Abstracts, Division of Biological Chemistry, 194th National Meeting of the American Chemical Society, August 30–September 4, 1987

S. J. Benkovic, Chairman; J. Peisach, Secretary
D. G. Gorenstein, Program Chairman; C. D. Poulter, Program Chairman Elect

MONDAY MORNING—SYMPOSIUM ON NEW METHODS IN NMR SPECTROSCOPY OF BIOLOGICAL MACROMOLECULES—D. G. GORENSTEIN, ORGANIZER, PRESIDING

1. NMR Investigations of the Effects of Amino Acid Modifications in Proteins. *John L. Markley.* Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin—Madison, 420 Henry Mall, Madison, WI 53706.

Developments in protein chemistry (lumped under the heading of protein engineering), NMR instrumentation (improved superconducting solenoids, electronics, and computers for data acquisition and processing), and NMR methodology (two-dimensional, multiple-quantum, and related techniques) present new approaches to the study of structure-function relationships in proteins. With proteins of $M_r < 7{,}000$, signal resolution and assignment (H-1, C-13, and N-15) can be carried out at natural abundance; with larger proteins, C-13 and N-15 enrichment may be advantageous. This talk will discuss current strategies in the protein NMR field and will present the work of members of my laboratory who have studied proteins with $M_r = 3{,}000$-23,000. Goals of individual projects have been to: (1) develop two-dimensional NMR methods for the study of larger proteins, (2) elucidate conformational changes resulting from selective peptide bond cleavage, (3) study hydrogen exchange mechanisms, and (4) correlate effects of single and double amino acid replacements on the structure, dynamics, and stability of an enzyme. (This work was carried out at the National Magnetic Resonance Laboratory at Madison with external support from NIH, NSF, and USDA.)

2. Drug–Protein Interactions: Details of the Interaction between Cyclosporin A and Cyclophilin As Revealed by High-Field 2D NMR Studies. *I. M. Armitage*, S. L. Heald, M. W. Harding, and R. E. Handschumacher. Departments of Molecular Biophysics and Biochemistry, Diagnostic Radiology and Pharmacology, Yale University, School of Medicine, New Haven, CT 06510.

In recent years, several new developments in nuclear magnetic resonance (NMR) spectroscopy methods have facilitated the study of drug:protein interactions. These include the use of 2 dimensional ^1H-^1H and, when specific isotope labeling is employed, ^1H-X (where X = ^{13}C,^{19}F etc.) chemical shift correlation, cross relaxation and magnetization transfer techniques. Using these techniques, we will discuss the general strategy used to elucidate the nature of the interaction between the potent immunosuppressant cyclosporin A (CsA) and its cytosolic receptor, cyclophilin. The talk will begin with a description of the allowed conformations of CsA in free solution and then present the current status of our assignment of specific domains of cyclophilin that participate in the drug binding site. While the emphasis will be on NMR techniques, reference will be made to the role that other biophysical and chemical modification methods have made in this study. (Grants: NIH DK18778 and ACS CH67).

3. ^{31}P and ^1H 2-Dimensional NMR Methodologies for Defining Structure and Dynamics of Double Helical Nucleic Acids. *David G. Gorenstein*, Josepha M. Fu, Claude R. Jones, Jim Metz, Vikram Roongta, and Steven Schroeder. Department of Chemistry, Purdue University, West Lafayette, IN 47907.

It is now possible to unambiguously assign all ^{31}P resonances in the ^{31}P NMR spectra of oligonucleotides by either two-dimensional (COLOC) NMR techniques or site-specific ^{17}O-labeling of the phosphoryl groups. Assignment of ^{31}P signals in the dodecamer duplex, $d(CGTGAATTCGCG)_2$ containing one base pair mismatch, and lac operator pseudo-fragment 14mer duplex $d(TGTGAGCGCTCACA)_2$, combined with several other recent assignments in the literature have allowed an analysis of the origin of the sequence specific variation in ^{31}P chemical shifts. The ^{31}P chemical shifts of duplex B DNA phosphates appear to correlate with the Calladine sum function for variation in the helical twist of the oligonucleotides. Using the time development of cross peak intensities in $^1H/^1H$ 2D-NOESY NMR spectra, we can semi-quantitatively determine the distance between various base and deoxyribose protons. From these NOESY-derived distances and constrained molecular mechanics calculations, structural and dynamic models for these duplexes can be determined.

4. Molecular Structure Determination via Complete Relaxation Matrix Analysis of 2D NOE Spectra and Molecular Mechanics: DNA Fragments. *Thomas L. James*, Anna Maria Bianucci, Brandan Borgias, Nagarajan Pattabiraman, Ei-ichiro Suzuki, Ning Zhou, and Gerald Zon. Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143.

To avoid the assumption of isolated spin pairs commonly used for analysis of NOEs, and consequent errors in distance determinations, we have been developing a complete relaxation matrix analysis (CORMA) for two-dimensional nuclear Overhauser effect (2D NOE) spectra. In contrast to the approximate approach, exact intensities in the 2D NOE spectrum are calculated for a given three-dimensional array of protons after diagonalizing the relaxation matrix. We have used CORMA to determine interproton distances quantitatively in small molecules, but our primary interest is in biopolymers. Consequently, some DNA fragments have been studied using phase-sensitive 2D NOE proton spectra. Experimental 2D NOE spectra obtained at a series of mixing times have been compared with CORMA-generated theoretical spectra for structural models based on either x-ray diffraction-derived molecular coordinates or coordinates derived from molecular energy refinement calculations, in particular, from use of the program AMBER. As the intensities in the 2D NOE spectra are quite sensitive to internuclear distances, this comparison of theoretical and experimental 2D NOE spectra has provided

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insights into details of sequence-dependent DNA duplex structure in solution. The distance information from the 2D NOE analysis has been augmented by limited torsion angle information derived from coupling constants obtained from double-quantum-filtered COSY spectra.

MONDAY AFTERNOON AND EVENING—SYMPOSIUM ON FREE ENERGY PERTURBATIONAL METHODS APPLIED TO CHEMICAL AND BIOCHEMICAL PROCESSES (COSPONSORED WITH PHYS)—P. A. KOLLMAN, ORGANIZER, PRESIDING

5. Free Energies in Solution, the Aqua Vitae of Computer Simulations. *William L. Jorgensen.* Department of Chemistry, Purdue University, West Lafayette, IN 47907.

Modern simulation methods are being applied to obtain free energies for many important processes involving organic ions and molecules in solution. In conjunction with Monte Carlo statistical mechanics calculations, importance sampling methods and thermodynamic perturbation theory are being used to study the free energy changes for conformational equilibria, ion-pair association, and various reactions in solution including prototypical S_N1, S_N2, and addition processes. Other applications of the methodology include computation of free energies of hydration and pK_a's for weak organic acids in water.

6. Simulations of Equilibria: Xe–Myoglobin Interaction and (Poly)peptide Conformation. *Jan Hermans.* Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27514.

This paper will describe application of molecular dynamics perturbation methods to two simple systems that model protein functions: ligand binding and conformation change. The binding of xenon to the hydrophobic interior of myoglobin has been simulated by an approach that allows calculation of the equilibrium binding constant. The value so obtained (as standard free energy change of −1.7 kJ/mole at 1 atm Xe) is within 2 kJ/ mole of the experimental value. Analysis of the trajectories shows that the protein adapts considerably to insertion of Xe, at a cost of 12 kJ/mole. This strain causes a slight enlargement at a secondary site where another Xe is known to bind with circa 5-fold lower K_b. Simulations give good results for the affinity at two of the three secondary sites. Solvent was not represented in the calculations for myoglobin. Conformational equilibria related to the helix-coil transition have been studied for the alanine dipeptide and for the terminal residues of a short helix. In these simulations an environment of (SPC) water was present. Results for the dipeptide agree reasonably well with experiment. Results for the helix indicate an asymmetry for the two helix ends. These simulations are therefore being extended to more interior residues.

7. Evaluating Catalytic Free Energy in Enzymes. *Arieh Warshel.* Department of Chemistry, University of Southern California, Los Angeles, CA 90089-0482.

An effective way of correlating the structures and functions of proteins is obtained by evaluating the solvation energies of the substrate changes in the different stages of the given processes.[1] Early microscopic calculations of such solvation effects reproduced catalytic free energies in a qualitative way, and predicted the crucial role of the protein dipoles[1] (hydrogen-bonds). However, such predictions could not be confirmed until the emergence of genetic engineering, which has provided crucial information about enzyme catalysis. Being motivated by this information we have started a systematic evaluation of catalytic free energies in genetically modified enzymes. A combination of our EVB method with free energy perturbation methods enables us to reproduce catalytic free energies and binding free energies in an extensive set of test cases. This includes the 216-226 Gly→Ala mutation in trypsin,[2] the 155-Asn→Thr, 155-Asn→Ala, 155-Asn-Leu mutations in subtilisin,[3] as well as the 102 Asp→Asn in trypsin and the 64-His→Ala in subtilisin. All these studies reproduce the corresponding experiment within 1 kcal/mol. The implication of our findings and their potential for rational enzyme design are discussed. (1) A. Warshel and S. Russell, *Q. Rev. Biophys. 17*, 283 (1984). (2) A. Warshel and F. Sussman, *Proc. Natl. Acad. Sci. U.S.A. 83*, 3806 (1986). (3) J.-K. Hwang and A. Warshel, *Biochemistry* (in press).

8. On the Role of Protonation of Methotrexate Bound to Dihydrofolate Reductase—A Free Energy Perturbation Study. *U. C. Singh* and P. A. Kollman. Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Rd., La Jolla, CA 92037, and Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143.

The role of the ionic interaction between the N1-protonated methotrexate and the negatively charged ASP_{27} of *E. coli* DHFR to the binding has been studied using Free Energy Perturbation Method. The free energy difference between the binding of protonated ligand bound to the wild type (ASP_{27}) and the unprotonated ligand bound to the mutant (ASN_{27}) has been calculated to be 0.7 ± 1.2 kcal/mole, compared to the experimental value of 1.8 kcal/mole (Howell et al., *Science* (1986)). The free energy difference between the binding of unprotonated ligand to the wild type and to the mutant has been calculated to be 3.9 ± 1.1 kcal/mole. The free energy of binding has been decomposed into several components, such as due to the enzyme surroundings and due to the salt bridge. The cause for the insignificant enhancement to the binding affinity due to the replacement of the neutral pair in the mutant by the salt bridge in the wild type is discussed in terms of various free energy components.

9. Nucleic Acid Hydration: Computer Simulation Studies. *David L. Beveridge.* Department of Chemistry, Wesleyan University, Middletown, CT 06457.

Environmental effects, including hydration and ion atmosphere, are well known to influence significantly the conformational stability of various forms of DNA, RNA and oligonucleotide prototypes thereof. In this presentation the information available on nucleic acid hydration from thermodynamic, spectroscopic and crystallographic investigations will be considered, and a series of computer simulations aimed at providing additional information about the subject will be presented. Theoretical descriptions of the hydration of nucleic acid bases, sugars and phosphates will be reported, followed by results on oligonucleotide hydration in crystals and in aqueous solution. The newly emerging techniques in free energy determinations via molecular simulation provide the possibility of novel computer experiments which can reveal the role of the hydration of various structural elements of the double helix with respect to conformational stability. Our current series of projects underway in this area will be described.

10. Biomolecular Recognition, Stability and Reactivity: Theoretical Predictions. *J. Andrew McCammon.* Department of Chemistry, University of Houston, Houston, TX 77004.

New computer simulation methods have made it practical to study thermodynamic and kinetic aspects of protein function at the level of atomic detail (1). These methods hold considerable promise as tools for the interpretation of experimental data and for the design of new molecules with desired activities. This lecture will provide an introduction to some of these new methods and describe several different applications. The latter will include predictions of the effects of chemical and genetic modifications on the affinity of certain enzymes and inhibitors. Ongoing studies of enzyme-substrate, antibody-hapten, and virus-drug interactions and of protein folding stability may also be described. (1) J. A. McCammon and S. C. Harvey, "Dynamics of Proteins and Nucleic Acids," Cambridge University Press, Cambridge (1987).

MONDAY AFTERNOON—POSTER SESSION—D. G. GORENSTEIN AND C. D. POULTER, PRESIDING

Enzyme Kinetics and Mechanisms

11. Studies on the Reaction Mechanism of Ketol Acid Reductoisomerase. Greg Mrachko and *Kim 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. This unique enzyme catalyzes two reactions: the migration of an alkyl group and the reduction of a ketone. The only co-factor required is NADPH. The enzyme reaction shows an absolute requirement for Mg^{2+}. In basic solution, the substrate for the reaction, 2-hydroxy-2-methylacetoacetate acid, undergoes a rearrangement where the carboxylate group migrates. Our overall goal is to understand how the enzyme catalyzes the migration reaction. We report preliminary studies on the reaction mechanism. Steady-state kinetic analysis and product inhibition studies reveal an ordered sequential mechanism where NADPH binds first and NADP dissociates last. Several beta-keto-alpha hydroxy acids were synthesized and tested as substrates. Comparison of V and V/K values for the compounds suggest that the migrating alkyl group acquire carbanionic character in the transition state. We also report ^{D}V and $^{D}(V/K)$ valuers as a function of pH for the reverse reaction. The pH variation of these two quantities suggest that a group ionizing near pH 7.5 contributes to the catalysis of the oxidation of the diol product.

12. Product Formation Curves of Enzymic Reactions and Their Relation to the Structural Properties of the Enzymic System. *J. Chrastil*. USDA, ARS, SRRC, P. O. Box 19687, New Orleans, LA 70179.

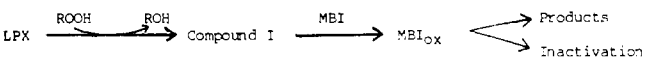
Many factors can influence product formation in enzyme catalyzed reactions. These factors are all related to the enzyme and its environment. Many physicochemical characteristics of an enzyme, for example, binding to other proteins, polysaccharides or lipids, and the presence of heterogeneous diffusion rate limiting structures, may be revealed from the product formation kinetics. The analysis of the entire enzymic time curves reveals accurately not only the Henri-Michaelis-Menten binding constants but also other structural characteristics of the enzyme system which cannot be determined from initial velocities. Kinetic data from the product formation curves are shown on specific examples where this alternate mathematical approach is needed.

13. Synergistic Binding of Thiamine Pyrophosphate (TPP) and Sulfonylurea Herbicides by Acetolactate Synthase (ALS). *L. M. Ciskanik* and J. V. Schloss. Central Research, Du Pont Co., Wilmington, DE 19898.

The stoichiometry of binding, and rate of release, of TPP by ALS isozyme II from Salmonella typhimurium (ALSII) was examined by use of [^{14}C]TPP and rapid gel filtration (Penefsky columns). ALSII that had lost activity of storage bound less [^{14}C]TPP, or its reduced analog tetrahydro-TPP (H_4TPP), than higher specific activity enzyme. ALSII with a specific activity of 10 units/mg maximally bound 0.18 mol [^{14}C]TPP/mol protomer (69,000 g), or 0.14 mol/mol [^{14}C]-H_4TPP, while enzyme with a specific activity of 19 units/mg bound 0.37 mol/mol [^{14}C]TPP or 0.39 mol/mol [^{14}C]H_4TPP. Assuming fully active enzyme binds 1 mol of cofactor per protomeric unit, it should have a specific activity of 50 ± 7 μmol of acetolactate formed/min/mg (37 °C). ALSII-bound [^{14}C]TPP exchanged with [^{12}C]TPP with a half-time of 4.9 ± 0.3 min. Addition of 1 mM sulfometuron methyl or 100 mM sodium pyruvate did not reduce the stoichiometry of [^{14}C]TPP binding, but did increase the half-time for exchange to 40 min or 22 min, respectively.

14. Suicide Inactivation of Lactoperoxidase by 2-Mercaptobenzimidazoles. *Daniel R. Doerge*. Department of Agricultural Biochemistry, University of Hawaii, Honolulu, HI 96822.

2-Mercaptobenzimidazole (MBI) and 1-methyl-2-mercaptobenzimidazole (MMBI) cause hydroperoxide-dependent suicide inactivation of lactoperoxidase (LPX). The inactivation, which is accompanied by changes in the visible spectrum, correlates with the S-oxidase activity of LPX. The formation of the corresponding benzimidazole products concomitant to loss of enzymatic activity suggests the partitioning of inhibitor-derived reactive intermediates between inactivation and turnover pathways. Two lines of evidence support this hypothesis: 2-^{14}C-MBI was synthesized and 1.0 mole of MBI binds covalently per mole of LPX which is inactivated; the stoichiometry of hydroperoxide consumption is correlated with LPX inactivation and product formation by the use of 5-phenyl-4-pentenyl-hydroperoxide. These results provide strong support for a mechanism of LPX-catalyzed formation of reactive S-oxygenated intermediates from 2-mercaptoimidazoles with subsequent covalent addition to the heme cofactor.



15. In Vitro Induced Reactivation and Phosphylation Studies Relative to in Vivo Oxime Efficacy in Organophosphorus Poisoning. *D. W. Hanke*, M. A. Overton, A. D. Wolfe, J. K. Marquis, C. N. Lieske, and C. K. Burdick. US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425.

No acetylcholinesterase (AChE) provides a reliable model to correlate in vitro oxime reactivation potency with in vivo efficacy for organophosphorus poisoning in mammals. Our studies with eel, fetal bovine serum (FBS), and bovine caudate nucleus (BCN) AChE support this accepted position (Hanke, Society of Toxicology meetings, 1985 and 1986). It may be that the unique pharmacokinetic and pharmacodynamic properties of the individual oximes and organophosphorus compounds are intrinsic to the divergent observations. Therefore, we examined the phosphylation constants of eel, FBS, and BCN AChE using tabun, sarin, soman, VX, 4-nitrophenyl methyl(phenyl)phosphinate, and 4-nitrophenyl chloromethyl(phenyl)phosphinate. Our studies were carried out at pH 7.60 at 25.0 °C. Our results indicate that, although

oxime potencies vary significantly with different inhibitors and different sources of enzyme, the kinetic constants for inhibition are remarkably similar. For example, the biomolecular phosphylation constant (k_i, M^{-1} sec^{-1}) for tabun was 5.26×10^4 for eel AChE, 6.18×10^4 for FBS AChE, and 4.78×10^4 for BCN AChE. The dissociation constants (k_d, M) for the same series were 1.83×10^{-5}, 3.26×10^{-5}, and 1.50×10^{-5} respectively.

16. Inhibition and Inactivation of Alcohol Dehydrogenase. *Vinit Kathardekar*, Grace C. Shiao, and Ronald E. Viola. Department of Chemistry, The University of Akron, Akron, OH 44325.

Yeast alcohol dehydrogenase is inhibited by a series of nitrilopropionamides with K_i values ranging from several millimolar to submicromolar depending on the nature of the substituents. The most potent inhibition was seen with dibromo-substituted and N-substituted amides and esters. Removal of ether the nitrile or the amide functional groups resulted in loss of inhibition. Incubation of alcohol dehydrogenase with these inhibitors resulted in a time dependent inactivation of the enzyme. This slow inactivation appears to be irreversible since no recovery of activity was observed on dilution of the enzyme into an assay mixture containing saturating levels of substrates. The substrate NAD was found to partially protect the enzyme by slowing the rate of inactivation. No protection was observed with ethanol. The mechanism of inhibition and inactivation of alcohol dehydrogenase by this class of compounds is being examined. This project is supported by a grant to R.E.V. from the Dow Chemical Company.

17. A Comparison of the Mechanism of Action of Native and Co^{2+}-Substituted Carbonic Anhydrase II. *K. A. Kogut* and R. S. Rowlett.

No abstract available.

18. 2′,5′-2-Azidoadenylate and 8-Azidoadenylate Trimer 5′-Triphosphates (2-$N_3p_3A_3$ and 8-$N_3p_3A_3$): Enzymatic Synthesis, Biological Properties and Photoaffinity Labelling of RNase L. S. W. Li, N. L. Reichenbach, K. Karikō, B. Haley, and R. J. Suhadolnik. Temple University School of Medicine, Philadelphia, PA 19140, and University of Kentucky, Markey Cancer Center, Lexington, KY 40536.

Photoreactive analogs of 2′,5′-A have been enzymatically synthesized from 2- and 8-azidoATP by 2′,5′-A synthetase from lysed rabbit reticulocytes (LRR) in 1.0 and 0.3% yields. The structures of the 2-$N_3p_3A_3$ and 8-$N_3p_3A_3$ were established by enzymatic hydrolyses and HPLC. The ability of the 2-$N_3p_3A_3$ and 8-$N_3p_3A_3$ to bind to and activate RNase L was compared to authentic p_3A_3 in radiobinding, core-cellulose and rRNA cleavage assays using RNase L in L929 cell extracts. The 2-$N_3p_3A_3$ and 8-$N_3p_3A_3$ can bind to and activate RNase L as well as does p_3A_3. 2-$N_3p_3A_3$ and 8-$N_3p_3A_3$ covalently cross-link to a single protein with a M_r of ~80,000 in L929 cell extracts following 30 sec. uv illumination, 0°. Cross-linking is decreased by p_3A_3, but not by ATP nor 8-azidoATP suggesting that the photolabelled protein is RNase L. The 2′,5′-azidoadenylate analogs provide probes to characterize the binding and activation domain(s) of RNase L. Supported by NSF research grant DMB84-15002.

19. Design of Mechanism-Based Inhibitors of Cholesterol Esterase. *Alfred M. Nyanda* and Daniel M. Quinn. Chemistry Department, University of Iowa, Iowa City, IA 52242.

Pancreatic cholesterol esterase (CEase) is thought to be involved in the absorption of dietary cholesterol. Catalysis by CEase proceeds through a serine enzyme mechanism that involves acylenzyme intermediate. The connection between serum cholesterol and atherosclerosis has been well documented and investigated. Because of this observation, we have synthesized phosphate and phosphonate derivatives of cholesterol for evaluation as potential irreversible inhibitors of pancreatic CEase (Scheme I). The synthetic methods and inhibition investigations will be discussed.

Scheme I

Y–X–Chol Chol = 3-XY-cholest-$\Delta^{5,6}$-ene
X = O or S
Y = $(EtO)_2P{=}O$, n-Pr(Cl)P=O

20. Design of a Nonpeptide Semirigid Inhibitor of Trypsin and Related Serine Proteases. *Siegfried H. Reich* and Paul A. Bartlett. Department of Chemistry, University of California, Berkeley, Berkeley, CA 94720.

Using the bovine pancreatic trypsin inhibitor-trypsin complex (X-ray) as a template, a nonpeptide, semirigid inhibitor has been devised using molecular mechanics calculations and molecular graphics modeling. The key residues (P_1'-P_3) in the natural inhibitor (BPTI) have been structurally and conformationally mapped in the proposed inhibitor. Application of this strategy to related serine proteases which bind other natural inhibitors in similar conformations as well as the design and synthesis of the nonpeptide inhibitor will be described.

21. Activation Parameters for CO_2 Hydration Catalyzed by Co^{2+}-Substituted Carbonic Anhydrase II. *R. S. Rowlett* and K. A. Kogut.

No abstract available.

22. Umbelliferyl Carbamates as Inhibitors of Cholesterol Esterase and Lipoprotein Lipase. *Patricia S. Schlom* and Daniel M. Quinn. Department of Chemistry, University of Iowa, Iowa City, IA 52242.

The lipases cholesterol esterase (CEase) and lipoprotein lipase (LpL) catalyze the hydrolysis of the water-soluble substrate p-nitrophenyl butyrate (PNPB.) This hydrolysis can be inhibited by the compound 4-methylumbelliferyl-n-butylcarbamate. Inhibition appears to be via a stable acyl enzyme intermediate rather than a carbamylated enzyme. Treatment of either CEase or LpL with the inhibitor does not result in formation of 4-methylumbelliferone. LpL inactivation is irreversible; reactivation does not occur in the presence of .05 M hydroxylamine at pH 7.3, nor does it occur after dialysis of the inhibited enzyme. The second order rate constants for LpL and CEase inhibitions are 2900 ± 13 M^{-1} s^{-1} and 1200 ± 130 M^{-1} s^{-1} respectively. These results are interpreted in terms of the known elements of active site structure and catalytic mechanism of these enzymes.

23. A Reexamination of the Acid-Catalyzed Exchange Rates of the Amide Protons of *d*-Biotin (B) and Its Methyl Ester (BE). *E. H. Serpersu*, A. S. Mildvan, T. Fox, D. C. Fry, and M. D. Lane. Johns Hopkins Medical School, Baltimore, MD 21205.

A previous paper from this laboratory reported the acid-catalyzed exchange of the 1′-NH of B and BE at 25° as displaying a second order dependence on $[H^+]^2$ suggesting a doubly protonated intermediate resulting from transannular

S to C=O bonding (*J.A.C.S. 107*, 7659). Because of our and Breslow's failure to detect transannular bonding by ^{13}C NMR and X-ray (*Bioorg. Chem. 14*, 249), kinetic measurements were repeated at 10°, where the decreased exchange rates can be measured more accurately, over the pH range 4.67 to 5.53, by transfer of saturation and T_1 measurements. Unlike the earlier findings, we do not detect rates second order in $[H^+]^2$. However we do observe kinetic orders in $[H^+]$ slightly greater than 1.0 at the 1′-NH for B (1.23 ± 0.07) and BE (1.30 ± 0.06), and orders indistinguishable from 1.0 at the 3′-NH of B (1.04 ± 0.05) and BE (0.96 ± 0.12). Lowering the pH produces a greater line-broadening of the 1′-NH than of the 3′-NH resonances of B and BE. Exchange of the 1′-NH is 7-fold faster than of the 3′-NH at pH 5.0. Our new results, if not due to a systematic error in the measurement of rapid NH at pH 5.0. Our new results, if not due to a systematic error in the measurement of rapid NH exchange rates, suggest a much smaller amount of a doubly protonated intermediate, and provide little evidence for transannular bonding in B and BE.

24. NMR Studies of Staphylococcal Nuclease (SN) and of Active Site Ca^{2+}-Ligand Mutants. *E. H. Serpersu* and A. S. Mildvan. Department of Biological Chemistry, Johns Hopkins Medical School, Baltimore, MD 21205.

Paramagnetic effects of Mn^{2+}, bound at the essential Ca^{2+} site of SN, on T_1 of the C2 proton resonances of His 8, 46, 121, and 124 yield distances of 21, 12, 14, and 17 Å, while the X-ray distances are 29, 11, 21, and 22 Å. The order of the distances is consistent with the location of Ca^{2+} in the X-ray structure if the resonances previously assigned to His 8 and His 121 (Jardetzky et al., *Cold Spr. Harb. Symp. 36*, 257) are reversed. The absolute distances suggest differing orientations of His side chains in solution and in the crystal. Second sphere Mn^{2+} to P distances, and inner sphere Co^{2+} to P distances are found in ternary SN complexes of 5′-TMP and 3′,5′-pdTp. Ternary Mn^{2+} complexes of the Ca^{2+}-ligand mutant D40G show Mn^{2+}-P distances 1.2-2.7 Å shorter than wild type, reflecting active site distortion. The NMR spectrum of the D21Y Ca^{2+}-ligand mutant shows small changes in chemical shifts and line widths from wild type, and overlap of His 46 and 121 resonances. Active site binding of Mn^{2+} is so weakened that a second site for Mn^{2+} is detected at distances of 8.3, 7.2 and 13.1 Å from His 8, 121 or 46, and 124, suggesting binding to Glu 75 and Asp 77. NMR thus reveals small structural differences between wild type SN and its Ca^{2+}-ligand mutants.

25. Nuclear Overhauser Effect (NOE) Studies of the Interaction of *S*-(D-Lactoyl)glutathione (LSG) with Glyoxalase I. *E. H. Serpersu*, B. Mannervik, and A. S. Mildvan. Department of Biochemistry, University of Stockholm, S-10691 Stockholm, Sweden, and Department of Biological Chemistry, Johns Hopkins Medical School, Baltimore, MD 21205.

Glyoxalase I catalyzes the isomerization of α-keto aldehyde adducts of glutathione to form thiol esters. Transferred NOE's from proton resonances of the enzyme to those of the enzyme-bound product, LSG, were used to identify active site residues within 4 Å of bound LSG. Distinct peaks in the action spectrum argue against spin diffusion. Negative NOE's to the glu-CγH_2 (−1.5%), gly-CH_2 (−1.0%), and to the lactoyl-CH_3 (−0.5%) are detected on irradiation of the proton spectrum of the enzyme at 6.5 ± 0.1, 7.0 ± 0.1 and 7.4 ± 0.1 ppm. The data suggest the proximity to bound LSG of at least two aromatic amino acids, one being tyr and the other trp, phe, or his. Experiments in H_2O do not reveal NOE's from the indole NH of trp to LSG, although chemical modification and fluorescence quenching studies indicate an active site trp (*Biochem. J. 197*, 67). Displacement of LSG from the active site by the potent competitive inhibitor *S*-(*p*-bromobenzyl)-glutathione caused the disappearance of the NOE's to the lactoyl-CH_3 group. The conformation of enzyme-bound LSG (*J.B.C. 259*, 11436) is compatible with a direct interaction with two aromatic residues.

26. A Study of the Oxidation–Reduction Properties of Soybean Lipoxygenase-1. *A. L. Shorter* and C. Kemal. Department of Pharmaceutical Research and Technologies, Smith Kline and French Laboratories, Swedeland, PA 19479.

Reduction of the catalytically active ferric soybean lipoxygenase (LO) to the inactive ferrous form by nordihydroguaiaretic acid, hydroquinone, and 2,6-di-tert-butyl-4-methylphenol was demonstrated by fluorescence, EPR and CD techniques. During the time required for reduction, there was no irreversible loss of enzyme activity. Hexachloroiridate(III) and tris(1,10-phenanthroline)iron(II) did not reduce LO or product any activity loss. Attempts to oxidize the ferrous enzyme to ferric by the following were not successful: hexachloroiridate(IV), trans-1,2-diaminocyclohexanetetraacetatomanganate(III), tris(1,10-phenanthroline)cobalt(III), 1,4-benzoquinone, tetrachloro-1,4-benzoquinone and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). Hexachloroiridate(IV), DDQ and p-benzoquinone were shown to irreversibly inactivate LO. These results indicate that although ferric LO is easily reduced, conversion of the ferrous enzyme to ferric by oxidants other than fatty acid hydroperoxides is difficult. This apparent specificity could be due to a high redox potential for LO and/or inaccessibility of the iron(II) site to oxidants other than hydroperoxides.

27. Enolphosphates as Active-Site-Directed Irreversible Enzyme Inhibitors. *Larry D. Sutton*, Monica Wu, Theodora Calogeropoulou, David F. Wiemer, and Daniel M. Quinn. Department of Chemistry, The University of Iowa, Iowa City, IA 52242.

Cholesterol esterase (CEase) and acetylcholine esterase (AChE) belong to a family of hydrolytic enzymes known as the serine hydrolases. In vivo, pancreatic CEase aids in intestinal lipid absorption by catalyzing the hydrolysis of cholesteryl esters and AChE catalyzes the hydrolysis of the neurotransmitter acetylcholine in the neuromuscular junction, resulting in the cessation of neural stimulation. In vitro CEase and AChE catalyze the hydrolysis of p-nitrophenylbutyrate and o-nitrochloroacetanilide, respectively, via the acylenzyme mechanism. Inhibition of these in vitro activities by the following enolphosphates has been studied: diethylcyclohexenolphosphate, diisopropylcyclohexenolphosphate and the diethyl enolphosphate of 2-tetralone. These inhibitors bind at the enzymes' active site serine, forming the phosphorylated analog of the acylenzyme intermediate. Diethylcyclohexenolphosphate inhibits porcine pancreatic CEase and eel AChE with second order rate constants of 74.2 ± 3.4 M^{-1} sec^{-1} and 796 ± 9 M^{-1} sec^{-1}, respectively and diisopropylcyclohexenolphosphate inhibits CEase with a rate constant of 7.61 ± .30 M^{-1} sec^{-1}. The structure-activity relationships of enolphosphate inhibition will be discussed in terms of active site binding.

28. Direct Demonstration of the Isomerization Component of the Monoterpene Cyclase Reaction. *Carl J. Wheeler* and

Rodney B. Croteau. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

The tightly coupled nature of the reaction sequence catalyzed by monoterpene cyclases has precluded direct observation of the topologically required isomerization of geranyl pyrophosphate to the presumptive, enzyme-bound, tertiary allylic intermediate, linalyl pyrophosphate, which ultimately cyclizes to the various monoterpene skeleta. By using a partially purified cyclase preparation and 2,3-cyclopropylgeranyl pyrophosphate, a substrate analog designed to uncouple the reaction sequence, the production of the corresponding tertiary homoallylic pyrophosphate isomer was demonstrated, thus providing direct evidence of the usually cryptic isomerase activity. A number of related products generated by reaction of cyclase with the analog were also identified, the structures and proportions of which were consistent with the intermediacy in catalysis of a cyclopropylcarbinyl cation:pyrophosphate anion pair. The results confirm mechanistic similarities in the enzymatic ionization and subsequent transformations of allylic pyrophosphate and cyclopropylcarbinyl pyrophosphate intermediates of isoprenoid metabolism.

29. Design of Human Leukocyte Elastase Inhibitors Based on Ovomucoid Third Domains. *Richard Wynn*, Soon Jae Park, and M. Laskowski, Jr. Department of Chemistry, Purdue University, West Lafayette, IN 47907.

Human leukocyte elastase (HLE) is a serine proteinase which has been implicated with proteolysis of lung elastin observed in pulmonary emphysema. Inhibitors for this enzyme are potentially of therapeutic value. To minimize possible side effects such inhibitors should be specific towards HLE relative to other serine proteinases. We have measured the association equilibrium constant (K_a) of HLE with 25 ovomucoid third domains. These data can be used to design the strongest (largest K_a) and the most specific inhibitors of HLE. Such designs assume that all effects are strictly additive. This assumption has been tested with 5 other serine proteinases and in general holds true, although there are several exceptions. The strongest inhibitor has a predicted K_a of 4.2×10^{11} M^{-1}. The most specific has a predicted K_a of 2.3×10^9 M^{-1} and is predicted to be at least 1000 times stronger for HLE than for 5 serine proteinases for which we have data; bovine α-chymotrypsin, porcine pancreatic elastase, subtilisin, *S. griseus* proteinases A and B. Some of the effects used in our inhibitor design can be rationalized on the basis of the 3-dimensional structure of the HLE-Turkey ovomucoid third domain complex (Bode et al., *EMBO J.* 1986, *5*, 2453–2458). Supported by NIH GM10831.

Proteins—General

30. In Vivo and in Vitro Metal Substitution in *Eco*RI. *Lena A. Basile*, Shanthi Paranawithana, and Jacqueline K. Barton. Department of Chemistry, Columbia University, New York, NY 10027.

Among the transition metal ions, it appears that zinc has been primarily chosen by nature to act at the interface between proteins and nucleic acids. In our laboratory, we have been investigating the role of zinc in the restriction endonuclease, *Eco*R1, a simple DNA binding enzyme. Previously, we reported that zinc appears to be an integral component of the wild-type enzyme with 1 zinc ion per protein monomer, and that the metal also appears necessary for the catalytic activity of the enzyme. *Eco*R1 isolated instead from an overproducing strain was seen to contain lower levels of zinc (zinc/protein ratio of 0.3–0.4). Metal enrichment of the medium, however, led to increasing zinc levels (≥ stoichiometric) and an increase in activity of the resultant enzyme. Cobalt substitution can also be effected. Metal substitution in vivo and in vitro support the importance of the metal to enzymatic activity. These results point to the fact that consideration must be given to metal availability, metal uptake, and metal incorporation into the product during the process of overproduction of a putative metalloenzyme.

31. A General Spectrophotometric Assay for Carboxylyases. *Dennis H. Burns* and D. John Aberhart. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We have developed a highly sensitive, rapid, spectrophotometric method to measure the activity of phosphopantothenoylcysteine decarboxylase (EC 4.1.1.36), one of five enzymes involved in the conversion of pantothenic acid into coenzyme A. The assay utilizes a coupled enzyme system whereby liberated CO_2 is trapped with phosphoenolpyruvate and phosphoenolpyruvate carboxylase (EC 4.1.1.31) to form oxalacetate, which in turn is reduced with malic dehydrogenase (EC 1.1.1.37) to l-malate concomitantly with the oxidation of NADH to NAD. The resultant decrease in absorbance at 340 nm accurately reflects the activity of the decarboxylase as compared to $^{14}CO_2$ analysis. The method is capable of detecting the liberation of as little as 4 nmol of CO_2 per minute and is shown to be effective in assaying the activity of several other carboxylyases.

32. Effect of Caffeine on Hepatic Phase II Enzyme Activities in Rats. *M. W. Chou* and A. Chou.

No abstract available.

33. Partial Sequence of the Light Chain of Botulinum Neurotoxin Type A. *B. R. DasGupta*, J. Foley, and R. Niece. Food Research Institute and Biotechnology Center, University of Wisconsin, Madison, WI 53706.

Botulinum neurotoxin (NT) is produced by *Clostridium botulinum* as 7 antigenically distinct types, e.g. A, B, E. The heavy chain (M_r 100,000) of the NT (M_r 150,000) binds to the presynaptic membrane and the light chain (M_r 50,000) appears to induce neuromuscular paralysis (*J. Biol. Chem. 262*, 2660, 1987). The following 46 residues from the N-terminal end were sequenced (Applied Biosystems Model 470A gas phase sequencer): P.F.V.N.K.Q.F.N.Y.K.D.P.V.N.G.V.D. I.A.Y.I.K.I.P.N.A.G.Q.M.Q.P.V.K.A.F.K.I.H.N.K.I.W.V. I.P.E-. Residues #1-17, that were known (*J. Biol. Chem. 260*, 10461, 1985) matched with the first 17 of the 46 residues now sequenced. Comparison between the N-terminal 44 residues of type B NT and type A revealed 10 residues at identical positions. Among the first 46 residues of tetanus NT (*EMBO J. 5* 2495, 1986) and type A NT 10 residues have identical positions. Between tetanus NT and botulinum type A and B NT eight residues (#1,7,9,11,12,18,24 and 44) are identical; the stretch, -K.A.F.K.I- around residue 35 is invariant in all three NTs. Another stretch, -D.P.V.N- (residues #11-14) is identical between type A and tetanus NT. Funded by NIH (NS17742).

34. Studies on a Vitamin K_2 Binding Protein. *G. H. Dialameh.* Department of Biochemistry, School of Medicine, University of Tehran, Iran.

We wish to report the isolation of a binding protein with apparent specificity for vitamin K_2 from chick liver microsomes

after the injection of (6,7-$^3H_2$)-menadione into the pectoralis muscle of the chicks. This microsomal vitamin K_2 binding protein (KBP) has been partially purified by Emalgen 913 extract and a combination of chromatography on Sephadex G-100, DEAE-cellulose and several different salting out procedures. When KBP was subjected to SDS-polyacrylamide gel electrophoresis one major radioactive band at 100,000 daltons was obtained. The radioactivity associated with KBP was extracted, purified and characterized as (6,7-$^3H_2$)-vitamin K_2 (Dialameh, G. H. *Int. J. Vit. Nut. Res. 48*, 131–135, 1978). The vitamin K_2 ligand is non-covalently bound to the protein and can be dissociated from KBP by boiling. Although the function of this protein is unknown, it is probably coenzyme complex form of the vitamin K_2 in the formation of the blood clotting factors. The mechanism and biological significance remain to be elucidated.

35. Chemical Properties and Biological Activities of Arginine Esterase from the Venom of Egyptian *Cerastes vipera*. *T. M. Farid* and A. T. Tu. Department of Biochemistry, Colorado State University, Fort Collins, CO 80523. M. F. El-Asmar, Kh. F. Ghounim, and F. M. Tash. Department of Biochemistry, Ain Shams Medical School.

An arginine esterase isolated from *C. vipera* was found to be a single polypeptide chain with a molecular weight of 38,000. It hydrolyzed *N-p*-Tosyl-L-arginine methyl ester (TAME), *N*-Benzoyl-L-arginine methyl ester (BAME), and *N*-Benzoyl-L-arginine ethyl ester (BAEE), but not *N*-α-Benzoyl-DL-arginine-*p*-nitroanialide. DFP and benzemidine inhibited the enzyme activity, but other inhibitors such as trasylol, trypsin inhibitors and EDTA had no effect. The enzyme was nonhemorrhagic and had no effect on capillary permeability increasing activity. It showed a potent platelet aggregation activity. The aggregation initiated by the enzyme was not through prostaglandin formation as indomethacine had no effect on aggregation. Inhibitors of the enzyme action also inhibited platelet aggregation. The enzyme showed kininogenase activity as shown by contraction of isolated gravid uterus. The enzyme also had blood coagulation promoting action.

36. The Distribution of PFK Isoenzymes in the Liver of Camel, Rat and Rabbit. *S. M. Khoja*. Department of Biochemistry, King Abdulaziz University, P.O. Box 9028, Jeddah, Saudi Arabia.

The distribution of phosphofructokinase isoenzymes have been compared among camel, rat and rabbit livers. Only a single phosphofructokinase isoenzyme is present in the camel liver as characterized with four different techniques, namely $(NH_4)_2SO_4$ precipitation, chromatography on DEAE-cellulose, electrophoresis on cellulose acetate and regulatory properties. All the three enzymes showed different physical and regulatory properties from each other. The $(NH_4)_2SO_4$-precipitation curves of the camel and rabbit enzymes were monophasic, whereas the rat enzyme was biphasic. Rabbit liver phosphofructokinase was slightly more anodic than the rat enzyme, whereas the camel enzyme was the least anodic as shown by the techniques of DEAE-cellulose chromatography and cellulose acetate electrophoresis. Partially purified camel liver phosphofructokinase showed different regulatory properties from the rabbit and rat isoenzymes as the apparent K_m values were 0.58, 0.45, and 0.82 mM, respectively.

37. Activation of Cyclic AMP Phosphodiesterase by Lugworm Proteases. G. R. Parker and *Y. M. Lin*. Department of Chemistry, Tennessee State University, Nashville, TN 37203.

Four activating activities of cyclic AMP phosphodiesterase were isolated from lugworm, *Arenicola cristata*, by extraction from cut worm segments and preparative flat-bed isoelectric focusing. The activators corresponded respectively to pI's of 4.4, 5.0, 5.2 and 5.4, their molecular weights were estimated to be 36,200, 30,500, 30,200 and 28,300. Homogeneous preparations of two of the activators were obtained by further purification with TSK G3000SW HPLC. The lugworm activators, in contrast to calmodulin, a well known phosphodiesterase activator, were heat-labile and unaffected by EGTA. Examination with various protease inhibitors indicates that the activation of phosphodiesterase by the lugworm activators was proteolytic in nature. The effects of divalent cations, temperature and pH on the activation were also studied. The lugworm activators were confirmed to be trypsin-like proteases by their hydrolysis of N-p-tosyl-L-arginine methyl ester, a synthetic trypsin substrate. One of the activators also hydrolyzed benzoyl-L-tyrosine ethyl ester, a chymotrypsin substrate.

38. Liquid Chromatography with Pulsed Amperometric Detection for the Microdetermination of the Carbohydrate Composition of Glycoproteins. Kyung B. Lee and *Robert J. Linhardt*. Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, IA 52242.

Many methods have been developed for the determination of carbohydrate moieties released from glycoproteins, but in terms of microdetermination they leave much room for improvement. Recently we described a method using a liquid chromatography system (Dionex) with pulsed amperometric detection for the quantitation of sugars comprising capsular polysaccharides (Bryan *et al., Appl. Environ. Microbiol. 51*, 1304 (1986)). This method has now been applied to several glycoproteins to quantitate both neutral sugars and N-acetylneuraminic acid. The method consists of chemical or enzymatic hydrolysis followed by direct analysis using liquid chromatography. Results of analysis on both characterized and uncharacterized glycoproteins including antithrombin III, heparin cofactor II, fetuin, transferrin and α_1-acid glycoprotein are presented.

39. Purification of Tryptophanase and Tyrosine Phenol-Lyase by Hydrophobic Chromatography on Sepharose CL-4B. *Robert S. Phillips* and Robert L. VonTersch. Departments of Chemistry and Biochemistry, School of Chemical Sciences, The University of Georgia, Athens, GA 30602. Edith W. Miles and Syed A. Ahmed. Laboratory of Biochemical Pharmacology, NIDDKD, National Institutes of Health, Bethesda, MD 20892.

The carbon-carbon lyases, tryptophanase [EC 4.1.99.1] and tyrosine phenol-lyase [EC 4.1.99.2], catalyze the reversible cleavage of L-tryptophan or L-tyrosine to indole or phenol, respectively, and pyruvate and ammonia. These enzymes are of mechanistic interest and are useful for synthesis of L-tryptophan, L-tyrosine, and analogues. We have examined the applicability of hydrophobic chromatography to the purification of these enzymes. Tryptophanase from *E. coli* B/1t7-A is adsorbed to underivatized Sepharose CL-4B in the presence of 1.8 *M* $(NH_4)_2SO_4$, and is eluted at 1 *M* $(NH_4)_2SO_4$. This procedure gives tryptophanase in >95% purity and 60% overall yield. In contrast, tyrosine phenol-lyase is not adsorbed to underivatized Sepharose CL-4B in 2.0 *M* $(NH_4)_2SO_4$. How-

ever, chromatography of tyrosine phenol-lyase from *Citrobacter freundii* (ATCC 29063) on Octyl-Sepharose CL-4B in 1 *M* $(NH_4)_2SO_4$ results in several-fold purification, even though the enzyme is only modestly retarded. Thus, hydrophobic chromatography is a valuable method for purification of the carbon-carbon lyases.

40. Spectroscopic Studies of Active and Inactive Forms of Tissue Plasminogen Activator. *C. S. Randall* and R. M. Gamrat. Department of Pharmaceutical Research and Technologies, Smith Kline and French Laboratories, Swedeland, PA 19479.

Infrared spectroscopy and circular dichroism (CD) have been applied to characterize the solution conformation of tissue plasminogen activator (tPA). Of special interest to us are conformational changes which can be related to a concomitant loss of enzymatic activity. In a study of thermal stability, tPA solutions incubated at temperatures ranging from 40° to 80 °C were examined spectroscopically and also measured for activity using the S-2251 chromogenic substrate assay. Samples exposed to higher temperature (>60 °C) exhibited large activity losses as well as strikingly different CD spectral features in the regions of 200-210 nm and 235-240 nm, respectively. CD thus appears to be a useful probe for following denaturation; it has also provided new insight into the secondary and tertiary structural components present in native tPA.

41. Physical and Chemical Properties of B-Protein. *A. W. Schweikert*, R. M. Macleod, W. D. Whybrew, and E. T. Bucovaz. Departments of Biochemistry and OB/GYN, University of Tennessee, Memphis, Memphis, TN 38163.

Patients with cancer have a protein in their serum which is named B-Protein. Because of the close correlation of B-Protein levels with the state of the malignancy, there is considerable interest in the isolation, characterization, physiological and biochemical investigation of this protein. B-Protein was purified in active form by gel filtration, affinity chromatography, ion exchange, and reverse phase HPLC. Antibodies were developed against the purified protein. Although similar to albumin electrophoretically, B-Protein contains 3-10% carbohydrates, differs in amino acid composition, peptide fragments following cyanogen bromide cleavage, and amino acid sequence. B-Protein may have as its precursor a protein normally found in serum, or it may be produced as the reflection of genetic modification due to cancer.

42. Covalent Coupling of Proteins to Microparticles. C. Jeffrey Wang and *Dinesh O. Shah*. Pandex Division, Travenol Laboratories, Inc., 909 Orchard Street, Mundelein, IL 60060.

Covalent coupling of lysozyme to amino-polystyrene particles utilizing a heterobifunctional coupling agent, SPDP, and to aldehyde particles was studied by using particle concentration fluorescence immunoassay (PCFIA). The results showed that these two methods could be used to couple lysozyme covalently to either amino-polystyrene or aldehyde particles. The modification of amino-polystyrene particles with SPDP offers the advantage that the resulting pyridyldisulfide particles can be used to react specifically with the sulfhydryl group of proteins, or they can be reduced further with dithiothreitol (DTT) to form sulfhydryl particles. The aldehyde particles can be used to couple to proteins covalently without any coupling agent. The activity of lysozyme coated particles depends upon the orientation of lysozyme on the particles. Higher activity was obtained when the amino groups of the lysozyme were utilized to couple to the particles covalently.

Biomolecular Spectroscopy

43. Application of FTIR Spectroscopy to Studies of Protein Adsorption in Aqueous Solutions. G. Zuber and *K. Benedek*. Smith Kline and French Laboratories, Swedeland, PA 19101.

The major practical drawback for any FTIR study of proteins is the undesirably large absorbance of water, which overlaps the absorbance of the Amide I resonances, the most useful IR region for protein studies. The removal of water by D_2O-H_2O exchange is time consuming, and requires delicate sample handling and preparation methods, special technical skills and/or instruments (membrane filters or lyophilizer). A simple sample preparation technique has been developed in our laboratory. We dilute the H_2O containing samples with identical volumes with different proportions of D_2O/H_2O. The effect of the amount of D_2O on the FTIR spectrum of model proteins will be displayed in conjunction with the necessary data acquisition and data handling parameters. The applicability of the method will be demonstrated with model protein solutions and a protein adsorbed to hydrosol. Finally, examples to solve "real life" analytical method developmental problems will be presented using FTIR spectroscopy in experimental vaccine formulations.

44. Utilization of Selective Deuteration in NMR Spectral Assignment of Deoxyoligonucleotides: 2D NOESY Analysis of Non-Self-Complementary Duplexes. *Charles K. Brush*, Michael P. Stone, and Thomas M. Harris. Department of Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37235.

Methods for assignment of deoxyoligonucleotide proton NMR spectra by two-dimensional nuclear Overhauser enhancement spectroscopy have been developed in recent years. Although a number of oligomers have been completely assigned, the potential for overlapping peaks to interfere with the establishment of connectivities is significant. A method is presented for the simultaneous exchange of deuterium for protons at the 8 position of purines and the 5 position of cytosines on a non-selfcomplementary deoxyoligonucleotide. The deuterated strand is combined with the protonated complementary strand to form a heteroduplex; the complexity of the resulting NOESY spectrum is reduced in the spectral regions critical for the identification of internucleotide NOEs. The procedure may be repeated with the complementary strand. The two heteroduplexes may then be back-exchanged to yield the fully protonated DNA duplex. Supported by funding from the NIH, ES-00267 and ES-03755.

45. Hemosiderin-like Deposits of Iron in Smokers' Lung Tissue. *D. F. Church*, T. J. Burkey, and W. A. Pryor. Biodynamics Institute, Louisiana State University, Baton Rouge, LA 70810.

The lung tissue of long-time cigarette smokers becomes black; this has been attributed to the accumulation of macrophages laden with the tarry particulate matter from the smoke. We have shown that cigarette tar contains a stable organic free radical that can be readily detected using electron spin resonance (ESR). Based on its g-value of 2.0035, we have identified the radical in cigarette tar as being due to a semiquinone in a low molecular weight polymeric matrix. We therefore expected that the dark pigments in smokers' lung tissues should show this same signal. However, we instead find

that the pigment contains a strong signal with a temperature-dependent g-value of 2.1–2.3. We tentatively identify this signal as being due to iron in a highly ordered form similar to that found in hemosiderin. We suggest that this finding could have profound implications. Since it has been shown that hemosiderin initiates lipid peroxidation in model studies, we hypothesize that these iron deposits could initiate a cascade of oxidative damage in smokers' lungs.

46. Determination of Structures of ^{13}C-Enriched Carbohydrate Derivatives by ^{13}C NMR. *Warren J. Goux*, T. L. Chick, S. Dhawan, and C. J. Unkefer. Department of Chemistry, The University of Texas at Dallas, P.O. Box 830688, Richardson, TX 75083-0688.

We have recently been investigating the possibility of using the carbonyl carbon resonances of peracetylated carbohydrate and the benzyl methylene carbon resonances of perbenzylated carbohydrate derivatives as structural reporter groups. In each of these cases, we have found that sets of resonances arising from substituents of a derivatized oligosaccharide are sensitive to the types of constituent residues present and to the types of glycosidic linkages made between neighboring residues. These resonances have been assigned to specific carbon substituents of known structures using a combination of COSY, ^{13}C-^{1}H shift correlation and INADEQUATE NMR spectroscopies. The formation of a library of such shifts may ultimately form a basis for the determination of unknown carbohydrate structures. Furthermore, the potential of ^{13}C-enrichment of the substituents suggests that the method may compliment existing ^{1}H NMR methods for determining structures of complex carbohydrates isolated in limited quantity, without the complications of severely overlapping resonances, spin-spin splitting and interference from the solvent signal.

47. Staphylococcal Nuclease Active Site Amino Acids: pH Dependence of Tyrosine and Arginine As Determined by NMR and Kinetic Studies. *Charles B. Grissom*, Masanobu A. Chinami, Denise A. Benway, Eldon L. Ulrich, and John L. Markley. Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

The variation of enzymatic activity with pH has been reexamined for staphylococcal nuclease (originally from the Foggi strain of *S. aureus* and overproduced in *E. coli*) and correlated with the pKa's of amino acids located near the active site. With deoxythymidine 5′-*p*-nitrophenylphosphate as substrate, one pKa in the V/K vs. pH profile is observed at 8.4 ± 0.3. This is in contrast to two pKa's observed at 8.4 and 9.2 with deoxythymidine 5′-*p*-nitrophenylphosphate 3′-phosphate as substrate [Cuatrecasas, P., Wilchek, M., and Anfinsen, C. B., *Biochemistry 8*, 2277 (1969)]. The ^{13}C resonances of the 5 arginines do not titrate with pK's less than 11. This eliminates active site residues Arg-35 and -87 as candidates for the acid/base functionality required for catalysis. Tyr-85 has a pKa of 9.4 ± 0.1 in H_2O and 0.3 M KCl. None of the other tyrosines have pKa's less than 10. The pKa of Tyr-85 is consistent with its proposed role of H-bond donor to the 3′-phosphate of the substrate. The temperature dependence of both pKa's in the V/K profile will also be reported. (Supported by NIH Postdoctoral Fellowship GM10903-02 to C.B.G.; NSF grant DMB-841022; and NIH grants GM35976 and RR02301.)

48. Solution Conformation of Retinoids Obtained from 2D NOE NMR Experiments. *Kenneth D. Hope*, Donald D. Muccio, Marcia I. Dawson, and Peter D. Hobbs. Department of Chemistry, University of Alabama at Birmingham, Birmingham, AL 35294, and Bio-Organic Chemistry Laboratory, SRI International, Menlo Park, CA 94025.

Retinoic acid and its analogs have been shown to be potential cancer chemopreventative agents. The conformation of these retinoids is important in discerning a proposed relationship between the conformation and biological activity. In this study we have investigated three conformationally restricted retinoids based on the structure of TTNN (Tetrahydrotetramethylnaphthalenylnaphthoic acid), and have determined the *syn* and *anti* conformation about the coannular bond by 2D NOE NMR experiments. Prior to these studies, spectral assignments of the compounds were made by homonuclear selective decoupling and 2D heteronuclear correlated experiments. The cross peak intensities from the 2D NOE experiments were obtained for several mixing times, which were less than spin-lattice and cross-relaxation times. A model, with two energy minima and jump times in the extreme narrowing range, was used to describe the motion about the coannular bond. These data were used to determine the proton-proton distances. A dihedral angle was determined from the distances. Nonplanar geometries were predicted for each compound, with the angular distortion increasing in TTNN retinoids with ortho-substituted methyl groups. This conformational change is consistent with angles predicted from our UV-VIS data based on the results from model biphenyl compounds. This work was supported in part by research grants CA 13148 (DDM), P01 CA 28103-08 (DDM), CA 30512 (MID), and CA 32428 (MID).

49. Time-Resolved Spectroscopy of Chiral Metal Complexes in Probing DNA Conformation. *Challah V. Kumar, Dorothy A. Swain*, Jacqueline K. Barton, and N. J. Turro. Department of Chemistry, Columbia University, New York, NY 10027.

Time-resolved emission of chiral ruthenium complexes bound to DNA provides a sensitive probe of DNA conformation. Moreover, chiral discrimination is apparent spectroscopically for several modes of binding. The time-resolved emission spectra of Δ- and Λ-$Ru(phen)_3^{2+}$ blue shift (~8 nm) and sharpen significantly when bound to B-form calf thymus DNA. Similar results are observed with Δ-$Ru(phen)_3^{2+}$ and B form poly(dGC), yet no sharpening of the spectrum is found with Λ-$Ru(phen)_3^{2+}$ under these conditions. The excited state of Δ-$Ru(phen)_3^{2+}$ in the presence of right-handed B form poly(dGm^{5}dC) decays as a distinct biexponential, consistent with two major binding modes: intercalation and surface binding. In contrast, Λ-$Ru(phen)_3^{2+*}$ decays as a single exponential, suggesting surface binding as the dominant mode for this isomer. With poly(dGm^{5}dC), thought to be in a left-handed helical form, a completely different behavior is observed. Δ-$Ru(phen)_3^{2+*}$ shows a predominantly single exponential decay, whereas Λ-$Ru(phen)_3^{2+*}$ shows a distinct biexponential behavior. Time-resolved polarized emission results indicate differences in the mobility of the two isomers bound to different DNA helical structures.

50. Magnetic Resonance Studies of Vanadium-51 Interaction with the Sodium–Potassium ATPase. *Carmen J. Lôpez*, Ileana Nieves, Germán Santiago, and Miguel Rodríguez. Department of Chemistry, University of Puerto Rico, Humacao, PR 00661.

The sodium-potassium ATPase plays a key role in the exchange of sodium and potassium across the cell membrane.

This ATP-dependent allosteric protein pumps Na^+ out of the cell and K^+ in, against their concentration gradients. Ouabain and vanadate have been found to be potent inhibitors of the enzyme action. The infusion of vanadate or ouabain to dogs decrease the renal excretion of sodium due to vasoconstriction of the renal tubules. Conversely, the simultaneous infusion of both inhibitors increase the renal excretion of sodium. At present, it is not known whether these effects are derived from a direct chemical interaction between the inhibitors, the enzyme and the inhibitors or from an indirect mechanism, such as the regulation of Ca^{2+} intracellular levels. In order to clarify these antagonic effects studies are undertaken to determine the chemical interaction of vanadate and the sodium-potassium ATPase using nuclear magnetic resonance spectra for ^{51}V. Preliminary results show a decrease in the intensity of the vanadium V polimeric species as a function of the enzyme concentration. Studies are underway to determine the line width and the effects of pH and magnesium concentration on the ^{51}V NMR spectra. (Supported by MBRS Grant No. S06-RR08216).

51. ^{13}C NMR Investigations of Enzyme Reactions. *N. J. Stolowich*, H. J. Williams, and A. I. Scott. Center for Biological NMR, Department of Chemistry, Texas A&M University, College Station, TX 77843.

In recent years the use of ^{13}C nuclear magnetic resonance spectroscopy has gained considerable attention to probe the reaction mechanisms catalyzed by enzymes. Employing ^{13}C enriched substrates, inhibitors, or cofactors, and in some cases using techniques such as cyroenzymology, the direct observation of enzyme bound intermediates is now possible. The findings of several reacent examples will be presented and their implications on the enzyme's proposed mechanism discussed.

52. Tentative Identification of a Factor III Oxidation Product and a Tetramethylated Uro'gen Adduct. *H. J. Williams*, N. J. Stolowich, and A. I. Scott. Center for Biological NMR, Department of Chemistry, Texas A&M University, College Station, TX 77843. G. Müller. Institüt für Organische Chemie, Biochemie, and Isotopenforschung, Universität Stuttgart, D-7000 Stuttgart 1, West Germany.

A new tetramethylated metabolite isolated from cultures of *Propionibacterium shermanii* has been tentatively identified using a combination of techniques including ^{13}C and INADEQUATE NMR and UV spectroscopy. An oxidation product of Factor III with possible synthetic uses has also been identified using the same techniques as well as ^1H detected ^{13}C polarization transfer spectroscopy.

53. A New Strategy for the Identification of Protein ^1H Spin Systems: Complete Assignment of the Blue Copper Proteins, Plastocyanin. *Peter E. Wright* and Walter J. Chazin. Department of Molecular Biology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037.

Detailed studies of the conformation and dynamics of proteins in solution by ^1H NMR spectroscopy require assignment of the spectrum as an essential first step. For studies of small proteins, Wüthrich and coworkers have developed a method for obtaining complete sequence specific assignment of the ^1H spectrum, which involves identification of the discrete ^1H amino acid spin systems, then subsequent assignment to a location in the sequence. We describe here a new approach to spin system identification, developed in response to the special requirements of applying the N.O.E. based sequence-specific resonance assignment method to proteins of 80 to 150 residues. There are two essential features to this strategy: the backbone amide proton, not the Cα proton, is used as a foundation for the identification of the spin systems; rather than tracing through the spin system in a stepwise fashion, spin systems are identified by relating *all* assignments directly to the backbone amide and, when necessary, to characteristic side chain terminal protons. Experimentally, this strategy is different because it places considerably greater reliance on spectra recorded in ^1H_2O and it requires the systematic application of relayed coherence transfer and spin-locking techniques to generate connectivities between spins that are not directly coupled to each other. The rationale and general features of this new strategy will be discussed in the context of the complete assignment of the ^1H NMR spectrum of the 99 residue, blue copper protein, plastocyanin.

Lipids and Membranes

54. Dietary Trans Fatty Acids Modulate Erythrocyte Membrane Fatty Acyl Composition and Insulin Binding in Monkeys. *D. E. Barnard*, E. Berlin, J. Sampugna, S. J. Bhathena, and J. J. Knapka. NIH, VRB, USDHHS, Bethesda, MD 20892, ARS, USDA, Beltsville, MD 20705, and University of Maryland, College Park, MD 20742.

Erythrocyte membrane linoleate (18:2ω6), oleate (cis 18:1ω9) and trans 18:1 isomer contents were modulated when twelve *Macaca fasicularis* monkeys were fed 10% fat, semipurified diets in which 48-49% of the cis monoenoic fatty acids were replaced with trans 18:1 isomers. Erythrocyte ghosts were prepared from blood samples drawn at three week intervals during the fifteen week study. Trans fatty acid feeding resulted in replacement of approximately one-half of the membrane oleate with trans 18:1 isomers and also increased 18:2ω6 content by 20%. Linoleate incorporation may provide a mechanism to maintain membrane fluidity and integrity. Membrane fluidity, by steady state DPH polarization, was indeed not affected by diet. Insulin binding was greater in ghosts from cis-diet fed monkeys. These results, and earlier studies with cis unsaturated fats in minipigs, rabbits, and human subjects, suggest that insulin receptor activity is dynamic requiring specific fluid membrane domains.

55. Hormonal and Dietary Control of LDL and HDL Fluidity and Fatty Acyl Composition in Women. *E. Berlin*, J. T. Judd, P. P. Nair, D. Y. Jones, and P. R. Taylor. ARS, USDA, Beltsville, MD 20705, and NCI, NIH, DHHS, Bethesda, MD 20892.

Fatty acyl composition of LDL and HDL phospholipid (PL) and cholesteryl ester (CE) fractions were subject to hormonal and dietary controls in healthy adult women. Plasma lipoproteins were isolated in 31 subjects fed diets with polyunsaturated to saturated (P/S) fatty acid ratios of 1.0 or 0.3. All were fed diets with 40% of energy as fat during four menstrual cycles, followed with 20% of energy as fat, diets for a similar period. Lipoproteins were sampled during the 4th cycle of each diet period at times of peak estrogen (follicular phase) and progesterone (luteal phase) secretion. In women fed P/S = 0.3 diets, decreasing fat intake lowered linoleate (18:2) and raised oleate (18:1) contents in LDL-PL, CE, and HDL-PL fractions in the follicular phase only. Subjects fed P/S = 1.0 diets showed similar effects on LDL-PL and CE fractions during both hormonal phases of the menstrual cycle but HDL-PL was only affected in the luteal phase. LDL and HDL fluidities, by DPH fluorescence, were directly related

to PL linoleate during the luteal phase alone indicating that fatty acyl control of lipoprotein fluidity is subject to hormonal influences.

56. Modulation of Eicosanoid Production in Vivo by Dietary Omega-3 Fatty Acids. A Pilot Study. *A. Ferretti* and V. P. Flanagan. Lipid Nutrition Laboratory, BHNRC, ARS, USDA, Beltsville, MD 20705.

Dietary supplementation with the fish oil concentrate MaxEPA resulted in the presence of prostaglandin $(PG)E_3$ in urine of a female subject (estimated 87 ng/24-h). The appearance of PGE_3 was concomitant with a reduction of PGE_2 levels (144 vs. 488 ng/24-h) and with extensive alteration of the fatty acid profiles of the PL, TG, FA, and CE fractions of plasma. This pilot study was conducted with a volunteer who ingested quantities of MaxEPA ranging from 10 to 50 g/day during four years. Identification of PGE_3 was by comparison of chromatographic behavior of two distinct derivatives of the natural PGE_3 with those of authentic material on OV-17 and SP-2330 columns, and by selected ion monitoring mass spectrometry. PGE_3, a cyclooxygenase metabolite of eicosapentaenoate, could not be detected in 24-h urine pools sixteen weeks after fish oil supplementation ended. The physiological impact of a shift of the renal PGE_2/PGE_3 balance has not yet been explored.

57. Dietary Levels of Trans Fatty Acids Sufficient To Cause Milk Fat Depression. *Beverly B. Teter*, Joseph Sampugna, and Mark Keeney. Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742.

Studies were initiated to investigate further the milk fat depression phenomenon, which we have observed in lactating mice fed *trans* fatty acids (tfa). Diets were prepared with decreasing amounts of tfa in the diet fat (15% to 0% of calories) to determine the minimum dietary level of tfa which would cause milk fat depression. Statistically significant ($p = 0.013$) milk fat depression was observed at tfa levels as low as 3% of the fat (0.6% of the calories) but not at 1.7% of the fat. Examination of mouse milk fat samples by light microscopy revealed that the fat globules in milk from mice consuming tfa had fewer large diameter globules than samples derived from mice fed no tfa.

58. Leakage of Fatty Acid Radicals during Lipoxygenase Catalysis. *L.-C. Yuan*, R. Krupinski-Olsen, and C. Kemal. Department of Pharmaceutical Research and Technologies, Smith Kline and French Laboratories, Swedeland, PA 19479.

The distribution of hydroperoxides from soybean lipoxygenase-1 catalyzed oxygenation of linoleate (LH) has been shown to vary as a function of LH and O_2 concentrations (pH 9). At low [LH] (20 M), only 13-hydroperoxy-(9*Z*,11*E*)-octadeca-9,11-dienoate (ZE13-LOOH) forms. At high [LH] (1.8 mM), after consumption of 200 μM O_2, three isomers of ZE13-LOOH form in a total yield of 35%. When α-tocopherol (40 M) is included, the rate of oxygen consumption is decreased by ca. 30%, and the yield of three isomers is reduced to 7%. In oxygen saturated solutions, the yield is also reduced to 15%. These results indicate that the isomers of ZE13-LOOH arise nonenzymatically, from autoxidation initiated by linoleate derived radicals released into solution from the active site, and that the rate of radical release is suppressed by increasing the concentration of O_2. In air saturated solutions, one α-tocopherol can consumed per ca. 28 molecules of O_2 taken up. Since each α-tocopherol can consume two peroxyl radicals, one LH derived radical must be released from the active site ca. once every 14 turnovers. Supported by NIH grant GM137286.

Nucleic Acids

59. Photoactivated DNA Cleavage in Vivo with $Rh(DIP)_3^{3+}$. *Mindy R. Kirshenbaum* and Jacqueline K. Barton. Department of Chemistry, Columbia University, New York, NY 10027.

Photoactivated DNA cleaving agents are being developed to probe local variations in DNA conformations in vivo. The complex tris(4,7-diphenyl-1,10-phenanthroline)rhodium(III), $Rh(DIP)_3^{3+}$, cleaves plasmid DNA efficiently upon irradiation at 360 nm. *Escherichia coli* cells, transformed with pBR322, when incubated with 10 μM $Rh(DIP)_3^{3+}$ concentrate (1000 fold) the rhodium complex within the cell, as measured by atomic absorption spectroscopy. Irradiation of the treated cells and subsequent plasmid isolation reveals DNA cleavage which changes as a function of irradiation time. The chiral metal complex recognizes and cleaves at conformationally distinct sites both in vitro and in vivo. Moreover, the distinctive sites mapped in vivo may be correlated with those found upon photoreaction in vitro.

60. Cloning, Sequencing, and Overexpression of Δ^5-3-Ketosteroid Isomerase (KSI) of *Pseudomonas testosteroni* in *Escherichia coli*. *A. Kuliopulos*, D. Shortle, and P. Talalay. Departments of Biological Chemistry and Pharmacology, Johns Hopkins School of Medicine, Baltimore, MD 21205.

A genomic library of *P. testosteroni* total DNA constructed from partial *Eco*R1 digests ligated to a λgtWES vector, was packaged into λ particles and probed with a 23-base oligonucleotide mixture [ATGAAC(T)ACC(A,T)CCG(C,A)-GAG(A)CAC(T)ATGAC] corresponding to the NH_2-terminal sequence of KSI (Benson et al., *J. Biol. Chem.*, *246*, 7514, 1971). Subclones derived from a recombinant phage containing a 5400 bp insert were sequenced and found to contain the desired 375 nucleotide open reading frame flanked at both ends by in-frame TGA termination codons. The DNA sequence agreed with the above 125-amino acid sequence except for residues 22, 24, 33 and 38 all of which coded for ASP rather than ASN. A 1370 bp fragment was inserted into pUC-19 vector and used to construct a strain of *E. coli* JM101 which overexpressed the KSI gene in the presence of isopropyl-β-D-thiogalactopyranoside. Cytosolic extracts of this strain contained a major protein band that migrated on SDS-PAGE with the KSI subunit and had 10% spec. act. of crystalline KSI. The chromatographic and kinetic properties, and inhibitor profiles of the enzyme were closely similar to those of pure KSI of *P. testosteroni*.

61. A Chiral Probe for the A-DNA Conformation Tris(tetramethylphenanthroline)ruthenium(II). *Houng Yau Mei* and Jacqueline K. Barton. Department of Chemistry, Columbia University, New York, NY 10027.

The design of conformation-specific DNA-binding molecules may be useful in exploring local variations in the secondary structure along the DNA strand. A chiral metal complex, tris(3,4,7,8-tetramethylphenanthroline)ruthenium(II), $Ru(TMP)_3^{2+}$, has been developed and shown to bind preferentially to A-form polynucleotides with enantiomeric selectivity. Coupling conformation-specific binding to metal-activated photoreaction yields conformation-specific DNA cleavage. Consistent with the binding results, $Ru(TMP)_3^{2+}$ cleaves the

A-form polymer upon irradiation while little cleavage is found for B-DNA. The observed enhancement of the photocleavage reaction in D_2O and inhibition by sodium azide is consistent with a singlet oxygen mediated reaction. Fine mapping experiments on a linear pBR322 restriction fragment have shown specific cleavage sites for this metal complex. These sites recognized by Λ-$Ru(TMP)_3^{2+}$ constitute 6-13 base pair homopurine-homopyridine stretches.

62. The B-DNA to Z-DNA Transition in Alkali and Tetraalkylammonium Salts Correlated with Their Effects on Solvent Structure. *R. S. Preisler.* Department of Chemistry, Towson State University, Towson, MD 21204.

Dissolved cations promote the B-to-Z transition in alternating purine-pyrimidine DNA sequences through a combination of effects, such as screening of phosphate repulsion, binding to DNA bases, and removing water of hydration from DNA. In an attempt to assess the importance of the last factor, the conformations of poly(dG-dC)·poly(dG-dC) and poly(dG-me^{5}dC)·poly(dG-me^{5}dC) were analyzed via ultraviolet spectroscopy and circular dichroism over a range of salt concentrations. Among the alkali halides, the efficiency in inducing the B-to-Z transition in poly(dG-dC) decreased with increasing atomic number from Na^+ to Cs^+, paralleling a decrease in cation interaction with water. No clear trend among these salts was observed with poly(dG-me^{5}dC), however. Increasing the alkali chain length from methyl to *n*-butyl in the tetraalkylammonium halides, which increases the solvent ordering effect of the cation, also enhanced their ability to promote the transition in both polynucleotides. The effects of nonaqueous solvents and a structure-forming anion were also studied. Work supported by a grant from the Faculty Research Committee, Towson State University.

63. High-Resolution Mapping of the DNA–Protein Interface in the *Eco*RI DNA Methylase System. *N. O. Reich* and M. Danzitz.

No abstract available.

64. Functional Analysis of Cysteines in the *Eco*RI DNA Methylase. *N. O. Reich* and L. DiMichele.

No abstract available.

65. Properties of the Nuclear Angiotensin II Receptor Revealed by Protein and DNA Studies. *Dale Seth, D. Lucio,* R. N. Re, J. Brown, and S. E. Bryan. Department of Biological Sciences, University of New Orleans, New Orleans, LA 70148.

Specific angiotensin II (AII) receptor binding sites in micrococcal nuclease generated nuclear fragments have been reported by this laboratory. Previous work has suggested that the AII nuclear receptors are proteins that bind DNA rather tightly. Furthermore, a discrete AII-binding nucleoprotein particle has been resolved on DNP gel electrophoresis. Among the experiments presented here are those carried out to reconstitute the nucleoprotein particle by interacting the AII-binding protein with DNA that has been transferred to nitrocellulose. Micrococcal nuclease generated nuclear fragments, subsequently digested with DNAase and RNAase, followed by a 24 hour dialysis have now been subjected to SDS-polyacrylamide gel electrophoresis. These gels treated with silver nitrate stain show four polypeptides ranging in molecular weight from approximately 36,000 to 66,000 daltons. Nondenaturing gel electrophoresis, gel permeation and affinity column chromatography studies of the hormone nuclear receptors have also been employed. Evidence for a possible link between copper and AII-nuclear receptor interactions is presented.

Mutagenesis and Carcinogenesis

66. Redox Cycling and Cigarette Tar-Induced DNA Damage. *Edward T. Borish,* Gary W. Winston, Walter A. Deutsch, Daniel F. Church, and William A. Pryor. Biodynamics Institute, Louisiana State University, Baton Rouge, LA 70803.

Aqueous extracts of cigarette tar consume dioxygen producing reduced oxygen species including superoxide, hydrogen peroxide and hydroxyl radicals. DNA that is exposed to cigarette tar does not support DNA synthesis in vitro. Cigarette tar introduces DNA single-strand breaks that are blocked by the presence of phosphoryl groups at the 3′-terminus. Kinetic studies reveal that the formation of an intermediate precedes the production of tar-induced DNA strand breaks. We postulate that components of tar bind to DNA and that hydroxyl radicals produced in a site-specific, metal-mediated pathway are primarily responsible for this DNA damage. Oxygen consumption by cigarette tar is enhanced by microsomal or mitochondrial electron transport systems. This is accompanied by enhanced oxy-radical production and we propose the involvement of redox cycling by guinonoid components of tar. Thus, the ability to redox cycle and bind to DNA increases the potential for cigarette tar to induce damage in crucial biological targets in vivo. These factors may contribute to the known carcinogenicity and cardiotoxicity of cigarette smoking.

67. Application of B-Protein as a Cancer Management Aid. *E. T. Bucovaz,* J. C. Morrison, W. D. Whybrew, and A. W. Schweikert. Departments of Biochemistry and OB/GYN, University of Tennessee, Memphis, Memphis, TN 38163, and Department of OB/GYN, University of Mississippi Medical Center, Jackson, MS 39216.

B-Protein, which appears in serum of cancer patients, was tested in retrospective and prospective studies as a cancer management aid. All patients included in the study had established metastatic disease other than or in addition to lymph nodes. The patients in both groups had received a variety of modalities of therapy. The retrospective study utilized B-Protein data in conjunction with CEA determinations. In the majority of cases, B-Protein data followed the medical history of the patient more closely than CEA results, and indicated the recurrence of the disease at a time when additional treatment might have been effective. The prospective study was conducted using only the B-Protein assay as a serum monitor. In this study, the B-Protein titer also was closely associated with the progression of the metastatic disease. Both retrospective and prospective studies showed that the serum titer of B-Protein is a reliable reflection of the course of the malignancy.

68. Mechanism of Increased Fibronectin Turnover after Transformation in Avian Tendon Cells. *Lai-Man Chan,* Camela J. Cobbins, Antony M. McCollum, and Syamal K. Ghosh. Department of Chemistry, Jackson State University, Jackson, MS 39217.

Transformation of primary avian tendon (PAT) cells by Rous sarcoma virus (RSV) leads to decreased levels of fibronectin (Fn). This work analyzes gelatin- and Fn-degrading proteases and examines the interaction of Fn with colla-

gen/gelatin. Both normal and transformed PAT cells contain two cell-associated Fn degrading proteases. Much stronger gelatin degrading proteases are found in normal and transformed cells; the majority of gelatinases are secreted into the medium. Transformed cells have at least two more gelatinases than normal cells. The gelatinases require Ca^{2+} and have a pH optimum between pH 7 and 9. In vitro studies demonstrate that the degradation of Fn is partially and specifically protected by gelatin and collagen, either in solution or when deposited on the substratum. Thus difference in the steady-state levels of Fn in normal and transformed PAT cells may be a consequence of changes in collagen levels. Supported by NIH RR08047 and DOE DEFG05-86ER 75274.

69. Benzoyl Peroxide-Induced Mitochondrial Damage. *Christopher H. Kennedy*, Gary W. Winston, Daniel F. Church, and William A. Pryor. Biodynamics Institute, Departments of Chemistry and Biochemistry, and Institute for Environmental Studies, Louisiana State University, Baton Rouge, LA 70803.

The free radical initiator benzoyl peroxide (BPO) is used as the active ingredient in acne medicines. BPO is a skin tumor promotor and enhancer. Since skin cells contain mitochondria and these organelles are a site of oxidative stress, we have studied the effect of BPO on mitochondria. When micromolar concentrations of BPO are added to mitochondria, inhibition of mitochondrial succinoxidase and NADH oxidase are observed. Uncoupling agents do not reverse the inhibition of respiration by BPO, indicating that BPO acts as an electron-transport chain inhibitor. BPO also induces rapid, large amplitude swelling of mitochondria; the swelling is not dependent upon the presence of respiratory substrate. No spin-trappable free radicals are observed when mitochondria are treated with BPO in the presence of the spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide, suggesting that this system is not an efficient generator of scavengable radicals. The mechanisms of BPO-induced respiratory inhibition and swelling will be presented.

70. Heparin, Boc-D-Phe-Pro-arginal, and Warfarin (Fibrin Antagonists) Inhibit Metastasis in an in Vivo Model. *Gerald F. Smith*, J. L. Sundboom, K. Best, P. D. Gesellchen, R. L. Merriman, R. Shuman, and B. L. Neubauer. Lilly Research Laboratories, Eli Lilly & Company, Indianapolis, IN 46285.

We have examined the hypothesis that tumor cells require fibrin formation in order to successfully metastasize because, if true, then agents which prevent fibrin formation would serve as a source of potential antimetastatic drugs. We have evaluated different types of fibrin formation antagonists for in vivo efficacy as anti-tumor agents in a rat PAIII prostatic adenocarcinoma model which measures spontaneous spread of tumor into lymph nodes (ileac and gluteal) and into the lungs. Warfarin, in daily oral doses, gave dose-dependent inhibition of pulmonary metastasis which correlated with anticoagulant effects and with factor II and factor VII blood levels. Heparin (15 unit/kg-h) and thrombin inhibitor Boc-D-Phe-Pro-Arginal (1 mg/kg-h) infused i.v. continuously during the 30 day test markedly prevented pulmonary and lymphatic metastasis. The anti-tumor efficacy of these dissimilar fibrin formation antagonists suggests that fibrin formation is essential for tumor spread and that novel anticoagulant agents (e.g., thrombin inhibitors) should be considered as a class of potential new antimetastatic agents.

General

71. Microbial Transformation of Over-the-Counter Antihistamines. *Eugene B. Hansen, Jr.*, Carl E. Cerniglia, Robert H. Heflich, Walter A. Korfmacher, Dwight W. Miller, and Kenneth J. Lambert. DHHS/PHS/FDA/NCTR, Jefferson, AR 72079.

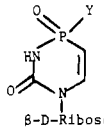
Interest in the toxicology of antihistaminic drugs occurred after the 1980 report of 100% liver tumor incidence induced in rats exposed to methapyrilene. We have applied microbial transformation methodologies to produce sufficient quantities of metabolites of pyrilamine, tripelennamine, methapyrilene, thenyldiamine, and triprolidine for structure elucidation and toxicological evaluations. *Cunninghamella elegans* grown in the presence of these drugs metabolized pyrilamine, tripelennamine, methapyrilene, and thenyldiamine to N-oxidized and O- and N-demethylated derivatives. Triprolidine was transformed predominantly to the hydroxymethyl derivative. These transformation products were isolated by HPLC and identified by their DCIMS and ^1H NMR properties. Pyrilamine, triprolidine, and their major fungal metabolites showed no appreciable mutagenic activity in *S. typhimurium* strains TA97, TA98, TA100, TA102, or TA104 with and without S9 activation. These results suggest that the fungal metabolism of these antihistamines occurs predominantly through metabolic pathways which do not result in mutagenic activation. In addition, the fungal antihistamine metabolites isolated in this study were similar to those previously reported in mammalian systems clearly indicating similarities between microbial and mammalian oxygenase systems.

72. Comparative Studies on the Metabolism of Doxylamine Succinate by Male and Female Fischer 344 Rats. *Claude L. Holder*, William Slikker, Jr., Walter A. Korfmacher, Harold C. Thompson, Jr., and Carl E. Cerniglia. HHS/FDA/NCTR, Jefferson, AR 72079.

The metabolism of doxylamine succinate, an ethanolamine type H_1 antagonist, was studied in the male and female Fischer 344 rat. The animals were housed two per cage and dosed by gavage with 13.3 and 133 mg/kg body weight with the test chemical. Seventy percent of the dose was eliminated in the first 24 h after dosing, and ninety-five percent of the dose was recovered after 72 h. A gradient HPLC system was used to isolate nine doxylamine metabolites, and the chemical structures of these metabolites were identified by mass spectrometry (MS) and nuclear magnetic resonance (NMR). Sex differences were observed in the excretion pattern for N-demethylated products of doxylamine as O-glucuronide conjugated and ring-hydroxylated nonconjugated metabolites. At the high dose, the male rats excreted 46.6 ± 1.2 (mean ± SE) percent of the dose as N-demethylated products, whereas the female rats excreted only 37.9 ± 1.8 percent of these metabolites ($p < 0.05$). At the low dose, a similar trend was observed (45.6 ± 2.8% male and 36.4 ± 2.6% female but was not significantly different. This observed sex difference in doxylamine metabolism/elimination should be considered in the interpretation of data from doxylamine succinate chronic bioassay studies in male and female Fischer 344 rats.

73. Chemoenzymatic Synthesis of 5,6-Dihydroxyindole Derivatives. *Mu-Ill Lim* and Dilip G. Patil. Clairol Inc., 2 Blachley Rd., Stamford, CT 06922.

5,6-Dihydroxyindole and its derivatives, important intermediates in the hair melanin biosynthetic pathway as well as purported monomer unit of the polymeric eumelanin, brown or black hair pigment, are believed to be produced from DOPA, dopamine, epinephrine and norepinephrine. The syntheses of 5,6-dihydroxyindole derivatives *3* were accomplished by enzymatic oxidation of catecholamines *1* with tyrosinase to aminochromes *2* and subsequent reduction.



1: Y = NH_2 2: Y = SPh

74. Reduction of Lumiflavin in Dioxane. *R. Marrero*, M. Aponte, R. Melendez, A. E. Perez, J. L. Reyes, and R. V. Toledo. Chemistry Department, Humacao University College, Humacao, PR 00661.

Lumiflavin (7,8,10-trimethylisoalloxazine) and certain derivatives have been reported to act as model compounds of enzymes containing flavin as a prosthetic group. Lumiflavin was reduced by sodium borohydride in freshly distilled dioxane and traces of added water to produce 1,5-dihydrolumiflavin under nitrogen. A quantitative spectrophotometric determination of reduced flavin with aqueous ferricyanide under nitrogen was performed. Rapid reoxidation of the 1,5-dihydroflavin was observed with concommitant production of hydrogen peroxide as determined by standard iodometric methods. A solution of Lumiflavin in dioxane was treated with the reduced form of nicotinamide adenine dinucleotide phosphate, NADPH. Results and implications on biochemical processes in low dielectric constant environments will be presented.

75. Spectral Properties of Alkoxyphenoxazone Compounds: Dependence on Concentration and Solvent. *Jean Rabovsky*, Elizabeth Heflin, Deloris J. Judy, and Natalie Sapola. Nat'l. Inst. Occup. Safety & Health, Div. Resp. Dis. Studies, Morgantown, WV 26505.

We have investigated the spectral properties of four alkoxyphenoxazone compounds, methoxy (MeO-)-, ethoxy (EtO-)-, pentoxy (PeO-)- and benzyloxy (BzO-)-phenoxazone (Ph) (used as substrates for cytochrome P450-dependent monooxygenase reactions). Wavelengths of maximum absorbance (λ_{max}) and extinction coefficients (ϵ)(mM^{-1} cm^{-1}) were measured in EtOH and aqueous solution (0.05 M Hepes buffer, pH 7.8) from 340–600 nm. All compounds exhibited linear dependence with concentration in EtOH with a λ_{max} = 460 nm and ϵ^{ROPh} = 20.6-25.2. Linearity was not observed at concentrations greater than 2 μM PeOPh and BzOPh and 10 μM EtOPh. In the case of PeOPh, at concentrations greater than 10 μM, two distinct peaks were observed: a low wavelength peak at 370 nm and a high wavelength peak at 425 nm. As the concentration approached the linear region, the high wavelength peak shifted to 480 nm and displayed an increase in ϵ^{PeOPh} from less than 10 to 22, while the peak at 370 nm (ϵ^{PeOPh} = 7) changed to a shoulder with no change in ϵ. The results suggest that phenoxazone compounds containing large bulky substituents, interact in aqueous solution to form a species with unique spectral properties.

76. Interactions of Pentose and Purine Analogs of Adenosine with Human Platelet Rich Plasma Suspensions. *B. H. Ragatz* and G. Modrak. Ft. Wayne Center for Medical Education. J. Papiez. Chemistry Department, IPFW Campus, Ft. Wayne, IN 46805.

Three pruine analogs [1-*N*-methyladenosine (1-Me Ad); 8-bromoadenosine (Br Ad); 2-chloroadenosine (Cl Ad)] and two pentose derivatives [3′-*O*-methyladenosine (3′-Me Ad); 2′,3′-dideoxyadenosine (DD Ad)] of adenosine (Ad) have been incubated alone with platelet rich plasma (PRP), or with PRP followed by adenosine 5′-diphosphate (ADP) additions at either 30 s or 5 min. As expected, none of these compounds induce platelet aggregation. Among the purine analogs, Cl Ad produced a dose dependent inhibition of ADP induced aggregation (2 × of Ad), and 1-Me Ad exhibited a weak, dose dependent inhibition (1/100 × of Ad). With the two pentose derivatives, no dose dependent inhibition was seen for rapid ADP additions. DD Ad showed a slight time dependent enhancement of inhibition (< 1/100 of Ad). The implications of goodness-of-fit of these five compounds at the platelet Ad receptor will be discussed.

77. Effects of Aerobic Exercise on Human Platelet Count and Activation. *B. H. Ragatz* and G. Modrak. Ft. Wayne Center for Medical Education, Indiana University School of Medicine, Ft. Wayne, IN 46805.

Platelet count (PC) and responsiveness to various aggregating agents [adenosine 5′-diphosphate (ADP); epinephrine (EPI); collagen; and ristocetin] were evaluated in healthy human males (age 19-45 years) before and following a 2.5 mile run. PC increased 13%. No significant change in onset or extent of reversible or irreversible ADP induced or EPI induced aggregation was observed. There was also no alteration in adenosine inhibition of ADP or EPI induced aggregation following this exercise. A slight increase in onset but a 7% decrease in magnitude of collagen induced aggregation was seen. Finally, a 90 s decrease in onset time but no change in magnitude of ristocetin induced aggregation was observed. The effects of aerobic exercise on PC and platelet activation will be discussed in light of these observations and previously published results which suggest plasma factor VIII levels also increase following short periods of aerobic exercises.

WEDNESDAY AFTERNOON—REPLIGEN AWARD IN BIOLOGICAL CHEMISTRY SYMPOSIUM—J. DIXON, PRESIDING

78. Oxidation Reactions Catalyzed by Metallo(III) Porphyrins. *Thomas C. Bruice.* Department of Chemistry, University of California, Santa Barbara, CA 93106.

Catalase, peroxidase, and cytochrome P-450 enzymes are raised to their biochemically important highest oxidation state by the transfer of an oxygen atom to apoenzyme bound iron(III) protoporphyrin IX. Oxidation of substrate results in the regeneration of the iron(III) protoporphyrin IX moiety. Spectral, crystallographic, and dynamic studies have defined the structures of the higher-valent intermediates of the enzymes (at least for catalases and HR peroxidase), the substrate specificity, and those aspects of enzyme mechanism which may be addressed by the use of isotopes and retention or non-retention of stereochemical integrity. Present studies are directed towards the synthesis of the higher-valent metallo-oxo porphyrin species and the determination of the details for both the formation of these species and the mechanisms and rates of their oxidation of substrate molecules. The lecture will describe the contribution of this laboratory to these investigations.

79. Towards the Design of Enzymes. *E. T. Kaiser.* Laboratory of Bioorganic Chemistry and Biochemistry, The Rockefeller University, New York, NY 10021-6399.

As part of our protein engineering effort we have been engaged in modifying enzyme active sites and structural regions by either site-directed mutagenesis or by chemical modification (semisynthetic enzymes). One of our target systems for the mutagenesis studies has been *E. coli* alkaline phosphatase. In our use of chemical modification we have introduced new coenzyme groups covalently at the active sites of enzymes like papain and glyceraldehyde-3-phosphate dehydrogenase, altering in some cases (e.g., flavopapain) the types of reactions catalyzed by the enzyme. Most recently, we have undertaken studies on the application of semisynthetic or mutant enzymes in synthesis. We are examining the properties of a mutant endopeptidase in ligating appropriate peptide active esters to give large peptide products. An important goal of our work is to construct small enzymes and redesigned analogs through peptide fragment synthesis-condensation.

80. Thymidylate Synthetase. *Daniel V. Santi.* Departments of Biochemistry & Biophysics and Pharmaceutical Chemistry, University of California, San Francisco, CA 94143.

Thymidylate synthetase (TS) catalyzes the reductive methylation of dUMP by 5,10-methylenetetrahydrofolate to give dTMP and dihydrofolate. Because this enzyme represents the sole de novo path to dTMP, it has been extensively studied and is the target for a number of drugs. The enzyme from different sources is highly conserved, with about 75 invariant amino acid residues in the protein from all sources. A considerable amount of information is available concerning the catalytic mechanism and inhibition of TS. The hallmark of catalysis is that the reaction involves the formation of a covalent bond between the 6-position of the substrate dUMP and a nucleophilic thiol of a cysteine group. Thymidylate synthetase is of additional importance because is serves as a paradigm for numerous other enzymatic reactions which involve electrophilic substitution reactions at the 5-position of pyrimidines. These include dUMP and dCMP hydromethylases, tRNA methylase, DNA-cytosine methylase, and other related enzymes. I will describe the background studies which have led to our understanding of the catalytic mechanism and inhibition of TS. I will also describe the 3-dimensional structure of TS from *L. casei*, which has recently been solved, and discuss aspects of the structure-function of TS.

81. Implications for Protein Function from Kinetic Analysis. *S. Benkovic*, C. Fierke, K. Johnson, and R. Kuchta. Department of Chemistry, The Pennsylvania State University, University Park, PA 16802.

Kinetic schemes and associated free energy profiles have been obtained for the *E. coli* dihydrofolate reductase and polymerase I (Klenow fragment) that predict the steady-state kinetic parameters. A key feature of the reductase sequence is that product dissociation follows a preferred pathway in which H_4 folate dissociates after NADPH replaces $NADP^+$ in the ternary complex. In contrast the polymerase sequence shows multiple dNMP incorporations before dissociation of the enzyme·DNA complex. Thus both these reactions are limited by enzyme·product dissociation. The internal equilibria $E{\cdot}H_2folate{\cdot}NADPH \rightleftharpoons E{\cdot}H_4folate{\cdot}NADP^+$ and $E{\cdot}DNAdNTP \rightleftharpoons E{\cdot}DNA^{+1}{\cdot}PP_i$ are 1800 and 2.5 respectively, although the chemical equilibria in both reactions are both $>10^3$. This marked difference may reflect the partial linkage in the case of the polymerase of ΔG to nonchemical processes requiring protein movement or conformation changes as required for maintaining fidelity in replication. Active site mutations on the dihydrofolate reductase at a conserved Phe-31 residue have revealed compensatory changes in the free energy profiles--increased product dissociation rates balanced by decreased rates of hydrate transfer. The more important function of Phe-31, however, is to maintain an active site environment that allows general acid catalysis at physiological pH.

THURSDAY MORNING—ELI LILLY AWARD FOR FUNDAMENTAL RESEARCH IN BIOLOGICAL CHEMISTRY SYMPOSIUM—S. J. LIPPARD, PRESIDING

82. Large DNA Technology and Its Applications. *Charles R. Cantor*, Jian-Bing Fan, Stephanie Klco, Mathew K. Mathew, and Cassandra L. Smith. Department of Genetics and Development, Columbia University, New York, NY 10032.

Methods have been developed that are capable of preparing intact DNA molecules as large as 9 million base pairs (6 billion Daltons), fractionating these by size, and cleaving these into discrete fragments for further analysis. These methods include schemes for preparation and enzymatic manipulation of DNA in solid state matrices, and techniques for separating such large molecules by agarose gel electrophoresis using pulsed electric fields. The principles and practice of these methods will be illustrated and examples will be shown of how they can be used to provide physical maps of the structures of entire chromosomes.

83. Synthetic Sequence Specific DNA Binding Molecules. *Peter B. Dervan.* Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.

The design of sequence specific DNA binding molecules has advanced in recent years due, in part, to the development of analytical techniques such as footprinting and affinity cleaving which allow rapid and precise analysis of hundreds of potential DNA binding sites. The construction of synthetic molecules that bind in the minor and major groove of DNA with incrementally increasing sequence specificity is the first step toward developing a set of rules for the three dimensional readout of double helical DNA.

84. DNA Binding Properties of Intercalator-Linked Platinum Complexes. *Stephen J. Lippard.* Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

The antitumor drugs *cis*-diamminedichloroplatinum(II) (*cis*-DDP), and $[Pt(en)Cl_2]$ bind covalently to double-stranded DNA, forming intrastrand crosslinks preferentially at d(GpG) and, to a lesser extent, d(ApG) sequences. At low levels of platinum binding, certain sequences of this kind are less preferred than others, as mapped by exonuclease III digestion. These sequence preferences can be altered by addition of ethidium bromide during the platination reaction, but not by added acridine orange or various phenanthridium analogs of ethidium. The molecule (AO(C6)-Pt, in which acridine orange is linked by a hexamethylene tether to dichloroethylenediamineplatinum(II), also shows altered regioselectivity for enzyme detectable DNA binding compared with $[Pt(en)Cl_2]$. The relative abilities of intercalators to modify platinum binding preferences are shown to be correlated directly with

the dissociation rates of the intercalators from their sites on DNA. The design of, and synthetic approach to, new intercalator-linked platinum complexes, formulated to address the fundamental question of whether only some or all platinum binding sites on DNA are enzyme detectable, will be discussed.

85. Targeting DNA Sites with New Probes and Reactions of Chiral Metal Complexes. *Jacqueline K. Barton.* Department of Chemistry, Columbia University, New York, NY 10027.

Chiral metal complexes serve as uniquely sensitive probes to examine the local variations in DNA conformation. We have designed complexes to distinguish left and right handed helices and are now exploring new means to obtain complexes which either recognize different sites along the DNA strand or show differing sequence-dependent reactivity. Once such probe is Λ-$Ru(TM)_3^{2+}$, which selectively recognizes local regions in the A-conformation. To extend the utility and reactivity of chiral metal complexes as conformationally-specific "nucleases", a double stranded cleaving agent has also been prepared. The complex maintains the features for site recognition characteristic of its analogue, Λ-$Co(DIP)_3^{3+}$, shown earlier to cleave DNA at conformationally distinct sites such as Z-DNA. But the novel chiral complex also contains two polyamine arms designed to bind metal ions and so deliver metal centered reactions to both DNA strands, yielding double stranded scission. An understanding of how these complexes discriminate between DNA sites may serve as useful reactive models for protein-nucleic acid interactions. Moreover, the chiral metal complexes may be used, much like site-specific enzymes, to map with remarkable specificity the distinctive conformations present along native DNA substrates in vitro and even within the cell.

THURSDAY AFTERNOON—PFIZER AWARD IN ENZYME CHEMISTRY SYMPOSIUM—H. FRAUENFELDER, PRESIDING

86. Mechanism of Suicide Inactivation of Beta-Lactamase by Penicillin Sulfones. *A. L. Fink*, L. Ellerby, P. M. Bassett, E. A. Chichester and M. Hsieh. Department of Chemistry, University of California, Santa Cruz, CA 95064.

The inactivation of beta-lactamase from *B. cereus* by several penicillin sulfones was investigated. The results with nafcillin sulfone are representative. Inactivation is optimal around pH 9, 37 °C. The inactive enzyme undergoes significant acid-catalyzed reactivation at pH 6 and below. Inactivation involves the formation of a covalently bound, chromophoric enamine. Tritium labeling indicated that the penicillamine sulfone moiety was not lost during inactivation. Inactivation involves two different processes, one under kinetic, the other thermodynamic, control. At low ionic strength the inactivation rate is first-order whereas at high ionic strength it becomes biphasic, the second process becoming much slower. This second process was shown to involve a major conformational change in the enzyme, as determined by far-UV circular dichroism, IEF and ANS-binding. The data suggest that formation of the enamine triggers a conformational change which results in inactivation by preventing deacylation. This mechanism is significantly different from that proposed for the *E. coli* enzyme with penicillanic acid sulfone (Brenner and Knowles, *Biochemistry*, *23*, 5833, 1984).

87. Structures of Site-Directed Mutants of Aspartate Aminotransferase. *Dagmar Ringe.* Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139. Douglas L. Smith. Eastman Kodak Company, Rochester, NY 14650.

Aspartate aminotransferase catalyzes the reaction: L-Asp + 2-oxoglutarate = oxaloacetate + L-Glu. The enzyme is central to amino acid metabolism and is the best studied of the vitamin b_6 (pyridoxal phosphate) requiring enzymes. The enzyme from *E. coli* has been cloned and expressed in *E. coli* (B. A. Malcolm & J. F. Kirsch, *Biochem. Biophys. Res. Commun. 132*, 915–921, 1985). The properties of the wild type and two engineered mutants have now been studied both enzymatically and crystallographically. In the first mutant, the lysine residue which forms a Schiff base to pyridoxal phosphate has been changed to alanine. Consequently, the enzyme can no longer function as a transaminase. However, it now acts as a decarboxylate for oxaloacetate. The crystal structure of this mutant will be described. In the second mutant, the arginine which forms one of the specificity sites has been changed to aspartic acid. The enzyme now acts as an arginine aminotransferase, albeit slowly. The crystal structure of this mutant will also be described. The structures of the mutants give some indication why the single mutations made create enzymes with different functionality although not very efficient. Analysis of these structures also suggest additional modifications to improve them.

88. Yeast Triose Phosphate Isomerase: What Can We Learn about Enzymatic Catalysis and Stability from a "Simple" Enzyme? Thomas C. Alber, Robert C. Davenport, Jr., Elias M. Lolis, Dagmar Ringe, and *Gregory A. Petsko.* Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

The reaction catalysed by the glycollytic enzyme triose phosphate isomerase is one of the simplest in metabolic biochemistry, and has been dissected both kinetically and energetically in great detail. The crystal structure of the enzyme is known, as are the structures of the enzyme-substrate and enzyme-transition state analog complexes. The genes for both the yeast and chicken muscle enzymes have been cloned, sequenced and expressed in *E. coli*. A number of site-specific mutants have been made and characterized, some crystallographically. The results from all of these studies suggest that this "simple" enzymatic reaction is catalysed by a number of factors in concert, among which are: general base catalysis, electrostatic stabilization of the transition state, steric destabilization of an unfavorable structure for the enediol intermediate, and steric desolvation of the active site. It appears to be incorrect to consider catalysis by this enzyme in terms of specific functional groups with precise chemical roles. Rather, the enzyme provides a catalytic surface on which many factors are brought to bear on the substrate. Further progress will require techniques not yet employed on this enzyme.

89. Protein Dynamics and X-ray Diffraction. *Hans Frauenfelder.* Department of Physics, University of Illinois at Urbana–Champaign, 1110 West Green St., Urbana, IL 61801.

Proteins are dynamic systems; their internal motion is essential for their biological function. To understand the relation of static and dynamic structure to function, both the spatial and the temporal characteristics of proteins must be explored. X-ray diffraction studies yield the spatial information, in particular the extent of the spatial fluctuations through the Debye-Waller factor, the flexibility of various parts through the thermal expansion, and the overall motions through com-

parison of the structures in different states. The corresponding temporal information is obtained through a multitude of techniques, for instance the observation of ligand binding after flash photolysis over a wide range of temperature and time, the monitoring of the proteinquakes that follow protein reactions, and the detection of changes in the infrared spectrum after perturbations. The combination of the spatial and temporal information yields rich insight into the dynamics of heme proteins.

90. Bacterial Enzymes Utilizing D-Alanine. *C. T. Walsh.* Departments of Chemistry and Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Mechanistic studies on bacterial enzyme generating or utilizing D-alanine for peptidoglycan biosynthesis will be discussed.

WEDNESDAY—EDWARD E. SMISSMAN–BRISTOL-MYERS AWARD IN MEDICINAL CHEMISTRY SYMPOSIUM (COSPONSORED WITH MEDI)—L. B. TOWNSEND, PRESIDING

Probing Enzyme–Inhibitor Complexes by Chemical Synthesis and ^{13}C NMR. *Daniel H. Rich*, Douglas Kalvin, Juergen Maibaum, Theo Hofmann, Mitsuo Oka, and Paul G. Schmidt. School of Pharmacy, University of Wisconsin—Madison, Madison, WI 53706, Department of Biochemistry, University of Toronto, Toronto, Canada, and Vestar Inc., 939 E. Walnut St., Pasadena, CA 91106.

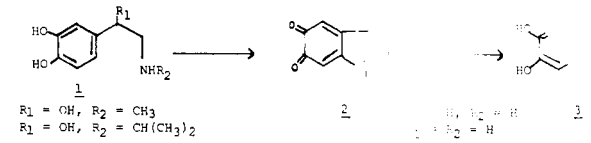
Tight-binding enzyme inhibitors are being used increasingly as lead compounds for the development of new drugs and for characterizing enzyme-ligand interactions and catalytic mechanisms. This talk will present recent data on the mechanism of inhibition of target proteases by analogs of the naturally occurring protease inhibitor, pepstatin. ^{13}C-labeled lysinestatone (LySto) has been synthesized and used to study the binding of this inhibitor to penicillopepsin by ^{13}C NMR. ^{13}C-Labeled derivatives of cysteinestatine (CySta) in conjunction with isotope directed NOE methods (IDNOE) are being used to determine nearest neighbor interactions in CySta derivatives bound to penicillopepsin. The use of these methods to "map" enzyme active sites by NMR will be described.

Inactivation of *E. coli* Ribonucleotide Reductase by N_3UDP. S. Salowe, M. Ator, and *J. Stubbe.* Department of Biochemistry, University of Wisconsin—Madison, Madison, WI 53706.

Incubation of ribonucleotide reductase (RNR) with substrate analog 2′-azido-2′-deoxyUDP (N_3UDP) results in enzyme inactivation and destruction of the tyrosyl radical cofactor in a single turnover. Studies using [β-^{32}P] and [azido-^{15}N]–labeled compounds have revealed the production of 1 equivalent each of PP_i, N_2, and uracil. When RNR is inactivated by [5′-^3H]N_3UDP, the B1 subunit of the enzyme is specifically radiolabeled with 1 equivalent of ^3H. The activity of this subunit is reduced by half, consistent with the modification of only one of the two active sites. The production of 0.2 equivalent of H_2O during inactivation of RNA by [3′-^3H]N_3UDP indicates that 3′ C-H bond cleavage occurs. Furthermore, an isotope effect upon RNR inactivation and tyrosyl radical loss is observed with [3′-^2H]N_3UDP, although the kinetics of these processes are complex. This remarkable sequence of events is accompanied by the formation of a new N-centered radical derived from N_3UDP [Sjöberg et al. (1983) *J. Biol. Chem. 258*, 8060-8067; Ator et al. (1984) *J. Am. Chem. Soc. 106*, 1886–1887]. A mechanism will be presented which accommodates the observed kinetics and products of the reaction and their relationship to normal substrate turnover.

Design, Synthesis, and Evaluation of Inhibitors of Nucleoside Metabolism. *Paul A. Bartlett*, Diana Burman, Uday Maitra, John M. Scholtz, and Gary W. Ashley. Department of Chemistry, University of California, Berkeley, CA 94720.

Consideration of the chemical mechanism of an enzyme-catalyzed transformation can be a crucial element in the design of a potent inhibitor. The synthesis of pyrimidine analogs containing a phosphorus atom at the 4-position of the heterocycle (I) leads to an effective reversible cytidine deaminase inhibitor, for example, presumably because the tetrahedral phosphorus mimics the unstable tetrahedral intermediate of the enzymatic transformation. In contrast, substitution of the phosphorus with a leaving group (2) affords an irreversible inhibitor of cytidine deaminase. Our progress towards understanding the manner in which these inhibitors interact with the target enzyme will be described, along with our progress toward the synthesis of multi-substrate analog inhibitors of carbamyl phosphate synthetase and purine nucleoside phosphorylase.



Award Address. Edward E. Smissman–Bristol-Myers Award in Medicinal Chemistry Sponsored by Bristol-Myers Company and Administered by the ACS Division of Medicinal Chemistry.

SUNDAY, MONDAY, AND TUESDAY—SYMPOSIUM ON METAL CLUSTERS IN PROTEINS (COSPONSORED WITH INOR)—L. QUE, J. PENNER-HAHN, K. D. KARLIN, AND B. A. AVERILL, PRESIDING

Tutorial: Morning Session

Synthetic Strategies for Modeling Dinuclear Copper and Iron Sites in Biological Systems. *Kenneth D. Karlin.* Department of Chemistry, State University of New York (SUNY) at Albany, Albany, NY 12222.

It has been well established that dinuclear metal centers, containing two copper or iron atoms in close proximity, are found in a variety of metalloprotein active sites. For copper, these include the dioxygen carrier protein, hemocyanin, the monooxygenase tyrosinase, and the copper oxidases, including laccase. Hemerythrin is a diiron containing O_2-carrier, while ribonucleotide reductase and purple acid phosphatases are also known to possess dinuclear iron centers. Bioinorganic chemists are actively involved in modeling studies, and the role of such investigations in establishing relevant coordination chemistry and in elucidating protein structure and function will be assessed. The main approaches utilized in biomimetic studies will be described, including those aimed at providing structural and/or spectroscopic and/or functional models. Examples of model systems which address each of these aspects will be highlighted, focusing primarily on those relevant to O_2-carrying proteins. The importance of systematic approaches in biomimetic studies will be emphasized and examples of what can be learned in this manner will be provided. Ligand design and synthesis will also be discussed.

Synthetic Strategies for Modelling Metal–Sulfur Sites. *B. A.*

Averill. Department of Chemistry, University of Virginia, Charlottesville, VA 22901.

Synthetic models for the metal-sulfur sites present in Fe-S proteins and nitrogenase have been developed in a number of laboratories. The most common approach to date has been spontaneous core assembly, involving reaction of iron salts, mercaptides, and sulfide or sulfur. This has yielded accurate models for the 1Fe, 2Fe-2S, and 4Fe-4S centers found in the "simple" iron-sulfur proteins, as well as tri- and hexanuclear clusters no found in proteins. Utilization of sterically hindered ligands has allowed generation of previously unstable oxidation states. In contrast, despite a great deal of effort, an accurate synthetic representation of the novel Mo-Fe-S cluster present in the FeMo-cofactor of nitrogenase has not yet been achieved. Spontaneous core assembly reactions utilizing Mo_4^{2-} have yielded only "linear" and "cubane" clusters containing the MoS_2Fe and $MoFe_3S_4$ units, respectively. Recent synthetic efforts have utilized systematic approaches to the design of clusters possessing specific structural features that may be more relevant to the structure of the FeMo-cofactor.

Aconitase: Evolution of the Active Site Picture. *Mark H. Emptage.* Central Research & Development Department, E. I. du Pont de Nemours & Co., Wilmington, DE 19898.

Aconitase, the second enzyme of the citric acid cycle, catalyzes the interconversion of citrate and isocitrate via a dehydration/hydration reaction. Only within the past few years has it been known that aconitase is an Fe-S protein. This result discredited the "ferrous wheel" model of aconitase, which featured a monomer iron active site, and raised questions concerning the role of a redox active prosthetic group in an enzyme which catalyzes no apparent redox chemistry. The answer to this question has required the combined information gleaned from several techniques; most notably, EPR, Mössbauer, and ENDOR spectroscopy. The results from these studies and others have led to the discovery of cluster structural interconversions controlling enzyme activity, new Fe-S cluster structures, and the direct coordination of substrate to one iron of a [4Fe-4S] cluster. Specific isotopic labeling has allowed a detailed picture of the substrate-cluster interaction from the point of view of both the Fe-S cluster using Mössbauer spectroscopy and the substrate using ENDOR spectroscopy. Finally, recent studies have produced several other enzymes that catalyze similar hydration and/or dehydration reactions which apparently utilize an Fe-S cluster.

Tutorial: Afternoon Session

Combining Electron Paramagnetic Resonance and Mössbauer Spectroscopy. *E. Münck.* Gray Freshwater Biological Institute, University of Minnesota, Navarre, MN 55392.

By performing EPR and Mössbauer spectroscopy on the same sample, or suitably prepared "parallel" samples, a wealth of information can be obtained. In this tutorial a variety of points will be discussed, including: Kramers vs. non-Kramers systems; shapes of Mössbauer spectra inferred from EPR; spin quantitations; separating contributions from different clusters in multi-cluster proteins; prospects for the near future.

Magnetic Circular Dichroism Studies of Iron–Sulfur Proteins. *Michael K. Johnson.* Department of Chemistry, University of Georgia, Athens, GA 30602.

Variable temperature magnetic circular dichroism (MCD) spectroscopy is being increasingly used to probe the electronic and magnetic properties of metal centers in metalloproteins. This paper discusses the experimental and theoretical aspects of MCD spectroscopy that are applicable to the study of metal clusters in proteins. Using simple iron-sulfur proteins such as rubredoxins and ferredoxins as examples, results are presented that illustrate the type of information that is available via detailed analysis of low temperature MCD data. This includes the resolution and assignment of electronic transitions, the identification of cluster-type, and the characterization of ground state properties such as spin state, effective g-values, and zero field splitting parameters. Finally the utility of low temperature MCD studies for selectively investigating the properties of discrete clusters in multicomponent enzymes is discussed using both the MoFe and VFe nitrogenase proteins from *Azotobacter vinelandii* as examples.

Resonance Raman Spectroscopy of Metal Clusters in Proteins. *Joann Sanders-Loehr.* Department of Chemical Sciences, Oregon Graduate Center, Beaverton, OR 97006.

Resonance Raman spectroscopy is of particular use for studying metal-ligand vibrational modes in metalloproteins containing chromophoric metal clusters. Intense ligand → metal charge transfer transitions give rise to substantial enhancements of Raman scattering intensities, thereby allowing metal-ligand and ligand vibrational modes to dominate the observed spectra. Specific ligands such as the phenolate group of tyrosine, the thiolate group of cysteine, and the imidazole group of histidine can be identified from the appearance of distinctive vibrational frequencies. In the binuclear copper protein, hemocyanin, imidazole assignments have been verified by frequency shifts accompanying deuterium exchange. For the oxo-bridged binuclear iron proteins, as well as the binuclear and tetranuclear iron-sulfur proteins, vibrations associated with the bridging ligands can be identified by replacement with 18-O (oxo) and 34-S (sulfido) isotopes. In the case of the iron-sulfur proteins such as the two- and four-iron ferredoxins, the pattern of spectral peaks provides information on the coordination geometry of the cluster. For the oxo-bridged binuclear iron proteins such as hemerythrin and ribonucleotide reductase, the frequency and intensity of the Fe-O-Fe symmetric stretch relates to Fe-O-Fe angle and the disposition of other ligands in the cluster.

Use of X-ray Absorption Spectroscopy for Characterizing Metal Clusters in Proteins: Possibilities and Limitations. *James E. Penner-Hahn.* Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055.

X-ray absorption spectroscopy has in the last ten years become a widely used technique for investigating the immediate structural environment of the metal sites in metalloproteins. The information which can be obtained from X-ray absorption, both in the extended X-ray absorption fine structure (EXAFS) and in the X-ray absorption near edge structure (XANES) regions will be reviewed. Different approaches to data analysis will be surveyed with emphasis on their relative ease of use, applicability, and reliability. Recent results, including studies of the multi-copper enzyme laccase will be presented in order to highlight both the potentials and the potential limitations of the technique.

First Session: Binuclear Centers Containing Copper

^{1}H NMR Spectra of Cu_2Co_2SOD. *I. Bertini.* Department of Chemistry, University of Florence, Florence, 50121 Italy.

The occurrence of magnetic coupling between metal ions with different electronic relaxation times provides further

relaxation mechanisms for the slow relaxing electrons (I. Bertini and C. Luchinat, NMR of Paramagnetic Species in Biological Systems, Benjamin/Cummings, Menlo Park, Ca, 1986). This aspect has been investigated through water ^1H nuclear magnetic relaxation measurements performed in an extended range of magnetic fields. In Cu_2Co_2SOD magnetic coupling causes a decrease in the electronic relaxation time of copper(II) from 10^{-9} to 10^{-11} s; the ^1H NMR spectra have been observed for all the protons of all histidines bound to both cobalt and copper. Analysis of T_1, T_2 and shifts at magnetic fields between 60 and 400 MHz has allowed us to perform a complete assignment of the signals and to estimate the correlation times in the coupled system. Structural differences among isoenzymes and mutants and reactivity with inhibitors have been studied. Implications for the mechanism are obtained.

Models for Copper Proteins: Reversible Binding and Activation of O_2 and the Reactivity of Peroxo and Hydroperoxo Dicopper(II) Complexes. *Kenneth D. Karlin*, Amjad Farooq, Richard W. Cruse, Y. Gultneh, Michael S. Haka, Brett I. Cohen, Phalguni Ghosh, Zoltan Tyeklar, and Jon Zubieta. Department of Chemistry, State University of New York (SUNY) at Albany, Albany, NY 12222.

The reactivity of Cu(I) centers with O_2 is of interest, in part due to the relevance to dioxygen-activating proteins such as hemocyanin (O_2 carrier) and tyrosinase (monooxygenase). The types of model systems which can bind dioxygen reversibly at low temperatures will be described. In the first one, a phenoxo-bridged dicopper(I) complex binds O_2 to give a peroxo-dicopper(II) species (*1*) in which the cycling between $Cu(I)_2$ and $Cu(II)_2$-(O_2^{2-}) forms can be followed spectrophotometrically; CO and PPh_3 can displace the bound O_2 ligand. The other system utilizes dinucleating ligands not possessing a potential bridging group for the copper ions, and the reversible binding of O_2 is accompanied by the formation of UV-VIS spectral features very similar to that of oxyhemocyanin. Possibilities for the structures of these O_2-complexes will be discussed. A protonated form of *1* (i.e., hydroperoxo species) can be generated; it can effect the oxygenation of PPh_3 and RSR. New developments in our system which is a model for copper monooxygenases will also be presented.

Binuclear Cu(II) Complexes as Catalysts for Oxygen Atom Transfer Reactions. Lawrence D. Margerum, Alice Fan Tai, and *Joan S. Valentine*. Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA 90024.

Our understanding of the mechanism of copper enzymes which react with dioxygen is limited. Type III copper proteins, such as hemocyanin and tyrosinase, contain a binuclear site which binds and/or activates dioxygen. While model studies using binuclear copper complexes have, in general, concentrated on mimicking the structural aspects of the active site, we have chosen to attempt to model the reactivity and mechanism of the oxygen atom transfer step. We have found that several different binuclear Cu(II) complexes catalyze the epoxidation of olefins by single oxygen atom donors in nonaqueous solution, while analogous mononuclear complexes are much less effective. The effect of structure, redox potential, and the nature of the substrate on the oxidation reactions will be discussed in the context of possible mechanisms of oxygen atom transfer using binuclear Cu sites.

Coupled Binuclear Copper Active Sites. *Edward I. Solomon.* Department of Chemistry, Stanford University, Stanford, CA 94305.

The active sites of a number of metalloenzymes contain a coupled binuclear copper unit which exhibit similar spectral features and interact with dioxygen. In our earler work on hemocyanin we generated a series of protein derivatives which enabled the active site to be systematically varied and subjected to a variety of spectroscopic probes. These studies have led to the spectroscopically effective model of the oxyhemocyanin and tyrosinase active site. Laccase is the simplest of the multi-copper oxidases which contains a Type 1, a Type 2, and a coupled binuclear (Type 3) copper center. Initially, our research focused on the Type 3 site in the Type 2 depleted (T2D) derivative. These studies have determined that the Type 3 site is similar to the coupled binuclear copper site in hemocyanin with respect to the presence of an endogenous bridging ligand responsible for antiferromagnetic coupling; however, there is a significant difference in that exogenous ligands do not bridge the two coppers at the Type 3 site in T2D. Finally, we have used low temperature MCD spectroscopy to define exogenous ligand binding to native laccase. This method allows a correlation between excited state absorption and ground state EPR spectral features. These studies indicate that one N_3^- simultaneously binds to both the T3 and the T2 centers, defining a new trinuclear copper active site.

Cytochrome *c* Oxidase. Sunney I. Chan. California Institute of Technology.

No abstract available.

Second Session: Metal–Oxo Sites

Active Sites of Binuclear Iron–Oxo Proteins. *Lawrence Que, Jr.* Department of Chemistry, University of Minnesota, Minneapolis, MN 55455.

Binuclear iron-oxo proteins are an emerging subclass of iron proteins whose metal sites in their oxidized forms consist of oxo-bridged diiron(III) units. The oxo bridge gives rise to strong antiferromagnetic coupling between the high spin iron(III) centers, which is the common physical property of the metal sites in this subclass. In this group are hemerythrin (an invertebrate dioxygen carrier), ribonucleotide reductase (mammalian and bacterial), and purple acid phosphatases. Hemerythrin is prototypical of this group; the crystal structures of the met, meta-azide, and oxy derivatives reveal a binuclear iron unit bridged by an oxo group and two carboxylates and terminally coordinated by five histidines. The sixth terminal site is available for coordination by exogenous ligands. The principal question to be addressed is to what extent this triply bridged diiron unit is conserved in semimethemerythrin azide, ribonucleotide reductase, and uteroferrin (a purple acid phosphatase from porcine uterus). Useful tools include NMR and EXAFS spectroscopy. Comparisons of the protein sites with synthetic complexes will also be made.

The Early Stages of Ferritin Core Formation. *Elizabeth C. Theil*, Dale E. Sayers, Boi H. Huynh, and Alain Fontaine. Departments of Biochemistry and Physics, North Carolina State University, Raleigh, NC 27695, Department of Physics, Emory University, Atlanta, GA 30322, and LURE, Université Paris-Sud, F-91405 Orsay Cedex, France.

The formation of the polynuclear, hydrous ferric oxide core of ferritin inside the hollow protein coat occurs in multiple stages, as first observed by Harrison et al. using visible

spectroscopy, suggesting a possible role for the protein in core nucleation. Monoatomic metal ions bind to the protein as shown, e.g., by Harrison and by Chasteen et al. using V and Mn as EPR probes. Recently, we demonstrated the participation of the protein in nucleation by detecting small clusters of Fe atoms bridged to each other and to the protein, using both x-ray absorption (XAS/EXAFS) and Mössbauer spectroscopy; the number of Fe atoms were limited to facilitate identification of the early stages of core formation (10 Fe/molecule compared to 4500 for a full core) (Yang et al., *Biochemistry 26*, 497, 1987). By collecting XAS data rapidly in the dispersive mode (30s scans), we also observed that the protein initially stabilizes large amounts of Fe(II) (480/molecule) at sites inaccessible to *o*-phenanthrolene, probably inside the protein; complete oxidation occurred in 16 h. (Part support, NIH GM34675 and DK20251).

Oxo-Bridged Polyiron Complexes. *Stephen J. Lippard.* Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

Iron oxo proteins have emerged as an important and distinct class of biological molecules known or believed to be responsible for such diverse functions as oxygen transport, reduction of ribo- to deoxyribonucleotides, acid phosphatase activity, methane monooxygenase activity, and iron storage. Insight into the structures, magnetic properties, spectroscopic and redox behavior, as well as the catalytic chemistry of the polyiron oxo cores in these proteins can be provided by the synthesis and characterization of small molecule analogs. Bridged binuclear complexes containing diiron(II,II), diiron(II,III), and diiron(III,III) units have been synthesized and studied as models for hemerythrin, ribonucleotide reductase, and methane monooxygenase. Molecules containing oxo-bridged Fe_3, Fe_4, and Fe_{11} have also been obtained in an approach to the formation and structure of the polyiron core in ferritin. Reactions of these oxo-bridged polyiron complexes including redox, bridging ligand exchange, and hydrocarbon oxidations will be described.

Synthetic Manganese–Oxo Clusters. *Karl Wieghardt.* Lehrstuhl für Anorganische Chemie I, Ruhr-Universität, D-4630 Bochum, Federal Republic of Germany.

A series of oxo/hydroxo bridged binuclear complexes of manganese (II,III,IV) containing additional acetato bridges and the macrocyclic ligand 1,4,7-triaza-cyclononane (L) or its *N*-methylated derivative have been synthesized and characterized. The cyclic voltammograms indicate reversible one-electron waves generating $Mn^{II}Mn^{II}$ and Mn_2^{III}, $Mn^{III}Mn^{IV}$ and Mn_2^{IV} dimers. A tris-(μ-acetato) complex, $[LMn^{II}(ac)_3Mn^{II}L]^+$, has been isolated. Preparations, structures and properties of the tetranuclear complex $[L_4Mn_4^{IV}O_6]Br_4$ 5.5 H_2O and of the mixed valent complex $[L_2Mn_2^{IV}(\mu\text{-}O)_2(OH)_2][Mn_3^{II}(C_2O_4)_4(OH_2)_2]\cdot 6\ H_2O$ will be discussed. These complexes will be shown to function as models for the water oxidizing metalloprotein photosystem III and pseudocatalase isolated recently from *Lactobacillus plantarum* (Fidovich et al., 1983).

Manganese in the Oxygen Evolving Complex of Photosystem II. *Gary W. Brudvig*, Warren F. Beck, and Julio C. de Paula. Department of Chemistry, Yale University, New Haven, CT 06511.

A polynuclear Mn complex functions in Photosystem II both to accumulate oxidizing equivalents and to bind water and catalyze its four-electron oxidation. The Mn complex can exist in five intermediate oxidation states called S_i-states (i=0-4). The S states are sequentially advanced via charge separation in the Photosystem II reaction center. EPR studies of the Mn complex in the S_2 state show that four Mn ions are required to account for its magnetic properties. From a simulation of the magnetic properties, we conclude that the Mn complex exists in a Mn_4O_4 cubane-like structure in the S_2 state. The EPR properties of the Mn center are altered by prolonged incubation in the dark, treatment with alcohols such as ethylene glycol, glycerol, or ethanol, and treatment with amines such as ammonia, methylamine, hydroxylamine, or N-methylhydroxylamine. The changes in EPR properties are interpreted in terms of changes in the exchange couplings between the four Mn ions that result from the coordination of exogenous ligands or protein conformational changes. On the basis of these results, structures are proposed for the five intermediate oxidation states of the Mn complex and a molecular mechanism for the formation of an O-O bond and displacement of O_2 from the S_4 state is suggested.

Third Session: Metal–Sulfur Clusters

Importance of Peptide Sequence in Electron Transfer Reactions of Oligopeptide Complexes of Iron–Sulfur Clusters. *Akira Nakamura* and Norikazu Ueyama. Department of Macromolecular Science, Osaka University, Toyonaka, Osaka, Japan 560.

The invariant peptide sequences found in many different ferredoxins are essential for their functions, e.g. electron transfer. We have systematically synthesized a series of oligopeptide model complexes of ferredoxins with those sequences nearest to the metal. The electrochemistry of these models indicates importance of NH--S hydrogen bonding and of hydrophobic environment created by the specific peptide sequences for control of the redox potential. Oxidative stability of Fe_4S_4 core of some of the peptide models upon reaction with dioxygen or other oxidants has been found to increase with an increase in the bulkiness and hydrophobicity of the peptide ligands. An efficient catalysis was found for cysteine-containing peptide/Fe_4S_4 complexes in electron transfer oxidation of benzoin or other substrates at ambient temperature in DMF.

Recent Results on Biologically Relevant Iron–Sulfur Clusters. *R. H. Holm*, M. J. Carney, J. A. Kovacs, R. Mukherjee, and T. D. P. Stack. Department of Chemistry, Harvard University, Cambridge, MA 02138.

Recent results on clusters containing the core units Fe_2S (**1**), Fe_4S_4 (**2**), Fe_4Se_4 (**3**), and VFe_4S_4 (**4**) will be presented. Matters to be discussed include synthesis (**1**–**4**), antiferromagnetic coupling modulated by bridge angle (**1**), ground spin state variability of reduced clusters ($S \geq 1/2$; **2**,**3**), site-specific functionalization (**2**) and regiospecific reactions (**2**,**4**). Certain of these aspects will be considered in relation to behavior and function of related clusters in metallobiomolecules.

A New Approach To Describe the Spin Coupling of Valence-Delocalized Systems and Novel MFe_3S_4 Clusters (M = Co, Zn, Cd). *E. Münck*, V. Papaefthymiou, K. Surerus, and J.-J. Girerd. Gray Freshwater Biological Institute, University of Minnesota, Navarre, MN 55392, and Laboratoire de Spectrochimie des Elements de Transition, Universite de Paris-SUD, F914505 Orsay.

Mössbauer studies of reduced Fe_3S_4 clusters have demonstrated the presence of an Fe^{3+} site and a valence-delocalized

Fe^{3+}/Fe^{2+} pair. Consideration of spin-coupling revealed that the spins of the delocalized dimer are "ferromagnetically" coupled. In order to describe the magnetism of the cluster, Heisenberg exchange as well as valence delocalization need to be considered. We have described the latter contribution with a new term, gleaned from Anderson-Hasegawa treatment of double-exchange. The new theory works well for Fe_3S_4 clusters. In order to assess the magnetism of Fe_4S_4 cubanes, we have studied the three novel clusters $CoFe_3S_4$, $ZnFe_3S_4$ and $CdFe_3S_4$. Since Zn^{2+} and Cd^{2+} are diamagnetic, considerable simplification of the spin-coupling problem, while retaining valence delocalization, has been achieved.

Nitrogenase: Overview and Recent Results. *E. I. Stiefel*, R. E. Bare, H. Thomann, T. V. Morgan, S. J. N. Burgmayer, and C. L. Coyle. Exxon Research and Engineering Co., Rt. 22 East, Annandale, NJ 08801.

This paper introduces the nitrogenase enzyme and discusses recent results on the extracted and in-protein iron-molybdenum cofactor (FeMoco) center of the enzyme. Nitrogenase catalyzes the six-electron reduction reduction of N_2 to $2NH_3$ concommitant with the evolution of H_2. Other unsaturated and reactive substrates are also reduced. ATP hydrolysis derives the process. The two protein components of the enzyme, [Fe] and [Fe-Mo], contain transition metal sulfide cluster. Recently, an alternative nitrogenase containing vanadium has also been reported.[1] [Fe-Mo] contains the unique FeMoco site upon which we focus. This site has been studied using microbiological, molecular genetic, biochemical, biophysical, chemical and synthetic modelling approaches. The extracted FeMoco is a metal sulfide cluster with unusual spectroscopic and magnetic properties. In our laboratory the relation of the extracted cofactor to the center in the intact FeMo protein has been probed by Electron Spin Echo Spectroscopy. Recent ideas on the structural nature of FeMoco will be discussed. (1) B. J. Hales, E. E. Case, J. E. Morningstar, M. F. Dzeda and L. A. Mauterer, *Biochem., 1986*, 25, 7251 and references therein.

The Synthesis and Characterization of Fe/Mo/S Clusters of Possible Relevance to the Active Center in Nitrogenase. *D. Coucouvanis*, M. G. Kanatzidis, and A. Salifoglou. Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055.

The synthesis of polynuclear Fe/Mo/S aggregates of the type $[Fe_6S_6(L)_6(Mo(CO)_3)_2]^{n-}$ (L = Cl^-, Br^-, I^-, OR^-; n = 3,4) is accomplished using the preformed $[Fe_6S_6(L)_6]^{3-}$ clusters as ligands for the $Mo(CO)_3$ unit. The halide series and the OR^- analogs have been characterized structurally and their electrochemical and electronic properties have been studied. These molecules are precursors for the $[Fe_6S_6(L)_6(Mo(CO)_3)]^{n-}$ 1:1 adducts that more than appropriately can be considered as structural analogs for the active site in nitrogenase. Our progress toward the synthesis of these compounds will be described and reaction chemistry aimed toward the introduction of higher oxidation state Mo ions in the $[Fe_6S_6(L)_6]^{3-}$ clusters will be discussed.

Clusters of Nitrogenase. *W. H. Orme-Johnson*, G. S. Harris, D. Wink, P. McLean, T. White, and T. Collet. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

The biological system for reducing dinitrogen to ammonia utilizes metal clusters in many phases of the process, from pyruvate oxidation, through electron transport, ATP coupled reduction of the catalyst, activation of N_2, and ultimately production of products. Most of these clusters appear to be variants on Fe_4S_4 centers. In the case of *ferredoxins* the protein-bound clusters rapidly self assemble from apoprotein and Fe and S containing "simple" precursors. In contrast, enzymes associated with N_2 fixation appear to use additional protein factors during the installation of these centers. Even more complex is the preparation of apoMoFe protein and the MoFe cofactor entity, the assembly of which require approximately four gene products each, following which the cofactor binds apoprotein to form MoFe protein *per se*. The nature of these assembly processes and the nature of products (cofactor and apoprotein) will be discussed. Supported by NIH (GM31574) and NSF (DM85-20687).

TUESDAY AFTERNOON—SYMPOSIUM ON THE CUTTING EDGE: THE INTERFACE BETWEEN BIOLOGY AND CHEMISTRY (DIVISION OF CHEMICAL EDUCATION)—P. SALTMAN, PRESIDING

Tired Blood, Brittle Bones and the Periodic Table. *Paul Saltman.* Department of Biology, University of California at San Diego, La Jolla, CA 92093.

Although 14 elements constitute less than 0.01% of our body weight, their presence in adequate amounts is essential for healthy growth and development. Most of these elements are transition metals who share chemical behavior and biological function. Their solubility, coordination and oxidation/reduction govern their biological activities of catalysis, respiration, transport and structural integrity. The pervasive incidence of iron and copper insufficiency is a factor of our own dietary perversities. The net result is iron deficiency anemia. How iron and copper are mutually interactive can be understood at the molecular level. The role of trace metals in general, and manganese and copper in particular with respect to bone metabolism, will be demonstrated. Manganese deficiency can lead to porotic and brittle bone development.

Probing Active Site Structure of Redox Metalloproteins. James A. Fee. Isotope and Structural Chemistry Group, Los Alamos National Laboratory, Los Alamos, NM 87545.

Trace elements such as Fe, Mn, Cu, and Mo often serve as essential cofactors of enzyme action. Indeed, understanding the biological functions of such enzymes at the molecular level is one goal of nutritional studies. Toward this end, the past two decades have seen the development of an array of powerful physical tools capable of revealing structure–function relations of metalloenzymes. In this talk, a few examples will be discussed in which Mössbauer spectroscopy, electron spin resonance techniques, and optical methods have been used in combination with specific isotope labeling to reveal details of active site structure. Supported by U.S.P.H.S. Grant GM35342 and the Stable Isotopes Program of the U.S.D.O.E. Office of Health and Environmental Research.

NMR Determination of Three-Dimensional Protein and Peptide Structures. *Peter E. Wright.* Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Recent advances in two-dimensional NMR methodology allow determination of the three-dimensional solution structures of proteins and other biomolecules. The essential first step in any NMR analysis of a protein is the assignment of ^1H resonances to specific amino acids in the sequence. New

strategies for making unambiguous spin system assignments will be described. Distance constraints obtained from nuclear Overhauser effect measurements and dihedral angle constraints from coupling constants are used to determine elements of secondary and tertiary structure. The complete three-dimensional structure in solution can be calculated using distance geometry or restrained molecular dynamics methods. The determination of protein structure from NMR data will be illustrated for the blue copper protein, plastocyanin (99 amino acids). Two-dimensional NMR spectroscopy can also be used to identify transient secondary structures in peptide fragments of proteins. The demonstration that certain small linear peptides can adopt highly preferred conformations in water has important implications for protein folding and for recognition by antipeptide antibodies.

Sea Serpents, Doorstops, Wood Nymphs, and Oriental Gambling Games: Concerto for Chemist vs. Biology. *Gregory A. Petsko.* Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

I will attempt to present the way a structural biophysical chemist looks at contemporary problems in biological chemistry. Particular attention will be paid to questions that are relevant to the emerging field of protein engineering. Among the questions considered will be: can we look directly at enzyme–substrate complexes at atomic resolution? Do we understand the rules of enzymatic catalysis and specificity in structural as well as chemical terms? How does protein flexibility affect protein function? Is there such a thing as *the* structure of any protein? What are we learning so far from our initial attempts at protein engineering? Although there will be a whimisical tone to the presentation, there will be an effort to provide solid answers for these questions. Examples will be drawn from studies on myoglobin, snake neurotoxins, glycolytic enzymes, and enzymes of amino acid metabolism.