2. J. D. Morrison, Ed., *Asymmetric Synthesis* (Academic Press, New York, 1985), vols. 1 through 5.
3. M. Nogradi, *Stereoselective Synthesis* (VCH Verlag, Weinheim, West Germany, 1986).
4. G. M. Coppola and H. F. Schuster, *Asymmetric Synthesis, Construction of Chiral Molecules Using Amino Acids* (Wiley, New York, 1987); J. W. ApSimon and T. L. Collier, *Tetrahedron* **42**, 5157 (1986).
5. D. A. Evans, J. Bartroli, T. L. Shih, *J. Am. Chem. Soc.* **103**, 2127 (1981). For a more recent review, see D. A. Evans, in *Stereospecificity in Chemistry and Biochemistry*, vol. 27 of the *Proceedings of the Robert A. Welch Foundation Conferences on Chemical Research* (Robert A. Welch Foundation, Houston, 1984), pp. 13–49.
6. T. Imai *et al.*, *J. Am. Chem. Soc.* **108**, 7402 (1986).
7. A. I. Meyers, *Aldrichimica Acta* **18**, 59 (1985), and references cited therein.
8. Y. Gao *et al.*, *J. Am. Chem. Soc.* **109**, 5765 (1987), and references cited therein.
9. For recent reviews, see K. E. Koenig, *Asymmetric Synthesis* J. D. Morrison, Ed. (Academic Press, New York, 1985), vol. 5, pp. 71–161; J. M. Brown and P. A. Chaloner, in *Homogeneous Catalysis with Metal Phosphine Complexes*, L. H. Pignolet, Ed. (Plenum, New York, 1983), pp. 137–165.
10. I. A. Williams *et al.*, *J. Am. Chem. Soc.* **105**, 31 (1983); I. A. Williams, *ibid.* **109**, 6299 (1987); H. Yamataka, S. Nagase, T. Ando, T. Hanafusa, *ibid.* **108**, 601 (1986). This study failed to evaluate the impact of water on the transition structure in the addition of amines to aldehydes.
11. S. T. Bachrach and A. Streitwieser, Jr., *J. Am. Chem. Soc.* **108**, 3946 (1986).
12. For several reviews on the mechanism of the addition of Grignard reagents to ketones, see E. C. Ashby, J. Laemmle, H. M. Neumann, *Acc. Chem. Res.* 7, 272 (1974); E. C. Ashby, *Pure Appl. Chem.* **52**, 545 (1980).
13. E. C. Ashby and R. S. Smith, *J. Organomet. Chem.* **225**, 71 (1982).
14. H. M. Neumann, J. Laemmle, E. C. Ashby, *J. Am. Chem. Soc.* **95**, 2597 (1973).
15. E. C. Ashby, J. Laemmle, H. M. Neumann, *ibid.* **90**, 5179 (1968); E. C. Ashby and R. S. Smith, *J. Org. Chem.* **42**, 425 (1977).
16. E. A. Jeffery, T. Mole, J. K. Saunders, *Aust. J. Chem.* **21**, 649 (1968); E. A. Jeffery and T. Mole, *ibid.* **23**, 724 (1970).
17. S. Pasynkiewicz and E. Sliwa, *J. Organomet. Chem.* **3**, 121 (1965). These workers appear to be the first to propose the bimetallic six-centered transition state $\mathbf{T_9}$.
18. M. B. Marx, E. Henry-Basch, M. P. Freon, *C. R. Acad. Sci. Paris, Ser. C* **264**, 527 (1967); M. B. Marx, *ibid.* **266**, 1646 (1968). No transition structures were proposed in these studies.
19. S. Masamune, R. M. Kennedy, J. S. Peterson, *J. Am. Chem. Soc.* **108**, 7404 (1986). For a lucid discussion of the general applications of this chiral borane in asymmetric synthesis, see S. Masamune, in *Stereochemistry of Organic and Bioorganic Transformations*, W. Bartmann and K. B. Sharpless, Eds. (VCH Verlag, Weinheim, West Germany, 1987), pp. 49–72.
20. J. D. Morrison, Ed., *Asymmetric Synthesis* (Academic Press, New York, 1985), vol. 2, chaps. 2 and 3, and references cited therein.
21. ———, *ibid.*, chap. 3, pp. 87–89.
22. S. Itsuno, K. Ito, A. Hirao, S. Nakahama, *Chem. Commun.* **1983**, 469 (1983); S. Itsuno, A. Hirao, S. Nakahama, N. Yamazaki, *J. Chem. Soc. Perkin Trans. I* **1983**, 1673 (1983); S. Itsuno, K. Ito, A. Hirao, S. Nakahama, *J. Org. Chem.* **49**, 555 (1984); S. Itsuno *et al.*, *J. Chem. Soc. Perkin Trans. I* **1985**, 2615 (1985).
23. S. Itsuno, Y. Sakurai, K. Ito, A. Hirao, S. Nakahama, *Bull. Chem. Soc. Jpn.* **60**, 395 (1987).
24. E. J. Corey, R. K. Bakshi, S. Shibata, *J. Am. Chem. Soc.* **109**, 5551 (1987); ———, C.-P. Chen, V. K. Singh, *ibid.*, p. 7925.
25. This illustration was rendered with the Macintosh program Chem-3D written by S. Rubenstein (Cambridge Scientific Computing, Inc., Cambridge, MA).
26. For two comprehensive reviews of this area through 1981, see G. Solladie, in (*2*), vol. 2, pp. 157–199; in (*3*), pp. 159–220.
27. N. Oguni and T. Omi, *Tetrahedron Lett.* **25**, 2823 (1984).
28. E. J. Corey and F. Hannon, *ibid.* **28**, 5233 (1987); *ibid.*, p. 5237.
29. A. A. Smaardijk and H. Wynberg, *J. Org. Chem.* **52**, 135 (1987).
30. K. Soai, A. Ookawa, T. Kaba, K. Ogawa, *J. Am. Chem. Soc.* **109**, 7111 (1987).
31. S. Itsuno and J. M. J. Frechet, *J. Org. Chem.* **52**, 4142 (1987).
32. M. Kitamura, S. Suga, K. Kawai, R. Noyori, *J. Am. Chem. Soc.* **108**, 6071 (1986).
33. For an excellent entry into the literature dealing with the structures of Lewis acid–carbonyl complexes, see: M. T. Reetz, *et al.*, *ibid.* **108**, 2405 (1986).
34. J. G. Noltes and J. Boersma, *J. Organometallic Chem.* **1**, 425 (1968); H. M. M. Shearer and C. B. Spencer, *Acta Cryst. B* **36**, 2046 (1980).
35. K. Soai, S. Niwa, T. Yamada, H. Inoue, *Tetrahedron Lett.* **28**, 4841 (1987).
36. R. K. Dieter and M. Tokles, *J. Am. Chem. Soc.* **109**, 2040 (1987), and references cited therein; S. H. Bertz, G. Dabbagh, G. Sundararajan, *J. Org. Chem.* **51**, 4953 (1986), and references cited therein; F. Leyendecker and D. Laucher, *Nouv. J. Chim.* **9**, 13 (1985).
37. E. J. Corey, R. Naef, F. J. Hannon, *J. Am. Chem. Soc.* **108**, 7114 (1986).
38. C. Chapuis and J. Jurczak, *Helv. Chim. Acta* **70**, 436 (1987).
39. K. Narasaka, M. Inoue, N. Okada, *Chem. Lett.* **1986**, 1109 (1986); T. R. Kelly, A. Whiting, N. S. Chandrakumar, *J. Am. Chem. Soc.* **108**, 3510 (1986).
40. K. Maruoka, T. Itoh, T. Shirasaka, H. Yamamoto, *J. Am. Chem. Soc.* **110**, 310 (1988).
41. Y. Ito, M. Sawamura, T. Hayashi, *ibid.* **108**, 6405 (1986); *Tetrahedron Lett.* **28**, 6215 (1987).
42. The author thanks his co-workers G. Fu, K. Woerpel, M. DiMare, and J. Gage for their insightful critique of this manuscript. The National Science Foundation, the National Institutes of Health, Eli Lilly, and Merck are acknowledged for their sustained research support.

The Interplay Between Chemistry and Biology in the Design of Enzymatic Catalysts

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Chemists and biologists are focusing considerable effort on the development of efficient, highly selective catalysts for the synthesis or modification of complex molecules. Two approaches are described here, the generation of catalytic antibodies and hybrid enzymes, which exploit the binding and catalytic machinery of nature in catalyst design. Characterization of these systems is providing additional insight into the mechanisms of molecular recognition and catalysis which may, in turn, lead to the design of tailor-made catalysts for applications in chemistry, biology, and medicine.

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RECENT DEVELOPMENTS IN THE DESIGN OF HIGHLY SELECTIVE catalysts are having an important impact on chemistry, both in our ability to efficiently synthesize and modify molecules, as well as on our efforts to understand the molecular interactions involved in ligand binding and catalysis. Chemists are becoming increasingly proficient in the synthesis of selective catalysts that complex and transform small molecules or structural motifs. Chiral transition metal complexes are proving to be useful general catalysts in organic synthesis. Notable examples include titanium-tartrate catalysts for the epoxidation of allylic alcohols (*1*) and chiral rhodium hydrogenation catalysts (*2*). Cavity-containing hosts are being derivatized with nucleophilic groups and cofactors in an effort to construct catalysts that mimic and generalize enzyme-catalyzed reactions (*3*). For example, bifunctional crown ethers and

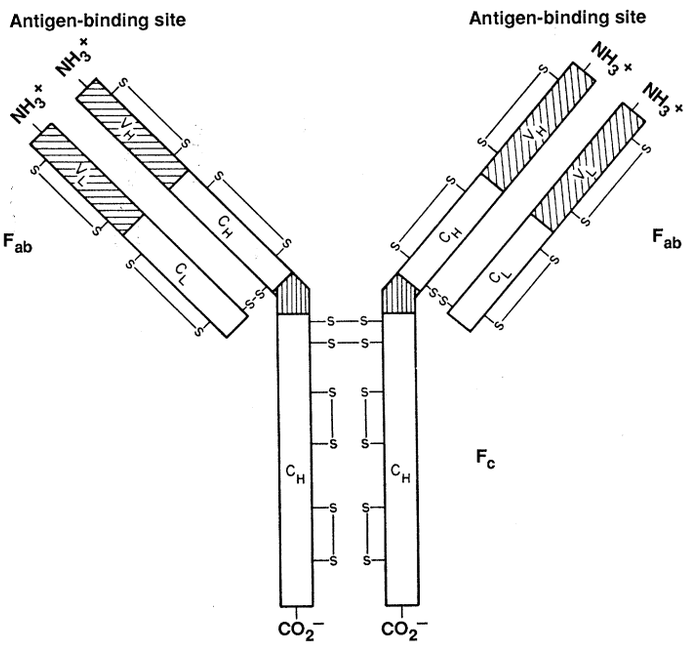

Fig. 1. Schematic representation of a human immunoglobulin. Subscripts H and L indicate heavy and light chains, respectively; V and C indicate variable and constant regions, respectively. Disulfide bonds are indicated (–S–S–) as are the Fab and Fc regions.

cyclodextrins have been developed that selectively hydrolyze and transaminate small substrates (*3*). Although catalysts of this sort have not yet proven generally useful in synthesis, they do have potential for isolating and studying mechanisms of recognition and catalysis. Enzymes themselves are being used with increasing frequency in organic synthesis (*4*).

However, as the size and complexity of substrates increase, our ability to generate tailor-made catalysts for rationally manipulating their structures becomes quite limited. The synthesis of such catalysts requires the generation of bifunctional molecules with binding sites capable of discriminating complex polyfunctional molecules as well as the appropriate chemical groups to carry out subsequent catalysis. To date, only limited success has been achieved in the rational design of receptors capable of selectively binding complex molecules. Most efforts have focused on modifying the specificity of existing enzymes by the method of oligonucleotide-directed mutagenesis (*5*). For example, the specificities of the proteolytic enzyme subtilisin (*6*) and the pyridoxal phosphate–dependent enzyme aspartate aminotransferase (*7*) have been successfully altered. Naturally occurring ligands and receptors have also been chemically modified with cofactors and redox-active metals to create selective catalysts. For example, peptides and oligonucleotides have been modified with EDTA·Fe(II) (*8*) to generate selective DNA-cleaving molecules, and papain has been modified with flavin to create nicotinamide-reducing catalysts (*9*).

The ability to design catalysts with predetermined specificites for reactions such as the selective cleavage, condensation, or modification of natural and synthetic polymers would be of great importance for drug design, molecular biology, and materials chemistry. The synthesis and characterization of these catalysts might provide additional insight into ligand receptor-recognition and catalysis. The purpose of this article is to describe two strategies being explored in our laboratory for the development of tailor-made catalysts: generation of catalytic antibodies and hybrid enzymes. Both approaches depend on an interplay between the principles and methods of chemistry and molecular biology.

Catalytic Antibodies

Key to the design of selective catalysts is the production of highly selective binding sites. With the advent of monoclonal antibodies (*10*) it has become possible to generate homogeneous ligand binding sites with enzyme-like affinities and specificities. Antibodies have been selectively generated against biopolymers such as nucleic acids, proteins, and polysaccharides; smaller multifunctional molecules such as steroids and prostaglandins; and synthetic polymers such as polypropylene. Antibodies bind ligands ranging in size from about 6 to 34 Å with association constants in the range of 10^4 to $10^{10} M^{-1}$ and as high as $10^{12} M^{-1}$ (*11*).

Antibody-ligand specificity has been well documented and is illustrated by the following examples: antibodies against *cis-N*-phenylmaleic acid monoamide bind the *trans* isomer with 10^3 lower affinity (*12*); antibodies generated against 3,17-androstanedione bind 3α,17-dihydroxyandrostane with 10^3 lower affinity (*13*); and antibodies against tetra-L-alanine bind glycine-(L-alanyl)$_3$ with 30-fold lower affinity (*14*). The ligand combining site of immunoglobulins (Igs) consists of six hypervariable regions, regions of extensive amino acid diversity, located in roughly the first 110 amino acids of the light (V_L) and heavy (V_H) polypeptide chains (Fig. 1) of the Ig (*15*). Combinatorial joining of the gene segments encoding the V_L and V_H genes and combinatorial association of different light and heavy chains generate a minimum of 10^8 different antibody molecules; mutations expand this baseline repertoire of receptors still further (*16*). This diversity has made antibodies one of the most important classes of receptors in biology and medicine today, with applications in diagnostics, drug delivery, and protein and nucleic acid purification and characterization. The development of viable strategies for introducing catalytic activity into antibody combining sites might, therefore, afford a general route to enzyme-like catalysts with tailored specificities.

A number of strategies can be envisioned for generating catalytic antibodies. Antibody specificity could be used to selectively stabilize transition state configurations or to overcome entropic barriers involved in orienting reaction partners. Catalytic groups, either amino acid residues or synthetic catalysts, might be introduced into antibody combining sites, or genetic selection might be used to generate catalytic antibodies. Our group's initial efforts to generate catalytic antibodies focused on the generation of antibodies specific for transition state analogs for hydrolytic reactions.

Transition state stabilization. An antibody generated to a haptenic group resembling the transition state configuration of a given reaction should lower the free energy of activation of the reaction by stabilizing the corresponding transition state relative to reactants or products (*17*). In fact, the active sites of many enzymes are complementary in structure and electronic distribution to rate-limiting transition states, as evidenced by studies of enzyme active sites (*18*) as well as enzyme inhibition by transition state analogs (*19*). We initially studied immunoglobulins specific for tetrahedral, charged phosphonate, and phosphate transition state analogs for the hydrolysis of carbonates and esters. We chose these well-characterized reactions in order to simplify mechanistic studies of ligand binding and catalysis. We have succeeded in characterizing antibodies that selectively catalyze two hydrolytic reactions: the hydrolysis of *p*-nitrophenyl *N,N,N*-trimethylammonioethyl carbonate **2** (*20, 21*) and the hydrolysis of methyl *p*-nitrophenyl carbonate **1** (*22*) (Fig. 2). Other investigators have generated a third catalytic antibody, against picolinic acid–containing phosphonate **6**, that hydrolyzes the analogous aryl ester **3** lacking the picolinic moiety (*23*).

A number of factors were considered in the design of this first generation of catalytic antibodies. Carbonates and esters undergo substantial structural and electronic change on the reaction pathway

from substrate to product, making possible differential binding and stabilization of the transition state (*24*). Moreover, the tetrahedral transition states for these hydrolytic reactions have been successfully mimicked with tetrahedral phosphonate, α-difluoroketone, and hydroxymethylene groups (*19*). Hydrolytic reactions do not necessarily require catalytically active side chains (such as a nucleophilic serine), simply attack of a binding site–accessible water molecule on the polarized carbonyl of the substrate. Additional considerations included the desirability of common recognition elements in both substrate and transition state, as well as diminished antibody affinity for the reaction products.

Since small molecules are not immunogenic unless coupled to a carrier molecule (*25*), methylene tethers were incorporated into the phosphonate transition state analogs in order to couple the haptens to the carrier proteins bovine serum albumin and keyhole limpet hemocyanin. The length of the spacer arm between the hapten and carrier was typically in the range of 6 to 8 Å, which should preclude any steric interference with carrier side chains. The haptens were coupled to the lysine side chains of the carrier proteins, and monoclonal antibodies were then elicited against the conjugates. Monoclonal antibodies, although more time-consuming to produce than polyclonal antibodies, offer the advantage of a reliable source of relatively large quantities of homogeneous Ig (in fact, we have been unsuccessful thus far in generating polyclonal catalytic antibodies) (*26*).

Monoclonal antibodies specific for tetrahedral transition state analogs **4** through **6** were found to selectively catalyze the hydrolysis of the corresponding substrates (S) to products (P) with kinetics consistent with the Michaelis-Menten rate expression 1 (*20–23*) (Table 1).

$$\mathrm{Ig} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{Ig{\cdot}S} \xrightarrow{k_{cat}} \mathrm{Ig} + \mathrm{P} \qquad (1)$$

The antibody-catalyzed reactions were inhibited by their corresponding transition state analogs. The inhibition constants (K_i's) for the transition state analogs are in every case substantially lower than the substrate Michaelis constants (K_m's), suggesting that transition state stabilization plays a role in catalysis. Antibodies specific for haptens **4** and **5** were found to be first order in hydroxide ion concentration. A comparison of the rates of the antibody-catalyzed reactions ($v_{Ig} = k_{Ig}[Ig{\cdot}S][OH]$) with the rates of hydroxide-dependent hydrolysis ($v_{OH} = k_{OH}[OH][S]$) afforded antibody rate accelerations in the range of 10^3 to 10^4. These accelerations are in the same range as those attributable both experimentally and theoretically to transition state stabilization in hydrolytic enzymes.

Another important feature of the antibody-catalyzed reactions is that they show high substrate specificity (*20–23*). For example, antibodies specific for phosphonate **4** did not catalyze the hydrolysis of methyl-2-nitrophenyl carbonate to any detectable extent, nor did antibodies specific for phosphonate **6** catalyze the hydrolysis of the corresponding acetamidophenylacetyl ester. Although at present the rates of these antibody-catalyzed reactions are low, they demonstrate the possibility of developing catalysts with binding sites tailored to the substrate of interest.

Biophysical studies are beginning to provide some insight into the mechanisms of the antibody-catalyzed reactions. Chemical modification experiments suggest that both tyrosine and arginine play a catalytic role in the antibodies specific for haptens **4** and **5** (*20–22*), whereas a histidine is thought to be involved in antibodies specific for **6** (*23*). Nuclear magnetic resonance (NMR) studies with transition state analogs containing nitroxide spin labels have identified positively charged and aromatic side chains in the combining sites of antibodies specific to hapten **4** (*27*). Electron spin resonance studies have demonstrated a high degree of steric complementarity between these spin labels and the antibody combining site (τ = 15

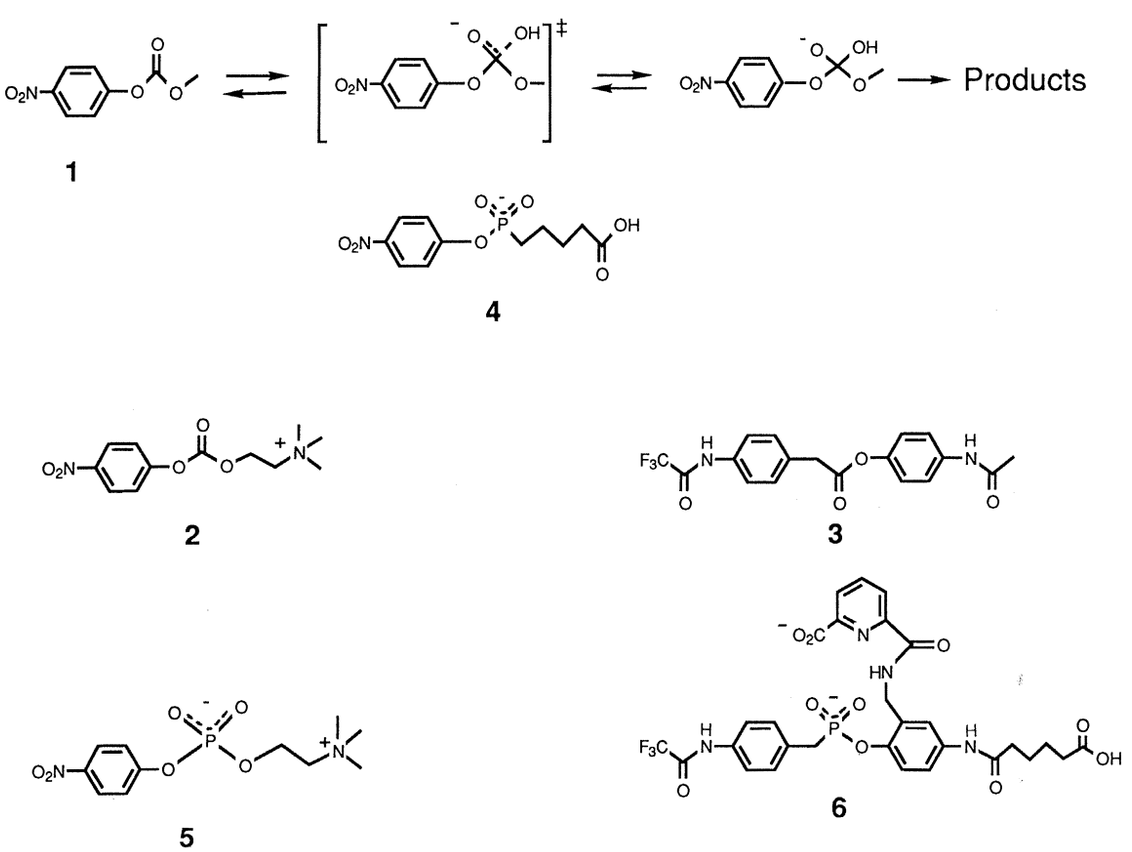

Fig. 2. Transition state analogs and the corresponding substrates (*20–23*).

Table 1. Catalytic parameters for antibody-catalyzed reactions.

Hapten	k_{cat} (min^{-1})	K_m (μM)	K_i (μM)	k_{lg}/k_{OH} ($\times 10^3$)
4	30	350	3	17.2
5 (T15)	0.32	708	55	9.2
(MOPC167)	0.4	208	5	12.0
6*	1.6	1.9	0.16	

*See (*23*).

nsec) and suggest that the depth of the combining site is between 9 and 12 Å (*28*).

The three-dimensional structure of the antibody McPC603, which is highly homologous to the phosphorylcholine-binding antibodies T15 and MOPC167, has been solved. This allows direct identification of the combining site residues (Fig. 3) (*29*). The hapten is bound in the cavity of McPC603, with the choline group deep in the interior and the phosphate toward the exterior, in contact with aqueous solvent. The heavy chain residues Tyr^{33}(H) and Arg^{52}(H), which are invariant in all of the phosphorylcholine-binding Igs sequenced to date (*30*), bind the phosphate via hydrogen bonding and electrostatic interactions with the phosphoryl oxygen atoms. NMR studies of McPC603 also suggest the partial formation of hydrogen bonds between the phosphate oxygens of phosphorylcholine and Tyr^{33}(H) and Arg^{52}(H) (*31*). The positive charge of the choline moiety is neutralized by the light chain residue Asp^{97}(L) and the invariant Glu^{35}(H), which are buried deep in the hapten binding cavity. In addition, there are van der Waals contacts between the hapten and Trp^{107a}(H).

The crystal structure clearly shows that the combining site of McPC603 is both sterically and electronically complementary to the tetrahedral, negatively charged phosphate moiety of phosphorylcholine. Inasmuch as this tetrahedral phosphate mimics the transition state for the hydroxide ion–catalyzed hydrolysis of **2**, the phosphorylcholine antibodies should be capable of polarizing the bound carbonate for attack of hydroxide ion in the rate-determining step. In light of the fact that the ground state structure of **2** differs substantially from the transition state configuration, the differential binding affinity of the Ig to these two species should be reflected in a lowered free energy of activation for reaction. In fact, the transition state analog 4-nitrophenyl phosphorylcholine is bound more tightly than the corresponding substrate by both MOPC167 and T15 (*20, 21*). However, the differential binding of T15 and MOPC167 to carbonate **2** and the transition state analog **5** does not fully account for the magnitude of the rate accelerations. Therefore, although the crystallographic and binding data strongly suggest that T15 and MOPC167 selectively stabilize the transition state configuration and thereby reduce the barrier to reaction, other factors are contributing to the rate acceleration. Stopped-flow experiments provide no evidence for a sudden burst of nitrophenolate ion release, ruling out attack by a nucleophilic combining site residue, such as Tyr^{33}(H), on the bound carbonate to generate a covalent carboalkoxy-antibody intermediate (*20, 21*). Moreover, there are no conserved residues such as a carboxylate or histidine that might act as a general base in T15 and MOPC167 (*30*). Mutagenesis studies on the combining site heavy chain residues of the Ig T15 (*32*) should help to define the roles these residues play in catalysis and will make it possible to selectively introduce histidine, aspartate, and cysteine residues to act as general bases and nucleophilic groups.

Antibodies elicited to phosphonate **6** were demonstrated by chemical modification studies to have an essential histidine in the combining site (*23*). This fact, combined with studies of altered substrates, led Tramontano *et al.* (*23*) to propose that histidine acts as a general base to activate combining site–accessible water. It is not clear at this point to what degree general base catalysis and transition state stabilization are responsible for catalysis in this system. If general base catalysis does prove to be important in the hydrolytic reaction, then rational strategies must be developed for generating antibodies with general bases or acids in the combining site. It should also be feasible to generate catalytic antibodies that will be based on other classes of transition state analogs, such as glycosidase, protease, ribonuclease, or deaminase inhibitors.

Proximity effects. What other strategies can be applied to the design of catalytic antibodies? If we again exploit the notions of enzymic catalysis, it should be possible to use antibody binding affinity to increase reaction rates by overcoming entropic barriers to reaction. The binding energy in this case might reduce translational and rotational motions for reaction by properly orienting the reactants in the antibody combining site. This approach should be applicable to intramolecular reactions such as macrocyclic lactonization reactions or intermolecular reactions such as amide bond forming or Diels-Alder reactions. In model studies, Bruice and Pandit (*33*) have demonstrated large increases in the rate of intramolecular anhydride formation from monoesters of dicarboxylic acids as rotational degrees of freedom in the substrate are reduced. Griffiths and Bender (*34*) have demonstrated that cyclodextrins can accelerate an intramolecular transesterification reaction by imposing orientational restriction on the reacting groups. Page and Jencks (*35*) have argued that each rotational degree of freedom frozen out in the ground state of an intramolecular process contributes a factor of 5 in rate acceleration.

We have used these ideas to guide us in the generation of antibodies that carry out the formal Claisen rearrangement of chorismic acid **7** to prephenic acid **8** (Fig. 4) (*36*). This thermal 3,3-sigmatropic rearrangement occurs through an asymmetric chair-like transition state **9** in which the carbon-oxygen bond is substantially broken while carbon-carbon bond formation has not occurred to any appreciable extent (*37–39*). The activation entropy and enthalpy of reaction have been determined to be −12.85 entropy units and 20.7 kcal/mol, respectively (*40*). The unimolecular rearrangement is catalyzed approximately 10^6-fold by the enzyme chorismate mutase at the branch point in the biosynthesis of aromatic amino acids in bacteria and plants (*41*). Although the enzymatic reaction has also been demonstrated to proceed through a chair-like transition state, the mechanism by which chorismate mutase accelerates the rearrangement remains poorly understood (*42*).

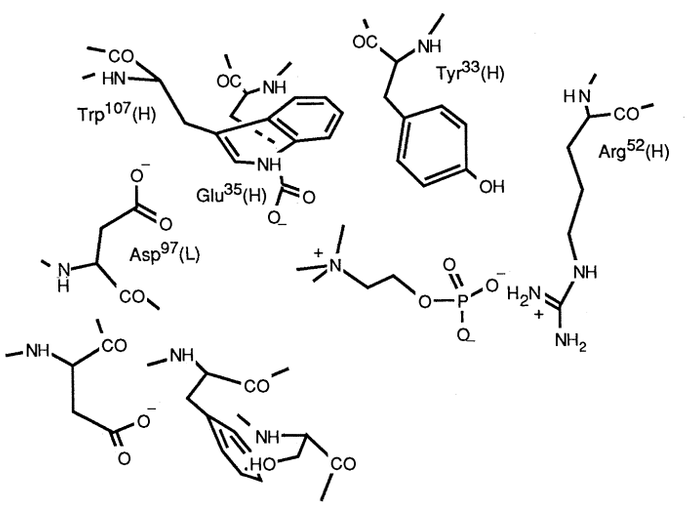

Fig. 3. Combining site of the phosphorylcholine binding antibody, McPC603 (*21*). [Reprinted from (*21*) with permission, copyright 1988 by Cold Spring Harbor Laboratory]

We have characterized the catalytic properties of antibodies specific to the chorismate mutase inhibitor **10** (*43*). To the extent that the bicyclic diacid **10** accurately simulates the conformationally restricted activated complex involved in the rearrangement of chorismic acid, antibodies specific for **10** should lower the entropic (and enthalpic) barrier to reaction. On the basis of the entropy of activation for the uncatalyzed reaction, one might expect a rate acceleration of 10^3 by an antibody that functions solely as an entropy trap (*40*).

An IgG has been identified that selectively catalyzes the Claisen rearrangement of chorismate to prephenate. The antibody-catalyzed reaction demonstrates saturation kinetics (k_{cat} = 2.7 min^{-1}, K_m = 260 μM; 10°C) and is inhibited by the bicyclic transition state analog **10** (K_i = 9 μM). The antibody accelerates the rearrangement by a factor of 10^4 (k_{cat}/k_{uncat}) over the thermal reaction. Characterization of the activation parameters and mechanism of this reaction will provide additional insight into the role rotational motion may play as a driving force in this antibody-catalyzed reaction.

Benkovic and co-workers (*44*) have demonstrated that an antibody elicited to transition state analog **12**, which is representative of a six-membered ring lactonization reaction, acts as a catalyst for the corresponding substrate (Fig. 5). The rate of the antibody-catalyzed reaction relative to the uncatalyzed reaction was 167. Again the antibody-catalyzed reaction was inhibited by the corresponding cyclic phosphonate. The antibody cyclization reaction was stereoselective, permitting isolation of the lactone in an enantiomeric excess of 94%. This finding also illustrates the high selectivity of antibody-ligand recognition and suggests that antibodies may be used to catalyze stereoselective chemical reactions.

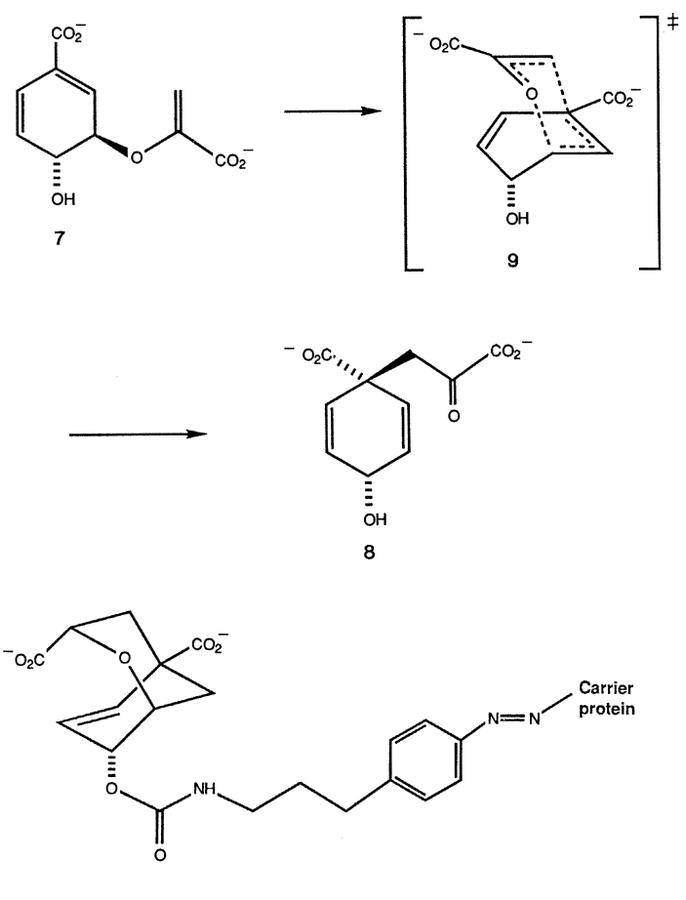

Fig. 4. Antibody-catalyzed Claisen rearrangement of chorismic acid to prephenic acid.

Although the unimolecular rearrangement and lactonization reactions described above are consistent with the idea that antibodies can reduce entropic barriers to reaction, much work remains to define the mechanisms by which these antibodies lower the free energy of activation for reactions. In addition, it will be of considerable interest to ask whether antibodies can efficiently catalyze bimolecular reactions such as selective peptide bond formation or Diels-Alder reactions. An important consideration in such reactions will be design of the appropriate haptens, so as to avoid significant product inhibition.

Introduction of catalytic groups. A third strategy whereby catalytic antibodies might be generated involves the site-specific introduction of catalytic residues into Ig combining sites. One might accomplish this by genetic methods, including site-directed mutagenesis and selection, by selective chemical modification, or by exploiting antibody-hapten complementarity. The last approach has been successfully applied to the generation of antibodies that catalyze the photocleavage of thymine dimers (*45*).

Thymine dimers are the major photolesions that result from irradiation of DNA with ultraviolet light. Organisms have evolved a number of systems to repair thymine dimers, including light-dependent photoreactivating enzymes (*46*). Although the mechanism of the enzymatic reaction remains poorly understood, it has been demonstrated in model systems that a number of sensitizers including indoles, quinones, and deazaflavins can photosensitize dimer cleavage (*47, 48*). One might, therefore, imagine that an antibody combining site, specific for thymine dimers and containing an appropriately positioned sensitizer, might act as a selective photoreactivating enzyme.

It has been amply demonstrated that many haptens containing a positively charged moiety induce a complementary negatively charged residue in the antibody combining site, and that haptens with polarized π systems induce a complementary aromatic residue in the combining site (*11*). The question arises whether we might expect antibodies generated against the π system of a *cis-syn* thymine dimer conjugate to contain a complementary tryptophan, a photosensitizer for dimer cleavage. We have isolated and characterized six monoclonal antibodies specific for a thymine dimer derivative, five of which efficiently cleave thymine dimers when irradiated with light at >300 nm (*45*). The antibody-catalyzed reaction is characterized by biphasic kinetics with a stoichiometric sudden burst of product release. The kinetics of the slow phase are consistent with the Michaelis-Menten rate expression 1. Mechanistic studies suggest the participation of a tryptophan residue in the cleavage reaction (antibodies that are not specific for thymine dimer, but that contain a combining site tryptophan, do not catalyze dimer cleavage).

Chemical modification might also be used to introduce synthetic catalytic groups, such as redox-active metals, nucleophilic groups, and cofactors into antibody combining sites. Relatively small hydrophilic and hydrophobic cavities have been derivatized with synthetic catalysts to produce enzyme mimics that accelerate hydrolytic and transamination reactions by specific substrate binding (*3*). More recently, Kaiser and co-workers have modified the binding site of the proteolytic enzyme papain with flavin analogs to create flavopapain (*9*). Sluka and co-workers (*49*) as well as Chen and Sigman (*50*) have modified small DNA-binding proteins with EDTA·Fe(II) and phenanthroline·Cu(II), respectively, to produce DNA-cleaving molecules. This same strategy, if applied to antibody combining sites, could afford semisynthetic catalytic antibodies with tailored specificities.

The key to this approach is the development of new mild methods for selectively modifying large, complex proteins with residues of unique reactivity (such as thiols) that can be subsequently deriva-

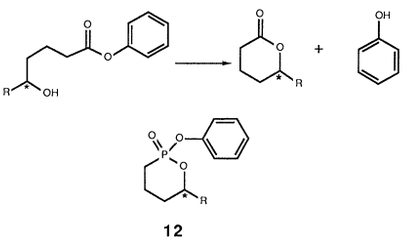

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Fig. 5. Antibody-catalyzed lactonization reaction with the corresponding transition state analog (*44*).

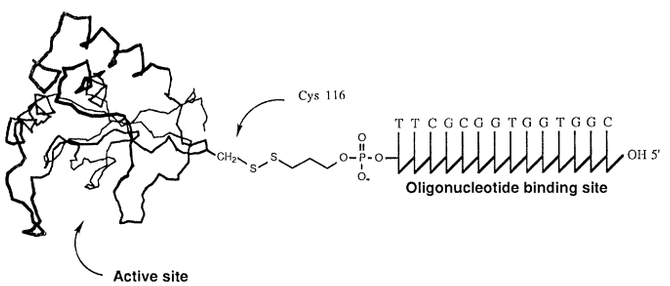

Fig. 6. Hybrid nuclease consisting of staphylococcal nuclease linked to an oligonucleotide binding site by a disulfide bond (*53, 54*). [Reprinted from (*54*) with permission, copyright 1988 by the American Chemical Society]

tized. We have recently developed a general chemical method for placing thiols selectively into the combining site of immunoglobulins based on the use of cleavable affinity-labeling agents (*50a*). It remains to be seen whether the thiol can be selectively derivatized with a synthetic catalytic group. Alternatively, site-directed mutagenesis might be used selectively to introduce a thiol into an antibody combining site. The development of efficient sytems for the expression of antibody binding regions in *Escherichia coli* would make this an attractive approach. In either case, the combination of antibody binding specificity with efficient synthetic catalysts might prove to be the most powerful approach toward the design of catalytic antibodies.

Hybrid Enzymes

We have described a number of efforts aimed at exploiting the specificity of natural receptors to generate novel catalysts with tailored specificities. It may also be possible to exploit nature's catalytic machinery in the design of new catalysts. One popular approach has been to redesign existing enzyme active sites by using the method of oligonucleotide-directed mutagenesis (*5*). Alternatively, it may be possible to add or replace entire binding or catalytic domains to generate hybrid enzymes with novel specificities (*51*). For example, selective fusion of peptide-specific binding domains to a catalytic domain that efficiently hydrolyzes amide bonds may produce sequence-specific peptidases; the selective fusion of nucleic acid–specific binding domains to a nonspecific phosphodiesterase may produce sequence-specific DNA- or RNA-cleaving enzymes. The recent report that the two functional domains of human and yeast phosphoglycerate kinase can be interchanged without impairing catalytic activity (*52*) is encouraging.

We chose to apply this strategy to the design of sequence-specific nucleases that cleave single-stranded RNA, single-stranded DNA, or duplex DNA at recognition sites of defined length and sequence (*53, 54*). A key requirement in the design of the catalyst was that the mechanism of cleavage involve hydrolysis of the phosphodiester bond, so that the termini of the resulting fragments could be enzymatically manipulated. Chemical catalysts capable of efficiently hydrolyzing the phosphodiester bonds of DNA do not yet exist. Moreover, there are no restriction enzyme equivalents for the selective hydrolysis of single-stranded RNAs and DNAs or for hydrolyzing large double-stranded DNAs (>100 kb) at unique sites. Such catalysts would be quite useful for studying nucleic acid structure and function as well as in mapping and sequencing.

The enzyme staphylococcal nuclease hydrolyzes the phosphodiester bonds of single-stranded RNA, single-stranded DNA, and duplex DNA relatively nonspecifically at regions rich in A-U or A-T, with rate accelerations of roughly 10^{15} above that of hydroxide ion (*55*). Selective fusion of an oligonucleotide binding domain, either single-stranded DNA or a DNA-binding protein, to this catalytic domain should generate a hybrid sequence-specific nuclease. Again, one faces the problem of selectively modifying and coupling large biopolymers with complex functional groups. The oligonucleotide binding site must be fused to staphylococcal nuclease such that the complexed substrate is functionally aligned with the catalytic residues. The coupling reaction must also be carried out with high selectivity and under mild reaction conditions. Staphylococcal nuclease is an ideal candidate for these studies; it is a well-characterized, stable enzyme consisting of a single polypeptide chain 149 amino acids in length (*55*). Enzymatic activity requires Ca^{2+}, which provides a mechanism for modulating the enzyme's action (*55*). The structure and mechanism of staphylococcal nuclease have been elucidated from a series of chemical, physical, and genetic studies. An x-ray crystal structure of a staphylococcal nuclease-diphosphothymidine (pTp)–Ca^{2+} complex has been determined to 1.5 Å (*56*). The pyrimidine ring of the inhibitor pTp fits into a hydrophobic pocket at the enzyme surface and the 5′-phosphate is near Arg^{35} and Arg^{87}. Glu^{43} is thought to act as a general base for activation of the attacking water molecule, whereas Arg^{35}, Arg^{87}, and Ca^{2+} stabilize the trigonal bipyramidal transition state configuration (*56, 57*).

We initially chose as our binding domain an oligodeoxynucleotide of defined sequence since oligodeoxynucleotides bind to single-stranded DNA or RNA with high specificity and affinity based on the Watson-Crick base-pairing interactions (Fig. 6) (*53, 54*). Moreover, oligodeoxynucleotides can be generated rapidly by solid-phase chemical methods, providing a large number of potential specificities. The three-dimensional structure of staphylococcal nuclease suggested that coupling of the 3′ terminus of the oligonucleotide binding domain to Lys^{116} or Val^{114} on the enzyme surface should align a hybridized substrate with the active site residues. The oligonucleotide was coupled to the enzyme via a disulfide linkage for two reasons: disulfide exchange reactions (i) proceed in high yield under mild conditions and (ii) can be carried out with high selectivity as a result of the absence of competing free thiols in DNA or staphylococcal nuclease. This strategy required the selective introduction of a free thiol at the 3′ terminus of the oligonucleotide and either residue 114 or 116 of the enzyme.

The 3′ terminal thiol of the oligonucleotide was introduced in the form of a disulfide linkage between the 3′ nucleotide and solid-phase support prior to the first step of solid-phase phosphoramidite synthesis (*58*). A flexible tether was incorporated to allow variability in the geometry of the hybridized substrate. After synthesis, deprotection of the oligomer, and cleavage of the disulfide, the free thiol was converted to 3′-*S*-thiopyridyl disulfide. A unique thiol was introduced into staphylococcal nuclease by replacement of Lys^{116} or Val^{114} with Cys by oligonucleotide-directed mutagenesis (*53, 54*). The Cys^{116} and Cys^{114} mutants obtained were found to have the same k_{cat} and K_m as the native enzyme, lacking any cysteines. The reduced Cys^{116} and Cys^{114} enzymes were cross-linked to the 3′-*S*-thiopyridyl oligonucleotides to produce the hybrid enzyme in high yield after cation-exchange chromatography.

The specificity of the hybrid enzymes was assayed by using ^{32}P

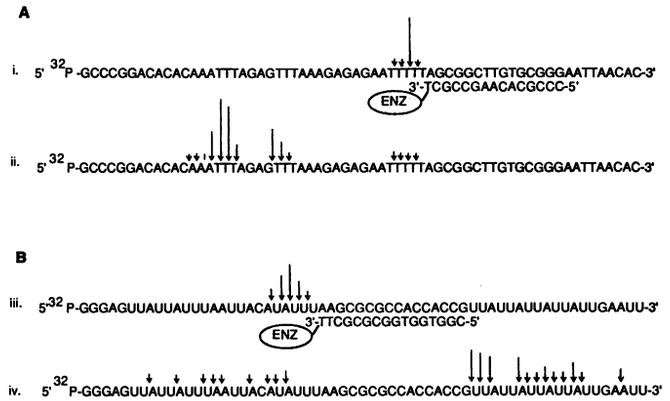

Fig. 7. Histogram of the cleavage of (**A**) single-stranded DNA and (**B**) RNA by hybrid nuclease. Arrows above sequence represent degree of phosphodiester bond cleavage; sequences i and iii show cleavage by the hybrid enzyme; sequences ii and iv show cleavage by native staphylococcal nuclease (*53, 54*). [(A) is reprinted from (*53*) with permission, copyright 1987 by the AAAS]

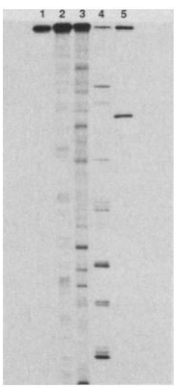

Fig. 8. Autoradiogram of a 6% denaturing polyacrylamide gel. Lane 1, M1 RNA 5′ end-labeled with ^{32}P; lane 2, ribonuclease T1 partial digest (G specific); lane 3, OH^- digest; lane 4, partial staphylococcal nuclease (K116 to C116) digest; lane 5, hybrid enzyme digest. The sequence of the oligonucleotide binding site is 5′-GGAGTTTACCGTGCCACGGACT-3′.

end-labeled single-stranded DNAs and RNAs (prepared by runoff transcription) of defined length and sequence (*53, 54*). The hybrid enzymes site-specifically cleaved the target nucleic acids adjacent to the oligonucleotide binding site (Fig. 7). In contrast, the underivatized mutant enzymes cleaved relatively nonspecifically at sites rich in A-T or A-U. In some cases, cleavage with the hybrid enzyme occurred at more than one phosphodiester bond contiguous to the combining site, most likely as a result of the flexible tether.

Can these hybrid enzymes site-specifically cleave larger natural RNAs or DNAs that contain significant secondary and tertiary structure? To answer this question, a hybrid enzyme was generated with a 22-nucleotide binding site that hybridizes to a hairpin loop in M1 RNA, the 377-nucleotide catalytic RNA subunit of *Escherichia coli*–ribonuclease P (*59*). Inspection of the autoradiogram illustrated in Fig. 8 reveals that the hybrid does in fact cleave one predefined phosphodiester bond, $A^{214}pA^{215}$. Moreover, the cleavage reaction can be carried out at room temperature after hybridization of the enzyme to M1 RNA at elevated temperature or directly at 75°C by adding Ca^{2+} and the hybrid enzyme to the M1 RNA (*60*).

The hybrid enzymes constructed to date, which consist of oligonucleotides 14 to 25 nucleotides in length, can be used to stoichiometrically cleave the phosphodiester bonds of single-stranded nucleic acids. However, because the hybrid enzyme binds and cleaves target sequences considerably faster than nonspecific sites, the use of shorter, nonhydrolyzable oligonucleotides with lower melting temperatures should lead to turnover. Moreover, fusion of domains that bind double-stranded DNA with staphylococcal nuclease could lead to hybrid enzymes capable of cleaving large genomic DNA. At the same time, mutagenesis of the amino acid residues in the nucleotide binding domain of the enzyme should make possible cleavage contiguous to G and C as well as T and A.

Prospectus

Two general strategies have been described for the design of selective catalysts: catalytic antibodies and hybrid enzymes. These approaches have been successfully applied to the generation of catalysts for acyl and phosphoryl transfer reactions as well as carbon-carbon bond forming and breaking reactions. Characterization of these systems has provided additional insight into the molecular mechanisms of recognition and catalysis. The challenge that now faces us is to extend these ideas to the design of catalysts for other reactions of general interest in chemistry, biology, and medicine. The realization of this goal will depend on an increasing interplay between chemistry and biology.

REFERENCES AND NOTES

1. K. B. Sharpless, S. S. Woodward, M. G. Finn, in *Selectivity—a Goal for Synthetic Efficiency*, W. Bartmann and B. M. Trost, Eds. (Verlag Chemie, Weinheim, Federal Republic of Germany, 1984), pp. 377–390.
2. R. Noyori *et al.*, *J. Am. Chem. Soc.* **109**, 5856 (1987).
3. R. Breslow, *Science* **218**, 532 (1982); J. Lehn, *ibid.* **227**, 849 (1985); D. J. Cram, *ibid.* **219**, 1177 (1983); see also *International Symposium on Bioorganic Chemistry*, R. Breslow, Ed. *Ann. N.Y. Acad. Sci.* **471** (1987).
4. G. M. Whitesides and C. H. Wong, *Angew. Chem. Int. Ed. Engl.* **24**, 617 (1985).
5. J. R. Knowles, *Science* **236**, 1252 (1987).
6. D. A. Estell *et al.*, *ibid.* **233**, 659 (1986).
7. C. N. Cronin, B. A. Malcolm, J. F. Kirsch, *J. Am. Chem. Soc.* **109**, 2222 (1987).
8. P. B. Dervan, *Science* **232**, 464 (1986); H. E. Moser and P. B. Dervan, *ibid.* **238**, 645 (1987).
9. E. T. Kaiser and D. S. Lawrence, *ibid.* **226**, 505 (1984).
10. G. Kohler and C. Milstein, *Nature (London)* **256**, 495 (1975); F. Seiler *et al.*, *Angew. Chem. Int. Ed. Engl.* **24**, 139 (1985).
11. D. Pressman and A. Grossberg, *The Structural Basis of Antibody Specificity*, (Benjamin, New York, 1968); J. W. Goodman, in *The Antigens*, M. Sela, Ed. (Academic Press, New York, 1975), vol. 3, pp. 127–187; A. Nisonoff, J. Hopper, S. Spring, *The Antibody Molecule* (Academic Press, New York, 1975).
12. K. Landsteiner and J. van der Scheer, *J. Exp. Med.* **59**, 75 (1934).
13. L. Milewich, C. G. Sanchez, P. C. MacDonald, P. K. Siiteri, *J. Steroid Biochem.* **6**, 1381 (1975).
14. I. Schechter, *Nature (London)* **228**, 639 (1970).
15. L. Amzel and R. Poljak, *Annu. Rev. Biochem.* **48**, 961 (1979).
16. S. Tonegawa, *Nature (London)* **302**, 575 (1983).
17. L. Pauling, *Am. Sci.* **36**, 51 (1948).
18. T. J. Borgford, T. E. Gray, N. J. Brand, A. R. Fersht, *Biochemistry* **26**, 7246 (1987); C. C. Blake *et al.*, *Proc. R. Soc. London* **167**, 378 (1967).
19. R. Wolfenden, *Annu. Rev. Biophys. Bioeng.* **5**, 271 (1976); P. Bartlett and C. Marlowe, *Biochemistry* **22**, 4168 (1983).
20. S. J. Pollack, J. W. Jacobs, P. G. Schultz, *Science* **234**, 1570 (1986).
21. S. J. Pollack and P. G. Schultz, *Cold Spring Harbor Symp. Quant. Biol.*, in press.
22. J. Jacobs, P. G. Schultz, R. Sugasawara, M. Powell, *J. Am. Chem. Soc.* **109**, 2174 (1987); J. Jacobs and P. G. Schultz, *Mol. Struct. Energetics*, in press.
23. A. Tramontano, K. D. Janda, R. A. Lerner, *Science* **234**, 1566 (1986).
24. W. Jencks, *Catalysis in Chemistry and Enzymology* (McGraw-Hill, New York, 1969).
25. B. Erlanger, *Methods Enzymol.* **70**, 85 (1980).
26. J. W. Goding, *Monoclonal Antibodies: Principles and Practice* (Academic Press, New York, 1986).
27. P. G. Schultz and D. Wemmer, unpublished results.
28. P. G. Schultz and D. Clark, in preparation.
29. D. Segal *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4298 (1974); Y. Satow, G. H. Cohen, E. A. Padlan, D. R. Davies, *J. Mol. Biol.* **190**, 593 (1986).
30. P. J. Gearhart, N. D. Johnson, R. Douglas, L. Hood, *Nature (London)* **291**, 29 (1981).
31. A. Goetz and J. H. Richards, *Biochemistry* **17**, 1733 (1978).
32. R. Perlmutter *et al.*, *Adv. Immunol.* **35**, 1 (1984).
33. T. C. Bruice and U. K. Pandit, *Proc. Natl. Acad. Sci. U.S.A.* **46**, 402 (1960).
34. D. W. Griffiths and M. L. Bender, *J. Am. Chem. Soc.* **95**, 1679 (1973).
35. M. I. Page and W. P. Jencks, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1678 (1971).
36. D. Jackson, S. Reich, R. Sugasawara, J. Jacobs, P. Bartlett, P. G. Schultz, unpublished results.
37. S. G. Sogo *et al.*, *J. Am. Chem. Soc.* **106**, 2701 (1984).
38. S. D. Copley and J. R. Knowles, *ibid.* **107**, 5306 (1985).
39. L. Addadi, E. K. Jaffe, J. R. Knowles, *Biochemistry* **22**, 4494 (1983).
40. P. R. Andrews, G. D. Smith, I. G. Young, *ibid.* **12**, 3492 (1973).
41. E. Halsam, *The Shikimate Pathway* (Wiley, New York, 1974).
42. W. J. Guilford, S. D. Copley, J. R. Knowles, *J. Am. Chem. Soc.* **109**, 5013 (1987).

43. P. A. Bartlett and C. R. Johnson, *J. Am. Chem. Soc.* **107**, 7792 (1985).
44. A. D. Napper, S. J. Benkovic, A. Tramontano, R. A. Lerner, *Science* **237**, 1042 (1987).
45. A. Cochran, R. Sugasawara, P. G. Schultz, unpublished results.
46. B. M. Sutherland, *Enzymes* **24**, 481 (1981).
47. S. E. Rokita and C. T. Walsh, *J. Am. Chem. Soc.* **106**, 4589 (1984).
48. C. Helene and M. Charlier, *Photochem. Photobiol.* **25**, 429 (1977).
49. J. P. Sluka, S. J. Horvath, M. F. Bruist, M. I. Simon, P. B. Dervan, *Science* **238**, 1129 (1987).
50. C. B. Chen and D. S. Sigman, *ibid.* **237**, 1197 (1987).
50a. S. J. Pollack and P. G. Schultz, unpublished observations.
51. The catalytic activity of enzymes has been selectively localized by the construction of chimeric and covalently modified antibodies. For example, see M. S. Neuberger *et al.*, *Nature (London)* **314**, 268 (1985).
52. M. T. Mas, C. Y. Chen, R. A. Hitzeman, A. D. Riggs, *Science* **233**, 788 (1986).
53. D. R. Corey and P. G. Schultz, *ibid.* **238**, 1401 (1987).
54. R. N. Zuckermann, D. R. Corey, P. G. Schultz, *J. Am. Chem. Soc.* **110**, 1614 (1988).
55. P. Cuatrecasas, S. Fuchs, C. Anfinsen, *J. Biol. Chem.* **242**, 1541 (1967); P. W. Tucker, F. Cotton, E. Hazen, *Mol. Cell. Biochem.* **22**, 67 (1978); W. Hörz and W. Altenburger, *Nucleic Acids Res.* **9**, 2643 (1981).
56. P. W. Tucker, F. Cotton, E. Hazen, *Mol. Cell. Biochem.* **23**, 3 (1979).
57. E. H. Serpersu, D. Shortle, A. S. Mildvan, *Biochemistry* **26**, 1289 (1987).
58. R. Zuckermann, D. Corey, P. G. Schultz, *Nucleic Acids Res.* **15**, 4403 (1987).
59. W. H. McClain, C. Guerrier-Takada, S. Altman, *Science* **238**, 527 (1987).
60. R. N. Zuckermann and P. G. Schultz, manuscript in preparation.
61. This work was supported in part by grants from the National Institutes of Health (IRO1AI24695-01), the Office of Naval Research (N00014-87-K-0256), and gifts from E. I. Du Pont de Nemours and Co. and Merck, Sharp and Dohme. We thank R. Sugasawara of IGEN, Rockville, MD, for preparation of antibodies.

Probing Structure-Function Relations in Heme-Containing Oxygenases and Peroxidases

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Structural factors that influence functional properties are examined in the case of four heme enzymes: cytochrome P-450, chloroperoxidase, horseradish peroxidase, and secondary amine mono-oxygenase. The identity of the axial ligand, the nature of the heme environment, and the steric accessibility of the heme iron and heme edge combine to play major roles in determining the reactivity of each enzyme. The importance of synthetic porphyrin models in understanding the properties of the protein-free metal center is emphasized. The conclusions described herein have been derived from studies at the interface between biological and inorganic chemistry.

NATURE USES METAL IONS EXTENSIVELY IN FUNCTIONAL AS well as structural roles. As biological catalysts, metal ions are of crucial importance in electron transfer reactions and in the activation and transport of small molecules such as dioxygen (O_2). Two major factors control the properties of metal ions in biological systems: (i) the structure of the metal, including the geometry of the complex and the nature of the ligands attached to the metal and (ii) the environment of the metal complex, including the polarity of the immediate surroundings and steric constraints on the accessibility of substrates to the metal and of the metal to the solvent. It was once thought that most, if not all, metal centers in enzymes had anomalous properties relative to isolated abiological metal complexes and were therefore in "entatic" or tensed states "poised for catalytic action" (*1*). Most metal complexes in proteins have structures that can be accurately reproduced in small molecule analogs outside the protein (*2*). Nonetheless, the protein environment clearly plays a crucial role in controlling the reactivity of the metal, and in some cases the protein can force metal ions into unusual geometries; the protein environment may be the determining factor controlling the activity of the increasing number of functionally distinct metalloproteins that have essentially identical metal centers. For example, cytochrome P-450 and chloroperoxidase have identical thiolate-ligated heme iron active sites, but P-450 is a mono-oxygenase, whereas chloroperoxidase halogenates substrates. Obviously, the final catalytic activity of a metal-containing system depends both on the structure of the metal and on the influence of the protein environment.

The importance of copper, iron, and zinc in biological systems has been evident for more than 50 years (*3*). The frequent requirement for other metals including cobalt, manganese, molybdenum, nickel, and vanadium in biochemical processes has been recognized (*4*). Even selenium has been found to be essential for the function of certain enzymes (*5*). In the late 1960s many inorganic chemists shifted from studying "pure" inorganic chemistry to investigating the biochemistry of metals. No longer were metalloproteins just proteins that happened to contain a metal. Instead, the metal became the focus of attention, the "built-in" probe.

In this article I address the question of how structure influences function in a system where enough is known about structure to begin to get answers to the questions. I examine results for four heme enzymes, cytochrome P-450, chloroperoxidase, horseradish peroxidase, and secondary amine mono-oxygenase, which have a number of common metal coordination structural features but different functional properties. The breadth of the field and lack of space prevent discussion of several "hot" bio-inorganic subjects such as metal-DNA interactions (*6*); the recent discovery of vanadium-containing enzymes (*7*); the involvement of nickel (*8*), manganese (*9*), and molybdenum (*10*) in the mechanisms of action of a growing number of metalloenzymes; and the role of copper (*11*) and nonheme iron in O_2 binding and activation (*12*), to name just a few.

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