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CONFERENCE PROCEEDINGS

Eleventh Enzyme Mechanisms Conference

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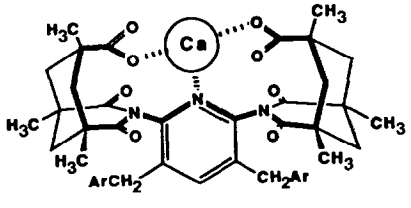
The eleventh biennial conference on enzyme mechanisms, organized by P. A. Bartlett (Chairman), J. A. Gerlt, J. V. Schloss, D. N. Silverman, and J. Stubbe, was held on January 6–8, 1989 at St. Petersburg Beach, Florida. Formal talks were given in each of six sessions on the general topics (1) Bioorganic Chemistry; (2) Enzymatic Reaction Mechanisms; (3) Radicals; (4) Nucleic Acid Enzymology; (5) Metabolic Pathways; and (6) Enzyme Structure and Function. 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, is given. Also, the titles and authors of the posters are listed. 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.

BIOORGANIC CHEMISTRY

In the initial presentation titled, "Stereoelectronic Effects in Complexation and Catalysis," J. Rebek, Jr., of the University of Pittsburgh summarized the work that he and his co-workers have been doing to model the characteristics of biological macromolecules using some new synthetic analogs that they have developed (*1–4*). These analogs contain a unique molecular cleft that incorporates carboxyls and other functional groups which can converge on other molecules held within. As illustrated by **1** and **2**, various examples of the synthetic materials can function



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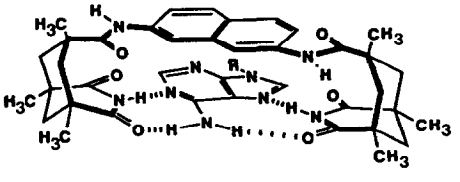

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as specific metal ion chelators or as receptors for organic molecules (adenine in the case of **2**). By using appropriate spacer elements, different shapes, sizes, and flexibility can be engineered into the structures so that molecules, which show high specificity for only one or a small group of similar ligands, can be constructed. Since the internal part of the cleft in these synthetic compounds is polar, while the outside is nonpolar, they can be used to extract polar molecules that they bind (as examples, metal ions, nucleosides, amino acids, and dipeptides) into organic solvents and will catalyze the transport of such polar molecules across membranes. In addition to their action as molecular chelates, the new structures also have permitted the examination of stereoelectronic effects at carboxyl oxygen. Finally, a major goal of the research is to develop molecules capable of catalysis, especially by concerted acid–base and nucleophilic catalysis. In his talk Dr. Rebek outlined his progress to date in this effort, specifically the catalysis of mutarotation by one of his model compounds.

The second lecture in the bioorganic chemistry session was given by T. C. Bruice of the University of California, Santa Barbara, and was titled "Mechanisms of Oxidations with Metalloporphyrins." The research he discussed (*5–8*) is aimed at distinguishing what mechanism is involved in the epoxidation of alkenes by various oxoporphyrin derivatives, reactions that presumably model similar transformations carried out by cytochrome P450 enzymes. Dr. Bruice began his talk by listing five mechanisms that various investigators have suggested for the initial attack of the oxoporphyrin on the alkene, namely (a) cycloaddition of the oxo metal to the alkene to give a four-membered metallocycle, (b) a radical attack by the oxoporphyrin on one end of the alkene to give an intermediate alkyl radical, (c) an electrophilic attack by the oxoporphyrin on one end of the alkene to give an intermediate carbocation, (d) an initial electron transfer from the alkene to the oxoporphyrin to give a charge transfer complex with the alkene existing as a cation radical, and (e) a concerted mechanism (oxenoid mechanism) involving the transfer of the oxygen of the oxoporphyrin to the alkene with no free intermediate. From results obtained using variously substituted alkenes, Dr. Bruice and his co-workers have been able to eliminate mechanisms a, b, and c as possibilities for most of the model reactions. Their results are most consistent with the rate-determining step for the epoxidation being either the formation of a charge transfer complex (mechanism d) or that the reaction is concerted (mechanism e) with some buildup of positive charge on the alkene in the transition state.

In the presentation titled "Rational Design of Enzymes," D. Hilvert of the Scripps Clinic, San Diego, described two complementary strategies that he and his group have been pursuing for developing specific biological catalysts (*9–11*). In one strategy they are converting existing, well-characterized proteins with defined binding pockets into new enzyme active sites. As an example, Dr. Hilvert described how they made the first artificial selenoenzyme by converting the essential serine residue of the bacterial protease subtilisin into a selenocysteine. He and his group are currently exploiting the properties of the novel selenol prosthetic group to probe enzyme mechanism, to model the naturally occurring redox enzyme, glutathione peroxidase, and to possibly develop catalysts that possess practical utility.

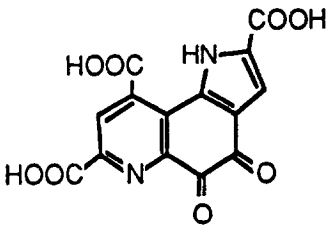
In the second approach, Dr. Hilvert and his co-workers are employing ration-

ally designed immunogens to generate antibodies that catalyze chemical reactions. Initially, they have targeted concerted transformations, since these are likely to be catalyzed through effects (induced strain and proximity) that antibodies are most likely to impart. They have prepared monoclonal antibodies against a transition state analog inhibitor of chorismate mutase and have found that some of the antibodies catalyze the formal Claisen rearrangement of (−)-chorismate to prephenate. The purified immunoglobins show the general features of real enzymes, including saturation kinetics, rate accelerations, and high enantioselectivity. Dr. Hilvert and his group have cloned and sequenced the heavy and light chain genes of one catalytic antibody and they are currently using molecular genetics to study and improve its catalytic efficiency. Progress to date, however, augurs well for the preparation of practical catalysts for use in research, industry, and medicine.

As the title indicates, the talk given by J. W. Frost of Purdue University on the "Molecular Disassembly of Organophosphonates: The Chemistry and Genetics of C–P Bond Cleavage" was concerned with the mechanism of the carbon–phosphorous bond cleavage, a reaction performed by microorganisms (for example, *Escherichia coli*) that can grow on organophosphonates (RPO_3H_2) as the sole source of phosphorous (*12–15*). One of the reasons that the reaction is of interest is because the herbicide glyphosate has such a structure. Working with whole cell cultures of *E. coli*, Dr. Frost and his co-workers could obtain no evidence that the metabolism of such compounds involved oxidative attack on the α-carbon of the organophosphonate. Rather, they found that alkanes and alkenes are the products that result from the alkyl group. This has led them to propose that the C–P bond cleavage results from initial attack on the phosphonate group, leading to a phosphoranyl radical that undergoes homolytic cleavage to metaphosphate and an alkyl radical. By this proposal the alkane and alkene arise from subsequent reactions of the alkyl radical. Unfortunately these reactions that occur in whole cells do not survive cell lysis so it has not been possible to confirm them in cell free systems. However, Dr. Frost and his group have been able to demonstrate that, when phosphoranyl radicals are generated in model reactions, they do cleave to metaphosphate (which can be trapped) and presumably alkyl radicals since alkanes and alkenes are formed. In investigations aimed at isolating the gene or genes involved in the *E. coli* metabolism of organophosphonates, Dr. Frost and his group have generated mutants that will not grow on organophosphonates as the sole phosphorous source and have located the position of the gene on a part of the *E. coli* genome that is not related to any other genetic function. Because isolation of active enzymes involved in the C–P bond cleavage has proved so intractable, it is hoped that the genetic approach to identifying the proteins that participate in this process will be more fruitful.

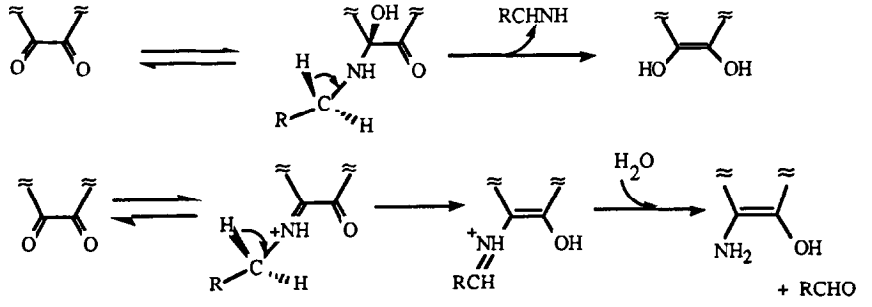
ENZYMATIC REACTION MECHANISMS

In her presentation titled "Pyrroloquinoline Quinone: A New Cofactor in Eukaryotic Enzymes," J. P. Klinman of the University of California, Berkeley, reviewed the evidence (*16–18*) that pyrroloquinoline quinone (PQQ) is an enzyme-



PQQ

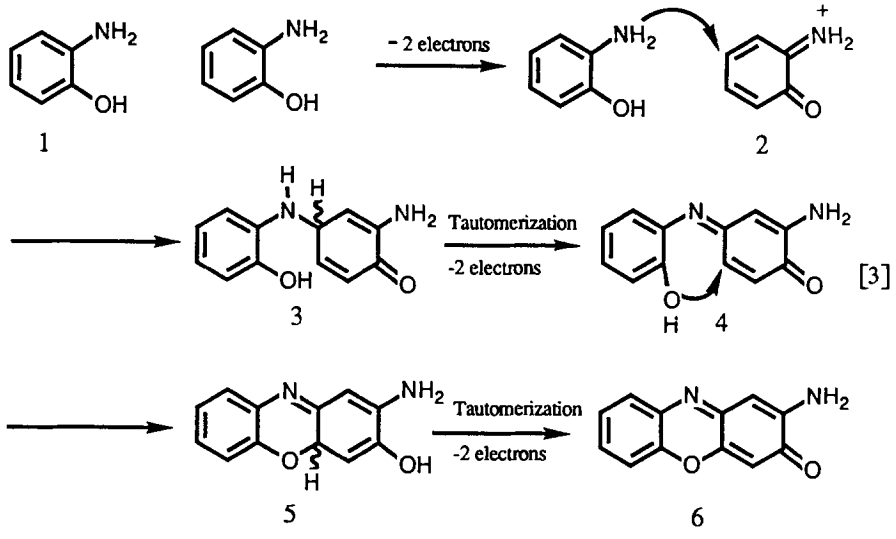
bound cofactor for various enzymes and then focused on studies that she and her group have been doing to elaborate more completely its role in the mechanism of the amine oxidases. From model studies, the two mechanisms that seem most likely for the involvement of this cofactor in the amine oxidase reactions are those outlined in Eqs. [1] and [2]. In both cases the resulting reduced cofactor would be



reoxidized by oxygen in subsequent steps. The main distinction between the two mechanisms is that the amino group is transferred to the cofactor in the mechanism of Eq. [2] (aminotransferase mechanism), whereas it is not in the mechanism of Eq. [1]. In their investigations Dr. Klinman and her co-workers have obtained a large amount of evidence indicating that the enzymic reaction proceeds by the aminotransferase mechanism (Eq. [2]). This evidence includes (a) reductive trapping of the substrate to the enzyme with $NaCNBH_3$, (b) demonstration that substrate oxidation involves loss of a proton from the α-carbon, and (c) detection of a stoichiometric transfer of nitrogen from substrate to cofactor following reduction with substrate under anaerobic conditions. Anaerobic rapid scanning stopped flow studies have also provided spectroscopic support for the involvement of enzyme-bound PQQ by the aminotransferase mechanism.

In the lecture titled "Mechanistic Studies on Phenoxazinone Synthase: Enzymatic Catalysis of an Aminophenol Oxidative Cascade" by T. P. Begley of Cornell University the mechanism of the enzyme involved in the final step of actinomycin biosynthesis (*19–21*) was discussed. The enzyme isolated from an overproducing strain of *Streptomyces lividans* is a copper containing enzyme with characteristics similar to those of tyrosinase and ascorbate oxidase. It catalyzes the seemingly complex overall oxidation (Eq. [3]) of variously substituted *o*-

aminophenols (**1**) to phenoxazinones (**6**); in the process the oxidant O_2 is reduced to water. In the research that Dr. Begley and his group have performed (*22*), they have shown that the enzymatic reaction follows the course illustrated in Eq. [3]



[3]

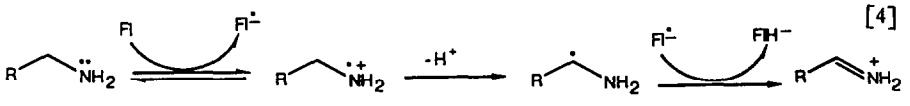
and that the enzyme is mainly involved in the steps leading to **4**. Thus, even though the overall reaction seems complicated, the enzymic role is really quite simple. It is required to catalyze the first two oxidations of *o*-aminophenols to quinoneimines and to bring the two initial reactant molecules together so that the first conjugate addition can occur. However, the subsequent tautomerizations and conjugate additions, as well as the final oxidation step, apparently all proceed nonenzymically.

The "Biochemistry of Selenium Incorporation into Enzymes" was the topic described by T. C. Stadtman of the National Institutes of Health, Bethesda. In her talk Dr. Stadtman focused on the mechanism of incorporation of selenocysteine into unique sites in proteins (*23*). Proteins known to have selenocysteine in a unique site included the bacterial enzymes, formate dehydrogenase, glycine reductase, and hydrogenase, and the animal proteins, glutathione peroxidase and a 75-kD protein found in liver, kidney, and serum. It now appears that, in the biosynthesis of such proteins, the selenocysteine residue is incorporated at a position coded on the gene by TGA, a codon that is frequently used as a termination codon but in these cases specifies the incorporation of selenocysteine. It has been known for several years that a unique tRNA that contains the anticodon to UGA (TGA) becomes aminoacylated with a serine residue and that this seryl-tRNA is readily phosphorylated to phosphoseryl-tRNA. Although not yet proven, it is now believed that this phosphoseryl-tRNA is somehow converted to selenocysteyl-tRNA which then incorporates selenocysteine into these selenoproteins at the site coded by TGA on the gene. One of the many questions which remain, however, is: how is TGA recognized as a reading frame codon when it is fre-

quently used as a termination codon in the biosynthesis of other proteins by these same biological systems?

RADICALS

In his presentation titled "Radical Intermediates in Monoamine Oxidase-Catalyzed Reactions," R. B. Silverman of Northwestern University described the use that he and his group have made of mechanism-based inactivators (*24*) to elucidate the catalytic mechanism of the flavoenzyme monoamine oxidase (MAO). From extensive investigations (*25, 26*) in which a variety of cyclopropyl- and cyclobutylamines were utilized as mechanism-based inactivators of MAO, Dr. Silverman and his co-workers have obtained strong evidence that the mechanism of the enzymic reaction is as outlined in Eq. [4]. Among the pieces of evidence for



this conclusion are the observations that the rings in the foregoing compounds undergo cleavage when they are acted upon by the enzyme, that enzyme inactivation is caused by ring-opened intermediates reacting with the flavin or amino acid side chains of the protein to form covalent adducts, and that intermediate radicals can be trapped with spin traps and analyzed by ESR. Dr. Silverman also described two new classes of MAO inactivators, namely, aminoalkyltrimethylsilanes (*27*) and aminoalkyltrimethylgermanes (*28*), that he and his group have developed, and he presented evidence that they cause inactivation by a radical mechanism as well. Finally, he reported on investigations indicating that 5-aminoalkyl-3-aryloxazolidinones inactivate MAO by a radical mechanism in which the oxazolidinone ring is cleaved with the formation of CO_2.

The first enzyme involved in the biosynthesis of the prostaglandins and their further metabolites was the topic of the presentation, titled "Studies on the Structure and Mechanism of Prostaglandin H Synthase," given by L. J. Marnett of Wayne State University. This important enzyme, which is the main target of nonsteroidal anti-inflammatory agents (aspirin, indomethacin, ibuprofen, etc.), is a hemeprotein which exhibits both a cyclooxygenase activity that oxygenates arachidonic acid to the hydroperoxy endoperoxide PGG_2 and a peroxidase activity that reduces PGG_2 to the hydroxy endoperoxide PGH_2. In their research Dr. Marnett and his group have obtained strong evidence that the peroxidase and cyclooxygenase reactions take place at two different sites on the enzyme surface (*29, 30*). The native enzyme with heme (Fe(III)-protoporphyrin IX) bound is resistant to trypsin cleavage, but when the heme is removed, limited tryptic digestion produces two fragments of molecular size 33 and 38 kDa. Reconstitution of the cleaved enzyme produces an enzyme with cyclooxygenase activity but no peroxidase activity. Furthermore, the cleaved enzyme binds one less heme molecule than the native enzyme. The trypsin cleavage occurs at arginine which is

present in the sequence His^{-250}–Tyr^{251}–Pro^{252}–Arg^{253}. Since (a) heme protects against trypsin cleavage, (b) peroxidase activity is lost on trypsin cleavage, and (c) the sequence at the point of trypsin cleavage is similar to that in many peroxidases (*31*), Dr. Marnett believes that heme bound at the trypsin cleavage site is responsible for the peroxidase activity of the enzyme. Dr. Marnett also speculated that the Try^{251} in the above sequence may be the tyrosine that is converted to the tyrosyl radical that others (*32*) have recently reported as the intermediate that initiates the cyclooxygenase activity by removing the 13-proS hydrogen from arachidonic acid.

In his lecture titled "The Radical Mechanism for Dopamine β-Hydroxylase," J. J. Villafranca of the Pennsylvania State University reviewed not only the evidence that this enzymic reaction proceeds by a radical mechanism, but he also summarized some recent data that clarify the environment of the copper in the enzyme and presented some results pertaining to the suggestion by others that PQQ is an enzyme-bound cofactor (*33–36*). Active dopamine β-hydroxylase is a tetramer composed of identical subunits, each of mw 72,500 and each containing two coppers. Recent spin-echo experiments, done in collaboration with J. Peisach, have shown that imidazoles are the main ligands of both coppers and that there are two different types of imidazoles acting as ligands. The best fit of the data indicates that each copper is bound to four imidazoles. Results from EXAFS studies (in collaboration with W. E. Blumberg) are also consistent with four imidazoles being bound to both the oxidized and the reduced forms of the enzyme. From NMR relaxation studies, it appears that, in addition, there is at least one rapidly exchanging water molecule bound per copper.

In the final part of his talk, Dr. Villafranca summarized the results of extensive experiments that he and his group have done which fairly conclusively show that PQQ is not a dopamine β-hydroxylase cofactor. The opposite conclusion, arrived at by others, had relied heavily on the observation that phenylhydrazine inactivates the enzyme; this was interpreted as being the result of phenylhydrazone formation with one of the carbonyls of PQQ. However, Dr. Villafranca and his coworkers showed that the inactivation by phenylhydrazine is a mechanism-based inactivation with the label from ^{14}C-labeled phenylhydrazine becoming attached to three different amino acids on the enzyme when it is inactivated.

NUCLEIC ACID ENZYMOLOGY

The presentation by P. Schimmel of the Massachusetts Institute of Technology on "Enzyme–RNA Recognition" focused on describing the experiments he and his group have done to determine the nucleotide determinants and kinetic parameters for the molecular recognition of tRNA's by the aminoacyl tRNA synthetases (*37, 38*). It turns out that for some tRNA's the anticodon is not essential for recognition by the cognate synthetase. From investigations with multiple sequence variations of an *E. coli* alanine tRNA, it was found that a single G3:U70 base pair is a major determinant for the identity of the tRNA. When this single base pair is introduced into other tRNA's, they can be aminoacylated with

alanine. From kinetic studies, it was found that the enzyme discriminates against other pairs (for example, A3:U70) through both a severely reduced k_{cat} and an elevated K_m. Because the enzyme discriminates on the basis of a small element (a single base pair) in the tRNA, Dr. Schimmel and his co-workers found that even a synthetic hairpin helix with only 7 bp is a substrate for the enzyme, but efficient aminoacylation still requires the presence of the G:U base pair. As Dr. Schimmel points out, these results have considerable implications for the molecular recognition, and evolution of identity, of tRNA's.

In the lecture on "RecA Protein-Promoted DNA Strand Exchange," M. M. Cox of the University of Wisconsin, Madison, described how the recA protein of *E. coli* is believed to promote a unidirectional strand exchange reaction coupled to ATP hydrolysis (39–41). The process is probably involved in recombination repair, in which a lesion is repaired by combining one good strand of DNA with another from a different dimer. The active species of the reaction is an extended nucleoprotein filament that forms on one of the two DNA molecules and can include thousands of recA monomers. On the basis of the current available evidence, a model for how this complex overall reaction proceeds is shown in Fig. 1. In this mechanism the protein filament promotes strand exchange by actively rotating one DNA molecule relative to the other. While the model explains many observations, including the energetic efficiency of the system, the structural relationship of the two DNA's undergoing exchange remains a major unresolved problem.

In his presentation titled "Enzymology of DNA Mismatch Correction," P. Modrich of Duke University described a methyl-directed mismatch correction system that *E. coli* uses to cope with DNA biosynthetic errors (*42–45*). Mismatch repair by this system is a postreplication event that responds to the state of methylation of d(GATC) sequences, with correction directed to the newly synthesized strand by virtue of its transient undermethylation at such sites. Dr. Modrich and his group have been able to reconstitute the system *in vitro* using eight near homogeneous activities, namely, *mutH, mutL,* and *mutS* gene products, DNA helicase II, SSB, DNA polymerase III holoenzyme, DNA ligase, and a 55-kDa stimulatory protein. Repair also requires ATP, NAD^+, Mg^{2+}, and the dNTP's. This defined system is capable of recognition and correction of seven of the eight possible base–base mispairs (all except C–C) and responds in a dramatic fashion to the state of the d(GATC) methylation, with the state of modification of a single d(GATC) site located 1000 bp from the mismatch being sufficient to determine the strand specificity of repair. Analysis of the purified *mut* gene products has demonstrated that the MutS protein binds to mismatched base pairs and that the MutH protein possesses an extremely weak d(GATC) endonuclease activity which incises an unmethylated DNA site 5′ to the dG of the tetranucleotide sequence. This endonuclease undergoes activation in a reaction that is dependent on MutL and MutS proteins, ATP, and the presence of a mismatch within the DNA substrate (Fig. 2). These findings indicate that the strand specificity of correction is determined by the strand specificity of the MutH-promoted incision event. Moreover, analysis of patterns of DNA synthesis associated with the correction reaction suggests that, at least in the case of some substrates, excision of the unmethylated

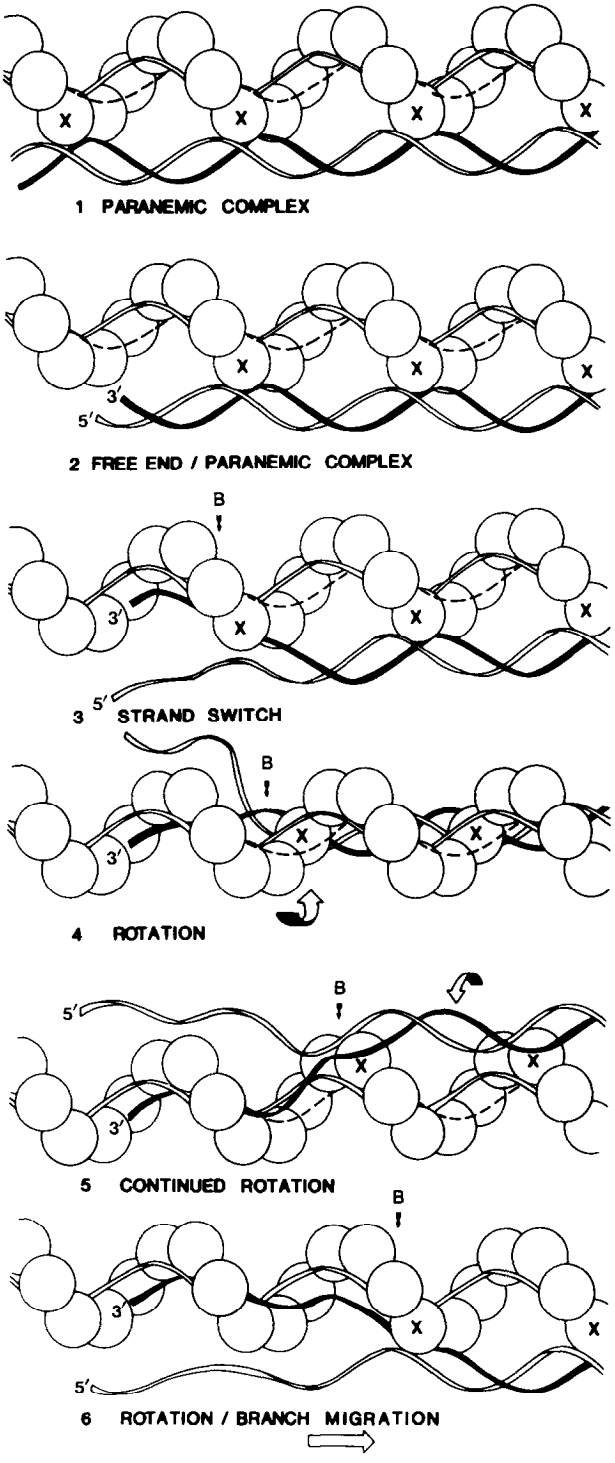


FIG. 1. Model for the mechanism of reactions catalyzed by the recA protein.

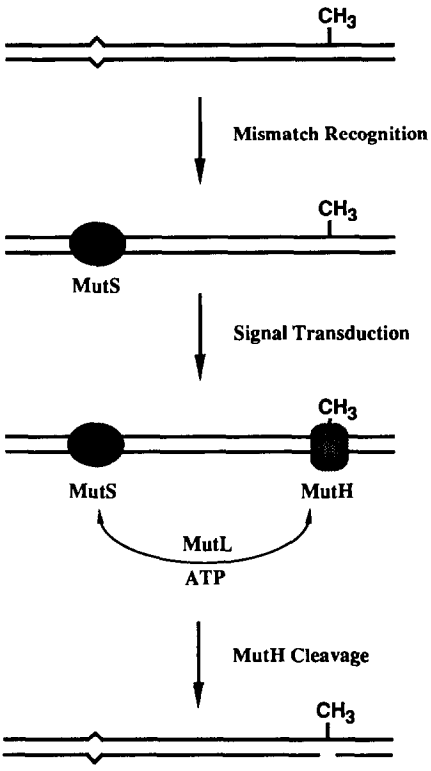


FIG. 2. Initiation of methyl-directed DNA mismatch correction.

strand proceeds 3′ to 5′ from the incised d(GATC) sequence toward the mispair, with the resulting gap repaired by DNA synthesis initiating in the vicinity of the mismatch.

In his lecture titled "Mechanisms by which DNA Polymerases Achieve Replication Fidelity," S. J. Benkovic of the Pennsylvania State University described the research that he and his group are doing, specifically with the Klenow fragment of *E. coli* DNA polymerase I (*46–49*). This enzyme acts not only as a polymerase but also as a pyrophosphorylase, and a 3′ to 5′ exonuclease. Using synthetic double-stranded oligonucleotides (for example, with 20 nucleotides on one strand and 13 on the other that are perfectly base-paired with the first 13 of the first strand), and investigating only the incorporation of one additional nucleotide by rapid quench techniques, Dr. Benkovic and his co-workers have defined the detailed kinetic mechanism for the polymerase reaction. The usual catalytic cycle involves (a) initial binding of the NTP to the enzyme–DNA complex, (b) a protein conformation change, (c) the actual formation of the new phosphodiester bond with formation of enzyme-bound pyrophosphate, and (d) release of pyrophosphate to regenerate the enzyme–DNA complex which would be ready for another cycle. The total discrimination against incorrect base pairs of 10^4 to 10^6 apparently

arises due to smaller effects on several of these steps. The discrimination on NTP binding (step a) is surprisingly small; the major effects are observed in steps c and d. If step d is slow, as it is if the incorrect base pair has been synthesized, then the exonuclease has a longer time to remove it. The rate of the exonuclease itself shows no discrimination for incorrect versus correct bases. Step c shows high discrimination by being slowed down both for putting an incorrect base in and also when adding a subsequent correct base after an incorrect one has been incorporated.

In another part of his lecture, Dr. Benkovic also described research his group is doing in attempts to define the relationship of the polymerase and exonuclease sites. From a solid-state three-dimensional structure determination by others the enzyme was found to exist in two domains, one the polymerase domain with a groove where DNA binds, and the other containing the exonuclease site which is 30 Å away. From experiments with oligonucleotides tagged at various points with a fluorescent or protein label, or crosslinked, Dr. Benkovic and his group have been able to show that in solution the exonuclease site is indeed ca. 30 Å from the polymerase site and, furthermore, that the exonuclease uses only single-stranded DNA. One of the problems that still remains, however, is that there is no kinetic evidence for the sliding of the DNA from the polymerase to the exonuclease site.

METABOLIC PATHWAYS

The initial lecture in this session was titled "Assembling and Restructuring Catabolic Pathways *in vitro*" and was given by K. N. Timmis of the University of Geneva, Switzerland. Dr. Timmis and his group are interested in developing organisms that will efficiently degrade pesticides, especially those that are aromatic compounds (*50–52*). Two different approaches that they have taken in an attempt to do this are (a) restructure an existing pathway by mutating existing proteins to eliminate nonpermissive steps, and (b) create a new pathway. As an example of the first approach, Dr. Timmis described how he and his co-workers were able to alter a pathway in *Pseudomonas putida* that degrades toluene so that it would now degrade 4-ethyltoluene. In the process they had to select for mutants for two of the enzymes in the pathway and also select for a mutant regulatory protein. However, when that was done the organism would now degrade 4-ethyltoluene. As an example of the second approach, Dr. Timmis described how he and his co-workers cloned into a strain of *Pseudomonas,* which was quite specific for the metabolism of 3-chlorobenzoate, two genes from other organisms so that the *Pseudomonas* would metabolize a much wider variety of aromatic compounds. The altered organism is now much more useful for treating waste streams. Dr. Timmis stressed that the organism carrying out the metabolism of a mixture of compounds should only have one pathway rather than two or more, because one pathway may produce suicide inhibitors for the other, and the organism will then not prosper. This is apparently the reason why many organisms having both the "ortho" and the "meta" pathways for catechol metabolism are

not very useful for metabolizing the mixtures of compounds encountered, for example, in waste streams.

In his presentation titled "Biosynthesis and Pharmacological Properties of Lipid A" C. R. H. Raetz of Merck, Sharp and Dohme Research Laboratories, Rahway, described the research he and his co-workers are doing to characterize this major outer membrane component of *E. coli* and other gram-negative bacteria (*53*). Lipid A (sometimes known as gram-negative endotoxin) is of interest because it is a potent pharmacological agent responsible for the complex immunostimulatory phenomena and shock that frequently accompany gram-negative infections. Lipid A is a β-1,6-linked disaccharide of glucosamine, phosphorylated at positions 1 and 4′, and acylated with *R*-3-hydroxymyristoyl moieties at positions 2, 3, 2′, and 3′. The *R*-3-hydroxymyristoyl groups at positions 2′ and 3′ are themselves acylated with laurate and myristate, respectively.

Although the structure of lipid A was not known until 1983, rapid advances in defining the steps in the enzymatic synthesis of this compound have occurred and these are outlined in Fig. 3. Dr. Raetz and his group have recently isolated mutants defective in UDP–GlcNAc acyltransferase and have demonstrated that these mutants are strikingly defective in their ability to synthesize lipid A *in vivo* under nonpermissive conditions. The availability of this and at least 10 other enzymes for generating radiolabeled substructures of lipid A has also facilitated structure/function studies and the development of new assays for studying the binding of lipid A to animal cell surfaces. They have also allowed Dr. Raetz' group to begin investigating the signal transduction mechanisms by which lipid A triggers the synthesis in macrophages of specific protein mediators such as interleukin-1 and tumor necrosis factor.

Part of the blood clotting cascade was the subject of the lecture, titled "Regulation of Thrombin-Catalyzed Activation of Fibrin Stabilizing Factor," given by J. A. Shafer of the University of Michigan. Among the numerous reactions that occur following injury to the vascular system (*54*), one is the crosslinking of initially formed fibrin gels by displacing ammonia from glutaminyl residues by lysyl residues. This transamidation reaction is catalyzed by factor XIIIa, which is the activated form of factor XIII. The conversion of XIII to XIIIa involves the removal of a 37-residue peptide and is catalyzed by thrombin. However, thrombin also is involved in the formation of the original noncrosslinked fibrin gel because it catalyzes the formation of fibrin from fibrinogen. Dr. Shafer and his group have focused on characterizing the interactions that ensure that the thrombin produced in the clotting cascade activates appropriate amounts of fibrin and factor XIIIa (*55–58*). These extensive studies have included determination of the steady-state kinetic parameters for the action of thrombin on fibrinogen and factor XIII both separately and in the presence of each other and their products. Among their findings is the observation that noncrosslinked fibrin is a potent activator of thrombin for the factor XIII to factor XIIIa conversion, whereas crosslinked fibrin is not. As Dr Shafer pointed out, this mode of feedback regulation of the activation of factor XIIIa may serve to ensure against wasteful and possibly deleterious generation of excess factor XIIIa after fibrin has been crosslinked. Another interesting observation made by Dr. Shafer and his group is that, when peptide is

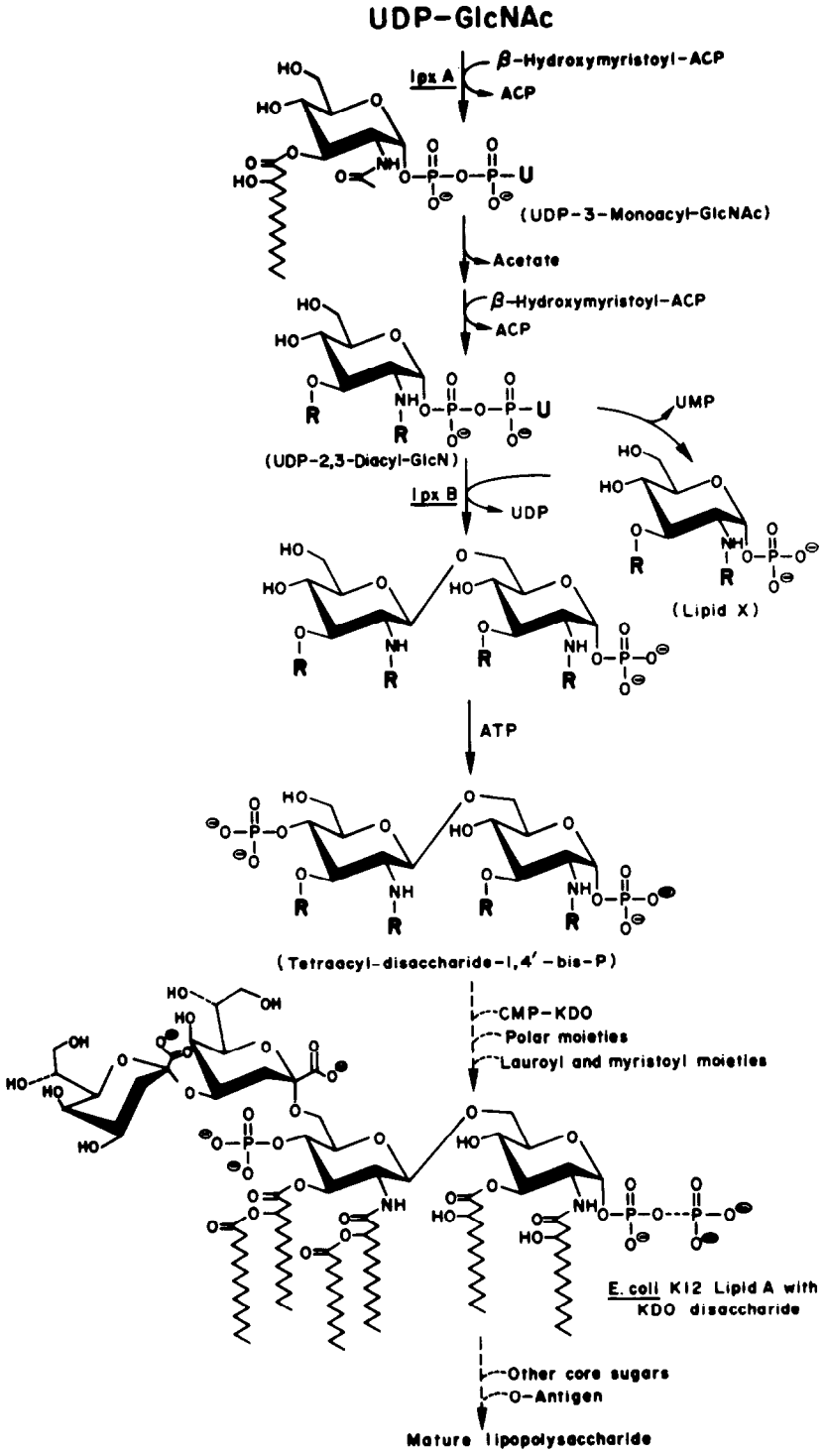


FIG. 3. The biosynthesis of lipid A and subsequent lipopolysaccharides.

released from only one of the two α-chains of factor XIII, the enzyme has full catalytic activity.

In the final presentation of the Metabolic Pathways session, R. G. Matthews of the University of Michigan described some studies she is doing in collaboration with F. C. Neidhardt on "Serine Catabolism and Heat Shock in *E. coli*." Earlier investigations (*59–61*) had indicated that, when *E. coli* is subjected to heat shock (42°C), a set of 15 polypeptides, termed heat shock proteins, are induced, triggered by an alternate σ factor for RNA polymerase that recognizes consensus sequences in the promoter regions of the heat shock polypeptides. Later studies (*62*) revealed that three of these heat shock proteins are abnormally induced in a *metK* strain of *E. coli*, a strain that shows a nutritional requirement for serine at elevated temperatures. The metabolic requirement for serine in this strain has been traced to an elevated level of L-serine deaminase activity, leading to accelerated conversion of serine to pyruvate and ultimately to excretion of acetate into the medium. Indeed it was found that all strains of *E. coli* examined have elevated levels of L-serine deaminase after temperature shifts that induce the heat shock response. Consequently, Dr. Matthews concludes that elevations in the rate of serine catabolism comprise part of the heat shock response in *E. coli* and that expression of three of the heat shock polypeptides is linked to changes in the rate of serine catabolism. Dr. Matthews postulates that probably the reason for the elevated deaminase activity on heat shock is to divert serine away from production of glycine and one carbon unit for nucleotide and protein synthesis, and toward pyruvate and acetate.

ENZYME STRUCTURE AND FUNCTION

In his lecture on "Site Directed Mutagenesis and Metalloenzyme Function" S. G. Sligar of the University of Illinois, Urbana, described extensive studies he and his group have done to probe structure–function correlations of various proteins, especially the heme proteins, myoglobin, cytochrome b_5, and cytochrome $P450_{cam}$, and the electron transfer protein, putidaredoxin (*63–67*). Some of the myoglobin mutants he and his co-workers have prepared, and the information they give about the myglobin structure, include (a) replacement of His^{64} and Val^{168} with a large number of other amino acids has given information about distal pocket effects, (b) replacement of Arg^{45} with Gly, Asp, Ser, and Asn points out the role of the salt linkage at one pocket access channel, and (c) replacing His^{93}, the normal axial ligand bound to the heme, with Cys and Tyr has clarified the importance of the axial ligand to function. Dr. Sligar emphasized that extensive biophysical studies, some of them in collaboration with other laboratories, have been necessary to determine the precise effects of the myoglobin mutations on protein function.

Dr. Sligar also described in his presentation how the site directed mutagenesis approach has proved useful to probe protein–protein interactions. Surface charge mutations in putidaredoxin and cytochrome b_5, together with fluorescence and NMR measurements, have allowed Dr. Sligar's group to generate a proposed

docked structure of a putidaredoxin–P450 electron transfer complex. Predicted from this model is an aromatic electron transfer path that includes the C-terminal tryptophan of putidaredoxin and Phe^{350} of P450. Replacement of the C-terminal Trp of putidaredoxin with Tyr or Phe yields an active protein–protein complex, but replacement with Val, Leu, Ile, Ala, Lys, Asp, or Arg results in an inactive reconstituted hydroxylase. Dr. Sligar also reported some recent results on the reengineering of hydroxylase specificity in the $P450_{cam}$ system by directed mutagenesis of several of the active site H-bonding and nonaromatic residues illustrated in Fig. 4.

In the final formal presentation of the symposium, H. Zalkin of Purdue University summarized a large amount of research that he and his co-workers, as well as others, have performed on the "Structure, Function and Mechanism for a Family of Glutamine Amidotransferase Enzymes" (*68–70*). Glutamine amidotransferases are a group of enzymes that provide the major route for the incorporation of nitrogen into biological molecules; they catalyze the transfer of the amide nitrogen of glutamine to various precursors in the biosynthesis of amino acids, purine and pyrimidine nucleotides, coenzymes, and amino sugars. All amidotransferases can utilize NH_3 as well as glutamine as the nitrogen donor.

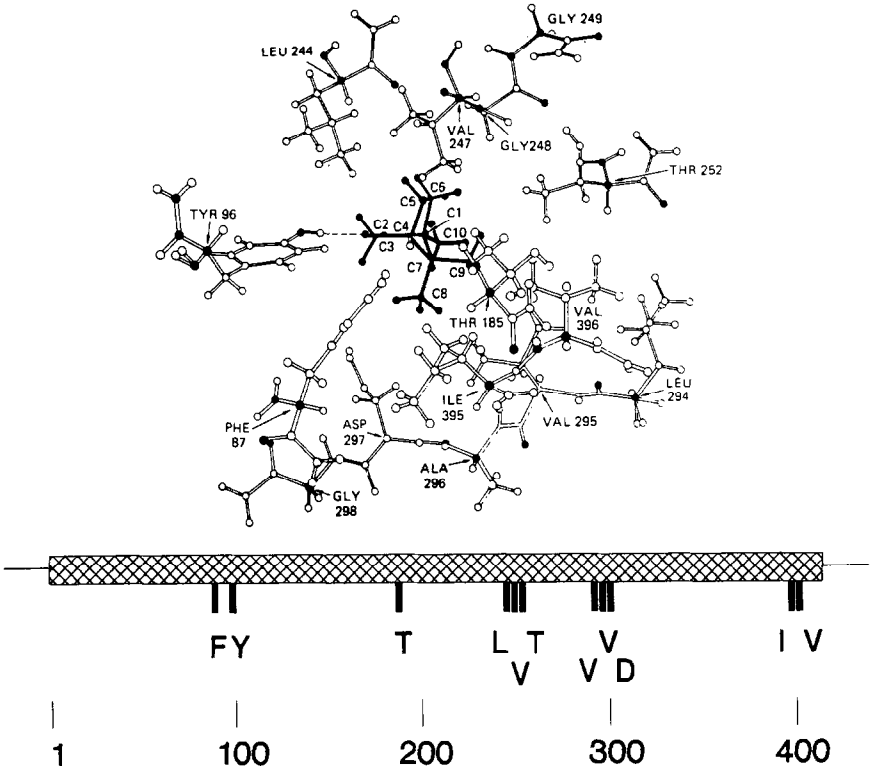


FIG. 4. Residues contacting camphor in its complex with cytochrome $P450_{cam}$.

Amidotransferases contain at least two functional domains, one concerned with glutamine amide transfer (GAT) and the other the utilization of nascent or exogenous NH_3 (aminator). GAT domains of ca. 200 amino acid residues have been identified in several enzymes by affinity labeling and sequence analysis and are of two types, designated as the G- and F-type GAT domains. Although the amino acid sequence in G-type GAT domains is different from that in the F-type, both types contain single conserved cysteine, histidine, and acidic residues (Glu in G-type and Asp in F-type) that are essential for GAT but not aminator function. The initial step in nitrogen transfer from glutamine is believed to involve formation of a covalent cysteinyl-glutamine intermediate. The active site histidine is thought to participate in formation of the covalent intermediate and the acidic residue probably functions in a subsequent step, either amide transfer or hydrolysis of the glutamyl-enzyme intermediate.

Some unique aspects of the amidophosphoribosyltransferase from *Bacillus subtilis*, that have been studied in collaboration with R. L. Switzer's laboratory (*71*), were also presented by Dr. Zalkin. This enzyme contains a [4Fe–4S] cluster and an N-terminal 11 amino acid propeptide. The recent results indicate that the FeS cluster is a site for an oxidative inactivation that precedes enzyme turnover and that the propeptide has no role in enzyme folding or maturation; presumably it is there to permit an essential overlap with an upstream gene in the *B. subtilis pur* operon.

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POSTER PRESENTATIONS

Orotidine Monoposphate Decarboxylase Catalysis: Evidence for a Noncovalent Mechanism, S. ACHESON, J. BARCLEY, M. E. JONES, AND R. WOLFENDEN, Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27599-7260.

Mechanism Based Inhibition of Dopamine β-Hydroxylase by Heterocyclic Aromatic Acetic Acids, K. ANDERSON, Z. WHITE, AND R. C. ROSENBERG, Department of Chemistry, Howard University, Washington, DC 20059.

Formation of the 1-(S-Glutathionyl)-2,4,6-Trinitrocyclohexadienate Anion at the Active Site of Glutathione S-Transferase, R. N. ARMSTRONG, G. F. GRAMINSKI, P. ZHANG, AND M. SESAY, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742.

N-Isopropyl Oxalhydroxamate as a Reaction-Intermediate Analog and Selective Inhibitor of Ketol-Acid Reductoisomerase in Bacteria and Plants, A. AULABAUGH AND J. SCHLOSS, Central Research and Development Department, E. I. du Pont de Nemours & Co., Wilmington, DE 19898.

Activation of Creatine Kinase Expressed in E. coli as an Insoluble Aggregate, P. C. BABBITT, B. L. WEST, I. D. KUNTZ, AND G. L. KENYON, Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94143.

Phosphinic Acid Inhibitors of Mung Bean Glutamine Synthetase, B. BOETTCHER, C. HAWELKA, C. JOHNSON, M. DOLSON, AND R. CHERPECK, Chevron Chemical Co., Richmond, CA 94804-0010.

The Effect of Ammonium Sulfate on the Heat Inactivation of Butyrylcholinesterase, S. BURGESS, Department of Chemistry, The University of North Carolina, Wilmington, NC 28403-3297.

Catalytic Reactions of Macrocyclic Nickel Complexes, C. J. BURROWS, J. S. ALBERT, H.-S. YOON, T. R. WAGLER, AND J. F. KINNEARY, Department of Chemistry, State University of New York, Stony Brook, NY 11794-3400.

Kinetics and Mechanism of Acetohydroxyacid Synthase, D. M. CHIPMAN, Ben-Gurion University of the Negev, Israel.

Substrate Specificity of Poliovirus 3C and HIV Proteinases, G. C. CHOW, P. BAX, R. INGRAHAM, P. PALLAI, AND M. T. SKOOG, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT 06877.

Oligosaccharyltransferase: N-Glycosylation of Synthetic Peptides and Characterization of the Glycopeptide Products, R. S. CLARK, S. BANERJEE, AND J. K. COWARD, Departments of Medicinal Chemistry and Chemistry, The University of Michigan, 428 Church St., Ann Arbor, MI 48109-1065.

Stereochemistry of the Porcine Plasma Amine Oxidase Reaction, A. A. COLEMAN, M. M. PALCIC, AND O. HINDSGAUL,* Departments of Food Science and *Chemistry, University of Alberta, Edmonton, Alberta, Canada.

Conservation of Active Site Residues in Inorganic Pyrophosphatases, B. S. COOPERMAN, L. F. KOLAKOWSKI, AND R. LAHTI, Department of Chemistry, University of Pennsylvania, Philadelphia, PA, and Department of Biochemistry, University of Turku, Finland.

Elementary Physicochemical Limitations on the Efficiency of the Glyoxalase Pathway, D. J. CREIGHTON, M. J. SHIH, AND J. W. EDINGER, Department of Chemistry, University of Maryland Baltimore County, 5401 Wilkens Avenue, Baltimore, MD 21228.

The Active Site of Triose Phosphate Isomerase, R. C. DAVENPORT, JR., Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Kinetic Characterization of Cloned HIV Proteinase, B. M. DUNN, Department of Biochemistry and Molecular Biology, College of Medicine, University of Florida, Gainesville, FL 32610.

Allosteric Interactions and the Channeling of Indole in the Tryptophan Synthase Bienzyme Complex, M. F. DUNN, K. HOUBEN, P. BRZOVIC, AND C. LEJA, Department of Biochemistry, University of California, Riverside, CA 92521.

Carnitine Acyltransferases: Mechanistic Deductions from Inhibition Studies with Rigid Analogs, R. D. GANDOUR, Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803-1804.

Covalent NADP-Aldehyde Adduct Formation Catalyzed by Bovine Kidney Aldose Reductase, C. E. GRIMSHAW, Research Institute of Scripps Clinic, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Deuterium Kinetic Isotope Effects for the Hydroxylation Reaction Catalyzed by α-Ketoisocaproate Dioxygenase, H. HAN AND R. C. PASCAL, JR., Department of Chemistry, Princeton University, Princeton, NJ 08544.

Use of Site Directed Mutants of Ribulose Bisphosphate Carboxylase/Oxygenase to Localize Role of Active Site Residues, F. C. HARTMAN, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831.

Introduction of a Cysteine Protease Active Site into Trypsin, J. N. HIGAKI AND C. S. CRAIK, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143.

HIV Reverse Transcriptase: Cloning and Expression in E. coli, D. J. HUPE, B. A. AZZOLINA, J. G. MENKE, AND D. L. LINEMEYER, Department of Biochemistry, Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065.

Site Directed Mutagenesis of E. coli Ribonucleotide Reductase, M. JOHNSTON, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

The ^{18}O Equilibrium Isotope Effect on the Deprotonation of ATP, J. P. JONES AND W. W. CLELAND, Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

Ligand-Directed Mutagenesis: Hydrogen Bonds of Remarkable Stability at the Active Site of Adenosine Deaminase, W. M. KATI AND R. WOLFENDEN, Department of Biochemistry, University of North Carolina, Chapel Hill, NC 27599.

Role of Oxygen during Horseradish Peroxidase Turnover and Inactivation, X. MA AND S. E. ROKITA, Department of Chemistry, State University of New York, Stony Brook, NY 11974.

Nitric Oxide Is an Intermediate in the Oxidation of L-Arginine to Nitrite and Nitrate by Macrophages, M. A. MARLETTA, P. S. YOON, R. IYENYAR,* C. D. LEAF,* AND J. S. WISHNOK,* Department of Medicinal Chemistry, University of Michigan, Ann Arbor, MI 48109-1065, and *Massachusetts Institute of Technology, Cambridge, MA 02139.

Studies of the Mechanism and Substrate Specificity of Papain Using Site Directed Mutagenesis, R. MENARD, A. G. STORER, C. PLOUFFE, R. DUPRAS, H. KOURI, D. Y. THOMAS, D. C. TESSIER, AND T. VERNET, National Research Council of Canada Biotechnology Research Institute, 6100 Royalmount Avenue, Montreal, Quebec, Canada H4P 2R2.

Iron Chemistry and the Mechanism of Lipoxygenases, M. J. NELSON, Central Research Department, E. I. du Pont de Nemours and Co., Wilmington, DE 19898.

Design, Synthesis, Overexpression, and Site Specific Mutagenesis of a Gene for Bovine (Pro)phospholipase A2, J. P. NOEL AND M.-D. TSAI, Department of Chemistry, The Ohio State University, Columbus, OH 43210.

Active Site Residues of Mitochondrial Phosphoenolpyruvate Carboxykinase, K.-C. CHENG, P. GUIDINGER, AND T. NOWAK, Department of Chemistry, University of Notre Dame, Notre Dame, IN 46556.

The β-Lactamase-Catalyzed Aminolysis of Depsipeptides: Proof of the Non-Existence of a Specific D-Phenylalanine-Enzyme Complex by Double Label Isotope-Trapping, S. PAZHANISAMY, Chemistry Department, Wesleyan University, Middletown, CT 06457.

Essential Cysteine and Tryptophan Residues in Tryptophan Indole-Lyase Studied by Site Directed Mutagenesis, R. S. PHILLIPS AND P. D. GOLLNICK, Department of Chemistry and Biochemistry, School of Chemical Sciences, University of Georgia, Athens, GA 30602, and Department of Biological Sciences, Stanford University, Stanford CA 94305.

Identification of the Redox-Active Thiols of Ribonucleotide Reductase from Corynebacterium nephridii, K. L. PLAISANCE AND H. P. C. HOGENKAMP, Department of Biochemistry, University of Minnesota, Minneapolis, MN 55455.

Tritium Labeling and Tritium NMR, or, How I Learned to Stop Worrying and Love the Bomb, G. D. PRESTWICH, Department of Chemistry, State University of New York, Stony Brook, NY 11794-3400.

A Transition State Analog for the Ribonuclease Reaction: Uridine-2,3,-cyclic Vanadate. The Story Retold, with a Somewhat Different Ending, W. J. RAY, JR., Purdue University, West Lafayette, IN 47907.

(PO_3)-Transfer in the Phosphoglucomutase Reaction: Is it Really a Largely Dissociative Process? W. J. RAY, JR., Purdue University, West Lafayette, IN 47907.

Inhibition of the Carboxyltransferase Site of Acetyl-CoA Carboxylase by Two Classes of Grass Selective Herbicides. A. R. RENDINA, A. C. CRAIG-KENNARD, J. D. BEAUDOIN, AND J. M. FELTS, Chevron Chemical Co., Research Agricultural Chemicals Division, Richmond, CA 94804-0010.

On the Relative Unimportance of Carbocation–Anion Pair Reactions in Water, J. P. RICHARD AND P. YEARY, Department of Chemistry, The University of Kentucky, Lexington, KY 40506-0055.

Interaction of Aryl- and Alkylsulfonamides with Carbonic Anhydrases I, II and III, R. S. ROWLETT, R. A. CARTER, A. CORBETT, M. ZELLER, AND T. KLYSA, Department of Chemistry, Colgate University, Hamilton, NY 13346.

Structural and Mechanistic Studies of E. coli β-Hydroxydecanoylthioester Dehydrase, J. M. SCHWAB, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.

Proton Transfer during Glycosidase Catalysis, T. SELWOOD AND M. L. SINNOTT, School of Chemistry, University of Bristol, England.

Trisnorsqualene Alcohol, a Potent Inhibitor of Vertebrate Squalene Epoxidase, S. E. SEN AND G. D. PRESTWICH, Department of Chemistry, State University of New York, Stony Brook, NY 11794-3400.

The Role of Histidine 64 in Carbonic Anhydrase II Studied by Site Directed Mutagenesis, D. N. SILVERMAN, C. K. TU, AND G. C. WYNNS, Department of Pharmacology and Biochemistry, University of Florida, Gainesville, FL 32610.

Chemical and Enzymatic Degradation of S-Adenosyl-L-methionine, J.-K. SON AND R. W. WOODARD, College of Pharmacy, The University of Michigan, Ann Arbor, MI 48109-1065.

Identification of Sorbitol-Phosphate in the Normal and Diabetic Mammalian Lens, B. S. SZWERGOLD, F. KAPPLER, T. R. BROWN, AND P. PFEFFER,* Department of NMR and Medical Spectroscopy, Fox Chase Cancer Center, Philadelphia, PA 19111, and *Eastern Regional Research Center, USDA, Philadelphia, PA 19118.

Biochemical Study of the Sex Pheromone Degrading Enzymes of the Tobacco Budworm Moth, M. L. TASAYCO AND G. D. PRESTWICH, Department of Chemistry, State University of New York, Stony Brook, NY 11794-3400.

A Novel Flavin Radical Cation and Its Proposed Intermediacy in Bacterial Bioluminescence, S.-C. D. TU, Department of Biochemical and Biophysical Sciences, University of Houston, Houston, TX 77004.

Multiple Isotope Effects for Malic Enzyme from Ascaris suum and Chicken Liver. 1. Studies with NAD(P), J. L. URBAUER, P. M. WEISS, S. R. GAVVA, B. G. HARRIS, W. W. CLELAND, AND P. F. COOK, Departments of Biochemistry, University of Wisconsin, Madison, WI 53706, and Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

Activation and Mechanistic Studies of L-Aspartase from Escherichia coli, R. E. VIOLA AND W. E. KARSTON, Department of Chemistry, The University of Akron, Akron, OH 44325.

Stoichiometry of Methoxyfluorane Metabolism in the Presence and Absence of Cytochrome B_5, L. WASKELL, K. KONOPKA, AND L. GRUENKE, Department of Anesthesiology and Liver Center, University of California, San Francisco, CA 94143.

Multiple Isotope Effects for Malic Enzyme from Ascaris suum and Chicken Liver. 2. Studies with Alternate Dinucleotides and a Chemically Modified Malic Enzyme, P. M. WEISS, J. L. URBAUER, S. R. GAVVA, B. G. HARRIS, P. F. COOK, AND W. W. CLELAND, Departments of Biochemistry, University of Wisconsin, Madison, WI 53706, and Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

Ketonization of 2-Hydroxymuconate, a Conjugated Enol, by 4-Oxalocrotonate Tautomerase, C. P. WHITMAN, College of Pharmacy, The University of Texas, Austin, TX 78712.

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