Abstracts, Division of Biological Chemistry, 199th National Meeting of the American Chemical Society, April 22–27, 1990

R. David Cole, Chair; Perry A. Frey, Chair Elect;
David S. Sigman, Program Chair; Vern Schramm, Program Chair Elect

MONDAY MORNING—1990 PFIZER AWARD IN ENZYME CHEMISTRY SYMPOSIUM IN HONOR OF JAMES WELLS—R. YOUNT, PRESIDING

1. Site-Directed Mutagenesis: Probes of Mechanism and Structure of Aspartate Aminotransferase. *J. Kirsch.* Department of Biochemistry, University of California, Berkeley, CA 94720.

No abstract available.

2. Engineering Proteins with Enhanced Thermal and Proteolytic Stability. *R. Sauer.* Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

No abstract available.

3. Deducing and Manipulating Structural Motifs in Proteins with Unsolved Structures. *Paul Schimmel.* Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Structural motifs in proteins with unsolved structures can be suggested by sequence comparisons with and functional relationships to proteins with known three-dimensional structures. Structural models based on such analyses lack credibility, particularly when sequence similarities to proteins with solved structures are limited. However, specific deletions and mutations can be introduced into a protein of unknown structure to test specific features of any structural model. These approaches have been used to develop models of structural units within some of the members of the aminoacyl tRNA synthetase class of enzymes. For a synthetase of unsolved structure, experimental evidence has been obtained for a large insertion into a predicted functional domain and for the location of an important segment of a substrate binding domain. Further applications have led to constructs which allow study of interdomain packing interactions and of chimeric proteins which have substituted a domain of one enzyme for that of another.

4. Engineering Specificity in Proteins. *J. Wells.*

No abstract available.

MONDAY AFTERNOON—ACS AWARD IN PURE CHEMISTRY SYMPOSIUM IN HONOR OF PETER SCHULTZ—W. DEGRADO, PRESIDING

5. De Novo Design of Helical Proteins. *William F. DeGrado.* E. I. du Pont de Nemours and Company, Central Research and Development Department, Experimental Station, Wilmington, DE 19880-0328.

Our group has recently adopted a synthetic approach to understanding the structural basis for protein function. To test some of the rules and concepts which are believed to be important for protein folding and stability, we are attempting to design some simple proteins which should fold into predetermined three-dimensional structures. Two types of helical proteins have been designed: the first is an idealized version of a four-helix bundle, a folding pattern found in the structures of a variety of natural water-soluble proteins including myohemerythrin, cytochrome *c*, and apoferittin, while the other class of design proteins is meant to mimic the structures of proteins which form ion channels such as the acetylcholine receptor. Although these two classes of proteins differ in their properties and function, they both are composed of bundles of α-helices. The synthesis and characterization of these proteins are currently underway and will be the focus of the talk.

6. Antibody Catalysis: Perspectives and Prospects. *D. Hilvert.* Departments of Chemistry and Molecular Biology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037.

The selectivity and diversity of the mammalian immune system can be exploited to generate antibody molecules with tailored catalytic activities. For example, we have used rationally designed transition-state analogues to prepare immunoglobulins that promote two concerted chemical reactions, a sigmatropic Claisen rearrangement and a Diels–Alder cycloaddition. These antibodies are proving to be valuable tools for evaluating how enzymes exploit binding energy to achieve large rate accelerations and exacting regio- and stereoselectivities. Related catalysts are likely to have significant practical utility, as Claisen rearrangements and Diels–Alder cycloadditions are among the most important transformations available to synthetic organic chemists for the construction of complex natural products, therapeutic agents, and synthetic materials of all kinds. Additional, ongoing studies directed toward the improvement of the generation of catalytic antibodies using molecular biology and genetics will also be discussed.

7. Protein Dissection Using Peptide Models. *Peter S. Kim.* Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142.

There is now experimental evidence indicating that some proteins can be broken into modules. These modules correspond to autonomous folding units and often are substantially smaller than a domain. Studies of subdomains allow one to dissect structure/function relationships in proteins and to begin understanding protein folding in structural terms. We have been studying two types of modules: (1) disulfide-bonded peptide pairs corresponding to protein folding intermediates of bovine pancreatic trypsin inhibitor (BPTI); (2) peptide dimers formed by the isolated "leucine zipper" motif, which corresponds to a coiled-coil structure found in several DNA binding proteins. In both cases, it is possible to obtain modules of proteins that show substantial folded structure in aqueous solution.

8. Interplay between Chemistry and Biology: Catalyst Design. *P. G. Schultz.* Department of Chemistry, University of

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California, Berkeley, CA 94720.

No abstract available.

TUESDAY MORNING—BIOCHEMISTRY OF TOXIC PROTEINS (SECTION A)—R. J. COLLIER, PRESIDING

9. Stability, Cytotoxicity, and Pharmacokinetics of Ricin A Chain Immunotoxins Prepared with Substituted 2-Iminothiolanes. *Stephen F. Carroll*, Dane A. Goff, Susan L. Bernhard, Ada Kung, and Patrick W. Trown. XOMA Corporation, 2910 Seventh Street, Berkeley, CA 94710.

Immunotoxins (antibody–toxin conjugates) represent an emerging class of pharmaceutical reagents capable of selectively eliminating antigen-bearing target cells in vitro and in vivo. The therapeutic potential of such agents depends largely upon properties intrinsic to the antibody, the toxin, and the linkage used for conjugation. Recently, much attention has been focused on the in vivo stability of such conjugates linked by a disulfide bridge. To investigate the role of bond stability on the properties of ricin A chain containing immunotoxins in greater detail, we have synthesized a series of sterically hindered cross-linking reagents based upon 2-iminothiolane (X2ITs) substituted at the 4- and/or 5-position (X2ITs). Depending upon the substituent(s), the in vitro stability of thiol-activated X2IT–antibody complexes was increased up to 8000-fold. Immunotoxins were prepared with the X2IT-derivatized monoclonal antibodies and the 30-kDa form of ricin A chain. The stability, cytotoxicity, and pharmacokinetics of these conjugates will be discussed.

10. Structure and Action of Ricin. *Jon D. Robertus*. Department of Chemistry, University of Texas, Austin, TX 78712.

The heterodimeric plant toxin ricin consists of a B chain lectin and an A chain enzyme held together by a disulfide bond. B chain binding to eucaryotic cell surface glycoproteins triggers endocytosis. The ricin A chain is then delivered to the cytoplasm where it inhibits ribosomes by specific N-glycosidation of a key adenine base. In this talk, the 2.5-Å X-ray structure of ricin is analyzed in terms of its known functions. Binding site residues are identified and their roles assessed for galactose recognition by B chain. Active-site residues involved with RNA recognition and hydrolysis by A chain are also analyzed. The structure has been used to direct genetic engineering of ricin. Some results will be discussed as will prospects for future design.

11. Structure–Function Analysis of IL-2-toxin: Fragment B Sequences That Are Required for the Delivery of Fragment A to the Cytosol of Target Cells. D. P. Williams, C. E. Snider, and *J. R. Murphy*. Department of Medicine, University Hospital, Boston, MA 02118, and Seragen Inc., Hopkinton, MA 01748.

We have used cassette and deletion mutagenesis to analyze the structural features of fragment B related sequences in IL-2-toxin that are necessary for the efficient delivery of fragment A to the cytosol of target cells. IL-2-toxin [Williams et al. (1987) *Protein Eng. 1*, 493] is a fusion protein in which the native diphtheria toxin receptor binding domain has been genetically replaced with human interleukin 2 (IL-2) sequences. While an intact disulfide bond between Cys461 and Cys471 is required for the cytotoxic action of native diphtheria toxin, this bond is not required for the targeted action of IL-2-toxin. The in-frame deletion of the 97 amino acids from Thr387 to His485 of IL-2-toxin increases both the potency and the apparent dissociation constant (K_d) of the fusion toxin for the high-affinity IL-2 receptor. In contrast, the in-frame deletion of either the 191 amino acids between Asp291 and Gly483 or the 85 amino acids between Asn204 and Ile290 results in a 1000-fold loss in potency. These regions contain the putative membrane spanning and amphipathic surface associating regions of fragment B, respectively.

12. Active-Site Studies of Diphtheria Toxin and *Pseudomonas Aeruginosa* Exotoxin A. R. John Collier and *Brenda A. Wilson*. Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.

Diphtheria toxin (DT) and *Pseudomonas aeruginosa* exotoxin A (ETA) are two highly potent bacterial toxins, which inhibit protein biosynthesis in sensitive eukaryotic cells by catalyzing the transfer of the ADP-ribose moiety of NAD to a posttranslationally modified histidine (diphthamide) of elongation factor 2 (EF-2). Although DT and ETA are highly divergent proteins, their catalytic domains share significant sequence homology. In both proteins, an important glutamic acid residue has been identified by photoaffinity labeling of the catalytically active fragments with NAD. X-ray crystallographic structure analysis of ETA indicates that this glutamic acid is located within the active-site cleft. Recent studies, using site-directed mutagenesis and various biochemical and biophysical methods, have led to the identification and characterization of this and other active-site amino acid residues involved in the NAD:EF-2 ADP-ribosyltransferase and NAD-glycohydrolase activities of DT and ETA. In particular, evidence is presented for the important role of this glutamic acid in enzymic catalysis.

TUESDAY MORNING—1990 REPLIGEN AWARD SYMPOSIUM IN HONOR OF HAROLD SCHERAGA: PROTEIN STRUCTURE AND FOLDING (COSPONSORED WITH THE DIVISION OF MICROBIAL AND BIOCHEMICAL TECHNOLOGY) (SECTION B)—L. GIERASCH, PRESIDING

13. Structures of Intermediates in Protein Folding. *Robert L. Baldwin*. Biochemistry Department, Stanford University, Stanford, CA 94305.

Stopped-flow amide proton exchange ($^1H \leftrightarrow ^2H$), followed by 2D ^1H NMR analysis after folding is complete, has been used to determine the locations of protected peptide NH protons in an intermediate preceding the rate-limiting step in the folding of ribonuclease A. The work of Dr. Jayant Udgaonkar shows that a highly structured folding intermediate is formed rapidly. All of the measurable peptide NH protons of the β-sheet, and also those in two of the three α-helices, are protected in the intermediate; for many protons the degree of protection exceeds 1000-fold. Only the protons of the N-terminal helix and of Glu 49 (which is not H-bonded in native ribonuclease A) remain unprotected, or weakly protected, in the folding intermediate. The results suggest that substantial native-like secondary structure forms rapidly and becomes highly protected before the rate-limiting step in folding is reached. An equilibrium folding intermediate of apomyoglobin, found at pH 4, 5 °C, has also been characterized by amide proton exchange. The results of Frederick Hughson, in collaboration with Dr. Peter Wright at Scripps, are consistent with part of the intermediate (the A, G, and H helices) being native-like and with the remainder being unfolded.

14. On the Origins of Structure in Globular Proteins. *Ken Dill* and Hue Sun Chan. University of California, San Francisco, San Francisco, CA 94143.

The principal forces of protein folding—hydrophobicity and conformational entropy—are nonspecific. A longstanding puzzle has therefore been, What forces drive the formation of the specific internal architectures in globular proteins? We find that any self-avoiding flexible polymer molecule will develop large amounts of secondary structure, helices, and parallel and antiparallel sheets, as it is driven to increasing compactness by any force of attraction among the chain monomers. Thus, structure formation arises from the severity of steric constraints in compact polymers. This steric principle of organization can account for why short helices are stable in globular proteins and why weakly unfolded proteins have been secondary structure. On this basis, it should be possible to construct copolymers, not necessarily by use of amino acids, which can collapse to maximum compactness in incompatible solvents and which should then have structural organization resembling that of proteins.

15. Protein Structure and Folding: Time-Resolved Protein Crystallography. *G. A. Petsko.* Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

No abstract available.

16. Protein Structure and Folding: Experimental and Theoretical Aspects of Protein Conformation. *H. A. Scheraga.* Department of Chemistry, Cornell University, Ithaca, NY 14853.

No abstract available.

TUESDAY AFTERNOON—SYMPOSIUM ON HYDROGEN TRANSFER (COSPONSORED WITH THE DIVISION OF ORGANIC CHEMISTRY) (SECTION A)—I. A. ROSE AND R. L. SCHOWEN, ORGANIZERS; E. GRUNWALD, PRESIDING

17. Proton Diffusion and Conduction in Enzyme Catalysis. *Irwin A. Rose.* Institute for Cancer Research, Fox Chase Cancer Research, Philadelphia, PA 19111.

Enzyme-bound active-site protons as T can be traced to substrate by a new pulse-chase method in which substrate is provided in a large diluting volume to enzyme that has been briefly exposed to TOH. Two kinds of behavior have been noted: T of the active site has been exchanged into a substrate replacing a proton donated to the enzyme in the case of triose-P and glucose-P isomerases. This internal exchange can involve more than one enzyme-bound T and presumably represents diffusion of the abstracted proton into a pool of protons in the active site. In addition, an active site that has been pulse-labeled with T can supply T to an active-site base for use in formation of product from a proton-deficient substrate. With fumarase at least three enzyme equivalents are trapped by fumarate, requiring several reaction cycles, and found in malate. Malate alone traps nothing, i.e., exchange labeling is not found. Presumably the enzyme provides a relay that conducts protons from medium to active site. In the case of fumarase the relay contains at least three hydrogens. Experiments with many enzymes suggest that active-site protons that can be transferred into reaction intermediates exchange with the medium only in the absence of substrate.

18. Principles of Proton-Transfer Reactions. *Steve Scheiner.* Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, IL 62901.

Ab initio quantum chemical calculations have been carried out to study the proton-transfer process in a number of different H-bonding systems. Groups which normally occur in organic and biological systems as participants in H bonds are represented by smaller model molecules. The calculations have revealed a number of fundamental principles that underlie the proton-transfer process. The energy barrier to transfer rises quickly as the two groups are separated from one another. Hence, the rate of transfer drops precipitously as the H bond is elongated. Angular characteristics of the H bond are intimately connected to the equilibrium position of the proton and can affect the relative stabilities of the AH–B and A–HB tautomers. These observations are explained on the basis of simple physical concepts.

19. Multiproton Catalytic Entities. *Richard L. Schowen.* Departments of Chemistry and Biochemistry, University of Kansas, Lawrence, KS 66045-0046.

Catalysis by acids and bases can occur with more than one protic site, as in bifunctional or tautomeric catalysis in nonenzymic systems and in charge-relay catalysis, "two-base" mechanisms, and other kinds of enzyme catalytic mechanisms. The prerequisites for these kinds of catalysis and their role in transition-state stabilization will be explored in terms of examples from catalysis by proteases, decarboxylases, and dehydrogenases. Among the techniques for detection and characterization of multiproton entities with isotope-exchangeable sites are solvent isotope effects in mixtures of protium and deuterium oxides (proton inventories).

TUESDAY AFTERNOON—SYMPOSIUM ON PHOSPHOLIPASE STRUCTURE/FUNCTION (SECTION B)—E. A. DENNIS, PRESIDING

20. Mechanism, Activation, and Inhibition of Phospholipase A_2. *Edward A. Dennis.* Department of Chemistry (M-001), University of California, San Diego, La Jolla, CA 92093-0601.

Phospholipase A_2 (PLA_2) catalyzes the hydrolytic release of the fatty acid at the *sn*-2 position of membrane lipids [E. A. Dennis (1983) *Enzymes (3rd Ed.) 16*, 307–353] and as such plays a key role in the control of arachidonic acid release for prostaglandin biosynthesis [E. A. Dennis (1987) *Bio/Technology 5*, 1294–1300]. Our laboratory has been studying both the "extracellular" PLA_2 which occurs in snake venom, mammalian pancreas, and other tissues as well as a "membrane-associated" PLA_2 from a macrophage-like cell line $P388D_1$. We will discuss new results on the relationship of protein structure, primary sequences, and evolution of the extracellular enzymes as well as the aggregation of the cobra venom PLA_2 to dimers or higher order aggregates in the presence of lipid substrate in accord with our "dual phospholipid model" for the mechanism of activation of PLA_2. Our laboratory has also developed a variety of novel inhibitors to study the detailed mechanism of action of PLA_2 including thioether–amide analogues of PE as substrate analogues as well as manoalide and manoalogue. New findings with these and other inhibitors will be reported.

21. Crystallographic Structure/Function Studies of Phospholipase A_2. *P. B. Sigler.* Department of Molecular Biophysics and Biochemistry and The Howard Hughes Medical Institute, Yale University, New Haven, CT 06511.

High-resolution crystallography of PLA_2 has been carried out with the aim of providing a stereochemical understanding

of the following mechanistic questions: (a) the role, if any, of dimerization in PLA_2 function; (b) the effect of binding calcium ion; (c) the interaction site for binding ion; (d) the mechanism of productive-mode binding of the substrate; and (e) the esterolytic events in atomic detail. We now have solved and refined to better than 2.0-Å resolution a variety of phospholipases A_2 (including the bee venom enzyme) both alone and in their functionally interesting complexes. By comparing close and distant homologues, we can infer the effect of sequence on structure. We can infer the role of dimerization by comparing closely related dimeric species of PLA_2 (e.g., *C. atrox*, and *A. p. piscivorus*). By comparing calcium-bound and calcium-free structures, we can infer the effect of calcium ion on conformation. We can infer the likely mechanism for binding and esterolysis by visualizing complexes bearing transition-state analogues. We will report our progress on all of these issues.

22. Molecular Details of the Activation of Phospholipase A_2 on Lipid Bilayer Surfaces. *R. L. Biltonen.* Departments of Biochemistry and Pharmacology, University of Virginia School of Medicine, Charlottesville, VA 22908.

Soluble phospholipase A_2 exhibits a time-dependent activation on the surface of lipid bilayer substrates. This activation process appears to require enzyme dimer formation as a necessary, but not sufficient, condition for activation. Furthermore, the activation is strongly coupled to dynamic cooperative structural fluctuations of the lipid surface. For example, the time required to achieve maximal activity is a minimum near the gel to liquid-crystalline phase transition temperature of the lipid. In many cases, the activation process appears to be strongly coupled to a highly cooperative structural transition associated with the existence of a second component in the bilayer. The use of computer modeling and kinetic, thermodynamic, and fluorescence spectroscopy techniques and computer simulation have allowed us to deduce the temporal sequence of events in the activation process. The results of these studies will be discussed. (Supported by grants from the National Institutes of Health, National Science Foundation, and Office of Naval Research.)

23. Interfacial Catalysis by Phospholipase A_2: Inhibition and Substrate Specificity Studies. *M. H. Gelb*, W. Yuan, and F. Ghomaschi. Departments of Chemistry and Biochemistry, University of Washington, Seattle, WA 98195. M. K. Jain. Departments of Chemistry and Biochemistry, University of Delaware, Newark, DE 19216.

Phospholipase A_2 is a challenging enzyme to study because it operates at a lipid/water interface. The water-soluble enzyme binds reversibly to the interface followed by a second step in which a phospholipid substrate binds to the catalytic site on the enzyme. Previous inhibition and substrate specificity studies have been misleading because the amount of enzyme bound to the interface has not been normalized. We have studied the action of the enzyme in the "scooting mode" in which the enzyme never leaves the interface. Results will be presented on the inhibition of phospholipase A_2 by transition-state phospholipid analogues. Studies on "absolute substrate specificity" will also be presented. Finally, attempts to study the inhibition of this enzyme by water-soluble inhibitors will also be discussed.

24. Perfecting an Enzyme: A Phospholipase A_2 with Significantly Improved Catalytic Activity. *M.-D. Tsai*, J. Noel, T. Deng, and M. Sundaralingam. Department of Chemistry, The Ohio State University, Columbus, OH 43210.

A gene coding for bovine pancreatic phospholipase A_2 was designed, synthesized, and expressed in *E. coli*. Site-directed mutagenesis was then used to probe the catalytic mechanism of the enzyme. Of particular interest are the mutants of lysine-56. Although this residue is not located at the active site, it has been suggested to be acylated by substrates during catalysis, as an obligatory step in the activation of the enzyme [A. G. Tomasselli, J. Hui, J. Fisher, H. Zurcher-Neely, I. M. Reardon, E. Oriaku, F. J. Kezdy, and R. L. Heinrikson (1989) *J. Biol. Chem. 264*, 10041]. Surprisingly, replacement of this residue by methionine not only did not eliminate activation but further enhanced the k_{cat} of the enzyme toward micellar phosphatidylcholine substrate by 10× and lowered its apparent K_m by 6×. Thus, the catalytic efficiency of the K56M mutant toward this natural substrate has improved by 60×. Several other mutants at position 56 also showed improved catalytic efficiency. The crystal structure of the K56M mutant is being solved. The possible structural and mechanistic basis of the improved catalytic activity will be discussed.

Wednesday Morning—Symposium on Hydrogen Transfer (Cosponsored with the Division of Organic Chemistry)—W. Bachovchin, Presiding

25. Redesigning Proteins To Understand Their Structure and Function. *C. S. Craik*, L. Evnin, R. Fletterick, J. Higaki, M. McGrath, C. Tsu, J. Vasquez, and M. Wilke. Departments of Biochemistry/Biophysics and Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446.

Genetic, biochemical, and biophysical methods are being used to explore the relationship between structure and function in the serine protease, trypsin. Site-directed mutagenesis is used to substitute specific amino acids in trypsin that are critical determinants for catalysis and substrate specificity. Enzymological and structural analysis of the variant enzymes provides an empirical basis for testing the role of geometry, hydrogen bonding, steric constraints, electrostatics, and transition-metal binding to the activity and architecture of the enzyme. Selected variants will be described that involve (1) replacement of the serine protease catalytic triad (Asp102, His57, and Ser195) with the thiol protease catalytic triad, (2) extended active-site replacements (Ser214 and Arg96), and (3) substitutions that directly influence substrate specificity (Asp189, Ser190, Gly216, and Gly226). The data base that is being developed is providing a better understanding of this essential class of enzymes and will lead to general strategies for controlling enzyme activity and for de novo protein design.

26. Proton Transfer in the Catalytic Mechanism of Carbonic Anhydrase. *David N. Silverman.* Departments of Pharmacology and Biochemistry, University of Florida, Gainesville, FL 32610.

The catalyzed hydration of CO_2 to form HCO_3^- and a proton is limited in rate by the transfer of the proton from zinc-bound water to solution for both isozymes II and III of carbonic anhydrase, with isozyme III 500 times less active. Replacement of residues by site-directed mutagenesis indicates that a histidine near the active site in both isozymes shuttles protons between the zinc-bound water and buffer in solution at a rate as great as 10^6 s^{-1}. Alternate pathways involving direct proton transfer between the metal site and buffer also contribute, and replacement of residues near the zinc enhances proton transfer in isozyme III to a rate as rapid as in isozyme II. Mutagenesis has revealed structure–function relations of

intramolecular proton transfer within the active-site cavity and of intermolecular proton transfer between the active site and buffers.

27. H-Bond Network Formation and Catalytic Mechanism in Human Carbonic Anhydrase I. *Angelo Vedani* and David W. Huhta. Biographics Laboratory, Departments of Chemistry and Biochemistry, University of Kansas, Lawrence, KS 66045.

Using molecular mechanics techniques, we have modeled details of metal coordination, H-bond network formation, and protein–solvent interactions in native, complexed, and Co(II)-substituted human carbonic anhydrase I. The studies are based on the 2.0-Å resolution X-ray crystal structure with the computational extension of the solvent shell and focus on the presence and activity of three potential proton-relay networks. These originate at one of the two zinc-bound water molecules and lead to Glu 106, His 64, or His 67, each of which has been identified as a potential terminal base for the zinc-bound water deprotonation. All proton-relay networks remain active when the natural substrate bicarbonate is present but are disrupted upon sulfonamide-inhibitor binding. The results of our study will be compared with NMR data as well as with ab initio and semiempirical calculations.

WEDNESDAY AFTERNOON—GENERAL POSTER SESSION/MIXER—D. S. SIGMAN, ORGANIZER

Biological Structure

28. Potentiation of Central and Peripheral Muscarinic Effects by an Imidazoindole. *P. K. Adhikary* and S. R. Pavuluri. Alvin C. York Veterans Administration Medical Center, Murfreesboro, TN 37130.

Pyridino(1,2-*a*)imidazo(5,4-*b*)indole (**1**) was tested for its central and peripheral cholinergic effects in unanesthetized mice. **1** was injected intraperitonially (ip) alone or in combination with muscarine, and the corresponding cholinergic symptoms (e.g., ataxia, tremor, salivation, and diarrhea) observed in the animals were recorded and compared with those responses produced by the ip injections of muscarine alone or saline (control) in mice. **1** did not produce any cholinergic symptoms when injected alone even at high dose levels. However, a combination dose of muscarine, 1 μm/kg, plus **1**, 1 μM/kg, produced cholinergic symptoms equivalent to the degree of response observed from the injection of 2 μM/kg of muscarine (100% dose response potentiation). Reduction of **1** dose level in the combination dose of muscarine, 1 μM/kg, plus **1**, 1 μM/kg, reduced the potentiation of muscarinic effects in a dose-dependent manner, but an increase of **1** dose level in the combination doses failed to increase cholinergic potentiation more than 100%. It appears that **1** does not induce cholinergic responses independently at experimental dose levels, but potentiates responses induced by muscarine.

29. Kinetic and Mechanistic Studies of Ion Transport by the L-Glutamate Receptor Ion Channel. *Gary Aistrup*, M. Szentirmay, E. Michaelis, T. Kuwana, and R. L. Schowen. Department of Biochemistry and Higuchi Biosciences Centers, University of Kansas, Lawrence, KS 66045.

A glutamate receptor–ion channel complex isolated from rat brain synaptic membranes can be reconstituted in artificial planar lipid bilayers in a Montel cell such that ion currents can be measured and related to the presence of agonists and antagonists and to other variables. Measurements of current fluctuations over periods of up to 30 min allow the calculation of kinetic parameters related to channel opening and channel closing, as well as the calculation of conductance levels. Samples so far examined indicate the existence of multiple conductance states or multiple conducting components. The agonists L-glutamate and quisqualate appear to produce responses in this system which are consistent with physiological observations.

30. Dynamics of Heme Proteins Studied by Quenching of an External Phosphorescent Probe. S. Beckham, M. M. Luchsinger, S. Bayles, A. Montrem, *Merlyn D. Schuh*, and T. M. Wright. Department of Chemistry, Davidson College, Davidson, NC 28036-1749.

The phosphorescence of 6-bromo-2-naphthyl sulfate (BNS) has been found to be quenched specifically by the heme group of cytochromes *c* and myoglobin. Quenching occurs by a short-range mechanism because the phosphorescence of complexes of 1-bromonaphthalene and β-cyclodextrin is unaffected by heme proteins. The quenching constant, k_q, has been measured as a function of pH and solvent viscosity. At pH 7 the value of k_q is about $^1/_{10}$ of the diffusion-controlled value for all proteins studied. The large values of k_q can be explained only if the heme group is more accessible to solution than is expected from X-ray crystallographic protein models. k_q decreases with increasing viscosity and with increasing pH. The former effect contradicts quenching by deep penetration of the BNS into the protein, and the latter effect is attributed to specific interactions between BNS and charged amino acid residues on the protein surface. The results are used to hypothesize that quenching occurs by surface insertion of BNS molecules into transient openings in the protein.

31. Ubiquitin-Mediated, ATP-Dependent Proteolysis of Ubiquitin–Calmodulin Conjugates Isolated from *Dictyostelium Discoideum*. *Kerry M. Black* and Edwin G. E. Jahngen. Department of Chemistry, University of Lowell, Lowell, MA 01854.

Ubiquitin plays an important role in ATP-dependent degradation of intracellular proteins. It has been found to be covalently conjugated to a wide variety of intracellular proteins in the presence of reticulocyte extracts. It is proposed that the formation of ubiquitin–protein conjugates functions in targeting the conjugate proteins for rapid degradation. The levels of ubiquitin–protein conjugates can be correlated to turnover rates in intact cells and in cell-free reticulocyte extracts. Preliminary studies have shown that *Dictyostelium discoideum* (AX3) contains ubiquitin and the ubiquitin conjugating system consisting of E1, E2, and E3. Calmodulin purified from *D. discoideum* has been shown to conjugate to ubiquitin from rabbit reticulocytes and human erythrocytes and then to be rapidly degraded by the ubiquitin-mediated degradative pathway. Present studies illustrate the presence of a ubiquitin-dependent protease in *D. discoideum* by following the degradation of isolated ubiquitin–calmodulin conjugates by Western blot techniques.

32. Engineering of a Semisynthetic Endonuclease Based on the λ Phage Cro Protein and Nuclease Activity of 1,10-Phenanthroline–Copper. *T. W. Bruice*, J. G. Wise, and D. S. Sigman. Molecular Biology Institute, University of California, Los Angeles, CA 90024-1570.

On the basis of crystallographic studies, the λ cro protein makes sequence-specific contacts to DNA via a helix–turn–helix format while the C-terminal segment interacts with the

minor groove of the operator sequence. An altered cro protein (A66C) has been generated by changing the C-terminal alanine residue to a cysteine by site-directed mutagenesis yielding a unique site of reactivity for alkylation by 5-(iodoacetyl)-1,10-phenanthroline (OP). By use of a mobility shift assay, the binding affinities of wt Cro, Cro (A66C), and OP-modified Cro (A66C) for a ^{32}P-labeled ds DNA fragment containing the high-affinity O_{R3} are equivalent. Addition of cupric ion, H_2O_2, and 3-mercaptopropionic acid to a gel slice containing the OP-modified Cro (A66C)–O_{R3} activates the nuclease activity of 1,10-phenanthroline–copper and leads to specific scission within each half-site. The observed scission sites are consistent with cutting from within the minor groove as predicted from the model of cro binding postulated by B. Matthews. Semisynthetic restriction enzymes based on the Cro protein will be developed.

33. Cancer Detection: Dual Binding Technique Used To Monitor Serum B-Protein. *E. T. Bucovaz*, J. C. Morrison, R. M. Macleod, and W. D. Whybrew. Departments of Biochemistry and Obstetrics and Gynecology, University of Tennessee, Memphis, Memphis, TN 38163, and Department of Obstetrics and Gynecology, University of Mississippi Medical Center, Jackson, MS 39216.

B-Protein is a general cancer marker which appears in serum. The standard assay is based on an interaction between B-protein and a radiolabeled low molecular weight protein of bakers' yeast, binding protein. Binding protein has 4′-phosphopantetheine as a prosthetic group. It was discovered that acyl carrier protein (ACP) of *E. coli*, which also has 4′-phosphopantetheine as a prosthetic group, will interact with B-protein. ACP appears to have a stronger affinity for B-protein than does binding protein. Other properties, however, favor the use of binding protein in the assay. It was observed that when radiolabeled ACP and unlabeled binding protein are present in the assay mixture, they bind in concert to B-protein in the absence of either ATP or an incubation step. This modification simplifies the procedure and improves the sensitivity of the assay.

34. Characterization and Kinetics of Carbon Monoxide Reactions for Hemoglobins from *Trachemys scripta elegans*. M. C. Chien, R. M. Vestal, and *R. C. Steinmeier*. Chemistry Department, University of Arkansas—Little Rock, Little Rock, AR 72204.

Among modern-day reptiles, the group Chelonia possesses one of the oldest evolutionary histories. *Trachemys scripta* (prior classification *Pseudemys*) is widely distributed across the U.S. and is capable of lengthy dives. Its erythrocytes contain two major and two minor hemoglobin components separable by IEF. We find the major components to have the β chain in common but to differ in the α chains, which are α^A or α^D, analogous to hemoglobins to many birds. Equilibrium binding of CO shows these hemoglobins to be of low affinity. Stopped-flow measurements of T-state CO association for HbA and HbD yield slow rates, which are further decreased by chloride, bicarbonate, ATP, or IHP. We also used flash photolysis to measure the distribution between R and T conformations as a function of ligation by CO. Activation energy measurements on CO association show marked enthalpy–entropy compensation. For *Trachemys* hemoglobins, CO dissociation by the microperoxidase method is faster than for human hemoglobin, differing by about 3-fold at pH 7. Thus, changes in both association and dissociation rate constants contribute to the kinetic origin of the reduced affinity for CO. (Grant support: NIH DK-40136, UALR Faculty Research Award.)

35. Purification and Kinetics of Ubiquitin-Activating Enzyme in *Dictyostelium discoideum*. *Gregory R. Chiklis* and Edwin G. E. Jahngen. Department of Chemistry, University of Lowell, Lowell, MA 01854.

Ubiquitin is a polypeptide of 76 amino acids that regulates many cellular functions through its ATP-dependent covalent linkage to target proteins. Since the ligation of ubiquitin to cellular proteins is catalyzed by a multienzyme system, we have begun our study by examining the activation of ubiquitin by the ubiquitin-activating enzyme (E_1). The activation is a two-step process where E_1 first adenylates the carboxy terminus of ubiquitin using ATP and Mg^{2+}. The adenylated molecule is then transferred via a thiol ester linkage to another site on E_1 with the release of AMP and PP_i. To study this, we have purified E_1 from the slime mold *Dictyostelium discoideum* by anion exchange, gel permeation, and a modification of the covalent affinity chromatography procedure of Ciechanover et al. [(1982) *J. Biol. Chem. 257*, 2537–2542]. To study the activation of ubiquitin by E_1, we employed a modification of the Ub amide synthesis assay to follow the [^{32}P]PP_i production [Pikart, C. M., & Rose, I. A. (1985) *J. Biol. Chem. 260*, 1573–1581]. This has allowed us to follow the burst as well as the steady-state turnover of [γ-^{32}P]ATP. Also, by the addition of formycin ATP to our reaction mixture, and its detection by reverse-phase HPLC, we have been able to correlate to the data obtained in the radiolabeled assay. Finally, we have attenuated and inhibited the activity of E_1 by addition of dithiothreitol and iodoacetimide, respectively.

36. Uptake and Interconversions of Purine Bases As Demonstrated by HPLC in the *Methanobacterium thermoautotrophicum*. *Debra J. Costa* and Edwin G. E. Jahngen. Department of Chemistry, University of Lowell, Lowell, MA 01854.

Purine nucleotides are a necessary requirement of all biological life as "energy sinks" for protein interaction and as key building blocks for nucleic acids. The biochemistry of purine base uptake and interconversion is well understood in many eukaryote and eubacteria, but scattered studies have elicited minimal information concerning archaebacteria. Preliminary data have suggested that the archaebacteria *Methanobacterium thermoautotrophicum* incorporates radioactive purine bases and, furthermore, that these bases are interconverted into purine nucleosides, nucleotides, and nucleic acids. The isolation of the nucleobases was done by means of TCA lysis for nucleotide pools and phenol extraction for RNA and DNA and then HPLC separation for individual nucleotides. By standard RNase phenol extraction techniques, DNA yield has been low; to rectify this, low-melting agarose plugs have been employed to separate RNA from DNA via an electric field. This has led to the determination of the relative quantities of radioactive purine bases that were incorporated into and interconverted by these organisms.

37. Role of Ubiquitin in *Dictyostelium discoideum* during Heat and Developmental Stress. *Corinne M. Cressman* and Edwin G. E. Jahngen. Department of Chemistry, University of Lowell, Lowell, MA 01854.

When *Dictyostelium discoideum* AX3 is taken from its normal growing temperature of 21–30 °C, major biochemical changes occur. In general, the majority of protein synthesis

ceases, and the organism initiates the synthesis of a group of proteins formally referred to as "heat shock proteins" (HSP). One of these HSPs has been identified as ubiquitin. Ubiquitin has also been identified as a developmentally regulated protein in *D. discoideum*. Gerisch et al. [(1988) *J. Cell Sci. 90*, 51–58] previously described the increase of ubiquitin mRNA in *D. discoideum* during heat and developmental stress. In this study, ubiquitin has been isolated and characterized from *D. discoideum*, and the change in the amount of ubiquitin during stress within the cells' subcellular fractions (cytosol, mitochondria, nucleus, and plasma membrane) has been determined by immunoassay. These results correspond to those found by Gerisch and confirm that ubiquitin is an important agent in the protection of the cell during heat and developmental stress.

38. Identification by Specific Aminolysis of the Sites of Hemoglobin Esterification by PAH Epoxides. *B. W. Day*,[1] P. L. Skipper,[1] S. Naylor,[2] R. R. Rich,[1] and S. R. Tannenbaum.[1] [1]Chemistry Department, Massachusetts Institute of Technology, Cambridge, MA 02139, and [2]MRC Toxicology Unit, Carshalton, England.

The hydrolytic instability of the carboxylic esters formed between human hemoglobin and *anti*-benzo[*a*]pyrene diol epoxide (BaPDE) with the resulting difficulty in characterizing the site of adduction on the protein prompted the development of a method to label the site with a proteolytically stable ligand which also introduced a unique functional group to aid subsequent isolation. Incubation of ^{14}C-BaPDE-alkylated globin with excess 3-amino-1,2-propanediol led to aminolysis of the ester bond formed between BaPDE and a carboxylate of the protein producing a dihydroxypropyl amide side chain on the protein. The protein was digested to yield tryptic peptides. A single vicinal diol-containing aminolysated peptide was isolated by boronate affinity chromatography. This peptide was characterized by amino acid analysis and sequencing, as well as by FAB-MS, and was shown to be the dihydroxypropyl amide of the α-chain fragment Thr-41 to Lys-56 (C6 to E5), in which Asp-47 (CE5) contains the adduct-forming carboxylate. This residue is located in the nonhelical region of the α-chain linking the C and E helices. The method was used to compare the sites of esterification between various PAH epoxides and mouse and human hemoglobins.

39. Lipid Peroxidation Initiation by Superoxide, Perhydroxyl, and Peroxyl Radicals: Comparative and Mechanistic Studies. *Thomas A. Dix*[1] and John Aikens.[2] Departments of Chemistry[2] and Biological Chemistry,[1] University of California, Irvine, CA 92717.

The initiation of lipid peroxidation by superoxide (O_2^-), perhydroxyl (HOO$^\bullet$), and peroxyl (ROO$^\bullet$) radicals has been investigated. O_2^- does not initiate lipid peroxidation in linoleic acid lipid dispersions, while HOO$^\bullet$ and ROO$^\bullet$ are good initiators. Low levels of linoleic acid hydroperoxides (LOOHs) in the lipid dispersions dramatically sensitize the lipids to HOO$^\bullet$- but not ROO$^\bullet$-initiated lipid peroxidation. The mechanistic differences between HOO$^\bullet$ and ROO$^\bullet$ and the role of LOOHs in the initiation process will be discussed. Lipid peroxidation is implicated in pathology; this study provides a mechanistic basis for HOO$^\bullet$ as a reactive derivative of O_2^- that participates in lipid peroxidation initiation in vivo.

40. Comparative DNA Western Blots of Microtubule Proteins Electrophoretically Fractionated in Different Gel Systems. *K. J. Dwyer* and K. A. Marx. Department of Chemistry, University of Lowell, Lowell, MA 01854.

Microtubules are an integral part of the mitotic spindle apparatus of all dividing eukaryotic cells. The microtubule proteins interact with the DNA containing kinetochore structure present in the centromeric region of all higher eukaryotic chromosomes during mitosis. The factors responsible for the interaction between the spindle microtubules and the kinetochore have not been determined; however, the DNA binding ability of certain microtubule proteins, the τ and high molecular weight MAPs, is well established. In this study we utilize these DNA/protein interactions in a comparison of the DNA binding efficiency of ^{32}P-labeled total calf thymus DNA in Western blots following electrophoretic fractionation in different gel systems. The gel systems we compare are (1) SDS–PAGE, (2) basic nondenaturing (pH 9.0), (3) basic + urea (pH 9.0), (4) acidic + urea (pH 4.0), and (5) acidic + urea + EDTA (pH 4.0). We chose to make these comparisons because the ionic detergent sodium dodecyl sulfate has been shown to lower or prevent the DNA binding ability of certain proteins [Wendler, I., & Grossbach, U. (1988) *Anal. Biochem. 172*, 29–38] in standard SDS–PAGE DNA Western blots. The protein binding species and DNA binding efficiency in the different gel systems will be correlated with the known protein binding species and protein denaturants in the respective gel systems. (Supported by NIH AI 24110 and University of Lowell Seed Money Grants.)

41. Solid-State NMR and GC/MS Studies of the Effect of Cephalothin on *B. subtilis* Cell Wall Metabolism. *T. M. Forrest*,[1] G. E. Wilson,[1] J. K. Hardy,[1] T. A. Lee,[1] J. Schaefer,[2] and Y. Pan.[2] [1]Department of Chemistry, University of Akron, Akron, OH 44325, and [2]Department of Chemistry, Washington University, St. Louis, MO 63130.

The molecular basis for the action of the β-lactam antibiotic cephalothin, on cell wall metabolism in the Gram-positive bacterium *B. subtilis*, was probed by solid-state NMR and GC/MS techniques. *B. subtilis* were grown on a synthetic medium containing L-[2-^{13}C,^{15}N]aspartic acid and D-[1-^{13}C]alanine in the presence and absence of a sublethal dose of cephalothin. At the antibiotic concentrations employed in this study, gross changes in morphology and growth behavior occurred. The level of labeled amino acid incorporation and the degree of label scrambling of the cell wall amino acids in the presence and absence of antibiotic were quantified by GC/MS. The degree of cell wall cross-linking in cells from the control and antibiotic growth were assessed by using data from GC/MS, CPMAS, and REDOR solids NMR techniques. The results provide insight into the effects of cephalothin on pathways of cell wall biosynthesis and metabolism.

42. Isolation and Characterization of the 1.688 g/mL Satellite DNA of *Dictyostelium discoideum*. *J. A. Freeman* and K. A. Marx. Department of Chemistry, University of Lowell, Lowell, MA 01854.

Like most other eukaryotic organisms, the slime mold *Dictyostelium discoideum* contains satellite DNAs. One satellite DNA, the 1.672 g/mL linear rDNA extrachromosomal palindrome, has been characterized, while a second satellite DNA (density = 1.688 g/mL) has not. We have studied this latter satellite DNA, initially by isolation in sequential cesium chloride density gradients using the drug netropsin to enhance resolution. The sequence organization of the purified satellite was determined by restriction enzyme

mapping and restriction fragment hybridization. FIGE electrophoretic fractionation of total *D. discoideum* DNA was performed, and high molecular weight bands were found. Satellite DNA sequences were found to hybridize to both the linear rDNA extrachromosomal palindrome (150 kb) as well as to four other very high molecular weight fractions (greater than 2 Mb), believed to be small intact chromosomes or very large chromosomal fragments. (Supported by NIH AI 24110 and University of Lowell Seed Money Grants.)

43. Immunochemical (ELISA) Demonstration of Z-DNA Antigenic Determinants in Purified Human, Calf, and Mouse Normal Crystalline Lens DNA Using Z-DNA Chiral Metal Complex Molecular Probes. *Claude E. Gagna.* Department of Anatomy, New York University Dental Center, New York, NY 10010.

The presence of left-handed Z-DNA sequences has been revealed for the first time in purified DNA from the human, calf, and mouse normal crystalline lens by using a novel technique involving the enzyme-linked immunosorbent assay (ELISA), with Z-DNA chiral metal complex binding and cleaving (photoactivation) molecular probes. The primary application of the Z-DNA chiral metal probes causes these probes to intercalate themselves into the DNA double helix, either directly blocking other Z-DNA probes (e.g., anti-Z-DNA antibodies) or cutting up the base pair sequences, in a manner such as enzymes. Therefore, the application of secondary applied anti-Z-DNA antibodies could not bind to any Z-DNA antigenic determinants, and ELISA results produced no significant Z-DNA immunoreactivity data. These results are further proof that the lens contains left-handed Z-DNA sequences or sequences with the potential to form Z-DNA which may act to regulate the gene function of the normal lens.

44. Z-DNA Immunoscreening of Two Human Eye Complementary DNA (cDNA) Libraries, Two Bovine Crystalline Lens cDNA Libraries, and One Rat Crystalline Lens cDNA Double-Stranded Library. *Claude E. Gagna.* Department of Anatomy, New York University Dental Center, New York, NY 10010.

Novel techniques involving the screening of complementary DNA (cDNA) (double-stranded sequences) libraries (with anti-Z-DNA antibodies) have revealed the presence of Z-DNA sequences. Two human eye cDNA libraries (λgt10, 0.4–3.2 kb insert size range, and λgt11, 0.4–2.8 kb), two bovine lens cDNA libraries (λgt10, 0.3–3.7 kb, and λgt11, 0.5–3.4 kb), and one rat lens cDNA library (λgt11, 1.1 kb) were immunoscreened. The cDNA library is an alternative strategy to the screening of genomic DNA libraries, since the genomic DNA library, besides containing genes, also contains noncoding DNA and thus no genes at all. The cDNA library represents only those DNA sequences that are transcribed into RNA. It is plausible to conclude that Z-DNA sequences or potential Z-DNA base pairs exist in the vertebrate crystalline lens and may be involved in the regulation of gene expression of both the normal and cataractous lens. To my knowledge, this is the first time in which Z-DNA sequences have been immunoscreened in any type of a cDNA library.

45. Elemental Composition of the Molybdenum–Iron Cofactor of Nitrogenase by X-ray Photoelectron Spectroscopy. *Alison B. Hickman,* David A. Wink, and William H. Orme-Johnson. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

The molybdenum–iron protein of nitrogenase contains four atypical Fe_4S_4 clusters and two molybdenum–iron cofactors (FeMoco) of approximate metal composition $MoFe_{5-8}S_{8-10}$ which are believed to be the sites of N_2 binding and reduction to NH_3. Recently, homocitrate (2-hydroxy-1,2,4-butanetricarboxylic acid) has been shown to be an organic moiety of FeMoco. Furthermore, our laboratory has developed a protocol which allows for the extraction of intact FeMoco from the molybdenum–iron protein into various organic solvents. We have applied the technique of X-ray photoelectron spectroscopy (XPS) to FeMoco and various FeS- and MoFeS-containing model compounds and have deduced the elemental composition of extracted FeMoco. This is an example of a novel application of a powerful surface technique to probe a biological system.

46. The Combination of DNA Methylation and H_1 Histone Binding Inhibits the Action of Restriction Nuclease on Plasmid DNA. Masao Higurashi and *R. David Cole.* Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

To investigate the roles of DNA methylation and H_1 histone in regulating the action of DNA binding proteins, well-ordered complexes were formed by slow dialysis of H_1 histone with either methylated or nonmethylated DNA. The sites methylated in the plasmids were CCGG. Methylation of cytosine in this site protects the DNA against *Hpa*II endonuclease but not against *Msp*I. However, when the methylated DNA is complexed to H_1, it is protected against *Msp*I. The protection of DNA afforded by the combination of H_1 binding and DNA methylation does not apply to RI, *Pst*I, or *Bam*HI sites, and so does not seem to be due to aggregation of the DNA by H_1 histone. Gel retardation assays indicated that the affinity of H_1 for methylated DNA was not detectably different from affinity for nonmethylated DNA. Probably methylated DNA when bound to H_1 is in a conformation that is resistant to *Msp*I endonuclease. Such conformational changes induced by DNA methylation and H_1 binding might affect the action of other DNA binding proteins.

47. NMR Spectroscopy of the *met* Repressor Protein and Its Complex with *S*-Adenosylmethionine. *David W. Hoffman,* Ronald C. Greene, and Leonard D. Spicer. Department of Biochemistry, Duke University, Durham, NC 27710.

The *met* repressor is a DNA binding protein which in concert with its corepressor *S*-adenosylmethionine (SAM) regulates production of the enzymes involved in methionine and SAM biosynthesis in *E. coli.* We are using NMR spectroscopy (1) to investigate the interaction of the *met* repressor protein with SAM and (2) to study structural features of this protein. Titrations of the *met* repressor protein with SAM, monitored by proton NMR, show that a weakly bound repressor–corepressor complex is formed. We are currently investigating the kinetics of this complex formation by saturation-transfer methods as a function of temperature and concentration to quantitatively determine the rate constant for SAM binding by the repressor protein. Assignment of the protein resonances is also in progress. This is a challenging problem due to the fact that this relatively large protein exists as a stable dimer. We are using a variety of techniques including isotopic labeling with deuterium and ^{15}N in this effort. Results will be presented in the context of a functional model for repressor activity.

48. Electrostatic Fields in Proteases: An Examination of Three-Dimensional Homology. *David W. Huhta*, Richard L. Schowen, and Angelo Vedani. Biographics Laboratory, Departments of Chemistry and Biochemistry, University of Kansas, Lawrence, KS 66045.

The electrostatic field in the active sites of a number of serine proteases whose coordinates are available from high-resolution X-ray studies (trypsin, chymotrypsin, elastase, subtilisin et al.) have been mapped to define regions of similar potential across this group. Potentials at points in a three-dimensional grid were calculated by using empirical (Coulombic) descriptions of the electrostatic interactions. The effects of substrate binding, tetrahedral intermediate formation, solvation, and counterions on the field were examined. Comparisons of serine protease active-site fields with those of cysteine proteases were also carried out.

49. Comparison of the DNA-Alkylating and Mutagenic Properties of *S*-Haloethyl Derivatives of Cysteine and Glutathione. *W. G. Humphreys*, D.-H. Kim, J. L. Cmarik, and F. P. Guengerich. Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37232.

The mutagenicity of ethylene dibromide (EDB) is dependent upon its conjugation to glutathione by the enzyme glutathione *S*-transferase. The conjugates thus formed can react with DNA and yield almost exclusively N^7-guanyl adducts. We have synthesized the *S*-haloethyl conjugates of cysteine and glutathione (as well as selected methyl ester and *N*-acetyl derivatives) and compared them on the basis of their ability to produce N^7-guanyl adducts with calf thymus DNA. The cysteine compounds were found to be far more reactive toward calf thymus DNA and yielded adduct levels more than an order of magnitude greater than those of the glutathione compounds. Adduct levels corresponded reasonably well with the net charge of the compound and were not affected by substitution of bromine for chlorine. The compounds were also tested for their ability to act as direct mutagens in the *Salmonella typhimurium* TA100 bacterial tester strain. They were all found to act as direct mutagens, but the levels of mutagenicity produced did not correlate at all with the levels of alkylation. In fact, *S*-(2-chloroethyl)glutathione was found to be the most potent mutagen, although it produced the lowest levels of alkylation, indicating that the guanyl adduct formed in this case seems to be unusually mutagenic. (Supported in part by USPHS Grants ES01590, CA44353, and ES05473.)

50. Functional Relationships between Ascorbic Acid and Uric Acid. K. F. McGowan, *J. D. Henderer*, S. A. Graham, and W. H. Church. Department of Chemistry, Trinity College, Hartford, CT 06106.

A biochemical role for uric acid in the development of the central nervous system was investigated. Ascorbic acid (AA) and uric acid (UA) levels were determined in plasma and brain tissue collected from rats in three different age groups: <16 days old (group I), 16–25 days old (group II), and >60 days old (group III). Group II plasma AA and UA increased by 120% and 429%, respectively, over group I. Group III AA levels decreased by 172% and UA levels increased by 42% vs group I. The plasma UA/AA ratio increased progressively across the age groups, from 0.392 in group I to 1.52 in group III. Brain tissue analysis revealed an increase in AA with age. Brain UA was detectable only in group III. Studies investigating the antioxidation role of AA and UA will be presented. The significance of the observed trends as they relate to nervous system development will be discussed.

51. Purification of Plasmid DNA from *E. coli* Alkaline Lysates by HPLC. *C. M. Mello*, E. G. E. Jahngen, and K. A. Marx. Department of Chemistry, University of Lowell, Lowell, MA 01854. J. Thayer, Dionex Corporation, Sunnyvale, CA 94086.

The use of recombinant DNA cloning techniques has proved to be invaluable to many investigators. However, some of these procedures are quite expensive and lengthy. For example, the preparation of plasmid DNA from host cells in a relatively pure form requires lysis of the cells, centrifugation to remove chromosomal DNA, and finally banding of DNA through a cesium chloride gradient in the presence of ethidium bromide. A typical plasmid preparation would take a number of days. Consequently, a need for a more expedient procedure without sacrificing purity exists. Recently, an HPLC technique has emerged which may allow the purification of plasmid DNA from alkaline lysates in less than an hour. An anion-exchange HPLC column, Omnipac NA100, manufactured by Dionex Corporation has shown base-line separation of the individual components present in crude plasmid preparations. The basis for this separation is the exchange of anions in the mobile phase with the negative phosphate backbone of the nucleic acids. Initial studies have applied up to 1 OD to the column without altering the separation. Further characterization of the plasmid DNA isolation from crude lysates is presently under investigation. (Supported by NIH AI24110 and University of Lowell seed grants.)

52. Purification Attempts of Putative Microtubule Associated Protein 2 from Heat-Stable Brain Protein by HPLC. *C. M. Mello* and K. A. Marx. Department of Chemistry, University of Lowell, Lowell, MA 01854.

Microtubule associated protein 2 (MAP2) is a 220000 M_r protein which copolymerizes with microtubules. In early studies it has been shown that MAP2 is thermostable and binds to tubulin and DNA. It is our belief that MAP2 may be involved in the attachment of microtubules to the kinetochore during mitosis. The traditional purification of MAP2 consists of microtubule preparation from bovine brain tissue followed by a heat denaturation step. This results in two microtubule species, MAP2 and τ. MAP2 is then separated from the proteins by gel filtration chromatography. An alternate procedure has also emerged which purifies MAP2 from total unfractionated, heat-stable brain protein followed by gel filtration chromatography. Gel filtration chromatography is a very long and tedious procedure. As a result we are attempting to purify MAP2 from heat-stable total brain protein by ion-exchange HPLC. Preliminary studies have shown partial purification on the basis of isoelectric point fractionation with an elution gradient of pH. The process, if successful, will allow the purification of MAP2 in approximately 20 min by HPLC techniques versus a number of hours by traditional column chromatography. (Supported by NIH AI24110 and University of Lowell seed grants.)

53. Preparation of an Oligonucleotide Adducted with Ethylene Dibromide Glutathione Conjugate. *T. Oida*, W. G. Humphreys, and F. P. Guengerich. Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37232.

Mutagenicity of ethylene dibromide (EDB) is known to result from glutathione conjugation followed by DNA binding at guanyl residues. *S*-(2-[N^7-Guanyl{d(ATGCAT)}]ethyl)-glutathione was synthesized to study the conformational effects of DNA adduct formation. The hexadeoxyribonucleotide d(ATGCAT) was prepared by using solid-phase phosphoramidate chemistry and mixed with an excess amount of *S*-(2-chloroethyl)glutathione at pH 7 and 37 °C. The reaction was monitored by ion pair HPLC using a mobile phase containing 4 mM tetrabutylammonium phosphate. One major peak and several minor peaks were detected, resulting in a 20% yield of the major peak and 40% of the original oligonucleotide. The major product was purified by HPLC using SAX and DEAE columns. The ^{1}H NMR spectrum of the major product indicates that the oligomer was labeled at the N^7 position of the guanine base with a stoichiometry of 1:1. The transition temperature in the UV melting curve of the adduct oligomer was similar to that of the original oligomer. These results suggest that the glutathione conjugate of EDB is the ultimate reactive metabolite. It forms DNA adduct under physiological conditions, and adduct formation does not affect the stability of the DNA double helix. Comparisons were made with *S*-(2-[N^7-guanyl{d(ATGCAT)}]ethyl)-*N*-acetyl-L-cysteine methyl ester, the guanyl adduct of which is much less mutagenic. (Supported in part by USPHS Grants ES01590 and CA44353.)

54. NMR Studies of Interactions between Serine Proteases and ^{13}C-Labeled Peptide Inhibitors. *Claudio Ortiz*, Charles Tellier, Howard J. Williams, Neal J. Stolowich, and A. Ian Scott. Center for Biological NMR, Department of Chemistry, Texas A&M University, College Station, TX 77843.

X-ray crystallographic data (E. Meyer, Jr., private communication) indicate that a stable complex is formed between the serine 195 hydroxyl of trypsin and the arginine carbonyl carbon of certain peptide inhibitors. To study this interaction, two pentapeptides, Leu-Leu-Arg-Sar-Tyr and Leu-Leu-Arg-Pro-Tyr, have been prepared with ^{13}C label at the carbonyl carbon of the arginine residue. NMR spectroscopy has been used to determine the nature of the covalent complex formed between the enzyme and each of the peptides. Information on the mechanism of inhibition of these peptides was obtained from chemical shift and pH titration data.

55. Chromatographic Determination of Amino Acid Side Chain–Side Chain Interaction Free Energies. *Thomas Pochapsky* and Quinton Gopen. Department of Chemistry, Brandeis University, Waltham, MA 02254.

An HPLC stationary phase (SP) which mimics the leucine side chain was prepared by treating microparticulate silica with isobutyltrimethoxysilane, forming a bonded layer of isobutyl groups on the silica surface. This SP was used for the chromatographic separation of a series of *N*-acetyl α-amino acid *N*-methylamide analytes using water as a mobile phase. Both hydrophobicity and steric factors are shown to be important for determining retention times. Differences in chromatographic retention are related to the difference in free energies of adsorption onto the SP for this series of analytes. The utility of these free energy values for describing solution interactions between amino acid side chains will be discussed, including their applicability to describing the initial stages of protein folding.

56. Identification of a β-Sheet Structure in Putidaredoxin by ^{1}H NMR. *Thomas C. Pochapsky* and Ye Xiao Mei. Department of Chemistry, Brandeis University, Waltham, MA 02254.

Two-dimensional ^{1}H NMR data permit the identification of an antiparallel β-sheet in putidaredoxin (Pdx), a 106-residue globular electron-transfer protein containing a 2-Fe 2-S cluster for which no crystallographic structure exists. The sheet consists of at least four five-residue strands, with distinctive αCH–αCH interstrand NOEs indicating antiparallel structure. The sequential assignment of proton resonances in oxidized Pdx will be described, as well as progress toward a three-dimensional solution structure of Pdx based on NOE constraints.

57. The Role of Electrostatics in Protein–Protein Recognition. *Karla K. Rodgers* and Stephen G. Sligar. Departments of Biochemistry and Chemistry, University of Illinois, Urbana, IL 61820.

A useful model system for studying protein–protein recognition is that of cytochrome b_5 (cyt b_5) and cytochrome *c* (cyt *c*). We have undertaken experiments combining site-directed mutagenesis with high-pressure techniques to study the role of the negative surface charges on rat liver cyt b_5 in binding to cyt *c* [(1988) *Science 240*, 1657]. We have continued these efforts to elucidate the possible orientations of the complex formation under various conditions. The affinity of the different cyt b_5 charge mutations with cyt *c*, as well as the ΔV upon complex dissociation obtained by high-pressure studies, was measured. From a comparison of the volume changes in the interactions of the charge mutants with cyt *c* versus that of the native cyt b_5, the precise residues of cyt b_5 that are involved in complex formation were identified. High-pressure results also provide evidence of more than one orientation present upon binding. (This work was supported through NIH Grants GM33775 and GM31756.)

58. Examination of the Structures of Membrane-Bound Glycolipids by Oriented Sample NMR Methods. *Charles R. Sanders II* and James H. Prestegard. Department of Chemistry, Yale University, New Haven, CT 06511-8118.

The conformation and dynamics of the disaccharide headgroups of glycolipid analogues β-dodecylmelibiose and β-dodecyllactose were examined by interpreting the deuterium NMR spectra arising from the deuterated analogues in structural terms using AMBER [S. S. Weiner et al. (1986) *J. Comput. Chem. 7*, 230–252] and the NMR Pseudoenenergy Approach [P. Ram and J. H. Prestegard (1988) *J. Am. Chem. Soc. 110*, 2383–2388]. The different glycosidic linkages between the terminal galactose and the β-alkyl glucoside of the two molecules (α-1–6 for the melibioside, β-1–4 for the lactoside) were found to result not only in significant differences in the relative configurations of the two sugars comprising the headgroups of the two but also in different orientations for the headgroups as a whole with respect to the membrane interface. The effect of the presence of both positively and negatively charged amphiphiles in the phospholipid matrix upon the structures of the glycolipids was also examined. Finally, the potential of the above methodology for use in studies of membrane receptor–protein interactions was also evaluated.

59. Proton Diffusion in the Active Site of Phosphoglucose Isomerase. *S. S. Seeholzer* and I. A. Rose. Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111.

No abstract available.

60. Effect of Histidine Mutation on the Active-Site Environment of Hydroxymethylbilane Synthase. A. I. Scott, *Karen R. Clemens*, Clotilde Pichon, and Neal J. Stolowich. Center for Biological NMR, Department of Chemistry, Texas A&M University, College Station, TX 77843.

Hydroxymethylbilane synthase [HMBS (EC 4.3.1.8)] is necessary for tetrapyrrole assembly in porphyrin biosynthesis and is thus ubiquitous in bacterial, plant, and mammalian metabolism. Site-specific mutagenesis of the only strictly conserved histidine residue in *E. coli* HMBS has yielded enzyme with significantly reduced catalytic activity. Replacement of His80 by Asn (H80N) causes a 70% reduction in activity, with retention of wild-type stability. This presentation will address the nature of the perturbation in the H80N mutant. The active-site environment and the overall conformation of the two enzymes will be analyzed by NMR spectroscopic methods and comparative kinetic studies, as well as classical biochemical techniques. HMBS contains a covalently bound dipyrrolic cofactor which serves as a primer for the head-to-tail polymerization of four substrate molecules. NMR T_1 relaxation parameters of the ^{13}C-enriched cofactor and ^1H–^1H and ^1H–^{13}C nuclear Overhauser effects of wild-type and mutant enzyme will be discussed with regard to the active-site conformation and cofactor mobility in each. Comparative inhibition kinetics using N-alkylated substrate analogues, as well as previously described inhibitors, will provide a test of the relative steric tolerance of the active site.

61. Reverse-Phase Separation of Protein D/L Amino Acids. *R. Shapira*. Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.

Amino acids liberated by acid hydrolysis of proteins were incubated briefly with *N*-acetyl-L-cysteine and *o*-phthalaldehyde to form fluorescent diastereoisomers (1-*N*-acetyl-L-alaninethio-2-D- and L-carboxymethylalkylisoindoles). The isomers were separated on a Waters 5μ Nova-PAK C_{18} Radial-PAK cartridge at pH 5.9 and a *n*-propanol gradient from 1% to 12% v/v and 2 mL/min. The amino acids eluted in the following order: D-Asp, L-Asp L-Ser, D-Ser, L-His, L-Glu, D-His, D-Glu, D-Thr, L-Thr, L-Arg, D-Arg, L-Ala, D-Ala, L-Tyr, D-Tyr, L-Val, L-Met, D-Met, D-Val, L-Ile, L-Phe, D-Phe, D-Ile, L-Leu, D-Leu, L-Lys, D-Lys. The separation between the isomers was complete; thus, less than 1% of the D isomers could be quantitatively measured in acid hydrolysates of human myelin basic protein.

62. Femtosecond Solution-Phase Dynamics of Excited-State Proton Transfer in 7-Azaindole. *P. Share*, M. Pereira, M. Sarisky, S. Repinec, and R. Hochstrasser. Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104-6323.

The dynamics of excited-state proton transfer of 7-azaindole in aprotic media has been investigated by using the fluorescence upconversion technique in a femtosecond laser system. The solute, which exists primarily as dimers in solution under the conditions employed, has been found to undergo photoinduced proton transfer by two different mechanisms. The first mechanism involves the formation of the 7-azaindole tautomer in the excited state, followed by relaxation to the ground-state tautomer with fluorescence emission centered at 500 nm. This excited-state process was found to occur on a time scale of 200 fs. The second mechanism involves direct conversion from the excited 7-azaindole dimer to the tautomeric ground state in a presumably concerted fashion. Transient absorption measurements indicate the appearance of the ground-state tautomer on a time scale similar to that found for the excited-state process.

63. Use of I_2 To Sequence RNA Containing Phosphorothioate. *D. Speckhard*, G. Gish, and F. Eckstein. Loras College, Dubuque, IA 52001, and Max Planck Institut Ex Medicine, Gottingen D3400, FRG.

The difference in reactivity between phosphate and phosphorothioate diesters is the basis of a chemical method for sequencing RNA. Phosphorothioate groups are incorporated into RNA transcripts enzymatically in four separate reactions, each with three natural ribonucleoside triphosphates and one α-thiotriphosphate. The transcripts are labeled with ^{32}P on the 5′ terminal. Selective cleavage under mild conditions is achieved by activation of the phosphorothioate with iodine, resulting in a hydrolytically labile phosphotriester. These conditions are appropriate for footprinting protein–RNA complexes. As examples, the sequence of yeast tRNA–Phe and preliminary footprinting results of the yeast tRNA–Phe–Phe synthetase are presented.

64. Photoaffinity Labeling of Rho by 8-Azido-ATP. Barbara L. Stitt and *InSug O.* Department of Biology, New York University, Washington Square East, New York, NY 10003.

Bacterial transcription termination protein rho hydrolyzes ATP during rho-dependent transcription termination. The molecular mechanism of coupling of the obligatory ATP hydrolysis to transcription termination remains unknown. To determine what amino acids form the ATP binding site, we used a photoaffinity analogue of ATP, 8-azido-ATP. 8-Azido-ATP is a substrate for rho, and the K_m and V_{max} were determined to be 18 μM and 1.5 μmol min^{-1} (mg of rho)$^{-1}$, respectively. When rho is incubated with 8-azido-ATP in >300-nm light, its ATPase activity is inactivated with pseudo-first-order kinetics. Inactivation is prevented in the presence of ATP, the normal rho substrate. The amino acid of the ATP binding site of rho that is modified by ^{32}P-8-azido-ATP was identified by sequencing of a labeled, HPLC-purified tryptic peptide.

65. Identification and Quantitation of the Fatty Acid Intermediates Using GC/MS. *C. S. Tamvakopoulos* and V. E. Anderson. Department of Chemistry, Brown University, Providence, RI 02912.

The identification, separation, and quantitation of the fatty acid oxidation intermediates have been achieved by gas chromatography/chemical ionization mass spectrometry. Because of the very low concentrations found in biological systems, negative ion chemical ionization has been investigated as a potential sensitive assay method for detecting these intermediates. The CoA thioesters are separated by solid-phase extraction by elution from charcoal followed by conversion to a pentafluorobenzyl derivative. The thioesters could be selectively hydrolyzed in base followed by conversion to the pentafluorobenzyl ester or converted to the *O*-pentafluorobenzyl hydroxamate directly through hydroxaminolysis. Quantitation of picogram amounts is achieved by addition of a CoA thioester standard of known concentration. The above method could be used to quantify the fatty acid oxidation intermediates in intact mitochondria (beef heart), tissue homogenates, and reconstituted fatty acid oxidation systems supplied with the necessary cofactors.

66. Solid-State NMR Investigations of the Mechanism of Proton Pumping in Bacteriorhodopsin: Tyrosinate Is Not Found in bR-568 or the M Intermediates. L. K. Thompson, A. E. McDermott, M. R. Farrar, and R. G. Griffin. Massachusetts Institute of Technology, Cambridge, MA 02139. C. Winkel and J. Lugtenburg. University of Leden, The Netherlands. R. S. Brown and *J. Herzfeld*. Brandeis University, Waltham, MA 02254.

The mechanism of light-driven proton pumping by bacteriorhodopsin (bR) is thought to include proton transfer among ionizable amino acids. In particular, FTIR and UV spectroscopies have yielded evidence for the presence of a tyrosinate (Tyr) residue in bR-568 and the M intermediate of the photocycle. A recent solid-state NMR study of [4′-^{13}C]Tyr-labeled bR and tyrosine model compounds demonstrated that a single Tyr would give a well-resolved, observable resonance in bR, shifted 9 ppm downfield from the Tyr resonance. Such a Tyr was not observed in the dark-adapted bR until the protein began to denature above pH 12. We have extended these studies to light-adapted bR (bR-568) and the two forms of M that are trapped at low temperature and pH 10, in the presence and absence of 500 mM guanidine hydrochloride. Although we cannot rule out small chemical shift changes corresponding to unusual Tyr interactions or hydrogen bonding, we can rule out the large chemical shift changes that would be associated with deprotonation.

67. The Regulation of Iron Absorption, the Importance of Molecular Components of the Intestinal Mucosal Cell. *R. W. Topham* and C. E. Eads. Department of Chemistry, University of Richmond, Richmond, VA 23173.

A number of factors implicated in the regulation of intestinal iron absorption have been simultaneously analyzed in control rats and control rats transferred to low-iron diets for various time intervals. Brush-border membrane vesicles from control rats transferred to low-iron diets showed increased iron uptake and a different distribution of iron between iron-binding components of the brush-border membrane. The distribution of iron between iron-binding components in the cytosol of the mucosal cells of these animals also differed markedly from that of controls. The activities of the intestinal ferroxidase and ferrireductase, enzymes implicated in determining the distribution of cytosolic iron between iron-binding components, were altered when control animals were transferred to low-iron diets. All of these changes appeared to be complete within 72 h after the transfer of control animals to a low-iron diet. These findings emphasize the speed and complex nature of the molecular changes that occur in the mucosal cell, permitting this cell to adjust iron absorption in relation to changes in the dietary iron content. (Supported by NIH Grant DK 38313.)

68. Nicotinate Phosphoribosyltransferase from *Salmonella typhimurium*. *A. Vinitsky*,[1] J. W. Foster,[2] and C. T. Grubmeyer.[1] [1]Department of Biology, New York University, New York, NY 10003, and [2]Department of Microbiology, University of South Alabama, College of Medicine, Mobile, AL 36688.

The *pncB* gene coding for nicotinate phosphoribosyltransferase (NAPRTase, EC 2.4.2.11) has been cloned from *S. typhimurium* and the enzyme overexpressed 1000-fold, allowing a high yield, three-step purification procedure. The reaction catalyzed by the homogeneous enzyme is (ATP) + NA + PRPP $\rightleftharpoons$ NAMN + PP_i + (ADP + P_i). As with other microbial NAPRTases, stoichiometric hydrolysis of ATP was found to stimulate NAMN synthesis, this stimulation being unusual among PRTases. The presence of ATP also increases the K_{eq} for NAMN synthesis reaction by a factor of 5000. Thus, NAPRTase presents a unique system for the study of energy coupling in ATP-linked reactions. We have found that the enzyme is phosphorylated by MgATP. The nature and role of the phosphorylated intermediate are under investigation.

69. Electrochemistry of Cytochrome c_3 from *D. desulfuricans* (NCIMB8372): Redox Behavior at Different Electrode Materials. *D. L. Wang*,[1] M. T. Stankovich,[1] L. H. Eng,[2] and H. Y. Neujahr.[2] [1]Department of Chemistry, University of Minnesota, 207 Pleasant St. S.E., Minneapolis, MN 55455, and [2]Biochemistry, Royal Institute of Technology, S-10044 Stockholm, Sweden.

The electron-transfer reaction (ETF) of the tetraheme cytochrome c_3 from *D. desulfuricans* (NCIMB8372) was investigated at a glassy carbon electrode (GCE), a pyrolytic graphite electrode (PGE), a mercury electrode, and a gold electrode by using cyclic voltammetry and differential pulse polarography. The ETF of cytochrome c_3 at the GCE and PGE is typical of a diffusion-controlled and reversible process. The overall average redox potential, $E_m = -297 \pm 5$ mV (SHE), agrees quite well with that obtained by mediated potentiometric titration, in which the proposed ETF mechanism was said to occur in four steps, with one-electron transfer at −260 mV (for one heme) and three-electron transfer at −330 mV (for three hemes). The cyclic voltammetric behavior indicated that cytochrome c_3 adsorbed onto the mercury electrode, and all electron transfers appeared to be reversible with the overall E_m value about 80 mV more negative ($E_m = -380 \pm 10$ mV) than that obtained at the GCE or PGE. This may indicate that cytochrome c_3 adsorbs in a highly oriented position on the Hg electrode. The electrode-material dependence of the cytochrome c_3 redox potential may be due to the conformational changes which occur when the cytochrome c_3 interacts with the Hg electrode.

Enzymology

70. *N*-Acetylmuramyl Dipeptide Functions by Association with Serotonin Receptors. *Fred C. Westall*, Daniel Ostercamp, and Darlene Hand. Institute for Disease Research, Alta Loma, CA 91701-8293, Harvey Mudd College, Claremont, CA 91711, and California State Polytechnic University, Pomona, CA 91768-4031.

N-Acetylmuramyl dipeptide (MDP), an immunoactive component of Freund's complete adjuvant and a potent sleep factor, can initiate several autoimmune diseases. When the sequence Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg (the Trp peptide of myelin basic protein) and MDP are injected into guinea pigs, allergic encephalomyelitis (EAE) is produced. Similarly, the hormone LHRH and MDP produce autoimmune castration. Previously we showed that MDP binds to both the Trp peptide and LHRH [(1986) *J. Inferential Deductive Biol. 2*, 1]. This binding is important for disease induction. Furthermore, both LHRH and Trp peptide bind serotonin [(1984) *Brain Res. Bull. 12*, 425]. Using both Redfield NMR and infrared spectroscopy, we find that encephalitogenic regions which do not bind serotonin do not bind MDP or produce EAE with MDP. Serotonin also associates with "MDP receptors" on macrophages. Moreover, *N*-acetylneuraminic acid, which is a component of other serotonin receptors, will itself bind to serotonin and MDP. We suggest that MDP, a component of gut bacteria, functions in vivo both

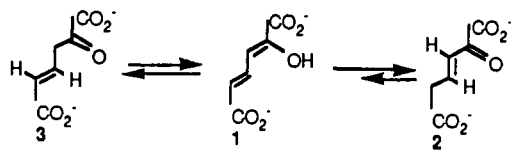
neurologically and *immunologically* through association with various specific serotonin receptors.

71. Rapid-Quench Kinetic Experiments on Mn^{2+}-Activated Glutamine Synthetase. *Lynn M. Abell* and Joseph J. Villafranca. Department of Chemistry, The Pennsylvania State University, 152 Davey Laboratory, University Park, PA 16802.

Glutamine synthetase from *E. coli* catalyzes the ATP-dependent formation of glutamine from glutamate and ammonia via a γ-glutamyl phosphate intermediate. The enzyme requires two divalent metal ions for catalysis. The physiologically important metal is Mg^{2+}; however, Mn^{2+} supports in vitro activity, though at a reduced level. The enzyme is regulated by a covalent adenylylation modification, and the metal specificity of the reaction depends on the adenylylation state of the enzyme. Unadenylylated glutamine synthetase shows activity with both Mg^{2+} and Mn^{2+}, while the adenylylated enzyme only shows activity with Mn^{2+}. While this switch in metal specificity upon adenylylation has been well-known for some time, very few experiments have been conducted to examine possible mechanistic differences between the two forms of the enzyme. Rapid-quench kinetic experiments on the unadenylylated enzyme with either Mg^{2+} or Mn^{2+} as the activating metal revealed that product release is the rate-limiting step. However, in the case of the adenylylated enzyme, the phosphate-transfer step is the rate-limiting step. The internal equilibrium constant for phosphate transfer is 2 and 5 for the unadenylylated enzyme, respectively, whereas that for the adenylylated enzyme is 0.1, indicating that phosphate transfer is less energetically favorable for this form of the enzyme. The phosphate-transfer step itself was investigated more closely by using the slow tight binding inhibitor phosphinotricin, which becomes phosphorylated in the slow inactivation step. (Supported in part by NIH Fellowship GM11994-03.)

72. Enzymatic Ketonization of the Dienol 2-Hydroxymuconate. *Bruce. A. Aird* and Christian P. Whitman. College of Pharmacy, The University of Texas, Austin, TX 78712.

4-Oxalocrotonate tautomerase (4-OT) catalyzes the ketonization of 2-hydroxymuconate (**1** to **2**). Recent studies



have shown that the chemical decay of **1** in aqueous solution (20 mM Na_2HPO_4, pH 7.3) results in a rapid ($t_{1/2} \sim 42$ s) drop in the absorbance at 295 nm, followed by a much slower decrease to equilibrium ($t_{1/2} \sim 18$ min). The initial drop corresponds to the rapid formation of **3**; the slower decrease stems from the formation of **2** from **1**. The rapid generation of **3** in solution raises the question of what the actual physiological substrate of 4-OT is. Our results indicate that **1** is the substrate and that **3** apparently enters the cycle passively through enolization to **1**. Additionally, results of preliminary kinetic studies suggest that the k_{cat}/K_m value for this reaction approaches the diffusion-controlled association rate. Further studies of the 4-OT reaction may provide insight into the energetics of enzymatic intermediates which proceed through dienol intermediates.

73. Overproduction and Characterization of Nitrogenase V. *Amy C. Anderson*, T. A. Collet, and W. H. Orme-Johnson. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

This presentation describes the first successful experiments to overexpress the nitrogenase V (*nif* V) protein as well as experiments to characterize the catalytic activity of the protein product. The experiments begin with the initial cloning of the *nif* V gene, which positions it on the same vector as the strong *tac* promoter. To bring the *nif* V gene and the *tac* promoter as close as possible, we used a unique method of promoter protection and T4 DNA polymerase exonuclease activity. This new construction has the *nif* V gene within 50 base pairs of the promoter and forces the cell to make the *nif* V protein well in excess of the wild type. The relationship of this protein to homocitrate synthesis will be explored. [This work was supported by National Institutes of Health Grant GM30943-06 (W.H.O.-J.).]

74. α-Proton-Catalyzed Exchange by Crotonase. *B. J. Bahnson* and V. E. Anderson. Department of Chemistry, Brown University, Providence, RI 02912.

Bovine liver crotonase catalyzes the stereospecific syn β-elimination of (3*S*)-3-hydroxybutyryl-CoA. The stereochemistry and the syn course of the reaction require that the *pro-R* α-proton be abstracted. Crotonase catalyzes the exchange of the *pro-S* α-proton of butyryl-CoA and octanoyl-CoA stereospecifically. The ^1H NMR monitored exchange of a single α-proton of butyryl-CoA was complete after 30 min in D_2O (pD 6.5, 1 μM crotonase) and stable for 30 h. Acyl-CoA dehydrogenase (porcine kidney, 20 μM), which catalyzes the exchange of the *pro-R* α-proton of butyryl-CoA stereospecifically, was added, and it exchanged the α-proton which was stable to crotonase-catalyzed exchange. The octanoyl-CoA exchange was measured similarly. Crotonase catalyzes the exchange of the α-proton of (2*R*)-*S*-methylmalonyl-CoA. The nonenzymatic exchange rate of the α-proton had a half-life of 3.5 h at pD 7.2, 0.01 M phosphate buffer. The crotonase-catalyzed exchange (1.5 μM enzyme) of (2*R*)-*S*-methylmalonyl-CoA was complete in 30 min under conditions identical with those of the nonenzymatic exchange measured. (This work was supported in part by NIH Grant GM36562.)

75. Effects of Inhibitors of Cytochrome $P\text{-}450_{17\alpha}$ on Steroid Production in Mouse Leydig Cells and Mouse and Pig Testes Microsomes. Alan M. Berg,[1] *Alison B. Hickman*,[1] Emily Miao,[2] Andrea Cochran,[1] Stephen R. Wilson,[2] and William H. Orme-Johnson.[1] [1]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, and [2]Department of Chemistry, New York University, New York, NY 10003.

Cytochrome $P\text{-}450_{17\alpha}$ catalyzes the oxidative cleavage of the 17,20-bond of progesterone and pregnenolone in the biosynthesis of androgens. We have studied the effects of several synthetic steroid inhibitors of $P\text{-}450_{17\alpha}$ on testosterone production in mouse Leydig cell suspensions. The estimated concentrations of inhibitor required to inhibit 50% of hCG-stimulated testosterone production (I_{50}) for 17β-(*N*-cyclopropylamino)androst-5-en-3β-ol (I) and 17β-(*N*-methylamino)androst-5-en-3β-ol (II) were 0.2 and 25 μM, respectively. 16α,17α-Methyleneprogesterone (III) and 17α-methylpregnenolone (IV) were each shown to have an I_{50} of approximately 0.6 μM. The I_{50} values for 21-chloro- (VI) and 21-bromopregnenolone (VIII) were found to be 3 and 5 μM, respectively. These synthetic steroids and 16α,17α-epoxyprogesterone (V) inhibited 17α-hydroxylase activity in adult mouse testis and neonatal pig testis microsomes in a concen-

tration-dependent manner. We deduced that these compounds inhibited 17,20-lyase activity since these compounds inhibited androstenedione and subsequent testosterone production stimulated by the addition of 17α-hydroxyprogesterone. [This work was supported by NIH Grants GM028358 (W.H.O.-J.) and GM31482 (S.R.W.).]

76. Kinetic Mechanism of Orotate Phosphoribosyltransferase from *Salmonella typhimurium*. *Mohit B. Bhatia*[1] and Charles Grubmeyer.[2] Departments of Chemistry[1] and Biology,[2] New York University, New York, NY 10003.

Orotate phosphoribosyltransferase (EC 2.2.4.10) catalyzes the formation of a N-glycosidic bond between α-D-5-phosphoribosyl 1-pyrophosphate (PRPP) and orotate to form orotidine 5-phosphate, a step in the de novo biosynthesis of the pyrimidine nucleotides. The plasmid pCG13, bearing the *Salmonella typhimurium* pyr^E gene under the control of a T7 promoter, harbored in the *E. coli* T7 polymerase host BL21-(DE3) directs the overproduction of OPRTase. Using a simple protocol, we have purified 300–400 mg of OPRTase to homogeneity from 60 g of cells. Initial velocity and product inhibition studies indicate that *S. typhimurium* OPRTase follows a random kinetic mechanism. This conclusion is supported by equilibrium isotope exchange kinetics. In addition, the enzyme catalyzes slow exchanges between OMP–orotate and PRPP–PP_i, in the absence of the other substrate–product pair. These partial reactions demand that an enzyme-bound 5-phosphoribosyl intermediate exist and are indicative of a stabilized oxycarbonium ion in the chemical mechanism.

77. Cloning of Alginate Lyase Gene, *alx*M, and Expression in *Escherichia coli*. *Bette Jo Brown*. Department of Chemistry, Michigan Technological University, Houghton, MI 49931. Lonnie O. Ingram and James. F. Preston III. Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611.

Marine bacterial isolates associated with pelagic species of *Sargassum* secrete alginate-degrading enzymes that cleave the (1→4) bond of the substrate by a β-elimination reaction. These alginate lyase enzymes have a preference for either the α-L-guluronic acid (GulA) sections or the β-D-mannuronic acid (ManA) sections of the carbohydrate polymer. A library of chromosomal DNA from a bacterium secreting a ManA-specific enzyme was prepared in pUC18. *Escherichia coli* TC4 was transformed with the plasmid containing 4–6 kb segments of the genomic DNA. Synthesis of the alginate lyase gene product was demonstrated by (1) zones of clearing on alginate–agarose plates exposed to transformed colonies and visualized by ruthenium red staining, (2) the presence of thiobarbituric acid reactive products resulting from the action of a chloroform-treated cell suspension on alginate, and (3) increase in the 232-nm absorbance from the Δ4,5 product of the transelimination reaction catalyzed by the supernatant of a spheroplast preparation of transformed *E. coli*.

78. Purification and Characterization of an Extracellular PHB Depolymerase from *Penicillium funiculosum*. *Cheryl L. Brucato*, Richard A. Gross, and Shan S. Wong. Department of Chemistry, University of Lowell, Lowell, MA 01854.

An extracellular poly(β-hydroxybutyrate) (PHB) depolymerase was isolated from *Penicillium funiculosum* (ATCC 9644) grown on PHB as the sole carbon source. Production of the enzyme paralleled the growth of the fungus and reached peak activity at the stationary growth phase. The enzyme was purified on a hydrophobic norleucine column after the extracellular culture medium was adjusted to 2 M ammonium sulfate. Pure enzyme was obtained on elution with buffer containing no salt. The polymerase did not bind to any anionic or cationic exchanger between pH 4 and 8, but bound strongly to Dowex 50W, indicating its very hydrophobic nature. It is a monomer with a molecular weight of 38 000 as shown by SDS–PAGE and gel filtration and exhibits a pH and temperature dependence with optimal activity at pH 5.0 and 30 °C. The polymerase also hydrolyzes *p*-nitrophenyl acetate and *p*-nitrophenyl butyrate. It does not, however, degrade poly(caprolactone) films. Its substrate specificity on PHB–poly(β-hydroxyvalerate) copolymer, and various modified forms of PHB, as well as its mode of degradation of these polymers will be discussed. (Supported in part by a Seed Money Grant from the University of Lowell administered by Dr. Leon Beghian, Associate Vice President for Research.)

79. Substrate Analogue Inhibitors of Glycinamide Ribonucleotide Transformylase. *Carol A. Caperelli* and B. Robert McKellar. Division of Pharmacology and Medicinal Chemistry, College of Pharmacy, University of Cincinnati Medical Center, Cincinnati, OH 45267-0004.

Glycinamide ribonucleotide transformylase (GAR TFase) catalyzes the transfer of the formyl group from 10-formyltetrahydrofolate to the terminal amino group of glycinamide ribonucleotide (GAR) to produce *N*-formyl-GAR and tetrahydrofolate in de novo purine biosynthesis. Several analogues of GAr, in which the terminal amino methylene group has been replaced by electrophilic or potentially electrophilic moieties such as CH_2Cl, CH_2Br, CH_2N_2, and CH_2CN, have been synthesized and evaluated with the purified enzyme. All of these analogues are reversible inhibitors of GAR TFase, competitive against GAR, with micromolar inhibition constants.

80. Time-Dependent Inactivation of Thiolase by Phosphine Oxide and Phosphinate Analogues of Acetoacetyl-CoA. *Jrlung Chen* and K. C. Calvo. Department of Chemistry, University of Akron, Akron, OH 44325.

Phosphine oxide and phosphinate analogues of acetoacetyl-CoA, DMPCOA and MPCOA, were synthesized and then purified on a Bio-Gel P2 column. Their inhibitive effects were studied on cytosolic acetoacetyl-CoA thiolase, an enzyme catalyzing a Claisen-type condensation between two acetyl-CoA molecules. Competitive inhibition studies showed that DMPCOA had a K_i of 73.5 μM and MPCOA had a K_i of 145 μM. Both behaved as inhibitors in a time-dependent manner. The thiolase activity was apparently irreversibly inhibited after incubation with DMPCOA for a long period. The enzyme activity could be recovered by dilution at different times right after incubation with the inhibitor.

81. Mechanistic Studies of Ketol Acid Reductoisomerase from *E. coli*. Srinivas K. Chunduru, Gregory T. Mrachko, and *K. C. Calvo*. Department of Chemistry, University of Akron, Akron, OH 44325.

The reductoisomerase enzyme catalyzes the third step in the biosynthetic pathway for the branched-chain amino acids. It catalyzes two separate reactions: an acetoin rearrangement and a reduction. The kinetic mechanism of the reaction has been investigated by using chiral substrates. The interaction of acetolactate and hydroxyketo isovalerate with Mn(II) and

Mn(II) reductoisomerase has been examined by measurements of ^{13}C longitudinal ($1/T_1$) relaxation rates. On the basis of the pH studies of the kinetic parameters, we propose an acid/base catalytic mechanism for the enzyme. We also discuss results of kinetic isotope effect, inhibition, inactivation, and alternate substrate studies.

82. Antibody-Catalyzed Bimolecular Reactions. *A. G. Cochran* and P. G. Schultz. Department of Chemistry, University of California, Berkeley, CA 94720.

In recent years, monoclonal antibodies have been shown to efficiently catalyze a variety of organic reactions, including ester hydrolysis, β-elimination, a Claisen rearrangement, and a photosensitized cyclobutane cleavage. We have recently found that antibodies specific for *N*-methylmesoporphyrin IX catalyze the chelation of zinc or copper by mesoporphyrin. In addition, antibodies elicited to a bisubstrate analogue accelerate the transamination of *p*-nitrophenylpyruvic acid by pyridoxamine. The design and characterization of these systems will be discussed.

83. Analysis of Nucleoside Metabolism during Positron Emission Tomography (PET) Imaging Studies of Brain Tumors with ^{11}C-Labeled Thymidine (TdR). *P. S. Conti*, J. Hilton, C. A. Magee, and J. H. Anderson. Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Methyl-^{11}C-labeled thymidine is currently being used clinically for brain tumor imaging with PET. Kinetic models designed to translate PET data and tissue radioactivity into DNA synthetic rates require an understanding of the short-term in vivo metabolism of TdR labeled with ^{11}C ($T_{1/2}$ = 20.4 min). HPLC procedures utilizing reverse-phase chromatography and a dilute phosphate buffer and methanol gradient mobile phase have been developed which afford separation of several radiolabeled catabolites of thymidine, including thymine, dihydrothymine (DHT), β-ureidoisobutyric acid (β-UIB), and β-aminoisobutyric acid (β-AIB), along with the mono-, di-, and triphosphorylated TdR. This system has permitted evaluation of the acid-soluble extracts from both plasma and tissue samples in canine experiments with [^{14}C]-TdR. Successful preliminary analytical studies, demonstrating rapid clearance of TdR from plasma by 10 min, with β-AIB being the major labeled plasma component by 5 min, suggest that this system is applicable to clinical studies with [^{11}C]TdR which require blood sample analyses.

84. Sequence-Selective Hydrolysis of Duplex DNA by an Oligonucleotide-Directed Nuclease. *David R. Corey* and Peter G. Schultz. Department of Chemistry, University of California, Berkeley, Berkeley, CA 94720.

Selective nucleases have been synthesied by fusing staphylococcal nuclease to an oligonucleotide via a disulfide linkage. The resulting hybrid enzymes hydrolyze RNA and DNA substrates adjacent to the site of hybridization. This methodology has been applied to the cleavage of double-stranded DNA, and hybrid nucleases have been shown to efficiently cleave supercoiled plasmids pUC19 and M13mp19. This was accomplished by introducing the oligonucleotide-directed hybrid nuclease into double-stranded DNA via D-loop formation using dilute base to partially denature the substrate. The use of topoisomerase I in conjunction with ethidium bromide introduces additional supercoiling into substrate plasmids and greatly increases the efficiency of the procedure. This method allows the stoichiometric cleavage of microgram amounts of substrate DNA and leaves 3′ and 5′ termini which can be enzymatically manipulated in subsequent reactions. Further work on this project will involve developing conditions which permit the selective hydrolysis of chromosomal DNA.

85. *Salmonella typhimurium* Quinolinate Phosphoribosyltransferase: Cloning, Overexpression, and Purification. *Andrea Dessen*,[1] Kelly Hughes,[2] and Charles Grubmeyer.[1] [1]Department of Biology, New York University, New York, NY 10003, and [2]Department of Microbiology, University of Washington, Seattle, WA 98195.

Quinolinate phosphoribosyltransferase (EC 2.4.2.19) catalyzes the formation of nicotinic acid mononucleotide from quinolinic acid and α-D-5-phosphoribosyl 1-pyrophosphate (PRPP) accompanied by release of CO_2 and pyrophosphate, a step in the de novo biosynthesis of NAD. The structure and mechanism of this enzyme are poorly understood. QAPRTase has been detected in human brain, and increased intracerebral levels of quinolinic acid have been linked to the neuropathogenesis of Alzheimer's disease, epilepsy, and AIDS dementia. We have cloned a 5-kb *AvaI–PstI* fragment containing the *Salmonella typhimurium nadC* gene in a T7 promoter plasmid and overexpressed the gene in a BL21(DE3) *E. coli* host. The enzyme has been purified to homogeneity by ion-exchange and affinity chromatography. SDS–PAGE indicates a subunit molecular weight of approximately 35 000, and size exclusion chromatography suggests a dimeric aggregation state. Enzymatic activity has an absolute requirement for Mg(II), with a pH optimum in the range 6.5–7.5. Amino acid and DNA sequence data, as well as chemical characterization, will be presented.

86. Ketonization of a Dienol by Steroid Isomerase. T. C. M. Eames, *D. C. Hawkinson*, and R. M. Pollack. Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742.

No abstract available.

87. The Mechanism of Klenow Fragment Misincorporation. *Bryan T. Eger*, Particia A. Benkovic, and Stephen J. Benkovic. The Department of Chemistry, The Pennsylvania State University, University Park, PA 16802. Robert D. Kuchta, The Department of Chemistry, The University of Colorado, Boulder, CO 80309.

Previous work from this laboratory has elucidated the minimal kinetic mechanism for nucleotide misincorporation by the Klenow fragment into a 9-mer primer/20-mer template DNA duplex (9/20-mer). Recent nucleotide discrimination data have shown that dATP binds to the Klenow–9/20-mer complex in two steps. These data along with the measurement of the rate of pyrophosphate release from the ternary species have required the expansion of the minimal mechanism. We have also addressed the question of how the length of the relatively short 9/20-mer duplex affects features of this mechanism. A duplex using a 30-mer primer/M13-mer template (30/M13-mer) with high homology to the 9/20-mer was chosen because it more closely models the longer duplexes found in vivo. Measurements of the nucleotide turnover, elemental effect, and pre-steady-state burst did not show significant length effects between the 9/20-mer and 30/M13-mer.

88. Effect of Ring Size on the Acidity of Cyclic Benzyl Ketones. *S. Eldin*, A. Ross, D. Whalen, and R. M. Pollack. Department of Chemistry and Biochemistry, University of

Maryland, College Park, MD 20742.

No abstract available.

89. Effects of Modifiers on the Esterase and Peptidase Activities of Carboxypeptidase A. *J. L. Frye* and J. F. Sebastian. Department of Chemistry, Miami University, Oxford, OH 45056.

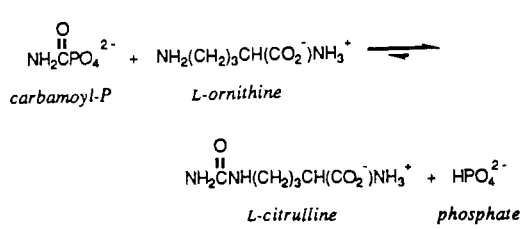
Carbobenzyloxyglycyl-L-5-[α-(aminophenyl)ethyl]-1(2)*H*-tetrazole (CbzGPT) is an activator of the carboxypeptidase A (CPA) catalyzed hydrolysis of carbobenzyloxyglycyl-L-phenylalanine [P. Hermann et al. (1987) *FEBS Lett. 212*, 83–86]. We have now found that CbzGPT is an uncompetitive inhibitor of ester hydrolysis. Thus, the trend for this enzyme that activators of peptide hydrolysis are uncompetitive inhibitors of ester hydrolysis encompasses three different types of anionic termini (carboxylate, sulfonate, and tetrazole anion). These results and the effects of alkylsulfonates and of adamantyl and silaalkyl derivatives on the esterase and peptidase activities of CPA will be discussed in detail.

90. Interaction of Dianionic Activators and Inhibitors with Bovine Carbonic Anhydrase III. *Nicholas J. Gargiulo*, Frank. A. Santoli, and Roger S. Rowlett. Department of Chemistry, Colgate University, Hamilton, NY 13346.

Data have been collected on the inhibition and activation of bovine carbonic anhydrase III (BCA) by various dianionic species such as SO_4^{2-}, SO_3^{2-}, HPO_4^{2-}, malonate, and oxalate. Both kinetic and NMR methods have been employed to define the binding nature of these species to BCA. The evidence is consistent with an anion binding site on the enzyme, possibly the positively charged Arg-67 residue located 9 Å away from the active-site Zn^{2+} ion. Sulfate and oxalate are partial inhibitors of BCA at pH 7.0, whereas SO_3^{2-}, HPO_4^{2-}, and malonate are activators of the enzyme under the same conditions. Both HPO_4^{2-} and SO_4^{2-} compete for a common binding site, which is not the binding site for N_3^- and other Zn^{2+} ion directed inhibitors of CA. We have noticed that the pK_b of a dianionic species influences whether it will inhibit or activate the enzyme and tentatively propose that activating dianionic species are acting as proton shuttle groups during catalytic turnover in the reaction $CO_2 + H_2O \rightleftarrows HCO_3^- + H^+$, speeding a partially rate determining proton-transfer step in the mechanism.

91. Role of Arginine-57 in Binding and Catalysis of Ornithine Transcarbamoylase. *J. O. Goldsmith* and Lawrence C. Kuo. Department of Chemistry, Boston University, Boston, MA 02215.

Ornithine transcarbamoylase, the first enzyme of the urea cycle, catalyzes the nucleophilic S_N2 attack of carbamoyl phosphate by L-ornithine forming L-citrulline and inorganic phosphate as products.



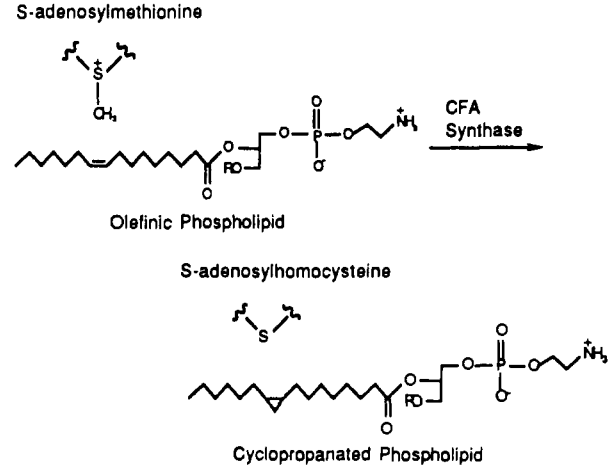
Arginine-57, located at the carbamoyl phosphate binding domain of the enzyme, has been implicated as a critical residue for efficient transcarbamoylation. To study the importance of Arg-57 in binding and catalysis, this residue has been excised and replaced by one of several amino acids varying in size and charge. Three site-directed mutant enzymes have been employed, replacing arginine, at amino acid residue 57, with glycine (loss of charge and bulk), histidine (loss of bulk), or glutamine (loss of charge). By comparing the kinetic and spectroscopic characteristics of the wild-type and mutant enzymes, we have been able to probe the mechanistic requirements of the enzyme. The replacement of arginine by glycine results in a decrease of 6 kcal/mol in entropy and a complete loss in the enzyme's ability to undergo a substrate-induced isomerization observed by UV difference spectroscopy. The replacement of arginine with histidine reveals that the protonation state of residue 57 dictates the reaction rate. This protonation state also governs the kinetic mechanism of the enzyme: with a protonated residue, the enzyme accepts the substrates in an ordered fashion with carbamoyl phosphate as lead substrate, but, with an unprotonated residue, the enzyme can bind either substrate first. The glutamine mutant is presently being studied to support the above conclusions. (Supported by NSF REU Grant CHE-8712942 and NIH Grant DK38089.)

92. Solvent Isotope Effects in Oxidative Decarboxylation of Pyruvate Catalyzed by Pyruvate Decarboxylase. *Gholam Hajipour* and Richard L. Schowen. Department of Chemistry, University of Kansas, Lawrence, KS 66045-0046.

The zwitterionic or enamine intermediate formed in the decarboxylation of pyruvate by the thiamin diphosphate dependent enzyme pyruvate decarboxylase (EC 4.1.1.1) can be oxidized by agents such as dichlorophenolindophenol (DCIP) with the diversion of product from acetaldehyde to acetate and formation of reduced DCIP. The decarboxylation reaction is activated by the slow binding of pyruvate at a regulatory site, followed by decarboxylation of pyruvate at the catalytic site; the oxidation reaction shows simple saturation kinetics in DCIP. In deuterium oxide solvent, the rate of oxidation increases [$k_{cat}[H_2O]/k_{cat}[D_2O] = 0.79$ at pH 6.2, 0.31 at pH 7.0; $(k_{cat}/K_m[H_2O])/(k_{cat}/K_m[D_2O]) = 0.63$ at both pHs], reflecting the decreased flux through the proton-transfer pathway to acetaldehyde.

93. In Vitro Studies of the Enzymatic Cyclopropanation of Fatty Acids in *E. coli*. *Sarah Iacobucci* and Marc d'Alarcao. Department of Chemistry, Tufts University, Medford, MA 02155.

The chemical mechanism of fatty acid cyclopropanation in *E. coli* is under investigation. In vitro studies with purified cyclopropane fatty acid synthase and several substrates will be presented. The implications of isotope exchange experiments will be discussed.



94. Antibodies as Redox Catalysts. *N. Janjic* and A. Tramontano. Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Antibodies may act as redox catalysts by binding diverse redox active dye molecules in proximity to a second substrate at the antigen binding site. Fluorescein and its derivatives define an appropriate scaffold for eliciting antibody sites capable of binding ligands representing fragments of the hapten. A tricyclic fragment resulting from bond disconnection at C9 of fluorescein is a model for various dye molecules. Xanthene and phenoxazine derivatives occupy an immunodominant subsite of the anti-fluorescyl antibody combining site. Both dye types are effective in a number of redox processes including oxidation of sulfite to sulfate and of thiols to disulfides. The reduced dyes are typically rapidly reoxidized in air and can therefore act as recyclable oxidative cofactors. The mechanisms of these reactions will be discussed, and the influence of specific monoclonal antibodies will be reported.

95. Search for Enzyme Activity in Prebiotic Synthesis. *Heng Jiang*, Sree Kumar, Yasuhiro Honda, and Cyril Ponnamperuma. Laboratory of Chemical Evolution, Department of Chemistry, University of Maryland, College Park, MD 20742.

In our study of chemical evolution, we have shown that a variety of nitrogenous polymers are produced by electric discharges on simulated primitive atmosphere. We have searched for possible enzymatic activity in these polymers. Polymers and combinations of Cu and Zn with these polymers were assayed for superoxide dismutase (SOD) activity by using cytochrome *c* reduction by hypoxanthine and xanthine oxidase. Combination of Cu, Zn, or both with polymers has no SOD activity. The reduction rates of cytochrome *c* are enhanced about 2–4-fold by the polymers or their combinations with Cu or Zn and inhibited by SOD (33.9–57.0%). On the other hand, the polymers can reduce cytochrome *c* directly only in the presence of cytochrome *c*. The reduction appears to be mediated by the polymers or their combination with metals. These results may be significant for our understanding of the origin of a prebiotic enzyme.

96. Engineering of Cathepsin B Selectivity into Papain. *Henry E. Khouri*, Daniel C. Tessier, Thierry Vernet, David Y. Thomas, and Andrew C. Storer. National Research Council of Canada, Biotechnology Research Institute, 6100 Royalmount Avenue, Montreal, Quebec, Canada H4P 2R2.

A synthetic gene coding for papain has been expressed in a baculovirus-insect cell system (Vernet et al., *J. Biol. Chem.*, submitted). Through site-directed mutagenesis several papain mutants have been produced and expressed in this system with the aim of investigating papain's mechanism (Mênard et al., *Biochemistry*, submitted). On the basis of amino acid alignment between papain and cathepsin B and by use of site-directed mutagenesis three simultaneous mutations into papain S_2 subsite were introduced (V133A, V157G, and S205E). Kinetic characterization of the papain triple mutant thus produced indicated that its S_2 subsite specificity has been modified to resemble that of cathepsin B.

97. Confirmation That *S*-Adenosylmethionine Is a Hydrogen Carrier in the Lysine Aminomutase Reaction. Role of SAM in the Reaction Mechanism. *James L. Kilgore* and D. John Aberhart. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Tritium transfer from the 5′ position of SAM to α- and β-lysine during enzyme-catalyzed equilibration of the isomeric amino acids was recently reported [Barniak et al. (1989) *J. Biol. Chem. 264*, 1357; Moss & Frey (1987) *J. Biol. Chem. 262*, 14859]. Previously, we could detect no deuterium transfer from substrate (α-lysine-3-d_2) to SAM [Aberhart (1988) *J. Chem. Soc., Perkin Trans. 1*, 343]. Transfer would only be detectable if excess SAM in solution equilibrates with the enzyme-bound cofactor [Zappia & Ayala (1972) *Biochim. Biophys. Acta 268*, 573]. An improved synthesis of (2-*RS*)-[3-^{3}H]lysine was developed, and this labeled substrate was used to reexamine cofactor lability in the functioning enzyme and the role of SAM as a hydrogen carrier. When we repeated the transfer experiment with high specific activity lysine at low ratios of SAM:enzyme, effficient transfer of label was observed. Attempts to detect the hypothetical intermediate 5′-deoxyadenosine will also be described.

98. A Potential Surface for Hydride Transfer between NAD^+ Analogues. *Y. Kim* and M. M. Kreevoy. Department of Chemistry, University of Minnesota, Minneapolis, MN 55455.

We have developed a triatomic collinear potential energy surface for hydrogen transfer between two 15-Da masses. This surface has been used to calculate reaction rates by variational transition state theory with a least action, large curvature tunneling approximation. These rates have been made to approximate a series of hydride-transfer reactions between NAD^+ analogues in solution. The experimental Marcus parameters, λ, W^r, τ, are approximately reproduced: $W^r \approx 0$, $\tau \approx 0.87$, $\lambda \approx 80$ kcal mol^{-1}. The calculated kinetic isotope effect is 5.7 for a typical symmetric hydrogen-transfer reaction. The calculated KIE decreases with increasing bond strength in symmetrical reactions: $\tau_D - \tau_H \approx 0.025$. All of this is in good agreement with experiment. Both zero-point energy effects and tunneling contribute significantly to the KIE.

99. Fluorescence and NMR Properties of the Sole Tyrosine (Tyr-14) in a Double Mutant of Δ^5-3-Ketosteroid Isomerase (KSI). *A. Kuliopulos*, P. Talalay, and A. S. Mildvan. Departments of Biological Chemistry and Pharmacology, Johns Hopkins School of Medicine, Baltimore, MD 21205.

The $10^{9.5}$-fold rate acceleration of steroid isomerization produced by KSI may be ascribed quantitatively to concerted general acid-base catalysis of substrate enolization and reketonization by both Tyr-14 and Asp-38 in a hydrophobic site [(1989) *Biochemistry 28*, 149; Xue et al., Abstract 128]. The double mutant Y55F + Y88F, which decreases k_{cat} and K_M by only 4-fold, retains as the only aromatic residues Tyr-14, 3 histidines, and 10 phenylalanines, consistent with UV absorption spectra and fluorescence activation and emission spectra. Increasing the pH halves the tyrosine fluorescence (λ_{ex} = 278 nm; λ_{em} = 307 nm), yielding an apparent pK_A for Tyr-14 of 9.7 ± 0.1. The substrate analogue 19-nortestosterone (NT) quenches the fluorescence of Tyr-14 by 98%, yielding a K_D of 0.9 μM. 1D and 2D (HOHAHA) NMR of the Y55F + Y88F mutant at 600 MHz, deuterating the Phe rings, permitted assignment of the coupled ring proton resonances of Tyr-14 at 6.66 (3,5H) and 6.83 ppm (2,6H). Deprotonation of Tyr-14 did not alter these shifts, but binding of NT induced downfield shifts of 0.12 and 0.06 ppm of 3,5H and 2,6H, respectively, possibly due to the deshielding effect

of the 3-keto group of the steroid. However, NT binding also produced +0.02 to −0.40 ppm changes in the chemical shifts of eight Phe residues, indicating steroid-induced conformation changes in the enzyme.

100. Active-Site Labeling of Dopamine β-Hydroxylase with Mechanism-Based Inhibitors. *Alok Kumar* and Joseph J. Villafranca. Department of Chemistry, The Pennsylvania State University, University Park, PA 16802. G. King Farrington. Repligen Corporation, One Kendall Square, Building 700, Cambridge, MA 02139.

Dopamine β-hydroxylase (DβH, EC 1.14.17.1), a copper-containing monooxygenase, catalyzes the hydroxylation of dopamine to form norepinephrine. The enzyme exhibits broad substrate specificity allowing for the design of numerous mechanism-based inhibitors. Recently, the active site of DβH has been labeled with four different radiolabeled mechanism-based inhibitors [[^{3}H]-6-hydroxybenzofuran, [^{3}H]-5-hydroxybenzofuran, 1-bromo-[1,1-^{3}H]-2-(4-hydroxyphenyl)-ethane, and [^{14}C]phenylhydrazine]. The radiolabeled peptides have been sequenced, labeled positions determined, and labeled amino acids identified by comparison of the peptide with either the sequence of the unlabeled peptide or the bovine cDNA sequence. In each case, an unlabeled modified peptide has been detected, suggesting an alternate inactivation route other than the covalent modification by the mechanism-based inhibitor. Here, we present the radiolabel syntheses, labeling of the enzyme, isolation, and finally characterization of the labeled peptides by peptide sequencing and FAB mass spectroscopy. (Supported in part by NIH Grant GM-29139.)

101. Isotope Effects on Binding of NADH to Lactate Dehydrogenase. *R. D. LaReau* and V. E. Anderson. Department of Chemistry, Brown University, Providence, RI 02912.

The isotope effects on the binding of both *pro-R* [4-^{2}H]-NADH and *pro-S* [4-^{2}H]NADH to lactate dehydrogenase have been shown to be 0.95 and 1.08, respectively, by using the method of remote-label ^{3}H/^{14}C ratio scintillation counting. These values demonstrate that relative to the coenzyme free in solution the *pro-R* C_4–H bond of NADH is more stiff and the *pro-S* C_4–H bond is less stiff when bound in the binary complex. Also, the relative redox potentials of NMN^+ and the dephosphorylated NR^+ were measured by forming the cyano adducts in an equilibrium solution to demonstrate that the presence of the 5′-phosphate moiety increases the reduction potential at the C_4 position of the mononucleotide. These results are used to support a model for dehydrogenase action in general which includes an intrinsic discrimination between the reduction potentials of the *pro-R* and *pro-S* hydrogens of NADH. (This work was supported by NIH Grant GM36562.)

102. Characterization of the Sulfhydryl Groups in Heparinase. *Deborah Leckband* and Robert S. Langer. Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139.

Heparinase, a 43 000 M_r enzyme, cleaves heparin sulfate in an endolytic fashion. Heparinase has been used in the study of anticoagulation, heparin structure, and development of an immobilized enzyme blood filter for the extracorporeal removal of heparin. Heparinase-like enzymes have been implicated in the release of mitogens from cell surfaces and the stimulation of neovascularization in tumor growth. Though this enzyme is of interest in several areas of biology, little structural information exists. The amino acid sequence is known. The enzyme contains 37 lysine residues, similar to other heparin binding proteins, which exhibit a high percentage of lysines. The enzyme also contains four cysteine residues. The location and function of the cysteine residues in heparinase are unknown. This study elucidates the location of the sulfhydryls and their functional significance in the molecule. The structural properties of the four cysteine residues were determined by sulfhydryl modification and radiolabeling. One cysteine residue is present in a region of the enzyme inaccessible to nonpolar molecules and not a heparin binding region. By use of metal affinity chromatography, this environmentally obstructed cysteine residue was found to be inaccessible to immobilized reactive groups. The three remaining cysteines were shown to be buried in the enzyme molecule. Temperature-dependent inactivation and partial denaturation of the enzyme performed in the presence and absence of reducing agents facilitated the determination of the structural significance of these residues.

103. Sequence-Specific Degradation of a DNA–RNA Hybrid by Bleomycin. *Jae S. Lee* and John W. Kozarich. Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742. JoAnne Stubbe. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

Bleomycin, an antitumor–antibiotic agent, has been demonstrated to cleave double-stranded DNA by the abstraction of the 4′ hydrogen on the deoxyribose of the DNA. Bleomycin has also been reported to specifically degrade the DNA strand of copolymer poly(rA)–poly(dT) hybrid [Haidle & Bearden (1975) *Biochem. Biophys. Res. Commun. 65*, 815–821; Krishnamoorthy, Vanderwall, Kozarich, & Stubbe (1988) *J. Am. Chem. Soc. 110*, 2008–2009]. We have prepared a DNA–RNA hybrid corresponding to the *Hin*dIII–*Bam*HI fragment of pBR322. The RNA strand of the hybrid is generated by RNA polymerase using the double-stranded DNA as the template, and the corresponding DNA strand of the hybrid is produced by the action of reverse transcriptase. Preliminary studies have shown sequence-specific degradation of the DNA strand of the DNA–RNA hybrid. Comparison of the degradation of the hybrid to the degradation of the DNA duplex of the same sequence will be discussed.

104. Turnover and Inactivation of Horseradish Peroxidase and Chloroperoxidase with Ortho-Substituted Phenols. Xiaoyan Ma and *Steven Rokita*. Department of Chemistry, State University of New York, Stony Brook, NY 11794.

Many enzymes classified as peroxidases have been shown to catalyze a broad spectrum of reactions. Substrate conversion may arise from alternative interaction with free or enzyme-bound intermediates and may proceed through one- or two-electron chemistry. The relative propensity of each potential pathway is now under investigation using bifunctional substrates that may partition into a number of diagnostic products. To date, the reactivity of both *o*-cyclopropyl- and *o*-ethynylphenol was found to mimic the behavior of the parent substrate, phenol, in the presence of the title enzymes. Compounds formed by radical coupling mechanisms dominated the product profile, and no modification of the ortho substituents was detected. Enzyme inactivation was also noted during the turnover process, but, again, the substituents did not appear to participate directly in this event.

105. Reactivity and Inhibition of Acylase I. *M. S. Matta* and Shenshen Dou. Department of Chemistry, Southern Illinois University, Edwardsville, IL 62026.

Aminoacylase I (EC 3.5.1.14) is a potential prototype enzyme for the design of potent inhibitors of zinc-containing hydrolase enzymes, including proteases. We have synthesized some chromogenic substrates and measured the inhibition of a number of reversible inhibitors of acylase I. All work was conducted at pH 7.6, 0.1 M Hepes, 25 °C. Furylacryloyl (Fa) L-amino acids FaMet, FaNor (DL), FaLeu, and FaPhe are substrates of the enzyme; k_c/K_m for FaMet = 362 000 M^{-1} s^{-1}. Respective relative k_c/K_ms for the substrates listed above are in the ratio 100:38:0.37:0.014. The *O*-furylacryloyl ester of racemic 2-hydroxycaproic acid is not a substrate. Inhibition studies were conducted against FaMet. A number of *N*-tosyl and *N*-mesyl amino acids gave competitive and mixed inhibition constants in the millimolar range. The slow substrate FaPhe was the best rapidly reversible inhibitor of FaMet hydrolysis, with K_i = 27 μM. Butylmalonic acid is a slow-binding inhibitor with complex kinetics. Potential transition-state analogue inhibitors such as butylboronic and phenylphosphonic acids exhibit no special affinity for the acylase enzyme.

106. Precursor to Mature Protein Conversion. Changes in Properties of Mitochondrial Aspartate Aminotransferase. J. R. Mattingly, Jr., F. Altieri, A. Iriarte, T. Wu, and *M. Martinez-Carrion.* School of Basic Life Sciences, University of Missouri—Kansas City, Kansas City, MO 64110.

The intact precursor protein of rat liver mitochondrial aspartate aminotransferase (pmAAT) has been isolated as a stable, dimeric protein which can be converted into the mature form (mAAT) upon import into intact, activated mitochondria [Altieri et al. (1989) *J. Biol. Chem. 264*, 4782]. This process can be mimicked in vitro by the action of trypsin. Under these conditions, trypsin hydrolysis is sequential, with cleavage first occurring after Arg(-22) then after Arg(-2). The specific enzymic activity of the resulting protein is increased 1.3-fold relative to that of undigested pmAAT. Trypsin-digested pmAAT is more resistant to thermal denaturation and the effects of guanidine hydrochloride than pmAAT. However, when the active-site-bound pyridoxal 5′-phosphate is removed from both forms of the protein, pmAAT shows higher temperature stability. Both apoproteins are stable in the cold and can be restored to the holoenzymes by addition of pyridoxal 5′-phosphate. The 29 amino acid signal peptide presequence is not a structurally inert appendage to the amino-terminal region of the mAAT. (Supported by HL38412.)

107. Mechanistic Studies of the β-Elimination Reactions Catalyzed by UV Endonuclease V from Bacteriophage T_4 and Endonuclease III from *E. coli*. *Abhijit Mazumder*,[1] John A. Gerlt,[1] Michael J. Absalon,[2] JoAnne Stubbe,[2] and Richard P. Cunningham.[3] [1]Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, [2]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, and [3]Department of Biological Sciences, State University of New York, Albany, NY 12222.

Using substrates that are stereospecifically labeled with ^3H in the 2-position of the abasic site, we have determined that the stereochemical consequences of the β-elimination reactions catalyzed by both UV endonuclease V and endonuclease III are syn, the result of abstraction of the 2-*pro-S* proton and formation of the trans geometric isomer of the α,β-unsaturated aldehyde reaction product. The same stereochemical course is observed when either single- or double-stranded substrates containing aldehydic abasic sites are used. The stereochemical courses of these β-elimination reactions require that the reaction proceed from either the free aldehyde or an activated derivative and not from the mixture of cyclic hemiacetals which predominates. Further mechanistic investigations of the reactions catalyzed by these enzymes are in progress.

108. Cytochrome $P\text{-}450_{scc}$ Mediated Oxidation of (20*S*)-22-Nor-22-thiacholesterol: Characterization of Mechanism-Based Inhibition. *Emily Miao*,[1] Chun Zuo,[1] Atsushi Nagahisa,[1] Benjamin J. Taylor,[1] Subhendu Joardar,[1] Chang Byon,[1] Stephen R. Wilson,[2] and William H. Orme-Johnson.[1] [1]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, and [2]Department of Chemistry, New York University, New York, NY 10003.

(20*S*)-22-Nor-22-thiacholesterol is found to be a potent competitive inhibitor of pregnenolone biosynthesis from cholesterol by purified, reconstituted cytochrome $P\text{-}450_{scc}$ from bovine adrenal mitochondria. The thermodynamic dissociation constant (K_d), determined by difference spectra, is 0.67 μM, while kinetic studies indicate an inhibition constant (K_i) of 0.8 μM. Further kinetic studies indicate that this compound is converted to a tighter binding inhibitor, (20*S*,22*R*)-22-nor-22-thiacholesteryl oxide, with high stereoselectivity. The sulfoxide diastereoisomer (20*S*,22*R*)-22-nor-22-thiacholesteryl oxide binds 10 times more tightly than (20*S*,22*S*)-22-nor-22-thiacholesteryl oxide with K_d values of 0.10 and 1.14 μM, respectively. Optical absorbance and EPR spectroscopy of enzyme–inhibitor complexes further indicate that the sulfoxide coordinates with the heme iron through the oxygen atom. [This work was supported by NIH Grants GM028358 (W. H.O.-J.) and GM31482 (S.R.W.).]

109. Electrostatic Effects on Carboxylate–Imidazolium Hydrogen Bonding in Models of the Asp–His Couple in Serine Proteases. *Nabeel A. R. Nabulsi*, Frank R. Fronczek, and Richard D. Gandour. Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803-1804.

Hydrogen bonding plays an important role in biological systems and has been studied extensively by crystallographic techniques. The electrostatic stabilization of the buried carboxylate in trypsin by three specially oriented dipoles was shown theoretically to be larger than the corresponding stabilization of an ionized carboxylate in aqueous solution, and Asp-102 will stay ionized at all relevant stages of catalysis. We have explored the microenvironment in crystalline models of the Asp–His couple and found that multiple hydrogen bonding to the carboxylate has a pronounced effect on the strength and stereoelectronics of the carboxylate in hydrogen bonding with imidazole.

110. Inactivation of Mandelonitrile Lyase by Phenylglyoxal. J. D. Nicolette and *E. J. Brush.* Department of Chemistry, Tufts University, Medford, MA 02155.

Mandelonitrile lyase (D-oxynitrilase; EC 4.1.2.10; MNL) catalyzes the stereospecific addition of cyanide to benzaldehyde forming (*R*)-mandelonitrile. Found exclusively in cyanogenic plants, MNL is responsible for catalyzing the central reaction in the release of cyanide from natural precursors, although the role of this reaction in plant metabolism is not clear. Mandelonitrile lyase requires FAD for activity, although the redox properties of FAD apparently play no role in the en-

zymatic reaction. To better understand the purpose of this curious reaction in plant metabolism and the role of the reversibly bound FAD, we have initiated an investigation on the chemical reaction mechanism of MNL. We have found that phenylglyoxal (PG) is a potent inactivator of the enzyme-catalyzed elimination of cyanide from D,L-mandelonitrile. At pH 5.5 the half-life for inactivation by 0.5 mM PG is 2 min. Protection from inactivation is afforded by benzoate (inhibitor, K_i = 8 μM) and D,L-mandelonitrile (K_m = 0.5 mM), suggesting that the inactivation reaction is directed at the active site. The related compounds methylglyoxal and butane-2,3-dione have no effect on MNL activity. Inactivation by PG also leads to an increase in the absorption spectrum of the enzyme at 350 nm. The characterization of this new chromophore, the inactivation mechanism, and the relationship of the inactivation reaction to the mechanism in the normal substrate reaction will be discussed. (Supported in part by the donors of the Petroleum Research Fund, administered by the American Chemical Society.)

111. Site-Directed Mutagenesis at Lysine 47 in *E. coli* Glutamine Synthetase Abolishes Inhibition by 5′-[*p*-(Fluorosulfonyl)benzoyl]adenosine. *Cushrow R. Parakh* and Joseph J. Villafranca. Department of Chemistry, The Pennsylvania State University, University Park, PA 16802.

Glutamine synthetase (GS) from *E. coli* is an important enzyme involved in the regulation of bacterial nitrogen metabolism. The enzyme catalyzes the ATP-dependent condensation of ammonia with glutamate to form glutamine and is inactivated by the ATP analogue 5′-[*p*-(fluorosulfonyl)-benzoyl]adenosine (5′-FSBA). Lysine 47 has been identified as the single amino acid covalently modified by this inhibitor per enzyme active site [Pinkofsky et al. (1984) *J. Biol. Chem. 259*, 9616–9622]. A related GS, from the cyanobacterium *Anabena* 7120, with valine as the amino acid analogous to lysine 47 in *E. coli* GS, is unaffected by 5′-FSBA. Site-directed mutagenesis has been carried out on *glnA*, the structural gene for *E. coli* GS. The procedure developed by Kunkel was used to change lysine 47 to glutamine and valine. The resulting GS mutants are not inactivated by 5′-FSBA, though the analogue is a competitive inhibitor with respect to ATP for both enzymes. A comparison of the kinetic characteristics of GS from *Anabena* 7120, *E. coli* wild-type, and mutants will be presented. (Supported in part by NIH Grant GM-23529.)

112. Use of Dithio Analogues of Coenzyme A in Assaying for Thiolase and HMG-CoA Synthase. *L. V. Penn* and V. E. Anderson. Department of Chemistry, Brown University, Providence, RI 02912.

Acetyldithio-CoA serves as a nucleophile in the condensation reaction of mitochondrial thiolase. The product of the reaction is the ene(thio)late of the β-ketodithioacyl-CoA. β-Ketodithioacyl-CoA has an extinction coefficient of 21 600 M^{-1} cm^{-1} at 358 nm that is independent of chain length. The pK_a of the product is 6.5, and the equilibrium constant for the reaction is 16-fold larger than that for the formation of the 3-oxoacyl-CoA product. These properties alleviate several difficulties present in the current assay, and the ready availability of the substrates provides a facile method for assaying for chain length specific thiolases. 3-Ketodithiobutyryl-CoA is a substrate for the β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase reaction. An apparent K_m of 14.4 ± 2 μM has been obtained with 200 μM acetyl-CoA. Using 3-ketodithiobutyryl-CoA as an alternate substrate provides a more sensitive assay for HMG-CoA synthase activity. (This work was supported in part by NIH Grant GM36562.)

113. Probes of Protein Kinase Specificity. Mary Prorok, Marianne Mendelow, Allen Salerno, and *David S. Lawrence*. Department of Chemistry, State University of New York at Buffalo, Buffalo, NY 14214.

Protein kinases have been implicated in the molecular events that constitute carcinogenesis. In addition, kinase-mediated protein phosphorylation is recognized to be an essential component in a myriad of biological phenomena in normal cells. In an effort to better understand the fashion by which protein kinases recognize substrates, we have sought to elucidate the noncovalent active-site interactions that control protein kinase specificity. Our initial efforts have focused on the cAMP-dependent protein kinase, and we have found, through the use of peptide-based molecular probes, that steric factors, hydrogen bonding, and electrostatic interactions are the forces responsible for the molecular recognition of both substrates and inhibitors.

114. Charge-Relay Catalysis of Acetylcholinesterase and Cholesterol Esterase Reactions. *D. M. Quinn*, L. D. Sutton, A. N. Pryor, and B. H. Lee. Chemistry Department, University of Iowa, Iowa City, IA 52242.

There is extensive sequence homology around residues S194, H435, and D79 (CEase numbering) among the cholinesterases and cholesterol esterase (CEase), which suggests not only that the residues are involved in catalysis but also that they may comprise a charge-relay system such as that of the serine proteases. The proton inventory technique has been used to probe putative charge-relay systems in acetylcholinesterase (AChE) and CEase catalyses. Reactions of nonphysiological substrates, such as phenyl esters, invariably have single-proton-transfer transition states. However, when biomimetic substrates are used (i.e., choline esters for AChE or phospholipids for CEase), the proton inventories are consistent with multiproton-transfer stabilization of transition states. Therefore, the catalytic function of cholinesterases and CEase appears to have converged on the same theme for prototropic transition-state stabilization as that of the serine proteases.

115. Inactivation of *E. coli* CTP Synthetase by Thiourea Dioxide. *James G. Robertson*, Louis J. Sparvero, and Joseph J. Villafranca. Department of Chemistry, The Pennsylvania State University, University Park, PA 16802.

Cytidine triphosphate synthetase catalyzes the formation of CTP from ATP, UTP, and glutamine. The enzyme from *E. coli* demonstrates substrate-induced positive cooperativity, and GTP acts as a cooperative allosteric activator. Substrates and GTP promote cooperative behavior by shifting a dimer–tetramer equilibrium toward the more active tetrameric form of the enzyme. Thiourea dioxide (TUD) was used to attempt chemical modification of potentially catalytically important lysine and histidine residues and was found to cause time-dependent inactivation in the range 2–20 mM. Enzyme was >97% inactive after 19 min in the presence of 20 mM TUD at pH 8.0. The replot of K_{inact} versus [TUD] was linear, demonstrating that no reversible complex forms prior to inactivation. From the replot, the second-order rate constant for inactivation was calculated to be 10.8 M^{-1} min^{-1}. A plot of log K_{inact} versus pH showed that inactivation involves a functional group with a pK_a of 7.3, which suggests an imidazole or α-NH_2 group. Protection experiments demonstrated that ATP prevents inactivation, whereas UTP and GTP appear to

enhance inactivation. In combination, however, equimolar concentrations of UTP and GTP prevented inactivation. Plots of $1/K_{inact}$ versus nucleotide concentration displayed cooperative behavior. Therefore, the data suggest that TUD may modify a site or sites on the enzyme that are involved in cooperative interactions induced by nucleotide binding.

116. Kinetic Evidence for Enzyme Channeling. *J. Rudolph*, Y. S. Cheng, and J. Stubbe. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

The first two enzymes in the de novo purine biosynthetic pathway are phosphoribosyl pyrophosphate amidotransferase (PRPP-AT) and glycinamide ribonucleotide synthetase (GAR-syn). They share the chemically unstable substrate/product phosphoribosylamine (PRA), which has a rate of decomposition of 1.16/min at pH 7.5, 37 °C [Schendel et al. (1988) *Biochemistry 27*, 2614–2623]. The instability of this common intermediate brings to question the efficiency of synthesis through these two enzymes and leads one to propose enzyme channeling. Preliminary in vitro kinetic studies of the coupled enzyme system have indicated a distinct absence of lab time, in contrast to models describing a noninteractive system. By Western blotting, the in vivo concentrations of PRPP-AT and GAR-syn were shown to be 1.8 ± 0.3 and 0.8 ± 0.3 μM, respectively, in cells grown on complex media, and 20.3 ± 4.1 and 3.2 ± 1.2 μM, respectively, in cells grown on minimal media. Current channeling studies have focused on the kinetics of the coupled assay under in vivo concentrations of enzyme on the rapid-quench time scale.

117. Photoaffinity Labeling of the Phenylalanine Hydroxylase Active Site. *B. J. Schuster* and S. J. Benkovic. Department of Chemistry, The Pennsylvania State University, University Park, PA 16802.

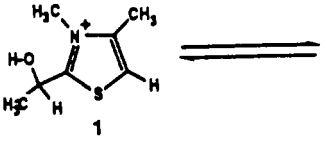
We have synthesized and characterized the photoaffinity label 5-[(3-azido-6-nitrobenzylidene)amino]-2,6-diamino-4-pyrimidinone, a pterin analogue, as a probe of the active site of rat liver phenylalanine hydroxylase. The label has been found to be a competitive inhibitor of the enzyme with respect to 6,7-dimethyltetrahydropterin, having a K_i of 8.8 ± 1.1 μM. The irreversible inactivation of rat liver phenylalanine hydroxylase by the photoaffinity label is both concentration and time dependent. Phenylalanine hydroxylase is covalently labeled with a stoichiometry of 0.8 label per enzyme subunit. 6-Methyltetrahydropterin and 5-deaza-6-methyltetrahydropterin protect against covalent labeling and enzyme inactivation, and this indicates that labeling occurs at the pterin binding site. The nature and specific location of covalent modification will be discussed.

118. Use of Solvent Isotope Effects on the Kinetics of Bacterial Lactate Dehydrogenase. *J. Seravalli* and R. L. Schowen. Department of Chemistry, University of Kansas, Lawrence, KS 66045-0046.

The lactate dehydrogenase from *Bacillus stearothermophilus* catalyzes the reduction of pyruvate by NADH with a temperature optimum of 55 °C. Previous studies of secondary isotope effects (Huskey, unpublished results, 1985) and site-directed mutants [Clarke et al. (1986) *Nature 324*, 699] have implicated involvement of active-site residue(s) in electrophilic catalysis of the hydride transfer to the carbonyl moiety of pyruvate. The solvent isotope effect ($k_{cat(HOH)}/k_{cat(DOD)}$) at pH 6.00 and 55 °C in the absence of the effector fructose 1,6-bisphosphate (FBP) is 2.9 ± 0.2, in agreement with the proposal of electrophilic catalysis of pyruvate reduction. Primary isotope effects and proton inventories give further information on the interplay between proton transfer and hydride transfer in the mechanism.

119. Normal Acid Behavior for Thiazolium C(α)–H Proton Transfer. James T. Stivers and *Michael W. Washabaugh*. Department of Biochemistry, Johns Hopkins University, Baltimore, MD 21205.

Carbon acids are believed to ionize slowly because of their weak acidity and large intrinsic barriers, which have been attributed to imbalance in the transition state between the development of resonance or solvation and C–H cleavage. Rate constants for C(α)–hydron transfer from 2-(1-hydroxyethyl)-3,4-dimethylthiazolium ion (**1**) were determined



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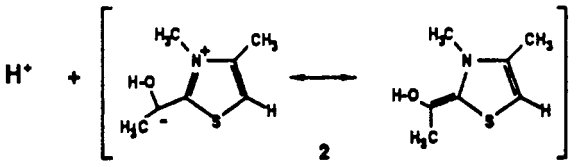

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by iodination of the C(α)-carbanion/enamine (**2**) at 25 °C and ionic strength 1.0 M in H_2O. C(α)–H transfer (pK_a = 21.8) shows general-base catalysis with $\beta \geq 0.85$ for bases of $pK_a \leq 15$. Primary isotope effects, k_H/k_D, of 1.0 for catalysis by buffer bases and hydroxide ion and the secondary isotope effect, k_{OD}/k_{OH}, of 2.5 for C(α)–H transfer are consistent with a very late transition state, the incursion of substantial internal return, and an intrinsic rate constant of $\geq 10^{9.5}$ s^{-1} for C(α)–H transfer. It is concluded that any electron delocalization and related transition-state complexity that might be present do not make a large contribution to the intrinsic barrier for C(α)–hydron transfer.

120. Organic Synthesis with Papain. A. C. Storer, *B. J. Gour-Salin*, P. Lachance, and L. Teasell. Biotechnology Research Institute, 6100 Royalmount Avenue, Montreal, Quebec, Canada H4P 2R2.

Peptide and amino acid analogues with nitrile functional groups are powerful reversible inhibitors of papain [Lucas, E. C., & Williams, A. (1969) *Biochemistry 8*, 5125–5135]. Recently, a number of workers (J.-R. Brisson, P. R. Carey, and A. C. Storer, unpublished results) have elucidated the structure of the enzyme-bound nitrile to be that of a thioimidate. The nitrile forms a covalent adduct with the thiol group of cysteine 25 in the active site of papain. At neutral pHs, the thioimidate is very slow to hydrolyze and can, therefore, undergo reactions with external nucleophiles. Herein, we wish to describe the NMR studies of the reactions of both amino and thiol nucleophiles with a variety of peptide nitrile–enzyme adducts. With amino nucleophiles amidines appear as intermediates, and this method has potential as a synthetic route to heterocycles. With thiol nucleophiles, papain acts as a nitrilehydratase, and this activity coupled with papain's well-characterized amidase activity results in the synthesis of a carboxylic acid from a nitrile.

121. A Rapid, Reproducible Assay for the Release of Fi-

brinopeptides A and B from Bovine Fibrinogen by Thrombin. *Joel K. Swadesh*. Polymer Laboratories Inc., 160 Old Farm Road, Amherst, MA 01002. Hiroko Sato. Kyoto University Research Center for Medical Polymers and Biomaterials, 53 Kawahara-cho Shogoin, Kyoto 606, Japan.

Fibrinopeptides A and B are released from bovine fibrinogen by thrombin, generating fibrin monomer and initiating blood clotting. Reversed-phase liquid chromatography has long been used to assay for the rate of release of fibrinopeptides A and B, but procedures for sample preparation have been cumbersome. The present poster demonstrates the use of a high-performance polymeric reversed-phase material (PLRP-S) in a convenient rapid, reproducible assay for bovine fibrinopeptides A and B. The dependence of the relative rates of fibrinopeptide release under various clotting conditions is examined.

122. Investigation of the Mechanism of the Asn-102 Mutant of Rat Trypsin Using pH-Dependence Studies and Solvent Isotope Effects. *R. Szawelski*, J. Bibbs, C. Craik, S. Johnson, and R. Schowen. Department of Chemistry, University of Kansas, Lawrence, KS 66045-0046, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, and Clarke College, Dubuque, IA 52001.

The pH–rate profile for the hydrolysis of anilide substrates by the Asn-102 mutant of rat trypsin, in which the Asp-102 group has been replaced by Asn, reveals that upon mutation the value of k_{cat} drops some 4000-fold, the pK_a of His-57 being lowered from 6.5 to 5.6; a hydroxide-catalyzed pathway asserts itself at alkaline pH. The reaction of the active enzyme with D-Ileu-Pro-Arg-pNA gives an isotope effect of 2.5 and a proton inventory which is "bowl-shaped", but in the mutant, the isotope effect falls to 1.7 and the proton inventory becomes linear. These data may be best explained, we believe, as a disruption of the Asp-His-Ser proton relay system upon mutagenesis, though other forms of multiproton catalysis are considered too.

123. Proton Inventories of the Neutral Methanolysis of *S*-Ethyl Trifluorothiolacetate. *K. S. Venkatasubban* and B. Byrd. Department of Natural Sciences, University of North Florida.

No abstract available.

124. Adenosine 5′-*O*-[(3-Bromo-2-oxopropyl)thiophosphate]: A New Affinity Label of an ADP Activator Site of Glutamate Dehydrogenase. *Marleen B. Walner* and Roberta F. Colman. Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716.

A new reactive nucleotide analogue, adenosine 5′-*O*-[(3-bromo-2-oxopropyl)thiophosphate] (AMP-S-BOP), has been prepared and characterized by UV, ^{31}P, and ^{1}H NMR spectroscopy, as well as by bromide and phosphorus analysis. Incubation of AMP-S-BOP (75 μM) with bovine liver glutamate dehydrogenase (GDH) (0.5 mg/mL) at pH 7.8 and 33 °C results in a 1.9-fold time-dependent increase in enzyme activity along with the incorporation of 0.5 mol of reagent/mol of enzyme subunit, i.e., modification of three subunits of the hexameric enzyme. The rate constant exhibits a nonlinear dependence on AMP-S-BOP concentration with a maximum k of 0.075 min^{-1} and K_I = 43 μM as measured over a concentration range 25 μM–3 mM. The addition of ADP, an activator of GDH, completely protects against both the increase in activity and covalent reagent incorporation. In contrast, GTP and NADH, allosteric inhibitors of GDH, have little effect on the rate constant. These results indicate that AMP-S-BOP functions as an ADP analogue and reacts specifically at an ADP regulatory site of glutamate dehydrogenase. (Supported by USPHS Grant DK 37000.)

125. Methionine Diimine, a New Inhibitor of Glutamine Synthetase. *F. C. Wedler*, B. W. Ley, A. Kumar, L. Rocca, and J. J. Villafranca. Departments of Molecular and Cell Biology and Chemistry, The Pennsylvania State University, University Park, PA 16802.

Inhibition of the glutamine synthetase reaction (L-Glu + NH_3 + ATP $\rightleftharpoons$ L-Gln + ADP + P_i) is important for both practical and mechanistic reasons. With the *E. coli* GS, L-Met-*S*-sulfoximine, Met(O) (NH), is a transition-state analogue (K_{is} = 1 μM vs Glu) and shows cooperative binding in suicide inhibition (I_{50} = 25 μM). Met-sulfone, Met(O)$_2$, binds 200 times more weakly and inhibits GS reversibly. Synthesis of a new analogue, Met-diimine, Met(NH)$_2$, allows one to probe the effects of replacing an oxygen on Met-SOX with an imino group. Met(NH)$_2$ exhibits K_{is} = 5 μM vs L-Glu, and in the suicide reaction I_{50} = 30 μM. The suicide inhibition rate vs [Met(NH)$_2$] is nonsigmoidal, whereas Met(O) (NH) showed positive cooperativity. Similar data were obtained with the *Anabaena* enzyme. These data provide further insights to transition-state structure and how subunit interactions (negative cooperativity) may participate in catalysis and turnover. [Supported in part by NIH Grants GM-26582 (F.C.W.) and GM-23529 (J.J.V.).]

126. Genetic and Biochemical Studies on the Formation of the *Klebsiella pneumoniae* Nitrogenase Components in *E. coli*. *Theresa C. White*, Georgianna S. Harris, Janice E. Flory, Thomas A. Collet, Karen Howard, and William H. Orme-Johnson. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

A binary plasmid system was used to produce nitrogenase components in *E. coli* and to define a minimum set of nitrogen fixation (*nif*) genes required for the production of the iron protein and the iron–molybdenum cofactor (FeMoco) reactivatable apomolybdenum–iron (apoMoFe) protein of nitrogenase. Systematic deletions of individual *nif* genes from plasmids which produce apoMoFe protein and/or Fe protein in *E. coli* established the genetic requirements for the production of these nitrogenase components. Formation of the Fe protein requires only the genes *nifHM+A*, while maximal formation of the apoMoFe protein requires the genes *nifHDK(T)YUSWZM+A*. ^{55}Fe radiolabel and immunodetection techniques showed that several of these gene products are involved in the assembly of the unique Fe_4S_4 clusters contained within the nitrogenase components whose structural proteins are encoded by *nifHDK*. It was also found that addition of FeMoco to apoMoFe protein increased the electrophoretic mobility of the protein, in a two-step fashion, to that of holoMoFe protein. However, deletion mutants lacking apoMoFe protein activity produce an immunoreactive tetrameric species comigrating with active apoMoFe protein which contains no detectable iron and does not undergo this same two-step mobility shift. (This work supported by the National Institutes of Health under GM30943.)

127. Characterization of the Irreversible Inactivation of Dopamine β-Monooxygenase by (*p*-Hydroxyphenethyl)trimethylsilane. *Z. W. White*, P. F. Hudrlik, and R. C. Rosenberg. Department of Chemistry, Howard University, Washington, DC 20059.

Dopamine β-monooxygenase (DBM, EC 1.14.17.1) is a copper-containing enzyme which catalyzes the benzylic hydroxylation of phenylethylamines. The mechanism of hydroxylation is believed to involve the transient formation of a benzylic free radical. (*p*-Hydroxyphenethyl)trimethylsilane (PHPTS) was designed to explore the nature of the benzylic intermediate by exploiting the enhanced stability of free radicals and cations formed β to silicon in organosilicon compounds. We have synthesized and investigated the effects of PHPTS on DBM and found that DBM is rapidly and irreversibly inactivated upon incubation with PHPTS. The inactivation is independent of time, is dependent on the PHPTS concentration, and does not require either ascorbic acid or oxygen. In addition, PHPTS appears to reversibly inhibit bovine liver catalase (a component of the DBM assay). The results of these investigations will be presented. [Support: American Heart Association (GIA-861232) and National Institutes of Health (5506-RR-08016).]

128. Concerted Rate-Limiting Enolization in the Reaction Catalyzed by Δ^5-3-Ketosteroid Isomerase (KSI). *L. Xue*, P. Talalay, and A. S. Mildvan. Departments of Biological Chemistry and Pharmacology, Johns Hopkins School of Medicine, Baltimore, MD 21205.

KSI catalyzes the isomerization of Δ^5-3-ketosteroids to Δ^4-3-ketosteroids by a conservative tautomeric transfer of the 4β proton to the 6β position using Tyr-14 as a general acid and Asp-38 as a general base [(1989) *Biochemistry 28*, 149]. On deuterating the 4β position (>97%) of the substrate k^H_{cat}/k^D_{cat} is 6.1 in H_2O and 6.3 in D_2O. The solvent isotope effect $k_{cat}(H_2O)/k_{cat}(D_2O)$ is 1.6 for both 4β-H and 4β-D substrates indicating a concerted rate-limiting step. On deuterating the 4α position (88%) of the substrate, the corrected $(V/K)^H/(V/K)^D$ is 1.11 ± 0.03, which decreases to 1.06 ± 0.01 when the 4β position is also deuterated, establishing these to be kinetic (rather than equilibrium) secondary isotope effects. Since a change in hybridization from sp^3 to sp^2 occurs at C-4 only during enolization, this constitutes a rate-limiting and concerted step. Concerted enolization is consistent with the anti orientation of Tyr-14 and Asp-38 with respect to the enzyme-bound substrate [(1989) *Biochemistry 28*, 149] and with the additive effects of mutations of these catalytic residues on k_{cat} [Kuliopulos et al. (1990) *Abstr. Biophys. Soc. Meet.* Feb 19–22].

129. Pre-Steady-State and Steady-State Kinetics of the Low Molecular Weight (17 000) Acid Phosphatase from Bovine Heart. *Zhong-Yin Zhang* and Robert L. Van Etten. Department of Chemistry, Purdue University, West Lafayette, IN 47907.

The complete time course for the hydrolysis of *p*-nitrophenyl phosphate catalyzed by an acid phosphatase was followed and analyzed successfully for the first time. A transient pre-steady-state stoichiometric "burst" of *p*-nitrophenol (formation rate constant of 48 s^{-1}, pH 7.0, 4.5 °C) is followed by a slow steady-state turnover (rate constant of 1.2 s^{-1}). Product inhibition studies indicate an ordered uni bi kinetic scheme for the hydrolysis. Partition experiments conducted for several substrates led to a constant product ratio. V_{max} was constant for these substrates, and the overall rate of hydrolysis was dramatically increased in the presence of alcohol acceptor. An enzyme-catalyzed ^{18}O exchange between inorganic phosphate and water occurred with $k_{cat} = 2.1 \times 10^{-3}$ s^{-1} at pH 5.0. These results were consistent with the existence of a phosphoenzyme intermediate in the catalytic pathway, and the breakdown of the intermediate is the rate-limiting step. K_s (10 mM), K_m (0.37 mM), and the rate constants for phosphorylation (k_2 = 624 s^{-1}) and dephosphorylation (k_3 = 24 s^{-1}) were determined at pH 5.0 by the "reduced flow" method. From the available equilibrium constants and rate constants, an energetic diagram for the reaction was constructed. (This work was supported by DHHS Grants GM 27003 and RR 01077.)

Thursday Morning—Ralph Hirschmann Award in Peptide Chemistry Symposium in Honor of Bruice Merrifield (Cosponsored with the Division of Organic Chemistry)—J. A. Smith, Presiding

130. Synthesis and Biological Activity of Conformationally Constrained Analogues of Growth Hormone Releasing Factor. *A. M. Felix*, C.-T. Wang, E. P. Heimer, V. S. Madison, D. Fry, V. Toome, R. M. Campbell, C.-M. Su, and T. F. Mowles. Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Conformational analysis (circular dichroism and molecular dynamics calculations based on NOE-derived distance constraints) have been carried out on growth hormone releasing factor, GRF(1–29)-NH_2, and related linear analogues, in 75% methanol (pH 6) and water (pH 3). These studies have provided important information on the preferred conformation which includes a central α-helical region that may be a critical element in the receptor-bound conformation. These observations provided insight into the design of a series of $i - (i + 4)$ monocyclic analogues of GRF(1–29)-NH_2 which can stabilize the α-helix in the GRF system. Attempts to stabilize the α-helical region further led to the design and synthesis of novel multiple $i - (i + 4)$ cyclized lactams with enhanced amphiphilic character which possess substantial in vitro and in vivo biological activity. N-Terminal structure modification (including desamino Tyr^1 and/or D-Ala^2 substitution) significantly improved plasma stability of the GRF analogues. The cyclic GRF analogues also exhibited enhanced resistance to enzymatic degradation and prolonged plasma half-life.

131. New Results from Thiol Capture Synthesis. *D. S. Kemp*, Nader Fotouhi, Robert I. Carey, and David R. Buckler. Department of Chemistry, Room 18-584, Massachusetts Institute of Technology, Cambridge, MA 02139.

The thiol capture strategy that has been reported previously has now been extended by the development of new families of acyl-transfer templates that exhibit enantioselectivity. The potential applicability to semisynthesis is demonstrated by clean coupling of deblocked 13- and 26-peptide fragments from the ROP sequence.

132. Synthesis and Mechanism of Action of Channel-Forming Antibacterial Peptides. *R. B. Merrifield*. The Rockefeller University, New York, NY 10021.

Several naturally occurring antibiotic peptides, including the cecropins, sarcotoxins, melittin, and magainins, have been synthesized by solid-phase methods and their antibiotic activities measured. Cecropin A is produced in the silk worm in a 64-residue precursor form which was synthesized. It is processed by loss of a 22-residue hydrophobic signal sequence, then, in two steps, by a dipeptidase to convert the propeptide into the active antibiotic. The C-terminal amide arises from a final glycine residue preceding the stop signal in the gene. Many replacement analogues have contributed to a better understanding of the structures required for activity. Recently, hybrid analogues of cecropin and melittin have led to even

more active antibiotics. The peptides form ion-conducting pores or channels in lipid bilayers and are thought to kill bacteria and the malaria parasite by related mechanisms.

133. Synthetic Peptide Vaccines: Design and Synthesis of Unambiguous Peptide-Based Vaccines. *James P. Tam.* The Rockefeller University, 1230 York Avenue, New York, NY 10021.

Chemically defined models, known as the multiple antigen peptide (MAP) system, are being designed and engineered specifically for synthetic peptide vaccines. MAPs comprise an oligomeric branching lysine as the core and multiple copies of peptide antigens as dendritic branches. Different peptide antigens such as B and T helper epitopes could be unambiguously incorporated and amplified by total chemical synthesis in a specific and deliberate manner. Furthermore, a built-in adjuvant is also included in the design of the MAP model to eliminate the need of an adjucant. Several general designs of these chemically unambiguous MAP models were tested in several infectious disease systems including malaria, hepatitis, and AIDS. High titers of antibodies that recognized both native proteins from which they were derived were elicited by immunization with MAPs. Furthermore, in the malaria model, the level of serum antibodies correlated with protection from malaria infection, and significant protection against challenge with 2000 sporozoites was observed in mice immunized with MAPs. Thus, a synthetic, multiple-epitope peptide-based immunogen with a chemically unambiguous structure may be a suitable candidate for the development of vaccines against infectious diseases.

134. Chemical Problems Encountered in the Development of Bioactive Peptides. *Daniel F. Veber*, Victor M. Garsky, Patricia K. Lumma, Roger M. Freidinger, Peter D. Williams, Debra S. Perlow, and Roger D. Tung. Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

The search for new lead structures at Merck has led to the discovery of bioactive peptides which present complex and novel problems in peptide synthesis. The platelet aggregation inhibitor, echistatin, is a 49-peptide having 4 disulfides which was isolated from the venom of the viper, *Echis carinatis*. The present state of peptide synthesis makes it feasible to synthesize such a structure rapidly and in the quantity necessary for detailed biological evaluation. Chemical synthesis can also play a role in probing the chemistry needed to develop a practical process using molecular biology approaches. A cyclic hexapeptide isolated from *Streptomyces silvensis* has given a novel class of oxytocin antagonist. Although smaller in size, it presents special challenges for chemical synthesis because of the presence of five N-substituted amino acids. Solution methods developed for slow peptide couplings have been adapted to solid-phase synthesis allowing rapid preparation of analogues directed at developing improved physical and biological properties.

THURSDAY AFTERNOON—ELI LILLY AWARD SYMPOSIUM IN HONOR OF GEORGE MCCLENDON (COSPONSORED WITH THE DIVISION OF INORGANIC CHEMISTRY)—H. GRAY, PRESIDING

135. NMR Studies of Cytochrome *c*. *W. Englander.* Department of Biochemistry, University of Pennsylvania, Philadelphia, PA 19104.

No abstract available.

136. Axial Ligand Replacement in Horse Heart Cytochrome *c* by Semisynthesis. A. L. Raphael, D. Wuttke, and *H. B. Gray.* Arthur Amos Noyes Laboratory, California Institute of Technology, Pasadena, CA 91125.

Semisynthesis has been employed to replace the axial methionine in horse heart cytochrome *c* with histidine. The reduction potential of the His 80 protein (cyt *c*–His 80) is 41 mV vs NHE (0.1 M phosphate, pH 7.0, 25 °C). The absorption spectra of oxidized and reduced cyt *c*–His 80 are very similar to those of the native protein in the porphyrin region, but the 695-nm band is absent in the oxidized His 80 protein. We are preparing cytochromes with other variations in the heme area to assess the importance of nuclear and electronic factors on electron transfers in a protein medium.

137. Long-Range Electron Transfer in Proteins. *B. Hoffman.* Department of Chemistry and Biochemistry, Northwestern University, Evanston, IL 60208.

No abstract available.

138. Bespoke Proteins: Tailoring for Fit, Function, and Durability. *George McLendon.* Department of Chemistry, University of Rochester, Rochester, NY 14627.

Examples of recent site-directed mutagenesis experiments with heme proteins, cytochrome *c*, and cytochrome *c* peroxidase will be discussed focusing on mutations which effect macromolecular recognition, redox potential and electron transfer in vivo and in vitro, and protein folding and associated thermal stability.