PROTEIN STRUCTURE, FOLDING AND DESIGN

Dale Oxender, Organizer

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Model Systems for Protein Structure-Function Studies

0628 DIRECTED MUTAGENESIS AS A PROBE OF DIHYDROFOLATE REDUCTASE STRUCTURE AND FUNCTION,
J. Kraut, J. E. Villafranca, E. E. Howell and J. N. Abelson, Department of Chemistry, University of California, San Diego and The Agouron Institute, La Jolla, CA 92037.

Structure-function relations in dihydrofolate reductase (DHFR) are being explored by the techniques of oligonucleotide directed site-specific mutagenesis, classical enzymology and x-ray crystallography. Several mutant versions of *E. coli* DHFR have been obtained by introducing specific base changes and overexpressing the cloned gene. These mutations are designed to answer a variety of questions raised by the refined, high-resolution crystal structures of three species of the enzyme and the considerable body of results from spectroscopy, enzyme kinetics and binding studies which already exists.

Mutant DHFRs in hand at this time include Asp-27 to Asn, Glu, Leu and Ser; Pro-39 to Cys; Gly-95 to Ala; Trp-22 to His; Trp-30 to Ser; Arg-44 to Thr; Ser-63 to Glu; and the double mutation containing both Thr-44 and Glu-63. The most thoroughly characterized of these are the Asp-27 to Asn and the Pro-39 to Cys mutants.

The Asn-27 mutant has a k_{cat} of about one-thousandth that of the wild type DHFR at pH 7.0, but k_{cat} increases rapidly with decreasing pH so that at pH 5 the k_{cat} of the mutant enzyme is one-fortieth of its maximal value for the wild type. Concurrently, however, as k_{cat} increases with decreasing pH, K_m also increases and the value of k_{cat}/K_m remains constant. This behavior is consistent with extensive nonproductive binding of substrate to the mutant DHFR at neutral pH which diminishes sharply at low pH. It suggests that protonated dihydrofolate is the productive substrate species for the mutant DHFR, while unprotonated dihydrofolate is the nonproductive species. It can be argued from these observations that the catalytic role of Asp-27 in the wild type enzyme is to donate a proton to the substrate in the transition state rather than to stabilize the protonated state by means of a charge-charge interaction.

The Cys-39 mutation was designed to introduce a disulfide bridge at Cys-85 into the DHFR molecule. We find that the disulfide bond is not made in the cytosol of *E. coli*, but can be made in vitro quite readily by oxidation with disulfide-exchange reagents like DTNB. The x-ray structure of the reduced Cys-39 mutant verifies our conclusions from modeling studies that the new cysteine side chain is well situated to make a disulfide bridge with Cys-85. Both the reduced and disulfide-bridged form of the mutant enzyme have activities that are comparable to the wild type. The disulfide-bridged form of the mutant DHFR shows enhanced resistance to unfolding by guanidine hydrochloride and decreased cooperativity of unfolding.

A set of three mutants has been constructed to investigate the preference of DHFR for the cofactor NADPH as opposed to NADH. One of these, a double mutant of Arg-44 to Thr and Ser-63 to Glu, shows a strong preference for NADH.

Directed Mutagenesis Studies of Protein Structure-Function

0629 SINGLE-STRANDED HEXAMERIC OLIGONUCLEOTIDE LINKERS: USE FOR IN VITRO MUTAGENESIS,
Francis Barany, Johns Hopkins University School of Medicine, Baltimore, MD 21205

A procedure has been devised for constructing two amino acid insertions using single-stranded oligonucleotide linkers. The method inserts a hexameric sequence into a (sticky end) restriction site (such as HpaII) and creates a new six base restriction site (such as EcoRI). The mutation can easily be localized by a restriction digestion. These linkers offer several advantages over conventional linkers: (i) they insert two amino acids without causing a frame shift; (ii) they can be used on both 5' and 3' sticky ends; (iii) they can only form the new site upon addition to double-stranded DNA, and hence can be easily recut by the second enzyme even in huge linker excess; (iv) most are compatible with biochemical or biological selection; and (v) the reaction can be completed in a single tube without purifying away the single-stranded linkers. There are 52 single-stranded linkers that can be applied to this strategy. A few of these have been synthesized and are currently being tested. Preliminary results show that conversion of SalI to ApaI, EcoRI, KpnI, SacI, or XhoI in the pBR322 tetracycline resistance gene is approximately 80% efficient, and all five newly generated genes retain tetracycline resistance. (This fortuitous result has created a new family of pBR322 vectors.) Other insertions into the tetracycline resistance gene indicate that the technique may be useful in mapping functional domains. Temperature sensitive mutations in the β-lactamase gene with altered specificity to various β-lactams have also been isolated.

0630 STUDIES OF A BACTERIAL SENSORY TRANSDUCER USING OLIGONUCLEOTIDE DIRECTED MUTAGENESIS, John Bollinger and Gerald L. Hazelbauer, Washington State University, Pullman, WA 99164-4660

Chemotaxis in E. coli involves excitation and adaptation. Both phases are mediated in part by membrane spanning transducer proteins. During adaptation these proteins are carboxylmethylated at specific, identified glutamate residues. Some of the methyl-accepting glutamates are created by deamidation of glutamines.

In the transducer protein Trg, there are at least five methyl-accepting glutamates, two of which result from deamidation. These sites are grouped in two tryptic peptides which are separated by almost 200 residues in the linear sequence of the intact protein. We are using oligonucleotide directed mutagenesis of the Trg gene to determine the role of each peptide and each site of methylation and of deamidation in the tactic functions of the Trg transducer.

0631 BIOLOGICAL ACTIVITIES OF RECOMBINANT IL-2 ANALOGS, T.C. Boone, V.R. Chazin, B. Anderson, H.R. Hockman, W.C. Kenney, M. Carter, T. Jones, K.K. Chen, W.R. Bacheller, M. Swan and B.W. Altrock. Amgen, Thousand Oaks, CA 91320.
A gene for human IL-2 was chemically synthesized and inserted into a plasmid for expression in E. coli. Codons chosen for the synthetic gene were those found in the highly expressed proteins of E. coli. The gene was expressed by an E. coli tryptophan synthetase promoter at a level of 30% of the total cellular protein. Restriction endonuclease sites were incorporated into the gene at convenient positions for easy alteration of the gene. One analog was made which eliminated the N-terminal 23 amino acids from the protein by replacing one restriction endonuclease fragment with another. This analog, which begins at an internal methionine, has no activity in a ^{3}H-thymidine incorporation assay using an IL-2 dependent murine T-cell line (CTLL). Ten analogs were constructed using oligonucleotide site directed mutagenesis. Four of these analogs were truncated at various sites near the C-terminus. These four analogs had less than 0.1% of the activity of the recombinant IL-2 in the CTLL assay. The remaining six analogs were constructed to replace the three cysteines with various other amino acids. Those experiments showed that the first two cysteines in the molecule are required for maximum activity in the CTLL assay, presumably because they form a disulfide bond. All the analogs were purified from E. coli extracts to at least 95% purity. To further study the structure-function relationship between IL-2 and its receptor, these highly purified analogs were tested in receptor binding studies by competing with I^{125} labeled human recombinant IL-2. These studies indicate the C-terminus is necessary for activity as well as receptor binding.

0632 SITE-DIRECTED MUTAGENESIS OF BACILLUS SUBTILISIN, P.N. Bryan, K.R. Furr, C.C. O'Neal, N. Vasantha and T.L. Poulos

We have used oligonucleotide-directed mutagenesis to introduce a number of amino acid substitutions into subtilisin from Bacillus amyloliquefaciens. Subtilisin is a major, extracellular serine protease. Two classes of mutations are being analyzed. The first, mutants of active site residues are being used to study the reaction mechanism and changes in substrate specificity. The second class involves the introduction of cysteine residues into subtilisin to create disulfide bonds. These are being examined for their influence on thermal and catalytic properties of subtilisin. Physical and catalytic properties of all these mutants will be discussed.

0633 DIRECTED MUTAGENESIS OF L. CASEI DHFR, R. Wayne Davies, Paul Sims, Steven G. Minter, Manda Gent and Julie Andrews, UMIST, Manchester, U.K. L. casei DHFR is well characterized by X-ray crystallography and NMR work. Together with the NMR group at the NIMR, Mill Hill, London, U.K., we have undertaken to study the effects of defined amino acid substitution on the structure and function of L. casei DHFR by biochemical and NMR analysis. The DNA sequence of the DHFR gene from a methotrexate-resistant strain of L. casei was completely determined, together with over 300 bp 5' and 3'. The DNA sequence showed two differences from the published protein sequence. The gene was then cloned in the E. coli high expression vector p PLc 28, and certain derivatives of this produce very large amounts of L. casei DHFR in E. coli. We have used an adaption of the 2-primer method of oligonucleotide-directed in vitro mutagenesis to introduce particular mutations leading to particular amino acid substitutions. Biochemical data from the first series of such mutant proteins will shortly be available.

0634 SITE-DIRECTED MUTAGENESIS OF RAT CARBOXYPEPTIDASE A (CPA): INVESTIGATION OF THE ROLE OF TYR248 IN CATALYSIS. S.J. Gardell, C.S. Craik, M.S. Urdea,* E.T. Kaiser** and W.J. Rutter, Hormone Research Institute, University of California, San Francisco, Ca 94143, *Chiron Research Laboratory, Emeryville, Ca 94608 and **The Rockefeller University, New York, N.Y. 10021

The phenolic hydroxyl group of Tyr248 of CPA has been tentatively identified as the proton donor during peptide hydrolysis. Site-directed mutagenesis of the rat CPA cDNA was utilized to test this proposed role. Hence, a CPA variant in which Tyr248 had been replaced by Phe (CPA-Phe248) and the wild type CPA (CPA-WT) were synthesized and secreted from yeast via the α factor-directed expression system (Brake et al. (1984). PNAS 81, 4642). Both proteins were purified to homogeneity. CPA-Phe248 exhibited virtually no change in kcat with the peptide substrate, hippuryl-L-phenylalanine; however, there is an ∿3 fold increase in the apparent Km. Hydrolysis of the ester substrate, hippuryl-DL-phenyllactate, was not significantly affected. In contrast, CPA-Phe248 had diminished activity toward another ester substrate, O-(p-chloro-trans-cinnamoyl)-L-β-phenyllactate. The peptidase activity of the CPA variant, unlike CPA-WT, was unaffected by tetranitromethane, a chemical reagent known to react specifically with Tyr248. In addition, CPA-Phe248 exhibited an ∿16 fold decrease in apparent affinity towards the potato carboxypeptidase inhibitor (PCI). These results indicate that the phenolic hydroxyl group of Tyr248 is not required for peptide hydrolysis although it may play a role in the binding of substrates and PCI.

0635 STUDIES OF THE MECHANISM OF THE REACTION CATALYZED BY STAPHYLOCOCCAL NUCLEASE A USING PRIMER DIRECTED SITE SPECIFIC MUTAGENESIS, John A. Gerlt, David W. Hibler, Mark A. Reynolds, and Neal J. Stolowich, Department of Chemistry, University of Maryland, College Park, MD 20742

Primer directed mutagenesis is being used to generate mutations in functional groups present in the active site of Staphylococcal nuclease A so that the roles of these groups in both catalysis and substrate binding can be better defined. The gene for the nuclease was cloned by Shortle, and in collaboration with M. Inouye (SUNY, Stony Brook) we have constructed an expression-secretion plasmid that directs the secretion of mature Staphylococcal nuclease A into the periplasmic space of E. coli. On the basis of Cotton's 1.5 A x-ray structure and chemical studies performed in this laboratory, the role of glutamate-43 in catalysis is presumed to be a general basic catalyst involved in assisting the in-line attack of water on the substrate. We have mutated this glutamate residue to an aspartate residue, and the mutant enzyme has a V/K 250-fold less than that of the wild type enzyme. Although this observation can be interpretted as providing strong evidence for the essential role of the glutamate carboxylate group in catalysis, we are devising methods that will allow us to evaluate whether the small level of enzymatic activity possessed by the mutant might be better explained by either alteration of active site tertiary structure or by infidelity in protein synthesis. The results of these studies and additional mutageneses will be presented. (Supported by NIH GM-34573 and the Alfred P. Sloan Foundation.)

0636 FUNCTIONAL STUDIES OF YEAST CYTOCHROME c PEROXIDASE BY SITE DIRECTED MUTAGENESIS, David B. Goodin, A. Grant Mauk and Michael Smith, Dept. of Biochemistry, 2146 Health Sciences Mall, University of British Columbia, Vancouver, B.C. V6T 1W5

For many years it has been known that the compound I intermediate of yeast cytochrome c peroxidase is markedly different from that of other peroxidases. The presence of a magnetically uncoupled free radical instead of the normally observed porphyrin pi-cation radical has implicated a reversibly oxidized amino acid only in the yeast enzyme. However, the identity and functional role of that residue remains controversial. Trp-51 is correctly positioned to take part in a proposed mechanism of catalysis (Poulos and Kraut (1980) J. Biol. Chem. 255, 8199). However, an alternate oxidized methionine residue has been implicated in ENDOR studies (Hoffman et al. (1981) J. Biol. Chem. 256, 6556). Both Trp-51 and Met-172 are replaced in other peroxidases by highly conserved, and less easily oxidized residues.

We have used oligonucleotide site directed mutagenesis to alter the Met-172 codon of the CCP gene to that of serine and cysteine. Mutagenesis and screening techniques were adapted for use with pEMBL vectors (Dente et al. (1983) Nucl. Acids Res. 11, 1646) as an alternative to the single stranded M13 system. The constructed mutants have been expressed in S. cerevisiae, and active CCP can be prepared for both mutants. Results will be presented on the characterization, spectroscopic, and functional properties of purified preparations of the mutant enzymes.

0637 SITE-DIRECTED MUTAGENESIS OF THE EcoRI ENDONUCLEASE, Patricia Greene, Norbert O. Reich, Judith McClarin, Herbert W. Boyer, and John Rosenberg, UCSF, San Francisco, CA 94143 and U. Pittsburgh, Pittsburgh, PA

The EcoRI endonuclease has been crystallized with its substrate and the X-ray structure has been solved to 3Å (Nature 309, 327, 1984). Sequence recognition is mediated by a series of hydrogen bonds between complementary surfaces of the protein and the major groove of the DNA. We have used the crystallographic analysis to design putative specificity mutations in the endonuclease and to predict possible new specificities that might arise. In particular, a glutamic acid residue which is postulated to interact with the N_6 positions of the adjacent adenines in the recognition sequence has been replaced with glutamic acid. The predicted recognition sequence would contain a guanine in place of one of the adenines. This alteration has been introduced into the endonuclease gene by means of mismatch primer mutagenesis. The expression and characterization of the mutant protein is underway.

0638 ALTERED INHIBITION PROPERTIES OF α1-ANTITRYPSIN VARIANTS CONSTRUCTED BY SITE-DIRECTED MUTAGENESIS. S. Jallat, L-H. Tessier, *R.G. Crystal, J-P. Lecocq and M. Courtney, Transgène S.A., Strasbourg, France and *Pulmonary Branch, NHLBI, Bethesda, MD, USA.

Human α1-antitrypsin (α1-AT) is a serine protease inhibitor that displays a wide range of inhibition rates with different enzymes (e.g. neutrophil elastase >> trypsin >> thrombin). Inhibition of neutrophil elastase in the lower respiratory tract is thought to be the major physiological role of α1-AT and this is corroborated by the high incidence of emphysema in individuals with hereditary α1-AT deficiency. Also, α1-AT can be inactivated by oxidation of a Met residue at the reactive centre and this may occur in smokers' lungs, leading to a protease/antiprotease imbalance and emphysema. The α1-AT reactive centre acts as a substrate analogue to trap and inactivate target enzymes. It is proposed that the rate of inhibition depends upon the similarity of α1-AT reactive centre to the preferred cleavage site of the protease. We have demonstrated this directly by designing α1-AT variants by *in vitro* mutagenesis of the cloned gene and expressing the products in bacteria. These new inhibitors have altered inhibition properties with respect to e.g. elastases, thrombin, and cathepsin G. In addition, we have constructed oxidation resistant variants that remain efficient antielastases. These may be particularly useful in the treatment of the α1-AT deficiency by replacement therapy.

0639 MODEL BUILDING AND MUTAGENESIS OF COLICIN E1, Cyrus Levinthal, Qui Rong Liu, Veronica Crozel, Francoise Levinthal and Richard Fine, Columbia University, New York, NY 10027

Molecular model building and oligonucleotide mutagenesis have been used to study the structure-function relationships of that portion of the colicin E1 protein which makes ion channels in phospholipid membranes. By introducing methionine codons to provide a site for CNBr cleavage we can vary the location of the amino-terminus of the channel peptide fragment. Similarly, by introducing stop codons the position of the carboxy-terminus can be varied. To date, the shortest active fragment has 129 amino acids. This size seems inconsistent with a model of the structure which is a barrel of six alpha helices each of which would have to be about 20 amino acids long in order to span the membrane. Additional mutations have been made which introduce changes in the number and location of charged residues within the active peptide. These also suggest that the structure is not made of alpha-helices.

We are currently testing the hypothesis that the channel is composed of a single layer β sheet with ten strands formed into a twisted barrel. The tests involve producing mutants each of which has a cys residue at different positions along the polypeptide chain so that sulfhydryl reagents can be used to determine whether particular residues face into the channel lumen or into the lipid region of the membranes.

0640 STRUCTURE-ACTIVITY STUDIES OF RECOMBINANT INTERLEUKIN 2 WITH MUTATION AT THE CYSTEINE RESIDUES, Shu-Mei Liang, David R.Thatcher and Bernard Allet, Biogen S.A., Geneva, Switzerland.
Recombinant human interleukin 2 (rIL2) has been purified to homogeneity from E.coli, harboring the plasmids encoded with IL2 gene. Peptide mapping of the rIL2 showed that cysteine residues at position 58 and 105 formed a disulfide bridge while cysteine residue at position 125 had a free sulphydryl group. Site specific mutagenesis procedures were used to modify the IL2 gene by changing or deleting each of the cysteine codons. Each modified protein was then purified to homogeneity and compared with the unmodified protein. Substitution of SER for CYS residue at position 125 retained full biological activity. Substitution of ALA for CYS residue at position 105 reduced biological activity 10 fold while substitution of ALA for CYS at position 58 reduced activity 100 fold. These results confirm that the disruption of the single disulphide of rIL-2 bridge produces a molecule of lower biological activity and the different levels of residual activity of the mutant proteins also indicate that the two constituent cysteine residues play an unequal role in the maintainance of the active structure. This hypothesis was confirmed by deletion of CYS 105 (activity retained) and deletion of CYS 58 (activity completly abolished). Deletion of the free thiol residue, CYS 125, completely destroys the biological activity of rIL-2. As deletion of neighboring PHE 124 or GLN 126 also produces biologically inactive protein, the conformation of this C-terminal portion of rIL-2 must be crucial for biological activity.

0641 EXPRESSION AND MUTAGENESIS OF BOVINE PANCREATIC TRYPSIN INHIBITOR, Cara Berman Marks and Stephen Anderson, Genentech, Inc., South San Francisco, CA 94080.

Bovine pancreatic trypsin inhibitor (BPTI) is a small protein that has been intensely studied physically, chemically, and theoretically as a model system for globular polypeptide domains. Its attractiveness as a model system could be further enhanced by the ability to modify genetically its amino acid sequence and hence its tertiary structure. For this reason, we have developed a heterologous expression system for the BPTI gene. We are using this expression system and site-directed in vitro mutagenesis to make amino acid substitutions in BPTI. The effects of several mutations on the structure and function of BPTI will be discussed.

0642 A NOVEL PROTEIN ENGINEERING SYSTEM, David Mead, Elzbieta Skorupa, and Byron Kemper, University of Illinois, Urbana, IL 61801
We have simplified the in vitro engineering of mutant RNAs and their corresponding proteins by constructing chimeric plasmids suitable for the efficient biosynthesis of large amounts of translationally active RNA and single or double stranded DNA (ssDNA, dsDNA). We cloned the intergenic region of bacteriophage f1 into the bacteriophage SP6 promoter plasmids, pSP64 and pSP65, in both orientations. Coinfection of E. coli with these chimeric SP6 promoter/phage f1 plasmids and the interference resistance phage, IR1, results in the replication and secretion of the pSP6•f1 plasmids as ssDNA. Thus, cDNA cloned in these vectors can be obtained as ssDNA for mutagenesis or sequencing purposes or as dsDNA for the transcription of μg amounts of mutant RNAs. The uncapped mRNA transcribed from bovine preproparathyroid hormone cDNA cloned in these vectors was efficiently translated in the reticulocyte cell-free system. In the presence of microsomal membranes, conversion of the resultant pre-proteins to pro-proteins was observed. We are using this in vitro protein engineering system for the structure-activity analysis of signal sequence mutants. This system eliminates the shuttling of DNA fragments to an M13 phage vector, since the mutated gene can be expressed in vitro directly from the plasmid used for the ssDNA mutagenesis.

0643 STRUCTURE AND FOLDING OF MUTANT E. coli TSASE α-SUBUNITS, D. L. Milton, R. M. Myers, and J. K. Hardman, University of Alabama, University, AL 35486.
Studies have been initiated to examine the folding behavior of mutants of the E. coli TSase α-subunit. The approach is to saturate the trpA gene (which encodes for this enzyme) with single-base pair alterations at potentially all sites in the gene and to examine selected mutant proteins that result for alterations in structure and folding properties. Mutants are obtained by random chemical mutagenesis (nitrous acid, formic acid, hydrazine, dimethylsulfate) in vitro of cloned, discrete, short regions of trpA. Mutagenesis conditions are such that 5-6 different amino acid substitutions can occur at each residue position in the polypeptide. To this end we have constructed mutagenesis vectors containing 3 trpA fragments: fragment I, containing the first 110-bp of the gene and encoding for residues 1 to 36; fragment II, containing the 111 to 207-bp region and encoding for residues 37 to 68; and fragment III, containing the 208 to 365-bp region and encoding for residues 68 to 122. The construction of additional vectors carrying fragments of the remaining portion of the gene is in progress. Following mutagenesis of ss-forms of these vectors and the conversion of the fragments to ds-forms, they are excised and isolated by urea/formamide gradient polyacrylamide gels. The mutated fragments are sequenced and substituted into an otherwise normal trpA gene contained in an over-expression vector. This vector was constructed such that the trpA gene is under control of the tac promotor and is maintained in a lac i^q strain. Upon induction with IPTG, these cells produce 25-30% of the total soluble protein as α-subunit. Thus any mutant protein selected can be readily obtained in sufficient quantity (20-30 mg/L cells) for study.

0644 STRUCTURE-ACTIVITY STUDIES OF RECOMBINANT-γ-INTERFERON, Bernd Otto, Michel Dayer, Marlis Hirschi, Bernard Mach, Ulrich Otto, Paul Wingfield and Bernard Allet, Biogen S.A., Geneva, Switzerland.
Recombinant human γ-interferon has been purified to homogeneity from E.coli bacterial cells, harboring the plasmid encoding the γ-interferon gene. This protein has been compared in respect to biological activities (antiviral, immunomodulating, antitumors in mice containing human renal tumors) as well as to the dimer structure with natural γ-interferon. In addition by using gentechnological methods we investigated how far we would shorten this protein from either the N-terminus of the carboxyl-end without affecting structure and biological activities.

0645 A STABILIZING DISULFIDE BOND ENGINEERED INTO T4 LYSOZYME BY SITE-DIRECTED MUTAGENESIS, L. Jeanne Perry, and Ronald B. Wetzel, Genentech, Inc. S.S.F., CA 94080
Using recombinant DNA/cloning techniques, including M13 site-directed mutagenesis, we have replaced the codon for Ile-3 with a Cys codon in the gene of the normally disulfide-free protein T4 lysozyme. The mutant protein, synthesized in E. coli, contains 3 cysteines: the newly introduced Cys-3 and the two wild-type cysteines Cys-54 and Cys-97. Oxidation in vitro generated a disulfide bond between Cys-3 and Cys-97. Both oxidized and reduced mutant exhibited full enzymatic activity. The disulfide-crosslinked form of T4 lysozyme (Ile-3 → Cys) was significantly more stable toward thermal inactivation than the wild-type protein. The activities and stabilities of this and other derivatives will be presented and discussed.

0646 **KINETIC AND THERMODYNAMIC STUDIES OF YEAST CYTOCHROME c ALTERED BY SITE-DIRECTED MUTAGENESIS AT AN INVARIANT PHENYLALANINE RESIDUE.**, Gary J. Pielak, A. Grant Mauk and Michael Smith, Dept. of Biochem., Univ. of British Columbia, Vancouver, B.C. V6T 1W5 Canada
Phenylalanine-87 (Phe-82 in the tuna numbering system) occurs on the surface of cytochrome c and is one of the conserved aromatic residues surrounding the heme. Although assigned a role in electron transfer, perhaps by controlling the polarity of the heme environment, interest in Phe-87 has been accentuated by a model for the cytochrome c-cytochrome c peroxidase (CCP) complex (Poulos and Kraut J. Biol. Chem. 255, 10322 (1980)). In this model, Phe-87 forms part of a a conduit for electron transfer between the two heme proteins.

Using site-directed mutagenesis the codon at position 87 has been changed to that of a Ser, Tyr or Gly. The mutated genes have been transferred into a yeast strain that lacks cytochrome c. The transformants grow on glycerol indicating that the mutated proteins are at least partially functional. Amino acid analyses confirm the mutations.

The mutant proteins are from 20 to 70% as active as the wild type in the CCP assay. The reduction potentials of the Ser and the Gly mutants are decreased about 50 mV while that of the Tyr mutant is unchanged with respect to the wild type protein. Inspection of the water accessible surface of a simulation of the Gly mutant suggests that this substitution opens the surface of the protein in the vicinity of the met ligand. These results demonstrate Phe-87 is not an absolute requirement for reaction of cytochrome c with either its oxidase or peroxidase and suggest that solvent has more access to the heme in the Ser and Gly mutant, than in the Tyr mutant and the wild type protein. A detailed steady state kinetic analysis and investigation of the tertiary structure of the mutant proteins are currently underway.

0647 RECOMBINANT METALLOTHIONEIN DOMAIN CODING SEQUENCES, Richard Pine and P.C. Huang Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD 21205
The structure-function relationship between the two domains of metallothionein and their corresponding exons is unclear. Fragments from metallothionein cDNA coding sequences and synthetic oligonucleotides were used to construct recombinant mutants which are the equivalent of a single putative exon. Isolated metallothionein coding sequence was digested with various restriction endonucleases to yield fragments which encode just the amino or carboxyl terminal ends of the native protein and fragments which contain either one of the two domain coding units. Ligation of the appropriate, isolated sequences produced clones in which each single domain coding unit is flanked by native initiation and termination sequences. Clones having various termini were constructed. Similar constructs were made with a double-stranded, synthetic DNA oligonucleotide, having the sequence 5'CTGAATTCGCGAAGCTTATGAG3', in place of either the native initiation or termination signals. Another class of recombinant mutants was created by inserting the linker 5'CCCGGG3', or its oligomers, to expand the distance by different lengths between domain coding units. A plasmid containing the phage lambda leftward promoter and the temperature sensitive CI857 repressor gene has been used as a vector for the inducible expression of these recombinant mutants. These recombinants and their protein/peptide products provide a basis for further studies to test whether metallothionein metal binding domains and their genes have evolved and now function independently or interdependently. This work was supported by grant NIH R01 GM32606

0648 SITE-SPECIFIC MUTAGENESIS OF *E. coli* ASPARTATE TRANSCARBAMOYLASE. Ellen A. Robey and H. K. Schachman. University of California, Berkeley, CA. 94720

Replacement of tyrosine 165 of the catalytic (c) chain of ATCase with serine produces a mutant ATCase with lowered enzymatic activity and altered regulatory properties. Kinetic analysis of the isolated mutant catalytic (C) trimer showed that V_{max}/K_m for aspartate was reduced 48 fold and K_d for carbamoyl phosphate was reduced by a factor of three. Hybrid trimers we'e formed from the mutant C trimers and C trimers which had been chemically modified at lysine 84. Although the mutant and chemically modified C trimers had low activity, (3% and 5% respectively of the specific activity of unmodified C trimer at 10 mM aspartate) hybrid trimers composed of both types of chains had 23% and 28% of the activity of unmodified C trimer. This restoration of activity approaches 33%, the level of activity that would be expected if one unmodified active site per trimer were present in the hybrids and supports the data from x-ray crystallography which indicates the active sites in ATCase are composed of residues from adjacent c chains in the C trimer. For the holoenzyme, the tyrosine 165 to serine substitution greatly reduces the homotropic effects of aspartate, however nucleotide effects are still present. Sulfhydryl reactivity and activation of enzyme activity by the bisubstrate analog PALA indicate that the allosteric equilibrium may be altered by the mutation.

0649 REDESIGNING TRYPSIN: ALTERATION OF CATALYTIC ACTIVITY AND SUBSTRATE SPECIFICITY, Steven O. Roczniak, Charles S. Craik, Robert Fletterick and William J. Rutter, Hormone Research Institute, University of California, San Francisco, Ca 94143
We describe a general system for the modification by site-specific mutagenesis and the subsequent expression of eucaryotic genes. Using this system we have replaced the aspartic acid residue at position 102 with an asparagine. The resultant mutant protein has similar Km values but 5% of the kcat values of the wild type enzyme in catalyzing the hydrolysis of Arg and Lys peptide substrates. (Work on ester substrates is now in progress.) Other amino acid changes have been made in the enzyme's specificity pocket. The glycine residues at positions 216 and 226 have been replaced with alanine residues. Computer graphic analysis suggested that these substitutions would selectively affect arginine and lysine substrate binding. While substrate selectivity (expressed as kcat/Km) for arginine and lysine was observed: trypsin (216 Gly→Ala)(Arg>>Lys); trypsin (226 Gly→Ala)(Lys>>Arg); and trypsin (216,226 Gly→Ala)(Arg>Lys); they were not dominated by the Km as predicted but rather by the kcat values. Other changes in the specificity pocket (e.g. 189 Asp→Glu and 180 Asp→Lys) will be discussed.

0650 SITE SPECIFIC MUTAGENESIS AND METALLOENZYME FUNCTION, Stephen G. Sligar, University of Illinois, Urbana, IL 61801
We seek to understand the molecular mechanisms of metalloenzyme function and the relationship of metal coordination and ligand environment to the specific chemical and biophysical activities and properties of these systems. We have defined the role of the uncommon cysteine residue in cytochrome $P\text{-}450_{cam}$ whose three dimensional X-ray structure has been solved by Dr. Poulos (Genex). The cytochrome $P\text{-}450_{cam}$ gene has been cloned and expressed in high yield, and the cysteine axial ligand to the heme iron replaced with both histidine and serine. This replacement reaction was conducted by cloning, made possible by the fortunate placement of two restriction sites flanking CYS-355 in the native sequence. Other CYS SER replacements are induced by single strand oligonucleotide-directed mutagenesis using pEMBL derived vector systems. A human betaglobin clone has allowed the converse experiment of replacing histidine with cysteine and is the subject of a collaboration in protein dynamics with Professor Frauenfelder's group in the Illinois Physics Department. In addition, we have synthesized *de novo* the gene coding for the production of the hepatic electron transport protein cytochrome b_5, and cloned the genes from *Pseudomonas* responsible for the production of the blue copper protein azurin and cytochrome c-551. With these systems in hand, a molecular understanding of inter-protein electron transport, metalloenzyme spectroscopy, and biological oxygen metabolism may be possible.

0651 ROLE OF TRYPTOPHAN 121 IN INTERLEUKIN 2 STRUCTURE AND FUNCTION, David R.Thatcher, Shu-Mei Liang and Bernard Allet, Biogen S.A., Geneva, Switzerland.
Human interleukin 2 [rIL-2] or T-cell growth factor contains a single tryptophan residue at position 121. This residue was changed to serine by site specific mutagenesis and the protein purified to homogeneity. The purified mutant protein was indistinguishable from human recombinant IL-2 on SDS-PAGE and gel filtration but was 10-100 fold less able to promote the proliferation of a T-cell dependent cell line. Intrinsic flourescence measurements on native and denatured rIL-2 indicated that TRP 121 is buried in a hydrophobic core, inaccessible to solvent. The side chain of TRP 121 therefore does not interact directly with the cellular system which triggers the proliferative response (receptor or associated molecule). The TRP to SER substitution must lower the efficacy of the interaction by destabilizing the conformational surface of the rIL-2 molecule.
The effect of the mutation is most probably a disturbance of the local conformation of the C-terminal sequence as this region of the molecule is essential for biological activity: when the TRP 121 codon was changed to a STOP codon by site specific mutagenesis, a completely inactive truncated molecule was produced.

0652 Site-Directed Mutagenesis of Fujinami Sarcoma Virus Reaveals That Tyrosine Phosphorylation of P130 Modulates Its Enzymatic and Biological Activities.
G. Weinmaster and T. Pawson. Dept. Microbiology U.B.C. Vancouver, B.C. Canada.

Fujinami sarcoma virus (FSV) encodes a 130 kd transforming protein (P130) which is phosphorylated at multiple tyrosine and serine residues in transformed cells, and which phosphorylates both itself and exogenous substrates at tyrosine in vitro. The major site of tyrosine phosphorylation within P130 is at residue 1073, which is located in the carboxy-terminal enzymatic kinase domain of P130. Oligonucleotide-directed mutagenesis of the cloned FSV genome has been used to alter the TAT codon for tyrosine (1073) to a TTT codon for phenylalanine, which cannot be phosphorylated. The mutant FSV can still transform rat-2 cells but does so with a very much longer latent period than the wild type FSV. Structural analysis of the mutant protein indicated that the principal site of tyrosine phosphorylation had indeed been lost, but that apart from the desired amino acid substitution it was indistinguishable from wild type P130. The mutant protein retains the ability to phosphorylate enolase at tyrosine in vitro, but its kinase activity for this exogenous substrate is 5-fold less than that of wild type FSV P130. To exclude the possibility that a non-specific second-site mutation was responsible for the functional impairment of mutant P130 we used site-directed mutagenesis to synthesize a revertant FSV genome. The revertant has regained the rapid transforming activity and higher level of P130 kinase activity of the wild type FSV. These data argue that tyrosine phosphorylation of this residue stimulates both the biochemical and biological activities of FSV P130, and may therefore, be important in its ability to transform cells.

0653 ALTERATION OF SUBSTRATE SPECIFICITY OF SUBTILISIN BY CASSETTE MUTAGENESIS, James A. Wells*, David B. Powers*, Jeff Miller+, Tom Graycar+, and David A. Estell+, Genentech, Inc.*, Genencor, Inc.+, South San Francisco, CA 94080.

Subtilisin is a good model system for studying structural determinants of substrate specificity by site-directed mutagenesis. We have cloned and sequenced the gene for this well characterized serine protease from B. amyloliquefaciens (Wells, J.A. et al. (1983) Nucleic Acids Res. 11, 7911-7924). The gene has been expressed and the protease secreted in large amounts in a protease deficient strain of B. subtilis.

Molecular modeling of the enzyme structure shows glycine-166 occupies a position at the bottom of the P-1 specificity pocket. Due to uncertainties in structural predictions, it was difficult to predict how specific amino acid substitutions would alter substrate specificity. Using a novel cassette mutagenesis method, all 19 amino acid substitutions have been made at position-166; the mutant proteins have been expressed and purified.

The Km against a synthetic substrate having phe at P-1 was found to vary as much as 100 fold (D-166 vs. F-166) with most neutral and small hydrophobics having reduced Km values. The turnover constant, kcat, varied as much as 600 fold (I-166 vs. G-166) indicating the binding pocket mutations did not simply affect formation of the Michaelis Complex (E.S). The catalytic efficiency (kcat/Km) varied 700 fold (W-166 vs. A-166).

Several of the mutants were evaluated against an arginyl P-1 substrate. Aspartate-166 and glutamate-166 showed dramatically reduced Km and nearly unchanged kcat compared to wild-type or their respective amid homologs against this trypsin substrate. These data show substrate specificity was significantly altered by mutagenesis of position-166. Data from other specificity pocket mutants will also be presented.

0654 CLONING, SEQUENCING, AND SITE-SPECIFIC MUTAGENESIS OF CREATINE KINASE-ENCODING cDNA

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UCSF, San Francisco, CA 94143

Creatine kinase is important for energy regulation in high energy-requiring tissues. The primary sequence of the enzyme from four species has been obtained in several laboratories by both direct protein sequencing and indirect cDNA sequencing. We have cloned and sequenced a full-length cDNA encoding creatine kinase from the electric organ of Torpedo californica. The inferred primary translation product of this cDNA is a 381 amino acid protein of molecular weight 42,941. In comparison to the inferred protein sequences of rabbit, rat, and chicken muscle creatine kinases, the Torpedo enzyme is 85%, 84%, and 83% homologous. The 18 amino acid sequence neighboring an iodoacetamide-sensitive CYS at 282 (numbering the initiating MET as -1) is 100% conserved in all four species. This high conservation between divergent species may imply strict requirements for the creatine kinase protein structure. Model building algorithms (Biochemistry 22 (1983) 4894) suggest that the C-terminal one-half of the protein, in which CYS 282 resides, is consistent with an α/β geometry; this suggestion will be tested by future crystallographic studies. To study the structural and catalytic contributions of this CYS, we have mutated our creatine kinase-encoding cDNA so that an ALA is encoded at 282. Also, the cDNA has been mutated at the initiating MET, to allow the expression of the enzyme in bacteria. The biological and chemical effects of these manipulations are now being examined.

0655 SITE-DIRECTED MUTAGENESIS AS TESTS FOR PROTEIN FOLDING MECHANISMS,

Terry B. White, Peter B. Berget, and Barry T. Nall, University of Texas Medical School, Houston, Texas 77025

The conservation of amino acid residues among homologous proteins may reflect requirements for specific residues in certain aspects of protein structure, function, and folding. Thus, understanding the reasons for the conservation of a given residue is a good foundation for understanding the relationship between amino acid sequence and protein structure and function. We propose that some conserved residues may be critical for the process of formation of tertiary structure. To test this proposal, we have altered conserved amino acid residues in yeast iso-2 cytochrome c by site-directed mutagenesis of the cloned gene and characterized the properties of protein folding in the mutant and wild type proteins.

0656 PENICILLIN G ACYLASE (E.C.3.4.1.11) SUBSTRATE SPECIFICITY MODIFICATION BY IN VITRO MUTAGENESIS,

John A. Williams, Timothy J. Zuzel, Pfizer Central Research, Groton, CT 06340

The acylase genes from E. coli W ATCC 9637 and a mutant (broA) with modified substrate specificity were cloned. Replacement subcloning of regions in the wild type gene with equivalent regions of the mutant gene localized the mutation to within a 480bp AccI/NruI internal fragment. A unique NcoI restriction site within this region in the wild gene was absent in the mutant gene fragment. DNA sequencing of this fragment identified the change as a transversion mutation of adenine to thymine. This mutation resulted in the conversion of a methionine in the wild type acylase to a leucine in the mutant acylase with the concomitant substrate specificity change. The ATG codon was subsequently changed by oligonucleotide mutagenesis to yield the following amino acids: phenylalanine, leucine, valine, alanine, threonine, glycine, histidine, lysine, glutamic acid, dileucine and divaline in place of methionine. All mutant enzymes except the lysine, glutamic acid, dileucine and divaline analogs were active on N-(3-carboxy-4-nitrophenyl)-phenylacetamide, a chromogenic substrate characteristic for the wild type acylase. However, only leucine, valine, alanine, threonine, glycine and histidine were active on N-(3-carboxy-4-nitrophenyl)-6-bromoadipamide, a chromogenic substrate characteristic for the broA mutant acylase. These results indicate that both size and chemical properties of the amino acid R group affected the resultant substrate specificity of the acylase.

Structure-Function Studies with DNA Binding Proteins

0657 DIRECTED MUTAGENESIS STUDIES WITH DNA BINDING PROTEINS AND GENE REGULATION SEQUENCES, M. H. Caruthers. L. P. Bracco, D. R. Dodds, S. J. Eisenbeis, R. Gayle, and J. Sutton, Department of Chemistry, University of Colorado, Boulder, CO 80309

The recognition of cro repressor for cro operator has been studied by altering the amino acid sequence of cro repressor and the DNA sequence of cro operator. Selected amino acid changes were introduced into cro repressor domains (the α-3 helix and the carboxyl terminus) where specific contacts between protein and DNA have been proposed (1). Altered cro proteins produced by mutants constructed in this manner were assayed for binding to lambda operator O_R3 *in vivo* (2). Mutations directed into the α-3 helix coding region of the cro gene produced altered cro proteins with a range of affinities for operator DNA. Certain altered cro repressors including the following had reduced affinity for cro operator: $Y_{26} \rightarrow$ P,L,D; $Q_{27} \rightarrow$ L,C,R; $S_{28} \rightarrow$ A,T; $N_{31} \rightarrow$ K; $A_{33} \rightarrow$ K; $R_{38} \rightarrow$ T. Other altered cro repressors such as the following had either higher or comparable affinity for cro operator: $T_{26} \rightarrow$ K; $N_{31} \rightarrow$ Q; $H_{35} \rightarrow$ K,Q,N,R; $A_{36} \rightarrow$ T,K. The cro carboxy terminus and how it interacts with cro operator was studied by deleting certain amino acids. The results show that the first four amino acids at the carboxy terminus (A_{66}, T_{65}, T_{64}, K_{63}) can be deleted without altering the affinity of cro repressor for cro operator. Removal of A_{66}-K_{62} however generates a cro repressor with reduced affinity for cro operator. These results support the hypothesis that cro repressor contacts cro operator through the α-3 helix and further suggest that contacts may also occur near the carboxy terminus (K_{62}). Additionally, these results suggest that certain amino acids (T_{26}, N_{31}, H_{34}, A_{36}) when substituted by others lead to cro repressors having comparable or higher affinity for cro operator. When these altered cro repressors are modeled by computer graphics, new or altered contacts with cro operator can be postulated. Research supported by NIH (GM21120).

1. D. H. Ohlendorff, W. F. Anderson, R. G. Fisher, Y. Takeda, and B. W. Matthews, *Nature* 298m 718-723 (1982).
2. S. J. Eisenbeis, M. S. Nasoff, S. A. Noble, L. P. Bracco, D. R. Dodds, and M. H. Caruthers, *Proc. Natl. Acad. Sci. USA*, in press.

0658 CRYSTALLOGRAPHIC STUDIES OF REPRESSOR/OPERATOR INTERACTIONS, Stephen C. Harrison, John Anderson, Cynthia Wolberger, Mark Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

The DNA-binding domain of repressor from bacteriophage 434 has been crystallized, bound to a 14 base-pair operator (1). Crystals of the cro-protein from 434 bound to the same operator have also been obtained. Progress in the structure determinations will be described. This work has been supported by NIH Grant GM29109.

(1) Anderson, J., Ptashne, M. and Harrison, S. C. (1984). "Co-crystals of the DNA-binding Domain of Phage 434 Repressor and a Synthetic Phage 434 Operator." Proc. Natl. Acad. Sci. USA 81, 1307-1311.

0659 STRUCTURE AND STABILITY OF MUTANT LYSOZYMES FROM BACTERIOPHAGE T4, Brian W. Matthews, Tom Alber, Markus G. Grütter, Terry M. Gray, Joan A. Wozniak and Larry H. Weaver, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The lysozyme from bacteriophage T4 is being used as a model system to determine the factors that influence that folding and stability of proteins. The three-dimensional structure of the protein is known (1) and lysozymes with modified properties arising from single amino acid substitutions have been obtained by classical selection techniques as well as by site-directed mutagenesis.

Several temperature-sensitive mutant lysozymes have been studied in detail. These include lysozymes with the substitutions Arg 96 → His, Met 102 → Thr, Ala 146 → Thr and Thr 157 → Ala (2,3). In each case the overall stucture of the mutant protein is very similar to that of wild type. Only for the substitution of Ala 146 by threonine is there clear evidence for structural changes of more than a few tenths of an angstrom unit that propagate away from the site of the substitution. Notwithstanding the observed small structural changes, thermodynamic measurements indicate that the mutant lysozymes have large changes in the enthalpy of unfolding that are largely compensated by changes in entropy (4).

In the case of the substitution Thr 157 → Ala it appears that several factors contribute to the alteration in stability including loss of hydrophobic interaction, loss of hydrogen bonding and changes in solvent structure. In order to differentiate between these and other effects a series of lysozymes has been constructed in which Thr 157 is replaced by other amino acids including Ala, Ser, Val, Gly, Leu, Ile, Asn, His, Arg, Glu and Asp.

In another approach to understanding protein stability, a method has been developed to screen for mutants of phage lysozyme that are more thermostable than the wild-type enzyme (5).

A number of the above mutant lysozymes have been isolated and some have been crystallized. Further analysis is underway.

(1) Remington, S.J., Anderson, W.F., Owen, J., Ten Eyck, L.F., Grainger, C.T. & Matthews, B.W. (1978) J. Mol. Biol. 118, 81-91.
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(3) Grütter, M.G., Weaver, L.H., Gray, T.M. & Matthews, B.W. (1983) In "Bacteriophage T4", C.K. Mathews, E.M. Kutter, G. Mosig & P.M. Berget, eds., 356-360.
(4) Hawkes, R., Grütter, M.G. & Schellman, J. (1984) J. Mol. Biol. 175, 195-212.
(5) Alber, T. & Wozniak, J.A. Proc. Natl. Acad. Sci. USA, in press.

0660 STRUCTURE OF DNA-EcoRI ENDONUCLEASE COMPLEX AT 3 ANGSTROMS RESOLUTION, John M. Rosenberg, Judith McClarin, John Grable, Christin Frederick, Cleopas Samudzi and Linda Jen-Jacobson, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260; Bi-Cheng Wang, Biocrystallography Laboratory, Box 12055, VA Medical Center, Pittsburgh, PA 15240; Herbert W. Boyer and Patricia Greene, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143.

The 3 A structure of a co-crystalline recognition complex between EcoRI endonuclease and the oligonucleotide TCGCGAATTCGCG will be reported. The DNA and the protein share a common (crystallographic) two-fold axis of rotational symmetry, as expected from the intrinsic symmetry of the recognition site (GAATTC). The DNA conformation within the complex is different from that found in the absence of protein suggesting that the set of conformational states which are accessable to protein-free DNA is expanded by the binding of sequence specific proteins. These include the torsional neo-1 kink which widens the major groove by unwinding the DNA by approximately 25° thereby facilitating contact between the edges of the purine bases and amino acid side chains. A second (neo-2) kink is located three base-pairs away which also facilitates access of protein to DNA and/or has a role in the hydrolytic mechanism of this enzyme.

A five stranded α/β structure forms the foundation of each endonuclease subunit with the strands of β-sheet and the α-helices oriented approximately perpendicular to the average DNA helix axis. Polypeptide loops at the carboxy edge of the β-sheet form a cleft which contains the segment of DNA backbone spanning the scissile bond. (DNA hydrolysis was inhibited via omission of Mg^{+2}). The cleft is complementary to one strand of double helical DNA and its shape determined by the intrinsic twist of β-sheet. This novel feature suggests that DNA and protein are intrinsically complementary at a fundamental level.

Sequence specificity is determined by "modular" interactions. One large symmetric module recognizes the inner tetranucleotide (AATT) while two additional symmetry-equivalent modules recognizes the outer base pairs. The inner module consists of two symmetry equivalent α-helices which project from the α/β units. Lysine and glutamic acid side chains at the ends of the α-helices hydrogen bond to the adenine residues thereby determining the specificity for the inner tetranucleotide. The outer module is formed by a separate segment of the polypeptide chain containing an arginine side chain which hydrogen bonds to guanine.

0661 PHAGE REPRESSORS, R. Sauer, J. DeAnda, F. Gimble, M. Hecht, H. Nelson, A. Pakula, A. Vershon and M. Weiss, Department of Biology, M.I.T., Cambridge MA 02139

The repressor proteins of bacteriophage λ and P22 provide good model systems for protein folding, protein-protein interactions, and protein-DNA recognition. We have been using genetic, biochemical, and physical methods to study the roles of specific repressor side chains in these processes. We will discuss mutations that increase and decrease protein thermal stability, dimerization, and operator and non-operator DNA binding.

Structure-Function Studies with DNA Binding Proteins

0662 MECHANISM OF DNA-SEQUENCE RECOGNITION BY CAP AND LAC REPRESSOR

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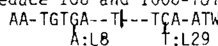
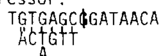
Figure 1 gives the sequence of the consensus DNA site for CAP; L8 and L29 are symmetrically related substitutions that reduce 100 and 1000-fold the affinity for CAP.



We have shown that substitution of Glu181 of CAP by Lys, Leu, or Val alters the DNA-sequence specificity of CAP. *In vitro* binding experiments with Glu181→Leu and Glu181→Val confirm that the mutant proteins interact tightly with L8, L29, and L8-L29, but fail to bind with normal affinity to the wild-type DNA site. The conformation of the mutant proteins is indistinguishable from that of wild-type CAP as assayed by: (a) reactivity to trypsin, chymotrypsin, subtilisin, and V8 protease, and (b) inter-subunit Cys178-Cys178 crosslinking. We conclude that Glu181 is the amino acid of CAP that contacts base pairs 7 and 16 of the DNA site.

Figure 2 presents the sequence of the lac operator; the indicated O^c mutations reduce 12 to 130-fold the affinity for Lac repressor.



Lac repressor contains a helix-turn-helix motif homologous in sequence and structure to that in CAP. Our objective was to identify the base pair of lacO contacted by Gln18 of Lac repressor, the residue positionally equivalent to Glu181 of CAP.

We have investigated the interaction with O^+, and with each O^c illustrated above, of mutationally altered Lac repressors in which Gln 18 was substituted by Gly, Ser, or Leu. We find that the Gln18→Gly mutant is unable to distinguish between the O^c base T and O^+ G at position "4" of lacO (K_{DO^c}/K_{DO^+} = 0.93). In contrast, the mutant protein discriminates O^c from O^+ by a factor of 13 to 23 at each other position. The same pattern of results is obtained with Gln18→Ser and Gln18→Leu.

Gln 18→Gly and Gln18→Ser also are unable to discriminate between O^c base A and O^+ base G at position "4". Gln18→Leu interacts at least 5-fold more tightly with O^c base A than O^+ G at position "4".

We propose that Gln18 contacts G:C base pair "4" of lacO. These data suggest that the interaction between DNA and the helix-turn-helix motif may be very similar or identical in CAP and Lac repressor.

0663 REPRESSORS HAVE EXCHANGEABLE α-HELICES THAT SPECIFY OPERATOR BINDING,
Robin P. Wharton and Mark Ptashne, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, Mass. 02138

We have reported that the putative DNA-binding α-helix of bacteriophage 434 cro portein can be substituted for the putative DNA-binding α-helix of 434 repressor to create a hybrid protein named repressor* (1). The specific DNA contacts made by repressor* are like those made by 434 cro protein.

We have extended this experiment by replacing the putative DNA-binding helix of 434 repressor with the putative DNA-binding helix of bacteriophage P22 repressor. Wildtype P22 repressor binds to P22 operators and not to 434 operators. We synthesized a segment of a 434 repressor gene that encodes an artificial α-helix; the amino acids that are predicted to lie on the inside of this helix (and therefore on the inside of the 434 repressor protein) were left as the appropriate amino acids of 434 repressor, and the amino acids that are predicted to lie on the outside of this helix (and are therefore presumably free to interact with DNA) were replaced with the appropriate amino acids of bacteriophage P22 repressor. We inserted this artificial gene segment into the remainder of the 434 repressor gene. The protein encoded by the resulting synthetic gene, named "434 repressor ('α3' P22 repressor)," binds specifically to P22 operators and not to 434 operators in vivo and in vitro.

(1) Wharton, R.P., Brown, E.L., and Ptashne, M. (1984). Substituting an α-helix switches the sequence-specific DNA interactions of a repressor. Cell 38, 361-69.

DNA Binding Proteins

0664 PROTEIN NUCLEIC ACID INTERACTIONS IN PRERIBOSOME BIOGENESIS,
François Amalric, Anne Martinel, Michèle Caizergues-Ferrer and Gérard Bouche, CRBGC, Toulouse 31062, France.
In eukaryotic cells, the ribosomal genes (200 to 400) were localized in a particular structure, the nucleolus. Two classes of proteins were involved in the first steps of preribosomes biogenesis! Most of the ribosomal proteins that were recovered in the nature cytoplasmic ribosomes and proteins specific of the nucleolus that were transitorely associated with the nascent ribonucleoproteins. Among them, several were immunologically related with a 100 kDa protein that is the major species. It's metabolism is clearly related to the rDNA transcription level. We have shown that a 130 kDa is the precursor of the 100 kDa. The two proteins possessed affinity for rDNA and pre rRNA. Competition between different rDNA fragments and random sequences, showed an increase in the affinity for rDNA by a factor 2.5. However due to the high concentrations of the proteins and of the rDNA sequences, a simple calculation demonstrated that in vivo, most of the rDNA could interact preferentially with the two proteins. To get an insight on the eventual function of these proteins in the transcription and maturation of pre rRNA, in vitro experiments have been carried out. Run off experiments were developped using cloned DNA containing the 5'end of rDNA as template. The 130 kDa and 100 kDa proteins have no effect on the specificity of initiation and on the amount of transcribed RNA. During the reaction the 100 kDa protein was cleaved in several defined peptides. The addition of leupeptin, a protease inhibitor, resulted in a complete and selective inhibition of rDNA transcription. The 130 kDa protein could bind to rDNA to keep the gene to be transcribed in an open structure. During the elongation of transcription, the 130 kDa would be cleaved and the maturation product (100 kDa) translocated to the nascent pre rRNA.

0665 TRYPTOPHAN BINDING PROPERTIES OF ESHERICHIA COLI TRYPTOPHAN APOREPRESSOR, · Dennis N. Arvidson, Can Bruce, and Robert P. Gunsalus, Microbiol. Dept. and Mol. Biol. Inst., UCLA, Los Angeles, CA 90024
Transcription of the trp trpR and aroH operons of E. coli is regulated by the trp repressor in response to the intracellular L-tryptophan concentration. We have purified the tryptophan aporepressor and find that it is homogeneous with respect to aggregation, existing as a dimer. Tryptopan binding properties of purified tryptophan aporepressor were studied by equilibrium dialysis and flow dialysis. Results obtained by these two methods were in agreement. The aporepressor dimer has two identical and independant binding sites for the corepressor L-tryptophan. The equilibrium dissociation constant was 41 μM at 21 °C in a buffer consisting of 10 mM potassium phosphate pH 7.4, .2 M potassium chloride, .1 mM EDTA and 5 % glycerol. The affinity of aporepressor for L-tryptophan decreased with increasing temperature and increased with increasing salt between .1 M and .4 M potassium chloride.

0666 STRUCTURES AND RELATIONSHIPS BETWEEN DIFFERENT PROTEINS THAT RECOGNIZE THE DNA SEQUENCE 5'-CC(A/T)GG-3', Ashok S. Bhagwat and Richard J. Roberts, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Several restriction endonucleases and methylases are known to recognize the sequence 5'-CC(A/T)GG-3'. Enzymes EcoRII, EclII, BstNI and ApyI are some of the examples. A natural E. coli plasmid N3 codes for the EcoRII restriction-modification system. EcoRII endonuclease cleaves this sequence before the first C unless the second C is methylated by the EcoRII methylase. E. coli chromosome carries a gene, dcm, that codes for a methylase with the same specificity as the EcoRII methylase. BstNI endonuclease, in contrast, cleaves the DNA between C and A regardless of whether the second C is methylated. We have cloned the genes for the EcoRII proteins and the dcm locus onto multi-copy plasmid and phage vectors. Using the cloned genes as probes in Southern blotting studies, we have found that although Enterobacter cloacae strain that codes for EclII carries sequences highly homologous to EcoRII genes, neither the cloned dcm nor the DNA from Bacillus Stearothermophilus strain N (BstNI) are very homologous to EcoRII. We are in the process of determining the structures of these proteins by sequencing the genes. We are also interested in making mutations in these genes to alter the DNA sequence specificity of the coded proteins.

0667 DNA POLYMERASE I: TOPOLOGY MEASURED WITH BzATP AND ANTIBODIES, W.E. Brown and J.B. Bodner, Carnegie-Mellon University, Pittsburgh, PA. 15213
Utilizing Pol I produced by the overproducing strain ATL100 (J. Biol. Chem. 259 10386-10392), studies have been started to identifiy amino acids in the multiple binding sites on the polymerase holo-enzyme. Two sites in particular are the focus of the analysis: the triphosphate and template binding regions. In the first case, the photo-activatable reagent 3'-O-(4-benzoyl)benzoyl-ATP (BzATP) is being used as an affinity label for the triphosphate site. Results with this reagent indicate parallel inhibition of both the polymerase and exonuclease activities. The polymerizing activity can be protected by competition with dATP but not by dAMP or template primer. Labeling is restricted only to the large fragment portion of the molecule and incorporation of labeled BzATP responsible for activity can be prevented by competition with dATP. Amino acids proximal to the triphosphate site and labeled by BzATP will be mapped. In the second set of studies, antibodies inhibitory to pol I have been isolated. These antibodies recognize the resolved CNBr peptides of pol I in Western blots. Affinity selected "pseudo-monoclonal" antibodies to each of the peptides are being used to determine the regions of the protein not involved in DNA binding and regions made accessible through prior binding to DNA. This work was supported by NIH grant GM-24688 to WEB.

0668 THE EFFECT OF AMINO ACID SUBSTITUTIONS ON THE STRUCTURE AND STABILITY OF THE N-TERMINAL DOMAIN OF LAMBDA REPRESSOR, Michael H. Hecht, Hillary C. M. Nelson, and Robert T. Sauer, Massachussets Institute of Technology, Cambridge, Mass, 02139

Mutations coding for many differrent single amino acid replacements in the DNA binding domain of phage lambda repressor were isolated and characterized. The thermal stabilities of purified mutant repressors were studied and compared with the temperature-sensitive activity of the mutants in vivo. In general, mutant proteins bearing solvent exposed substitutions have thermal stabilities identical to wild type, whereas buried substitutions reduce stability. In one case, a single amino acid substitution increases the thermal stability of repressor.

Same-site and second-site revertants of mutations in the N-terminal domain have been isolated and characterized. These revertants restore activity by introducing favorable interactions that are not found in the wild type protein. The molecular basis for restoration of activity is interpreted in terms of the crystal structure of the N-terminal domain.

0669 DNA BINDING MUTATIONS IN THE Tn10 tet REPRESSOR, Paul J. Isackson and Kevin P. Bertrand, University of California, Irvine, CA 92717

The tetracycline resistance determinant in transposon Tn10 consists of two genes, the tetA resistance gene and the tetR repressor gene, that are transcribed from divergent promoters. In the absence of tetracycline, tet repressor inhibits transcription of tetA and tetR by binding to two adjacent operator sites that overlap the tet promoters. Previous sequencing studies had identified a region of tet repressor (amino acids 27-46) that is homologous to the "helix-turn-helix" regions of λ repressor, Cro, CAP, and a number of other sequence-specific DNA-binding proteins. We isolated trans-dominant tetR mutants ($tetR^{-d}$) that encode mutant repressors which are deficient in operator binding, but retain some capacity to form dimers with, and thereby inactivate, wild-type repressor monomers. The mutants were isolated on a tetR-containing plasmid by transforming a $tetR^{+}$ tetA-lacZ fusion strain with hydroxylamine-mutagenized tetR plasmid DNA, plating on lactose MacConkey agar, and picking weakly Lac^{+} colonies amongst a background of Lac^{-} colonies. DNA sequence analysis of 50 independent $tetR^{-d}$ mutants identified seven different mutations, six of which are in the region of "helix-turn-helix" homology. These results provide strong support for the proposal that this region of tet repressor makes direct contacts with tet operator DNA.

0670 FUNCTIONAL ROLE OF THE N-TERMINAL DOMAIN OF ECORI ENDONUCLEASE, L. Jen-Jacobson, D. Lesser & M. Kurpiewski, Dept. Biol. Sci., Univ. Pittsburgh, Pittsburgh, PA 15260

Proteolysis of Eco RI endonuclease-DNA complexes selectively deletes defined segments of an N-terminal domain. We find that residues 4-28 are essential for DNA scission, but not for high-affinity sequence recognition or homodimer formation.

In the absence of DNA containing the recognition site d(GAATTC), EcoRI endonuclease is rapidly and completely degraded by proteases. In the presence of 12 or 13 bp cognate DNA, the 31 kDa subunits are quantitatively converted to large fragments which are entirely resistant to further proteolysis. DNAs lacking the recognition sequence do not protect the endonuclease. The identities and functional properties of the purified proteolytic "deletion derivatives" are:

Protease	Protected	Properties of Fragment		
	Region	DNA Cleavage	Binding	Homodimer
None	1-276	Yes	Yes	Yes
Trypsin	29-276	No	Yes	Yes
Chymotrypsin	27-276	No	Yes	Yes
V8 Protease	12-276	Nicking?	Yes	Yes
Lys-C	3(4)-276	Yes	Yes	Yes

All potential protease targets in the N-terminal domain remain accessible. We infer that this domain lies in the periphery of the enzyme-DNA complex. Protection of the C-terminal 90% of the molecule by bound cognate DNA suggests that a general "tightening" of the endonuclease conformation accompanies sequence-specific binding.

0671 RELATING STRUCTURE TO FUNCTION FOR DNA POLYMERASE I OF E. COLI, Catherine M. Joyce, David Ollis, Thomas A. Steitz and Nigel D.F. Grindley, Yale University, New Haven, CT 06510

The three-dimensional structure of the Klenow fragment of DNA polymerase I has recently been solved to 2.8A resolution, allowing fitting of the primary sequence to the electron density map. The molecule has two domains. The smaller (N-terminal) domain contains the dNMP binding site, which is probably related to the 3'-5' exonuclease active site. The larger domain contains a cleft that we hypothesize to be the DNA binding site. We are following several lines of investigation to assist our interpretation of the structure:

1) Footprinting studies indicate that Klenow fragment covers about 12 bp of duplex DNA upstream of the primer terminus. This conclusion is supported by our observation that a restriction fragment having only 11 bp of duplex DNA, together with 4-nucleotide 5' extended ends, is a good substrate in the primed synthesis reaction. Additional contacts extend at least 4 nucleotides beyond the primer terminus.
2) Sequence characterization of the polA5 and polA6 mutations, which affect the polymerase-DNA interaction, indicates that the changed amino acids map on or close to the surface of the proposed DNA-binding cleft.
3) By sub-cloning, we have overproduced separately the two domains of the Klenow fragment. We plan to determine whether either domain has enzymatic or substrate binding activity.
4) We are using oligonucleotide-directed mutagenesis to make specific changes in the dNMP binding region, in the hope of locating residues involved in the 3'-5' exonuclease activity.

The results of these studies will be presented.

0672 PHOTOAFFINITY LABELING OF A NUCLEIC ACID HELIX-DESTABILIZING PROTEIN, Richard L. Karpel, Valerie Yrttimaa Levin and Boyd E. Haley*, Dept. of Chemistry, University of Maryland Baltimore County, Catonsville, MD 21228 and *Dept. of Biochemistry, University of Wyoming, Laramie, WY 82071

In order to delineate the nucleic acid binding site on helix-destabilizing (single strand-specific) proteins (HDPs), we have synthesized the photoaffinity label, ^{3}H-poly(A,8N$_3$A). This material quenched the intrinsic tryptophan fluorescence of the T4 HDP, gene 32 protein (32P) in a manner similar to that observed with other polynucleotides. Addition of sufficient NaCl significantly reversed the effect. However, irradiation at 254 nm of 32P-poly(A,8N$_3$A) mixtures prior to addition of salt prevented reversal by NaCl, suggesting that the label was covalently bound to the protein. 32P-^{3}H-poly(A,8N$_3$A) mixtures subjected to short periods of irradiation (5 min, 9000 erg mm^{-2} or less) formed high molecular weight complexes, which on SDS polyacrylamide gels were radioactive and stained with Coomassie Blue R. Under the same conditions, ^{3}H-poly(A) failed to label 32P, though cold poly(A) was observed to competitively inhibit labeling by ^{3}H-poly(A,8N$_3$A). When subjected to the action of Staph. nuclease after irradiation but prior to electrophoresis, the ^{3}H-poly(A,8N$_3$A) radioactivity was seen to migrate just behind the 32P monomer. Preliminary results suggest that the affinity-labeled material could be separated from unlabeled 32P on single-stranded DNA-cellulose, and from free poly(A,8N$_3$A) on DEAE-Sephacel. We are currently attempting to locate the label within the 32P sequence, and are exploring the interactions of this useful photoaffinity probe with other HDPs.

0673 PATTERN RECOGNITION AND THE STRUCTURAL DETERMINATION OF PROTEINS, Susan M. Kilkowski and Peter A. Kollman, University of California, San Francisco, CA, 94143

The structures of cro, cI, and cII repressors, CAP, and the EcoRI restriction endonuclease are predicted using a pattern recognition program which uses as its sole input the protein sequence and a set of structure parameters. The parameter set combines information about hydrophobicity with steric and electrostatic constraints and when displayed spatially indicates secondary structure and gives residue-by-residue information about which amino acids along a particular piece of secondary structure will be buried. This method suggests folding pathway differences between proteins and yields information about whether each residue has primarily a structural role or contributes to specificity (site-specific binding to DNA) within its class (DNA-binding proteins). Structure determination by pattern recognition is a general method that has been applied successfully to other classes of proteins.

0674 NUCLEOTIDE SEQUENCE OF THE *BSU*RI RESTRICTION-MODIFICATION SYSTEM,
Antal Kiss,* Gyorgy Posfai,[+] Janos Posfai,[+] Pal Venetianer[+] and Richard J. Roberts,* *Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 and [+]Biol. Res. Cent. Hung. Acad. Sci. 6701 Szeged, Hungary

The GGCC-specific *Bsu*RI system of *B. subtilis* R has been cloned in *E. coli* using selection based on the expression of the methylase. Expression in *E. coli* of the *Bsu*RI endonuclease was demonstrated by phage infection experiments and by showing the presence of *Bsu*RI endonuclease activity in cell-free extracts of the clone. The nucleotide sequence of the genes coding for the *Bsu*RI enzymes has been determined. The two genes are oriented in the same direction with the endonuclease gene being upstream from the methylase gene. The coding regions of the two enzymes are separated by 784 bp. Comparison of the amino acid sequences derived from the DNA sequence revealed no homology between the two proteins. However, regions of homology were found between the *Bsu*RI methylase and two other GGCC-specific DNA methylases, the *Bsp*RI and SPR methylases.

0675 ANALYSIS OF THE FUNCTIONAL DOMAINS OF THE DNA-BINDING PROTEIN OF ADENOVIRUS TYPE 5.
Geoffrey R. Kitchingman and Cheryl O. Quinn, St. Jude Children's Research Hospital, Memphis, TN 38101.
The single-stranded DNA-binding protein (DBP) of adenovirus is involved in the regulation of DNA synthesis, and of RNA synthesis at the levels of transcription, mRNA stability and RNA processing. We are utilizing a combined structural and genetic approach to determine the regions of this 72,000 dalton protein that are involved in each of these functions.

Sequences of the DBPs from several adenovirus serotypes have been determined and compared to identify regions that have been highly conserved - presumably these represent regions important for the function and/or structure of the protein. We have identified three exactly conserved regions of 9, 9 and 12 amino acids in the carboxy-terminal 44K domain of this protein that have some of the features of prokaryotic single-stranded DBPs. Using oligonucleotide-directed site-specific mutagenesis, we have changed the aromatic and charged amino acids in each of these conserved regions to uncharged, neutral amino acids and are in the process of determining the affects of these mutations on several of the functions of the adenovirus DBP. These functions include the ability of the protein to bind to single-stranded DNA and the ability of the DBP to help adeno-associated virus grow, a function that probably mimics one that occurs during lytic adenovirus growth.

0676 COOPERATIVE BINDING OF DNA AND ATP TO DNA GYRASE,
Anthony Maxwell and Martin Gellert, LMB, NIADDK, National Institutes of Health, MD 20205

The DNA supercoiling catalyzed by DNA gyrase is carried out at the expense of ATP hydrolysis, and DNA greatly stimulates the ATPase activity of the enzyme. Although closed circular DNA is necessarily the substrate for DNA supercoiling, other forms of DNA will also serve as cofactors for ATP hydrolysis. Using linear DNA molecules of different lengths, we have found that while those of 100 bp or more are effective cofactors for the gyrase ATPase reaction, molecules less than 70 bp are ineffective under the same conditions. However, when short DNA molecules (<70 bp) are employed at much higher concentrations, significant stimulation of the gyrase ATPase reaction is observed; the dependence of the ATPase on the concentration of small DNA fragments is sigmoidal. These data are supported by binding studies of DNA to DNA gyrase and are consistent with the interaction of DNA with 2 sites on the enzyme, with positive cooperativity between the 2 sites. In the presence of long DNA molecules (>100 bp) the initial rates of ATP hydrolysis by DNA gyrase do not show the usual hyperbolic dependence upon substrate (ATP) concentration. Instead, v against s curves are sigmoidal, indicating the cooperative binding of 2 ATP molecules to the enzyme prior to hydrolysis. A model for the interaction of DNA and ATP with DNA gyrase is suggested.

0677 STRUCTURE OF DNA-EcoRI ENDONUCLEASE COMPLEX AT 3 ANGSTROMS RESOLUTION, Judith McClarin, John M. Rosenberg, John Grable, Christin Frederick, Cleopas Samudzi and Linda Jen-Jacobson, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260; Bi-Cheng Wang, Biocrystallography Laboratory, Box 12055, VA Medical Center, Pittsburgh, PA 15240; Herbert W. Boyer and Patricia Greene, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143.

The main structural features of the EcoRI endonuclease interaction with the oligonucleotide TCGCGAATTCGCG are revealed in the 3 Å electron density map of the co-crystalline recognition complex. The protein consists of two subunits related by a molecular (and crystallographic) two-fold axis. Each subunit is an α/β structure and a mini domain. The α/β structure contains five stranded β sheet surrounded by helices. The average helix axis is in the plane of the β sheet allowing the intrinsic twist of the β sheet to form a cleft containing segments of the backbone of DNA.

Sequence specificity is determined by two pairs of symmetry related modules. The inner tetranucleotide, AATT, is recognized by a large symmetric module where the specific interactions cross the molecular two-fold such that residues from both subunits are physically adjacent in three dimensions. The inner module consists of a pair of lysine and glutamate residues which project from α helices. The outer module, which recognizes a GC base pair contains an arginine side chain.

0678 MOLECULAR MODELLING OF PROTEIN-NUCLEIC ACID INTERACTIONS, Joseph J. McDonald, Roswell Park Memorial Institute, Buffalo, N. Y., 14263

Computer modeling techniques are used to study the interaction of proteins with nucleic acids. The methods utilize both information from genetic and chemical modification experiments, as well as macro molecular constructural constraints. These techniques, in conjunction with theoretical energy calculations, have been applied to the study of lac and cro repressor-operator systems. Several modecular models of the putative helical segment of cro interacting with its OR3 operator have been examined[1]. This has been done by taking advantage of the super secondary structural motif which appears in a number of aligned sequences of DNA-binding proteins, as well as the DNA/ protein backbone complementarity (i.e., isogeometry and iso periodicity of DNA and protein backbones, as well as the spatial fitting of interacting sites). The models which have been examined are reflective of possible intermediate conformations experienced by the repressor in recognition of the operator sequence. The predicated interactions between lac and its operator agree with those reported for lac, as proposed by Matthews & coworkers, which were based upon the sequence alignment with the cro repressor.

[1] Rein et al, J. Biomolecular Structure and Dynamics 1:1051 (1983)

0679 EcoRI METHYLASE: ACTIVE SITE MAPPING VIA PHOTOAFFINITY LABELING, Norbert O. Reich, Herbert W. Boyer and Patricia Greene, Univ. of California, San Francisco 94143
The EcoRI modification enzyme methylates the sequence d(GAATTC) in double-stranded DNA at the two central adenines to yield 6-methylaminopurine. Our aim is to determine which portions of the monomeric protein are responsible for S-[methyl]-adenosyl-L-methionine (SAM) binding. DNA-protein interactions are also being investigated via photolysis of the DNA-protein-SAM complex. S-[methyl-^{3}H]-adenosyl-L-methionine alkylates the EcoRI methylase upon irradiation with 254 nm light. Comigration (on an SDS-PAGE gel) of radioactivity and protein after SDS treatment and boiling are consistent with covalent linkage. No radioactivity comigrates with the protein without irradiation. No retention of radioactivity results when the methylase is pre-incubated with cold SAM, irradiated, and then treated with [methyl-^{3}] SAM and irradiated. Current work is aimed at analyzing which amino acid(s) are methylated. The use of SAM as a specific photoaffinity label in characterizing the EcoRI methylase active site appears to be useful.

0680 LAC REPRESSOR TRANSCRITIONAL CONTROL: TWIST AT THE DNA BINDING SITE, Henri M. Sasmor and Joan L. Betz, University of Colorado Health Science Center, Denver, Colorado
Analysis of a set of lac operator mutants, as well as the recent finding of a symmetric lac operator sequence, indicates that the repressor protein attempts to make symmetric contacts at its specific binding site. In order to favorably align and facilitate these contacts on the natural operator sequence, the protein alters the DNA structure. The conformational perturbation of the DNA effected by repressor binding in the specific mode was investigated using a gel electrophoresis system and a series of specific poly-operator DNA molecules. It was demonstrated that DNA structural changes occur and that this perturbation is propogated into adjacent sequences. Additionally it was found that specific sequences, immediately external to the central 21 bp known to be important in protein binding, contribute stabilizing energy to the binding interaction in vitro as well as in vivo. These results suggest that lac repressor binding specifically to the lac operator twists the DNA structure; the generated DNA conformational change is propogated into the adjacent sequences and this perturbation affects the contiguous RNA polymerase binding and transcriptional initiation sites. In part, this DNA structural change helps mediate transcriptional control at this site. Preliminary evidence utilizing the tetracycline promoter of pBR322 suggests that such in vivo control at transcriptional sites can be effected by the repressor protein. The symmetry of the DNA binding site is compatible with the projected repressor protein structure (envisaged by analogy to the known structures of several other site specific DNA binding proteins) and provides a functional mechanism to effect transcriptional control.

0681 EUKARYOTIC SITE-SPECIFIC RECOMBINATION IN VITRO: INTERACTION OF THE FLP PROTEIN OF THE YEAST 2 MICRON PLASMID WITH ITS RECOMBINATION SITE, Julie F. Senecoff, Robert C. Bruckner, and Michael M. Cox, Department of Biochemistry, College of Agriculture and Life Sciences, University of Wisconsin-Madison, Madison, WI 53706.
The recombination site for the yeast FLP protein promoted recombination reaction consists of a 34 base pair region of the 2 micron plasmid DNA. This region includes two 13 base pair inverted repeats separated by an 8 base pair spacer. Changing the size of the spacer region abolishes recombination. A third copy of the 13 base pair repeat, lying immediately adjacent to the site, is unnecessary for recombination. In vitro, recombination sites with deletions of up to 3 but not more than 5 base pairs from one repeat still undergo efficient recombination. During the recombination reaction, the FLP protein cleaves the DNA within the recombination site. This cleavage occurs at the boundary of the spacer and the inverted repeat, generating an 8 base pair staggered cut. The protein becomes covalently attached to the DNA via a 3' phosphate and generates a 5' protruding terminus.

0682 COOPERATIVE DNA BINDING OF DROSOPHILA HSTF: IMPLICATIONS FOR SYMMETRIC AND DYNAMIC INTERACTIONS, David J. Shuey and Carl S. Parker, Division of Chemistry, California Institute of Technology, Pasadena, California 91125
Heat shock response in Drosophila is a well studied regulatory system involving both transcriptional and translational control. Upon temperature elevation, the transcription of a set of seven genes is induced while transcription of other genes is repressed. A key component of this response in Drosophila appears to be a heat shock gene-specific transcription factor (HSTF). The HSTF has been shown to bind to a position upstream from the TATA homology of many of the heat shock genes by DNAse I footprinting (Parker and Topol, 1984). Deletion studies indicate that this protected region of the hsp 70 promoter actually consists of two contiguous HSTF binding sites and that the HSTF binds to these two sites cooperatively.
Dimethyl sulfate (DMS), a methylating agent specific for N7 of guanine and N3 of adenine, has been used as a probe to study HSTF-DNA interactions. In DMS-footprinting experiments the protein-DNA complex is formed followed by exposure to DMS. Subsequent methylation-specific cleavage of the DNA reveals residues whose reactivity has been decreased or, in some cases, enhanced by the presence of bound HSTF. The pattern observed displays close contacts, in both the major and minor groves, exhibiting marked rotational symmetry within each binding site suggestive of a multimeric HSTF unit. Interference studies, involving DNA methylation prior to protein binding, support these findings. Certain residues protected in the DMS-footprinting studies, when methylated prevent specific binding of the HSTF. In addition, a subset of these critical contacts in those complexes where one binding site is occupied differ when both binding sites are occupied. This suggests protein conformational changes may occur upon cooperative binding to the second site.

0683 CONSERVATION OF BIOCHEMICAL AND BIOLOGICAL PROPERTIES OF MAMMALIAN AND YEAST RAS PROTEINS, Irving, S. Sigal, Deborah DeFeo-Jones, Gretchen L. Temeles, Jackson B. Gibbs, Jill S. D'Alonzo, Kelly Tatchell, Douglas R. Lowy and Edward M. Scolnick, Merck Sharp & Dohme Research Laboratories, West Point, PA. 19486.

The mammalian ras oncogenes Harvey, Kirsten and N-ras encode homologous 21,000 dalton polypeptides (p21). Transforming variants of these proteins have amino acid substitutions at positions 12, 59 and 61. The yeast *Saccharomyces cerevisiae* also has 2 essential ras related genes SC1 and SC2 which encode proteins of 309 and 322 amino acids, respectively, in which the initial 172 NH_2-terminal amino acids are 62% homologous with that of p21. The biochemical properties of both mammalian and yeast ras protein species have been studied using bacterially expressed proteins. The normal forms of p21 and SC1 form GTP complexes which undergo hydrolysis to the GDP complexes. The GTPase activity of both p21 and yeast ras proteins are reduced by those amino acid substitutions leading to oncogenic transformation. These observations suggest that the [ras protein * GTP] complex is a positive growth effector which deactivates on hydrolysis to [ras protein * GDP]. Expression of Harvey p21 in yeast complements the deletion of the yeast ras genes. The 161 amino acid NH_2-terminal domain of yeast ras proteins when fused to the 24 amino acid COOH-terminus of p21 transforms NIH 3T3 cells when "oncogenic" amino acid substitutions are present.

0684 A RAMAN DYNAMIC PROBE FOR ELUCIDATION OF PROTEIN-DNA INTERACTION SITES IN VIRUSES AND REPRESSOR-OPERATOR COMPLEXES. George J. Thomas, Jr. and James M. Benevides, Department of Chemistry, Southeastern Massachusetts University, North Dartmouth, MA 02747

Laser Raman spectroscopy has been employed as a probe of deuterium exchange of purine 8C-H groups in nucleic acids exhibiting different secondary structures and different hydrogen bonding schemes. DNA and RNA secondary structures significantly and specifically retard the rate of purine 8C-H exchange. The retardation factor (R) is greatest for the A helix (9.5 ± 1), intermediate for the B helix (2.8 ± 0.6) and smallest for the Z helix (1.5), thus permitting the three authenticated DNA structures to be distinguished from one another using the Raman dynamic probe. An extraordinarily large retardation of exchange (R 200) occurs in structures containing Hoogsteen base pairing, attributable to the effect upon the exchange mechanism of hydrogen bonding at the purine 7N acceptor site. These results indicate that 8C-H exchange may be exploited, not only to detect Hoogsteen interactions, but also to identify hydrogen bonding roles of purine 7N sites in complexes of DNA or RNA with nucleic acid binding proteins. The feasibility of the method has been evaluated in applications to enveloped genomes of DNA and RNA viruses and to repressor-operator complexes.

Equilibrium Raman data which reveal specific hydrogen bonding interactions of key amino acids side chains in dimers of lambda (cI) repressor will also be discussed.

Supported by N.I.H. Grants AI11855 and AI18758.

0685 TRYPTOPHAN REPRESSOR PROTEIN INTERACTIONS WITH L-TRYPTOPHAN, Eldon L. Ulrich, Michael J. Tsapakos, Paul V. Haydock, Jill Zeilstra-Ryalls, and Ronald L. Somerville, University of Wisconsin-Madison, Madison, WI 53706 and Purdue University, W. Lafayette, IN 47907

The structure of the *Escherichia coli* Trp repressor and its interactions with L-tryptophan are being investigated by one- and two-dimensional ^1H nuclear magnetic resonance spectroscopy. First order peak assignments have been made based on the chemical shift and coupling patterns of resonances observed in 2D correlated spectra. ^1H NMR spectra of the aporepressor (repressor lacking bound L-trp) contain several sharp lines, assigned to one tyrosine and several alanine and threonine residues, superimposed on an envelope of broad resonances. These amino acids may be located in a flexible solvent exposed structure similar to that observed for other DNA binding proteins. Few N-H peaks are observed with samples in ^2H_2O indicating that water is able to penetrate much of the protein structure. Addition of L-trp affects the chemical shift of methyl peaks assigned to one valine, a leucine or isoleucine, and one threonine or alanine and the line widths of several other protein resonances. The apo and holorepressor appear to have significant although not large structural differences. The interaction between the repressor protein and L-trp causes up-field shifts in the resonances for the $C^{\delta 1}$, $C^{\zeta 1}$, $C^{\zeta 2}$, and C^{η} protons and a downfield shift in the $C^{\varepsilon 1}$ proton peak.

0686 NMR OF LAMBDA CRO REPRESSOR: SEQUENTIAL ASSIGNMENTS, STRUCTURAL INFERENCES
Paul L. Weber, David E. Wemmer, and Brian R. Reid, University of Washington, Seattle, WA 98195

We have almost completed the sequential NMR resonance assignments for lambda phage cro repressor. Several different recent techniques, including phase-sensitive NOESY, optimized RELAY, and double quantum coherence spectroscopy were used to generate these results. The use of these experiments in assigning the rather crowded cro spectrum is discussed. The NOE data obtained from the NOESY experiments were also used to assess the conformation of cro in solution, in particular its secondary structure and the geometry of the bend between the α_2 and α_3 helices. Future work involves investigating the structure of the cro repressor-operator DNA complex; preliminary findings will be presented.

0687 POLYPEPTIDE OLIGOMERS ORGANIZE NUCLEAR RIBONUCLEOPROTEINS, J. Wooley, S. Chung, J. Wall and W. LeStourgeon, NSF, NIH, Brookhaven Natl. Labs, and Vanderbilt Univ.
Nuclear pre-messenger ribonucleoprotein complexes termed 40S monoparticles or RNPs, released from mammalian nuclei by mild RNase action, contain 6 major polypeptide species, termed A1 (32KD), A2 (34KD), B1 (36KD), B2 (37KD), C1 (42KD), and C2 (44KD), respectively. A1, A2 and C1 are equimolar and 3-fold more abundant than B1, B2 and C2. Determination of the mass of RNPs (globular, somewhat irregular, complexes about 220A in diameter) by scanning transmission electron microscopy, coupled with the RNA/protein ratio and protein stoichiometry, indicates that there are about 50 polypeptides per individual RNP. Zero-length and other cross-linking reagents produce notably three homotrimeric species, $(A1)_3$, $(A2)_3$, and $(C1)_3$; heterologous A1-A2, A2-B1, and C1-C2 species are also formed. Further *in vitro* RNase digestion of RNPs rapidly releases a new 44S nuclease-resistant complex,or NRC (uniform in size,about 180A in diameter),containing only 200 nucleotides of RNA along with A2 and B1 quantitatively recovered in their original 3:1 molar ratio. The mass of the NRC is about the same (2MD) as that of the original monoparticle; EM counting experiments indicate that 4 RNPs give rise to 1 NRC. Chemical crosslinking of NRCs produces A2 trimers, and $(A2)_3$B1 tetramers.The (A2) B1 tetramer is released free in solution by treatment of the NRC with 0.6M NaCl. Exhaustive RNase digestion of NRCs produces a left-handed, helical (pure-protein) filament (160A in width, about 600A pitch, and of indefinate length). Thus, our studies suggest the presence of internal symmetry within RNPs, that is, there is a complex oligomeric protein structure, repeated several times, which serves to fold the nascent RNA.

Techniques for Site-Directed Mutagenesis

0688 PROTEIN-DNA INTERACTIONS IN THE SITE-SPECIFIC RECOMBINATION SYSTEM OF BACTERIOPHAGE, Arthur Landy, Wade Bushman, John Thompson, Lina Vargas, Ellen Woodland and Sam Yin, Brown University, Providence, RI 02912

Our research interests include protein-DNA and protein-protein interactions in the site-specific recombinogenic structure of bacteriophage lambda called the "intasome". This structure is built upon a 240 bp length of DNA with specific binding sites for three different proteins. One of these proteins, Int (integrase), recognizes two different families of binding sites: one set of sites flanks the region of strand exchange and the other set is in the distal arms of the att site(s). The arms of the att site(s) also contain 2 binding sites for Xis (excisionase) and 3 binding sites for IHF (integration host factor). We shall discuss various strategies that have been, and/or could be, useful in studying the structural and functional interactions of these complex reactive structures.

0689 FUNCTIONAL ANALYSIS OF MUTATED H-2 GENES, E. McLaughlin-Taylor, C.G. Miyada, A.A. Reyes and R. B. Wallace, Beckman Research Institute of the City of Hope, Duarte, CA 91010, USA

The mutant histocompatibility gene H-2K^{bm1} differs from its parent gene H-2K^{b}, in 7 nucleotide positions which are clustered in a small region of the C1 exon. These seven changes result in 3 amino acid substitutions (152: Glu-Ala; 155-156: Arg-Leu- Tyr-Tyr), which give rise to strong allogeneic cell mediated responses and effect the loss of H-2K^{b} restriction sites. We are examining the functional role of the individual residues of the H-2K^{bm1} mutation by using site directed mutagenesis to construct mutant H-2K^{b} genes with specific base substitutions. The functional significance of these substitutions is analysed by T-cell recognition of the mutated gene products expressed in mouse L-cells following DNA mediated gene transfer. Plasmid or bacteriophage vectors containing the H-2K^{b} gene have been constructed and subjected to mutagenesis. The mutagenesis involves the use of synthetic oligonucleotides, bearing the appropriate mutant sequence, as primers on the circular plasmid or bacteriophage DNA template. The primer-template complex is extended *in vitro* to produce a partial heteroduplex. This heterduplex is used to transform E. coli and is resolved into mutant and wild type molecules by DNA replication. The mutant DNA containing colonies are identified by dried gel hybridization with the same oligonucleotide (^{32}P-labelled) used for the mutagenesis. Using this approach we have successfully dissected the H-2K^{bm} mutant by creating, *in vitro*, two mutant H-2K^{b} genes. Mutant H-2K$^{bTyr-Tyr}$ has substitutions at position 155-156 from Arg-Leu to Tyr-Tyr, leaving position 152 unchanged. The reciprocal mutant, H-2K^{bAla}, has position 152 mutated from Glu to Ala leaving positions 155-156 unchanged. Cloned cells lines expressing H-2K$^{bTyr-Tyr}$, and H-2K^{bAla} gene products have been identified by radioimmunoassay. The functional significance of the individual base substitutions represented by these two mutant H-2K^{b} genes is assessed by their ability to serve as efficient target structures for H-2K^{b} and H-2K^{bm1} specific cytolytic T-lymphocyte.

Unfolding and Refolding Reactions of Proteins

0690 MONOMOLECULAR α-HELIX FORMATION BY SHORT PEPTIDES IN WATER, Robert L. Baldwin*, Kevin R. Shoemaker*, Peter S. Kim*, David N. Brems*, Susan Marqusee*, Eunice York†, John M. Stewart†, *Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305, †Department of Biochemistry, University of Colorado School of Medicine, Denver, CO 80262.

Salt-bridge and helix-dipole models have been tested as explanations for the charged-group effect on the stability of the helix formed by C-peptide (residues 1-13 of ribonuclease A) in water. The procedure is to synthesize analogues of C-peptide containing single amino-acid substitutions for each of the residues containing a charged group with a pK between 2 and 7. Then these peptides are used to find the charged groups responsible for the bell-shaped curve of helix stability versus pH shown by C-peptide between pH 2 and pH 7.

The results show that Glu2^{-} and His12^{+} are needed for optimal helix formation and account for the strong pH dependence of helix stability. Glu9 is needed neither for optimal helix formation nor to explain the bell-shaped curve of helix stability versus pH. These results are not explained by the Zimm-Bragg model and host-guest data for α-helix formation, which require that Glu9 should be as effective as Glu2 in stabilizing the helix and which indicate that His^{+} is a strong helix breaker.

Since the results show that a Glu9^{-}···His12^{+} salt bridge is not needed but His12^{+} is needed for optimal helix formation, they argue against a salt bridge model for the charged-group effect. Both the present results and an earlier finding that an α-COO^{-} group at the C-terminus destabilizes the C-peptide helix may be explained by a helix dipole model. In this model a charged group stabilizes or destabilizes the helix by interacting with a nearby pole of the α-helix macrodipole. Glu2^{-} and His12^{+} stabilize because they are close to poles of opposite charge; an α-COO^{-} group at the C-terminus destabilizes because it is close to a pole of like charge.

As pointed out earlier, the C-peptide helix is much more stable than predicted by the Zimm-Bragg equation and host-guest data. We note here that this property is shared by an $(Ala)_{20}$ helix in the EAF and AEF block copolymers studied by Ihara, S., Ooi, T. & Takahashi, S. (1982) Biopolymers 21, 131-145. The problem of the unexpected stability of the C-peptide helix is discussed in light of this finding.

0691 Folding Kinetic Studies of Staphylococcal Nuclease and Site-Directed Mutants by Magnetization Transfer NMR. R.O. Fox*, P.A. Evans# and C.M. Dobson#.
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Magnetization transfer (MT) NMR experiments have been carried out to determine the equilibrium folding kinetics of staphylococcal nuclease in the thermal unfolding transition. Nuclease exists in at least two native folded structures in slow exchange on the NMR time scale. Two native structures are in chemical exchange with each other and with the unfolded population in the thermal transition zone. A minor native species unfolds at a rate ten times greater than the major folded form. Further experiments are in progress to determine whether the minor folded structure is an equilibrium folding intermediate. We are using magnetization transfer kinetic experiments to study the role of amino acid sequence information in the folding of nuclease. Using these experiments sequence variations which alter thermal stability of nuclease can be understood as changes in folding and/or unfolding rate constants. As a first step in this analysis we have attempted to determine the role of the single cis-peptide bond in nuclease (lys-116-pro117) in the native state heterogeneity. In pursuit of this goal we have cloned and sequenced the gene for staphylococcal nuclease and have expressed nuclease in _E. coli_ as 30-50% of total protein. A number of point mutations have been made in the residues surrounding the 116-117 peptide bond by primer-directed mutagenesis. One of these substitutions pro-117 → gly results in an increase in both the thermal stability of the native state and the interconversion rate of the native states.

0692 EFFECTS OF A COVALENT CROSS-LINK ON THE FOLDING PATHWAY OF BOVINE PANCREATIC TRYPSIN INHIBITOR (BPTI), David P. Goldenberg and Thomas E. Creighton, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH U.K.

A circular form of BPTI has been prepared by linking the N- and C- termini, which are in close proximity in the native conformation, together in a peptide bond. After being reduced, the circular protein can refold to the native conformation, and the pathway of folding has been analyzed by identifying the disulphide-bonded intermediates, as was done previously for the unmodified protein.

The stabilities of the native state and the folding intermediates, relative to the unfolded reduced protein, have been measured and compared with those for the respective forms of the uncross-linked protein. Surprisingly, the cross-link does not significantly stabilize the native conformation, apparently because the native protein is slightly strained by the cross-link. These results demonstrate that the extent of stabilization of a protein by a cross-link depends upon effects on the native conformation as well as the effect on the entropy of the unfolded chain: cross-links between groups rigidly held in the correct orientation in the native conformation are expected to contribute the most to stability.

The coss-link between the termini does not affect the overall stability of the one-disulphide intermediates, but does promote the formation of a second disulphide bond, indicating that the termini are not brought together until the two-disulphide stage of the folding pathway. These results illustrate the way in which a particular interaction can have different effects at different stages of folding.

0693 ^{1}H-NMR CHARACTERIZATION OF RCAM-BPTI EQUILIBRIUM FOLDING INTERMEDIATES,
Peter S. Kim*, David E. Wemmer†, Michael D. Beck*, Jannette Carey* and Robert L. Baldwin*, *Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305. †Department of Chemistry, University of Washington, Seattle, WA 98195.

We have confirmed an earlier observation (1) that the cooperativity of unfolding at acid pH, is reduced substantially in RCOM-BPTI, as compared to BPTI. RCOM-BPTI is made by reduction of the 14-38 disulfide bond in BPTI, followed by carboxymethylation of the SH groups. This chemical modification does not affect the overall structure of the protein, and RCOM-BPTI is still very stable (the t_m at pH 2.5 is $\sim 60°$ C). Different resonance lines in the ^{1}H-NMR spectrum of RCOM-BPTI show different apparent melting temperatures at pH 2.5. In contrast, resonance lines in the spectrum of BPTI show coincident thermal denaturation curves at acid pH.

The cooperativity of thermal unfolding is also reduced in RCAM-BPTI, a related derivative in which the 14-38 disulfide has been reduced and blocked by carboxamidomethylation. When RCAM-BPTI is unfolded with urea (pH 1.5, 48°C), different resonance lines show non-coincident transitions. The results indicate that intermediates are populated in the equilibrium unfolding transitions of RCAM-BPTI. Characterization of the intermediates will be discussed.

(1) Wüthrich, K., Roder, H. and Wagner, G. (1980) in _Protein Folding_ (R. Jaenicke, ed.) Elsevier, pp. 549-564.

0694 FOLDING MECHANISM OF HOMOLOGOUS RIBONUCLEASES, Franz X. Schmid, Herbert Krebs and Rainer Jaenicke, Biochemie II, Universität Regensburg, 8400 Regensburg, West Germany.

The fast and slow refolding reactions of a number of homologous pancreatic ribonucleases have been examined. Refolding kinetics of these proteins were similar, in particular a native-like intermediate, I_N, was shown to be populated on the slow refolding pathway of most ribonucleases. Folding rates are apparently correlated with the stability of the folded state.

0695 EVIDENCE FOR A TRANSIENT INTERMEDIATE IN THE FOLDING OF THIOREDOXIN, Earle Stellwagen, Robert Kelley, Christopher Bryant and Jane Wilson, University of Iowa, Iowa City, IA 52242

Thioredoxin purified from *E. coli* is a small single chained protein having no dissociable cofactors and only a single intrachain disulfide bond bridging a reverse turn in the crystallographic structure. The protein reversibly unfolds in a single cooperative transition between 2 and 3 guanidine hydrochloride (Gdn) at pH 7 and 25. This conformational change occurs in a single kinetic phase whose relaxation time is strongly dependent on [Gdn] above 2 M. Refolding in [Gdn] between 1 and 2 M occurs in at least two kinetic phases whose relative amplitudes depend upon the observation probe employed: effective volume, far ultraviolet circular dichroism, near ultraviolet absorbance or tryptophan fluorescence emission. Multimixing protocols indicate both the generation of slow phase refolding material in the denatured state as well as the transient accumulation of a native-like folding intermediate.

New Techniques in X-ray Crystallography

0696 THE CRYSTALLIZATION OF BIOLOGICAL MACROMOLECULES, Gary L. Gilliland, Physical Chemistry Dept., Hoffmann-La Roche Inc., 340 Kingsland St., Nutley, NJ 07110
Advances in the hardware and software used in data collection and crystallographic computations continues to decrease the time and effort required for the three-dimensional structure determination of single crystals of biological macromolecules. One necessary aspect of a structure determination, the conditions for the growth of large single crystals, must still be empirically determined. To facilitate the search for the appropriate crystallization conditions, a procedure will be presented. This procedure is based upon a survey of the crystallization condition data from over 1000 crystal forms of more than 600 biological macromolecules. The collected data of this survey is being submitted to the Brookhaven Protein Data Bank for general distribution.

0697 MACROMOLECULAR CRYSTALLOGRAPHY AT THE CORNELL HIGH ENERGY SYNCHROTRON SOURCE, CHESS, Keith Moffat, Donald Bilderback, Robert Hunt, Wilfried Schildkamp, Brenda Smith and Marian Szebenyi, Section of Biochemistry, Molecular and Cell Biology, and CHESS, Cornell University, Ithaca, New York 14853.
A synchrotron x-ray source such as CHESS emits an extremely intense white, pulsed, largely plane-polarized x-ray beam from which x-rays of any desired peak wavelength and bandpass may be selected by suitable x-ray optics. At CHESS, a focussed x-ray beam with bandpass $\sim 10^{-3}$ at 8 keV is 3 orders of magnitude more intense than that from a rotating anode x-ray generator. This permits the ready examination of very small, weakly diffracting crystals, or those with very large cell dimensions, which are impossible to study with a conventional x-ray source. The usual first products of initial crystallization attempts are microcrystals, and much biochemical effort must then be expended in enlarging them to a size suitable for x-ray examination. However, protein crystals with a volume as low as 10^5 cubic microns have been studied at CHESS, which are the most weakly scattering crystals ever examined. For larger crystals such as those of tetragonal lysozyme a complete, 2.5 A oscillation data set has been obtained in only 15 minutes total beam time, with individual exposures around 15 seconds. Finally, Laue techniques which utilize highly polychromatic radiation yield integrated intensities with a stationary crystal, and require exposures as low as 450 ms. This technique offers promise for time-resolved crystallography of short-lived structural intermediates.

0698 THE HEAVY ATOM DERIVATIVE PROBLEM and HOW TO BUILD A FLOW CELL, Gregory A. Petsko, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
Current methods for the preparation of isomorphous heavy-atom derivatives will be reviewed, with particular emphasis on new compounds that have been introduced in the last five years. A discussion will be given of the best methods for derivatizing any particular amino acid that has a reactive functional group, and also there will be a discussion of the methods available for introducing such functionality into proteins that do not have any. Methods for the location and refinement of heavy-atom sites will be evaluated, and a general strategy for the entire procedure will be proposed. In a second, live presentation, I will build flow cells until boredom (on either my part or that of the audience) sets in. I will distribute plans for yoke construction and demonstrate the design and assembly of flow cells for heavy-atom survey work and low-temperature enzyme-substrate binding experiments. There may be an opportunity for selected members of the audience to practice flow-cell construction under my supervision.

0699 CRYSTALLOGRAPHIC REFINEMENT AND GRAPHICS FITTING AS PROTEIN STRUCTURAL TOOLS, Janet L. Smith and Wayne A. Hendrickson, Dept. of Biochemistry & Molecular Biophysics, Columbia University, New York, NY 10032

Our understanding of protein structure has been greatly improved by the results of accurate crystallographic refinement of protein models. The development of several powerful refinement programs, the revolution in interactive molecular graphics, and rapid communication between refinement and graphics fitting have allowed improvement of existing protein models and modeling of ordered solvent to become routine practices in many laboratories. Our research on myohemerythrin illustrates the development of graphics fitting and refinement. The initial model building was done by a semi-automatic procedure, which, although successful without interactive graphics, was hindered by a lack of visual feedback. Refinement of the myohemerythrin model has proceeded apace with our development of stereochemically restrained least-squares refinement. Following a period of graphics fitting at a remote facility, an in-house interactive graphics system, well integrated with our refinement capability, has facilitated experimentation in refinement and investigation of ideas about myohemerythrin activity. Our recent observations of conformational heterogeneity in crambin, erabutoxin B, myohemerythrin and lamprey hemoglobin have arisen directly from accurate refinements conveniently linked to interactive graphics. A set of geometric-analysis programs has greatly simplified modeling both this flexibility and solvent structure. Finally, the refinement/graphics system has helped us position hydrogen atoms in crambin as we use our diffraction data to their limit.

0700 PHASE DETERMINATION COMBINING SOLVENT FLATTENING AND POSITIVITY WITH ISOMORPHOUS REPLACEMENT AND ANOMALOUS SCATTERING METHODS, Bi-Cheng Wang, VA Medical Center, Box 12055, Pittsburgh, PA 15240 and Crystallography Department, University of Pittsburgh, Pittsburgh, PA 15260.

A method for effectively utilizing the principles of solvent flattening and positivity in phase determination will be discussed. Prior to the application of these principles, using an automated procedure, the method determines the location of the ordered regions in the crystal. This information then serves as a crucial geometrical constraint in the rebuilding of the electron density in the crystal. This re-building process can either be called a filtering process which emphasizes the basic philosophy of removing noise, or by the more commonly used terms of solvent flattening and density positivity. With the concept of filtering the potential of the method for resolving phase ambiguity is easily understood, as the false phase solutions obtained from the SIR or the single-wavelength anomalous scattering methods can be regarded as a form of noise in the reciprocal space. Through repeated filtering and Fourier transformation this noise can, in principle, be removed. The method can also be used for phase extension. Results on applications of the method to known and unknown structures will be presented.

Model Systems for Protein Structure Function Studies

0701 INVESTIGATIONS OF STRUCTURE-FUNCTION RELATIONSHIPS BY SITE-SPECIFIC MUTAGENESIS OF TRYPSIN AND CARBOXYPEPTIDASE GENES. Charles S. Craik, Stephen J. Gardell, Steven Roczniak, Robert Fletterick & William J. Rutter, Hormone Research Institute, University of California, San Francisco, Ca 94143

Amino acid replacements have been made by site-directed mutagenesis of cloned trypsin and carboxypeptidase A genes and the resultant genes expressed in mammalian cells and yeast, respectively. Wild type and mutant enzymes have been isolated and their properties studied.

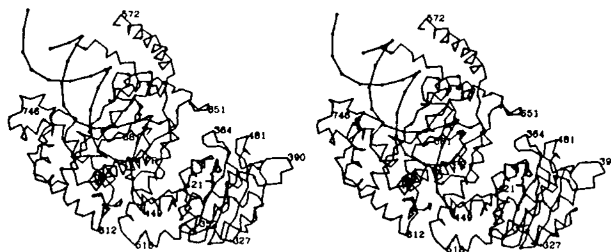
Three systems have been investigated: (1) The active site of carboxypeptidase: Tyr248→Phe248 results in increased Km but no change in kcat for peptide substrates. (2) The active site of serine proteases: Asp102→Asn102 does not affect Km but lowers kcat for peptide substrates and increases kcat for ester substrates. (3) Investigation of the substrate binding pocket of trypsin: The glycine residues have been replaced with alanine residues at positions 216 and 226, both located within the substrate binding cavity. Substrate selectivity (expressed as kcat/Km) for arginine and lysine was observed, trypsin (216G→A)(R>>K); trypsin (226G→A)(K>>R) and trypsin (216,226G→A)(R>K) but were not dominated by the Km as predicted by computer graphics analysis but rather by kcat values. Mutants containing alanine at position 226 exhibit dramatically decreased kcat's and an altered (trypsinogen?) conformation that can be converted to a trypsin-like structure on binding with a substrate analogue. Other binding pocket modifications are presently being characterized: trypsin (189D→K), (189D→E) and CPA (255I→E).

These experiments demonstrate the power of the site-specific mutagenesis as a means for investigating protein structure-function relationships and the general primitiveness of our knowledge.

0702 STRUCTURE OF THE LARGE FRAGMENT OF *E. coli* DNA POLYMERASE I COMPLEXED WITH dTMP: T.A. Steitz[†], D. Ollis[†] and P. Brick[†*], [†]Dept. Mol. Biophys. Biochem., Yale Univ., New Haven, CT 06511; [*]Present address: Biophysics Group, Imperial College, London, SW7 2AZ, UK.

A model of the large fragment of *E. coli* DNA polymerase I (Klenow fragment) complexed with d-TMP has been built by fitting its amino acid sequence into a 3.3 Å electron density map[1] (Fig. 1). The Klenow fragment structure consists of two domains, the smaller of which binds Zn-dTMP. The 5' phosphate interacts with a metal ion that is bound to the protein by three carboxylate side-chains. The 3'-OH is buried and H-bonded. This site appears to be the product of the editing 3' to 5' exonuclease reaction. The most striking feature of the larger domain is its hand-like shape which forms a deep crevice having dimensions appropriate to bind double stranded B-DNA. A two helix feature penetrates into this crevice and can fit into the DNA major groove thus restricting the possible positions of the DNA. Two mutations that affect DNA binding and processivity lie in this cleft. Extensive amino acid sequence homologies exist between T7 DNA polymerase and Klenow fragment in those polypeptides that form the cleft[2]. In collaboration with N. Grindley and C. Joyce, changes in the protein are being made to test the function of the domains and eliminate the 3' to 5' exonuclease activity.

1. Ollis, D., Brick, P., Hamlin, R., Xuong, N.G., Steitz, T.A. (1984) Nature, in press.
2. Ollis, D., Kline, C. and Steitz, T.A. (1984) Nature, in press.



Protein Folding

0703 MITOCHONDRIAL AND CYTOPLASMIC FORMS OF MALATE DEHYDROGENASE, Leonard Banaszak, Steven Roderick and Jens Birktoft, Washington University, St. Louis, Mo 63110.
The recently completed molecular structure of mitochondrial malate dehydrogenase, mMDH, is being compared with the cytosolic enzyme, sMDH, with the aim of identifying features which would explain their different chemical properties. First, the molecular interactions between the coenzyme, NAD, and both sMDH and mMDH are being studied. As in most of the dehydrogenases, the interactions of the dinucleotide, NAD, with the protein appear to involve mainly hydrogen bonding with atoms of the polypeptide chain. Differences in the amino acid sequence may, therefore, have relatively little effect on the affinity of either mMDH or sMDH for the coenzyme. In addition, mMDH exists in multiple oligomeric states ranging from a monomer to a tetramer. Subunit:subunit interactions in the mMDH dimer are thought to be similar to those in the sMDH dimer, but mMDH undergoes a pH-dependent dissociation to monomers (pK 5.3). By studying the molecular structure of mMDH and sMDH at the subunit:subunit interface, the molecular basis of this dissociation should be identifiable. The ability of mMDH to form molecular complexes with the metabolically adjacent enzymes in the citric acid cycle and malate shuttle has been reported. Models of the complex between mMDH and either citrate synthase or aspartate amino transferase are being studied using crystal structure coordinates. Lastly, both mMDH and sMDH are synthesized in the cytosol but mMDH in its precursor form has an additional 24 residue leader sequence on the NH_2-terminal end. A comparison of the number and spatial distribution of amino acid types on the external surface of the two enzymes is being made with the aim of evaluating structural factors which could play a role in mitochondrial protein import.

0704 DENSITY IN SUBMOLECULAR REGIONS OF GLOBULAR PROTEINS, Diana J.S. Beardsley and Walter J. Kauzmann*, Princeton University*, Princeton, NJ 08544, and Harvard Medical School, Boston, MA 02115
The folding of secondary structure elements to form a protein's three dimensional conformation is not well understood. We have studied packing in the interior of five globular proteins by calculating densities within submolecular regions of each molecule. Cylindrical regions (6-69 $Å^3$ in size), centered at the C-N peptide bond and perpendicular to the peptide plane, were defined for a series of peptides in internal beta pleated sheets in lysozyme, ribonuclease-S, subtilisin-BPN', carboxypeptidase-A, and alpha-chymotrypsin. The mass of each nearby atom or group was distributed evenly throughout a sphere of radius equal to its van der Waals radius. Integration of the volume of intersection of each spherical atom group with a given submolecular region allowed calculation of a "density" in g/cm^3 for each region. Density was found to be inhomogeneous when specific submolecular regions with a volume of 10-20 $Å^3$ were compared. In beta structure, these packing densities were 5% lower than the average internal density of the proteins. Packing was most effective when hydrophobic side chain groups from distant residues were sterically able to interdigitate between the side chains of neighboring residues. In subtilisin and carboxypepidase, dense regions were located toward, but not at, the molecular surface. High density regions were also noted at the base of each enzyme's catalytic site with lower density near the substrate binding groove. This approach to packing density does not require assumptions regarding the surface of the molecule and allows study of submolecular regions where packing interactions are likely to predominate over bonded interactions.

0705 EQUILIBRIUM DENATURATION OF PITUITARY AND RECOMBINANT DERIVED BOVINE GROWTH HORMONE, David N. Brems, Scott M. Plaisted, Henry A. Havel, and E. Wayne Kauffman, The Upjohn Co., Kalamazoo Michigan, 49001
In order to probe the relationship between chemical structure and conformational stability, the guanidine hydrochloride denaturation of bovine growth hormone was investigated. Denaturation was monitored by a large number of spectroscopic and physicochemical methods. The data demonstrate that the denaturation of bovine growth hormone is a multistate process with several stable equilibrium intermediates. The conformational stability of pituitary and recombinant derived hormone is very similiar. Work is underway to further characterize these equilibrium intermediates and to illucidate their role in the kinetic pathway of folding.

0706 STRUCTURAL STUDIES ON BACTERIORHODOPSIN, Christie G. Brouillette, Donald D. Muccio, and Teresa K. Finney, UAB Medical Center, Birmingham, AL 35294.

We have studied bacteriorhodopsin (BR) in the purple membrane of H. halobium by DSC and temperature dependent visible spectroscopy, intrinsic fluorescence and CD as a function of pH (5-11), temperature (25-110°C) and to a limited extent, ionic strength. Visible spectroscopy (and CD) has been most informative about factors effecting the protein's chromophore. Our fluorscence studies have added information on BR's tryptophan residues as well as information on the retinal moiety. DSC has been very useful in linking the spectroscopic data together, and in quantitating our results. Collectively, these techniques have revealed three major temperature dependent transitions in BR (labeled R, X, and D). The high temperature transition is due to the irreversible denaturation of the protein and is, therefore, labeled "D". The low temperature transition is associated with the conversion of the species absorbing at 560nm, bR560, to one absorbing at 460nm, bR460, (determined from the difference spectra maximum), and features an isobestic point at about 510nm. This transition is completely reversible (hence the designation "R"). The reversible transition of bR560 to bR460 was shown to be derived from the acid-base equilibrium between the protonated and unprotonated retinal Schiff base. (Druckmann, et. al, (1982) Biochemistry 21, 4953-4959.) Two features of our studies on the purple membrane stand out: 1) the cooperativity of both the R and D transitions that results from significant inter-protein interactions, and 2) the dramatic effect pH has on the transition enthalpy, Tm and cooperative unit, which suggests an important role for titratable groups in the structure of BR. Our studies support the hypothesis that polar interactions control membrane protein stability.

0707 ANTISERA AGAINST SYNTHETIC PEPTIDES AS PROBES OF FELINE PANLEUKOPENIA VIRUS STRUCTURE, Jonathan Carlson[1], Keith Rushlow[2], Alistair McNab[2], Scott Winston[2], and William Hahn[3], [1]Department of Microbiology/Environmental Health, Colorado State University, Ft. Collins, CO 80523, [2]Syngene Products and Research, Inc., Ft. Collins, CO 80524, [3]Department of Anatomy, University of Colorado Health Sciences Center, Denver, CO 80262

Feline panleukopenia virus (FPV) is an autonomous parvovirus which causes a drop in leukocyte count and severe gastroenteritis in cats. Parvoviruses are small, non-enveloped icosahedral viruses that cause a variety of diseases in a number of mammalian species including man. Parvovirus virions contain three proteins whose amino sequences form a nested set sharing a common carboxyterminus. We have cloned and determined the nucleotide sequence of the gene for the FPV capsid proteins. Nineteen peptides of an average length of fifteen amino acids were synthesized from the predicted capsid protein sequence. The peptides were coupled to either keyhole limpet hemocyanin or thyroglobulin and used to immunize guinea pigs. Antisera against the peptides were tested for antibodies against FPV in ELISA. Most of the anti-peptide sera reacted with SDS denatured FPV indicating that the peptides were immunogenic. However, antisera to only a few of the peptides reacted with intact virus particles. The difference in binding to native and denatured virus must reflect the accessibility and conformation of peptide sequence in the virus structure. A detailed knowledge of the structure and antigenic determinants of this class of virus may allow the rational design of preventative and therapeutic strategies against them.

0708 A MODEL FOR THE FOLDING OF THE LIGHT-HARVESTING COMPLEX II APOPROTEIN OF Lemna gibba, P.R.Chitnis, E.Harel, B.Kohorn, G.Karlin-Neumann, E.M.Tobin and J.P.Thornber, Dept. of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

The LHC II apoprotein from Lemna gibba is coded by members of a multigene family. Two of these genes have been cloned and sequenced[1]. We have used David Eisenberg's procedures[2] to predict from amino acid sequences a model for conformation and orientation of LHC II polypeptide chain in a lipid bilayer. It includes three transmembrane α-helices with which all of the chlorophyll molecules are postulated to be associated. According to the model the proportion of the protein outside the lipid bilayer on the stromal side is much greater than previously envisaged. The model is also applicable to LHC II apoproteins from other higher plants. The features of this model and some biochemical evidence supporting it will be presented. We plan to test functional domains predicted by this model by studying the in vitro uptake and incorporation into isolated chloroplasts of in vitro mutagenized LHC II polypeptides.

Research supported by grants from NIH, NSF and USDA.

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2. Eisenberg, 1984, Annu.Rev.Biochem. 53:595-624

0709 BIOSYNTHESIS OF NERVE GROWTH FACTOR: ROLE OF SOLUBLE PROTEASES, Joan C. Dunbar and Ralph A. Bradshaw, Department of Biological Chemistry, California College of Medicine, University of California, Irvine, CA 92717

Mouse submandibular nerve growth factor (mNGF) consists of a hormonal active subunit (β) and two serine proteases (α and γ), of which only the latter is catalytically active. The mature form of βNGF consists of two 118 amino acid polypeptides that are released from precursor molecules as deduced from cDNA sequence studies. Prior to final folding, processing is required at both termini with a large (∿20 kDa) fragment removed from the N-terminus and a dipeptide (Arg-Gly) from the C-terminus. A Lys-Arg sequence precedes the amino terminal serine residue of βNGF and another arginine residue precedes the C-terminal dipeptide. Since the specificity of the γ-subunit is for Arg-X bonds, it is a potential candidate for both cleavages. In contrast to mNGF, the NGF complex isolated from guinea pig (gp) prostates contains no esteropeptidase activity. Prostate extracts do, however, contain one major soluble esteropeptidase with a specificity similar to mγNGF. The amino acid sequence shows a high degree of similarity to α- and γ-NGF subunits and to the kallikrein family of serine proteases. The esterase is a single polypeptide chain and contains the catalytic histidine, aspartic and serine residues as well as the residues which determine substrate cleavage preference. It readily cleaves tosyl-arginine-methyl ester (TAME) but is unable to cleave benzoyl-arginine-p-nitroanilide (BAPNA) - the normal substrate used to identify γ-NGF. The kallikrein also differs from mγNGF in that it does not form stable complexes with βNGF. The significance of these differences and the role of the two endopeptidases in the processing of NGF remains to be determined. Supported by USPHS NS 19964.

0710 A MODEL FOR A SIX-STRANDED BETA TUBE, D. Eisenberg, P. Pryciak, and F.A. Eiserling, Molecular Biology Institute, University of California, Los Angeles, CA 90024

Short barrels formed from 5 to 13 strands of peptide chain in the beta configuration, are frequent motifs in globular proteins (1), and systematic conformational studies of such structures have been made (2). We have built a model for a closely related tube, capable of infinite length, formed from 6 parallel peptide chains in beta-like configurations. The search for such a structure was inspired by the probable existence of a length-determining protein complex in bacteriophage T4 tail tubes, perhaps having similar properties (3).

Our structure is formed from 6 extended strands, each repeating in backbone phi-psi angles after six residues. The strands run roughly parallel and are hydrogen-bonded laterally to each other, but not every potential hydrogen bond is formed: each strand makes four hydrogen bonds to its neighbor, and then skips two. The strands are related to each other by a 6-fold axis. The amide N of residue 1 on strand 2 hydrogen bonds to the carbonyl oxygen of residue 2 on strand 1. Residue 4 forms no hydrogen bonds, and residues 1 and 3 form only 1 each. Phi-psi angles for all but residue 3 are close to those found by Salemme and Weatherford (2), for twisted parallel beta sheets, phi=$-110\pm10^{\circ}$, psi=$120\pm10^{\circ}$, but those for residue 3 are about phi=-165° and psi=130°.

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0711 INTERMEDIATES IN THE FOLDING OF RIBONUCLEASE A. Anthony L. Fink and Roger G. Biringer, Division of Natural Sciences, University of California, Santa Cruz, CA 95064.

The folding of ribonuclease A has been investigated at subzero temperatures using aqueous methanol cryosolvents. The presence of the relatively more hydrophobic cosolvent results in the stabilization of partially-folded intermediate states. The kinetics of refolding at -15°C of some nitro-tyrosine derivatives of RNAse A were compared to those for unmodified protein. Inhibitor binding and catalytic activity were also studied as a function of refolding. In each case multiphasic kinetics were observed, and revealed that different areas of the protein folded at different rates. Reduced, alkylated, enzyme was used as a control. No significant difference was observed at pH* 3 and 6. The data are consistent with the detection of partially-folded intermediates, formed late in the folding process. A model, taking proline isomerization into consideration, and having a minimum of two such intermediates, in each of two converging pathways is proposed.

0712 ELECTROSTATIC FIELDS INSIDE PROTEINS, Michael K. Gilson, Alex Rashin, Richard Fine, and Barry Honig, Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168th Street, New York, N.Y. 10032

A comprehensive, classical approach to calculating electrstatic interactions in proteins is presented. The protein is modelled as a region of low dielectric constant with spherical charges embedded within it, surrounded by an aqueous solvent of high dielectric constant which may contain a simple electrolyte. The approach is comprehensive because it explicitly treats all the electrostatic interactions in this classical model. In particular, the treatment permits calculation of solvent screening, polarization forces, and self-energies (which are related to solvation energies). Calculations based on this approach lead to several major conclusions: 1) Solvent polarization has a large effect on pairwise electrostatic interactions, with a marked dependence on the positions of the charges. This position dependence makes it impossible to devise an expression for the effective dielectric constant which will be both simple and accurate. 2)Self-polarization energies are also large; the work of moving a charge from solvent to the protein interior is on the order of 30 kcal/mole. 3) Solvent polarization effects are far less important for electrostatic interactions involving dipoles. In conclusion, this work highlights the importance of electrostatic energy contributions as a determinant of protein structure, and offers a relatively simple way of obtaining quantitative information on these contributions.

[Supported by NIH GM30518]

0713 ANALYSIS OF THE HIGH AFFINITY LIPID BINDING SITE IN PYRUVATE OXIDASE, Lowell P. Hager, Susan E. Hamilton and Michael A. Recny, University of Illinois, Urbana, IL 61801

Pyruvate oxidase, an E. coli peripheral membrane protein, catalyzes the TPP-dependent oxidative decarboxylation of pyruvate to form acetate, CO_2 and reduced FAD. The catalytic activity of pyruvate oxidase is dramatically enhanced either by binding lipid amphiphiles to a high affinity site on the reduced form of the enzyme or by proteolytically hydrolyzing a small peptide (the "α-peptide", Mr=2,000) from the carboxy terminus of the enzyme. Proteolytic activation of pyruvate oxidase must be carried out on the reduced form of the enzyme in the presence of TPP; when TPP is omitted from the reaction mixture, proteolysis occurs at a different site and cleaves a 9,000 M.W. peptide (β-peptide) from the carboxy terminus. A number of observations suggest that proteolytic activation and lipid binding are mutually exclusive processes which involve the same or closely related sites. For example, proteolytically activated enzyme will not bind lipids and the lipid activated enzyme is resistant to proteolysis. Further support for the "common site" hypothesis comes from affinity labeling studies with ^{14}C-lauric acid. Carbodiimide activation of ^{14}C-lauric acid produces an affinity-labeled, activated enzyme. Tryptic digestion of the affinity-labeled enzyme releases a ^{14}C-labeled β peptide from the carboxy terminus. The ^{14}C-β peptide and the α peptide have been isolated and the α peptide has been sequenced. Model studies on the α peptide have been initiated in order to further define the high affinity lipid binding site in pyruvate oxidase.

0714 ELECTRONIC ROLE OF ACTIVE SITE RESIDUES IN RIBONUCLEASE A, Karen Haydock and Leland C. Allen, Chemistry Dept. Princeton University, Princeton, NJ 08544

The molecular mechanism of catalysis by RNase A has been investigated by performing high-level electronic structure calculations on model systems representing the active site residues and substrate, both individually and in combination. Our computational procedure has allowed us to separate the effects of individual components and elucidate the role of each residue at each step in the mechanism. The reaction is known to proceed through a 2',3'-cyclic intermediate which is subsequently hydrolyzed, resulting in 5'-hydroxyl and 3'-phosphate cleavage products. Although numerous high resolution X-ray and neutron diffraction structures of RNase A and its complex with various substrate analogs have been reported, they have not resolved all questions concerning the positions, protonation states, and hydrogen bonding arrangements of the active site residues. For example, while the original crystal structures showed His 12 hydrogen bonding to the substrate's 02'-hydroxyl, implicating it as the residue responsible for deprotonation and protonation, in more recent structures it hydrogen bonds to the phosphate group, with Lys 41 hydrogen bonding to the 02'. By comparing the geometry and energetics of these and other interactions at each state in the reaction, we have tested the validity of alternative mechanisms and produced a plausible reaction pathway for RNase A catalysis.

0715 INFLUENCE OF -S-S-BONDS AND SINGLE AMINO ACID MODIFICATIONS ON THE STABIlity of BPTI, H.-J. Hinz and H. Wenzel, Biochemie II, Universität Regensburg, FRG

Quantitative information on the enthalpic and entropic contributions to protein stability of specific amino acid modifications or disulfide bridges is scarce. Knowledge of these thermodynamic parameters is, however, highly desirable from a variety of aspects. They serve as standard values for theoretical treatments, are instrumental in understanding the delicate balance of forces in proteins and enzymes and may be useful in designing new proteins having tailor made properties.
We studied the exceptionally well characterized BPTI in order to render possible a structural interpretation of the model independent thermodynamic quantitites. It was found by direct microcalorimetric measurements that against wide spread believe disulfide bridges do not only decrease conformational entropy of the random state but also increase energetic interactions of the native state.
Specific modifications of amino acids can increase or decrease enthalpy and Gibbs energy of the inhibitor, which in turn has significant consequences for its biological function.

0716 EFFECTS ON THE NH_2-TERMINAL REGIONS OF LOSING THE LOOSE ENDS IN A COENZYME DEPENDENT SYSTEM. Ana Iriarte and M. Martinez-Carrion. University of Navarre, Pamplona, Spain and Virginia Commonwealth University, Richmond, Virginia 23298.

Proteolytic loss of the NH_2-terminal region of the isozymes of the pyridoxal phosphate (PLP) dependent aspartate transaminase induces a catalytic loss and conformational changes. The effect of various proteases varies and the loss in activity is produced after removal of a 19 amino acid NH_2-terminal peptide. Differential scanning calorimetry shows that new conformations of lower thermal stability are produced. These arise from the creation of at least 2 distinct core proteins missing the amino terminal region. Spectrometric (UV, CD, NMR, FTIR) investigations give indications that the contact amino acids at the active site retain affinity for PLP and the enzyme's substrates and their analogs. Partial catalytic events can be followed in this system. None of the amino acids in the sections removed by protease form part of the catalytic site, yet they overlap the hydrophobic region of each companion subunit in these dimeric proteins. Rigidity over several structural domains in the remaining core protein appears altered as consequence of losing the NH_2-terminal portion. The resulting core protein becomes available to endoprotease action to which native protein is resistant.

0717 SOLUTION STRUCTURE OF PROTEINS, P.A. Kosen, H. Naderi, S. Manogaran, V. Basus, P.G. Schmidt, and I.D. Kuntz, University of California, San Francisco, CA 94143

Recently, it was demonstrated that high field ^1H-NMR difference spectroscopy of lysozyme mono-specifically labeled with the oxidized and reduced forms of nitroxide spin labels could be used to determine distances between the spin labels and various lysozyme protons (Schmidt, P.G. and Kuntz, I.D. (1984) Biochemistry 23, 4261.) By using algorithms from distance geometry, the distances uniquely determined the positions of the spin labels in lysozyme. A more general application of this approach would be to determine the total conformation of a macromolecule in solution. To this end, monospecific spin labeled bovine pancreatic trypsin inhibitor (BPTI) derivatives are being prepared by nonspecific reaction of spin labels directed against various reactive sites of BPTI, followed by chromatographic separation. These BPTI derivatives are being analyzed by one- and two-dimensional NMR difference spectroscopy to identify numerous intramolecular proton distances. Selective spectra will be presented and assessed for the potential of spin labeling in conjunction with distance measurements and distance geometry as a tool for general solution conformation studies.

0718 A STATISTICAL TECHNIQUE FOR PREDICTING MEMBRANE PROTEIN STRUCTURE,
Leslie A. Kuhn and John S. Leigh, Jr., University of Pennsylvania, Philadelphia, PA 19104

The transmembrane segments of a membrane protein may be predicted from a membrane propensity profile of its amino acid sequence in which the amino acids are replaced by their frequencies of occurrence in a number of putative transmembrane segments and the resulting sequence is smoothed with a running average. In such a profile transmembrane segments appear as extended, positive peaks. When this technique was applied to a pool of 10 previously studied membrane proteins, the predicted intra- and extramembrane structures agreed 93.6% on a residue-by-residue basis with the previously suggested structures. This algorithm has been applied to predict the transmembrane segments in subunits I, II, III, IV, V, VIa, VII, VIIIa, and VIIIb of beef cytochrome c oxidase and has also been used as a tool for studying structural homology between different species' cytochrome oxidase subunits I, II, and III and cytochrome b proteins.

0719 REFOLDING OF SERINE PROTEINASES,
Albert Light, Purdue University, Lafayette, IN 47907

We have been studying the folding pathway of pancreatic serine proteinases. The mixed disulfide of the fully reduced protein and glutathione is used to study the renaturation process. The progress of refolding is followed by 1) regain of enzymatic activity, 2) polyacrylamide gel electrophoresis, 3) isoelectric focusing, and 4) HPLC. We have demonstrated the formation and disappearance of intermediate species in refolding trypsinogen, chymotrypsinogen, and chemically modified derivatives. We find that trypsinogen and chymotrypsinogen refold at the same rate and probably by identical mechanisms. Furthermore, we have evidence that the two domains refold independently of one another and prior to the formation of the intermolecular disulfides and the development of the active site.

0720 ALKALINE ISOMERIZATION AND SLOW REFOLDING IN YEAST ISO-2 CYTOCHROME C.
John J. Osterhout, Jr. and Barry T. Nall, University of Texas Medical School, Houston, Texas 77225

The kinetic properties of the alkaline isomerization reaction, τ_{pH}, of yeast iso-2 cytochrome *c* and the slow protein folding reactions detected by absorbance, τ_{1a}, and fluorescence, τ_{1b}, have been studied. All three phases are found to have activation enthalpies in the range expected for proline isomerization: $\Delta H\dagger$ = 27 Kcal/mole for τ_{1a}, 21 Kcal/mole for τ_{1b}, and 28 Kcal/mole for τ_{pH}.

Double-jump (unfolding, then refolding) kinetic experiments are used: 1) to monitor the rates of equilibration between fast and slow refolding forms of the unfolded protein, and 2) as a test for the involvement of proline isomerization in the pH induced conformational change in the folded protein. Both slow protein-refolding phases, τ_{1a} and τ_{1b}, appear to be generated by slow equilibration between unfolded species, as expected for proline isomerization. The pH induced phase, τ_{pH}, does not seem to involve proline isomerization, since the species which generate this phase are: 1) produced within the mixing time of a double (pH) jump experiment, and 2) sensitive to high concentrations of guanidine hydrochloride.

0721 CLONING AND INVESTIGATION OF BACILLUS AMYLOLIQUEFACIENS EXTRACELLULAR RIBONUCLEASE,
Chris Paddon and Robert W. Hartley, NIH, Bethesda, MD 20205
The extracellular ribonuclease of Bacilus amyloliquefaciens (barnase) is a small enzyme (M_R:12,382;110 residues) which contains no disulfide bonds, divalent cations or other non-peptide moieties (Hartley, R.W., 1977, JBC 252, 3252-3254). The atomic structure of barnase has been determined by X-ray crystallographic studies (Mauguen, Y. et al., 1982, Nature 297, 162-164). The natural inhibitor of barnase, barstar, is produced intracellularly in B. amyloliquefaciens. Barnase is of interest both as a secreted protein and as a model system for protein folding studies.

Initial attempts to isolate the barnase gene from libraries of B. amyloliquefaciens chromosomal DNA produced in E. coli and B. subtilis were unsuccessful using either a mixed oligonucleotide probe to a portion of the gene, or direct production of barnase. This may indicate that barnase expression is lethal in the absence of barstar. An alternative strategy to clone barnase using transposon (Tn917) insertional mutagenesis of B. amyloliquefaciens to generate a barnase mutant followed by cloning of the transposon in E. coli with its associated flanking DNA regions was successful. The transposon has been shown to have inserted in the barnase gene by DNA sequence analysis, the barnase gene has thus been cloned in an inactive form. Further analysis of the barnase gene will be presented. The gene will be expressed, possibly in an inactive form by in vitro mutagenesis of the active site, allowing further protein folding studies to be undertaken.

0722 MOUSE APOLIPOPROTEIN E cDNA SEQUENCE: ANALYSIS OF INTERNAL HOMOLOGY,
Tripathi Rajavashisth, John S. Kaptein, Karen L. Reue, Stuart Rich and Aldons J. Lusis, University of California, Los Angeles, CA 90024
Apolipoprotein E (apo E) is responsible for the binding of VLDL and chylomicron remnants to cellular receptors thereby effecting their removal from the circulation. We have isolated and determined the sequence of a nearly full-length cDNA clone of C57Bl/6J mouse apo E. The clone encodes 284 amino acids and the entire 3' untranslated region of 112 nucleotides. Comparison with the sequences of human and rat apo E reveals a high degree of conservation. There are two regions, however, which in each species is characterized by unique insertions and deletions. The resulting reading frame shift is most extensive in mouse and could alter an entire potential amphipathic α-helix. Analysis of the sequence homologies within apo E reveals that the entire sequence is made up of repetitive units despite an intron interruption. The most primitive unit appears to be an 11-nucleotide repeat within higher order repeats of 22 or 33 nucleotides. These repeats are read in different reading frames in various portions of the molecule; hence, at the amino acid level, the homologies are not always apparent. We postulate that apo E and those other apolipoproteins related to it have arisen by duplication and subsequent modifications of an 11-nucleotide unit or multiples thereof. Furthermore, insertions and deletions in one particular region have been tolerated because the alternate amino acid sequence yields comparable structural features.

0723 ANALYSIS OF IMMUNOGLOBULIN VARIABLE REGION STRUCTURE BY ELECTRON MICROSCOPY,
Kenneth H. Roux, Florida State University, Tallahassee, FL 32306
Immune complexes composed IgG and Fab antibody were negatively stained with uranyl formate and examined by immunoelectron microscopy. The Fab antibody fragments were directed to idiotypes and allotypes of the variable region of the target IgG thus revealing their relative location and orientation with respect to the remainder of the IgG molecule. Rabbit variable region allotypes are characterized by multiple amino acid substitutions distributed in two clusters within a 115 amino segment. The electron micrographs clearly show multiple allotypic determinants scattered over the surface of the variable domain. In contrast, antibody to idiotypes are restricted to the distal terminus of the molecule in the area of the antigen combining site. Thus, the various conformations of the complexes are concordant with the predicted locations of allotypes and idiotypes based on primary amino acid sequence comparisons and three dimensional models of the variable region.

0724 EFFECT OF CARBOHYDRATE ON THE FOLDING OF THE α-SUBUNIT OF THE BOVINE GLYCOPROTEIN HORMONES.

Thomas W. Strickland, John G. Pierce, UCLA School of Medicine, Los Angeles, CA 90024, Arlen R. Thomason, Amgen, Thousand Oaks, CA, and John H. Nilson, Case Western Reserve University, Cleveland, Ohio.

In order to study the role of carbohydrate in the folding of the bovine glycoprotein hormone α-subunit, the ability of completely reduced and denatured α-subunit forms containing different amounts of carbohydrate to fold *in vitro* was determined. The presence of native structure was assessed by a conformationally sensitive α-subunit radioimmunoassay and by the ability of the α-subunit to combine with β-subunit to form a hormone with *in vitro* biological activity. As reported previously (J. Biol. Chem. 251, 6392, 1976), the fully glycosylated α-subunit reforms its native structure quantitatively. α-subunit which has been partially deglycosylated by treatment with trifluoromethanesulfonic acid such that it retains only the chitobiose core of each asparagine linked oligosaccharide also refolds to form a native-like structure in high yield. However, α-subunit which is synthesized in *E. coli* and is thus completely devoid of carbohydrate yields only 2-3% of native-like material. Although low, 2-3% of native-like subunit is greater than the 0.1% native material which would result from random formation of the five disulfide bonds of the α-subunit. Thus it appears that while carbohydrate is not an absolute requirement for folding of the α-subunit, its presence greatly increases the yield of properly folded material.

0725 EVOLUTIONARY SIMILARITY AMONG PEPTIDE SEGMENTS IS A BASIS FOR PREDICTION OF PROTEIN FOLDING

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Short segments of polypeptide, from a protein for which the primary sequence but not the three-dimensional structure is known, are compared to a library of known structures. The basis of comparison is the odds with which residues in the unknown segment might have been substituted through evolution for residues in segments from the library of known structures. Segments from known structures that are similar in sequence to those from the unknown protein are often found to be similar in three-dimensional structure to one another and to the true structure of the segment from the unknown protein. This provides a basis for prediction not only of secondary structure type, but also of actual three-dimensional structure.

0726 MODEL SUBSTRATE FOR LEADER PEPTIDASE—A SYNTHETIC 73 AMINO ACID RESIDUE PEPTIDE, M13 PROCOAT,

J. M. Tomich, S. J. Horvath, M. R. Emerling, M. S. Tomich, W. D. Nelson, L. E. Hood, W. Wickner* and J. H. Richards, Braun Laboratories, California Institute of Technology, Pasadena, California 91125 and *The Molecular Biology Institute, University of California at Los Angeles, Westwood, California 90024.

As suggested in the "membrane trigger hypothesis" (W. Wickner, *Ann. Rev. Biochem.* 48: 23-45, 1979), the presence of a leader sequence confers a water soluble conformation upon secretory or membrane proteins during and after synthesis in the cytosol. The bulk of the information needed to bring about secretion through or insertion into membranes is encoded in the soluble conformation induced by the presence of an intact leader sequence. Another feature of this hypothesis is the requirement that the leader sequence be removed enzymatically by leader peptidase to make the process irreversible.

A peptide based on the amino acid sequence of phage M13 procoat has been synthesized by a much improved solid-phase synthetic method. In this method each amino acid coupling is performed at >98.8% efficiency in less than two hours. The M13 procoat sequence (73 amino acids) was synthesized with an overall yield of about 50%. The purity of the isolated peptide was checked by 20% polyacrylamide gel electrophoresis and sequence analysis in a gas-phase sequenator. The synthesis yielded 1.5 gm of peptide.

Structural analysis of the peptide reveals that the protein can adopt various conformations and degrees of oligomerization. These states appear to be concentration and pH dependent. The monomer is soluble up to 0.25 mg/ml. As initially isolated, the monomer is resistant to cleavage by leader peptidase obtained from *E. coli*. Upon heating, followed by slow cooling the peptide is readily hydrolyzed by the peptidase suggesting that the interaction with leader peptidase requires a specific three-dimensional conformation of the preparations.

0727 FOLDING OF PARATHYROID HORMONE: EXPERIMENTAL EXAMINATION OF A THEORETICAL MODEL,
James E. Zull and A.L. Frelinger, Case Western Reserve Univ., Cleveland, Oh, 44106
Parathyroid hormone is a single chain 84 amino acid polypeptide. We have proposed a model for the folding of this hormone which allows predictions concerning the chemical propreties of specific residues in the chain. One prediction is that the met residue at position 8 in bovine PTH (bPTH), should be less accessible to solvent than met-18. This prediction was confirmed by study of the rate of H_2O_2 oxidation using HPLC to identify forms of the hormone oxidized at each methionine. Residue 18 is oxidized more rapidly than met-8 in both the native hormone and in a biologically active fragment, 1-34 bPTH. When oxidation is conducted in 3M Guanidine HCl, this difference is eliminated in 1-34 and reduced but not eliminated in 1-84. For both peptides, increasing the ionic strength (NaCl) of the reaction medium greatly increased the difference between the two methionine's reactivity. A second prediction from our model is that all of the lys residues should be equally accessible, but that the N-terminal amino group may be burrried. The reactivity of the amino groups is 1-34 bPTH with methyl acetimidate was therefore examined using CNBr cleavage and HPLC to identify amino group modification. The results indicate that the lys residues in 1-34 bPTH are all equally reactive, but the N-terminal residue is less reactive. However, the reactivity of all the amino groups in the hormone was increased in denaturing solvents. These results suggest that our model for the structure of PTH has some validity, but that it needs to be modified to accomodate the apparent significant inaccessibility of the lysine residues, as well as the N-terminus.

Theory and Dynamics of Protein Folding

0728 KINETIC PATHWAYS OF PROTEIN FOLDING,
Thomas E. Creighton, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

Protein folding transitions of individual domains are cooperative; consequently, the only method currently capable of elucidating the folding pathways of individual domains is to use the disulphide bond interaction to trap the inherently unstable partially-folded intermediates that accumulate kinetically. The folding pathways of bovine pancreatic trypsin inhibitor (BPTI) (1), several homologues (2), and ribonuclease T_1 (C.N. Pace, unpublished) have been determined in this way. The BPTI pathway was unexpected in that it occurs most readily via rearrangements of two-disulphide intermediates (1); this is now believed to reflect the very high stability of the BPTI folded conformation (2), because the rate-limiting step in refolding of this, and other, proteins involves a distorted, high-energy form of the final folded conformation (3), and less stable proteins do not require such rearrangements (2). A more detailed understanding of the BPTI folding transition should be obtained from the conformational properties of the trapped intermediates and the folding of modified forms of the protein. The amino acid sequences of at least 23 homologues of BPTI from widely varying organisms provide a useful description of the constraints on the amino acid sequence of proteins with the BPTI folded conformation.

The unique information about the relative free energies of the various disulphide interactions during folding has led to a useful empirical description of folded conformation stability in terms of the effective concentrations of interacting groups (4). The intramolecular nature of all interactions within a folded protein means that they can contribute to net stability of the folded state, in spite of the existence of similar intermolecular interactions between the unfolded protein and the solvent. This can account qualitatively for the lower enthalpy of the folded state, even though the hydrophobic effect provides a positive contribution.

Isolation of a genomic DNA segment coding for BPTI (5) has led to its expression in E.coli (D.P. Goldenberg, unpublished). This should permit production of useful modifications of BPTI and elucidation of the pathway of folding in vivo.

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Predictions of Protein Folding

0729 THEORY FOR THE FOLDING AND STABILITY OF GLOBULAR PROTEINS, Ken A. Dill, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143

The preference of a protein for the globular state is principally the result of a balance of two forces: the association of solvophobic residues to minimize their contact with solvent, on the one hand, opposed by the conformational entropy of the chain, on the other. We consider a chain of n residues to be comprised of $n\phi_h$ solvophobic and $n(1-\phi_h)$ solvophilic units. Through use of a simple mean-field lattice method, we develop statistical mechanical theory for the free energy of folding as a function of composition and chain length. The theory predicts a relatively sharp transition as a function of temperature or solvent character. Molecules which are too short or too long or which have too few solvophobic residues are predicted not to fold. Globular molecules should have a large solvophobic core, but there is an entropic tendency for some residues to be "out of place", particularly in small molecules. For long chains, molecules comprised of globular "domains" are predicted to be thermodynamically more stable than spherical molecules. The number of accessible conformations in the globular state is calculated to be tens of orders of magnitude smaller than is often supposed.

0730 AMPHIPHILICITY AND HYDROPHOBICITY IN PROTEIN FOLDING, D. Eisenberg, R.M. Weiss, T.C. Terwilliger, and S. Eshita, Molecular Biology Institute, University of California, Los Angeles, CA 90024

The relationship of protein amino acid sequence to three-dimensional structure may be described in terms of hydrophobic moments. These moments are useful for understanding folding in terms hydrophobic and hydrophilic segments of the peptide chain, and for prediction of structure from sequence for protein segments that are highly hydrophobic or highly amphiphilic.

The zeroth hydrophobic moment is defined as the sum of the hydrophobicities of the amino acid residues of the segment, and is the analog of the net charge of a cluster of point charges. The first moment, or "hydrophobic moment" is the analog of the electric dipole moment of a cluster of charges. Just as the electric dipole moment measures the asymmetry of the charge distribution, the hydrophobic moment measures the amphiphilicity of the structure. A large hydrophobic moment indicates that a structure is predominantly hydrophobic on one side and predominantly hydrophilic on the other. The hydrophobic moment can be considered in more general terms as a Fourier transform: Suppose that a protein sequence is described as a one-dimensional function, with a numerical hydrophobicity assigned to each amino acid. Then the hydrophobic moment of a segment of the sequence is given by the magnitude of the Fourier transform of the hydrophobicity function. The hydrophobic moment calculated in this way is a function of the periodicity of the protein folding. For example, a highly amphiphilic alpha helix contains hydrophobic side chains every 3.6 residues on average.

Several applications of these ideas will be presented, including: (1) Detection and classification of possible membrane penetrating alpha helices from amino acid sequences; (2) Detection from sequences of highly amphiphilic helices; and (3) Analysis of the amino acid sequence of diphtheria toxin.

0731 MOLECULAR RECOGNITION IN GLOBULAR PROTEINS, George D. Rose and Richard H. Lee, Department of Biological Chemistry, Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033.

The phenomenon of molecular recognition depends strongly upon surface complementarity between associating molecular units, analogous to the assembly of a three-dimensional jigsaw puzzle. The driving force for association is solvent entropy which increases when protrusions of the irregular surface of one subunit fit snugly into invaginations of the other, squeezing out intervening water.

To model this process, an algorithm is presented that automatically finds the prominent topographic surface features of rigid units in proteins of known structure. Using this algorithm, molecular docking can be reduced to a tractable problem by a docking strategy based upon exhaustive trial of combinations of surface features.

The interaction energy between associating units is a function of the surface area buried between them. To measure these interaction energies, a new empirical scale of hydrophobicity will be derived.

Design and Synthesis of Model Peptides and Proteins; Conformational Analysis; Structure in Extreme Environments

0732 STAPHYLOCOCCUS AUREUS V8-PROTEASE CATALYSED REFORMATION OF PEPTIDE BONDS: AN APPROACH TOWARDS SEMISYNTHESIS OF COVALENT ANALOGS OF THE α-CHAIN OF HEMOGLOBIN S.
R. Seetharam, and A. S. Acharya. Rockefeller University, New York, NY.
The design and preparation of new covalent analogs of the α and/or β^s chains either with two or more mutations in them, or with ^{13}C labelled amino acid at a given site will facilitate attempts to delineate the co-operative interactions (coupling) of intermolecular contact regions of deoxy Hb S during gelation. Towards this objective, we have investigated the synthetic potential of Staphylococcus aureus V8 protease, especially since this enzyme selectively hydrolyses the peptide bond Glu(30)-Arg(31) of the α-chain. The two complementary fragments α_{1-30}, α_{31-141}, and heme interact to maintain the native-like structure of the α-chain. On extensive digestion, three more peptide bonds of the α-chain are also hydrolysed (i.e., 23-24, 27-28, and 47-48). HPLC analysis of this digest yields α_{1-23}, α_{1-27}, α_{1-30}, and α_{31-47}. The synthetic potential of V8-protease has been investigated by incubating an equimolar mixture of α_{1-30} and α_{31-47} with the protease at 0°C in the presence of an organic co-solvent, n-propanol. A new chromatographically distinct component is generated: this has been identified by amino acid anlysis and tryptic peptide mapping as α_{1-47}. Demonstration of the potential of V8-protease to reform the peptide bond 30-31 in the fragments, coupled with the fact that α_{1-30} and α_{31-141} interact to form a fragment complementing system, opens up a new avenue for the semisynthesis of novel covalent analogs of the α-chain which could be used in studying the intermolecular contact regions of Hb S. (Supported by NIH Grant HL-27183. ASA is an Established Fellow of NYHA.)

0733 THE CATALYTIC PATHWAY OF CARBONIC ANHYDRASE. Leland C. Allen and Karen Haydock, Princeton University, Department of Chemistry, Princeton, NJ 08544

What are the roles of the essential zinc ion and neighboring active site residues of carbonic anhydrase in catalyzing the hydration of carbon dioxide to bicarbonate? Many conflicting mechanisms have been proposed, based on a variety of kinetic and spectroscopic studies. We have performed molecular orbital calculations to discriminate between the alternative mechanisms and to model the reaction pathway. Our results suggest that zinc is 5-coordinate, with a hydroxyl ion ligand binding CO_2. A cyclic transition state is stabilized by the hydrogen bond chains Arg 246...Glu 106...Thr 199 and His 64...water. The release of HCO_3^- is dependent on a proton transfer involving the His 64...water chain.

0734 MEDIUM RESOLUTION HYDROGEN EXCHANGE, ELECTROSTATIC MODELLING AND SINGLE SITE MUTANT STUDIES OF QUATERNARY STRUCTURE CHANGE IN E. COLI ASPARTATE TRANSCARBAMYLASE
Norma Allewell, David Burz and Mary Glackin-Sundell, Wesleyan University, Middletown, CT. 06457; James Matthew, Genex Corporation, Gaithersburg, MD 20877.
Allosteric regulation in E. coli aspartate transcarbamylase depends upon transmission of signals between active sites and between active and regulatory sites. Medium resolution hydrogen exchange methods, electrostatic modelling and single site mutants have been used to define the mechanism by which signals are transmitted between subunits. Average tritium exchange rates from peptic peptides in c_3 and r_2 have been determined in the presence and absence of effectors (PALA, a bisubstrate analog, and CTP, an allosteric inhibitor, respectively) with the methods developed by Rosa and Richards (1979) and Englander et al (1983). Both ligands produce a net slowing of exchange (Lennick and Allewell, 1981); however, there are significant differences between individual peptides. Calculation of the electrostatic potential following Matthew and coworkers (1979) indicates that docking of the negatively charged substrates is faciliated by a positive potential energy surface near the active site while a region of negative potential on r_2 is involved in c:r interactions. Single site mutants in which a hydrogen bond between c_3 subunits in c_6r_6 has been eliminated have been constructed and are being analyzed. The mutant in which Tyr 165 is replaced by Phe has reduced affinity for L-Asp but responds normally to CTP and ATP. Tm for thermal denaturation of c_3 is the same as the wild type.

Supported by NIH grant AM-17335.

0735 STRUCTURE FUNCTION STUDIES WITH THE LEUCINE-BINDING PROTEINS FROM E. COLI. Tammy K. Antonucci, Robert Landick, and Dale L. Oxender, Dept of Biological Chemistry, Univ. of Michigan, Ann Arbor, MI 48109-0010.
The branched chain amino acid transport system of E. coli includes two periplasmic binding proteins. We are using these two protein components as model systems for structure function studies. We have cloned the genes for both proteins and determined their DNA sequences. The sequences are 80% homologous suggesting that these two genes are the products of gene duplication. Both proteins contain 23 amino acid signal sequences required for their export into the periplasmic space. One of the proteins binds the L-isomers of leucine, isoleucine, and valine (LIV-BP) and the other is specific for L-leucine (LS-BP) although the latter has considerable affinity for D-leucine. The LIV-BP has been crystallized and the three dimensional structure has been determined. The LIV-BP is composed of two structural domains with a cleft between where the substrate is bound. We are using in vitro mutagenesis techniques to determine the structural requirments for the following three functions of the two binding proteins: 1) substrate binding specificity; 2) interaction with membrane components; and, 3) the structural requirments for export into the periplasmic space. By deleting portions of the 3' terminus of the gene for LS-BP we have shown that only the N-terminal portion of the protein is required for export. Gene fusion experiments have shown that the N-terminus can be fused to tryptophan synthetase and effect the export of the hybrid protein across the cytoplasmic membrane of E. coli. We are examining additional differences between these closely related proteins to better understand the relationship between protein structure and function. (This research is supported by NIH Grant 11024.)

0736 SURFACTANT INTERACTIONS WITH PIGMENT-PROTEIN COMPLEXES OF THE CHLOROPLAST THYLAKOID MEMBRANE, Ronald Bartzatt, UCSD Cancer Center T-011, La Jolla, Ca. 92093
The interactions of anionic, nonionic, and zwitterionic surfactants on exposed components of the thylakoid membrane were examined. Difference spectroscopy at long wavelength (greater than 650 nm) shows that chlorophyll molecules are perturbed by surfactants. As the surfactant concentration increases interaction between the membrane and the surfactants changes. Addition of increasing amounts of surfactants to an aqueous solution results in a decrease in the surface tension at the air/liquid interface until the critical micelle concentration is reached. At surfactant concentrations below that for formation of micelles gross membrane structure is little affected. Correlating the maximal chlorophyll absorption with surface tension it is found that for nonionic and zwitterionic surfactants the change in chlorophyll maximal absorption is a function of surface tension only. At low concentrations of surfactants, interactions of monomeric surfactant molecules and membrane occur largely at the membrane/solution interface and appear to be specific for components in Photosystem I. This data shows surface-exposed pigment-protein complexes appear to be susceptible to surfactant-mediated change in the environment at the membrane surface.

0737 NMR AND CIDNP STUDIES OF THE APO- TO CALCIUM BOUND CONFORMATION OF BOVINE MILK ALPHA-LACTALBUMIN, Lawrence J. Berliner and Keiko Koga, Department of Chemistry, The Ohio State University, Columbus, Ohio, 43210, U.S.A.

The α-lactalbumins are calcium binding proteins with binding constants in the nanomolar range. We have characterized the apo- and calcium forms by high resolution proton NMR and laser photo CIDNP at 500 MHz. There are numerous resonances which shift upon calcium binding, suggesting that the conformational change is distributed over a significant portion of the protein structure. CIDNP experiments indicate that two exposed Tyr residues become inaccessible to the solvent upon calcium binding. NOE measurements in the region of a hydrophobic box encompassing Ile 95, Trp 104, Trp 60 and possibly Tyr 103 show that some inter-residue distances change slightly upon calcium binding.

Supported in part by USPHS Grant HD 17270.

0738 ENHANCING FUSION PROTEIN EXPRESSION AND RECOVERY BY MODIFICATION OF PROTEIN STRUCTURE.
Michael L. Bittner, Edith Y. Wong, Edwin Rowold Jr., Beverly A. Reitz, Triprayar V. Ramabhadran and Gwen G. Krivi, Monsanto Co., St. Louis, MO 63167

Bioproduction of small peptides in *E. coli* is currently achieved by producing proteins which are a fusion of a larger protein (usually bacterial in origin) and the desired small peptide. Incorporated into the fusion protein is some characteristic which will allow cleavage of the desired peptide from the carrier moiety. By studying the expression of the oligopeptide hormone somatostatin in *E. coli* we have determined that one can obtain very considerable differences (>1000 fold) in the levels of expression by fusing a given oligopeptide to a variety of carrier proteins or even to different segments of the same carrier protein. Through studies of one particular carrier protein, the *E. coli* recA protein, we have identified a very hydrophilic segment of the recA protein which is both the major antigenic determinant of this protein and required for the solubility of recA:oligopeptide fusion proteins. By incorporation of this hydrophilic segment of the recA protein into a fusion protein, a product is produced which is both soluble and recognizable to monoclonal antibodies prepared against recA protein.

0739 MONOCLONAL ANTIBODY PROBES FOR SELECTED DOMAINS ON THE Escherichia coli recA Protein
Michael A. Blanar, Ted Nilson, Jonathan B. Rothbard*, Alvin J. Clark, and Alexander E. Karu, *Stanford University, Palo Alto CA 94305 and University of California, Berkeley CA 94720

To facilitate studies of the structure and function of E. coli recA protein (recA), we recently performed a computer analysis which indicated that recA belongs to the "parallel α/β" class of proteins, and predicted a general arrangement of α-helices and β-strands to form a stable core (Blanar et al., CSHSQB 49, 1984). We have now prepared synthetic peptides representing six regions external to the putative core structure: 1-15 (A), 30-45 (B), 116-128 (C), 129-141 (D), 152-164 (E), and 309-321 (F). Peptide B spans sites of the recA441 and recA629 mutations, E spans the recA1 mutation, and C includes cys_{116}, implicated in recA ATPase. On immunoblots, monoclonal antibodies (MAbs) to peptide A recognized products of three cloned truncated recA gene fragments and the recAam123 peptide. A MAb made against intact recA protein reacted with different proteolytic fragments of recA441 and recA730 proteins, demonstrating that recA730 is a structural gene mutation. The computer-generated model predicts that peptides B and D lie on one side of the α/β core and that peptides C and E lie on the opposite side. Experiments using MAbs are in progress to test this prediction, and to determine the effects of the antibodies on the enzymatic and cofactor-binding properties of the recA protein. [Supported by American Cancer Society Grant NP237 and U.S. Office of Naval Research Contract N00014-81-C-0570.]

0740 PROBING THE FUNCTION OF DOMAINS OF THE SMALL SUBUNIT PROTEIN OF RUBISCO,
Hans J. Bohnert[1], Elizabeth Seftor[1], Bernd Reiss[1] and Peter H. Schreier[2], University of Arizona[1], Tucson, AZ 85721 and MPI Züchtungsforschung, 5000 Köln, West Germany

In the process of constructing fusion protein genes which consist of the 5'-coding part of the small subunit gene of Rubisco and the entire coding region of neomycin-phosphotransferase (nptII) we found that such fusion proteins are expressed after agrobacterium-mediated plant transformation and that the protein was transformed to the chloroplast and processed. For one construction which contains part of the mature small subunit coding region we have circumstantial evidence that the (SSU-npt) fusion protein is assembled into holoenzyme. We intend to follow this approach in order to identify SSU-domains using deletions in the gene and site directed mutagenesis.

0741 ACTIVE HYBRIDS FORMED BETWEEN INTERFERON-β AND INTERFERON-α,
Paul G. Boseley, Alan G. Porter, Leslie D. Bell, Andy G. Stewart, Graham H. Catlin, Ivan J.D. Lindley, John R. Adair, C. Hynes, N. Warburton and John Smith, Searle Research, G.D. Searle & Co. Ltd., High Wycombe, Bucks. HP12 4HL, England

Computer prediction studies (Sternberg and Cohen, Int. J. Macromol., 4, 137-144 (1982)) have suggested that IFN-β and IFN-α share a similar tertiary structure. These predictions have been tested by replacing specific structural domains with equivalent IFN-α segments. This was accomplished by ligating chemically synthesized oligonucleotides into specific areas of the IFN-β gene using convenient, naturally occurring restriction sites. The examples given demonstrate that the antiviral, antiproliferative, and immunoregulatory properties of the hybrid IFNs are retained even after replacement of large portions of the IFN-β molecule.

0742 PROGRESS IN UNDERSTANDING ADENOVIRUS ARCHITECTURE,
Roger M. Burnett, Jan van Oostrum and Michael M. Roberts, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, New York, 10032.

The icosahedral adenovirus virion is formed from at least ten different polypeptides and has a total molecular mass of 175 x 10^6 daltons. The detailed architecture is being explored by a combination of X-ray crystallography, electron microscopy and biochemistry. A 2.9 Angstrom electron density map of the major coat protein, hexon, has given a preliminary chain tracing, which is being refined by correlation of the density with the known sequence. The majority of the 967 amino acids have already been located. The molecular morphology has facilitated the interpretation of high resolution electron micrographs of various capsid fragments, which reveal the organization of the outer shell. The remarkably simple symmetry permits hexons in the four topologically distinct locations to maintain a large fraction of identical inter-hexon bonds. A biochemical analysis of the virion proteins has provided the absolute stoichiometry for the different structural polypeptides, defined the organization of the vertex region, and indicated how a puzzling symmetry mismatch at the vertex can be overcome. The dissociation pattern of the capsid may be explained by the location of polypeptide IX as a cement. The results suggest that larger units than the polypeptide should be considered as structure units in viral architecture. Large, weakly-interacting, building blocks permit accurate assembly, with stability conferred by the later addition of a different structural component. The controversy surrounding the recent demonstration of an all-pentameric polyoma capsid is resolved using the new approach. (Supported by NIH Grant AI 17270).

0743 EXPRESSION AND SECRETION IN *E. COLI* OF BOVINE GROWTH HORMONE FUSED PRECISELY TO THE SIGNAL SEQUENCE OF β-LACTAMASE,
Andrew D. Charles, Alison King, ICI Corporate Bioscience Group, Runcorn, Cheshire, England.

We have constructed a plasmid containing the gene for mature bovine growth hormone (BGH) fused precisely to the portion of the β-lactamase gene containing the promoter and 23 amino acid signal sequence. The resultant hybrid βla-BGH gene expresses pre-βla-BGH *in vitro* but produces no protein *in vivo*. Other studies [1,2] have shown that nucleotide sequences in the 5' portion of the gene for mature BGH block expression in constructs where the gene is fused to a *trp* promoter and ATG codon. It was suggested that this block is exerted at the translational level. We have constructed in-phase deletions and introduced single base changes by way of site-specific mutagenesis in this 5' region in order to determine the features of the BGH sequence regulating expression and secretion of the hybrid βla-BGH.

[1] P. H. Seeburgh, S. Sias, J. Adelman, H. A. deBoer, J. Hayflick, P. Jhurani, D. V. Goeddel, H. L. Heyneker (1983) DNA, 2: 37-45.

[2] H. A. deBoer, P. H. Seeburg, H. L. Heyneker (1983) U.K. Patent GB 210611A.

0744 ALPHA 4, A SYNTHETIC PROTEIN, W.F. DeGrado, W. Wilcox, and D. Eisenberg, Experimental Station, E.I. DuPont DeNemours and Co., Wilmington, DE 19898 and Molecular Biology Insitute, University of California, Los Angeles, CA 90024

An artificial protein, ALPHA4, of four antiparallel alpha helices has been designed and is being synthesized by chemical methods. It resembles the four alpha helical bundle class of naturally occuring proteins, described by Weber and Salemme (1).

To simplify synthesis, ALPHA4 has been designed in 9 modular units: four identical helices, three identical linkers which each join two of the helices, and N- and C- terminal units. Thus the entire structure of 90 residues can be represented as:

N-term + Helix + Link + Helix + Link + Helix + Link + Helix + C-term

Each helix consists of 16 residues designed to fold into a highly amphiphilic alpha helix, with leucyl resides along one face and lysyl and gutamyl residues along the opposite face. In models, the leucyl residues from four helices pack tightly in a hydrophobic interaction, exposing all charged side chains to solvent. One leucyl residue in each chain is somewhat exposed, and it is expected that methylene groups from one of the charged side chains will mask it from solvent.

In the first stage of work, the helix has been synthesized and purified, and x-ray grade crystals are being sought from unlinked helices. In a second stage, it is planned to join two helices with a simplified link consisting of five glycyl residues.

(1) P.C. Weber and F.R. Salemme (1980) Nature, 287:82-84.

0745 ANTIBODIES RAISED AGAINST PORCINE PARVOVIRUS PROTEIN FRAGMENTS - THE EFFECT OF ANTIGEN PRESENTATION, G.M. Fox, A. Banks, M. Caruthers, J. Bruszewski, S. Hu, S. Suggs, G.A. Bitter, and D. Langley. Amgen, Thousand Oaks, CA 91320.

Many laboratories have used recombinant DNA techniques to express viral proteins in *E. coli* for potential use as subunit vaccines. So far, few *E. coli*-produced materials have been capable of eliciting antibodies which neutralize the infectivity of the target virus. In many cases, it is possible that the proper linear determinants are in fact present but the bacterially produced proteins are folded in such a way that the relevant regions are not on the surface or are held in a conformation such that they are not properly recognized. We have been concerned with the production of a subunit antigen which is able to elicit neutralizing antibodies against porcine parvovirus (PPV). We have cloned and expressed in *E. coli* several fragments of the PPV genome. Several methods of antigen presentation have been explored using the most hydrophilic portion of the viral capsid protein coding region. We have: 1) expressed the region as a fusion with the *E. coli* trp E gene; 2) expressed a similar region using a synthetic promoter and initiation codon so that no foreign protein is attached; 3) expressed a larger region - as in 2) but with an additional 160 C-terminal amino acids; 4) synthesized a peptide whose sequence is contained within the hydrophilic region. The materials described above have been injected into rabbits and the resulting sera have been tested for the ability to bind PPV and neutralize viral infectivity.

0746 SYNTHESIS CONFIRMATION: SOLID PHASE SEQUENCING ON THE GAS PHASE SEQUENCER J. Lawrence Fox, Yangkil Kim, and Virender K. Sarin, Department of Molecular Biology, Abbott Laboratories, North Chicago, Illinois 60064

Automated Edman degradation has been successfully used for determining the primary structure of numerous peptides and proteins. Quantitative solid phase Edman degradation has great potential use for amino acid sequence analysis of synthetic peptides assembled on resin support by the Merrifield procedure. We report here the combined use of a modified gas phase sequencer program and our improved reverse phase HPLC analysis for PTH amino acids to carry out the sequence analysis on synthesized peptide resins. This approach is far more sensitive than using glass beads on the conventional solid phase sequencer. The peptide was assembled on copoly (styrene-1% divinylbenzene) resin beads at an initial substitution of 0.54 mmol/g. On a routine basis, 10-15 resin beads are used, and a repetitive yield $\geq$95% is obtained; as far as 4 beads can be successfully sequenced. The HPLC PTH-amino acid analysis is sensitive down to subpicomole quantities. This procedure offers a sensitive and rapid analytical tool for checking the purity of peptides as they are being assembled on solid support. This poster will discuss the utility of preview sequencing for testing the integrity of syntheiszed peptide on solid support and its implications in following the course of a synthesis. It should greatly facilitate synthesis of peptides.

0747 DESIGN OF DNA SEQUENCE-SPECIFIC BINDING PEPTIDES, Alan D. Frankel and Carl O. Pabo, Johns Hopkins School of Medicine, Baltimore, MD 21205

As part of our project to design novel proteins and peptides with defined structures, we have tried to design a peptide that would bind to a specific DNA sequence. Through a variety of model-building studies, we tried to pick a peptide sequence which would bind to the sequence, 5'GAATTC. This peptide is 21 residues long and is predicted to be a single alpha helix with hydrogen bonds to each of the 6 base pairs and several hydrophobic and phosphate contacts. We synthesized the gene encoding this peptide and inserted it into a plasmid containing a protein, LE', such that a fusion protein would result with a methionine residue at the junction. This plasmid was inserted into E. coli and the resulting fusion protein was expressed as approximately 30% of the total cellular protein. The peptide was purified by cyanogen bromide cleavage of the partially purified fusion protein followed by paper chromatography and HPLC using a C18 resin. We are currently studying the DNA binding properties of this peptide by restriction endonuclease inhibition and by DNA footprinting methods, and are studying the conformation of the peptide by circular dichroism. In addition, we are synthesizing a second peptide with similar proposed sequence-specific contacts but with the predicted alpha helical conformation stabilized by dimer formation via a hydrophobic surface and a disulfide bond.

0748 **ANALYSIS OF THE STRUCTURE, FUNCTION AND INTRACELLULAR TRANSPORT OF AN INTEGRAL MEMBRANE PROTEIN - THE HAEMAGGLUTININ OF INFLUENZA VIRUS.** M.-J.Gething, M.Roth, C.Doyle, S.Sharma and J.Sambrook. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.11724, U.S.A.

In recent years, we have used the haemagglutinin (HA) of influenza virus as a model to study eukaryotic integral membrane proteins. Our major interests are two-fold; firstly to correlate structure with function by identifying and analysing the protein domains or epitopes involved in receptor recognition, enzyme activity and antigenicity, and secondly to understand the mechanisms that determine the route of transport and final destination of nascent glycoproteins in eukaryotic cells. HA is the best characterized of all integral membrane proteins: its three-dimensional structure is known and the location of its major antigenic sites, the points at which it is glycosylated, its organization into trimeric structures and its orientation with respect to the membrane have been defined. The cloned gene for HA can be expressed with very high efficiency in mammalian cells, and the protein produced from the wild-type gene is displayed on the cell surface in a glycosylated form that is both biologically and antigenically active. It is therefore feasible to introduce mutations into the cloned HA nucleotide sequence, to express the altered genes in eukaryotic cells and to analyse the phenotype of the mutant proteins. Experiments will be described in which sequences encoding the three hydrophobic domains (the signal sequence, the fusion peptide and the transmembrane anchor) and the cytoplasmic tail of the HA molecule have been altered, deleted or exchanged for those of other eukaryotic membrane proteins.

0749 USE OF NON-GENETIC CODE AMINO ACIDS IN SINGLY SUBSTITUTED LINEAR GRAMICIDINS TO INVESTIGATE TRANSMEMBRANE CHANNEL FUNCTION, Roger E. Koeppe II, University of Arkansas, Fayetteville, AR 72701; and Olaf S. Andersen, Cornell University Medical College, New York, NY 10021.

Oligonucleotide-directed mutagenesis is limited to those amino acids which are specified by the genetic code. By contrast, chemical synthesis of "mutant" polypeptides offers the possibility of introducing non-genetic code amino acids for particular investigations, e.g. comparisons of the functional effects of essentially isosteric side chains of different polarities (such as Phe vs. o-, m-, p-F-Phe). We have used a semisynthetic approach, consisting of degradation from the formyl-N-terminal followed by re-synthesis, to introduce variant amino acid side chains into the monovalent cation-selective channel-forming pentadecapeptide, gramicidin A. Even though the side chains are not in direct contact with the permeating ions, the single-channel conductances for Na^+ and Cs^+ through gramicidin channels are markedly affected by changes in the physico-chemical characteristics of the side chains. The maximal single-channel conductance for Na^+ is decreased up to ten-fold when a polar side chain is present at position #1 in gramicidin. Furthermore, the selectivity for Cs^+ over Na^+ is increased when a polar side chain is at position #1. Because the transmembrane channels are dimers of gramicidin, it is possible to observe hybrid channels formed between the natural and modified gramicidins. These hybrid channels suggest that the observed conductance changes are not due to gross changes in channel structure, but rather to subtle ion-side chain interactions over distances of about 5-10 Å.

0750 DISTANCE APPROACHES TO PROTEIN STRUCTURAL ANALYSIS AND PREDICTION, Michael N. Liebman, Dept. of Pharmacology, Mt. Sinai School of Medicine, New York, N.Y., 10029

Experimental observations support the idea that the three-dimensional structure of a protein should be predictable from its constituent amino acid sequence, but as yet no single method or group of methods produce a consistently correct conformation for a given amino acid sequence. A simple computational method, the Linear Distance Plot (LDP), is described that provides for the rapid analysis of protein structure at the secondary structural level using atomic coordinates available from X-ray crystallography and based on distance relationships along the polypeptide backbone. We present the technique and examples of its use in several areas, including: 1) analysis and identification of secondary structural components and boundary definition; 2) identification of new patterns of secondary structure that are complex in conformation but observed in different proteins; 3) examination and comparison of secondary structural elements to classify sites and classes of conformational perturbation; 4) assignment of structural insertions and deletions upon comparison of two macromolecules, to contrast with sequence homology; 5) assignment of a structural sequence to permit searching of protein structures for 'structurally homologous' regions using amino acid sequence analysis techniques; and 6) development of an expanded structural syntax for use in correlation with our circular dichorism data base. These approaches are being used to formulate an algorithm for the prediction of protein structure based on the use of artificial intelligence techniques, pattern recognition and image processing.

0751 CLONING OF A RHESUS MONKEY LIVER ALCOHOL DEHYDROGENASE ISOZYME, David R. Light, Mark S. Dennis, Chung-cheng Liu and Carol E. Morita, Genentech, Inc. 460 Pt. San Bruno Blvd., S.S.F., CA 94080

A number of mammalian liver alcohol dehydrogenases have been characterized with respect to turnover and substrate specificity. In general, this type of ADH will recognize a variety of primary and secondary aldehydes and alcohols as substrates and while members of this group do not have identical substrate specificities their substrate preferences are broad and overlap each other. Several mammalian ADH proteins have been partially or completely sequenced and the sequence of a partial clone of human ADH has been published (Duester et al. (1984) Proc. Natl. Acad. Sci. 81, 4055-4059). Since the crystal structure of a mammalian ADH from horse is known, this type of ADH is well suited for studies of dehydrogenase structure, function and design. We report the cloning and complete sequence of a mammalian ADH isozyme from Rhesus monkey liver.

0752 A MODEL SYSTEM FOR THE STUDY OF LIPID-PROTEIN INTERACTION, J. Lowe, J. Sacchettini, L. Banaszak, and J. Gordon, Washington Univ. School of Medicine, St. Louis, MO 63110

Rat liver fatty acid binding protein (FABP) is a 14.6 kDa polypeptide which is abundantly represented in the cytosol of hepatocytes and enterocytes. 60% of liver cytoplasmic long chain fatty acids are affiliated with this protein. Each mole of purified protein contains approximately 1 mole of fatty acid (FA). Evidence points to a physiologic role for FABP in the uptake and/or intracellular compartmentalization of FA's. Because of its small size, FABP may provide a good model for the study of the molecular details of fatty acyl-protein interaction. We have therefore established a prokaryotic expression system in order to analyze these interactions by site-directed mutagenesis of cloned FABP cDNA. The coding sequence of FABP cDNA was linked with synthetic oligonucleotides to the control regions of the E. coli expression vector pPLc245. FABP purified from the strain of E. coli containing the chimeric plasmid represented approximately 1% of bacterial proteins. Partial amino acid sequence determination confirmed the integrity of the protein. Lipid analysis revealed a heterogeneous population of non-covalently bound, C14 - C24 FA, similar to the saturated FA population associated with hepatocyte FABP. Analysis of wild type and mutant FABPs using a solid phase binding assay indicated that the COOH-terminal region of the protein is important for its fatty acyl binding properties. In addition, a full length human FABP cDNA was isolated and sequenced. Comparison of the rat and human sequences revealed identity at 82% of the amino acid residues, and conservation of amino acid types at most of the non-identical positions. Secondary structure predictions indicated that the FABPs have features which distinguish them from serum albumin and the apolipoproteins.

0753 DENATURATION OF APOLIPOPROTEIN A-I BY HYDROSTATIC PRESSURE, William W. Mantulin and Henry J. Pownall, Baylor College of Medicine, Houston, Texas 77030

Apolipoprotein A-I (apoA-I) is the most abundant protein in human plasma high density lipoprotein. It is a surface active protein, which is also water soluble with a concentration dependent self-association. The molecular weight is 28,400 D and the amino acid sequence (243 residues) is known. Like other apolipoproteins apoA-I is structurally adaptive, as manifested by a low free energy of unfolding (2-4 kcal/mol) calculated from calorimetric data or chemical denaturation. We have used high hydrostatic pressure with fluorescence detection to compress and denature apoA-I in the presence and absence of GdmCl. By monitoring changes in apoA-I tryptophanyl fluorescence polarization we detect a midpoint in the pressure denaturation curve at 1.1 kbar, corresponding to a negative volume change of -53 $\pm$ 5 ml/mol. Small concentrations of GdmCl promote pressure induced perturbation of secondary and tertiary structure (confirmed by fluorescence spectral data) with a concomitantly larger volume change (-91 $\pm$ 9 ml/mol). The calculated free energy change for apoA-I unfolding by hydrostatic pressure is 1.8 kcal/mol. In conclusion, denaturation of apoA-I by pressure results in a compression of the protein's three dimensional structure (a negative volume change), probably by elimination of internal packing defects. (HL 27104)

0754 STRUCTURAL STUDIES ON HUMAN PLASMA APOLIPOPROTEINS. John B. Massey and Henry J. Pownall, Baylor College of Medicine and The Methodist Hospital, Houston, TX 77030.

The human serum apolipoproteins are surface-active polypeptides that solubilize the lipids circulating in plasma. The association of apolipoproteins with a water-phospholipid (PC) interface occurs with the development of an α-helical structure in which polar and non-polar faces are formed. The helix presumably locates at the interface with the polar face directed toward the aqueous phase and non-polar face penetrating into the lipid phase. To correlate apolipoprotein structure and function, we have studied apolipoprotein A-II in solution and associated with PC. ApoA-II contains two identical polypeptide chains of 77 amino acid residues which are linked by a disulfide bond at residue 6. In solution, the protein contains approximately 30% α-helical structure which changes to 70% when associated with PC. Kinetics of trypsin hydrolysis, where the release of individual peptides was followed by reversed phase HPLC, indicated that the lipid associated protein is hydrolyzed 1000 times slower than in solution which suggests that there are no unstructured regions susceptible to trypsin. Spectroscopic and chemical modification measurements of tyrosine residues determined their location in either the lipid or aqueous phase. Theoretical analysis using the helical amphipathic moment method of Eisenberg indicates several regions of high helical amphiphilicity. A combined approach of physical-chemical studies and structure prediction methods suggests a model for the secondary and tertiary structure of this protein at the lipid-water interface. For apolipoproteins, the amphipathic helical moment analysis is a promising method of correlating structure with function and for the design of model lipid-associating peptides.

0755 ENGINEERING OF PROCHYMOSIN ALTERS ITS ACTIVATION PROPERTIES, Michael T. McCaman, Diana Begley, and John King, CODON, Brisbane CA

Bovine prochymosin encoded on a bacterial plasmid has been purified and activated after its synthesis in *E. coli*. Prochymosin is an acid protease zymogen which is autocatalytically activated and processed at acid pH to form pseudochymosin (at pH 2) or chymosin (at pH 4.5), enzymes which clot milk. Proteolytic processing normally occurs with activation and results in the removal of 27 or 43 NH_2-terminal residues at pH 2 or 4.5, respectively. By genetic manipulation of the DNA sequence encoding prochymosin, we have constructed several altered zymogens with unusual activation properties. We altered the amino acid coding sequence surrounding the pH 2 and 4.5 processing sites to create DB20 and DB45 prochymosins, respectively. The DB20 zymogen is not processed at pH 2 but is partially and reversibly activated. Its pH 4.5 processing and activation occur normally. The DB45 prochymosin is neither processed nor activated at pH 4.5 but is activated normally at pH 2.

These engineered zymogens demonstrate that; 1) proteolytic processing is not essential for activation, 2) activation can be a (partially) reversible process, and 3) that the two zymogen processing sites seem to operate in an independent fashion.

0756 Abstract: STRUCTURE OF THE COMPLEX OF SUBTILISIN NOVO AND THE INHIBITOR CI-2 FROM BARLEY SEEDS, C.A. McPhalen and M.N.G. James, Medical Research Council of Canada Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

The molecular replacement method, with subtilisin BPN' as a search model, has been used to obtain an electron density map for the complex between the serine protease subtilisin novo and the inhibitor CI-2 from barley seeds. The structure of the inhibitor is currently being determined from this map. CI-2 is a member of the potato inhibitor 1 family, a group of serine protease inhibitors lacking disulfide bridges, and is the first member of this family to have its crystal structure determined. In the structures of other serine protease inhibitors, the stability provided by disulfide bridges is thought to be important in the mechanism of inhibition. Since CI-2 lacks these bridges, its structure may provide useful information on protein stability in general.

0757 GLUTATHIONE REDUCTASE - CORRELATION OF X-RAY STUDIES WITH KINETIC DATA, Emil F. Pai, Elke Horn and Georg E. Schulz, MPI for Med. Res., D-6900 Heidelberg, West-Germany

Glutathione reductase is a ubiquitous flavoenzyme. Its sequence and threedimensional structure at high resolution are known. The stereochemistry of its catalysis has been deduced from structural analyses of several reaction intermediates. During these studies numerous analogues and fragments of the two substrates NADPH and GSSG have been soaked into crystals of glutathione reductase. The complexes were analyzed using difference-Fourier metods.

As a first step to relate the results of this "static" method to the behaviour of the enzyme in solution we measured inhibition constants of substances which had been shown to bind to the NADPH-site of the crystalline enzyme, analogues with a modified nicotinamide ring or fragments of the NADPH-molecule. Later, compounds which had not been used in the crystallographic work were also included. Finally, Michaelis constants for several modified substrates were determined.

The kinetic results correlate well with the X-ray experiments. The enzyme clearly distinguishes between the binding of an oxidized and a reduced nicotinamide ring. The absence or presence of a 2'-phosphate certainly determines the strength of binding in a dominant way. Structural changes in the adenine part of reduced coenzymes had smaller effects on binding to the enzyme than the corresponding alterations on oxidized coenzymes.

0758 IN VITRO AND IN VIVO STUDIES OF ACYLATED APOLIPOPROTEINS. Henry J. Pownall, Gabriel Ponsin, Antonio M. Gotto, and James T. Sparrow. Baylor College of Medicine, Houston, TX 77030.

Apolipoproteins are important components of plasma lipoproteins that solubilize lipids, activate lipolytic enzymes, and contain determinants for receptor mediated endocytosis. A series of acylated apolipoprotein analogs with the structure, R'_n- SSLKEYWSSLKESFS where R' represents an acyl group with n=4 to 18 carbons has been synthesized and tested. The equilibrium constant for association of the peptides with reassembled high density lipoproteins (R-HDL) increased with increasing acyl chain length according to a predictable pattern in which each methylene unit contributes a constant increment to the free energy of association. When bound to R-HDL the peptides are highly helical and activate plasma lecithin: cholesterol acyltransferase. The rate of turnover and tissue sites of degradation of each peptide in the rat were determined. The plasma lifetime increased with acyl chain length; concurrently the tissue sites of degradation shifted from the kidney to the steroidogenic tissue, a picture that is consistent with the metabolism of the peptide as a component of rat HDL. These results demonstrate that increasing peptide hydrophobicity by acylation increases its affinity for HDL and targets the peptide to HDL receptors.

0759 STRUCTURE-LUMINESCENCE CORRELATIONS IN PROTEINS. F. G. Prendergast, J. Wick*, G. Ford*, and K. Ward*. Department of Pharmacology, Mayo Foundation, Rochester, MN 55905 and *Department of Chemistry, University of Wisconsin, Parkside, Kenosha, WI 53141.

Although inferences can be drawn from fluorescence data regarding the structure and mobility of proteins, there have been few attempts to determine how the structure is correlated with fluorescence in proteins. We have used measurements of fluorescence spectra, lifetimes, quantum yields, and anisotropy, and molecular graphics depictions (Connolly and Barry surfaces) in an attempt to make such correlations. The tryptophan fluorescence and crystal structures of horse liver alcohol dehydrogenase, Staphylococcal nuclease, melittin, myoglobin (apomyoglobin fluorescence), azurin, RNAse-T_1, elastase, and Streptomyces griseus proteinase A were examined. Solvent accessibility clearly plays a major role in determining the spectral properties, but in some instances emission spectra are shifted and lifetimes altered without evidence of significant solvent access. The data show clearly the role of the protein matrix in determining fluorescence properties. Also, while the molecular "packing" in the protein clearly influences fluorophore mobility (hence fluorescence anisotropy), the major effect seems to be on amplitude of motion rather than rate. Supported by NIH Grant GM 30178. F.G.P. is an Established Investigator of the American Heart Association and a Searle Foundation Scholar.

0760 A Genetic Approach to Secondary Structure Analysis of Coliphage Lysis Proteins, R. Raab, D. Maretea, G. Neal, S. Morham, and R. Young, Medical Biochemistry , TAMU College Station, TX 77843-1112

We have cloned the lethal lysis genes S and E (of λ and φx174, respectively) under lacPO control. We have selected and sequenced mutants in each gene and analyzed the effect of the codon changes using hydrophobicity and secondary structure algorthims. None of the changes have any effect on the hydrophobicity profile. However, many of the changes alter the Chou-Fasman prediction for small domains in the molecule form alpha helix in favor of beta pleated sheet or beta turn. We have used this mutational information and the probable structural homologies between these two lysis control proteins to develop a model for S and E gene product secondary structure. In addition, fusions to the lacZ gene have been constructed at various points in the E and S genes. The properties of these fusion genes suggest that fusions within membrane-spanning domains if cytoplasmic membrane proteins lead to inactive beta-galactosidase and lon-dependent instability. In contrast, fusions to probable aqueous domains are fully active as beta-galactosidase, even though the molecule is embedded in the membrane by the amino-terminal membrane-spanning region and also fully active as phage lysis genes. These results suggest there are signals in the lysis gene sequence which promote penetration of the membrane; these signals are completely different from classic signal sequences.

0761 SYNTHETIC PEPTIDE ANALOGS OF THE AMPHIPATHIC HELIX: STRUCTURE-FUNCTION STUDIES, Jere P.Segrest, G. M. Anantharamaiah, Christie G. Brouillette, B. Hong Chung and Charles F. Schmidt, UAB Medical Center, Birmingham, AL. 35294.

The amphipathic helix, originally described by us, is accepted as the quintessential lipid-associating domain of the exchangeable plasma apolipoproteins (apo). The amphipathic helix model defines a general α-helical domain containing opposing polar and nonpolar faces. Further, the polar face contains a specific distribution of charged residues. In recent studies we have shown that a computer-designed 18 amino acid synthetic peptide analog of the amphipathic helix, dimerized by proline, closely mimics apo A-I (the major protein of high density lipoproteins) in its interactions with lipids. Specifically, a combination of electron microscopy, nondenaturing gradient gel electrophoresis, ^{1}H-NMR and differential scanning calorimetry provides strong evidence for a peptide annulus-bilayer disc structure for peptide/phosphatidylcholine (PC) complexes; these complexes are of the same size as comparable weight ratio apo A-I/PC complexes. Further, this peptide analog competes with apo A-I for binding to high density lipoproteins and produces activation of lecithin:cholesteryl acyl transferase (LCAT) comparable to or greater than that achieved by apo A-I. Studies of other peptides indicate that the position of the charged residues on the polar face plays a major role in the lipid affinity and biological mimicry of an amphipathic helix, and deletion of a single residue from the center of an amphipathic helix domain distorts the resultant potential amphipathic helix structure such that lipid affinity and LCAT activity are markedly decreased. The results of these studies suggest that simple peptide analogs of the amphipathic helix represent a useful strategy for the correlation of the structure of apo A-I with function. Further, since plasma levels of apo A-I have a strong inverse correlation with risk for atherosclerosis, peptide mimics of apo A-I could prove useful as pharmacological agents.

0762 THE CONFORMATIONS OF WHEAT GLUTEN PROTEINS, Peter R. Shewry, Arthur S. Tatham, Benjamin J. Miflin and Peter Belton, Rothamsted Experimental Station, Harpenden, UK. and Food Research Institute, Norwich, U.K.

Wheat gluten is a mixture of at least 50 water-insoluble polypeptides which are associated by disulphide bonds, hydrophobic interactions and hydrogen bonds. It is a cohesive mass which exhibits the unusual physical properties of elasticity and viscous flow. In order to understand the molecular basis of these properties we are studying the conformations of whole gluten, gluten fractions and individual polypeptides by nuclear magnetic resonance and circular dichroism spectroscopy, and by computer prediction. On the basis of these studies we propose that elasticity is conferred by a group of polypeptides which are present in disulphide bonded polymers and have a conformation rich in repetitive β-turns. This is similar to the structure proposed for the mammalian connective tissue elastin.

0763 IONIC STRENGTH DEPENDENCE OF HELIX FORMATION BY C-PEPTIDE ANALOGUES, Kevin R. Shoemaker*, Peter S. Kim*, Susan Marquesee*, Eunice York#, John M. Stewart# and Robert L. Baldwin*, *Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305, #Department of Biochemistry, University of Colorado School of Medicine, Denver, CO 80262.

Studies of helix formation by C-peptide analogues have shown that the results can be partially explained by a helix dipole model. A charged group stabilizes the helix if it is close to a pole of opposite charge, or destabilizes if it is close to a pole of like charge. In order to test the helix dipole model, we have studied the ionic strength dependence of helix formation by these analogues. To discriminate charge shielding effects from specific ion effects, different ions in the Hofmeister series have been used in these studies.

In 0.1 M NaCl, peptides lacking either Glu 2 or His 12 fail to show detectable helix formation. At higher ionic strengths, these peptides show partial helix formation, suggesting that mobile counterions can interact with the poles of the helix dipole and stabilize the helix in a manner similar to bound charged groups.

The ionic strength dependence of helix formation varies strikingly as individual charged amino acids are replaced with uncharged residues. The relationship between these results and the work of Ihara et al. (Biopolymers 21, 131-145 [1982]) on helix formation by $(Ala)_{20}$ in block copolymers will be discussed.

0764 USE OF MUTANTS TO STUDY LOCAL EFFECTS VERSUS LONG-RANGE COUPLING IN HEMOGLOBIN, Francine R. Smith and Gary K. Ackers, Dept. of Biology, The Johns Hopkins Univ., Baltimore, MD 21218.

The free energy of cooperativity in human hemoglobin is largely comprised of contributions from interactions at the $\alpha^1\beta^2$ intersubunit contact region, including the intersubunit contacts $\alpha^1\beta^2$, $\alpha^2\beta^1$ and $\alpha^1\alpha^2$ (Pettigrew et al., (1982) Proc. Natl. Acad. Sci. USA 79, 1849). A major question then is that of how much coupling exists between the residue sites within this regulatory interface region. We have investigated this issue by studying hybrids of normal, mutant, and chemically-modified hemoglobins. We have developed methods whereby one can quantitatively determine the energetic properties of hybrid molecules in equilibrium with their parent species. The results imply that the "perturbations" resulting from structural modification at the various sites are "additive", i.e. they exhibit pairwise independence. This result holds over all the combinations of modifications tested. This finding is independent of the nature and location of the structural perturbation within the hybrid molecule. There thus appears to be no direct long-range coupling within the interface itself. In contrast, perturbations at the heme brought about by the binding of oxygen or other ligands lead to large and simultaneous alterations in the interactions between residue sites within the contact region. We thus have found indirect coupling among these sites through the perturbations at the heme. These results illustrate the powerful use of single-site modifications to investigate 1) the structural location of functional events and 2) structural pathways for coupling and transmission of functional events within a protein.

0765 PRODUCTION OF METHOTREXATE RESISTANT MUTANTS OF MOUSE DHFR BY IN VIVO SELECTION IN B. SUBTILIS, J. Thillet, T. Grange, B. Ribadeau-Dumas, R. Van Rapenbush, J. Jami and R. Pictet, Institut Jacques Monod, Paris, France.

We have developed a system to produce methotrexate resistant mutants of DHFR. A plasmid, pQS4, was constructed which placed the DHFR cDNA under the control of a modified promoter of β-lactamase which improves the efficiency of DHFR synthesis in both B. subtilis and E. coli. Consequently an overproduction of the wild type mouse DHFR enzyme was obtained in both strains. B. subtilis containing pQS4 was cultured in the presence of increasing amounts of methotrexate (MTX)-At 10 mM MTX, different mutant plasmids conferring MTX^R phenotypes were isolated. Kinetic studies of their DHFR activity indicated that some of these plasmids coded for enzymes with activities different from the wild type. One of them (pQS6) was particularly studied. It exhibited a modified K_M for DHF (5.10^{-5} M instead of 0.8 10^{-5} M) and a modified ID50 for MTX (10^{-6} M instead of 5 10^{-9} M). The mutated cDNA was sequenced and a punctual mutation was found which corresponds to a glutamine 35 → proline substitution in the protein. In the wild type enzyme, this glutamine is located in a stretch of α helix. Its replacement by a proline, an amino acid known to disrupt such a configuration, may affect the spatial position of the phenylalanine 34 which is known to be involved in contact with some substrate analogues. Because of the facility to select for mutations in bacteria, the system we described should help to accumulate a repertoire of similar mutations useful for the elucidation of the structure-function relationship of the enzyme.

0766 Antibody Crystallization on Phospholipid Films: Dynamics and the Effects of Antibody Conformation. E.E. UZGIRIS, General Electric Research and Development Center, Schenectady, NY 12301

Monoclonal antibodies form 2-D crystals when bound to haptinated phospholipid monolayers in physiological conditions and at ambient temperatures[1]. IgG_1 forms two crystal phases: A linear strand phase and a high order hexagonal phase. The relative distribution of these two phases is dependent on temperature, pH, and salt concentration. This dependence is one which is associated with protein intramolecular interactions rather than lipid-lipid or lipid-protein interactions for a number of reasons: 1) Polyclonal antibodies against the hapten DNP do not organize into any crystal structure for any of the experimental conditions used. 2) Slightly denatured Ig, (through storage at 4°C for example) do not readily crystallize and a shift in the temperature dependence for forming the hexagonal phase is observed. 3) There is no pH driven transition in crystallization tendency for IgE anti-DNP but a transition to disorder is observed at above 30°C. No such transition exists for IgG_1. Observation of the dynamics of crystal growth shows a clear and marked dependence on pH and temperature which is in accord with the results of long term incubations. It is found that high pH retards crystal growth very significantly for IgG_1 but not for IgE. Also, the crystal growth rate of 4°C stored IgG_1 is greatly reduced over fresh IgG_1 (-80°C stored). Furthermore, it is found that the linear phase of IgG_1 is an extremely rapidly forming phase but one that is metastable against the hexagonal phase.

[1] E.E. Uzgiris & R.D. Kornberg. Nature 301 134-136 (1983)

0767 ^1H NMR STUDIES OF BOUND AND UNBOUND MONOCLONAL ANTIBODIES TO CANINE ADENOVIRUSES I AND II, R. A. Van Deusen[1], S. Lacelle'[2], R. D. Scott[3], C. A. Whetstone[4], and B. C. Gerstein[2], National Veterinary Services Laboratories, P.O. Box 844, Ames, IA 50010[1]; Ames Laboratory, U.S. Dept. of Energy and Dept. of Chemistry, Iowa State University, Ames, IA 50011[2]; Dept. of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011[3]; and National Animal Disease Center, P.O. Box 70, Ames, IA 50010[4].

The ^1H nuclear magnetic resonance (NMR) spectra of monoclonal antibodies specific for proteins of canine adenoviruses I and II were obtained in the absence and presence of antigen. A comparison of "difference spectra" is presented and reasons for the observed differences are postulated.

All of the experimental preparations were stored at -20 C for several months, after which new NMR spectra were obtained. The nature of observed differences between spectra of fresh versus stored preparations are discussed.

0768 MOLECULAR ANALYSIS OF CALMODULIN'S ENZYME ACTIVATOR AND DRUG BINDING ACTIVITIES.
D.M. Watterson, T.J. Lukas, D.M. Roberts and R. Crea*, Howard Hughes Medical Inst. and Vanderbilt Univ., Nashville, TN and *Creative Biomolecules, S. San Francisco, CA.

Calmodulin is a calcium binding protein that has multiple in vitro biochemical activities, including the activation of specific enzymes and the stoichiometric binding of a series of drugs and dyes that can serve as antagonists of calmodulin function. Our analysis of calmodulin structure from a wide variety of animal and plant species has shown that calmodulins from vertebrate, slime mold, higher plant and algal species have 85% sequence identity. Most of the differences are conservative changes. These limited number of structural changes result in quantitative functional differences in the activation of some enzymes, such as NAD kinase, but do not appear to drastically alter the activation of other enzymes, such as phosphodiesterase. We have also re-investigated the stoichiometry of calmodulin's interactions with phenothiazines and other inhibitors of calmodulin function and shown that: 1) calmodulin binds 4-6 moles of chlorpromazine per mole of protein, 2) structurally unrelated drugs such as W-7, chlorpromazine and phenoxybenzamine may bind to common domains on calmodulin and 3) methionyl residues on the hydrophobic face of proposed amphipathic α-helices are the sites of labeling of calmodulin by phenoxybenzamine.
Based on this extensive database of calmodulin structure and function, we have designed and made a synthetic gene that codes for a calmodulin. We have expressed the gene and analogs of the gene, and are attempting to characterize the products in order to directly elucidate the structural basis of calmodulin's activities.

0769 STRUCTURAL ANALYSIS OF CARBOXYPEPTIDASE A AND ITS COMPLEXES WITH INHIBITORS AS A BASIS FOR MODELING ENZYME RECOGNITION AND SPECIFICITY, Harel Weinstein, Michael N. Liebman and Carol A. Venanzi, Dept. Pharmacology, Mount Sinai School of Medicine of CUNY, New York, N.Y. 10029.

A combination of novel methods of macrostructural analysis and theoretical chemistry were applied to study the metalloenzyme carboxypeptidase A and its complexes with two inhibitors: glycyl-L-tyrosine and a protein inhibitor isolated from potato. The methods of macrostructural analysis applied to the study of the crystal structures of these molecules include Structural Superposition, Distance Matrix Analysis and Linear Distance Plot analysis, and the approach is complemented by the examination of computed physicochemical properties including electrostatic potential surfaces, bulk hydrophobicity and complementarity of van der Waals surfaces. The structural analysis identified folding domains in carboxypeptidase A that relate to such domains in carboxypeptidase B and are involved in the conformational changes following complexation with the inhibitors. The nature of these conformational changes and their relation to the physicochemical properties suggest their role in determining recognition and reactivity characteristics of the enzyme. Some inhibitor-induced changes in structure were shown to occur in regions in which the interacting molecules are not in contact, while some of the contact regions, such as the active site, incur only minor perturbation. The analysis reveals relations between the structural organization of the protein and its function.

Computer Graphics and Molecular Modelling

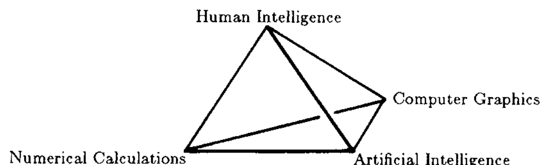
0770 WGS: A FLEXIBLE COMBINATION OF INTERACTIVE AND SOLID MOLECULAR MODELLING,
Jane M. Burridge, Peter Quarendon, Andy J. Morffew and Stephen J.P. Todd, IBM UKSC, Athelstan House, St. Clement St, Winchester, Hants SO23 9DR, England.

The application of the Winchester Graphics System to molecular modelling will be described. The system allows the user to:

- produce a wide variety of representations of protein structure. These include ball-and-stick, space-filling, peptide planes, molecular surface and secondary structure representations, on both calligraphic and raster display devices.
- display any calculable electronic property of a structure, as colour mapped onto the surface of a solid representation of that structure.
- produce animated sequences of data sets. These might include calculated dynamics simulation coordinate sets, different electrostatic potentials or electric field strengths around a protein, or different colour look-up tables.
- investigate and query the structure of interest, using real-time manipulation and orientation, displaying the answers to the questions in graphical fashion.

0771 **FUTURE DEVELOPMENTS IN MOLECULAR GRAPHICS**, R. Langridge, T. E. Ferrin, C. Huang, G. Couch, K. R. C. Arnold, N. Pattabiraman, P. Bash, D. Kneller, and T. Klein-Miller, Computer Graphics Laboratory, University of California, San Francisco, CA 94143

Interactive three dimensional real-time color computer graphics is the most efficient means of visualisation and manipulation of complex three dimensional information. At the UCSF Computer Graphics Laboratory we are integrating our graphical techniques, as exemplified by the highly interactive **MIDAS** (Molecular Interactive Display And Simulation), with real-time numeric calculations and the symbol manipulation, heuristics and exploratory programming techniques of artificial intelligence to develop tools for research on biomolecular structures and interactions, drug design, protein engineering and genetic engineering. The components of the system are visualised schematically below.



All components interact, but it is no accident that the human user is placed at the apex, for while the computer is crucial, it is only by interaction with the human user that the tools can be developed and used creatively. The realisation of this concept in silicon, copper and code will be described. Supported by NIH RR-1081 and DAAG29-83-G-0080.

0772 INTERACTIVE MULTI-PORT COMPUTER GRAPHICS MODELING, Keith D. Watenpaugh, The University of Washington, Seattle, WA 98195 and The Upjohn Company, Kalamazoo, MI 49001.

A device-independent/device-intellegent program has been developed using moderate cost graphics devices and the TEMPLATE software subroutine library that provides fexible and convenient ways to both exam molecular models and illustrate them for presentations. The program (MOMUS *, Modeling Multi-port System) can display a number of structures or the same structure in a number of ways. There is complete freedom to use line drawings, space-filling models or ball-and-stick models along with bi-colored ribbon backbone tracings or other backbone representations. The picture may consist of multiple viewports for comparing different molecules, the same molecule in different orientations, or the molecules using different types of models. The various viewports may be selectively erased and rebuilt while studying other viewports. A few of the options are: positioning of viewports; background color selection; reflective light sources; title fonts and colors; bond and atom colors and sizes; atom and residue text sizes, colors and positioning; and completely arbitrary selection of atoms and residues for coloring, text, sizes, and other attributes. Algorithms used for the displaying of the different modeling types, and how the program is used to studying molecular structure are presented.

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* Momus Gr. Myth. Ridicule personified as a mocking and censorious god.

0773 TOWARDS COMPUTER AIDED PROTEIN DESIGN, Shoshana Wodak, Philippe Delhaise, Michel Bardiaux, Philippe Alard, Daniel Van Belle, Bruno Marchal, Université Libre de Bruxelles, Belgium.

With recent progress in genetic engineering techniques and in experimental methods for studying 3-d structure and function of proteins, rational approaches to the design of modified or new proteins will have to be developed. Molecular modelling by computer (in its largest sense) will be indispensable in elaborating these approaches. It will require sophisticated hardware and software. But software with all the science behind it is bound to play a major role. This software will have to combine interactive molecular graphics, with conformational energy calculations, energy minimization and Molecular Dynamics simulations. It will have to incorporate computer reasoning procedures as well as efficient data-base handling.

We wish to describe modest software development efforts which go in this direction.

Design and Synthesis of Model Peptides and Proteins

0774 DESIGN OF TIGHT BINDING PEPTIDE INHIBITORS OF CALMODULIN. DeGrado, W. F., Thompson, K. F., +Cox, J.A., Wolfe, H.R., Jr., E. I. Du Pont de Nemours & Company, Experimental Station, E328/B32, Wilmington, DE 19898, +University of Geneva, Geneva, Switzerland.

Calmodulin is a protein which interacts in a calcium-dependent manner with a variety of enzymes, peptides, and hydrophobic drugs. Our initial attempt to elucidate the structural features responsible for its interactions involved the design of peptides which mimicked the structures, hydrophobicity, and charge distributions of a group of naturally occurring calmodulin binding peptides. An analysis of the sequences of these peptides suggested that although they displayed little exact sequence homology to one another, they all contained a sequence capable of forming a basic amphiphilic α-helix, which might be the common structural feature underlying their shared ability to bind calmodulin. The importance of this structural feature was tested by synthesizing the peptide FMOC(LeuLysLysLeuLeuLysLeu)$_2$ (FMOC = N-α-fluorenylmethyloxycarbonyl), the quintessence of a basic amphiphilic α-helix. This peptide bound calmodulin competitively with phosphodiesterase in a calcium-dependent manner with a 3 nM dissociation constant. In contrast, FMOC(LeuLysLysLeuLeuLysLeu) which has a much lower helical potential due to its decreased length, bound calmodulin with reduced affinity (0.15 μm). Also, FMOC(LeuGluGluLeuLeuGluLeu)$_2$ which forms acidic amphiphilic α-helices failed to bind calmodulin.

These results suggested that basic, amphiphilic peptides bind to an acidic, hydrophobic region on calmodulin. To identify this region, a three-dimensional model of calmodulin was constructed by interactive computer graphics, starting from the backbone atom coordinates for intestinal calcium-binding protein. For each of calmodulin's two internally homologous domains, appropriate side chains were introduced onto this backbone and the geometry of each structure was optimized by energy minimization using AMBER[1]. The resulting two domains were combined based upon their complementarity and upon the known physicochemical properties of calmodulin. The model for calmodulin contains one shallow hydrophobic crevice on each domain which is flanked by a region of highly concentrated negative charge. These sites are likely candidates for the peptide and target enzyme binding site. Based on the topography of the site found in domain two, which is anticipated to have the highest affinity for synthetic peptides, new peptides were synthesized which have picomolar dissociation constants for calmodulin. Further characterization of the interactions of these peptides with calmodulin by biophysical and chemical methods is currently being pursued.

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0775 THE BIOLOGICAL AND PHYSICAL PROPERTIES OF DESIGNED PEPTIDES AND PROTEINS, Emil Thomas Kaiser, Laboratory of Bioorganic Chemistry and Biochemistry, The Rockefeller University, New York NY 10021

A major goal of research in our laboratory is the design of enzymes, including both their active and binding sites. This problem though is complicated by the difficulties in predicting tertiary structure from primary amino acid sequence. Therefore, as a step toward the solution of the problem of protein design, in one of our approaches we have focussed on important binding regions of surface active proteins and peptides, where in many instances we can ignore tertiary structure, to a first approximation.

For the biological and physical properties of peptides and proteins that act at membrane surfaces amphiphilic secondary structural regions have been found to play very significant roles. Our model building in such systems is based on the premise that if the biological and physical properties of a peptide or protein are dependent on the secondary structural features of a particular region, then in building a model for that region, it may be possible to design a system having minimal homology to the natural system but possessing the potential to form a very closely related secondary structure. Thus, for a particular region of a peptide or protein a specific amino acid sequence may not be crucial but rather a given type of secondary structural feature may be necessary. In that case it is often feasible to reproduce that secondary structure using an amino acid sequence which is relatively non-homologous to the natural sequence. We have prepared peptide models for a large variety of systems, ranging from apolipoproteins through toxins to peptide hormones, where the models have minimal homology in their amphiphilic secondary structural regions to the corresponding regions of the naturally-occurring systems but simulate very well their biological and physical behavior.

Special Lecture

0776 HOMOTROPIC EFFECTS IN THE REGULATORY ENZYME ASPARTATE TRANSCARBAMYLASE.
William N. Lipscomb, Dept. of Chemistry, Harvard University, Cambridge, MA 02138

The three dimensional structure of Escherichia coli aspartate carbamoyltransferase (EC 2.1.3.2) complexed with N-(phosphonacetyl)-L-aspartate(PALA) has been solved using multiple isomorphous replacement techniques. At 2.9A resolution the R factor is 0.29 for the 32,000 unique reflections. The large conformational changes induced by PALA include separation of the two catalytic trimers by 12A, relative reorientation of these trimers about the molecular three-fold axis by 10° and reorientation of the regulatory dimers by about 15°. New polar interactions develop between equatorial domains of the catatylic subunits and the zinc domain of the regulatory subunits. Extensive changes in tertiary structure occur in the interface between catalytic trimers involving residues 230-245, in the region of Lys 84 and in the region where the bisubstrate analogue PALA binds. Each active site involves Ser 81 and Lys 84 from an adjacent catalytic chain within the catatylic trimer. His 134 is a potential functional residue near PALA in the structure. Ser 52 is adjacent to the phosphonate of PALA, although no chemical evidence of phosphoryl enzyme exists in the normal mechanism.

Conformation Analysis and Probes of Protein Structure

0777 PROTEIN STRUCTURE FROM ANOMALOUS X-RAY SCATTERING
Wayne A. Hendrickson, Department of Biochemistry & Molecular Biophysics, Columbia University, New York, NY 10032

The crystallographic 'phase problem' is the central conceptual difficulty in structure determination from diffraction data. Both amplitudes and phases of diffracted waves are needed for the Fourier reconstruction of a structural image, but phase information is lost in the observational interaction. The classical procedure for evaluating the phases of diffraction from protein crystals is by the method of isomorphous replacement. This method requires multiple crystal preparations and data collections and, in practice, precision of the resulting phases is limited. There are clear advantages to methods that allow one to derive both the phase and amplitude information from diffraction data on a single crystalline species. Recent developments in the measurement and use of anomalous scattering from protein crystals offers a new possibility for rapid structure determination. Appropriately designed experiments can isolate the contributions of the few anomalous-scattering centers from among the many normal-scattering light atoms in a protein structure. The phase shifts that accompany the anomalous x-ray scattering from these distinctive centers permit a solution of the 'phase problem'. Crambin was the first protein structure to be determined directly from the data of a single crystal - in this case from the rather weak scattering of sulfur atoms. These experiments were conducted at a single wavelength with a conventional x-ray source. A number of other protein structures have also been determined by this method of resolved anomalous scattering. The continuously tunable radiation from synchrotron x-ray sources permits a much more powerful and definitive exploitation of anomalous scattering. We have recently conducted a demonstration of these capabilities in phase determination of lamprey hemoglobin from multiple wavelength measurements of anomalous dispersion in diffraction from native iron atoms (Hendrickson, Smith, Phizackerley, Merritt and Love, unpublished results). These results coupled with previous experience indicate that exceptionally accurate and rapid structural determinations can be made from area detector measurements of anomalous scattering synchrotron facilities.

0778 VIBRATIONAL ANALYSIS OF PROTEIN STRUCTURE, S. Krimm, Biophysics Research Division, University of Michigan, Ann Arbor, MI 48109

The infrared and Raman spectra of a polypeptide molecule contain information on its three-dimensional structure. In order to extract this information, it is necessary to achieve a detailed understanding of the vibrational spectrum, namely to demonstrate that the observed bands are assignable to the normal vibrations expected for a given structure. While a normal mode calculation is generally feasible, given a structure and a vibrational force field, the results are meaningful only if the force field is capable of high predictability. Our refined force fields for β-sheet (1,2) and α-helix (3) polypeptides reproduce the frequencies of these structures to within ± 5 cm^{-1} (4), and can therefore provide meaningful distinctions between calculated modes of different conformations. These force fields also account satisfactorily for transition dipole moments (5) and intensities (6). We have used this method of vibrational analysis to study structural problems involving peptides (7), polypeptides (8), and proteins (9). This work will be summarized, and results of recent studies presented.

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0779 **ONE- AND TWO-DIMENSIONAL SPECTRAL ANALYSIS OF THE CONSEQUENCES OF SINGLE AMINO ACID REPLACEMENTS IN PROTEINS, John L. Markley,* William M. Westler, Gilberto Ortiz-Polo, R. Krishnamoorthi, and M. Albert Thomas, Purdue Biochemical Magnetic Resonance Laboratory, Purdue University, West Lafayette, IN 47907, and *Department of Biochemistry, University of Wisconsin, 420 Henry Mall, Madison, WI 53706**

The traditional approach of using homologous sequences to elucidate the role of specific amino acid residues becomes more meaningful as the number of differences are minimized, with the limit being alteration of a single residue. The set of ovomucoid third domains sequenced to date (1) contains 17 pairs of proteins that differ by a single substitution. These protein domains, which have a maximum of 56 amino acid residues, are inhibitors of serine proteinases. Laskowski and coworkers have determined the sequence dependence of the association constant for inhibitor-proteinase interaction (2), the X-ray structures of an isolated inhibitor (3), and the X-ray structure of an inhibitor-serine proteinase complex (4). For small proteins in solution, NMR spectroscopy offers a means of obtaining detailed information about each residue and its response to changes in the protein sequence (5). The ability to extract this information from NMR spectra (6) has been aided by recent progress in spectrometer technology (higher magnetic fields, more sensitive signal detection, more sophisticated computers) and experimental strategy (new homo- and heteronuclear pulse sequences including two-dimensional Fourier transform NMR spectroscopy). The first step in our NMR studies has been to compare several pairs of proteins differing by one or a few amino acid replacements: one and two dimensional homonuclear (^1H) and two-dimensional heteronuclear ($^{13}C\{^1H\}$) NMR methods have been used. These experiments have resulted in assignments to resonances from the sites of amino acid substitution. The second step has been to use the sequential NMR assignment strategy of Wüthrich and coworkers (7) to extend these assignments to adjacent regions of the molecule. The third step in this research is to investigate the consequences of specific amino acid replacements on protein structure, dynamics, and chemical reactivity. Our results with ovomucoids provide examples of sequence effects on pK_a' values and mobilities of side chain groups.

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Protein Structure in Extreme Environments

0780 THE STABILITY OF PROTEINS FROM EXTREME THERMOPHILES, Roy M. Daniel, Thermophile Research Laboratory, Biological Sciences, University of Waikato, Hamilton, New Zealand.

Proteins from extreme thermophiles are in general more thermostable than those from thermophiles, which are in turn more thermostable than those from mesophiles. The stabilisation of proteins against thermal denaturation is conferred by small changes in the amino acid sequence[1] (although other mechanisms, such as the binding of metal ions, do occur[2]). Such changes can affect stability without any obvious structural alteration[3], by giving rise to a relatively small number of additional intra-molecular interactions[4].

The upper temperature limit for life is unknown, but there is little evidence that it is governed by the stability of proteins. We have isolated an enzyme with an activity half life of 30 min at 110°C from an extreme thermophile, and have no reason to suppose that substantially more stable enzymes do not exist.

Protein thermostability correlates with resistance to denaturing agents such as detergents, organic solvents, and urea[5]. Less obviously, thermostable proteins resist proteolysis[6]. The correlation of thermostability with other properties has also been suggested.

This overall robust character of proteins from extreme thermophiles is useful. They make good subjects for techniques which might denature more labile proteins; they allow investigation of proteins which, when derived from mesophiles, are particularly labile; and they allow investigation of properties over a wide temperature range. The stability of enzymes from extreme thermophiles also has commercial applications[7], and the techniques which can be used to enhance the usefulness of less stable enzymes (such as immobilisation, cloning, site-directed mutagenesis, treatments to enhance stability) can also be applied to those from extreme thermophiles.

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0781 **RAPID ENZYME IMPROVEMENTS THROUGH GENETICS,** **David I. Hirsh, Robert Hageman, and Hans Liao, Synergen, Inc., Boulder, CO 80301**

We have developed genetic methods for increasing the thermostability of enzymes using cloning vectors that replicate stably in *E. coli* and in the thermophilic microorganism *Bacillus stearothermophilus*. We have been able to select thermostable variants of kanamycin nucleotidyl transferase (KNTase), which confers resistance to kanamycin. The wild-type enzyme is inactive above 50°C. The gene encoding KNTase was cloned into one of the vectors and transformed into *B. stearothermophilus*. Kanamycin-resistant survivors were selected at 63°C. Six independently derived survivors have a thermostable KNTase with a half-life of 15 minutes, compared to the wild-type enzyme half-life of 30 seconds at 60°C. DNA sequencing reveals that these thermostable variants have converted Asp to Tyr at position 80. One of the thermostable variants was further stabilized through a second round of selection at 70°C; it contains an additional change of Thr to Lys at position 130 and has a half-life of >60 minutes at 60°C. Another thermostable kanamycin-resistant survivor contains an enzyme that appears to be cold labile *in vitro*. These methods for generating thermostable variants of enzymes are generally applicable to improving other properties and to different classes of proteins.

0782 WHY DO ENZYMES IRREVERSIBLY INACTIVATE AT HIGH TEMPERATURES? Alexander M. Klibanov and Tim J. Ahern, Laboratory of Applied Biochemistry, Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

One of the major obstacles in industrial biocatalysis is that all enzymes eventually lose their catalytic activity as the temperature is increased [1]. Attempts to stabilize enzymes have been severely hampered by the lack of understanding of that process: while mechanisms of reversible thermal denaturation of enzymes are well established, those leading to irreversible thermoinactivation heretofore remained obscure [2]. Using hen egg-white lysozyme and bovine pancreatic ribonuclease as model enzymes, we have recently found a detailed answer to the title question. The processes causing irreversible inactivation of lysozyme and ribonuclease at 90°-100°C in the pH range of relevance to enzymatic catalysis are deamidation of asparagine residues, hydrolysis of peptide bonds at aspartic acid residues, destruction of S-S bonds, and formation of incorrect (scrambled) structures. Relative contributions of these processes depend on the nature of the protein and on the pH. Irreversible inactivation of lysozyme at 100°C is brought about (i) at pH 4, by a combination of deamidation and hydrolysis; (ii) at pH 6, by deamidation; and (iii) at pH 8, by a combination of deamidation, formation of incorrect structures and destruction of disulfide bonds. Irreversible inactivation of ribonuclease at 90°C is caused (i) at pH 4, by hydrolysis; and (ii) at pH 8, by a combination of formation of incorrect structures and destruction of S-S bonds. The aforedescribed processes are of a general nature, and thus both demarcate the upper limit of thermal stability of enzymes and afford rational strategies of enzyme stabilization by chemical modification or protein engineering. Also, since water participates in all the reactions resulting in thermoinactivation, dehydration should greatly stabilize enzymes; this conclusion was confirmed in separate experiments demonstrating that enzymes are much more thermostable in organic solvents than in aqueous solutions [3].

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0783 STRUCTURE-FUNCTION STUDIES OF THE BACTERIOPHAGE T4 DNA POLYMERASE: ISOLATION OF MUTANTS AFFECTING ACCURATE DNA SYNTHESIS, Linda J. Reha-Krantz, Department of Genetics, University of Alberta, Edmonton, Alberta, T6G 2E9

The T4 DNA polymerase has proven to be a good model system for the study of prokaryote DNA polymerases because it has been possible to correlate the in vivo mutation rates of DNA polymerase mutants with the in vitro - determined enzymological properties of the mutationally altered polymerases. Novel T4 DNA polymerase mutants have been isolated recently by a procedure designed to select mutants with high spontaneous mutation rates. In contrast to temperature sensitive DNA polymerase mutants that are located at numerous, random sites in the DNA polymerase gene, mutator mutants are located in clusters at just 3 sites. The positions of the clusters have been determined by recombination with two- and three-factor crosses and by marker rescue with plasmids containing cloned portions of the DNA polymerase gene. Some of the mutants have been shown to have a defective 3' → 5' exonuclease activity and other mutants have been shown to be defective in the insertion of correct deoxynucleotides during DNA synthesis. Some of the mutants are generalized mutators, while other mutants enhance the frequency of transversion, transition or frameshift mutations preferentially. Because the mutator mutants are clustered and because they alter enzymatic activities of the DNA polymerase, it is likely that these mutations are associated with the active sites of the enzyme.
Second-site mutations in the DNA polymerase that suppress the temperature sensitivity, but not the mutator activity of mutator DNA polymerases, have also been isolated. Further experiments will be to determine the DNA sequence of the mutator mutants and the suppressor mutants. (Research supported by NSERC, the Alberta Cancer Board and the Alberta Heritage Foundation for Medical Research.)