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CONFERENCE PROCEEDINGS

Ninth Enzyme Mechanisms Conference

GORDON A. HAMILTON

Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania 16802

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The ninth biennial conference on enzyme mechanisms, organized by E. H. Cordes (Chairman), S. J. Benkovic, J. W. Kozarich, J. R. Knowles, and D. Silverman, was held on January 3–5, 1985, at the Innisbrook Resort in Tarpon Springs, Florida. Three formal talks were given in each of six sessions on the general topics: 1, Peptide Chemistry; 2, Redox Chemistry; 3, Mechanisms; 4, Cofactors; 5, Complex Structures; and 6, Biosynthesis at the Enzyme Level. In addition, two poster sessions were held with a total of over 50 posters being presented. In the following, a brief synopsis of each of the major talks, as well as a few select references to recent work in the area of the talk, are given. Also, the titles and authors of the posters are listed along with a few references to material related to some of the posters. It is hoped that this brief summary of the meeting not only will indicate the types of studies currently of interest to mechanism oriented bioorganic chemists, but also will serve as a starting point for those who wish to delve more deeply into the topics that were discussed.

PEPTIDE CHEMISTRY

In the initial presentation, E. T. Kaiser of Rockefeller University summarized the work that he and his co-workers have been doing to model the secondary (and to some extent the tertiary) structure of some natural proteins and peptides, including hormones (*1–3*). They have focused especially on modeling peptides that act at interfaces, specifically the lipid–water interface. Such peptides are referred to as amphiphilic peptides, i.e., they have both extended hydrophobic and hydrophilic regions, but these structural features only become apparent when the peptides form a unique secondary structure, such as an α-helix or a β-strand. Dr. Kaiser and his co-workers have found that several model peptides, with little overall sequence homology to some natural peptides and proteins, but which have conserved the original peptides' amphiphilic characteristics when they assume the predicted secondary structure, do indeed have similar chemical and biological activities to the natural peptides. In some cases the model peptides are even more biologically active than the natural peptides themselves. Such investigations have strongly implied that apolipoprotein-A-I, the principal lipoprotein of high-density lipoprotein, functions *in vivo* as an amphiphilic α-helix, as does the peptide hormone, calcitonin. On the other hand, preliminary results suggest that fibronectin,

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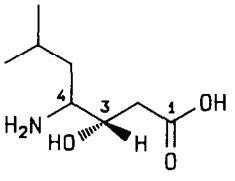
a relatively large (220 kDa per subunit) vertebrate glycoprotein involved in cell adhesion, functions biologically by forming an extended amphiphilic β-strand.

The work that Dr. Kaiser presented especially emphasized the importance of using synthetic model peptides to answer some fundamental questions concerning binding (including hormone–receptor binding) in complex biological systems. Also, it clearly demonstrates, as perhaps one should have expected, that a specific secondary (and probably also tertiary) structure of conformationally labile peptides is involved in their biological activities. More importantly, however, the work points the way to new approaches to determine what that specific conformation may be.

Dr. Kaiser and his group synthesize their model peptides by chemical procedures employing solid-phase peptide synthesis methods. During his talk he also briefly summarized a new solid-phase method he and his co-workers have developed whereby relatively long peptides (40 amino acids or more) are prepared by sequentially combining peptide units of approximately five amino acids or so, with each intermediate being fully characterized. By this technique he can not only move around in the model peptide short amino acid sequences at will but, more importantly, can be sure that the final structures are as they are supposed to be. This procedure (*4–6*) could potentially be an important improvement in the method of peptide synthesis, especially for longer peptides.

The inhibition and mechanism of aspartyl proteinases (*7–9*) was the topic discussed by D. H. Rich from the University of Wisconsin in his presentation. It has been known for some time that aspartyl proteinases, such as pepsin and cathepsin D, are very effectively inhibited (K_i of 10^{-10} to 10^{-11}) by pepstatin. This naturally occurring peptide has the sequence isovaleryl-Val–Val–Sta–Ala–Sta, where Sta has the structure illustrated below.



statine (Sta)

One of the goals of the research of Dr. Rich and his group is to understand in detail what makes pepstatin such a good inhibitor of pepsin so that similar principles can be applied to the design of better inhibitors for renin, an aspartyl proteinase that plays a very important role in the regulation of blood pressure in humans.

Dr. Rich summarized evidence from his own and other laboratories that the statine residue of pepstatin functions as a transition-state analog inhibitor, with the 3(*S*)-hydroxy group of the statine residue taking the place of one of the hydroxy groups of the suspected tetrahedral intermediate. His research group has been especially involved with synthesizing and studying the inhibitory power of several peptstatin analogs, but the conclusions they have reached from such studies have been confirmed by X-ray investigations of enzyme–inhibitor complexes. By coupling this information with what is known about the specificity of renin, Dr. Rich, in collaboration with D. F. Veber (see below), has been able to prepare very effective inhibitors for renin (*9*).

Another aspect of his research that Dr. Rich summarized is the use he has made of ketone isosteres of good pepsin inhibitors to obtain details concerning how the enzyme catalyzes its reaction. The research was designed to answer the following: Does one of the two aspartyl residues at the active site form a covalent bond with the substrate in going to the tetrahedral intermediate or do they merely catalyze the addition of water to the substrate? The ketone isosteres used were either peptides containing statone (where the 3-hydroxy group of statine has been oxidized to the ketone) or a statone homolog which has one extra carbon so that it better mimics the peptide substrate. By using ^{13}C NMR techniques and by studying the reaction of the inhibitor with pepsin in either D_2O or $H_2{}^{18}O$, he and his group obtained strong evidence that pepsin carries out its reaction by catalyzing the addition of water (and not an enzyme carboxyl group) to the carbonyl group. Presumably other aspartyl proteinases function in the same way.

The goal of the research described by D. F. Veber, of Merck, Sharp & Dohme Research Laboratories, is to obtain medically useful peptides or peptide analogs. In designing such compounds, he emphasized that one must be concerned not only with their specificity and potency, but also with their stability in biological systems. The approach used in much of the work of Dr. Veber and his group is to build conformationally constrained molecules by synthesizing cyclic analogs of physiologically active peptides. The conformational constraint serves a dual purpose: it usually makes the molecules less susceptible to degradation in biological systems, and it also gives information concerning the likely conformation of the peptide that is required for physiological activity. In his talk Dr. Veber summarized the application of these ideas to the design of potent somatostatin analogs (*10–12*) and described more recent work that he and his collaborators have been doing in an attempt to define more exactly what the active conformations of some of these cyclic analogs are. This has involved detailed NMR investigations combined with computational methods to generate the lowest energy conformations. With one somatostatin analog their sophisticated approaches led not to one unique conformation but gave 12 possible solutions. Nevertheless, these will be useful in designing new analogs which should allow them in the future to zero in on the most likely physiologically active conformation.

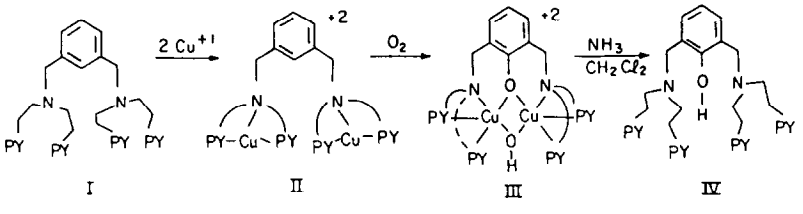
In another part of his presentation Dr. Veber summarized the research they have done in developing potent renin inhibitors containing statine (*9*), and applying principles similar to those discussed above to prepare stable cyclic analogs of these inhibitors. Although many of these inhibitors, like the somatostatin analogs, are not degraded in biological systems, unfortunately they are rapidly excreted and so have not proven useful as drugs. How to avoid the rapid excretion is a problem yet to be solved.

REDOX CHEMISTRY

All three presentations in this session were devoted to discussing the redox chemistry displayed by some metal complexes. Most of the research described was performed using nonenzymic systems, but with systems that are thought to be reasonable models for reactions catalyzed by various metalloenzymes.

K. D. Karlin of the State University of New York at Albany summarized in his talk the work that he and his collaborators have been doing in attempting to model the various reactions carried out by proteins that contain a dinuclear copper site. Such proteins include hemocyanin, which is a dioxygen carrier, and tyrosinase and dopamine-β-hydroxylase, which are monooxygenases involved in O_2 activation (*13*). In their work, Dr. Karlin and his group have been successful in modeling both types of reactions.

The monooxygenase model system that Dr. Karlin described is the reaction of the dinuclear Cu(I) complex **II** (Py refers to an α-pyridyl group) with O_2, a reaction which has been shown to yield **III** in high yield. The observed stoichiometry ($Cu : O_2$, 2 : 1) as well as results obtained using isotopically labeled O_2 demonstrate that this reaction is directly analogous to that catalyzed by the copper monooxygenases (*14*). Dr. Karlin also presented other results which give some insight into

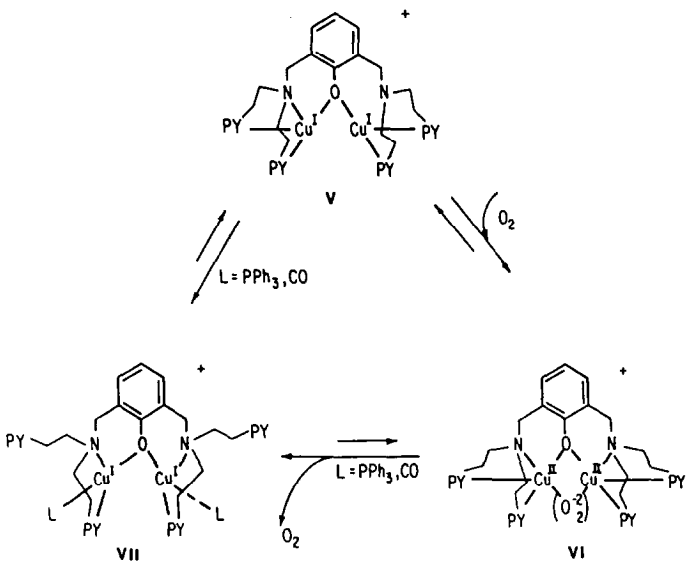


the mechanism of the reactions. He and his group have found that treating a dinuclear Cu(II) derivative of **I** with H_2O_2 gives high yields of the oxygenated product **III** as well. By contrast, neither the reaction of O_2 with a Cu(I) monomeric analog of **I** nor the reaction of H_2O_2 with a Cu(II) monomeric analog gives hydroxylated products. Together the evidence suggests that a peroxo-bridged dinuclear Cu(II) unit is involved as an intermediate in the conversion of **II** to **III** (*15*). It is believed that a similar peroxo-bridged species may also be involved in the enzymic reactions.

Dr. Karlin and his co-workers have modeled the reversible O_2 binding displayed by hemocyanin by using the binuclear Cu(I) compound **V**. On reaction of O_2 with **V** a dinuclear Cu(II)-peroxo complex **VI**, which is stable at low temperature, is formed (*16*). The binding of O_2 to **V** is quasi reversible; cycling between **V** and **VI** can be achieved and followed spectrophotometrically. Additional evidence for the reversibility of O_2 binding is the observation that treatment of **VI** with CO or PPh_3 leads to the formation of **VII** with quantitative release of O_2. Further characterization of these and related peroxo-bridged complexes should help to clarify what factors determine whether such a species reverses readily to O_2 (as in hemocyanin) or acts as a hydroxylating agent (as in the monooxygenases).

The subsequent two lectures given by T. C. Bruice of the University of California, Santa Barbara, and by J. T. Groves of the University of Michigan were both concerned with various mechanistic aspects of reactions catalyzed by cytochrome *P*-450 enzymes. Although both groups of investigators are interested in the same enzymic reaction, the approaches being used and the questions being asked are considerably different, and so their work is largely complementary.

It is now generally agreed that the actual species which hydroxylates various



substrates in the cytochrome *P*-450-catalyzed reactions is an oxo iron (Fe=O) species in which the iron has a formal valence of + 5. This species is usually formed in the enzymic reactions by a complicated sequence of steps ultimately involving the addition of O_2 and two electrons to the resting form of the enzyme which is an Fe(III) species. However, the putative enzymic Fe=O intermediate can apparently also be formed by treating the Fe(III) form with peroxides or other two-electron oxygenating reagents. The breakdown of an Fe(III) peroxide complex (Fe—OOR) to the Fe=O species and ROH (R=H, alkyl, acyl, etc.) is probably involved in both the usual enzymic cycle with O_2 as the oxidant as well as in the reactions where peroxides are used as oxidants.

In his presentation Dr. Bruice (*19–22*) focused especially on the mechanistic details of the Fe—OOR to Fe=O conversion, on the development of a new reagent for generating the Fe=O species, and on the characteristics of the reaction of this Fe=O compound with various substrates. The research that Dr. Groves described (*23–29*) was largely concerned with determining the mechanism of the oxygen transfer event and with the detailed structural features of oxo metalloporphyrin compounds. However, he also gave a few results that are relevant to the mechanism of Fe=O formation.

One of the main questions concerning the conversion of the enzymic Fe—OOR to Fe=O is whether the oxygen–oxygen bond scission occurs by a heterolytic or homolytic mechanism. By correlating the reactivity of a number of peroxides with tetraphenylporphin (TPP) complexes of Mn(III), Cr(III), Co(III), and Fe(III), Dr. Bruice and his co-workers have concluded that, with model compounds, the cleavage usually occurs by a heterolytic mechanism, the only exception being some of the reactions of Fe(III).TPP. With this latter compound the evidence suggests there is a change in mechanism from one that is heterolytic when the leaving group (ROH) has a $pK_a < 10$ to a homolytic mechanism when the leaving

group has a $pK_a > 10$. Dr. Groves in his presentation interpretated related results that he and his co-workers obtained using the tetramesitylporphin complex of Fe(III) as indicating only a heterolytic mechanism. However, there is no inconsistency in the results obtained by the two groups of investigators because the peroxides (peroxy acids) employed by the Groves group all have a leaving group $pK_a < 10$. The interesting finding that the mechanism can change, especially since it is observed with the metal encountered on the enzyme, seems to leave open the possibility of either mechanism for cytochrome *P*-450 itself. Further studies with modified porphin derivatives are thus needed to clarify what other factors favor each mechanism.

One of the problems in attempting to get good quantitative data for the reaction of Fe=O with various substrates is that most reagents used for its preparation lead to destruction of the porphyrin ring. Dr. Bruice and his group have found that if *p*-cyano-*N*,*N*-dimethylaniline-*N*-oxide is used as the oxygen donor this problem is alleviated, apparently because the Fe=O does not stay around long enough for it to destroy itself. If there is no other organic substrate for the Fe=O to react with, it reacts with the *p*-cyano-*N*,*N*-dimethylaniline product of the initial oxygen transfer reaction. Dr. Bruice and his co-workers have carried out a detailed kinetic and product study of this aniline oxidation and are now in a position to quantitatively compare the reactivity of the Fe=O with many organic compounds. Included in the findings obtained to date is the observation that the Fe=O transfers oxygen to alkenes approximately three orders of magnitude more rapidly than *m*-chloroperbenzoic acid does. This is to be contrasted with earlier results from Dr. Bruice's laboratory that the 4*a*-peroxide of flavin (which some have suggested as the oxygen transfer agent in flavin monooxygenase reactions involving phenol substrates) transfers oxygen approximately three orders of magnitude less rapidly than *m*-chloroperbenzoic acid. Such results not only indicate that Fe=O is a very reasonable possibility for the enzymic hydroxylating reagent in cytochrome *P*-450, but they also imply (at least to this author) that it is unreasonable to suggest that the flavin 4*a*-peroxide is the actual hydroxylating agent in phenol monooxygenases.

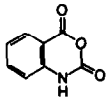
In their investigations of the oxygen transfer step from oxo metal species, Dr. Groves and his group have extensively utilized internal competition experiments. In other words they use reactants that can give two or more products; by determining what the ratio of products is, and how it changes with reagent or conditions, they infer what the mechanism of the oxygen transfer must be. Initially they considered four possible mechanisms by which the oxygen transfer might occur; namely (a) direct insertion by an oxenoid species, (b) initial H-atom abstraction, (c) initial electron transfer, and (9) for the case of alkenes, initial addition of the reagent to one end of the alkene. Unexpectedly, they found that none of these mechanisms seems to apply alone. Thus, in a case where allylic rearrangement could occur, they found, using cytochrome *P*-450 itself as well as various model metalloporphyrin systems, that the results are not consistent with either (a) or (b), but seemed to require a combination of the two. Even more surprising results were obtained when *trans*-1-deuteriopropene was used as substrate and the epoxide products were analyzed for deuterium content and stereochemistry. They found not only that some model complexes led to epoxide product with complete

retention of configuration while others caused considerable equilibration, but also that a large amount of the deuterium was lost to solvent during epoxidation by cytochrome *P*-450 itself. The explanation Dr. Groves offered for these results is that he believes that the Fe=O initially adds to the alkene to give a four-membered ring metallocycle which can reversibly rearrange to a metallocarbene (thus leading to proton loss to the solvent) before eventually decomposing to the epoxide and Fe(III) species. Such a mechanism could explain a number of other unusual results that have appeared in the literature and is clearly an exciting new development in the field.

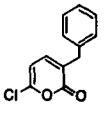
Dr. Groves also reported that he and his co-workers have successfully isolated a model Fe=O species that is stable enough to have various physical measurements performed on it. From EXAFS studies it has been found that the iron–oxygen bond distance is 1.6 Å. Mossbauer spectroscopy indicates that the iron is present as Fe(IV), so presumably the porphyrin ring exists as a cation radical as earlier investigations by others implied that it would. The spectral investigations suggest the system is present in the 3/2 spin state.

MECHANISMS

In the initial talk on mechanisms, R. H. Abeles of Brandeis University summarized research that he and his group have been doing (*30–33*) in attempts to develop effective suicide and transition-state analog inhibitors of various proteases. They earlier found that isatoic anhydride derivatives are good inhibitors of serine proteases because the acyl enzyme loses CO_2 and *o*-aminobenzoyl derivatives of the various enzymes deacylate very slowly (half times of hours). Knowing the specificity of thrombin, they were able to make a derivative of isatoic anhydride which is a very effective thrombin inactivator and which will prolong blood clotting fourfold *in vitro*. Unfortunately, however, the inhibitor is very rapidly hydrolyzed *in vivo* by a metal-containing enzyme and therefore it has not proved to be an effective therapeutic agent.



isatoic anhydride



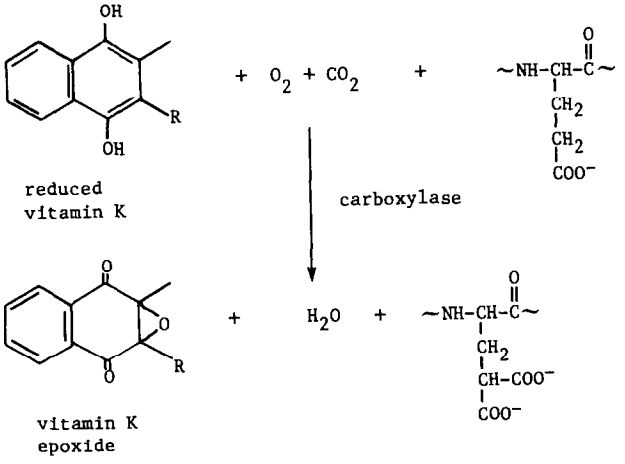
3-benzyl-6-chloropyrone

The inhibition of chymotrypsin by 3-benzyl-6-chloropyrone has turned out to be more complicated than originally thought. Dr. Abeles and his group had designed this compound to have specificity for chymotrypsin and to generate, when the acyl enzyme is formed, an acid chloride that might then inactivate the enzyme by reacting with some other enzymic group. Although the enzyme is inhibited by this compound, it is now clear that it does not occur by the mechanism predicted. Subsequent work, including an X-ray structure analysis of the inactive enzyme, has shown that the acylated enzyme has a free carboxylate group, and that it deacylates slowly because it is an α,β-unsaturated ester with the carboxylate forming a salt bond with an enzymic histidine residue. The slow step in the

deacylation involves isomerization of the α,β-unsaturated acyl enzyme to a β,γ-unsaturated ester that then hydrolyzes rapidly. This research is an elegant indication that even when the ultimate conclusions are not in accord with the original rationale for doing the work, interesting serendipitous findings can still result.

In another aspect of their work, Dr. Abeles and his group have been attempting to design better protease inhibitors that function by forming relatively stable adducts with the uniquely reactive nucleophilic group associated with such enzymes. These inhibitors have been referred to as transition-state analogs although that is somewhat of a misnomer; when bound to the enzyme they mimic a high-energy intermediate (the tetrahedral intermediate) rather than a transition state. In any event, aldehydes, with structural features similar to natural substrates, had been known to be reasonably effective such inhibitors whereas normal ketones are not because their adducts are much less stable than those of structurally related aldehydes. The problem with aldehydes, however, is that one can build-in enzyme specificity on only one side of the reactive carbonyl group while many proteases show specificity for groups on both sides of the reacting peptide bond. To get around these problems Dr. Abeles and his co-workers have been preparing several ketones that are fluorinated in an α position and which bear a structural analogy to natural substrates of various enzymes; the α-fluoro groups increase the stability of ketone adducts substantially (at least three orders of magnitude for a trifluoromethyl ketone relative to the methyl ketone). In this way they have obtained good inhibitors (inhibition constant of 10^{-6} to 10^{-11} M^{-1}) for several hydrolases, including carboxypeptidase, angiotensin-converting enzyme, renin, and cholinesterase. This is a promising new approach to the possible development of therapeutically useful hydrolase inhibitors.

The characteristics of the microsomal vitamin K-dependent carboxylase (*34–37*) were summarized in the talk given by J. W. Suttie of the University of Wisconin. This enzyme plays an important role in the blood-clotting process; specifically it catalyzes the formation of approximately 10 γ-carboxyglutamyl residues in the N-terminal region of prothrombin, one of the proteins involved in the blood-clotting cascade. The overall reaction the carboxylase catalyzes is summarized in the following equation:



In vivo the vitamin K epoxide is returned to the reduced form in a sequence of enzyme-catalyzed steps involving thiols and NADPH.

Unfortunately the carboxylase has proved very difficult to purify so all the mechanistic work has been done with microsomes or detergent-solubilized microsomes. Nevertheless several of the characteristics of the reaction have been determined. It is known that the epoxide oxygen is derived from O_2, that the pro-*S* hydrogen of the glutamyl residue is removed in forming the product, and that ^3H exchange into the glutamyl residue occurs in the absence of CO_2. This latter result suggests that a γ-glutamyl carbanion is an intermediate in the overall reaction, but other than that the detailed molecular mechanism remains a mystery.

Using synthetic peptides it has been determined that Glu–Glu sequences are the most reactive and that aspartyl residues are a couple of orders of magnitude less reactive than glutamyl residues in comparable peptides. One interesting recent finding is that the form of prothrombin found in plasma is probably not the substrate *in vivo*. A slightly larger and more basic form of prothrombin found in microsomes has a *Km* approximately two orders of magnitude less than plasma prothrombin, and C-DNA studies confirm that the initially synthesized form has a basic N-terminal end. Dr. Suttie summarized evidence that this basic end changes the conformation of the reacting region thus making it more susceptible to carboxylation. It is believed that an amphiphlic helix is formed and that the γ-carboxyglutamyl residues are then generated on one face of the α-helix. This finding emphasizes what Dr. Kaiser implied in his earlier presentation that such secondary structures may be important to many different areas of biochemistry.

In the following talk, M. H. O'Leary of the University of Wisconsin described some new developments in heavy isotope (especially ^{13}C) technology and the use of such methods in determining the characteristics of some representative enzymic and nonenzymic reactions (*38–40*). As an example of a new technique he recommended the use of $^{13}C^{18}O_2$ in experiments designed to determine whether CO_2 or HCO_3^- is the actual substrate in a reaction. The interpretations of many previous experiments using other techniques have been equivocal not only because of the relatively rapid hydration–dehydration reaction but also because adventitious CO_2 or HCO_3^- can modify the results. Starting with $^{13}C^{18}O_2$ in $H_2{}^{16}O$ it is easy to distinguish whether CO_2 or HCO_3^- is the reactant in, for example, a carboxylation reaction because all the ^{13}C-labeled product will have both oxygens labeled with ^{18}O if CO_2 is the reactant whereas only two-thirds of the oxygens will be ^{18}O if HCO_3^- is the reactant. In this way it was unequivocably shown that HCO_3^- is the substrate for PEP carboxylase.

Dr. O'Leary pointed out that the conclusion from a number of isotope fractionation and equilibrium isotope experiments is that the heavier isotope concentrates in the more constrained (bonded, solvated, etc.) environment. Thus, $^{13}CO_2$ is less soluble in H_2O than $^{12}CO_2$ ($K^{12}/K^{13} = 1.00110$) because H-bonding with water weakens the C=O bonds. However, ^{13}C causes an increase in the CO_2 hydration constant ($K^{13}/K^{12} = 1.0090$) and favors the components on the right hand side of the following equilibrium:

$$R^{12}CO_2^- + {}^{13}CO_2 \rightleftharpoons R^{13}CO_2^- + {}^{12}CO_2 \qquad (K = 1.0027)$$

From kinetic experiments it was shown that ^{13}C slows down both the CO_2 hydration and HCO_3^- dehydration steps, but the latter to a greater extent. With PEP carboxylase, k^{12}/k^{13} varies from 1.0029 at pH 7 to 0.9997 at pH 10, indicating some change in the rate-determining step with pH. For ribulose bisphosphate carboxylase-oxygenase, on the other hand, k^{12}/k^{13} is large (1.0264 to 1.0297) and not affected much by pH. Interestingly, however, this reaction also shows a deuterium isotope effect (with deuterium on the 3 position of the substrate k_H/k_D on V/K is approximately 2.5) and k^{12}/k^{13} is lower (1.020) when the 3-deuterated substrate is used. Such results not only indicate that both the D removal and carbon–carbon cleaving and forming steps are partially rate determining, but they also demonstrate the power of heavy isotope techniques in determining fine details of mechanisms.

COFACTORS

Selenium biochemistry (*41–43*) was the topic summarized by T. C. Stadtman of the National Institutes of Health, Bethesda. This element, which is an essential nutrient for animals, is present in normal mammalian cells at a concentration of 0.1 to 1 μM. Humans need 1–3 μequiv per day but higher amounts (10-fold or greater) are quite toxic and can be lethal. Approximately 10 proteins that contain selenium have now been characterized; most have been isolated from microbial sources but one, glutathione peroxidase, is present in animals and plays a major role in peroxide detoxification reactions. In some proteins (as examples, glutathione peroxidase, glycine reductase, and formate dehydrogenase) the selenium is present as selenocysteine, while others (thiolase and β-hydroxybutyryl-CoA dehydrogenase) contain the selenium as selenomethionine, and one example (nicotinic acid hydroxylase) is now known where the selenium appears to exist as selenide (it is removed as dialkylselenide on alkylation). Dr. Stadtman also summarized very recent results she and her group have obtained indicating the presence of selenium as a 2-selenouridine derivative in several bacterial tRNAs (*42, 43*).

Those enzymes that have selenium as selenocysteine contain the residue at one specific site in the amino acid sequence. Consequently, in those cases there is the presumption that the selenium is involved directly in the catalysis. This seems especially likely for glutathione peroxidase where selenocysteine's greater degree of ionization, higher nucleophilicity, and lower redox potential would be expected to make it a considerably better catalyst for the reaction than its sulfur analog. However, the role of selenium in tRNA and in selenoproteins that do not contain selenocysteine is unknown. Its function in those enzymes containing selenomethionine is particularly obscure because the selenomethionine is not at one specific site in the amino acid sequence but, rather, is present in fractional amounts at several methionine sites. A further current unknown is how the selenium is incorporated into the selenoproteins, whether it is by a post-translational modification or by some other mechanism. As implied by Dr. Stadtman's presentation, there are many unanswered questions in the field of selenium biochemistry and thus it is a field worthy of more concentrated study.

The subject of the presentation given by S. J. Lippard of the Massachusetts Institute of Technology was the detailed mechanism of action of the anticancer drug, *cis*-diaminedichloroplatinum(II) (*44–48*). In 1983 this was the leading anticancer drug used in the USA; it is especially effective against testicular cancer, where it has raised survivability from approximately 12 to 95%. Dr. Lippard summarized considerable evidence which suggests that it acts by binding to DNA, specifically to the N-7 positions of two adjacent guanines in the DNA sequence. In the process the two chlorides are displaced. The *cis* isomer of the drug is a more potent anticancer compound than the *trans* isomer apparently because it reacts more rapidly with DNA and because the *trans* compound is not capable of forming crosslinks with adjacent G's. It will, however, react with two G's separated by another nucleotide. Dr. Lippard believes that the *cis* isomer is such a good drug because the formation of the crosslink with the two adjacent G's does not require gross conformational changes in the rest of the helix and thus the modified DNA is probably difficult for the cellular repair enzymes to detect and excise. In another aspect of their work Dr. Lippard and his collaborators have used antibodies to platinum DNA adducts to show that tumor cells *in vivo* form the same platinum–DNA structures as those well-characterized ones formed *in vitro*.

The role of pyridoxal 5′-phosphate in phosphorylase catalysis was the topic discussed by D. J. Graves of Iowa State University (*49–55*). This cofactor is found in all animal α-glucan phosphorylases and yet its function has remained a mystery; clearly its role is different than in most other enzymes where it is found because reduction of the cofactor–phosphorylase Schiff base bond with borohydride does not inactivate the enzyme. It has been suggested that the 5′-phosphate group may act as a nucleophile (*49*), in electrostatic catalysis (*49, 50*), as an electrophile (*51, 52*), or an acid–base catalyst (*53, 54*), but proof is lacking for its exact role. It is known from X-ray crystallographic studies that the coenzyme is bound in a hydrophobic crevice close to the glucose phosphate binding site but there is no evidence of any covalent adduct formed with the coenzyme during turnover. Dr. Graves reported that studies in his laboratory using phosphorylase reconstituted with pyridoxal in the presence of phosphate analogs and other anions (*55*) suggest that the electrophilic or acid–base mechanism alone cannot explain the coenzyme involvement in catalysis. Very recent ^{19}F NMR experiments using phosphorylase reconstituted with fluoropyridoxal phosphate suggest that the environment around the ring differs in the phosphate and glucose 1-phosphate complexes. Dr. Graves believes that a possible role for the coenzyme in conformational transitions required for substrate binding or product release should receive further consideration.

COMPLEX STRUCTURES

The initial lecture in this session was given by G. G. Hammes of Cornell University, who described the work that he and his group have been doing to characterize the elementary steps in the reaction mechanism of chicken liver fatty acid synthetase (*56–58*). This multienzyme complex catalyzes the synthesis of palmitic

acid from acetyl-CoA, malonyl-CoA, and NADPH in a sequence of steps involving seven distinct enzymic activities, namely, acetyl transacylase, malonyl transacylase, β-ketoacyl synthase, β-ketoacyl reductase, dehydratase, enoyl reductase, and thiolesterase. The sequence of reactions is initiated by putting an acetyl group on the enzyme catalyzed by the acetyl transacylase; the fatty acid chain is built up by cycling several times through the next five enzymic activities, and it is terminated when the palmitic acid is hydrolyzed off by the thiolesterase. The enzyme complex has three sites to which an acyl group can become attached, an unusually reactive serine hydroxyl that initially accepts the acetyl or malonyl group, a pantetheinyl thiol where the acyl group resides during most of the individual chemical steps, and a cysteinyl thiol which acts as a storage depot for the shorter-chain acyl groups. This site cannot accommodate any acyl group longer than C_{14} so when the C_{16} compound is made at the pantetheinyl site the sequence of reactions is terminated by action of the thiolesterase.

Among the numerous kinetic findings described by Dr. Hammes, the following seem of special interest: the enzyme is quite efficient since the overall turnover number for C_{16} acid formation is approximately 1 sec^{-1}; coenzyme A is an inhibitor at low concentrations but an activator at high concentrations, the activation apparently being due to the removal of acyl groups that get on the wrong enzymic acyl binding site; and all the enzyme activities have similar turnover numbers so that no single enzyme activity is clearly rate determining. In another aspect of their work Dr. Hammes and his group have been able to characterize the reaction intermediates with respect to chain length, mode of binding to the enzyme, and the specific nature of the chain (ketone, alcohol, unsaturated, or saturated) by quenching the enzyme during catalysis and analyzing the resulting reaction intermediates. The results obtained were used to determine the acyl-binding sites (described above) where the individual steps occur. Furthermore, the finding that the 3-keto compounds comprise most of the intermediates detected when NADPH is the limiting substrate indicates that the reduction of the 3-keto intermediates is considerably slower than reduction of the unsaturated intermediates. Also, the distributions of chain lengths suggest that the initial condensation and reduction steps are slower than the analogous reactions with longer-chain-length intermediates.

During his presentation Dr. Hammes also summarized what is known about the stereochemistry of the individual enzyme-catalyzed steps. The condensation of acetyl and malonyl groups proceeds with retention of configuration at C-2. Interestingly, the reduction of acetoacetyl to D-3-hydroxybutyryl proceeds with transfer of the pro-4*S* hydrogen of NADPH, while the reduction of crotonyl to butyryl occurs with transfer of the pro-4*R* hydrogen of NADPH to the pro-3*R* position of butyryl and transfer of a solvent proton to the pro-2*S* position. The elimination of water from hydroxybutyryl is syn with the pro-*S* hydrogen from C-2 being removed.

In the following presentation, O. N. Rosen of the Sloan Kettering Cancer Research Institute described studies that she and her group have been doing on the structure and function of the insulin receptor (*59–61*). The receptor purified from placenta has an $\alpha_2\beta_2$ structure with subunit molecular weights of 135K and

90K, respectively. The α-subunit has the binding site for insulin while the β-subunit is a tyrosine-specific protein kinase which is stimulated by insulin; in this process the β-subunit is itself phosphorylated on tyrosine. Since it is believed that this insulin-stimulated activity is intimately associated with the mechanism by which insulin functions *in vivo,* it has been studied in considerable detail. Among the findings obtained are the following: insulin stimulates the kinase by increasing V_{max}; other peptides and proteins, such as histones and IGG, can also act as kinase substrates; from studies with synthetic peptides it is found that the kinase is not very specific for the sequence around tyrosine, although nearby negatively charged groups favor the reaction; the purified receptor binds insulin with a K_D of 5 nM, and half-maximal insulin binding correlates with half-maximal kinase activity; phosphorylation of the receptor does not alter insulin binding; and the phosphorylated receptor does not require bound insulin to be a kinase. Others have noted that one of the effects of insulin on cells is to increase the amount of glucose transport protein in the plasma membrane. However, Dr. Rosen and her collaborators found that this was not the result of phosphorylation of some precursor by the insulin receptor because such phosphorylation does not occur during the biosynthesis of the transport protein. Another known effect of insulin is to cause stimulation of ribosomal protein phosphorylation, in this case on serine residues. Dr. Rosen and her group have investigated this specific kinase (M_r 60,000) but she pointed out that it is still not clear how insulin causes its effect on this and other intracellular enzymes. A mechanism that can explain how the insulin-stimulated phosphorylation of its receptor could lead to the resultant intracellular effects has recently been suggested by the present author (*62*).

In another aspect of their work Dr. Rosen and her group have been attempting to isolate the message for the insulin receptor from various sources, especially insects, so that genetic approaches to finding out how phosphorylation of the receptor affects intracellular events can be applied. Part of the message for the receptor from placenta has been obtained and sequenced; it appears to have considerable similarity to the epidermal growth factor receptor.

It has been known for some time that GTP is required in order to detect a hormonal effect on the activities of various membrane bound enzymes, especially adenylate cyclase. The focus of the talk given by E. M. Ross of the University of Texas, Dallas, was to describe in molecular terms how the GTP, hormone, and proteins present in this complex overall system interact to cause the observed effects (*63–66*). Since most of his group's work has been done using adenylate cyclase (C) stimulated by isoproterenol (H) in the presence of β-adrenergic receptors (R), GTP, and the GTPase protein, conclusions derived using these compounds will be summarized. However, it appears that very similar mechanisms pertain to other systems that involve GTP and related GTPase proteins.

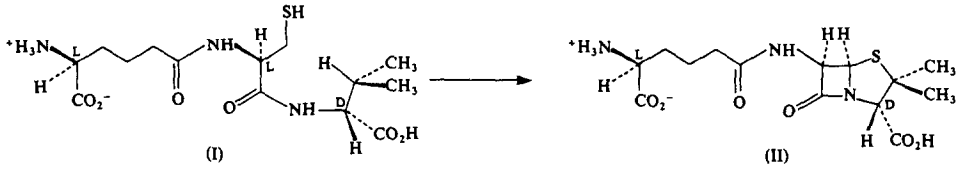
Stimulation of C by H requires the presence of R, GTP, and the GTPase protein. Dr. Ross presented evidence that this is accomplished by the R · H complex interacting with the GTPase protein in the presence of GTP to generate a species that can activate C. The actual C activator is one subunit (the α subunit) of the GTPase protein which in its inactive state contains three different types of subunits ($\alpha\beta\gamma$). The specific role of the R · H complex is apparently to speed up the

breakdown of the $\alpha\beta\gamma$ protein to α subunits and $\beta\gamma$ complexes. This requires the binding of GTP or a GTP analog to the α subunit. When GTP is used it gets hydrolyzed to GDP and P_i during the normal activation process but hydrolysis is not a prerequisite to activation; only the binding of a G-type nucleotide is required.

A mechanism such as the one proposed allows for considerable amplification of a signal because each R · H complex can cause the cleavage of several GTPase proteins and each activator α subunit, in turn, can cause many turnovers of the adenylate cyclase. Possibly for this reason this appears to be a general mechanism that occurs in several other systems as well. Dr. Ross pointed out that the $\beta\gamma$ subunits of these other GTPase proteins appear to be the same but the α subunits differ because they interact with different receptors and enzymes. Thus, for some inhibitory hormones the α subunit eventually interacts with a phospholipase while for transducin, the GTPase protein in rod outer segments, the α subunits interact with the light receptor. Clarifying in more detail the chemical mechanism by which these GTPase proteins function will therefore have relevance to several different areas of biochemistry.

BIOSYNTHESIS AT THE ENZYME LEVEL

In the initial lecture in this session J. E. Baldwin of Oxford University described the work that he and his group have been doing on the mechanism of penicillin biosynthesis. Earlier work (*67*) showed that cell-free extracts from *Cephalosporium acremonium* are capable of converting the tripeptide δ-(L-α-aminoadipoyl)-L-cysteinyl–D-valine (**I**) into isopenicillin N (**II**) and a homogeneous enzyme that



catalyzes this reaction has now been obtained from these extracts (*68*). This enzyme, called isopenicillin N synthase, has a M_r of approximately 37,000 and has no known cofactor, but the presence of O_2, Fe(II), and ascorbate is required for the reaction to proceed. From stoichiometric studies it was found that 1 mol of O_2 is consumed in the conversion of **I** to **II** so the O_2 reduction product must be H_2O (four H's are removed from **I** in the reaction). It is known that the two new bonds formed to the β-carbons of the cysteinyl and valine residues are formed with retention of configuration in the normal reaction. A study of kinetic deuterium isotope effects (on V and V/K) has shown that the reaction is stepwise and involves preliminary β-lactam formation (*69*). By modification of the valine moiety of the precursor, Dr. Baldwin and his group found that both five- and six-membered ring products can be produced simultaneously and, furthermore, that sometimes these products are formed with retention of configuration and at other

times with inversion. Such results imply that the reactions are proceeding by a free radical mechanism. If the valine is replaced by an unsaturated amino acid residue then the enzyme functions as a monooxygenase (*70*). To explain the results Dr. Baldwin proposed that an initially formed iron dioxygen complex and its subsequent decomposition products abstract H atoms from the substrate with iron–carbon bond formation also being involved.

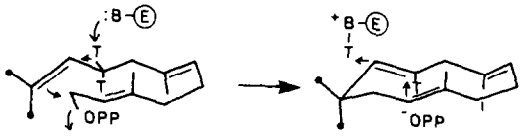
In a subsequent presentation, C. A. Townsend of Johns Hopkins University described results pertaining to the mechanism of a further step in β-lactam antibiotic synthesis, namely, the conversion of penicillin N (**III**) to deacetoxycephalosporin C (**IV**) and eventually to cephalosporin C. This reaction is catalyzed by

III IV

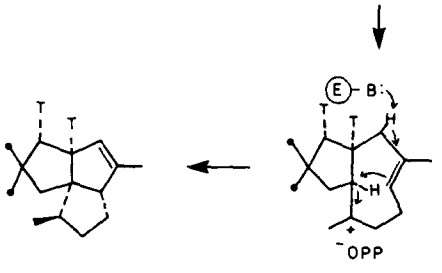
growing cultures of *C. acremonium*. The question he and his group have addressed is the stereochemistry for the conversion of a chiral methyl group of **III** into the new methylene position of **IV**. Previous investigations had indicated that DL-(3*R*,4*R*)- and (3*R*,4*S*)-[4-^{2}H, 4-^{3}H]valine could be prepared and their structures established unambiguously (*71, 72*). Furthermore, it was known from earlier work that incorporation of these chiral methyl substrates would label the β methyl of penicillin N and, upon oxidative ring expansion, label the C-2 methylene of cephalosporin C. The main problem was to determine the stereochemistry of the resultant C-2 methylene group. For this Dr. Townsend and his group developed a kinetic assay to determine the distribution of tritium at the diastereotopic C-2 methylene positions. The radiolabeled cephalosporin C isolated from the growing cultures was converted to *N*-*t*-butoxycarbonyldiacetylcephalosporin C-1β-oxide, and the loss of tritium radioactivity was monitored in aqueous buffer under conditions of constant temperature, pH, and ionic strength. Control experiments demonstrated the validity of treating the time-dependent tritium loss as parallel first-order processes from whose intercepts at $t = 0$ the initial relative populations of radioisotope at the diastereotopic C-2 methylene positions could be determined. Unexpectedly the distributions from both (*R*)- and (*S*)-methyl substrates were found to be equal. That this outcome did not result from a kinetic isotope effect of unity was clearly shown in a series of double-label (^{3}H and ^{14}C) experiments. Consequently, the oxidative ring expansion of penicillin N to deacetoxycephalosphorin C must proceed with complete stereochemical scrambling (*73, 74*). Using a different analysis technique others having confirmed this fining, although they originally came to a different conclusion (*75, 76*). The finding strongly implies that the ring expansion occurs by some kind of radical mechanism.

Some characteristics of the biosynthesis of sesquiterpenes and monoterpenes by cell-free systems was the topic discussed by D. E. Cane of Brown University (*77–82*). Farnesyl pyrophosphate (**V**) is the precursor of the sesquiterpenes, and

one of the reactions he and his group have been investigating is its conversion into pentalenine (**VI**). From stereochemical and labeling studies they have concluded that the reaction probably proceeds as illustrated and, furthermore, that the entire transformation is carried out by one enzyme.



V



VI

A monoterpene system that Dr. Cane and his collaborators have been investigating is the biosynthesis of borneol pyrophosphate (the precursor of camphor). One oddity is that an enzyme system from sage converts geraniol pyrophosphate into borneol pyrophosphate while an enzyme system from tansy converts the same precursor into the enantiomer of borneol pyrophosphate. Dr. Cane and his group have been interested in determining at what stage the sterochemical distinction is made by these two enzyme systems. They found that the immediate precursor of both products is not geraniol pyrophosphate but its isomer, linalool pyrophosphate, the isomer with the pyrophosphate group on the 3-position. Interestingly, a racemic mixture of linalool pyrophosphate is converted by either the enzyme from sage or that from tansy into a racemic mixture of the borneol pyrophosphates. In other words, the stereochemical distinction is made not at the carbon–carbon bond-forming steps but in the conversion of the achiral geraniol pyrophosphate to the chiral linalool pyrophosphate; apparently the enzyme from one source makes the *R* precursor while that from the other the *S*. Such results suggest that other interesting surprises await those who delve more deeply into these terpene biosynthetic systems.

CONCLUSION

Ranging as they do from studies aimed at determining very fine details of the mechanisms of enzymic and model reactions, to attempts to understand at a

molecular level some quite complex biological systems, and to attempts to utilize such information for practical (especially pharmacological) purposes, the topics covered in this conference represent well the present thrust of much of mechanistic bioorganic chemistry. Because of the power of the chemical techniques being applied to these biological problems, and because of the potential payoffs to society, it is clearly a field now coming into its own.

POSTER PRESENTATIONS

An Artificial Acyl-enzyme (Cinnamoyl-chymotrypsin) and an Artificial Enzyme (Chymotrypsin), M. L. BENDER, R. C. GADWOOD, I. M. MALLICK, V. T. D'SOUZA, K. HANABUSA, J. LEE, P. CHALABI, AND T. O'LEARY, Northwestern University, Evanston, Illinois.

Vitamin-K-Dependent Carboxylase: Stoichiometry of Oxygen Uptake, Vitamin K Epoxide and γ-Carboxyglutamic Acid Production, L. CANFIELD-SANDER, Texas A&M University, College Station, Texas.

Isotope Effects and pH Studies of NAD-Malic Enzyme, P. F. COOK AND D. M. KIICK, North Texas State University, Denton, Texas.

The Active Site Structure of Yeast Inorganic Pyrophosphatase (83–85), B. S. COOPERMAN, University of Pennsylvania, Philadelphia, Pennsylvania.

Metal Interactions with Beef Heart Mitochondrial ATPase, S. G. DAGGETT AND S. M. SCHUSTER, University of Nebraska, Lincoln, Nebraska.

Isolation and Characterization of an Agrobacter β-Glucosidase, T. DAY AND S. G. WITHERS, The University of British Columbia, Vancouver, British Columbia, Canada.

pH Dependence of Catalysis by the Aspartyl Proteases, B. M. DUNN, J. KAY, M. VALLER, M. JIMINEZ, AND C. ROLPH, University of Florida, Gainesville, Florida.

Analogs of the Autoinducer of Vibrio fischeri *Luciferase*, A. EBERHARD, C. A. WIDRIG, AND P. MCBATH, Ithaca College, Ithaca, New York.

Reversible and Irreversible Inhibition of β-Lactamase, A. L. FINK, P. BASSETT, K. BEHNER, E. CHICHESTER, AND L. ELLERBY, University of California, Santa Cruz, California.

NMR Studies of the Mechanisms of Adenylate Kinase and DNA Polymerase I, D. C. FRY, L. J. FERRIN, S. A. KUBY, AND A. S. MILDVAN, The Johns Hopkins University, Baltimore, Maryland.

The Free-Aldehyde Group of Aldoses and Aldose Reductase, C. E. GRIMSHAW, Scripps Clinic and Research Foundation, La Jolla, California.

Structure–Function Studies on the β-Oxidation Enzymes, B. GUSTAFSON, C. ROJAS, AND J. T. MCFARLAND, The University of Wisconsin–Milwaukee, Milwaukee, Wisconsin.

Lipid Activation of Pyruvate Oxidase: Identification of the Lipid Binding Site, S. E. HAMILTON, M. RECNY, AND L. P. HAGER, University of Illinois at Urbana–Champaign, Urbana, Illinois.

An Electronic Mechanism for Carbonic Anhydrase (86, 87), K. HAYDOCK, C. COOK, AND L. C. ALLEN, Princeton University, Princeton, New Jersey.

Formation of the Active Site in the Last Stages of Folding of Carbonic Anhydrase, R. W. HENKENS, K. H. GROOVER, R. A. SHEFFEY, AND T. J. WILLIAMS, Duke University, Durham, North Carolina.

Isotope Effects in Catalysis by Yeast α-Glucosidase. A Probable Failure of the Antiperiplanar Lone Pair Hypothesis in Glycosidase Action, L. HOSIE AND M. L. SINNOTT, University of Bristol, Bristol, England.

A Mechanism Based Protein Fragmentation in CoA Transferase, J. B. HOWARD, University of Minnesota, Minneapolis, Minnesota.

Structure and Mechanism of the Nitrogenase Fe-Protein, J. B. HOWARD, University of Minnesota, Minneapolis, Minnesota.

The Roles of Metal Ions in Yeast Mitochondrial ATPase, W. T. JENKINS, Indiana University, Bloomington, Indiana.

Affinity Labeling of Dihydrofolate Reductase, R. A. JOHANSON AND J. HENKIN, Abbott Laboratories, North Chicago, Illinois.

The Two Nonequivalent Flavins in Sarcosine Oxidase, M. S. JORNS, Hahnemann University, Philadelphia, Pennsylvania.

Do Trifluoromethyl Substituents on Enzyme Substrates and Inhibitors Carry Water Molecules Into Enzyme Active Sites?, J. W. KELLER AND B. J. HAMILTON, University of Alaska, Fairbanks, Alaska.

Thio- and Amino-Sugars and Their Phosphorylated Derivatives as Probes of Enzyme Reaction Mechanisms, W. B. KNIGHT AND W. W. CLELAND, University of Wisconsin, Madison, Wisconsin.

^{13}C NMR Evidence for Hemiacetal Formation in an Aldehyde Transition State Analog Complex of α-Chymotrypsin (88) K. LAI, D. SHAH, P. ABUAF, AND D. G. GORENSTEIN, University of Illinois, Chicago, Illinois.

Inhibition of Pyridoxal Phosphate Dependent Enzymes by Dihydroaromatic Substrate Analogs of GABA (89, 90), B. LIPPERT, B. W. METCALF, R. RESVICK, J. ADAMS, J. P. BURKHART, AND G. W. HOLBERT, Merrell Dow Research Institute, Cincinnati, Ohio.

Electrostatic Effects on the Associative Rate Constant of NADH and Liver Alcohol Dehydrogenase, C. LIVELY AND J. T. MCFARLAND, The University of Wisconsin–Milwaukee, Milwaukee, Wisconsin.

^{13}C NMR Characterization of the Thiohemiacetals Formed on Inhibition of Papain with D- *and* L-*N-Acetyl-[1-^{13}C]phenylalanyl Glycinal,* N. E. MACKENZIE, J. P. G. MALTHOUSE, S. GRANT, AND A. I. SCOTT, Texas A&M University, College Station, Texas.

Methionine Biosynthesis: Characterization and Affinity Labeling, J. H. MANGUM,* M. SMITH,* AND W. AWAD, JR.,† *Brigham Young University, Provo, Utah, and †University of Miami, Miami, Florida.

Identification of Metal Ligands in a Protein by Two-Dimensional NMR Spectroscopy: Hydrogen-1 to Cadmium-113 Connectivity in Cadmium Plastocyanin, J. L. MARKLEY,* C. L. KOJIRO,* D. LIVE,† AND D. COWBURN,† *Purdue University, West Lafayette, Indiana, and †Rockefeller University, New York, New York.

Enzymatic Properties of NAD Analogs Containing Modified Sugars, T. M. MARSCHNER, O. MALVER, B. L. KAM, AND N. J. OPPENHEIMER, University of California, San Francisco, California.

Reductive Trapping of Bromopyruvate Inactivated KDPG Aldolase. Peptide Studies Supporting Affinity Crosslinking, H. P. MELOCHE, J. NEWITT, AND E. L. O'CONNELL, Papanicolaou Comprehensive Cancer Center, University of Miami School of Medicine, Miami, Florida.

Acid Inactivation of Human Serum Butyrylcholinesterase, S. K. MILLER, University of North Carolina, Wilmington, North Carolina.

Biosynthesis of Riboflavin: Studies on 6,7-Dimethyl-8-ribityllumazine Synthase (91, 92), G. NEUBERGER, P. NIELSEN, AND A. BACHER, Universitat Munchen, Munich, West Germany.

Inhibition of Tryptophan Synthase and Tryptophanase by the Reaction Intermediate Analogs, Oxindolyl-L-Alanine and 2,3-Dihydro-L-tryptophan, R. S. PHILLIPS, E. W. MILES, AND L. A. COHEN, National Institutes of Health, Bethesda, Maryland.

Allenic Amino Acids as Mechanism-Based Inactivators of PLP-Dependent Enzymes, D. H. PLIURA, A. C. CASTELHANO, K. C. HSIEH, G. J. TAYLOR, AND A. KRANTZ, Syntex, Inc., Mississauga, Ontario, Canada.

Multinuclear Magnetic Resonance Studies of Enzyme-Bound Intermediates: Phosphoglucomutase, G. I. RHYU, W. J. RAY, JR., AND J. L. MARKLEY, Purdue University, West Lafayette, Indiana.

^{31}P NMR Studies on the Phosphoglucomutase Reaction Mechanism, G. I. RHYU, W. J. RAY, JR., AND J. L. MARKLEY, Purdue University, West Lafayette, Indiana.

Specific Mutagenesis for Site Saturation, J. H. RICHARDS, J. WU, S. C. SCHULTZ, AND W. J. HEALEY, California Institute of Technology, Pasadena, California.

3-Furylacryloyl-1,2-dipalmitoyltriacylglyceride—A Chromophoric Substrate of Lipoprotein Lipase, C. ROJAS AND J. T. MCFARLAND, The University of Wisconsin-Milwaukee, Milwaukee, Wisconsin.

Synthesis and Characterization of a New Semisynthetic Enzyme, Flavolysozyme, S. ROKITA AND E. T. KAISER, The Rockefeller University, New York, New York.

Inhibition of Dopamine-β-hydroxylase by Hydralazine and 2-Hydrazinopyridine, R. C. ROSENBERG, Howard University, Washington, D.C.

Binding of Inhibitors to Carbonic Anhydrases from the Skeletal Muscle of Mammals and Primitive Vertebrates, G. SANYAL,* E. R. SWENSON,† AND T. H. MAREN,‡ *Mayo Foundation, Rochester, Minnesota; †Mount Desert Island Laboratory, Salisbury Cove, Maine; and ‡University of Florida, Gainesville, Florida.

Binding of Amphiphilic Peptides to Calmodulin, G. SANYAL, L. MCDOWELL, AND F. G. PRENDERGAST, Mayo Foundation, Rochester, Minnesota.

Kinetic Isotope Effects in a V_{max} Mutant of AMP Nucleosidase, V. L. SCHRAMM, D. W. PARKIN, AND F. MENCH, Temple University, Philadelphia, Pennsylvania.

4-Amino-2(X-methyl)2-butenoic Acids: Substrates and Potent Inhibitors of γ-

Aminobutyric Acid Aminotransferase, R. B. SILVERMAN, S. C. DURKEE, AND B. J. INVERGO, Northwestern University, Evanston, Illinois.

Mechanism of Inactivation of Monoamine Oxidase by 1-Phenylcyclopropylamine and 1-Phenylcyclobutylamine (93–95), R. B. SILVERMAN AND P. A. ZIESKE, Northwestern University, Evanston, Illinois.

Glutathione Peroxidase is Neither Required nor Kinetically Competent for Conversion of Arachidonic Acid to 5-HETE, M. T. SKOOG, J. S. NICHOLS, AND J. S. WISEMAN, Merrell Dow Research Institute, Cincinnati, Ohio.

The C-6 Proton of Tetrahydrobiopterin Derives from Water not NADPH (96, 97), GARY K. SMITH AND C. A. NICHOL, The Wellcome Research Laboratories, Research Triangle Park, North Carolina.

^1H and ^{31}P NMR Studies of the Conformation of ATP Analogs at the Active Site of (Na^+ + K^+)-ATPase (98), J. M. McD. STEWART, D. KLEVIKAS, AND C. M. GRISHAM, University of Virgina, Charlottesville, Vigrinia.

Active Site Specificity in Phosphorylase (99, 100), I. P. STREET AND S. G. WITHERS, The University of British Columbia, Vancouver, British Columbia, Canada.

Acyl-CoA Dehydrogenase: Reduction by Substrate and Reoxidation via Interflavin Electron Transfer, COLIN THORPE, University of Deleware, Newark, Delaware.

Role of Cobalt-Bound Water in the Catalysis of the Hydration of CO_2 by Co(II)-Substituted Carbonic Anhydrase, C. K. TU AND D. N. SILVERMAN, University of Florida, Gainesville, Florida.

Product Turnover Effects in the Soybean Lipoxygenase Reaction, P. VANDENBERG, J. COLANDUONI, AND M. J. GIBIAN, Seton Hall University, South Orange, New Jersey.

Substrate Activation of L-*Aspartase*, R. E. VIOLA AND W. E. KARSTEN, The University of Akron, Akron, Ohio.

Kinetic Mechanism and Intermediates of Salicylate Hydroxlase (101), L.-H. WANG AND S.-C. TU, University of Houston, Houston, Texas.

Participation of Cytochrome P-*450 Form 2 + 5 and Cytochrome* b_5 *in the Microsomal Metabolism of Methoxyflurane*, L. WASKELL, E. CANOVA-DAVIS, J. CHIANG, AND R. PHILPOT, University of California, San Francisco, California.

The Mechanism of Chloride Activation of Peroxidatic Reactions Catalyzed by Chloroperoxidase, T. C. WHITE, G. M. YEN, R. GOLDOWSKI, N. SUN, Y. KHAKOO, AND R. D. LIBBY, Barnard College, New York, New York.

^{31}P NMR Relaxation Studies in Glycogen Phosphorylase Coenzyme Phosphate Immobilization upon Activation (102, 103), S. G. WITHERS,* N. B. MADSEN,† AND B. D. SYLEES,† *University of British Columbia, Vancouver, and †University of Alberta, Edmonton, Canada.

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