Abstracts, Division of Biological Chemistry, 212th National Meeting of the American Chemical Society, August 25−29, 1996

Rowena Matthews, Chair; Jack F. Kirsch, Chair Elect;
John M. Schwab, Program Chair; Kenneth A. Johnson, Program Chair Elect

SUNDAY MORNING—1996 REPLIGEN AWARD FOR CHEMISTRY OF BIOLOGICAL PROCESSES, HONORING WILLIAM P. JENCKS—J. KIRSCH, PRESIDING

1. Mechanism of the Molecular Chaperone GroEL. *Alan R. Fersht*. Centre for Protein Engineering, University of Cambridge, Cambridge CB2 1EW, England.

Two of the many seminal contributions of W. P. Jencks to enzymology and biochemistry are his rationalization of the use of binding energy in catalysis and the production of rules for the coupling of ATP hydrolysis and vectorial processes. The molecular chaperone GroEL is involved in the folding of proteins *in vivo*. It uses ATP hydrolysis somehow in the process. We present evidence for a mechanism: "the ATPase-gated and -pulsed folding and annealing cage". The chaperone has a resting state which is a holoenzyme with the co-chaperone GroES and ATP, a state which binds denatured proteins weakly. The hydrolysis of ATP is coupled to a conformational change that increases the affinity for denatured states and so catalyzes the unfolding of misfolded states. On the rebinding of ATP, the chaperone reverts back to a fast folding state.

2. Redesign of the Zinc Binding Site in Carbonic Anhydrase. *C. A. Fierke* and D. W. Christianson. Biochemistry Department, Duke University Medical Center, Durham, NC 27710; and Chemistry Department, University of Pennsylvania, Philadelphia, PA 19104.

The methods of molecular biology, enzymology, and structural biology have been combined to probe the catalytic zinc binding site in carbonic anhydrase II. This molecular dissection yields the following lessons pertinent to the evolution of structure, affinity, and function in zinc binding sites: (1) Alterations and augmentations of the direct metal ligands modulate zinc affinity but not the tetrahedral geometry of the site. (2) Optimal ligand−metal separation and favorable side chain torsion angles for metal ligands mainly determine metal affinity. (3) Indirect ligands modulate metal affinity, zinc−water stability, and metal binding kinetics. (4) The neutral ligands and hydrogen bond network maintain the electrostatic environment of the active site and are essential for high catalytic activity. (5) Hydrophobic residues surrounding the metal site position the zinc ligands for optimal affinity and activity. We are modifying these features of this zinc binding site to vary the metal ion affinity, specificity, and equilibration kinetics for optimization of a metal ion biosensor.

3. Biological Catalysis: From Ribozymes to RAS. *D. Herschlag*. Department of Biochemistry, Stanford University, Stanford, CA 94305-5307.

The discovery of catalytic RNA modified the definition of an enzyme to include RNA enzymes or ribozymes. We have taken an approach that can be considered "comparative biochemistry," in which catalytic strategies of RNA and protein enzymes are compared. Concepts first elaborated for protein enzymes by Jencks have been generalized to RNA enzymes through investigation of phosphoryl transfer reactions catalyzed by the *Tetrahymena* group I ribozyme and the smaller hammerhead ribozyme. These enzymes use binding interactions away from the site of chemical transformation to facilitate catalysis. These interactions provide an entropic advantage for the reaction at the ribozyme's active site and are also used to provide electrostatic destabilization of the reactants. Relief of this destabilization in the transition state then provides a rate enhancement. The specific mechanism of electrostatic destabilization uncovered with the *Tetrahymena* ribozyme may also be used by protein enzymes. In general, RNA enzymes appear to be quite tractable to energetic analysis.

4. Inhibitors of Serine and Thiol Proteases Based on S′ Interactions. *Robert H. Abeles*, Ricky Baggio, Yi-Qun Shi, and Yong-qian Wu. Department of Biochemistry, Brandeis University, 415 South St., Waltham, MA 02254.

The inhibitor (R-COOL) reacts with the target enzyme to generate an acyl-enzyme which hydrolyzes slowly ($t_{1/2}$ > 1 h). HO-C_6H_4-COO-Ser(Chym.) (I) is an example of a slowly hydrolyzing acyl-enzyme. Slow hydrolysis is due to the relatively high electron density on the carbonyl group of the acyl moiety resulting from the inductive effect of the OH group. I cannot be generated from HO-C_6H_4-CO-OCH_3, because of the high electron density on the carbonyl group. A *p*-NO_2-phenyl ester could be used, but is undesirable because of its instability. The rate of reaction of the inhibitor (k_{on}) with the enzyme can be greatly increased by incorporating a leaving group (L in R-COOL) which interacts with S′ subsites. Upon forming the acyl-enzyme, the leaving group departs, and has no effect on subsequent events. Other methods of increasing the electron density on the carbonyl group will be described. Slow hydrolysis of the acyl-enzyme can also be obtained through geometric effects. This approach to the design of inhibitors can also be applied to enzymes which hydrolyze glycosides.

5. The Chemistry of Movement. *William P. Jencks*. Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254-9110.

Coupled vectorial processes connect a chemical reaction, such as the hydrolysis or synthesis of ATP, with a physical reaction, such as muscle contraction, active transport of ions, and oxidative phosphorylation. An understanding of these remarkable reactions requires a clear−cut distinction between

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the kinetics, thermodynamics, and specificities of these processes. The coupling between chemical and physical reactions is generally brought about by alternating changes in the specificities of chemical and physical partial reactions. These changes in specificity ensure that neither the chemical reaction of ATP hydrolysis nor the physical reaction of movement can occur unless the other also occurs; i.e., the chemical and physical reactions are tightly coupled.

SUNDAY AFTERNOON—POSTER SESSION—J. M. SCHWAB, ORGANIZER, PRESIDING

6. 750 MHz NMR Study of the Structure of a DNA Dodecamer Duplex Containing a cis-syn Thymine Dimer. *Kathleen McAteer*,[1] Michael A. Kennedy,[1] Yueqing Jing,[2] and John S. Taylor.[2] [1] Pacific Northwest National Laboratory, Richland, WA 99352, and [2]Department of Chemistry, Washington University, St. Louis, MO 63130.

Exposure of DNA to ultraviolet light results in the formation of a variety of photoproducts, one of which is the cis-syn thymine dimer. This lesion is biologically relevant because of its correlation with mutation and skin cancer. To gain a better understanding of the structural basis for enzyme recognition of the dimer and its repair, we have analyzed the three-dimensional structure of DNA dodecamer duplex containing a cis-syn cyclobutane dimer. The two-dimensional NMR spectra reveal a number of unusual features at and close to the region of the dimer. The modification also had an effect on the phosphate backbone at the dimer position as shown by ^{31}P data.

7. Solution Structure of a DNA Decamer Containing the Antiviral Drug Ganciclovir. *M. Foti* and B. I. Schweitzer. Walt Disney Memorial Cancer Institute at Florida Hospital, Orlando, FL 32825. S. Marshalko and G. P. Beardsley. Yale University School of Medicine, New Haven, CT.

The antiviral agent 9-(1,3-dihydroxy-2-propoxymethyl)guanine (ganciclovir, DHPG), is an acyclic analog of deoxyguanosine and is used to treat herpes simplex type 1 (HSV-1), type 2 (HSV-2), and cytomegalovirus (CMV). DHPG has been shown to be incorporated into viral DNA and inhibit further DNA replication. To investigate the structural, physicochemical, and biochemical consequences of DHPG incorporation into DNA, we have developed methodology for chemical synthesis of a DNA containing enantiomerically pure (S)-DHPG. Using ^{1}H and ^{31}P NMR experiments acquired at 600 MHz, a high-resolution structure has been determined for the DNA containing DHPG. Molecular dynamics calculations reveal a DNA structure where the ganciclovir is stacked in the helix and participates in normal base pairing. However, significant structural changes in the sugar−phosphate backbone are observed and provide a possible mechanism for the drug's action.

8. Structural Studies of RNA Oligonucleotides Containing Modified Nucleosides. *D. R. Davis*, P. C. Durant, R. K. Kumar, M. Sundaram, and E. Botros. Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84112.

The structure and dynamics of the anticodon region of $tRNA_3^{Lys}$ were studied using NMR spectroscopy. $tRNA_3^{Lys}$ is the RNA primer for HIV reverse transcriptase, and modifications in the anticodon region have been shown to be important for recognition by the protein. Modifications have also been shown to strengthen specific RNA−RNA interactions between the tRNA primer and HIV genomic template RNA. To investigate the nature of these interactions, we have synthesized a series of RNA hairpin stem loops that serve as model systems for the anticodon of $tRNA_3^{Lys}$. These stem loops contain the various natural modifications found in the native tRNA. The unmodified RNA stem loop is extremely flexible, and the modifications play important roles in stabilizing the structure and making the molecule conformationally rigid. The structure and dynamics will be discussed in the context of interactions with HIV template RNA and the normal role of tRNA during protein synthesis.

9. The Effect of RNA Secondary Structure on the Kinetics of Polymerization Catalyzed by HIV-1 Reverse Transcriptase. Zucai Suo and *Kenneth A. Johnson*. Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802.

The effect of template secondary structure on polymerization catalyzed by HIV RT is not clear. In this study, we designed and synthesized an RNA template with a stable hairpin structure. The hairpin structure was unwound and read through by HIV-1 RT slowly, and several intermediate products from both DNA elongation and RNA degradation were observed—RT seems to pause during processing of these substrates. Pausing is due to weak substrate binding. In fact, only a small fraction of RT forms a productive complex with these substrates. RNase H activity was not important for RT to read through secondary structure since wild type and RNase H-deficient RT had similar activities in the time span from 6 ms to 15 min. RT also pauses during polymerization of the analogous DNA template. HIV nucleocapsid protein inhibits full-length product synthesis slightly. RNA secondary structure has no effect on AZT inhibition *in vitro*.

10. Identification of Atomic Groups in the tRNA Acceptor Stem Needed for tRNA Synthetase Discrimination. Penny J. Beuning, Hongjian Liu, Li-Ping Yap, and *Karin Musier-Forsyth*. Department of Chemistry, University of Minnesota, Minneapolis, MN 55455.

To maintain the fidelity of protein synthesis, 20 aminoacyl-tRNA synthetases must discriminate among the approximately 60 cellular tRNAs. Both positive and negative recognition elements contribute to the so-called "identity set" of each tRNA. In this work, we probe specific functional groups in the terminal (1:72) base pair of the acceptor helix of model RNA duplex substrates, as well as in semisynthetic full-length tRNAs. We identify atomic groups that contribute significantly to both positive and negative synthetase discrimination. Base analog substitutions suggest that major groove elements are responsible for positive recognition of the first base pair by *E. coli* prolyl-tRNA synthetase, and are also important in discrimination by *E. coli* alanyl-tRNA synthetase. Our study offers direct support for the hypothesis that the overlapping recognition sites of tRNAs allow nucleotides to make positive contacts with cognate synthetases and negative contacts with noncognate enzymes.

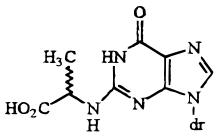
11. New Reagents for the Efficient Synthesis of Phosphorothioate-Containing Oligoribonucleotides. Qinghong Xu,[1]

Karin Musier-Forsyth,[1] Robert P. Hammer,[2] Brian W. Burke,[1] and George Barany.[1] [1]Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, and [2]Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803.

Phosphorothioate analogs of the phosphate moiety are of considerable interest in nucleic acid research. We have shown recently that 1,2,4-dithiazolidine-3,5-dione (DtsNH) and 3-ethoxy-1,2,4-dithiazoline-5-one (EDITH) are effective sulfurizing reagents in the synthesis of oligodeoxyribonucleotides [Xu, Q., *et al*. (1996) *Nucleic Acids Res*. *24*, 1602−1607]. The present work shows that DtsNH and EDITH are also effective in sulfurizing RNA oligomers. The methodology has been proven for the automated synthesis of oligoribonucleotides, of length 6−20 bases, containing the phosphorothioate substitution either at a single site or at all positions. EDITH is especially effective, and may be used at low concentrations and for short reaction times. Using this new methodology, we have incorporated single and multiple phosphorothioates into semisynthetic $tRNA^{Pro}$ molecules. Studies on the effects of these substitutions on *E. coli* prolyl-tRNA synthetase recognition are underway.

12. Methylglyoxal Induces DNA Modification: A Model System of DNA Mutagenesis. *Y. Al-Abed*,[1] A. Lee,[1] D. Reis,[1] C. Di Stefano,[2] H. Liebich,[2] A. Papoulis,[1] and R. Bucala.[1] The Picower Institute for Medical Research, Manhasset, NY 11030, and [2]Medizinische Klinik und Poliklinik der Universität, Tübingen D-72076, Germany.

Methylglyoxal (α-oxopropanal; MG) forms *in vivo* by elimination of phosphate from dihydroxyacetone phosphate and glyceraldehyde 3-phosphate and also by the Maillard process (advanced glycation). Increased levels of MG *in vivo* during hyperglycemia have been reported, suggesting that this reactive 3-carbon dicarbonyl may contribute to tissue damage and some of the long-term complications of diabetes. We recently described a novel nucleotide base modification (CEdG) produced by the incubation of guanine with glucose,



CEdG

Amadori product, or MG [Papoulis, A., *et al*. (1995) *Biochemistry 34*, 648]. Plasmid DNA modified by MG *in vitro* was also found to be mutagenized after its introduction and replication in murine lymphoid cells. DNA was incubated with MG under physiological conditions followed by enzymatic digestion to single nucleosides and subjected to GC-MS after trimethylsilylation. The GC-MS profile verified the presence of CEdG. Further studies for detection of CEdG *in vivo* by isolation of DNA from the tissue of diabetic and aged subjects are under investigation.

13. Tetrad Forming Oligonucleotides: Correlation between Ion-Selective Folding and Activity as an HIV Inhibitor. *N. Jing*,[1] R. F. Rando,[2] and M. E. Hogan.[1] [1]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030, and [2]Aronex Pharmaceutical Corporation, The Woodlands, TX 77380.

Previously, we presented evidence for inhibition of HIV-1 infection by treatment with phosphodiester oligonucleotides containing only G and T bases. Additional studies have suggested that such oligomers are potent inhibitors of HIV-1 integrase, *in vitro*. The highest activity was obtained using a 17mer, referred to as T30177, with composition G_{12}-T_5. Here, we have explored the sequence dependence of the intramolecular folding mechanism, in a set of 4 closely related 16−17 base oligonucleotide homologues, with sequences in the range G_{10-12}-T_{4-7}. The original T30177 compound was included, along with three derivatives which were designed so as to alter the structure of loop domains, while keeping the pair of G-tetrads intact. Based on thermal denaturation, CD, and kinetic analysis, we show that a single base alteration within the loop or tail domains can produce a very large change in folding stability. The K^+ ion dependence of these data suggested a preliminary model wherein the loop and tail domains interact to form stable metal ion-binding sites. A 16mer derivative (T30695) was designed within the context of that model, with the intent of enhancing the interaction between K^+ and the 5′ terminus of the oligomer. We have shown that T30695 folding is indeed more stable than other members of the group and is highly specific for K^+, as assessed from the ion dependence of thermal denaturation, CD spectra, and UV-detected folding kinetics. The stability and activity data are found to be highly correlated, as a function of sequence alteration, suggesting that formation of the stable intramolecular fold may be a prerequisite for both integrase inhibition and anti-HIV-1 activity.

14. DNA Binding by an L-2-Quinoxalylalanine-Containing Tandem β-Turn Peptide Motif. *Xiaofen Huang*, John R. Dobbins, N. Murali, and Eric C. Long. Department of Chemistry, Indiana University−Purdue University Indianapolis (IUPUI), Indianapolis, IN 46202.

The carboxy-terminal domain (CTD) of RNA polymerase II consists of multiple repeats of -(Ser-Pro-Thr-Ser-Pro-Ser-Tyr)$_n$- that contain two overlapping β-turns within each Ser-Pro-Xaa-Xaa site; these motifs may interact with DNA through the intercalation of their tyrosine rings. We have examined in detail the interaction of a synthetic analogue of this motif containing an additional tyrosine at its amino terminus (NH_2-Tyr-Ser-Pro-Thr-Ser-Pro-Ser-Tyr-$CONH_2$) with calf thymus DNA (^{1}H NMR, viscometry, and fluorescence spectroscopy). These analyses indicate that the peptide binds to DNA through nonclassical intercalation involving a simultaneous partial stacking of the tyrosine rings between the DNA base pairs. To further examine, and perhaps improve upon this DNA binding interaction, we have substituted Gly or D-Ala at the $i + 2$ positions of each Ser-Pro-**Xaa**-Xaa site (to increase the population of β-turns within this motif). In addition, a synthetic amino acid, L-2-quinoxalylalanine, was substituted for Tyr at each of the terminal positions. The resulting structures closely resemble members of the quinoxaline class of bis-intercalating antitumor antibiotics. Studies involving the analysis of the effect(s) of these substitutions on DNA binding and recognition will be presented.

15. Interactions of Tryptophan Containing Peptide Analogs with DNA. *P. Lugo-Ponce*, C. Robledo, and M. Vera. Department of Chemistry, University of Puerto Rico, Mayagüez, P. R. 00680.

Protein-induced distortions in DNA structure that enhance the interaction of proteins to specific DNA targets are

involved in the regulation of transcription. Some protein components have the ability to bind to specific sequences, and others can locally bend or kink the DNA, unwinding it over as much as a full turn of helix. Recent studies from Werner and co-workers, of the complex of the ETS1, from a family of transcription factors, to DNA, show intercalation of its side chain Trp^{28} displacing a C-G base pair with stacking of the invading tryptophan ring over the C-G base pair. In this complex, the indole ring of Trp^{28} is orthogonal to the displaced cytosine and guanine bases. Hydrogen bonding interactions in which the protons of the tryptophan ring act as acceptors and the π electrons of the displaced bases as donors were observed. Studies with oligopeptide models show that stacking interactions of aromatic amino acid residues with DNA are enhanced when more than one aromatic residue is present. Studies have shown that the aromatic residues next to N-terminal lysine establish stronger stacking interactions than those further away. The peptides synthesized and studied are symmetrical peptide analogs with two tryptophan residues next to lysyl residues spaced by either a diaminopropane or a diaminoacetone residue. The symmetrical peptides have two N-terminals and no C-terminal end. These peptides are expected to engage in bis-stacking interactions with nucleic acids. The complexes of these bis-partial intercalators with nucleic acids were studied by fluorescence and NMR spectroscopy. (Supported by NIH/MBRS Grant S06 GM08103-22.)

16. Molecular Modeling Studies of the Interaction of Peptides Containing Aromatic Amino Acid Residues with Poly(dA-dT)$_2$. *Marco A. De Jesús Ruiz*, Marisol Vera, Gustavo López, and Cynthia Robledo. University of Puerto Rico Mayagüez Campus, P.O. Box 5000, Mayagüez Puerto Rico 00681.

The interactions of proteins with nucleic acids can alter their folded structure. Studies of these interactions by theoretical or spectroscopic techniques such as molecular modeling or nuclear magnetic resonance are often difficult since it is a very complex system. Therefore, small peptides serve as a model system for the study of these interactions. A series of dipeptides of general structure lysine-X-NH_2, where X is alanine, phenylalanine, or *p*-nitrophenylalanine, with poly(dA-dT)$_2$, where A and T correspond to adenine and thymine base pairs, were studied by molecular modeling, in order to compare the theoretical results with available experimental data. The modeling studies were performed using the Tripos SYBYL 6.1 software. The electrostatic and stacking interactions were analyzed by molecular dynamics in order to determine its contribution to the stability of the system. The free peptide and peptide/nucleotide docked structure were studied to determine the most probable conformations and possible interaction sites. The use of molecular modeling for the study of more complex systems such as Lys-X-Y-X-NH_2 and Lys-X-Y-X-Lys where Y corresponds to a diaminoacetone or alanine residue was considered. (Project funded by NIH/MBRS Grant S06-GM08103.)

17. Studies of the Interactions of Oligopeptide Amides Containing Aromatic and Lysyl Residues with Nucleic Acids. *J. L. González-Román*, M. Vera, and C. Robledo. Department of Chemistry, University of Puerto Rico, Mayagüez Campus, Mayagüez, P. R. 00680.

Many different types of studies are in progress to understanding the molecular basis of protein−nucleic acid complexes. The control and regulation of transcription are dependent on protein-induced distortions in DNA structure. Proteins involved in the regulation of the transcription process, such as the TATA box-binding protein (TBP) and *E. coli* purine repressor protein (PurR), are able to intercalate side chains into the minor groove of DNA either by hydrophobic or by stacking interactions. These interactions can kink or bend the DNA structure. Oligopeptides containing aromatic amino acids and basic residues can be used as model systems in which to study both stacking and electrostatic interactions with nucleic acids. Oligopeptide amides were synthesized in which a C-terminal lysyl was added on the sequences of oligopeptides previously synthesized and studied. In addition, oligopeptides with naphthylalanine residues were synthesized. Studies of these peptides with CT-DNA were performed by ^{1}H-NMR, equilibrium dialysis, and Tm. Results will correlate the extent of stacking of the aromatic amino acid with the number of lysyl residues. The results of ^{1}H-NMR, equilibrium dialysis, and Tm studies will be presented. (Project funded by NIH/MBRS Grant SO6GM081032-22.)

18. Folding of Carbonic Anhydrase Probed by Amide Hydrogen Exchange Detected by Electrospray FT-ICR Mass Spectrometry. Zhongqi Zhang, Weiqin Li, *Shenheng Guan*, and Alan G. Marshall. Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Florida State University, 1800 E. Paul Dirac Dr., Tallahassee, FL 32310.

The unique capability of mass spectrometry to distinguish different conformational states in an H/D exchange experiment has led to its recognition as a complementary vehicle for elucidating protein folding. Only Fourier-transform ion cyclotron mass spectrometry (FT-ICR MS) can resolve isotopic distributions for individual charge states of most electrosprayed proteins. Here, we report labeling of human carbonic anhydrase II with deuterium during the folding process, and determine the sites and extent of deuterium substitution of backbone amide hydrogens by FT-ICR mass spectrometry, by use of fragmentation of multiply-protonated molecular ions in gas phase. [Work supported by the NIH (GM-31683), the NSF (CHE-94-13008), and the National High Magnetic Field Lab at Florida State University.]

19. Quantitation of H/D Exchange in Peptides and Proteins: Deconvolution of Natural Isotopic Abundance Distributions by Least Squares and Maximum Entropy Methods. Zhongqi Zhang, *Shenheng Guan*, and Alan G. Marshall. Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Florida State University, 1800 E. Paul Dirac Dr., Tallahassee, FL 32310.

The rate and extent of deuterium incorporation offer a powerful probe of protein shape in solution and gas phase. However, in order to determine the number of deuterium atoms in a peptide or protein from its mass spectrum, one must eliminate the "natural" isotopic distribution due to less-abundant isotopes of C, S, O, N, etc. Here, we compare least-squares and MEM for experimental FT-ICR mass spectra of MALDI-generated gas-phase peptide ions subjected to up to 1 000 000 collisions with D_2O vapor. We can deconvolve deuterium distributions from mass spectra

with overlapping natural isotopic distributions corresponding to incorporation of 1, 2, 3, ... deuteriums. [Work supported by the NSF (CHE-94-13008), the NIH (GM-31683), and the National High Magnetic Field Lab at Florida State University.]

20. Higher-Order Structure of Gas-Phase Peptides from Gas-Phase H/D Exchange Experiments. Zhongqi Zhang, Weiqin Li, *Shenheng Guan*, and Alan G. Marshall. Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Florida State University, 1800 E. Paul Dirac Dr., Tallahassee, FL 32310.

Matrix-assisted laser desorption/ionization (MALDI) generated peptide ions may be trapped at high neutral pressure for an extended period by broadband low-amplitude multiphase quadrupolar irradiation/axialization. As many as 1 million collisions of peptide ions with neutral gas molecules may occur during 2 h of trapping at 5×10^{-6} torr of D_2O pressure. We find that peptides of similar molecular weight can differ by orders of magnitude in their H/D exchange rates. The experimental results combined with molecular simulation studies allow for probing of higher-order peptide structure. [Work supported by the NIH (GM-31683), the NSF (CHE-94-13008), and the National High Magnetic Field Lab at Florida State University.]

21. Matrix-Assisted Laser Desorption/Ionization (MALDI) FT-ICR Mass Spectrometry of Oligosaccharides. Touradj Solouki, Bruce B. Reinhold, Catherine E. Costello, Matthew O'Malley, Shenheng Guan, and *Alan G. Marshall*. Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Lab, Florida State University, 1800 E. Paul Dirac Dr., Tallahassee, FL 32310.

MALDI Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry offers high mass measurement accuracy and high mass resolving power. Here we demonstrate that nondestructive FT-ICR detection allows for multiple detection and stepwise fragmentation of oligosaccharides, *i.e*., remeasurement of parent and selected fragment ions so as to dramatically improve sensitivity. We use stepwise sustained off-resonance irradiation (SORI) collisionally induced dissociation (CID) to obtain MS and MS^n mass spectra from a single laser shot to yield molecular weight and to assign glycosyl linkages and branching topology of carbohydrate polymers from a single batch of ions. [Work supported by the NSF (CHE-93-22824 and CHE-94-13008) and the National High Magnetic Field Lab at Florida State University.]

22. FK506 Binding Protein Conformation from Protein Amide Hydrogen Exchange Determined by Electrospray FT-ICR Mass Spectrometry. Zhongqi Zhang, Weiqin Li, *Shenheng Guan*, and Alan G. Marshall. Center for Interdisciplinary Magnetic Resonance, National High Magnetic Field Laboratory, Florida State University, 1800 E. Paul Dirac Dr., Tallahassee, FL 32310.

In a protein H/D exchange experiment, isotopic distributions for a deuterated protein and its fragments, resolved by a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS), reveal not only their deuterium contents but also how the deuterons are distributed among different protein molecules—a unique advantage of mass spectrometry which makes it complementary to NMR for understanding protein hydrogen exchange. Here, we examine hydrogen exchange behavior, both in gas phase and in solution, of human recombinant FK506 binding protein (FKBP) and its mutants under different conditions, thereby elucidating the stability and folding pathway of FKBP. [Work supported by the NIH (GM-31683), the NSF (CHE-94-13008), and the National High Magnetic Field Lab at Florida State University.]

23. Identification of Modification Sites in Poly(ethylene glycol)−Protein Conjugates by Peptide Mapping and Mass Spectrometry. *Ziping Wei*, Sunitha Menson-Rudolph, Pradip Ghosh-Dastidar, Deepak Jain, and Basant Sharma. The R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ 08869.

No abstract available.

24. Use of Multi-Wavelength UV/Visible Spectroscopy To Monitor Protein Structure. *S. Narayanan*,[1] R. L. Potter,[1] and L. H. Garcia-Rubio.[2] [1]Department of Chemistry and [2]Department of Chemical Engineering, University of South Florida, Tampa, FL 33620.

The optical density of a system is composed of both absorption and scattering components. With the advent of a computer-based algorithm that accounts for the absorption and scattering components of macromolecules, it is possible to obtain a precise absorbance coefficient without scattering interference. The algorithm employs *light scattering spectral deconvolution* which involves the spectrum being separated into its component chromophoric amino acids along with information on macromolecular size/dimensions and, potentially, molecular morphology of the protein. The algorithm validates multi-wavelength UV/vis spectroscopy as a quantative diagnostic tool in the study of protein structure and in particular is used to follow conformational changes in human serum albumin after perturbation by pH and chaotropic agents.

25. Electrostatic Control of Glucose Oxidase: Theory vs Experiment. *Judith G. Voet* and Kaori Shingledecker. Department of Chemistry, Swarthmore College, Swarthmore, PA 19081.

The dissociation constant of an essential acidic group on glucose oxidase from *Aspergillus niger* (K_4) is extremely sensitive to ionic strength (I). In previous work [Voet, J. G., *et al*. (1981) *Biochemistry 20*, 7182−7185], we showed that increasing I from 0.025 to 0.225 decreases $pK_{4,obs}$ from 8.2 to 7.3. At that time, this behavior was analyzed using the Debye−Hückel theory and the assumption that the enzyme was a homogeneously charged sphere. The essential residue was predicted to be a His with an intrinsic pK_a of 6.7. Here we report the use of the enzyme's 3-D structure [Hecht, H. J., *et al*. (1993) *J. Mol. Biol. 229*, 153−173] and the computational program DelPhi (gift of Kim Sharp) to analyze the dependence on I of the pK_a of several different His residues in the protein. An attempt is made to identify the specific His residue responsible for the I-dependent behavior based on agreement of the calculated model with experiment. Since the 3-D structure is of the deglycosylated enzyme, we also compare the I-dependent behavior of the original enzyme with that of the deglycosylated form.

26. Structural Studies of Insulin in Solution: The Effect of Co-Solvents in Aggregation State of the Protein. *Katayoun*

Amini and Frank Marí. Department of Chemistry and Biochemistry, Florida Atlantic University, P.O. Box 3091, Boca Raton, FL 33431.

Insulin is responsible for the regulation of glucose uptake in mammalians. Insulin can exist as a complex mixture of monomers, dimers, tetramers, and higher order oligomers depending upon the concentration of the protein. However, the active form of the hormone is the monomeric protein. In this study, we report the effect of the concentration and the use of organic co-solvents such as trifluoroethanol, acetonitrile, acetic acid, and methanol in the aggregation state of insulin. A combination of GFC-HPLC and NMR methods (1D spectra, 2D-NOESY, and TOCSY) is utilized to determine the association state of insulin and the effects on the 3D structure of the protein. In water solutions, Zn−insulin exhibits significant aggregation at concentrations above 500 μM, whereas Na−insulin is mostly monomeric under these conditions. The use of co-solvents significantly reduces the amount of aggregates found for similar conditions in water solutions. The results from these studies and a comparison with previous structures in solution will be discussed.

27. Changes in the Secondary Structure of β-Trypsin Induced by Increasing Temperature. *M. H. Brumano*, Q. Wang, and H. E. Swaisgood. Department of Food Science, North Carolina State University, Raleigh, NC 27695-7624.

Changes in the secondary structure of β-trypsin resulting from heat denaturation were monitored by circular dichroism (CD) spectroscopy. Far-UV CD spectra of the enzyme were taken in the presence and absence of calcium ions over the range of 184−260 nm. When Ca^{2+} was not present, the calculated secondary structure indicated an 11% decrease in antiparallel β-sheet as temperature increased from 25 to 65 °C. In the presence of Ca^{2+} above 45 °C, only a 4% decrease in antiparallel β-sheet was observed. Thus, binding of Ca^{2+} by β-trypsin prevented loss of β-sheet structure due to the heat effect. The CD estimation of secondary structure (9% α-helix, 41% antiparallel β-sheet, 2% parallel β-sheet, 18% turns, and 29% others) at 25 °C is in excellent agreement with X-ray results.

28. New Thermodynamic Stability of Chymotrypsinogen A, α-Chymotrypsin, and Ribonuclease A in Glycerol. *P. W. Chun*. Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL 32610-0245.

A method is described for evaluating the temperature-invariant enthalpy, $\Delta H°(T_o)$, for chymotrypsinogen A and ribonuclease A in an aqueous glycerol solution in the standard state, in order to determine the effect of glycerol on the thermodynamic stability of these two proteins. Chymotrypsinogen A has a temperature-invariant enthalpy of 210 kcal mol^{-1} at low pH in the absence of glycerol. In 10% aqueous glycerol solution, $\Delta H°(T_o)$ is 239 kcal mol^{-1}; in 40% glycerol, this value is 249 kcal mol^{-1}. The temperature-invariant enthalpy of α-chymotrypsin dimerization is only 33 kcal mol^{-1}, while that required for the conformational transition of ribonuclease A at low pH is approximately 60 kcal mol^{-1}. Solvents such as glycerol tend to lower the melting temperature, $\langle T_m \rangle$, in chymotrypsinogen A at low pH, possibly due to preferential interaction in the associated state, rather than a conformational thermal transition from the native to the denatured state. Using the Planck−Benzinger thermal work function, it is possible to determine the types of thermodynamic compensation taking place in these systems, that is, cases in which (i) thermodynamic compensation is dominated by the temperature-invariant enthalpy; (ii) balanced thermodynamic compensation between $\Delta H°(T_o)$ and the heat integrals; (iii) thermodynamic compensation among $\Delta H°(T_o)$, the heat integrals, and $T\Delta S°(T)$, typical of biological systems; and (iv) thermodynamic compensation dominated by $T\Delta S°(T)$ and the heat integrals.

29. An Independently Folding Peptide Model of the HIV Principle Neutralizing Determinant. *Kristin L. Fairbank* and Gregory R. Moe. Department of Chemistry, University of Delaware, Newark, DE 19716.

A 12 amino acid peptide, BB-PND, having a sequence similar to that of the V3 loop of the HIV surface glycoprotein gp120 was designed to adopt a hairpin-like structure in water. The structure determined by NMR spectroscopy shows that BB-PND folds into an extraordinarily compact, loop structure that is stabilized by hydrophobic interactions. The principles used in the design of BB-PND and of several other previously described examples may have many applications for the rational design of independently folding peptide mimetics of loop structures in larger proteins.

30. Probing Bioactive Conformation of Yeast α-Factor Using Peptidomimetic Conformational Constraints. *Y. Larry Zhang*,[1] H. Rao Marepalli,[1] Huifen Lu,[2] M. Greg Abel,[2] Jeffrey M. Becker,[2] and Fred R. Naider.[1] [1]Department of Chemistry, City University of New York, New York, NY 10314, and [2]Department of Microbiology, University of Tennessee, Knoxville, TN 37996.

Analogs of the tridecapeptide α-mating factor of the yeast *Saccharomyces cerevisiae* (WHWLQLKPGQPMY) containing a variety of conformational restrictions from selected combinations of constraints, e.g., Pro, D-residue, N-methylated residue, and γ-lactam, in place of the natural Pro-Gly motif were synthesized and bioassayed. Most of the restricted anlogs exhibited an enhanced bioactivity in causing growth arrest of *MAT***a** cells compared with the wild-type pheromone and an excellent receptor affinity in replacing the prebound tritiated α-factor from the receptor. However, constraints which mimic a type II β-turn always render the resulted analogs a better biological response and a much higher receptor affinity than those which mimic a type II′ β-turn do. Structural analysis of analogs with special biochemical and biophysical interests was carried out in solution phase using circular dichroism, 2D-NMR, and computer dynamics.

31. Important Factors in the Stability of the Three-Domain Structure of *Limulus* Hemocyanin Subunits. *R. Topham*, L. Strong, S. Tesh, and C. Bonaventura. Chemistry Department, University of Richmond, Richmond, VA 23173, and Duke University Marine Laboratory, Beaufort, NC 28516.

The crystal structure of subunit II of *Limulus* hemocyanin shows that the oxygen-binding, dinuclear copper center is located in domain 2 and that two disulfide bonds are attached to a loop of domain 3 that bridges both domains 1 and 2. A Ca^{2+}-binding site is located in domain 3, and an anion-binding site is located at the interface of domains 1 and 2. It has been proposed that the disulfide bonds, Ca^{2+} binding,

and anion binding could play important roles in the stability of the oxygen-binding site contained in domain 2. Reduction of the disulfide bonds results in the gradual loss of the oxygen-binding capacity in all eight subunit types that compose native *Limulus* hemocyanin. This provided a convenient chemical probe to determine the effects of subunit assembly, Ca^{2+} binding, and anion binding on the stability of the active sites of the individual subunits. Ca^{2+} binding and subunit assembly increased the stability, while anion binding diminished the stability of the active sites of many of the subunits. These results suggest that subunit assembly, Ca^{2+} binding, and anion binding affect the stability of the local tertiary structure of individual subunits as well as altering the oxygen affinity and stability of the native molecule. (Supported by NSF Grant DMB 9011992, NIEHS Grant ESO1908, and a Faculty Research Grant from the University of Richmond.)

32. Selectively Labeled Hemes Reveal Subunit Inequivalence in Time-Resolved Resonance Raman Study of Photolyzed CO-Hemoglobin. *Cynthia Rajani* and James R. Kincaid. Department of Chemistry, Marquette University, Milwaukee, WI 53233.

All previous time-resolved resonance Raman (TR^3) and transient optical studies of the primary photoproduct of COHb have been plagued by the interpretational ambiguity arising from the fact that the observed shifts represent an average value arising from the four individual hemes present in the tetramer (1−4). In the present study, using hybrids which contain a deuterated heme in either the alpha or beta subunits, two frequencies for ν_{19}, 20 cm^{-1} apart, could be observed in the RR spectra for each hybrid. The results showed a 3 cm^{-1} downshift for ν_{19} for the alpha subunits of Hb* relative to the deoxy form and only a 1 cm^{-1} downshift for the beta subunit. These data are consistent with the previously reported (average) 2−3 cm^{-1} downshifts in ν_{19} for native Hb* (1, 2).

33. Surface Enhanced Raman Studies of Dipeptides with an N-Terminal Lysyl Residue. *Maribel Morales*, Alberto Santana, Cynthia Robledo, Samuel Hernández, Marisol Vera. Department of Chemistry, University of Puerto Rico Mayagüez Campus.

When a molecule is adsorbed on a metal surface, its Raman intensity is enhanced by as much as 10^6-fold. This phenomenon, surface enhanced Raman scattering (SERS), can be used to obtain the vibrational spectrum of a sample at very low concentrations. SERS spectroscopy has great potential for application to biological molecules. The principal objective of this work is to develop a method for the spectroscopic Raman study of dipeptides with an N-terminal lysyl residue at low concentration using silver colloids. Conventional Raman requires concentrations in the 0.1 M range. The preparation of an adequate silver colloid is essential to observe the enhancement expected with SERS. Several methods for their preparation were evaluated and the resulting colloid solutions tested with pyridine. A change in color was observed when the colloid was adequate for further SERS studies. A series of aqueous amino acids and dipeptides with an N-terminal lysyl residue were then added to the colloid and their SERS spectra recorded with a Jobin Ybon T-64000 Raman spectrometer with an argon ion laser at 514 nm. The resulting spectra, and quantitation of the observed enhancement, as compared to conventional Raman, will be presented. (Project funded in part by NIH/MBRS Grant S06-GM08103 and NSF/RIMI Grant HRD-9550705.)

34. Conformation of Lividomycin A at the Active Site of an Antibiotic Modifying Enzyme. *Michael Mohler*, James R. Cox, and Engin H. Serpersu. University of Tennessee, Knoxville, TN 37996-0840.

A large number of aminoglycoside modifying enzymes have been found in a variety of bacterial species leading to their compromised clinical efficacy. 1- and 2-dimensional NMR techniques were used to determine the conformation of the aminoglycoside antibiotic lividomycin A bound to an aminoglycoside antibiotic modifying enzyme, 3′-phosphotransferase [APH(3′)-IIIa]. Complete proton chemical shift assignments were obtained for lividomycin. NOESY spectra were obtained for free and enzyme-bound lividomycin A. Observed NOEs were used as distance constraints in energy minimization studies. Although the overall conformation of lividomycin A was similar to the conformations of other 4,5-disubstituted aminoglycosides, butirosin and ribostamycin, unlike these antibiotics, no stacking arrangement of the primed and double-primed rings of enzyme-bound lividomycin was observed.

35. Investigations into the Mechanism for Suramin as an Inhibitor of cAMP-Dependent Protein Kinase. *M. L. Sanders*. Department of Chemistry, SUNY, Buffalo, NY 14260.

Suramin, a poly-sulfonated naphthylurea, inhibits a vast number of enzymes including protein kinases and phosphatases. Although suramin is a potent inhibitor, not much is known about its mechanism of inhibition. Suramin is believed to bind to substrates, thus preventing them from entering the active site of enzymes. It is also possible that suramin is reversibly binding to the enzyme, causing a conformational change in the enzyme and prohibiting substrate binding. A comparison of two substrates for protein kinase A was conducted to determine the mechanism for suramin binding. The substrates chosen were LRRRRFSG-amide and LRRFSLG-amide. IC_{50}s were 25 μM and 240 μM, respectively. IC_{50}s and UV studies verify that the positive charges on the substrate are important to suramin's function as an inhibitor. UV studies also indicate that suramin binds to the substrate LRRRRFSG-amide, thus forming a complex. Inhibition patterns using LRRFSLG-amide show competitive results for K_i versus ATP. Thus, suramin is likely to have two possible mechanisms for inhibition, making it a very potent inhibitor for many settings.

36. cAMP-Dependent Protein Kinase Inhibitor Study. *Mary E. Koszelak* and David S. Lawrence. Department of Chemistry, SUNY at Buffalo, Buffalo, NY 14260.

Protein kinases are enzymes which phosphorylate protein substrates in order to regulate cellular activity. One of the simplest and best known kinases is the cyclic-AMP-dependent protein kinase. This enzyme phosphorylates serine and threonine residues within a protein. Based on previously conducted substrate specificity studies, peptides containing different functionality were tested as inhibitors of PKA. IC_{50}s were obtained using the substrate LRRRRFSG-amide. Inhibitors which demonstrated low IC_{50}s were then assayed to determine their K_i values.

37. Reevaluation of the Link between Enzyme Mechanism and Reaction Stereochemistry for a Dehydratase−Allylic Isomerase. *John M. Schwab*,[1] Janet L. Smith,[2] Minsun Leesong,[2] and B. K. Sathyanarayana.[3] [1]Department of Medicinal Chemistry and Pharmacognosy and [2]Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, and [3]ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, P.O. Box B, Frederick, MD 21702-1201.

Escherichia coli β-hydroxydecanoyl thiol ester dehydrase equilibrates thiol esters of (*R*)-3-hydroxydecanoic acid, (*E*)-2-decenoic acid, and (*Z*)-3-decenoic acid. From the stereochemical courses of the dehydration and allylic rearrangement, as well as widely-accepted hypotheses concerning enzyme mechanisms and reaction stereochemistry, it has been assumed that a single active site group mediates proton transfers at C-2, C-3, and C-4 of the substrate. X-ray crystallography has now shown that histidine-70 and aspartate-84′ are the only polar amino acids in the largely hydrophobic active site. Molecular modeling has been conducted to evaluate a one-base mechanism with H70 as the catalytic group, as well as a novel two-base mechanism involving H70 and D84′. The two-base mechanism appears to provide superior orbital alignment, with minimal motion of the enzyme during catalysis. This may result in greater catalytic efficiency than if a single base must access all sites on the substrate where proton transfer takes place. It is concluded that current thinking about the link between enzyme mechanism and reaction stereochemistry should be reevaluated.

38. Glucarate Dehydratase: A *Stereorandom* Enzyme from the Enolase Superfamily. *David R. J. Palmer* and John A. Gerlt. Department of Biochemistry, University of Illinois at Urbana−Champaign, Urbana, IL 61801.

Glucarate dehydratase (GlcD) is a member of a superfamily of enzymes which includes enolase and mandelate racemase (MR), among others. These enzymes catalyze a reaction initiated by proton abstraction from a carbon atom adjacent to a carboxylate group. Sequence alignment demonstrates that the active site of GlcD retains all of the active site residues of MR implicated in catalysis, including basic residues able to abstract *R*- and *S*-protons (His345 and Lys213 of GlcD, respectively). We have demonstrated that this racemase-like active site character results in a loss of enzyme stereospecificity: GlcD can catalyze the dehydration of both D-glucarate and its 5-epimer, L-idarate, with similar turnover numbers (3 s^{-1} and 4 s^{-1}, respectively) to yield the same product: 4-*deoxy*-5-ketoglucarate. It is our hypothesis that glucarate dehydration initiates via deprotonation of C-5 (which has *R*-configuration) by H345 and idarate dehydration via deprotonation of C-5 (which has *S*-configuration) by K213. Site-directed mutagenesis experiments are underway to investigate this further. (Supported by Grant GM-52594.)

39. Cloning and Sequence of *Pseudomonas fluorescens* Kynureninase. *S. V. Koushik*, R. A. McGraw, and R. S. Phillips. Departments of Chemistry, Biochemistry, and Molecular Biology, College of Arts and Sciences, and Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602.

Kynureninase (EC 3.7.1.3) is a pyridoxal 5′-phosphate (PLP) dependent enzyme involved in the tryptophan degradation pathway of *Pseudomonas fluorescens*. In humans, a similar enzyme, 3-hydroxykynureninase, has been implicated in the etiology of numerous neurological diseases including epilepsy and AIDS-related dementia. We have cloned and sequenced the gene coding for kynureninase from *P. fluorescens* using a restriction site PCR technique [Sarkar *et al*. (1993) *PCR Methods Appl*., 318−322]. The gene codes for a protein of 416 aa with a calculated mol wt of 45 920. The protein sequence showed 28% identity to rat liver kynureninase and a similar homology with a yeast ORF previously deposited in Genbank. PILEUP analysis of the three protein sequences exhibits a highly conserved region which corresponds to the active site of rat liver kynureninase. Based on this, we conclude that Lys227 in *Pseudomonas* kynureninase is the PLP binding site. [Funded by a grant to R.S.P. from the NIH (GM 42588).]

40. Determination of the Role of Arginine-381 of Tyrosine Phenol-Lyase by Crystallography, Site-Directed Mutagenesis, and Kinetic Analysis. *Baktha*v*atsalam Sundararaju*,[1] Robert S. Phillips,[1] Tatyana V. Demidkina,[2] and Paul Gollnick.[3] [1]Department of Chemistry and Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602, [2]Englehardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia, and [3]Department of Biological Sciences, State University of Buffalo, Buffalo, NY 14260.

The X-ray structure of tyrosine phenol-lyase (TPL) complexed with a substrate analogue, 3-(4′-hydroxyphenyl)propionic acid, shows that Arg381 is located in the substrate binding site and forms a hydrogen bond with the 4′-OH of tyrosine. To evaluate the role of Arg381 in TPL catalysis, we prepared mutants with alanine (R381A) and with isoleucine (R381I). The TPL activity of R381A has been reduced by 10^{-4}-fold compared to the wild type, but it also has a small amount of tryptophan indole-lyase activity, which is completely absent in wild type and R381I TPL. R381A and R381I TPL react with *S*-(nitrophenyl)-L-cysteine, β-chloro-L-alanine, *O*-benzoyl-L-serine, and *S*-methyl-L-cysteine, and exhibit k_{cat} values similar to wild-type TPL. Rapid-scanning stopped flow spectroscopic analyses also show that wild-type and mutant TPLs can bind both tyrosine and tryptophan and form quinonoid complexes. R381A TPL shows only a small decrease in k_{cat}/K_m for tyrosine at lower pH, in contrast to wild-type TPL, which shows two pK_as of about 7.8. Thus, it is possible that Arg381 is one of the catalytic bases previously observed in the pH dependence of k_{cat}/K_m of TPL with L-tyrosine. [Supported by grants from the National Institutes of Health to R.S.P. (GM42588), and from the Fogarty International Foundation to R.S.P. and T.V.D. (TW00106).]

41. A Role for Histidine 264 in Substrate Binding by Avian HMG-CoA Synthase. I. Misra and *H. M. Miziorko*. Medical College of Wisconsin, Milwaukee, WI 53226.

H197N, H264N/A, and H436N avian cytosolic HMG-CoA synthases have been overexpressed in *E. coli*, isolated, and kinetically characterized. While H197N and H436N enzymes behave like wild-type enzyme, H264N and H264A synthases exhibit significant differences. Their K_m for acetyl-CoA is not substantially altered, but catalysis is diminished (∼25-fold slower). Efficient catalysis of partial reactions argues that H264N/A retain structural integrity. It is, therefore, significant that H264N/A synthases exhibit ∼100-

fold increases in K_m for acetoacetyl-CoA (AcAc-CoA). To test whether H264*AcAc-CoA interaction involves the thioester carbonyl, turnover of *S*-(3-oxobutyl)-CoA(OBS-CoA), a thioether-containing alternative substrate, was investigated. Wild-type synthase exhibits ~100-fold lower V_{max} and 25-fold higher K_m than observed with AcAc-CoA. When OBS-CoA replaces AcAc-CoA, H264A synthase exhibits a comparable effect on catalysis but no inflation of K_m. These data suggest that avian HMG-CoA synthase's H264 binds to AcAc-CoA's thioester carbonyl. (Supported by NIH Grant DK-21491.)

42. Investigation of the Function of an Active Site Histidine in HMG-CoA Lyase. *J. R. Roberts*,[1] G. A. Mitchell,[2] and H. M. Miziorko.[1] [1]Medical College of Wisconsin, Milwaukee, WI 53226, and [2]Hopital Ste-Justine, Montreal, Quebec H3T1C5, Canada.

HMG-CoA lyase is inactivated by diethyl pyrocarbonate (DEPC); activity can be fully restored by incubation with hydroxylamine. Protection against DEPC inactivation is afforded by a substrate analogue, suggesting an active site location for a DEPC target. Included in the inherited defects that map within the HMG-CoA lyase gene is a point mutation that results in an arginine substitution for histidine-233, one of only two invariant histidines. These observations prompted a functional test of the importance of H233. The mutant lyases H233R, H233A, and H233D were overexpressed in *E. coli*, isolated, and kinetically characterized. Substitution of H233 results in diminution of activity by ≈4 orders of magnitude. K_m values of the mutant lyases for HMG-CoA are comparable to the value measured for wild-type enzyme, indicating that these enzymes retain substantial structural integrity. In H233D, DEPC targets one less histidine than measured using wild-type lyase. The data support assignment of a catalytic role to H233. [Supported in part by NIH Grants R37DK-21491 (H.M.M.) and F32DK-09018 (J.R.R.).]

43. Characterization of Phosphoribulokinase−Nucleotide Binary Complexes: Evidence for the Structural Integrity of Catalytic Mutants. *J. A. Runquist*, H. A. Koteiche, and H. M. Miziorko. Medical College of Wisconsin, Milwaukee, WI 53226.

R. sphaeroides phosphoribulokinase (PRK) forms stable binary complexes containing stoichiometric levels of ATP or of the spectroscopically active substrate analogs, fluorescent trinitrophenyl-ATP (TNP-ATP) or spin-labeled ATPγSAP. Nucleotides dissociate slowly from the binary complexes. Analogs are displaced by ATP, suggesting that they bind at the active site. Catalytic mutants D42A and D169A [Charlier *et al.* (1994) *Biochemistry 33*, 9343] were evaluated and found competent to form stable binary complexes which contain stoichiometric levels of either TNP-ATP or ATPγSAP. These observations indicate that both D42A and D169A PRK mutants retain substantial structural integrity and contain a full complement of substrate binding sites. The data underscore the significance of the mutants' 10^5-fold diminution in catalytic activity and qualify the carboxyl groups of D42 and D169 as participants in the chemistry of ribulose 1,5-bisphosphate synthesis. (Supported by USDA NRI/Photosynthesis 93-373069181.)

44. Kinetic Mechanism of Nicotinate Phosphoribosyltransferase. *J. Gross*, M. Rajavel, and C. Grubmeyer. Department of Biochemistry and Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140.

Jencks [(1980) *Adv. Enzymol. 51*, 75−106] has posited that ATP-driven conformational coupling requires an obligatory intertwining of the steps of ATP hydrolysis with those of the coupled reaction. Coupling of ATP hydrolysis to nicotinic acid mononucleotide (NAMN) synthesis catalyzed by nicotinic acid phosphoribosyltransferase (NAPRTase) follows these rules. We have used steady-state kinetics, equilibrium gel filtration, isotope trapping, and rapid quenching techniques to determine the kinetic mechanism of the coupled NAMN synthesis reaction. NAPRTase autophosphorylates at a rate of 4.8 s^{-1}. The K_d for α-D-5-phosphoribosyl-1-pyrophosphate (PRPP) was 0.5 μM in the presence of ATP with no PRPP binding observed in the absence of ATP. k_{on} for PRPP and nicotinic acid (NA) binding to E−P were measured at 1.3×10^5 M^{-1} s^{-1} and 7.5×10^6 M^{-1} s^{-1}, respectively. Isotope trapping indicated PRPP binding can precede that of NA. The rate constants of the phosphoribosyl transfer step (>300 s^{-1}) and E−P hydrolysis (5.5 s^{-1}) were determined by adding NA to the preformed E−P·PRPP complex, and demonstrate that NAMN and PP_i formation precedes E−P hydrolysis. The investigation of the kinetic mechanism has delineated an obligatory order of events in which the ATP hydrolysis reaction does not proceed to completion without commensurate progress of the NAMN synthesis reaction. (Supported by NSF Grant DMB-9103029.)

45. Catalytic Base in Hypoxanthine-Guanine Phosphoribosyltransferase (HGPRTase). *Y. Xu*,[1] J. Eads,[2] J. C. Sacchettini,[2] and C. Grubmeyer.[1] [1]Department of Biochemistry, Temple University School of Medicine, 3420 N. Broad St., Philadelphia, PA 19140, and [2]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461.

HGPRTase catalyzes the formation of IMP and GMP from their respective bases hypoxanthine (Hx) and guanine (Gua). Based on the crystal structure of human HGPRTase [Eads, J. C., Scapin, G., Xu, Y., Grubmeyer, C., & Sacchettini, J. C. (1994) *Cell 78*, 325−334], Lys 165 or Asp 137 were proposed to act as a general base/acid. The pH profile of k_{cat} in the reverse reaction (IMP pyrophosphorolysis) revealed an essential acidic group with p*K* of 7.9, whereas the pH profile of k_{cat} for IMP formation was flat. We have constructed and purified two mutant HGPRTases. D137N displayed a 17-fold decrease of k_{cat} in the forward reaction with Hx as substrate, a 200-fold decrease of k_{cat} with Gua, and a 250-fold decrease of k_{cat} for IMP pyrophosphorolysis. D137N showed lower K_D for both IMP and PRPP. The pH profiles of k_{cat} for D137N were severely altered. The k_{cat} for K165Q was decreased by 2-fold in the forward reaction and was unchanged in the reverse reaction. The K_m and K_D values showed K165Q binds substrates weaker than the wild-type enzyme. Pre-steady-state experiments with K165Q indicated the phosphoribosyl transfer was fast in the forward reaction, as observed with the wild type. The pH profile of k_{cat} in the reverse reaction for K165Q remained the same as the wild type. In conclusion, Asp137 appears to act as the general catalytic base/acid for HGPRTase, and Lys165 makes ground-state interactions with substrates. (Supported by NIH Grant GM-52125.)

46. Synthesis and Properties of 8-CN-Flavins and Reconstituted Flavoproteins. *Yerramilli V. S. N. Murthy* and Vincent Massey. Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48105.

It is known that the oxidation−reduction potential of the flavin and the catalytic properties of flavoproteins are related. So in principle by manipulating the redox potential of the flavin, it is possible to modulate the catalytic activity of a flavoprotein. Introduction of electron-withdrawing groups at the 8-position of the flavin shifts the potential to more positive values. We synthesized a riboflavin derivative with a cyano group at the 8-position and converted it enzymatically to the FAD and FMN levels. The redox potential was found to be some 170 mV more positive than that of the native flavin. In the present work, the effect of replacement of the native FMN of the Old Yellow Enzyme with 8-CN-FMN is compared with respect to the physicochemical and catalytic properties of the two enzyme forms, particularly in reactions with α,β-unsaturated ketones.

47. Spectroscopic Properties of *E. coli* UDP-*N*-Acetylenolpyruvylglucosamine Reductase. Joseph Yanchunas, Jr.,[1] Milton J. Axley,[2] Robert Fairman,[1] and *James G. Robertson*.[1] [1]Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543, and [2]Naval Medical Research Institute, Bethesda, MD 20889.

The properties of tightly bound FAD in purified uridine diphosphate *N*-acetylenolpyruvylglucosamine reductase were analyzed by circular dichroism (CD) and UV−visible spectroscopy. The CD spectrum of bound flavin exhibited peaks at 364, 464, and 495 nm. The protein was reversibly unfolded and refolded from 9.8 M urea, and refolded enzyme incorporated FAD and catalyzed full activity. The extinction coefficient of bound FAD was $\epsilon_{464} = 11\,700$. Anaerobic reduction with dithionite was complete at 1 equiv. Anaerobic reduction with NADPH also was essentially complete at 1 equiv and produced a long-wavelength absorbance band characteristic of an FAD−pyridine nucleotide charge transfer complex. No evidence for a flavin semiquinone was obtained. Photochemically reduced enzyme was reoxidized by titration with either $NADP^+$ or UNAGEP. Reoxidation by $NADP^+$ reached a chemical equilibrium, whereas reoxidation by UNAGEP was stoichiometric. Binding of $NADP^+$ or UNAGEP to the oxidized form of the enzyme produced a dead-end complex that could be titrated by following a 10 nm red shift in the absorption spectrum of the bound FAD. The K_d of $NADP^+$ for oxidized enzyme was $0.7 \pm 0.3\ \mu$M, and the K_d of UNAGEP was $2 \pm 0.3\ \mu$M. No spectral changes were observed in the presence of a 40-fold excess of UNAM either aerobically or anaerobically. These studies have identified spectral signals for five steps in the kinetic mechanism and have indicated that product formation is essentially irreversible.

48. Construction, Purification, and Characterization of Recombinant D-Ribulose-5-phosphate 3-Epimerase from *Spinacia oleracea*. *Frank W. Larimer*, Tse-Yuan S. Lu, and Claude D. Stringer. Protein Engineering Program, Biology Division, Oak Ridge National Laboratory, Box 2009, Oak Ridge, TN 37831.

A full-length cDNA clone encoding spinach chloroplastic D-ribulose-5-phosphate 3-epimerase (RPE) was isolated from a lambda library by use of a homologous, radiolabeled hybridization probe. This probe was prepared by PCR amplification from the total library with primers based on the sequence of the potato *RPE* gene [Teige *et al*. (1995) *FEBS Lett*. *377*, 349−352]. Subclones were constructed to produce the transit protein as well as the mature chloroplastic protein in *Escherichia coli*. Both proteins are produced as active enzymes and reach levels approximating 10% of the soluble protein, 200-fold higher than the level of the endogenous *E. coli* RPE. The recombinant mature RPE has been purified to near-homogeneity by successive hydrophobic and ion-exchange chromatography. Based on subunit molecular weight and *N*-terminal sequence, the recombinant RPE is identical to the authentic enzyme from spinach. (Research sponsored by the Office of Health and Environmental Research, USDOE, under Contract DE-AC05-96OR22464 with Lockheed Martin Energy Research Corporation.)

49. Kinetic Studies of UDP-Galactose 4-Epimerase: The Effect of Uridine Nucleotide Binding on Catalysis. *Yijeng Liu* and Perry A. Frey. Institute for Enzyme Research, University of Wisconsin−Madison, Madison, WI 53705.

UDP-galactose 4-epimerase (EC 3.1.5.2) catalyzes the interconversion of UDP-galactose and UDP-glucose. Spectroscopic data explicitly indicate the occurrence of a conformational change of epimerase upon uridine nucleotide binding. In the presence of both glucose and UMP, the epimerase-bound NAD^+ can be reduced to NADH, which inactivates the enzyme. The mechanism for this reaction at pH 7.0 was studied, and the kinetic parameters were obtained. In order to quantitate the effect of UMP on the activation of epimerase toward reduction, the initial rates in the absence of UMP were measured under anaerobic conditions using highly active enzyme. A large discrepancy is observed between the rate constants in the presence and absence of UMP, and the activation energy difference due to uridine nucleotide binding is calculated.

50. The pH Titration of the Low-Barrier Hydrogen Bond in Chymotrypsin−Inhibitor Complexes. *Constance S. Cassidy*, Jing Lin, and Perry A. Frey. Institute for Enzyme Research, University of Wisconsin−Madison, Madison, WI 53705.

The involvement of a low-barrier hydrogen bond in the mechanism of chymotrypsin has been postulated. Evidence for this mechanism includes the low-field peak of the protonated triad. Also, chymotrypsin complexed with the inhibitor *N*-Ac-L-Leu-Phe-$COCF_3$ exhibits a chemical shift value of 18.7 ppm, and the estimated pK_a of the proton bridging His^{57} and Asp^{102} in the complexed triad is greater than 10.5. The ^{1}H NMR pH titration of chymotrypsin complexed with *N*-Ac-L-Leu-Phe-$COCF_3$ was reexamined. In addition, similar studies were carried out on the enzyme complexed with *N*-Ac-Phe-$COCF_3$. The low-field chemical shift values for the proton bridging $His^{57}-Asp^{102}$ in the two complexes are 18.9 and 18.6 ppm, and the pK_a values are elevated by approximately 5 and 4 pK units, respectively, upon complexation with inhibitor. Binding interactions may therefore facilitate the formation of a low-barrier hydrogen bond in the enzyme−inhibitor complexes.

51. Proton Transfer Reactions of Ribulose-1,5-bisphosphate (RuBP) Carboxylase/Oxygenase. *Mark R. Harpel* and Fred

C. Hartman. Protein Engineering Program, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-8080.

The ϵ-amine of Lys166 of RuBP carboxylase/oxygenase facilitates enolization of RuBP (the initial step in overall catalysis) more than 100-fold, despite being too remote from C-3 of RuBP to be the primary proton acceptor [Knight *et al*. (1990) *J. Mol. Biol. 215*, 113]. To clarify the roles of Lys166, we have analyzed products formed by mutants and chemically-rescued variants from both RuBP and isolated carboxylated reaction intermediate (CKABP). Lys166 mutants slowly convert RuBP to 1-deoxy-D-glycero-2,3-pentodiulose 5-phosphate, indicative of enolization followed by β-elimination. Thus, Lys166 not only promotes enolization but also forward processing of enediol. Restoration of carboxylase activity to K166C by aminoethylation increases CO_2/O_2 specificity and also enhances pyruvate formation, which reflects β-elimination of the terminal *aci*-acid intermediate [Andrews & Kane (1991) *J. Biol. Chem. 266*, 9447]. The previously uncharacterized product of CKABP hydrolysis by K166G [Lorimer & Hartman (1988) *J. Biol. Chem. 263*, 6468] is shown to be pyruvate, rather than 3-phosphoglycerate, as normally formed by protonation of the terminal intermediate. These results are consistent with Lys166 as the terminal proton donor, as deduced by crystallography, and demonstrate multiple roles for this residue in catalysis. (Supported by USDOE under Contract DE-AC05-96OR22464 with Lockheed Martin Energy Research Corporation.)

52. The Hydrolysis of Cellulose by *Trichoderma reesei* CBHI and -II in Relation to the Coupled Vectorial Process Hypothesis. *Nizar S. Sweilem* and Michael L. Sinnott. Department of Chemistry, m/c 111, University of Illinois at Chicago, 845 W. Taylor St., Chicago, IL 60607.

Cellobiohydrolyases I and II (CBHI, CBHII) from *Trichoderma reesei* are involved in the hydrolysis of native, highly crystalline cellulose. The hypothesis that these enzymes use the free energy of the hydrolysis of a glycosidic link to disrupt the cellulose crystallite and therefore should be able to catalyze the reverse reaction to drive the thermodynamically disfavored synthesis of a β-glucan link using cellulose crystallization energy was tested for CBHI and CBHII. Cellobiose labeled with ^3H at C1 and the highly crystalline *Acetobacter xylinum* cellulose were incubated with the enzymes CBHI and -II to test for the incorporation of [^3H]cellobiose into the cellulose. The appearance of ^3H in the cellulose was monitored by scintillation counting. The incorporation of labeled cellobiose into the cellulose crystallite was evident in comparison to the control BSA. The "Snap back" hypothesis was also tested with CBHI by monitoring the incorporation of [^3H]-1,5-anhydrocellobiitol into cellulose. Thus, these results on the incorporation of ^3H into cellulose support the coupled vectorial process hypothesis for the cellobiohydrolases from *Trichoderma reesei*.

53. Purification and Characterization of a Carbon−Sulfur Lyase (Aryl Desulfinase) from *Rhodococcus* sp. IGTS8 Involved in Desulfurization of Dibenzothiophene. *Gregory T. Mrachko* and Kevin A. Gray. Energy BioSystems Corp., 4200 Research Forest Dr., The Woodlands, TX 77381.

Biocatalysts are being developed at Energy BioSystems Corp. to remove sulfur from sulfur-containing organic molecules found in fossil fuel without decreasing the carbon content of the fuel. A metabolic pathway has been discovered in the Gram-positive organism *Rhodococcus* sp. IGTS8 consisting of four enzymes which convert dibenzothiophene (DBT), a major organosulfur constituent in middle distillate, to 2-hydroxybiphenyl (HBP) and sulfite. The final enzyme in this pathway (encoded by *dsz*B) catalyzes a reaction of which there are few examples involving the formation of sulfite from 2-(2-hydroxyphenyl)benzenesulfinic acid to yield HBP. The desulfinase has been purified and characterized both kinetically and physically. We report on chemical modification and other enzyme mechanism studies of this protein. The reaction is presented in light of chemical precedents, and the results are summarized in the form of a proposed enzyme reaction mechanism.

54. Biochemical Characterization of the Biodesulfurization of Dibenzothiophene by *Rhodococcus* sp. Strain IGTS8. *Kevin A. Gray*, Olga Pogrebinsky, Gregory T. Mrachko, and Charles H. Squires. Energy BioSystems Corp., 4200 Research Forest Dr., The Woodlands, TX 77381.

The Gram-positive organism *Rhodococcus* sp. IGTS8 catalyzes the removal of sulfur from organic components in fossil fuels (middle distillate). One of the major classes of sulfur-containing molecules in middle distillate are the alkylated dibenzothiophenes. Unsubstituted dibenzothiophene (DBT) is the usual model compound for studies on this class of molecules. A pathway has been discovered that utilizes a multienzyme system of which the first enzyme, DBT monooxygenase (encoded by *dsz*C), converts DBT to DBT-5,5-dioxide in two discrete steps. The third step involves the conversion of DBT-5,5-dioxide to 2-(2-hydroxyphenyl)-benzenesulfinic acid (PPS) catalyzed by DBT-5,5-dioxide monooxygenase (encoded by *dsz*A). The final enzyme, DSZB, catalyzes the removal of $-SO_2$ from PPS to form HBP and sulfite. The first two enzymes in the pathway require molecular oxygen, NADH, FMN, and an NADH−FMN oxidoreductase while the third enzyme requires no cofactors. The results of purification and kinetic and physical characterization of each enzyme are reported.

55. Oxidation of Phenols, Anilines, and Benzenethiols by Fungal Laccases: Correlation between Activity and Redox Potentials as well as Halide Inhibition. *Feng Xu*. Novo Nordisk Biotech, 1445 Drew Ave., Davis, CA 95616.

A comparative study has been performed with several fungal laccases for the oxidation of a series phenols, anilines, and benzenethiols and the inhibition of halides. The K_m and k_{cat} have been correlated to the structure of substrates. The change in log (k_{cat}/K_m) was found proportional to the difference between the one-electron redox potentials of the enzyme and substrate. The correlation indicates that the first electron transfer from substrate to laccase is governed by the outer-sphere mechanism. Compared to the electronic factor, the steric effect of small *ortho*-substituents was found unimportant. The inhibition potency order of $F^- > Cl^- > Br^-$ is attributed to limited accessibility of the T2 site in laccase. Although the enzymes studied have homologous primary sequences and predicted similar backbone structures, the difference exhibited by each enzyme in interacting with individual substrates or inhibitors suggests the existence of structural variation in their functional domains.

56. Kinetic Characterization of Soybean Root Nodule Urate Oxidase. *Kalju Kahn* and Peter A. Tipton. Department of Biochemistry, University of Missouri−Columbia, Columbia, MO 65211.

Urate oxidase catalyzes the oxidation of uric acid with concomitant reduction of O_2 to H_2O_2. We have investigated its kinetic properties using an oxygen electrode. Analysis of initial velocity kinetic patterns revealed a sequential mechanism for substrate binding. Xanthine inhibition patterns suggest that substrates bind in an ordered manner with urate first. Both V_{max} and V/K_{urate} pH profiles defined an apparent pK_a value of 6.2; deprotonation of the group with this pK_a is required for activity. The pK_i profile for the competitive inhibitor 9-methyluric acid was pH-independent over the pH range 6.0−9.5. These data suggest the presence of a base on the enzyme which is required for catalysis but not binding. The pK_i profile for xanthine shows that only the monoanion can bind to the enzyme; thus, the urate monoanion is predicted to be the substrate.

57. Mechanistic Studies of 6-Deoxyerythronolide B Hydroxylase by Site-Directed Mutagenesis. *O. Han* and C. R. Hutchinson. Department of Genetic Engineering, Chonnam National University, Kwangju 500-757, Korea.

The 6-deoxyerythronolide B hydroxylase is a soluble cytochrome P450 and catalyzes hydroxylation at C-6 of 6-deoxyerythronolide B. The amino acid sequence deduced from the DNA sequence revealed that an Ala-245 was present in the position corresponding to the highly conserved threonine. In an effort to elucidate the function of Ala-245 and to reexamine the catalytic role of the hydroxyl group of Thr in the mechanism of oxygen activation, we characterized catalytic properties of A245T and A245S mutants. The hydroxyl group of threonine seems to interact with iron−dioxygen species based on observations that the A245T mutant oxidizes NADPH most efficiently and decomposes hydrogen peroxide significantly.

58. Comparison of Prenyl Transferase Activities in Adult and Larval Lepidopteran Corpora Allata. Stephanie E. Sen, *D. Clifford Brown*, and Gregory J. Ewing. Department of Chemistry, Indiana University−Purdue University at Indianapolis (IUPUI), Indianapolis, IN 46202.

Lepidopteran juvenile hormone (JH) is a complex mixture of sequiterpenoids, consisting of methyl epoxyfarnesoate and four structurally related homologs. The relative amounts of these materials are in continual flux, varying according to insect age and developmental state. One possible source of JH homolog regulation is the presence of prenyl transferases that have varying substrate specificity. We have previously characterized larval prenyl transferase from the corpora allata of the lepidopteran *Manduca sexta* and have studied its substrate requirements. Recently, similar studies with the adult developmental stage have been performed, and differences between the larval and adult enzymatic activities have been noted. The results of these studies and the possible role of prenyl transferase in regulating JH homolog titers will be presented.

59. Investigation of Arginine Kinase in *Xenopus laevis* Oocytes. *M. W. Fasano* and R. L. Potter. Department of Chemistry, University of South Florida, Tampa, FL 33620.

The current paradigm of phosphagens localizes creatine kinase exclusively to vertebrates and arginine kinase (AK) exclusively to invertebrates. Paradoxically, it was recently shown that both of these enzymes have activity in the muscle cells of an (invertebrate) echinoderm, and our laboratory has immunological evidence which supports the presence of arginine kinase in *Xenopus laevis* oocytes (a vertebrate). Analysis of oocyte homogenates, using an antibody against shrimp AK, reveals a band at approximately 38 000, the approximately molecular weight of many AK isozymes. The task of assaying directly for AK activity in these oocytes has proven difficult due to the lack of specificity of the standard AK assay which ultimately measures inorganic phosphate. We are currently in the process of revising this assay by replacing nonspecific phosphate hydrolysis and spectrometric phosphate determination with thin-layer chromatography analysis of phosphoarginine. This will allow us to more effectively probe oocytes for the presence of AK activity.

60. Characterization of Acid- and Base-Extracted Collagens. *S. Patel* and R. Potter. Department of Chemistry, University of South Florida, Tampa, FL 33620.

Collagen is one of the major structural proteins found in animals and has been used extensively in the medical industry as a biomaterial. Since its properties can be modified by its method of preparation, it is important to characterize and understand the changes that occur during modification or processing. Methods of collagen preparation vary considerably depending on both the commercial considerations and the physical properties desired. Two common methods of collagen preparation require treatment of the collagen with relatively strong acid or base. To better understand and ultimately control the degree of modifications, we have investigated the use of protease peptide cleavage profiles as a way of tracking extract-induced changes. We show that the cleavage profiles of acid- and base-extracted collagens are significantly different. Thermolysin treatment shows the most rapid cleavage, being completed in approximately 30 min, and yields very small peptides. Thrombin fragmentation proceeds at a slower rate, producing larger peptides that are stable even at 24 h. Endoproteinase Arg-C also produces a unique pattern, but there are a larger number of peptide fragments produced compared to either thrombin or thermolysin.

61. Novel Cytoplasmic Sugar Modification at Potential Protein Phosphorylation Sites: Proliferative-Induced Changes in *N*-Acetylglucosamine Modification. *Chad Slawson*,[1] Paul Baekey,[2] and Robert Potter.[1] [1]Department of Chemistry, University of South Florida, and [2]Department of Pathology, H. Lee Moffitt Cancer Center, Tampa, FL 33620.

Recently, a novel sugar modification, *N*-acetylglucosamine, located on cytoplasmic and nuclearplasmic proteins has been found to be potentially analogous to protein phosphorylation [Kearse, Kelly, & Hart, G. W. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 1701−1705]. *N*-Acetylglucosamine, *O*-GlcNAc, is a possible protein regulatory modification much like a phosphorylation event. Our laboratory has detected changes in the level of *O*-GlcNAc modification in *Xenopus laevis* oocytes during progesterone-stimulated maturation. A significant increase in the level of *O*-GlcNAc modification was found on a small number of cytoplasmic proteins following

stimulation. Additionally, examination of proteins modified with *O*-GlcNAc in matched normal and tumorous breast tissue revealed increases in the level of monoglycosylated proteins in the tumor tissue. Interestingly, similar proteins were modified in both oocytes and various breast tissues although the relative level of modification on individual proteins was different.

62. UV/Vis Spectroscopic Measurements as Indicators of Platelet Quality. *Y. Mattley*,[1] S. Orton,[2] G. Leparc,[2] R. Potter,[1] and L. Garcia-Rubio.[3] [1]Department of Chemistry, [2]Florida Blood Services, and [3]Department of Chemical Engineering, University of South Florida, Tampa, FL 33620.

The necessity for high-quality whole blood and blood components is a major concern for the blood banking industry and the hospitals that transfuse these products. There is a large body of evidence suggesting that high-quality, functional platelets are necessary to alleviate spontaneous hemorrhage and other symptoms associated with decreased platelet count. In an effort to improve upon the quality assurance methods currently employed, we are investigating the use of UV/vis spectroscopy to monitor and quantify platelet quality. Previous work in our laboratories has demonstrated that a typical multiwavelength UV/vis spectrum includes information pertaining to cell size, number, and quality. The ability to extract this information from a single, rapid measurement makes UV/vis spectroscopy a powerful characterization tool. Preliminary simulations have demonstrated that we can detect changes in platelet number and aggregation using UV/vis spectroscopy. In addition, we have observed experimental spectral differences between platelets stored for varying lengths of time. We are currently investigating the physicochemical basis for these differences and their correlation to platelet quality.

63. Mitogen-Activated Protein Kinase (MAPK) Is Used as a Potential Marker for the Detection of Human Cell Proliferation and Differentiation. *Stephen A. Whelan, Jr.*,[1] Paul Baekey,[2] Charles Cox,[2] Nancy Lowell,[2] and Robert Potter.[1] [1]Department of Chemistry and [2]H. Lee Moffitt Cancer Center, University of South Florida, Tampa, FL 33620.

The early detection of abnormal cell proliferation and differentiation increases the effectiveness of the current cancer treatments and extends the longevity of the individual. We investigated the possibility of using mitogen-activated protein kinase (MAPK) as an indicator for tumorogenesis in human cells. Immunoblot data from three patients suggest that there is a higher quantity of MAPK present in tumorous breast tissue compared to matched normal tissue. In addition, we have examined a broad range of tumor tissues in an immunohistochemistry study. Most tissues as expected demonstrated an increase in the quantity of MAPK in tumorous tissue as compared to matched normal samples. Interestingly though, the lymph node, brain, and testes had especially high quantities of MAPK in tumors. We are currently extending these studies.

64. The Proposed Role of the B-Proteins as Anti-Apoptotic Agents in the Carcinogenic Process and in Other Processes. *E. T. Bucovaz*. Department of Biochemistry, University of Tennessee, Memphis, Memphis, TN 38163.

The titer of cancer-induced B-protein, which appears in the serum of individuals with a malignancy, is closely aligned with the progression of the disease. A correlation, as that identified with cancer, has been observed between patterns of B-protein production during pregnancy and tissue repair. It has been demonstrated based on purification protocol, amino acid composition, and other characteristics that two different B-proteins are produced by the body. An aberrant cell-induced B-protein is produced in response to cancer cells dividing abnormally. The other B-protein is produced by normal cells dividing in an abnormal manner, such as pregnancy or tissue repair. The ratio of cell proliferation vs cell death, normally a homeostatic mechanism for maintaining the body's cellular integrity, is controlled by anti-apoptotic processes of the body. In the case of cancer, an increase in the serum titer of B-protein signifies a more conducive environment for growth and division of cancer cells, and as the cancer cells grow and divide, the serum titer of B-protein increases. Thus, a perpetually expanding cycle is established that only can be interrupted either by eradicating the malignancy or by inhibiting the production of the aberrant cell-induced B-protein. The proposed role of the B-proteins is to create a conducive environment for the abnormal growth and division of cells (normal and aberrant) by inhibiting the apoptotic processes.

65. Inhibition of the *in Vitro* Formation of Irreversibly Sickled Cells Using Antioxidants. *Naomi F. Campbell*. Department of Chemistry, USA Comprehensive Sickle Cell Center, University of South Alabama, Mobile, AL 36688.

Sickle hemoglobin (HbS) polymerizes under conditions of low oxygen tension, producing sickle-shaped erythrocytes. Reversibly sickled cells return to the normal biconcave shape upon reoxygenation and depolymerization of HbS, but irreversibly sickled cells (ISCs) retain the deformed shape. Increased oxidative activity and reduced levels of antioxidants and reducing agents in the sickle erythrocytes could cause oxidative damage to the red cell membrane resulting in the formation of ISCs. We generated ISCs (~40% ISCs generated versus noncyclic control sample) using cyclic oxygenation/deoxygenation of low-density (45% Percoll layer) sickle erythrocytes for 16 h at 37 °C. Next, we incubated low-density sickle erythrocytes in the presence and absence of α-tocopherol and ascorbate using the aforementioned conditions. The cells incubated with α-tocopherol showed a 40% decrease in the formation of ISCs, and the cells incubated with ascorbate showed a 56% decrease in ISC formation. The correlation between ISC formation and oxidative damage to red cell membrane will also be discussed.

66. Myocardial Protection with Nitroxyl (NO^-) and Nitric Oxide ($^\bullet NO$) Donors. *J. Joseph*, E. A. Konorev, R. J. Singh, N. Hogg, and B. Kalyanaraman. Biophysics Research Institute, Medical College of Wisconsin, Milwaukee, WI 53226-0509.

Nitric oxide ($^\bullet NO$) donors have been shown to be protective against myocardial ischemia and reperfusion. $^\bullet NO$ is a free radical with an odd electron in the antibonding π orbital. It can readily be converted to the nitroxyl anion (NO^-) that will dimerize to form nitrous oxide (N_2O). Cytochrome *c* oxidase has been shown to reduce $^\bullet NO$ to NO^-. N_2O can also be formed during reaction between $^\bullet NO$ and protein and small molecular weight thiols. The objective of this study is to evaluate and compare $^\bullet NO$ and NO^- donors

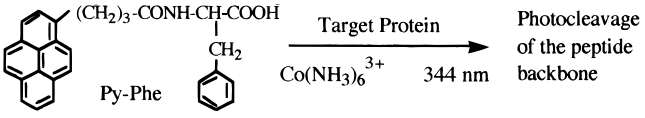
as cardioprotective agents in isolated Langendorf-perfused rat hearts. Results indicate that both *S*-nitrosoglutathione (•NO donor) and Angeli's salt ($HN_2O_3^-$, NO^- donor) protect the heart against myocardial ischemic injury. Nitronyl nitroxide counteracts the protective effects of GSNO and Angeli's salt. Direct ESR studies revealed the formation of nitrosylmyoglobin in intact hearts subjected to GSNO and Angeli's salt. We conclude that the cardioprotective action of GSNO and Angeli's salt may be mediated by either •NO or N_2O.

67. Spectroscopic Studies Characterizing the Complexes Formed by Reacting Nitric Oxide with *Chromatium vinosum* HiPIP. *Sandra L. Haberichter* and Kristene K. Surerus. Department of Chemistry, University of Wisconsin−Milwaukee, Milwaukee, WI 53201.

Cv HiPIP reacts with nitric oxide to form iron−nitrosyl complexes. The reaction was followed spectrophotometrically using NO released from spermine−NONOate ($t_{1/2}$ = 105), DEA-NONOate ($t_{1/2}$ = 7.7 min), or a saturated aqueous solution of NO. Following reaction of HiPIP with a 40-fold excess of saturated NO solution, the charge transfer band centered at 390 nm shifts to 355 nm. Reacting the HiPIP with a 40-fold excess of spermine−NONOate for 90 min or 40x DEA−NONOate for 20 min resulted in a bleaching of the 390 nm charge transfer band with a slight shift to 365 nm. The EPR spectrum of the resulting complex, regardless of the source of NO, had *g*-values at 2.04 and 2.02 which are characteristic of a dinitrosyldithiol−iron complex which is observed at liquid nitrogen temperatures. A new signal, not previously reported for iron−nitrosyl complexes, was observed with a *g*-value of 3.06. This signal is not seen at temperatures greater than 17 K. Currently work is in progress to better characterize the chemical species responsible for this signal.

68. Chemical Proteases: Site-Specific Photocleavage of Proteins. *Apinya Buranaprapuk* and C. V. Kumar. Department of Chemistry, University of Connecticut, Storrs, CT 06269-4060.

Site-specific photocleavage of proteins was achieved with the fluorescent probe Py-Phe. The fluorescent probe binds to a variety of proteins and cleaves the backbone when exposed to purple light, in the presence of cobalt(III)hexaammine chloride (CoHA). This is the first report of the



photocleavage of proteins, site-specific or not. The singlet excited state of Py-Phe is expected to sensitize the photodecomposition of CoHA to generate amine radicals, at the probe binding site. Amine radicals, similar in their reactivity to hydroxy radicals, can cleave the protein backbone via hydrogen abstraction from the α carbon of the peptide backbone. The resulting radical undergoes cleavage to give the protein fragments. This working hypothesis is under investigation. Bovine serum albumin (BSA), lysozyme, and myoglobin are cleaved efficiently by this methodology. No reaction occurred without Py-Phe, or CoHA, or light. Absorption, fluorescence, and circular dichroism experiments indicate the strong interaction of Py-Phe with the target proteins. The protein fragments were separated by polyacrylamide gel electrophoresis (SDS−PAGE).

69. Direct Electrochemical Study of Recombinant Nitrate Reductase Flavin Domain. M. J. Barber and A. J. Trimboli. Department of Biochemistry, University of South Florida, Medical College, Tampa, FL 33612. S. Nomikos and *E. T. Smith*. Department of Chemistry, Florida Tech, Melbourne, FL 32901.

Direct electrochemistry of the recombinant flavin domain of spinach assimilatory NADH:nitrate reductase (EC 1.6.6.1) was performed using a pyrolytic graphite electrode. The reduction potential ($E^{\circ\prime}$) of the FAD/$FADH_2$ couple was determined to be −268 mV versus SHE by square wave voltammetry. Cyclic voltammetry indicated an $E^{\circ\prime} = -257$ mV, and an $n = 2$ redox process with single waves in both reductive ($E_{p,c} = -272$ mV) and oxidative ($E_{p,a} = -242$ mV) directions. The peak current was directly proportional to (scan rate)$^{1/2}$, which indicated a reversible, diffusion-controlled electron transfer process. In contrast, $E^{\circ\prime} = -230$ mV was determined for free FAD. NADH:ferricyanide assays indicated no loss of catalytic activity following electrochemistry. The reduction potential of the flavin domain was apparently shifted ($E^{\circ\prime} = -192$ mV) and the peak current increased after NAD^+ was added. An $E^{\circ\prime} = -261$ mV was observed for the flavin domain after addition of nicotinamide. These values agree reasonably well with $E^{\circ\prime} = -280$ mV ($n = 2$) obtained for the nitrate reductase flavin domain from potentiometric titrations, and the results also indicated product complex formation influenced the flavin reduction potential. (Supported by the NIH and the USDA.)

70. A Temperature-Controlled Direct Electrochemical Investigation of Recombinant *Clostridium pasteurianum* Rubredoxin. *E. T. Smith* and S. Nomikos. Chemistry Department, Florida Tech, Melbourne, FL 32901. Q. Zeng, D. M. Kurtz, Jr., and R. A. Scott. Chemistry Department, University of Georgia, Athens, GA 30602.

Rubredoxin (Rd) is a low molecular weight iron−sulfur protein believed to be involved in microbial electron transport chains. The equilibrium electron transfer properties of recombinant *C. pasteurianum* Rd were examined by cyclic voltammetry directly at a pyrolytic graphite electrode utilizing a temperature-controlled electrochemical cell. The formal reduction potential ($E^{\circ\prime}$) of *Cp* Rd was determined to be −55 mV at 25 °C versus SHE, and the change in reduction potential of recombinant *Cp* Rd with temperature ($\Delta E^{\circ\prime}/\Delta T$) was determined to be −1.5 mV/°C from 10 to 50 °C. A continuum dielectric electrostatic model was used to reproduce the experimentally determined temperature-dependent reduction potential. This model was used to empirically derive a range of appropriate values for the protein dielectric constant ($\epsilon_{protein}$). In the case of *Cp* Rd, a temperature-dependent $\epsilon_{protein} = 5$ at 25 °C could be used to simulate the temperature-dependent reduction potential. Lower values of the dielectric constant (e.g., $\epsilon_{protein} = 4$ at 25 °C) could not be used to reproduce the experimental data.

71. Requirements in Quinone-Induced Lipid Peroxidation of Phosphatidylcholine Vesicles. *A. E. Alegría* and G. Santiago. Department of Chemistry, UPR-Humacao, CUH Station, Humacao PR 00791.

Quinones are cytotoxic compounds which are known to undergo redox cycling with the consequent production of oxygen radicals. Thus, one of the possible consequences of this process is lipid peroxidation initiated by reactive oxygen species. However, the quinone physical properties and structural requirements to induce lipid peroxidation have not been elucidated unambiguously. The extent of lipid peroxidation enhancement or inhibition by quinones in 3:1 egg-yolk phosphatidylcholine (PC)/linoleate (mol/mol) multilamellar vesicles, at pH 7.4, in the presence of Fe^{2+}−ascorbate, was measured. The quinones under study here comprise large ranges of octanol/buffer partition constants, one-electron redox potentials, and Fe^{3+} binding constants, as well as large differences in structure. All the quinones inhibited the Fe^{2+}−ascorbate-induced lipid peroxidation in the range of 2−6 mol % of quinone, relative to PC, with the exception of doxorubicin and daunorubicin at 6 mol % quinone. In general, no correlation was found between any of the quinone properties indicated above and the extent of lipid peroxidation inhibition/enhancement. However, for anthracycline-type quinones (ATQs), a correlation was found between their octanol/buffer partition constants and the extent of lipid peroxidation inhibition at 6 mol % of quinone. Thus, the iron-chelating features of ATQs united to their moderate hydrophilicity are more important requisites for lipid peroxidation enhancement than other factors.

72. Is Peroxynitrite a Source of Hydroxyl Radical Mediated Injury *in Vivo*? *Claire E. K. Richeson* and K. U. Ingold. Steacie Institute for Molecular Sciences, National Research Council of Canada, Ottawa, Ontario, K1A 0R6, Canada.

Peroxynitrite, formed by the reaction between the superoxide radical and nitric oxide, is a molecule of great biological interest. Although stable at alkaline pH, peroxynitrite undergoes rapid first-order decomposition below pH 7.4. The mechanism of peroxynitrite decay, which is believed to cause pathological damage *in vivo*, is disputed. One possibility is decomposition to a hydroxyl radical and a nitrogen dioxide radical. The aim of this work has been to identify and analyze methanesulfinic acid (MSA), the known major product of the reaction between dimethyl sulfoxide (DMSO) and hydroxyl radicals. Several potential methods for analyzing MSA were explored with ^{13}C NMR being the most advantageous. Preliminary results indicate that no more than a 2% yield of MSA based on peroxynitrite is obtained from peroxynitrite decomposition in the presence of a large excess of DMSO. Hence, no more than 2% of *free* hydroxyl radicals are formed from peroxynitrite. This work, as well as future experiments studying peroxynitrite decay, will be presented.

73. The Effect of Metal Ions on Zebra Mussel Arginase. *Calvin D. Tormanen*. Department of Chemistry, Central Michigan University, Mt. Pleasant, MI 48859.

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) is widely distributed in living organisms. The effect of metal ions on arginase was studied in the zebra mussel, a new exotic species of mollusk that was recently introduced into the Great Lakes. Arginase activity was found in both soluble and membrane-bound forms in the zebra mussel. Like all other arginases, zebra mussel arginase required Mn^{2+} for activity. In addition to Mn^{2+}, arginase was also activated by Ni^{2+} and Co^{2+}. The activation by Ni^{2+} was time-dependent, unlike the activation by Mn^{2+} and Co^{2+}. Several bivalent and trivalent metal ions were inhibitors of the enzyme. The K_m of the enzyme was similar with or without Mn^{2+}. Dialysis of the soluble arginase with or without prior activation by Mn^{2+} caused a large loss in enzyme activity which could not be recovered by incubation with Mn^{2+}, Ni^{2+}, or Co^{2+}.

74. Synthesis and Characterization of the Cadmium Peptide 49−61 of Rabbit Liver Metallothionein-II. *Amalia Munoz*, David H. Petering, and C. Frank Shaw III. Department of Chemistry, University of Wisconsin−Milwaukee, Milwaukee, WI 53201.

Protein folding of small proteins usually requires disulfide bonds or metal ions to stabilize the tertiary structure. Metallothionein (MT) is a small protein (61 amino acids) which lacks tertiary structure as the apoprotein, but folds into a 2-domain structure in the presence of seven Zn(II) or Cd(II). From previous NMR and crystallographic studies, it is known that the last four cysteines (50, 57, 59, 60) in the MT sequence are bound to the same metal ion (Cd-I). It is proposed that this is the nucleation site for the metal binding that leads to the folding of the protein. The peptide (Ac-Ile-Cys-Lys-Gly-Ala-Ser-Asp-Lys-Cys-Ser-Cys-Cys-Ala-COOH), corresponding to the last 13 amino acids of the rabbit liver MT-II, was synthesized according to the Merrifield solid phase method. The cadmium−peptide complex was characterized by ^{111}Cd-NMR, ES-MS, UV−vis, and amino acid analysis. Kinetic studies (with DTNB) were also carried out to compare the reactivity of the peptide−CD fragment with the holoprotein. This poster will discuss the characterization of the cadmium peptide and its comparison to the holoprotein.

75. Withdrawn.

76. Efflux is a Mechanism for Zinc Resistance in *Neurospora crassa*. *V. S. K. R. Vepachedu* and P. Maruthi Mohan. Department of Biochemistry, Osmania University, Hyderabad 500 007, India.

A zinc-resistant strain (ZNR2) of *Neurospora crassa* obtained by adaptation of wild type on zinc toxic medium shows 2-fold greater resistance to zinc than does wild type. The resistant strain shows reduction in energy-dependent uptake and increased efflux of zinc compared to wild type. The studies with radioactive zinc have shown that the metabolic inhibitors sodium azide and KCN do not cause any suppression of efflux, indicating the efflux is independent of metabolic energy. The efflux is not due to cell damage, and the mycelia show efflux even at low concentrations where the viability is not affected. The cell surface binding is less in the resistant strain than in the wild type when cells are metabolically active, but increases to 40% more than wild type in mycelia treated with sodium azide. These data, for the first time, show increased efflux as a mechanism of resistance to zinc toxicity in fungi.

77. Inhibition of a Bicarbonate and Sodium Ion Co-Transport Protein by Competitive and Noncompetitive Inhibitors. *Roger A. Egolf*,[1] Steven M. Grassl,[2] Michael H. Sroka,[1] Assy Yacoub,[1] and Hussam Yacoub.[1] [1]Department of Chemistry, Pennsylvania State University, Fogelsville, PA 18051, and [2]Department of Pharmacology, College of

Medicine, State University of New York Health Science Center, Syracuse, NY 13210.

A membrane-bound anion transport protein found in the proximal convoluted tubules of the kidneys is responsible for the active co-transport of bicarbonate ions along with sodium into the blood. The inhibition of this protein by compounds which bind to it can be measured by ^{22}Na uptake studies. We have found two classes of inhibitors of this protein, one which inhibits the transporter competitively, with the percent inhibition dependent upon bicarbonate concentration. This class includes 4,4′-dinitrostilbene-2,2′-disulfonate di-potassium salt (DNDS) and structurally related compounds. The other class inhibits transport noncompetitively with bicarbonate, and includes halogenated salicylates and halogenated 4-nitrophenols. This paper reports the synthesis and inhibitory properties of these compounds.

78. Isolation and Characterization of Biliprotein Complexes from Jellyfish (*Physalia physalis* and *Velella velella*). *Cliff I. Ross* and Frank Mari. Department of Chemistry and Biochemistry, Florida Atlantic University, 777 Glades Rd., Boca Raton, FL 33431.

Physalia physalis (Portuguese man-of-war) and *Velella velella* (by-the-wind-sailor) are brightly pigmented members of the pleustonic community of tropical and subtropical waters (Zaitsev, 1964; David, 1967) whose range occasionally extends into temperate areas. The toxic properties of the nematocysts of these organisms have been previously described (Lane, 1960), but the biochemical nature of their characteristic pigmentation has not been extensively investigated (Ball & Cooper, 1947; Herring, 1970). A more detailed study has been carried out in order to determine the relationship between the biochemistry and function of the pigments of *Physalia* and *Velella*. Previous work has indicated that the coloration of *Physalia* is due the presence of biliproteins. Bilins are tetrapyrrole chromophores found covalently attached to biliproteins that are responsible for color. A biliprotein mixture has been isolated from *Physalia* and *Velella* collected off the Florida Atlantic coast line. The isolation procedure included ammonium sulfate precipitation, gel filtration, ion exchange chromatography, and gel electrophoresis. Further separation was conducted via HPLC. Final analysis of the biliproteins and their prosthetic groups was carried out by NMR spectroscopy, UV−vis, fluorescence, and FT-IR. The results from the isolation and characterization procedure and a comparative analysis of the pigmented proteins found in these organisms will be discussed.

79. Isolation and Structural Studies of Neurotoxic Peptides from the Venom of Atlantic Cone Snail Species (*Conus* sp.). *Cliff I. Ross*, Robert Stangarone, and Frank Marí. Department of Chemistry and Biochemistry, Florida Atlantic University, 777 Glades Rd., Boca Raton, FL 33431.

Cone snails (genus *Conus*) are venomous and predatory molluscs. The venom is composed of small (10−30 amino acids) peptides with highly specific functions. Olivera *et al*. [Myers, R. A., Cruz, L. J., Rivier, J. E., & Olivera, B. M. (1993) *Chem. Rev. 93*, 1923−1936; Hopkins, C., Grilley, M., Miller, C., Shon, K. J., Cruz, L. J., Rivier, J., Yoshikami, D., & Olivera, B. M. (1995) *J. Biol. Chem. 270*, 22361−22367] have done extensive studies on the properties of the venom extracted from several *Conus* species collected in the Indo-Pacific region. However, very little is known about *Conus* species found off the Atlantic coast. The purpose of this study is to thoroughly analyze the venoms found in Floridian cone snails. Specimens of *Conus spurius* (The Alphabet Cone) were collected off the Florida Keys by SCUBA. The venom apparatus of these organisms was dissected out and disrupted. The venom was isolated by centrifugation, gel permeation chromatography, ion exchange, and reversed-phase HPLC. Several peptides with molecular weights ranging between 1.5 and 2 kDa have been isolated using this procedure. These peptides have been characterized using UV/Vis, FTIR, and NMR. The results from the characterization and structural determination of these peptides and possible structure−activity relationships will be discussed.

80. The Biosynthesis of Bisdechlorogeodin in *Penicillium glabrum* and *Ovadendron sulphureo-ochraceum*. *Koren A. Holland*, Jennifer Mazzola, and Prasad V. Kambli. Department of Chemistry, Gettysburg College, Gettysburg, PA 17325.

Examples of secondary metabolites with significant biological activity include taxol, bleomycin, and tetracycline. Therefore, the study of secondary metabolic pathways contributes significantly to the foundation from which novel pharmaceuticals are developed. Sulochrin oxidase is a secondary metabolic enzyme which catalyzes the oxidation and cyclization of sulochrin, a benzophenone, to bisdechlorogeodin, a seco-anthraquinone, in the presence of dioxygen. Sulochrin oxidase has been characterized as a blue copper oxidase (Nordlov & Gattenbeck, 1982). The most intriguing aspect about this particular system is that (+)-bisdechlorogeodin is produced from the *P. glabrum* enzyme while the (−) isomer is produced from the *O. sulphureo-ochraceum* enzyme. Elucidation of the mechanism, the active site configuration, and, eventually, the gene sequence from each source will allow us to further define the unique differences. Progress toward these goals will be presented in detail.

81. Synthesis and Biological Properties of Cobalt−Uric Acid Complex. *V. R. Vepachedu*. Biochemistry Department, Agricultural College, Andhra Pradesh Agricultural University, Awsaraopet 507301, India.

The ureides allantoin and allantoic acid were found to be the chief storage and translocatory forms of nitrogen in legumes. Though much has been studied about uricase, which forms allantoin from uric acid (UA), no specific, strong inhibitor of uricase is known. Studies wherein the metal complexes made with the substrates acted as dead end inhibitors of the respective enzymes indicated that this may also help in studying the kinetics of uricase. Hence, to study the metabolic importance of ureides, a metal complex of the uric acid was thought to be of some use in acting as a probe. In that process, a cobalt−uric acid complex has been synthesized and characterized. It was found to differ from the other complexes of uric acid. The other effects of the Co−UA complex on the induction and action of uricase were studied.

Monday Morning—Glycobiochemistry—H.-W. Liu and C.-H. Wong, Organizers, Presiding

82. Molecular Studies on UDP-Muramic Acid Biosynthesis in Bacterial Peptidoglycan Assembly. *Christopher T. Walsh*.

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115.

The first three enzymes in bacterial cell wall assembly encoded by the GlmU, MurA, and MurB genes convert UTP, glucosamine, acetyl-CoA, and PEP to UDPGlcNAc, enolpyruvyl-UDPGlcNAc, and D-lactyl-UDPGlcNAc (UDP-muramic acid) sequentially. Mechanistic and structural studies have been carried out on each enzyme. GlmU is a bifunctional acetyl transferase coupled to a uridylyl transferase. MurA transfers the enolpyruvyl group from PEP to the 3′-OH of UDPGlcNAc via a tetrahedral adduct intermediate. MurB is an NADH-utilizing flavoprotein reductase which carries out an anti addition of a hydride to C_3 and a proton at C_2 of enolpyruvyl-UDPGlcNAc to yield the lactyl ether in the UDP-muramic acid on which the peptidyl chain of the PG layer is subsequently assembled.

83. Enzymatic Synthesis of Lipopolysaccharide. *Christian R. H. Raetz*. Department of Biochemistry, Duke University, Durham, NC 27710.

Lipid A, the hydrophobic anchor of lipopolysaccharide (LPS) in Gram-negative bacteria, constitutes the outer monolayer of the outer membrane [Raetz, C. R. H. (1993) *J. Bacteriol. 175*, 5745]. Because of its potent (pM) immunostimulatory activity, lipid A is also known as endotoxin. Lipid A is a β, 1′-6-linked disaccharide of glucosamine, acylated at positions 2, 3, 2′, and 3′, and phosphorylated at positions 1 and 4′. In earlier studies, we determined that nine unique *E. coli* enzymes convert the precursors UDP-GlcNAc, acyl-acyl carrier protein, and CMP-Kdo to the minimal LPS (having the composition Kdo_2-lipid A) that supports cell growth. Most of the enzymes are now available in recombinant form. Some have been used for synthesis of lipid A analogs. Several are targets for development of new antibacterial agents, including the first enzyme, UDP-GlcNAc 3-*O*-acyltransferase, which has recently been crystallized. Its X-ray structure was determined to 2.6 Å resolution [Raetz, C. R. H., & Roderick, S. L. (1995) *Science 270*, 997−1000]. The enzyme is a trimer displaying a new type of protein secondary structure, termed a left-handed parallel β-helix (LβH). A hexad repeat specifies the LβH domain. Structural studies of the enzymes that assemble lipid A will provide fundamental new insights into protein/lipid and protein/carbohydrate recognition.

84. Some Unusual Oligosaccharides in Plant and Insect Glycoproteins and Their Biological Significance. *Yuan C. Lee*,[1] N. Takahashi,[2] S. Su,[3] and M. Betenbaugh.[4] [1]Department of Biology, Johns Hopkins University, Baltimore MD 21218, [2]GlycoLab, Nakano Vinegar Co., Handa City, Japan, [3]Veterans General Hospital, Taipei, Taiwan, and [4]Department of Chemical Engineering, Johns Hopkins University, Baltimore, MD 21218.

A Bermuda grass antigen, BG60, is a glycoprotein that contains an unusual oligosaccharide, Manα(1,6)[Manα(1,3)]-Manβ(1,4)GlcNAcβ(1,4)[Fucα(1,3)]GlcNAc, as the major carbohydrate constituent (ca. 70%). The carbohydrates in BG60 appear to be involved in immunochemical reactions. Although insect cells are commonly known to produce only high mannose type oligosaccharides, they can produce complex or hybrid type *N*-glycosides under certain conditions. For example, human IgG secreted by baculovirus-infected insect cells contain mostly complex type oligosaccharides, but the intracellular IgG contains mostly high mannose type oligosaccharide chains.

85. Protein Glycosylation Specificity and Function. *Barbara Imperiali*. Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.

The first committed step in the biosynthesis of all *N*-linked glycoproteins is catalyzed by the membrane-associated enzyme oligosaccharyl transferase and involves the cotranslational transfer of a complex carbohydrate from a dolichol-linked pyrophosphate donor to the carboxamide side chain of an asparagine residue. The primary peptide sequence requirements for the process include a minimum -Asn-Xaa-Ser/Thr- tripeptide recognition motif. This transformation is intriguing in that it formally involves the nucleophilic attack by an asparagine primary amide nitrogen, and displays remarkable selectivity considering the competing functionality in the peptidyl substrates in which the "reactive" asparagine is localized. This presentation will describe our recent progress toward understanding the sequence specificity and structural consequences of the *N*-linked glycosylation processes.

86. Kinetic Studies of Oligosaccharyltransferase-Catalyzed Glycosylation: Structural Modification of the Carbohydrate Donor and Peptide Acceptor Substrates. *J. K. Coward*, B. S. Gibbs, Y.-L. Liu, and X. Fang. Department of Chemistry and College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.

In the biosynthesis of *N*-linked glycoproteins, oligosaccharyltransferase (OST) catalyzes the *cotranslational* glycosylation of growing polypeptides as they exit the ribosomal complex enroute to the endoplasmic reticulum. The glycosylation "sequon" consisting of the amino acid sequence -NXT/S- is necessary but not sufficient for facile glycosylation. In this contribution, we present steady-state kinetic data on the OST-catalyzed glycosylation of the peptide Bz-NLT-NH_2 by either the natural, complex lipid-linked oligosaccharide (LOS) or the chitobiose-containing lipid disaccharide (LDS). Comparison of two donor substrates, LOS and LDS, indicates that the latter is only ca. 3−10-fold less active (V_{max}/K_m) than the former. In contrast, each member of a series of synthetic lipid disaccharides in which dolichol is replaced by a shorter, more homogeneous lipid component is inactive as an OST substrate. Acceptor substrates ranging in size from ca. 13 kDa (ribonuclease, α-lactalbumin) to several tetra- and tripeptides have been compared in terms of their substrate activity relative to Bz-NLT-NH_2. Included in this group are a series of peptides derived from external invertase, a glycoprotein containing 14 sequons, only 7 of which are always glycosylated.

Monday Afternoon—Glycobiochemistry—H.-W. Liu and C.-H. Wong, Organizers, Presiding

87. Mechanism Based Inhibition of Ribonucleotide Reductases. D. J. Silva,[1] W. van der Donk,[1] C. Lawrence,[1] G. Gerfen,[1] S. Licht,[1] V. Samano,[2] M. J. Robins,[2] and *J. Stubbe*.[1] [1]Department of Chemistry, MIT, Cambridge, MA 02139, and [2]Department of Chemistry, Brigham Young University, Provo, UT 84602.

Ribonucleotide reductases (RNRs) catalyze the conversion of nucleotides to deoxynucleotides in all organisms. Stopped-

flow UV−vis spectroscopic studies and rapid-freeze quench EPR studies have been used to examine the interaction of 2′,2″-difluorocytidine nucleotides and 2′-vinylenecytidine nucleotides with RNRs in an effort to obtain evidence for nucleotide radical intermediates and the ability of the thiyl radical to initiate nucleotide reduction by 3′ hydrogen atom abstraction. The use of the rapid-freeze quench method, in contrast to quenching on the second scale, is essential to obtain evidence for the initially generated radical intermediates.

88. Mechanistic Studies of the Biosynthesis of Ascarylose. *Hung-wen Liu*. Department of Chemistry, University of Minnesota, Minneapolis, MN 55455.

The penultimate step in the biosynthesis of ascarylose, a 3,6-dideoxyhexose found in the O-antigen of *Yersinia pseudotuberculosis*, is the C-3 deoxygenation reaction catalyzed by two enzymes—CDP-6-deoxy-L-*threo*-D-*glycero*-4-hexulose-3-dehydrase (E_1) and CDP-6-deoxy-L-*threo*-D-*glycero*-4-hexulose-3-dehydrase reductase (E_3). In our efforts to elucidate the mechanism of this novel C−O bond cleavage event, we have carried out a multifaceted study to fully characterize the catalytic properties of these two enzymes. Our results indicated that the reaction is initiated by a dehydration followed by a reduction involving stepwise electron transfer. Further analysis revealed the presence of a free radical intermediate with physical characteristics most closely resembling an uncoupled phenoxy radical. Various chemical as well as spectroscopic methods allowed us to monitor the kinetic course and to analyze the chemical nature of this organic radical intermediate. These results provide compelling evidence for the participation of a redox-active PMP pseudo-*ortho*-quinonoid intermediate, establishing an unprecedented role of coenzyme B_6 in redox catalysis. Evidence in support of the radical mechanism will be presented and the possible nature of the radical intermediate will be discussed.

89. The Biological Interconversion of Galactose and Glucose. *Perry A. Frey*. Institute for Enzyme Research, University of Wisconsin−Madison, Madison, WI 53705-4098.

The interconversion of galactose and glucose units in all living cells takes place at the level of the nucleotide sugars and is catalyzed by the enzymes of the Leloir Pathway. UDP-galactose 4-epimerase and galactose-1-P uridylyltransferase catalyze essential reactions in this process. The mechanisms of action and structures of these enzymes will be described. The epimerase contains tightly bound NAD^+ and catalyzes the reaction UDP-Gal ⇌ UDP-Glc. The mechanistic role of the nucleotide portion of substrate and product in the mechanism of epimerization will be described, as well as the detailed binding interactions between the enzyme and nucleotides. The uridylyltransferase catalyzes UMP-group transfer by a ping-pong mechanism in the reaction Gal-1-P + UDP-Glc ⇌ Glc-1-P + UDP-Gal. The intermediate is a uridylyl-enzyme, in which the UMP-group is bonded to His^{166} of the *E. coli* enzyme. The reaction mechanism and the importance of zinc and iron in maintaining the structure of the enzyme through coordination with histidine, cysteine, and glutamate residues will be described. (Supported by Grant GM30480 from the National Institute of General Medical Sciences.)

90. Glycosynthases: Engineered Glycosidases Which Synthesize, but Do Not Degrade, Oligosaccharides. *S. G. Withers*, Q. Wang, L. Mackenzie, and R. A. J. Warren. Protein Engineering Network of Centres of Excellence and Departments of Chemistry and Microbiology, University of British Columbia, Vancouver, B.C., Canada V6T 1Z1.

Glycosidases are enzymes which catalyze the hydrolysis of glycosidic bonds, doing so either by a direct displacement or by a double-displacement mechanism involving a covalent glycosyl-enzyme intermediate. The use of the latter class of enzymes to synthesize glycosides has been reasonably extensively explored, the most useful method involving a transglycosylation process. This requires the interception of the normal glycosyl-enzyme intermediate with a sugar acceptor in place of water, such that a new glycosidic bond is formed. However, this approach suffers from the severe problem that the product formed is necessarily a substrate for the enzyme; thus, careful timing is necessary for acceptable yields. We have largely overcome this problem by the use of specific glycosidase mutants which can synthesize glycosidic bonds, but cannot hydrolyze them. Results on this new approach will be presented, along with our attempts to effect control over regiochemistry.

91. Studies on the Mechanism and Inhibition of Sialyl Lewis X-Mediated Cell Adhesion. *C.-H. Wong*. Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037.

This lecture will present our recent work on the study of selectin−carbohydrate interaction, with particular emphasis on the development of inhibitors targeting sialyl Lewis X-mediated cell adhesion. Both carbohydrates and carbohydrate mimetics of sialyl Lewis X have been designed, and their inhibition of E-selectin has been evaluated. In addition, the mechanism and inhibition of α-1,3-fucosyltransferase V involved in the biosynthesis of sialyl Lewis X will be presented.

92. Glycals in Organic Synthesis: The Evolution of New Strategies for the Convergent Assembly of Oligosaccharides and Other Glycoconjugates. *S. J. Danishefsky*. Kettering Chair and Director, Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Ave., Box 106, New York, NY 10021, and Professor of Chemistry, Columbia University.

It is becoming increasingly apparent that oligosaccharides are intimately involved in important biological processes. Carbohydrates have been implicated as specific recognition elements in the immune response, in cell−cell adhesion, and in the attachment of pathogenic species. Given the significant roles these structures play in biology, the ability to assemble them in homogeneous form continues to be of substantial importance. The utilization of glycals has emerged as a powerful method for the construction of oligosaccharides and glycoconjugates. Glycal methods have been used to assemble many complex polysaccharides in solution, and more recently much progress has been made in the area of polymer-supported synthesis. In addition, methodology has been developed for the synthesis of glycopeptides on a solid support.

TUESDAY MORNING—1996 ELI LILLY AWARD IN BIOLOGICAL CHEMISTRY, HONORING GREGORY L. VERDINE—S. L. SCHREIBER, PRESIDING

93. Chemical Approach to Understanding and Controlling Signal Transduction. *Stuart L. Schreiber*. Department of Chemistry and Chemical Biology, 12 Oxford St., Cambridge, MA 02138.

Insights into signaling pathways and other cellular processes have resulted from studies of cell-permeable, organic molecules identified from natural sources and designed and synthesized in the laboratory. This lecture will present results of studies using such molecules to understand and control intracellular signaling pathways—the chemical genetics approach. These low molecular weight ligands cause either a conditional loss of function following binding to the products of wild-type alleles or a gain of function following binding to the products of rationally designed conditional alleles. Examples are seen in studies of immunophilin−natural product complexes that led to the identification of calcineurin as a mediator of T cell receptor signaling and of FRAP as a mediator of signaling that links mitogenic pathways to the cell cycle machinery. A family of cell-permeable ligands that induce intracellular proteins to associate, developed in collaboration with Gerald Crabtree, has been used to regulate transcription and signal transduction (including pathways emanating from the T cell receptor and the apoptosis-inducing Fas antigen), and other cellular processes such as intracellular protein degradation and translocation. Finally, we have been using protein-structure-based combinatorial chemistry to discover cell-permeable ligands to any protein target. Such a capability is required in order for chemical genetics to have the broad generality of classical genetics-based methods for studying protein function.

94. Exploring the Biology of Transition Metals. *R. D. Klausner*. National Cancer Institute, 31 Center Dr., MSC 2590, Bethesda, MD 20892-2590.

The requirement that virtually all cells exhibit for iron poses enormous challenges in the uptake, utilization, and control of the metal. Central to this control is the ability to sense available levels of iron and to translate that into mechanisms for the regulation of the expression and function of genetic networks. In complex eukaryotic cells, two iron sensors regulate multiple genes at the level of the fate of mature transcripts. These iron regulatory proteins (IRPs) are capable of binding iron, in one case as an iron−sulfur cluster. Iron binding results in the loss of high-affinity RNA binding for specific target structures called iron responsive elements. A genetic approach to solve the iron uptake problem in eukaryotes using *Saccharomyces cerevisae* has revealed a requirement for copper-dependent oxidases in the biochemical process of the movement of iron across membranes. The inter-relationship between copper as a cofactor in iron transport and the control of iron uptake appears to be conserved through eukaryotic evolution. Our genetic approach in a simple eukaryote has allowed us to map and identify the molecular components of iron uptake and control.

95. Pyranosyl-RNA. *A. Eschenmoser*. Laboratory of Organic Chemistry, ETH, CH-8092 Zürich, Switzerland.

Pyranosyl-RNA ("p-RNA") is the oligonucleotide system isomeric to RNA that contains ribopyranosyl instead of ribofuranosyl units and phosphodiester bridges between positions 4′ → 2′ instead of 5′ → 3′. The study of the chemical properties of p-RNA oligonucleotides is part of a comprehensive experimental investigation aiming at a chemical rationalization of nature's evolutionary choice of RNA and DNA as genetic systems. Experiments reveal that purine-pyrimidine pairing in p-RNA is not only stronger than in RNA, but also more selective with respect to pairing modes. This exceptional selectivity is one of the reasons for p-RNA's apparently superior potential to replicate without enzymes. The lecture will illustrate and discuss this potential.

96. Keepers of the Code. Studies on Proteins That Decorate and Mend the Genome. *G. L. Verdine*. Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138.

Numerous proteins attend to the genome, preserving the integrity of the genetic information reposited therein and modifying the covalent structure of DNA so as to increase its information content. Our program aims to uncover the structural basis for substrate recognition and catalysis by these keepers of the code. Analysis of the reaction mechanisms employed by DNA-modifying proteins serves as a basis for the design of molecules that subvert the normal catalytic pathway, leading to the formation of long-lived protein−DNA complexes that can be analyzed in detail through high-resolution structural methods. This approach has yielded insights into the processes through which proteins transfer methyl groups to and from DNA and catalyze the excision of damaged bases. These studies have revealed the dramatic ways in which proteins distort DNA in order to gain access to functionality that is otherwise inaccessible in a canonical B-form duplex. Aspects of these and related studies will be discussed.

TUESDAY AFTERNOON—CHEMISTRY OF GENE REGULATION—T. KODADEK, ORGANIZER, PRESIDING

97. Biochemical Mechanisms of Homeodomain Protein Action in *Drosophila* Embryos. *M. D. Biggin*, R. Austin, A. Carr, D. Dalma-Weiszhausz, Z. Liang, A. TenHarmsel, J. Toth, and J. Walter. Department of Molecular Biophysics and Biochemistry, Yale University, 266 Whitney Ave., New Haven, CT 06520.

Many of the genes regulating *Drosophila* development belong to a family of transcription factors which share a homologous DNA binding domain, termed the homeodomain. In vitro, repression of transcription by the homeodomain protein *even-skipped* (*eve*) involves cooperative binding to high and moderate affinity DNA sites and bending of the DNA between the sites. In vivo UV cross linking experiments support this result, showing that *eve* protein binds to a surprisingly broad array of genes and DNA sites in *Drosophila* embryos, including many moderate affinity sites. Further experiments suggest that while the broad DNA recognition properties of *eve* protein in vitro are likely to be important determinants of this protein's distribution on DNA in vivo, they are not sufficient to fully explain binding in vivo. The other factors influencing binding in vivo and the functional role of widespread DNA binding by *eve* and other homeodomain proteins are being investigated.

98. Mechanistic Studies of Eukaryotic Transcriptional Regulation. *Michael R. Green*. Program in Molecular

Medicine, University of Massachusetts Medical School, 373 Plantation St., Worcester, MA 01605.

The synthesis of mRNA in eukaryotic cells is a complex process that responds in a precise way to many biochemical signals. A central goal of molecular biology is to understand how transcription is regulated. Many of the proteins involved in this process will be discussed here. These general transcription factors (TFs), along with RNA polymerase II, constitute the core of the transcription machinery. Emphasis will be placed on how these factors communicate with gene-specific activators that mediate their activity. The mechanisms of how these activators bind their sites sequence specifically will also be considered.

99. Multilayered Control of Intracellular Receptor Function. *K. R. Yamamoto*, N. Kralli, J. A. Lefstin, W. Matsui, D. B. Starr, J. R. Thomas, and M. d. M. Vivanco. Department of Cellular and Molecular Pharmacology, UCSF, San Francisco, CA 94143-0450.

Intracellular receptors receive extracellular signals and regulate specific gene transcription. We describe three novel levels of control over glucocorticoid receptor (GR) activity. First, Pdr5p was discovered in yeast as an ABC transporter that exports particular steroids. Pdr5p-like proteins appear to act in some but not all mammalian cells, providing a potential mechanism for cell-specific signaling. Second, the GR zinc-binding region contains determinants that govern whether a remote portion of the molecule will form an activation or repression domain upon different GR−GRE interactions. Mutations isolated in both response elements and GR itself support the view that response elements serve as allosteric ligands that confer distinct GR conformations, and therefore functions. Third, transformation or immortilization of various cell types is accompanied by a striking and selective increase in the activities of intracellular receptors such as GR and RAR. Thus, normal primary cells may have a heretofore unrecognized capability to limit the magnitude of induction of gene expression. Overall, we conclude that the activities of intracellular receptors are influenced at multiple levels, including access to ligands, genomic interaction sites, and higher order controls that may relate to cell growth and differentiation phenotypes. We suggest that these multiple forms of cellular signaling information are integrated by the receptors, and therefore that they serve as key determinants of the cell- and gene-specificity of receptor action.

100. New Methods for the Study of Multiprotein Transcription Complexes in Vitro and in Vivo. *Thomas Kodadek*. Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712.

To facilitate detailed mechanistic studies of transcriptional regulation, we have developed two new methods to study protein−protein and protein−DNA interactions in vivo and in crude lysates. First, a "plasmid titration" method is presented, in which the DNA-binding properties of a transcription factor can be studied in a quantitative fashion in living yeast cells. This method has been used to elucidate the effect of other transcription factors on GAL4 protein−DNA interactions. Second, a novel protein cross-linking method is presented. In this procedure, a cross-linking reagent is delivered specifically to a particular location in a large complex through association with a peptide receptor fused to the target protein. When activated, the cross-linking reagent reacts only with nearby factors. This greatly simplifies interpretation of the results relative to a traditional cross-linking experiment, since only proteins in the immediate vicinity of the peptide receptor−cross-linker conjugate are affected. Application of this technology to the study of interactions between activators and intact transcription complexes is discussed.

Wednesday Morning—1996 Pfizer Award in Enzyme Chemistry, Honoring P. Andrew Karplus—R. Matthews, Presiding

101. The Growing Importance of Mass Spectrometry in Studies of Structure/Function in Proteins. *Kenneth A. Walsh*. Department of Biochemistry, University of Washington, Seattle, WA 98195.

Virtually all proteins appear to be posttranslationally modified by covalent additions or deletions during their biosynthesis or subsequent regulation. These chemical changes serve diverse ends, such as introduction of prosthetic groups, stabilization of protein structures, and coding of cellular targeting. Literally hundreds of different posttranslational events have been identified; of particular concern are recombinant proteins expressed in heterologous cells where different processing enzymes may yield unexpected products. Although some posttranslational modifications can be predicted from motifs observed in DNA sequences, the most generally applicable detection techniques rely on mass spectrometry in the electrospray (ESI-MS) or matrix-assisted laser desorption-time of flight (MALDI-TOF) mode. A difference between the mass predicted from the DNA and that observed for the protein leads to digesting the protein and examining, in a coupled HPLC/MS system, peptides that do not fit DNA predictions of mass. Tandem MS/MS techniques or postsource decay methods can then identify the modification and its locus.

102. Catalytic Mechanism of Human Purine Nucleoside Phosphorylase. *Steven E. Ealick*. Section of Biochemistry, Molecular, and Cell Biology, Cornell University, Ithaca, NY 14853. Mark D. Erion. Gensia, Inc., 9360 Towne Center Dr., San Diego, CA 92121.

Purine nucleoside phosphorylase (PNP) catalyzes the reversible phosphorolysis of purine nucleosides to the free base and ribose 1-phosphate. Human PNP functions in the purine salvage pathway and is specific for inosine, guanosine, and other 6-oxopurine nucleosides. Interest in PNP derives from its role as a target for chemotherapy and from its potential use as a laboratory catalyst for nucleoside synthesis. The structures of human PNP and of various PNP/ligand complexes were analyzed using X-ray crystallography, and residues potentially involved in catalysis were identified. These residues were systematically evaluated using site-directed mutagenesis and enzyme kinetics. The results of mutagenesis, kinetics, molecular modeling, and X-ray crystallography were used to propose a substrate-assisted catalytic mechanism. The transition state involves stabilization by a conserved Asn residue and an oxocarbenium ion that is stabilized by the substrate phosphate. A substrate-induced conformational change is used to position the key residues in the active site. Our studies show that the nucleoside binds in a nonstandard conformation and the phosphorolysis may

be assisted by ligand strain. The mechanism also features a catalytic triad consisting of Glu, His, and substrate phosphate. The mechanism for PNP-catalyzed phosphorolysis is distinct from that used by nucleoside glycosidases to cleave the glycosidic bond but may be generalizable to other phosphorylases.

103. Urease Bi-Nickel Metallocenter Assembly. *Robert P. Hausinger*. Department of Microbiology, Michigan State University, East Lansing, MI 48824.

Urease possesses a bi-nickel metallocenter that is bridged by a carbamylated lysine residue. The novel metallocenter can be formed by providing CO_2 and nickel ion to the apoprotein; however, the level of in vitro activation is low, and several other metal ions inhibit this process. Cellular biosynthesis of active urease requires the participation of four accessory proteins. Three of these proteins (UreD, UreF, and UreG) appear to function in complexes with urease apoprotein, whereas UreE appears to function as a nickel-binding peptide. This talk will describe the nickel-binding properties of UreE and include a comparison of the activation properties of urease apoprotein versus the UreD−urease, UreD−UreF−urease, and UreD−UreF−UreG−urease apoprotein species. In addition, I will describe evidence for the presence of a UreD−UreF−UreG complex separate from urease and suggest how it may be integrated into the cellular urease activation machinery.

104. 70 Years of Crystalline Urease: What Have We Learned? *P. Andrew Karplus*. Section of Biochemistry, Molecular, and Cell Biology, Cornell University, Ithaca, NY 14853.

The crystallization of jack bean urease by James B. Sumner in 1926 helped define the fundamental proteinaceous nature of enzymes. The jack bean urease structure has not yet been solved, but last year, we determined the crystal structure of a bacterial urease, that from *Klebsiella aerogenes*, revealing the geometry of the protein and the novel bi-nickel active site, and indicating an unexpected homology with the enzymes adenosine deaminase and phosphotriesterase. Combining the structural information with kinetics and mutagenesis studies has led us to propose a urea binding mode, and to conceive a catalytic mechanism that differs in detail from the orthodox mechanism. Further structural and biochemical studies of wild-type and mutant ureases are now underway to test these ideas. I will discuss these issues and new complexities of urease enzymology uncovered by our studies.

Wednesday Afternoon—Biological Chemistry of Copper—T. V. O'Halloran, Organizer, Presiding

105. Copper and Iron in Yeast: Mechanistic, Regulatory, and Physiologic Interactions. *D. J. Kosman*. Department of Biochemistry, SUNY, Buffalo, NY 14214.

In *Saccharomyces cerevisiae*, high-affinity Cu uptake requires expression of the *CTR1* gene, encoding a Cu transporter. High-affinity Fe accumulation requires the expression of *FET3*, a multi-Cu ferroxidase homologous to ceruloplasmin. The uptake of either metal requires reduction from the -ic to the -ous valent state by plasma membrane metal reductases encoded by *FRE1, 2*. Mac1p mediates Cu regulation of *CTR1* and *FRE1* transcription, while Aft1p mediates Fe regulation of *FET3* and *FRE2* transcription. Deletion of either *MAC1* or *CTR1* leads to a Cu deficiency that results in downstream Fe and consequent heme deficiencies. Strains that become Fe-deficient via any mechanism exhibit pleiotropic phenotypes that can be ascribed to loss of heme-based signaling pathways and *trans*-activators, *e.g.*, Hap1, a heme-dependent positive activator of *CYC1* (iso-1 cytochrome *c*), and *CTT1* (catalase). Both activities are strongly reduced in *mac1*Δ, *ctr1*Δ, and *fet3*Δ strains because of the Fe and heme deficiencies. Thus, these mutant strains are respiration-deficient and peroxide-sensitive due to the loss of transcriptional activation of genes encoding respiratory and antioxidant functions. The heavy metal and thermal sensitivity of these mutants also correlate to the loss of transcriptional activation of stress response genes such as *UBI4*, which encodes the stress response polyubiquitin, and *DDR2*, encoding a DNA damage repair function. The critical role that Cu plays in Fe metabolism in yeast and the essentiality of Fe for normal aerobic growth explain the tight, overlapping regulation of Cu and Fe metabolism by the metals themselves. (Supported by NIH Grant GM46787).

106. The Role of the *Saccharomyces cerevisiae ATX1* Protein in the Metabolism of Copper Ions and Oxygen Radicals. Valeria C. Culotta. Department of Environmental Health Sciences, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205.

Oxidative damage in yeast cells lacking the copper/zinc superoxide dismutase (SOD) can be suppressed by overexpression of the *ATX1* (anti-oxidant) gene. *ATX1* causes cells to accumulate elevated levels of copper, which in turn circumvents the need for copper/zinc SOD. The *ATX1* polypeptide bears striking homology to the MerP mercury transporter of bacteria and contains a single copy of the well-conserved MTCXXC metal binding motif found in both bacterial and eukaryotic metal transporters. Our studies strongly indicate that *ATX1* utilizes this motif to bind copper ions and to transport the metal from the cytosol to intracellular compartments of the cell. We have created yeast strains that lack *ATX1*; these cells show increased sensitivity toward O_2^- and are also defective in the copper-mediated pathway of ferrous iron transport. Hence, *ATX1* plays a dual role in oxygen radical and transition metal metabolism.

107. Interactions of the Copper Metalloregulatory Protein CUP2 with the Promoter of the Yeast Copper Metallothionein Gene. *Thomas D. Tullius*. Department of Chemistry, The Johns Hopkins University, Baltimore, MD 21218.

The product of the *CUP1* gene of the yeast *Saccharomyces cerevisae* is a metallothionein which protects against high concentrations of copper. Copper(I) is bound to the protein in a multinuclear, cysteine thiolate-bridged cluster. A second copper(I) cluster-containing protein, CUP2 (also known as ACE1), regulates the expression of the yeast metallothionein gene at the level of transcription. CUP2 binds upstream of the *CUP1* gene to a site called upstream activation sequence c (UASc). This DNA sequence has approximate inversion symmetry and binds two molecules of CUP2. Previous mutational studies found that the upstream-most half-site is most important for transcriptional regulation. We have prepared DNA molecules having either a single upstream or downstream UASc half-site or two palindromically-related upstream or downstream half-sites to determine the affinity of CUP2 for each half-site. We find by quantitative gel

mobility shift experiments that CUP2 binds to the upstream half-site with 8-fold higher affinity, and that there is no cooperativity in binding of two CUP2 molecules to the UASc. We have used hydroxyl radical footprinting and missing nucleoside experiments to compare the binding of wild-type CUP2 and the ace1 mutant protein, which lacks one cysteine [and one copper(I) ion from the cluster]. We use these data to relate the differences in binding we observe to the mode of interaction of CUP2 with DNA.

108. Copper−Dioxygen Reactivity in Synthetic Coordination Complexes. *Kenneth D. Karlin.* Department of Chemistry, The Johns Hopkins University, Baltimore, MD 21218.

Copper-containing proteins are critically involved in a variety of redox roles, including the transport of dioxygen (hemocyanins; arthropods & molluscs), monooxygenation of substrates (e.g., R−H → R−OH), the reduction of O_2 to H_2O_2 or H_2O with concomitant substrate oxidation, superoxide dismutation (i.e., in Cu−Zn SOD), electron transport, and the reduction of nitrogen oxides such as NO_2^- and N_2O. In addition, copper ion mediated oxidative processes may contribute to various disease states, via damage caused to protein or nucleic acid constituents. Copper(I)−dioxygen interactions are critical to most of these processes; our goals include the elucidation of fundamental aspects of the relevant coordination chemistry; the influence of ligand-donor type, coordination number, and geometry; and how such factors allow for reversible binding and/or substrate oxygenation, or control Cu_n-O_2 structure. Synthetically derived systems which reversibly bind O_2 will be surveyed, especially those utilizing mononucleating tetradentate ligands. When bulky or binucleating analogues are utilized, the O_2-binding stoichiometry or kinetics−thermodynamics of O_2 reaction are altered. The activation of O_2 for oxygenation of a hydrocarbon substrate (i.e., on the ligand which binds copper ions) will be reviewed. Recent examples suggested to involve dicopper complex mediated oxo-transfer will be described.

109. The Structure of Human Ceruloplasmin at 3.0 Å Resolution: The Beginning of the End of an Enigma. *P. Lindley*, I. Zaitseva, V. Zaitsev, A. Ralph, and G. Card. CCLRC Daresbury Laboratory, Warrington, WA4 4AD, U.K.

Ceruloplasmin is a member of the multi-copper oxidase family of proteins which includes laccase and ascorbate oxidase. Sequence homology also suggests that it is structurally related to blood clotting factors V and VIII. The protein comprises a single polypeptide chain of 1046 amino acid residues and up to 4 glycan chains (7−8% by weight), giving an overall molecular weight of some 132 kDa. The X-ray structure of human ceruloplasmin at a resolution of 3.0 Å reveals that the molecule is comprised of six cupredoxin-type domains arranged in a triangular array. There are six integral copper atoms, three of which form a trinuclear cluster sited at the interface of domains 1 and 6, and three mononuclear sites in domains 2, 4, and 6. The mononuclear copper in domain 6 and the trinuclear cluster form a four-copper oxidase center almost identical to that found in ascorbate oxidase and strongly suggesting an oxidase role for ceruloplasmin. The mononuclear coppers in domains 4 and 6 are typical “blue” type I copper centers; in domain 2, the copper is probably in the reduced state. The trinuclear cluster contains a pair of type III spin-paired coppers and a type II copper and is bound by eight histidines. Ceruloplasmin has long been known as “the enigmatic blue plasma protein” since its precise functions have not been defined. The X-ray structure confirms the probability that the protein is multifunctional and suggests strong evidence for ferroxidase and antioxidant activity. The putative role of ceruloplasmin in iron metabolism and other functional aspects will be discussed.

110. The Chemistry of Copper Chaperones. *T. V. O'Halloran*, R. A. Pufhal, G. Munson, and D. Huffman. Department of Chemistry and Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL 60208-3113.

Copper is an essential nutrient that can also disrupt the function of biopolymers at low concentrations. A variety of fatal human diseases are known to involve defects in copper metabolism, and several genes have recently been shown to be essential for microbial copper homeostasis. However, little is known about intracellular chemistry, biochemistry, or cell biology of proteins that operate by modulating the intracellular concentration or availability of this element. We are studying a subgroup of these proteins that appear to act as small chaperones for copper. Sequence analysis of such genes including those that encode ATX1, PcoC, and PcoE reveals amino acid motifs that may act as receptor sites for this metal. Few of these motifs are similar to those found in established Cu-enzymes but are now being found in other proteins that appear to play roles in copper homeostasis. Studies of the structure, inorganic chemistry, and mechanisms of these Cu-chaperones, as well as the related multi-copper oxidases and metalloregulatory proteins, are underway and reveal parallels between the copper circuitry in eukaryotic and prokaryotic cells.

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