monoclonal antibodies may be obtained from hybridomas grown by both passage as ascitic tumors or by *in vitro* cultivation. One striking characteristic of all antibodies, irrespective of their source, is their high degree of biochemical heterogeneity. We have analyzed the heterogeneity of CEV124, a murine IgG1 directed against the CEA antigen. The cation exchange chromatographic patterns of CEV124 obtained from ascites culture show primarily a single peak of IgG while the patterns of antibody obtained from *in vitro* culture are more complex, having three main peaks. Biochemical characterization of the IgG present in these three peaks indicates that the molecular basis for the observed differences in cation exchange behavior resides in the C-terminal sequence of the heavy chains and is due to the variable presence of a lysine residue at that position in the sequence. Three methods have been developed to process the heterogeneous forms of the antibody obtained from *in vitro* culture into a single form: incubation of cell culture supernatants at acidic pH, treatment of cell culture supernatants with ascites fluid, and limited proteolysis by carboxypeptidase B. For all three procedures the mechanism responsible for elimination of the cation exchange heterogeneity appears to be removal of C-terminal lysine residues. These methods should allow preparation of significant quantities of essentially homogeneous antibodies for research, diagnostic, and therapeutic uses.

A 406 HIGHLY LOCALIZED CLEAVAGE OF SINGLE STRANDED DNA USING AN ARTIFICIAL ENDONUCLEASE, Michael Mecklenburg[1], Stephen Cliffe[2] and Klaus Mosbach[1,2], 1) Department of Pure and Applied Biochemistry, Chemistry Center, University of Lund, P.O. Box 124, S-221 00 Lund Sweden, 2) Institute of Biotechnology, Swiss Federal Institute of Technology, ETH-Höngger-berg, CH-8093 Zurich, Switzerland.

The covalent attachment of the copper chelating tetrapeptide, gly-gly-his-phe, to the 5' terminus of the deoxynucleotide 5'-C-A-G-C-T-G-G-A-C-G-T-C-G-G-T-T-3' via an azide linkage is described. The sequence is complimentary to a 16-nucleotide sequence in the single str-anded phage M13mp8. In the presence of copper(II) and DTT, we have demonstraited that the oligonucleotide adduct site-specifically cleaves single stranded M13mp8 DNA which contains the complimentary sequence to that of the adduct. And that the resulting single stranded DNA is biologically intact as judged by its ability to serve as template DNA for the primer extension reaction of klenow. The cleavage piont is located symmetrically about the 5' end of the oligonucleo-tide adduct and covers a range of 16 nucleotides. Use of this adduct as a method for potentiating anti-sense inhibition is discussed.



Submitted to EMBO Journal.

A 407 REVERSIBLE UNFOLDING OF O^6-METHYLGUANINE-DNA METHYLTRANSFERASE (ADA) OF E. COLI, S. Mitra, W. D. Behnke[1], P. L. Chong[2] and D. Bhattacharyya, Protein Engineering Program, Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN 37831, [1]Dept. Biochem., Univ. Cincinnati Sch. of Medicine, Cincinnati, OH 45267, [2]Dept. of Biochem., Meharry Med. Coll., Nashville, TN 37208

The 39kDa Ada protein of *E. coli* has the central role in alkylation damage repair in DNA. The protein stoichiometrically accepts alkyl groups at two specific cysteine residues located in two domains connected by a hinge. The acceptor residue in the C-terminal domain dealkylates O^6-alkylguanine and O^4-alkylthymine, and alkyl transfer to the N-terminal domain from phosphotriesters converts the protein into a transcription activator of its own and other alkylation repair genes. The protein has about 20% each of α-helix and β-sheet contents. The protein is unstable at 37° in the absence of DNA and its two domains are differentially inactivated by urea and guanidine. CD and fluorescence data show denaturant-induced loss of all but 10-20% of the structure. However, the structure and activity of the protein are rapidly restored by the removal of urea. Heat-inactivated and even coagulated Ada protein can be fully reactivated by first dissolving it in 6M urea followed by removal. These results are consistent with a model of kinetic control in folding of the active form of Ada protein initiated by a core structure. (Research supported by USDOE under contract DE-AC05-840R21400 with the Martin Marietta Energy Systems, Inc.)

A 408 MOLECULAR MODELING AND FLUORESCENCE SPECTROSCOPY IN A STRUCTURE-ACTIVITY INVESTIGATION OF RENIN INHIBITORS HAVING C-TERMINAL P_1-P_1' PSEUDODIPEPTIDYLCARBOXAMIDE FUNCTIONALITIES, Tomi K. Sawyer, Boryeu Mao, Dennis E. Epps, Donald T. Pals, Linda L. Maggiora, Douglas J. Staples, John H. Kinner and Clark W. Smith, The Upjohn Company, Kalamazoo, MI 49001. Recently, the 3-D structure of H-D-His-Pro-Phe-His-Phe[CH_2NH]Phe-Val-Tyr-OH (U-70531E) complexed to the active site of the aspartyl protease, rhizopuspepsin, has been determined by x-ray crystallography at a resolution of 1.8Å. Structure-activity analysis of U-70531E has shown that specific N- and/or C-terminal chemical modifications effects markedly enhanced potency against human renin. For example, Ac-Ftr-Pro-Phe-His-Phe[CH_2NH]Phe-NH_2 (U-71908E) was 1000-fold more potent than U-70531E to inhibit human renin *in vitro*. To explore the molecular basis for such renin•inhibitor interactions, we have utilized molecular modeling and fluorescence spectroscopy methods to systematically probe the structure-conformation relationships of specific ligand•enzyme complexes or compounds (free of enzyme) in solution. First, a comparison of U-70531E at each of the two aspartyl proteases (*i.e.*, x-ray crystallographic structure of rhizopuspepsin and a computer- graphics model of human renin, CKH-RENIN) was performed by molecular modeling. Secondly, compounds of the generic formula Ac-Ftr-Pro-Phe-His-Xaa-Yaa-NH_2 (*e.g.*, Xaa-Yaa = LeuΨ[CH_2NH]Val or LeuΨ[$CH(OH)CH_2$]Val) were studied by molecular modeling to CKH-RENIN and by structure-activity *in vitro*. Finally, fluorescence spectroscopy of Ac-Ftr-Pro-Phe-His-PheΨ[CH_2NH]Tyr-NH_2 (U-81736E) was performed in solution to explore its structure-conformation properties based on intramolecular resonance energy transfer. This multidisciplinary strategy has provided insight into the rational design of pseudopeptidic inhibitiors of renin.

A 409 EXPRESSION AND SITE-SPECIFIC ALTERATION OF cGMP PHOSPHODIESTERASE γ-SUBUNIT FROM CATTLE RETINA. N. P. Skiba, I. P. Udovichenko, V. A. Bondarenko, E. P. Shirokova, S. A. Zozulya, V. M. Lipkin. Branch of Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Scienses, Pushchino, 142292, USSR
Retinal rod outer segments contain a phosphodiesterase (PDE), specific for cyclic GMP, important for visual signal transduction. Enzyme consists of two large catalytic subunits - α and β, and small γ-subunit, which is the internal inhibitor of the enzyme. Studies on the structure and function of PDE are of particular relevance to the understanding of the signal transduction mechanism. Plasmids for direct expression of γ-PDE in *E. coli* were constructed by a combination of standard techniques. Plasmids contain one and two copies of coding region of γ-PDE gene under control of a termoinducible Pr promoter of bacteriophage λ. Expression γ-PDE product was detected only in protease deficient strains, because extensive degradation of the strongly basic γ-PDE by bacterial proteases. Expressed γ-subunit was fully functional as determined by inhibition cGMP hydrolysis test. γ-PDE mutant forms produced by oligonucleotide directed mutagenesis are under funtional study.

A 410 COMPUTATIONAL TOOLS FOR THE STUDY OF PROTEIN STRUCTURAL HOMOLOGY, Arthur L. Williams, Jr. & Rulei Ting, ImClone Systems, Inc., 180 Varick Street, New York, NY 10014.
The tertiary structures of proteins are evolutionarily more conserved than their amino acid sequences and their DNA sequences. Functionally equivalent macromolecules, while divergent in sequence, tend to have similar tertiary structures. The major sequence and structural differences often occur in surface loops between the conserved secondary structural elements (the α-helices and β-strands). Structural equivalence of residues in proteins has been commonly identified by structural superposition methods that calculate r.m.s. differences of equivalent C_α atoms. This study utilizes image analysis techniques and a dynamic programming procedure to systematically compare the C_α backbone structure of proteins. Generally the common structure of functionally similar proteins is composed of conserved secondary structural motifs. The method presented here first identifies these motifs using an image analysis procedure on the distance matrices (Phillips, 1970) derived from the C_α's. It then assesses the similarity of the motifs by a dynamic programming algorithm that compares their linear distance plots (Liebman *et al.*, 1985). It differs from structural superposition and sequence alignment methods in two ways; (1) it allows for variability in the size of equivalent secondary structures and (2) it is independent of translation/rotation of the three dimensional structure. The application of this method to the NAD binding proteins and the serine proteases will be presented.