From Molecular Diversity to Catalysis: Lessons from the Immune System

Peter G. Schultz and Richard A. Lerner

By combining the enormous molecular diversity of the immune system with basic mechanistic principles of chemistry, one can produce catalytic antibodies that allow control of reactions in ways heretofore not possible. Mechanistic and structural studies of these antibodies are also providing insights into important aspects of enzymatic catalysis and the evolution of catalytic function. Moreover, the ability to rationally direct the immune response to generate selective catalysts for reactions ranging from pericyclic and redox reactions to cationic rearrangement reactions underscores the chemical potential of this and other large combinatorial libraries.

The rational design of selective receptors and catalysts remains a challenge for chemists and biochemists alike. In contrast, nature has produced enzymes that catalyze a huge array of reactions with exquisite selectivity and antibodies that bind virtually any molecule with high affinity. Nature has solved many of the complex problems associated with molecular recognition and catalysis by generating and screening large libraries of proteins, either on an evolutionary time scale or over the course of a few weeks during the immune response. Can the principles and tools of chemistry be used to guide these complex biological processes to generate new molecules with novel functions?

One of the first examples in which the chemical potential of large libraries was exploited was the use of transition-state theory to select catalysts from among the large population of antibody molecules (*1*). Since these early experiments, which involved relatively simple transformations, the reactions catalyzed by antibodies have increased in complexity and degree of difficulty (*2*). At the same time, the strategies for generating antibody catalysts have become increasingly sophisticated. Recent structural and mechanistic studies are showing that the chemical notions used to generate catalytic antibodies are indeed reflected in their active site structures (*3–5*). Today, there is interest in screening libraries not only of antibodies but also of Fab fragments (*6*), RNAs (*7*), peptides (*8*), synthetic organic molecules (*9*), and even solid-state materials (*10*) for interesting properties and functions. This review sets out to illustrate the productive interplay of chemistry and biology in generating new chemical function from the immune system.

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Immunological Versus Natural Selection

Evolving catalytic activities. The immune system is capable of both generating tremendous molecular diversity, through the combinatorial association of variable (V), joining (J), and diversity (D) genes with subsequent affinity maturation, and rapidly screening this diversity for a desired specificity (*11*). There are many parallels between these processes and the natural evolution of enzymes. However, whereas antibodies are selected on the basis of affinity for stable antigens, enzymes generally evolve maximum affinity for high-energy, transient transition states (*12*). Consequently, in order to tap the catalytic potential of antibodies, the immune system must be provided with information relating to the rate-limiting transition state of a particular reaction. One strategy for accomplishing this involves the use of a stable analog of the transition state ($TS^{\ddagger}$) as the immunogen (*1*). This effectively directs immunological evolution along the same pathway as enzymic evolution; the end result is an antibody with catalytic activity.

Consider, for example, the enzyme ferrochelatase, which catalyzes the insertion of Fe^{2+} into protoporphyrin as part of the heme biosynthetic pathway (*13*). A potent inhibitor of this enzyme is the bent porphyrin *N*-methylprotoporphyrin (**1**) (*14*), which is thought to resemble the transition state for porphyrin metallation in which distortion of the macrocyclic ring system facilitates binding of metal ions. Consequently, antibodies generated against a bent *N*-alkylmesoporphyrin should act as a ferrochelatase. To test this notion, Cochran and Schultz generated monoclonal antibodies against the mesoporphyrin derivative **1** (Fig. 1) (*15*), resulting in the isolation of the catalytic antibody G1-A12. Both enzyme and antibody can insert a variety of divalent transition metals [for example, Zn(II), Co(II), Cu(II), and Mn(II)] into porphyrins with similar turnover numbers (k_{cat}). The calculated value of k_{cat} for ferrochelatase with Zn(II) is 800 $hour^{-1}$, compared with the experimentally determined k_{cat} value of 80 $hour^{-1}$ for the antibody (*15*). The reported Michaelis constants (K_m) for the enzyme range from 10 to 70 μM, compared with 50 μM determined for the antibody (Cu^{2+}), and both the enzyme and antibody have comparable affinities for *N*-methylporphyrin (*13–15*). These similarities between antibody and enzyme both support the hypothesis that the enzyme has evolved through natural selection to bind a distorted heme structure and provide evidence that the immune response can be similarly directed.

Perhaps the most ubiquitous and well-characterized class of enzyme-catalyzed reactions are the acyl transfer reactions. These reactions have been an attractive target for antibody catalysis (*16, 17*) and again allow us to assess the degree to which antibodies can recapitulate the properties of enzymes. Consider, for example, two different anti-

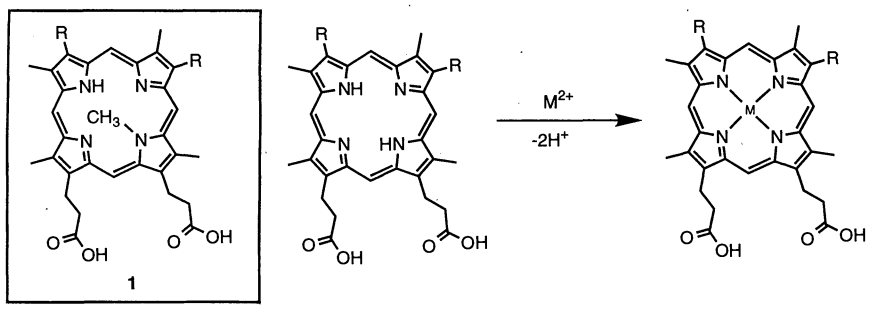

Fig. 1. Antibody-catalyzed porphyrin metallation reaction.

bodies that catalyze related transesterification reactions involving secondary alcohols (*18*, *19*). One antibody was raised against the corresponding phosphonate diester and the other against a phosphonate monoester transition state analog. Both antibodies were found to be remarkably efficient catalysts with effective molarities (the effective concentration of substrate in an uncatalyzed reaction that affords the same rate as the catalyzed reaction) on the order of 5×10^4 to 10^6 M (*18*, *19*). The efficiency and selectivity of these reactions are reflected by the fact that both antibodies exclude water from participating in the reaction, even though water is present in both cases at a concentration about 10^5 times that of the more hindered secondary alcohol, a characteristic of many enzyme-catalyzed reactions. As is also the case with many acyl transfer enzymes, such as the serine, aspartyl, and metalloproteases, the two antibodies function by means of different mechanisms. The differential binding affinity of one antibody to the phosphonate diester relative to substrates appears to account for a large fraction of the catalytic advantage, underscoring the importance of entropic factors in biological catalysis. In contrast, the second transesterification reaction proceeds through a ping-pong mechanism involving a covalent acyl-antibody intermediate (*18*). Moreover, the formation of the acyl intermediate appears to depend on an induced fit phenomenon. The above results suggest that these two catalytic antibodies can in fact be viewed as primitive enzymes following two distinct evolutionary pathways. Recently, the ideas used to generate these catalytic antibodies have been extended to the generation of antibodies that catalyze peptide bond formation (*20*, *21*), raising the intriguing possibility of using antibodies as tools for polypeptide synthesis.

Structural studies. Structural studies support the notion that catalytic antibody active sites evolve in response to the mechanistic information contained in the structure of the immunogen (*3*–*5*). Consider, for example, the antibody 17E8, which was raised to phosphonate **2** (Fig. 2) (*4*). This antibody catalyzes the hydrolysis of the corresponding formyl norleucine phenyl ester **3** with a k_{cat} of 223 min^{-1} and rate acceleration (k_{cat}/k_{uncat}) of 2.2×10^4. The x-ray crystal structures of the antibody–transition state analog complex and Michaelis complex (in which an amide analog of the substrate is bound) have been recently solved (*4*, *22*). These structures reveal a recognition pocket in the active site for specific binding of the side chain of the amino acid substrate as well as the light chain lysine residue (Lys^{97}), which likely stabilizes the negatively charged transition state, much like the oxyanion hole found in serine proteases. A comparison of the structures of the Michaelis complex and the transition state analog complex shows the increased binding interactions that occur as the reaction progresses toward the transition state configuration. The antibody active site has a Ser-His catalytic dyad proximal to the phosphorous of the bound hapten, resembling the Ser-His-Asp catalytic triad of the serine proteases. The positions of the antibody and enzyme side chains relative to the acyl group undergoing nucleophilic addition are virtually superimposable (Fig. 3), suggesting that the antibody resembles in many respects a primitive serine protease. Steady-state hydroxylamine partitioning experiments lend support to a catalytic mechanism involving rate-limiting formation of a covalent acyl-antibody intermediate. By introducing additional catalytic groups into the active site, as was done in the case of the phosphorylcholine-binding antibody S107, one might be able to further enhance the activity of this catalytic antibody (*23*, *24*).

A second hydrolytic antibody (48G7) has recently been crystallized, and the structure of the antibody-hapten complex has been determined to high resolution (2.0 Å) (*25*). This antibody was raised against a nitrophenyl phosphonate transition state analog and catalyzes the hydrolysis of the corresponding esters and carbonates. Both structural and mutagenesis data support the importance of transition state stabilization in the mechanism of this catalytic antibody. The genes encoding both antibody 48G7 and the germline precursor have been cloned, sequenced, and expressed. The equilibrium and kinetic binding constants for the transition state analog, as well as the K_m and k_{cat} values for a number of substrates, were determined for both the germline antibody and antibody 48G7, providing us a glimpse into the immunological evolution of this catalytic antibody. The germline antibody has an affinity for the TS‡ analog that is less than 10^{-4} that of antibody 48G7 and has significantly diminished catalytic activity in comparison to antibody 48G7. However, the mutations that occurred during affinity maturation, three in the light chain and six in the heavy chain, are not in the active site, indicating that secondary structural interactions can play a key role in the evolution of catalytic (and binding) function, perhaps by preorganizing a particular active site geometry. The structure of a third esterolytic antibody has also been solved and shows a number of structural similarities to antibodies 48G7 and 17E8 (*5*). However, a detailed analysis of this catalytic antibody awaits structural characterization of the hapten-antibody complex.

Another comparison between enzyme- and antibody-catalyzed reactions can be made in the case of the conversion of chorismic acid to prephenic acid catalyzed by the enzyme chorismate mutase. This 3,3-sigmatropic rearrangement, which is a key step in the biosynthesis of aromatic amino acids in plants and bacteria, proceeds through an asymmetric chairlike transition state (*26*). One might expect that an antibody-combining site complementary to the conformationally restricted transition state would accelerate this rearrangement, again by acting as an "entropy trap." Antibodies 1F7 and 2E11 elicited to derivatives of a bicyclic transition state inhibitor of chorismate mutase increase the rate of the rearrangement 10^2-fold (*27*) and 10^4-fold (*28*), respectively, over the uncatalyzed reaction, compared with the approximately 10^6-fold acceleration (k_{cat}/k_{uncat}) of the enzyme. Whereas antibody 2E11 (*28*) functions primarily by increasing the entropy of activa-

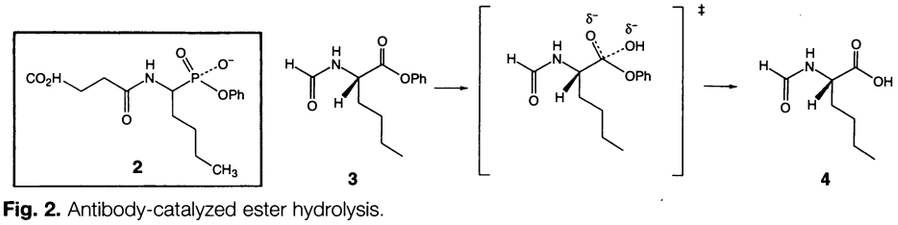

Fig. 2. Antibody-catalyzed ester hydrolysis.

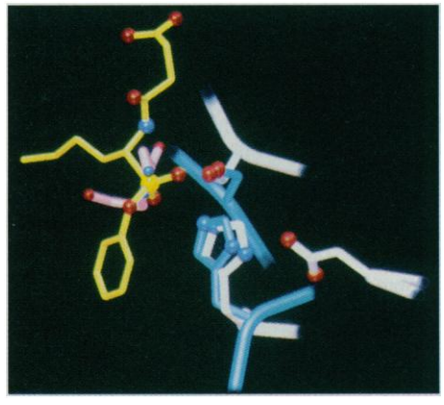

Fig. 3. The active site of antibody 17E8 superimposed on the active site of trypsin complexed with bovine pancreatic trypsin inhibitor (BPTI). The catalytic residues are on the right side with backbone and side chain atoms of trypsin in white and pink, respectively, and backbone and side chain atoms of 17E8 in yellow and red, respectively. The phosphonate hapten **2** is shown in yellow with the phosphorous atom in red (*4*).

tion $\Delta S^{\ddagger}$ for the reaction ($\Delta S^{\ddagger} = -1.2$ cal mol^{-1} K^{-1}, $\Delta H^{\ddagger} = 18.3$ kcal mol^{-1}), antibody 1F7 (*27*) primarily lowers the enthalpy of activation $\Delta H^{\ddagger}$ ($\Delta S^{\ddagger} = -22$ cal mol^{-1} K^{-1}, $\Delta H^{\ddagger} = 15$ kcal mol^{-1}), and the enzyme affects both ($\Delta S^{\ddagger} = 0.0$ cal mol^{-1} K^{-1}, $\Delta H^{\ddagger} = 15.9$ kcal mol^{-1}) (*26*).

The x-ray crystal structures of the enzyme and antibody 1F7 have been solved (*3*). The structural data suggest that the enzyme and the antibody stabilize the same conformationally restricted chairlike transition state. The overall shape and charge complementarity between the combining sites and the transition state analog result in binding of the correct enantiomer of chorismate in the conformation required for reaction. Differences in the number and nature of specific interactions available for restricting conformational entropy and stabilizing the highly polarized transition state may account for the observed lower (10^{-4} times) activity of the antibody 1F7 relative to that of the natural enzymes. Structural information on antibody 2E11, which is intermediate in activity between antibody 1F7 and the enzyme, should shed additional light on the evolution of this enzymatic activity.

The x-ray structures described above show that the properties of catalytic antibodies do indeed reflect the mechanistic information contained in the hapten structure. Other mechanistic studies also bear this out. For example, it has been shown that by raising antibodies to a positively charged hapten, one can generate an antibody with a complementary negatively charged glutamate residue in the combining site capable of efficiently catalyzing an elimination reaction of a related β-fluoroketone substrate (*29*). Recently, Hilvert and co-workers have exploited charge complementarity to generate very efficient catalytic antibodies that also operate by means of general base catalysis (*30*). An antibody specific for a positively charged 2-aminobenzimidazole was found to catalyze the decomposition of the corresponding benzisoxazole to give 2-cyanophenol with greater than 10^8-fold rate acceleration. In addition to demonstrating the large catalytic advantages that can be realized with antibodies, this experiment also begins to define the degree to which optimally positioned general bases and acids can contribute to enzymatic catalysis.

Naturally occurring catalytic antibodies. Although in each example described above the catalytic antibody evolved in response to a synthetic immunogen, recent reports suggest that the immune system can produce catalytic antibodies in the absence of such immunogens (*31*, *32*). For example, autoimmune antibodies isolated from patients with systemic lupus erythematosus were found to hydrolyze DNA with an apparent k_{cat} value of 14 min^{-1} and K_m value of 43 nM (*31*). These kinetic constants, which were obtained with highly purified Fab fragments, are remarkably close to those of restriction enzymes such as Eco RI. Unraveling the mechanism by which these antibodies are generated during the immune response could prove extremely useful in the generation of other effective antibody catalysts for biological and biomedical applications.

Evolving New Functions

Pericyclic reactions. Because the immune system responds rapidly to a given set of chemical instructions, one can attempt to evolve antibodies that catalyze reactions for which enzymes have yet to be found. Concerted pericyclic reactions, which include cycloaddition reactions, sigmatropic rearrangements, and electrocyclic ring closure reactions, represent one such opportunity. These reactions have not only received a greal deal of theoretical and mechanistic attention from chemists, they have also found many applications in organic synthesis (*33*). There is only one example of enzymic catalysis of what is formally a pericyclic rearrangement, the 3,3-sigmatropic rearrangement (Claisen rearrangement) catalyzed by chorismate mutase (*26*). However, the enzymatic reaction is largely asynchronous and may even involve a stepwise mechanism (*26*). Consequently, pericyclic reactions represent an ideal target to test the degree to which catalytic antibodies can be generated for reactions that are rare or do not occur in nature.

The first such reaction to be considered was the Diels-Alder reaction (*34*, *35*), which in its simplest form consists of the reaction between butadiene and ethylene to yield cyclohexene (*36*). The transition state involves a highly ordered cyclic array of interacting orbitals in which carbon-carbon bonds are broken and formed in a single concerted step. As a result of the stringent alignment of orbitals in the transition state, an unfavorable entropy of activation ($\Delta S^{\ddagger}$) on the order of -30 to -40 cal mol^{-1} K^{-1} is generally observed. The design of a hapten for this reaction should again exploit the notion of proximity effects as well as incorporate a mechanism for minimizing product inhibition. One general strategy for catalyzing the Diels-Alder reaction is illustrated by the antibody-catalyzed cycloaddition of the acyclic diene **5** to *N*-phenylmaleimide **6** to yield the cyclohexene product **7** (Fig. 4) (*35*). Hapten **8** is based on a bicyclo[2.2.2]octene skeleton in which the ethano bridge locks the cyclohexene ring into a conformation resembling the highly ordered transition state of the Diels-Alder reaction. Because the reaction product does not contain this hydrophobic bridge and has a conformation distinct from that of the hapten, it was expected to bind less tightly in the antibody-combining site, minimizing product inhibition. In fact, an antibody generated to hapten **8** catalyzed the formation of the Diels-Alder adduct with a k_{cat}/K_m value of 900 M^{-1} s^{-1} (diene); product was bound with a dissociation constant (K_D) of 10 μM, which can be compared with a K_D of 126 nM for the hapten.

Gouverneur and co-workers then asked whether a similar approach could be taken to generate antibodies that selectively catalyze formation of the Diels-Alder products resulting from either endo or exo attack of the dienophile on the diene (*37*). For reactions under kinetic control, the endo pathway is typically favored over the exo pathway as a result of secondary orbital interactions in the transition state. Consequently, antibody binding energy must be used to control the relative energy of transition states, in addition to the relative energies of the transition state and substrate. The reaction chosen for study was the cycloaddition between *trans*-1-*N*-acylamino-1,3-butadiene (**9**) and *N*,*N*-dimethylacrylamide (**10**) (Fig. 5). In the absence of a catalyst, the reaction proceeds under aqueous conditions to give an 85:15 mixture of the endo **11** to exo adduct **12**. A bicyclo[2.2.2]octene framework was again used to mimic the boatlike transition state for the pericyclic reaction. Hapten **13** mimics the endo approach in the transition state in which the amide group of the dienophile is oriented toward the π orbitals of the diene. Conversely, the exo transition state, in which the dienophile substituent is oriented away from the π system, is mimicked by hapten **14**. These two haptens were used to generate antibodies that catalyze the exclusive formation of either the endo or exo adducts (*37*). The effective molarities for the antibody-cata-

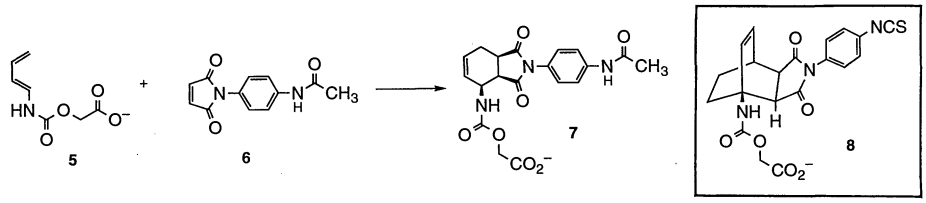

Fig. 4. Antibody-catalyzed Diels-Alder reactions.

lyzed reactions were 4.8 and 18 M, respectively, and in each case the degree of enantioselectivity was greater than 98%.

Another well-studied pericyclic reaction for which enzymic catalysis has yet to be demonstrated is the Cope rearrangement (*38*). To generate catalysts for this reaction, antibodies were raised against the diphenyl-substituted cyclohexane derivative **15**. This hapten is expected to mimic the six-membered ring transition state for this concerted rearrangement (Fig. 6) (*39*). Four antibodies were isolated that catalyze the oxy-Cope rearrangement of diene **16** to **17**, one with a k_{cat}/k_{uncat} value of 5300. An analysis of K_m, k_{cat}, and the inhibition constant K_i for the four antibodies suggests that hapten **15** does indeed induce catalysts by acting as a mimic of the cyclic transition state. These studies of the Diels-Alder and oxy-Cope reactions underscore the ability of the immune system, when guided by carefully defined mechanistic criteria, to evolve catalysts for a wide range of chemical transformations, some of which are heretofore unknown in nature.

Unnatural cofactors. Many enzymatic reactions depend on a small set of nonpeptidyl cofactors such as hemes, flavin, pyridoxal, and nicotinamide. Chemists have developed their own set of chemical auxillaries, including metal hydrides, transition metals, and Lewis acids, that perform similar functions. The large number and utility of the cofactors available to the chemist raises the question whether antibodies can be developed that use these chemical "cofactors" in addition to enzymatic cofactors for catalysis (*40*). One such example is the antibody-catalyzed oxygenation reaction of a sulfide to the corresponding sulfoxide (*41*). The monooxygenase enzymes responsible for this transformation use flavin or heme cofactors that typically require NADPH (NAD, nicotinamide adenine dinucleotide) for cofactor regeneration (*42*). Hsieh and co-workers (*41*) asked the question whether the inexpensive oxidant $NaIO_4$ could be used to carry out this reaction, eliminating the need for cofactor recycling, a significant barrier to the use of many enzymes in synthesis (Fig. 7).

Hammett σ-ρ studies, solvent isotope effects, and pH-dependence studies on sulfide oxidation by periodate suggest that the transition state for this reaction resembles that depicted in Fig. 7. Consequently, aminophosphonic acid **18** was designed to generate antibodies that catalyze the conversion of sulfide **19** to sulfoxide **20**. Because hapten **18** contains an amine that is protonated at physiological pH, antibodies specific for **18** were expected to stabilize the incipient positive charge on sulfur present in the transition state. A phosphonic acid moiety was introduced into the hapten to provide a binding site for the periodate ion. Antibody 28B4.2 was isolated, which catalyzes the $NaIO_4$-dependent oxidation of sulfide **19** with a k_{cat} value of 8.2 s^{-1}; no inactivation was observed as a result of antibody oxidation. The turnover number and catalytic efficiency of this antibody are comparable to those of the corresponding enzymes (*42*).

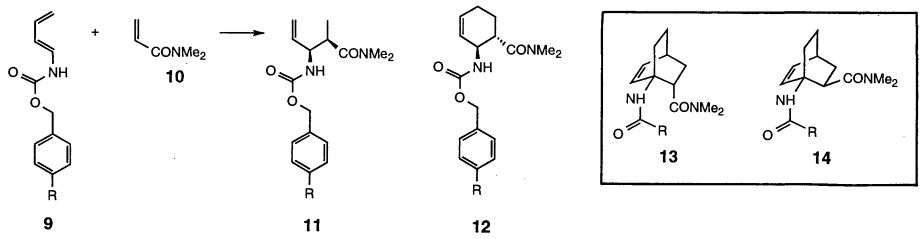

Fig. 5. Antibody catalysis of an exo Diels-Alder reaction.

A second example of antibody catalysts involving an "unnatural cofactor" is the antibody-catalyzed reduction of ketones to the corresponding alcohols (*43*, *44*). The enzymatic reduction of carbonyl groups to alcohols usually requires the cofactor NADH or NADPH. Chemical reductions, on the other hand, are usually carried out with inexpensive and versatile metal hydrides, such as $NaBH_4$ or $LiAlH_4$. In an effort to generate antibodies that catalyze the metal hydride–dependent reduction of ketone **21**, antibodies were generated against *N*-oxide **23** (Fig. 8). Antibodies raised against this hapten were expected to stabilize the tetrahedral transition state arising from nucleophilic attack of hydride on the carbonyl group, as well as provide a site to accommodate the reductant. The chiral active site of an antibody specific for one of the two enantiomers of hapten **23** should also discriminate between the enantiotopic faces of a prochiral substrate, affording high stereoselectivity. Antibody 37B.39.3 was found to catalyze the reduction of substrate **21** with a k_{cat}/K_m value of 1.9×10^3 M^{-1} min^{-1} (R = Et) (*44*). As expected, the antibody stabilized one of two possible enantiomeric transition states to give the *S* alcohol in 96% enantiomeric excess. The reduction of ketones containing branched and aryl substituents, including the highly symmetrical 1-nitrophenyl-3-phenyl-2-propanone, also showed high enantioselectivity. Moreover, the antibody catalyzed the reduction of diketone **24** regioselectively with greater than 75:1 selectivity for one of the two nearly equivalent ketone moieties and with high stereoselectivity, affording the *S* enantiomer of hydroxy ketone **25** in 96% enantiomeric excess and a 94% overall yield. In contrast, the uncatalyzed reaction afforded roughly a statistical mixture of eight products. This straightforward strategy for catalyzing carbonyl reduction may find general applicability to the regio- and stereoselective reduction of a broad range of compounds and may be useful for reactions not amenable to existing biological or chemical approaches. Moreover, this antibody-catalyzed reaction and others (*45*) illustrate the degree

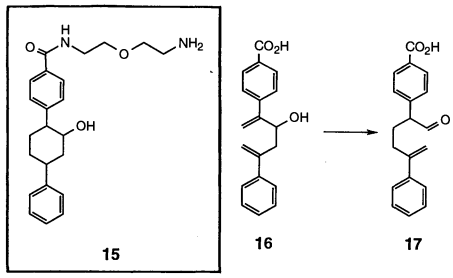

Fig. 6. Antibody-catalyzed oxy-Cope rearrangement.

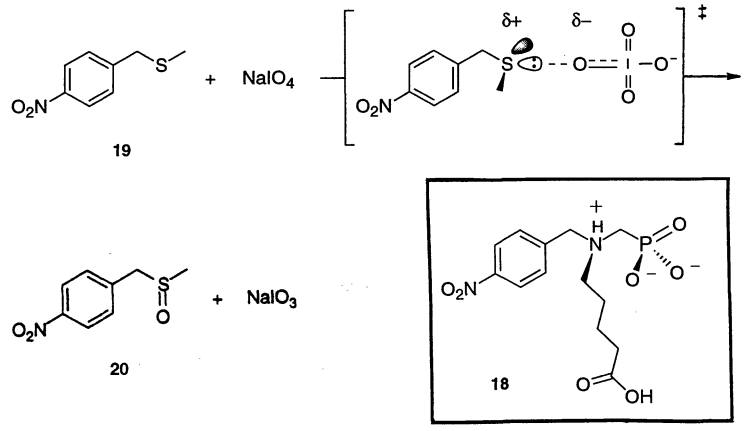

Fig. 7. Antibody-catalyzed, periodate-dependent oxygenation reaction.

to which antibodies can selectively bind and stabilize one of many nearly isoenergetic transition states on a reaction coordinate.

Other examples of antibodies that use novel cofactors include the peroxycarboximidic acid–dependent oxidation of unfunctionalized alkenes to yield epoxides (*46*). The enantioselectivity for this reaction was greater than 98% and exceeds that which can be achieved with the heme-dependent enzyme chloroperoxidase.

Difficult Chemical Transformations

Recently, the field of catalytic antibodies has begun to focus on catalytic transformations that are difficult to carry out using existing chemical methods. These include reactions that have been termed disfavored, that is, kinetically controlled reactions in which the products arise not from the lowest energy transition state (favored) but from a higher energy transition state (disfavored). In practice it has proven difficult to chemically discriminate and control the relative energies of these two transition states.

We have already seen that antibodies can modulate the relative energies of the transition states leading to the exo and endo products of a Diels-Alder reaction. Another disfavored reaction that has been successfully catalyzed by antibodies is the 6-endo-tet cyclization of the epoxyalcohol illustrated in Fig. 9 (*47*). This antibody-catalyzed reaction is formally a violation of Baldwin's "rules" for ring closure reactions (*48*), which state that the preferred product arising from the 180° transition state geometry of an intramolecular nucleophilic substitution reaction is the 5-exo-tet product. In order to catalyze 6-endo-tet cyclization, it was necessary to generate an antibody that not only lowers the energy barrier for epoxide ring opening but also overcomes the entropic barrier and strain necessary to bring the hydroxyl group into a geometry that favors a six-membered (disfavored) versus five-membered (favored) ring transition state geometry. It was anticipated that hapten **28** would generate a combining site that would stabilize both the developing charge in the breaking C–O bond and the six-membered ring geometry (*47*). The difference in dipole between the hapten and reaction product was expected to minimize product inhibition. Two antibodies generated against *N*-oxide **28** were found to catalyze the regioselective ring opening of epoxide **29** to form the six-membered ring product **31**. Comparison of the k_{cat} value with that of the uncatalyzed reaction was not possible because only the five-membered Baldwin ring closure product was formed in the absence of antibody. In addition, only the *S*,*S* epoxide was a substrate for antibody-catalyzed pyran ring formation.

Antibodies have also been generated that catalyze a disfavored syn elimination reaction of the acyclic substrate **32** to the (cis) Z olefin **33** (*49*) (Fig. 10). For acyclic systems, antiperiplanar elimination is generally favored over syn elimination (*50*). Moreover, all accounts of acyclic syn elimination provide a trans (*E*) olefin when the competing anti elimination suffers significant destabilizing steric interactions enroute to the alternative cis olefin. In fact, of the four possible elimination pathways (anti to trans, anti to cis, syn to trans, and syn to cis), syn elimination to a cis olefin is regarded as the least favored transformation (*50*). Consistent with these generalizations, substrate **32** undergoes anti elimination to give exclusively the trans olefin **34** in aqueous buffer. Hapten **35** was designed to generate antibodies that would bind and lock substrate **32** in an eclipsed conformation, such that subsequent

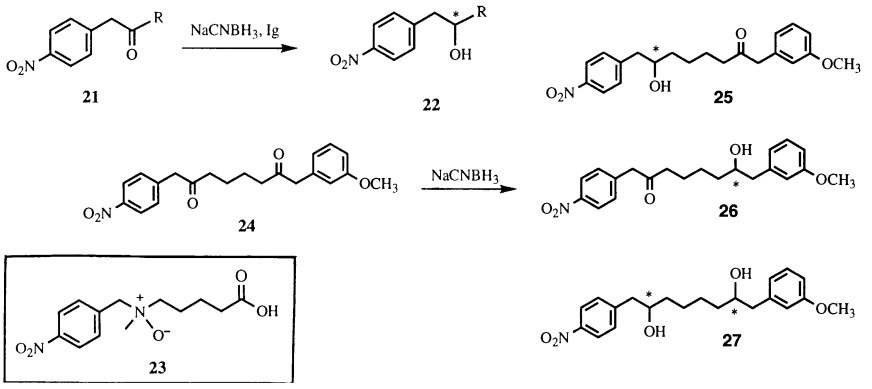

Fig. 8. Antibody-catalyzed, borohydride-dependent ketone reduction.

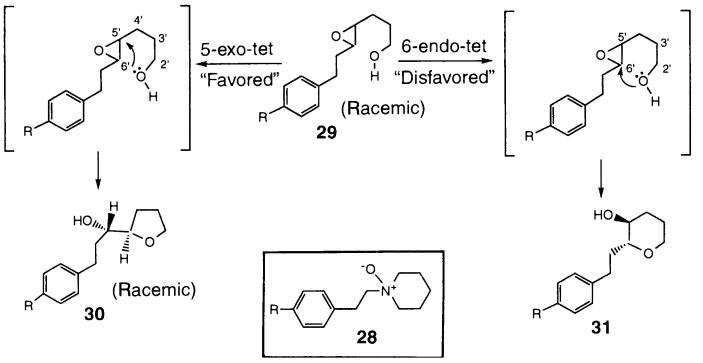

Fig. 9. Antibody catalysis of an anti-Baldwin cyclization reaction.

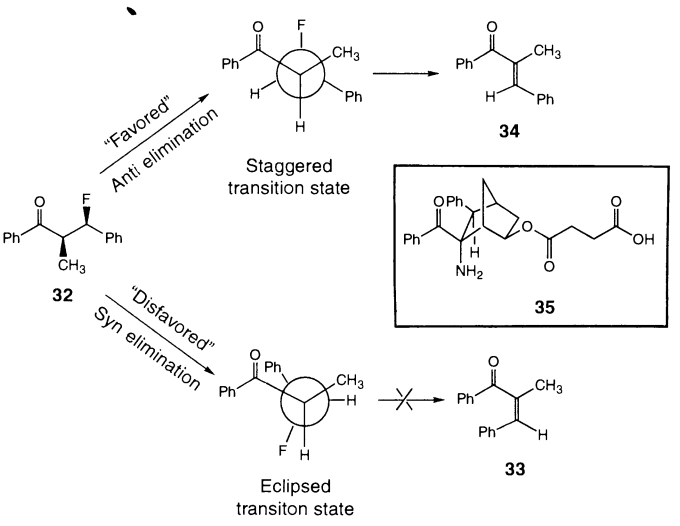

Fig. 10. Antibody catalysis of a disfavored elimination reaction.

elimination would occur syn to afford selectively the disfavored cis olefin **33** (Fig. 10). A primary amine was introduced in a position corresponding to the α-keto proton of substrate **32** to induce a general base in the antibody-binding pocket. An antibody specific for **35** was found that catalyzed exclusively the syn elimination of substrate **32** to the disfavored product **33**. Again, the rate acceleration (k_{cat}/k_{uncat}) for this antibody-catalyzed reaction could not be determined, because in the absence of antibody, formation of the cis product was immeasurably slow under the reaction conditions. An energy difference of up to a 5 kcal mol^{-1} has been estimated (*49*) between the free energy $\Delta G^{\ddagger}$ for the anti and syn elimination reactions of substrate **32** to products **34** and **33**. The complete reversal of reaction pathways as illustrated by these two antibody-catalyzed reactions suggests that even more difficult transformations, possibly even "disallowed" reactions, may be amenable to antibody catalysis.

Many other examples are appearing in which antibodies provide the chemist a high degree of control over the outcome of chemical reactions. Recently it was shown that antibodies can effectively exclude solvent from participating in reactions. Keinan and co-workers showed that an antibody generated against a quaternary ammonium ion hapten was able to catalyze the cyclization of a hydroxyethyl enol ether to the corresponding ketal in water with high enantiomeric purity (*51*). Ordinarily, ketal formation does not occur under aqueous conditions; the oxocarbonium ion intermediate is rapidly trapped by water to yield a hemiketal and ultimately a ketone. Antibodies have also been reported that control the syn:anti ratio of the oxime product that results from condensation of hydroxylamine with a ketone (*52*) as well as the formation of 3-hydroxyoxepane rings from simple hydroxy epoxides (*53*). A series of esterolytic antibodies have been generated that are able to resolve each of four diasteromeric esters (*45*), and esterolytic antibodies have been reported that are able to regioselectively deprotect an acylated carbohydrate (*54*). Finally, antibodies generated against a quaternary ammonium hapten catalyze the stereoselective hydrolysis of an enol ether under aqueous conditions to give the corresponding aldehyde with greater than 98% enantioselectivity (*55*).

Future Directions

Novel transformations. Antibody catalysis will undoubtedly be extended to many other interesting classes of reactions. For example, recently it was reported that antibodies can catalyze a cationic cyclization reaction (*56*). Antibody 4C6, which was generated against hapten **36**, catalyzes the cationic rearrangement of **37** to cyclohexene **38** (2%) and *trans*-2-dimethyl-phenylsilyl cyclohexanol **39** (98%) (Fig. 11). Such a narrow distribution of products is surprising because cationic cyclization reactions conducted under solvolysis conditions usually yield a plethora of products. The almost singular production of the cyclized product **39** was attributed to the antibody's ability to both enforce a pseudocyclic transition state geometry and trigger the reaction under conditions so mild that there is no detectable background reaction. These studies will undoubtedly lead to efforts aimed at larger multiring cyclization reactions.

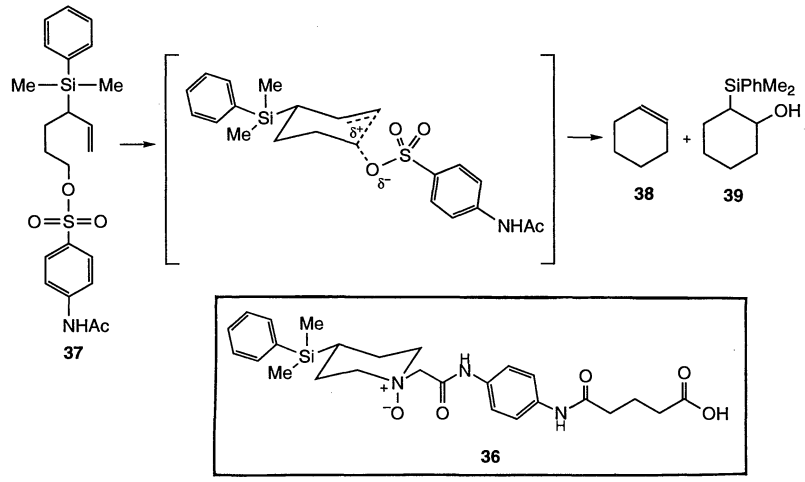

Fig. 11. An antibody-catalyzed cationic cyclization reaction.

New strategies. We will continue to see the development of new approaches for generating catalytic antibodies. One recent approach involves the use of mechanism-based screens in which covalent modification by a hapten is used to identify antibodies with an appropriately positioned nucleophile group in the active site (*57*, *58*). This approach has been applied to the generation of antibodies that catalyze the aldol condensation of an enolate with an aldehyde to give the corresponding β-hydroxyketone, a reaction at the heart of organic synthesis. Class I aldolase enzymes specifically catalyze this reaction through a complex mechanism that involves the formation of a covalent Schiff base formed between an active site lysine residue and the ketone substrate followed by enamine formation, condensation with aldehyde, and hydrolysis.

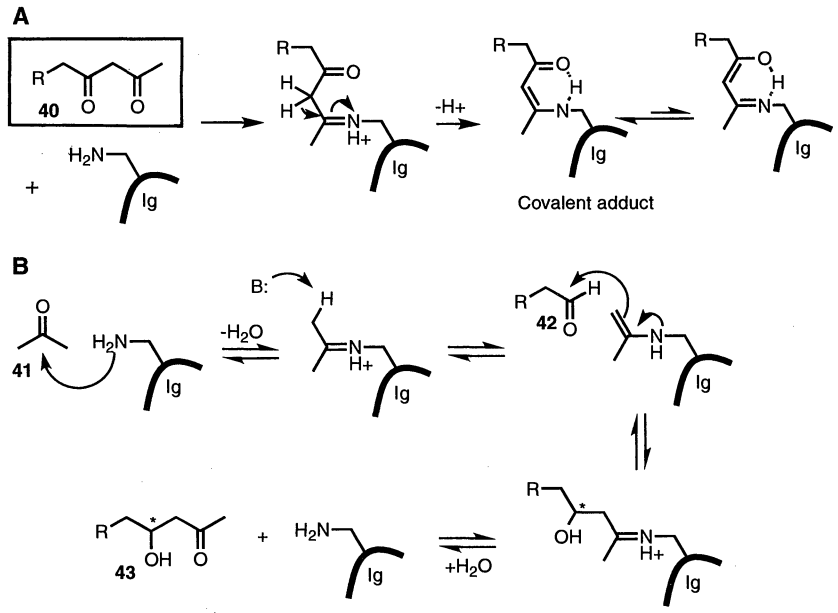

Fig. 12. (**A**) Mechanism-based screen for an active site lysine residue. (**B**) The corresponding antibody-catalyzed aldol condensation. Ig, immunoglobulin.

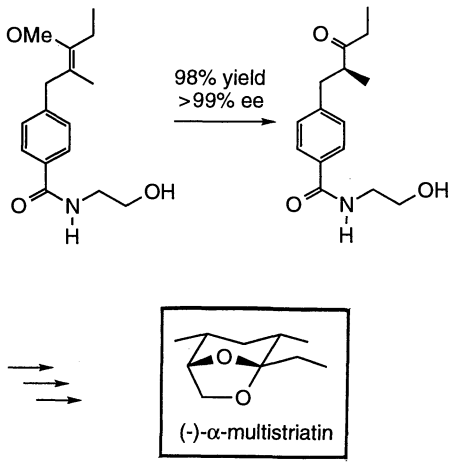

Fig. 13. Synthesis of (–)-α-multistriatin in which the key first step is catalyzed by an antibody (ee, enantiomeric excess).

To generate antibodies that might function by a similar mechanism, Wagner and co-workers designed diketone hapten **40** (Fig. 12). This hapten can form a stable vinylogous amide by reaction with a primary amine, such as that of a lysine side chain, in an antibody combining site. It was anticipated that the immunological selection process would select for clones that form such covalent adducts because of their apparent high affinity for antigen, and thereby elicit an active site nucleophile.

Two antibodies that form such adducts were identified and both are efficient catalysts for the aldol condensation of **41** (acetone) with **42** (2-phenylpropionaldehyde); antibody 33F12 provides a rate acceleration $[(k_{cat}/K_m)/k_{uncat}]$ of greater than 10^9-fold. By preferentially catalyzing the addition of the enamine to the *si* face of the aldehyde, regardless of the stereochemistry at the 2-position, the antibody produces β-hydroxyketones in high (>95%) diastereomeric excess. In some cases the inherent stereochemical preference of the reaction is reversed, affording the normally disfavored product in diastereomeric excess as high as 83%. This reaction underscores the mechanistic complexity that can be generated with appropriately designed hapten.

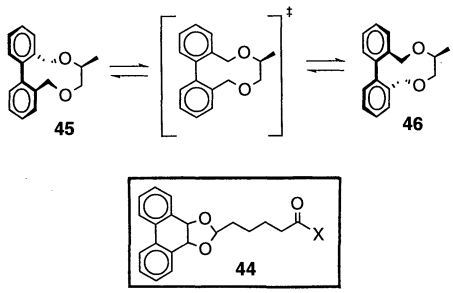

Fig. 14. An RNA-catalyzed isomerization reaction.

In addition to strategies involving synthetic immunogens, more biologically oriented approaches are being developed for generating catalytic antibodies. For example, the hydrolytic antibody 48G7 has been efficiently expressed in *Escherichia coli* for use as a model system to demonstrate the feasibility of using genetic selections based on complementation of cofactor-deficient auxotrophs as a general strategy to enhance catalytic activity (*59*). Similar approaches are being developed for selecting antibodies with enhanced activity that catalyze biosynthetic reactions, such as the conversion of chorismic acid to prephenic acid (*60*) and the decarboxylation of orotate (*61*). For both reactions, antibodies have been found that complement auxotrophs lacking the corresponding biosynthetic enzymes. In the latter case, this complementation was used as the basis for screening a library of antibodies generated from a mouse immunized with a synthetic immunogen.

Other interesting strategies have also appeared for generating catalytic antibodies including the development of rapid plate assays for screening large numbers of antibodies directly for catalysis (*62*, *63*). For example, Green and co-workers used an antibody specific for a reaction product to screen hybridoma supernatants for catalysts (*63*). Transgenic mice have been generated that produce antibodies with a high percentage of metal ion–binding light chains (*64*), an approach that may facilitate the generation of catalytic antibodies that use metal ions and other cofactors. Catalytic antibodies with cholinesterase activity have also been generated by immunizing mice with a monoclonal antibody directed against the active site of acetylcholinesterase (*65*). The catalytic efficiency of this antibody ($k_{cat}/k_{uncat} = 4 \times 10^8$) is quite high, suggesting that this approach may prove useful for generating structural and functional variants of enzymes.

Applications. It is likely that we will begin to see applications of antibody catalysis to practical problems in chemistry and medicine. Already there have been reports in the literature of antibody-catalyzed cocaine hydrolysis (*66*) and antibody-catalyzed prodrug activation (*67*, *68*). In the latter case, the use of a catalytic antibody makes possible prodrug activation by a reaction not catalyzed by endogenous enzymes. In addition, research efforts are focusing on methods for efficiently generating polyclonal catalytic antibodies in the hope that someday one may be able to actively immunize and produce therapeutic catalytic antibodies (*69*).

The recent demonstration that enantioselective conversions can be carried out by antibodies on multigram scale (*70*) as well as efforts aimed at extending antibody catalysis to organic solvents (*71*) should facilitate the use of antibodies in organic chemistry. A recent report describes the use of a catalytic antibody to carry out the key synthetic step, the enantioselective protonolysis of an enol ether, in the total synthesis of (−)-α-multistriatin, the aggregation pheromone of the European elm bark beetle (Fig. 13) (*72*). This reaction, which was carried out in greater than 99% enantiomeric excess and 98% chemical yield, was followed by 10 chemical steps with all four asymmetric centers originating from the chirality achieved through the use of the catalytic antibody.

Limitations. Given that one can generate catalysts for many reactions (even those that may have complex mechanisms), attention has naturally focused on the practical aspects of the technology. The ability to use antibody catalysts in organic synthesis, medicine, and industry reduces to the same general issues as do other enzymes, such as turnover, accessibility, and cost. Whereas early antibody catalysts achieved only modest rate accelerations, more recently developed catalysts have enhanced rates, sometimes rivaling those of natural enzymes. Still, we must improve the rates of those catalysts thought to be of practical significance by improving methods for generating and screening antibodies. There are also many classes of reactions that remain to be surveyed; effective haptens must be designed in order to catalyze such reactions. It is not clear, however, whether some of the more complex behavior of enzymes such as allosteric activation can be replicated with antibodies. Another aspect of most antibody catalysts is their high specificity. In many instances this is a great asset, but for some applications, for example in organic synthesis, one would rather have a catalyst that will carry out a given type of transformation regardless of the structure features surrounding the reactive center. This is less likely with antibody catalysts and we envision that most often they will be customized for specific transformations and will only use substrates that have related structural features. Cost efficiency and availability of antibodies must be improved. In terms of availability, the monoclonal antibody and combinatorial library methodology have turned antibodies into reagents like any other in that homogeneous and immortal source of the reagent exists and the reagent can be transferred from laboratory to laboratory or sold. However, the diversity of the immune system dictates that catalysts induced by different investigators may differ in terms of fine specificities. This is generally an advantage so long as free exchange and cataloging procedures are commensurate with

the growing list of catalysts, particularly those of interest for organic synthesis. The current high cost of antibodies is being addressed with improved bacterial and yeast expression systems and expression in plants and their seeds. In addition, some practical problems associated with the use of antibodies may be overcome by making scaffolds that carry out the same functions of antibody catalysts (*73*).

From antibody libraries to materials science. Finally, many of the ideas that form the basis for the work described in this review are being applied elsewhere (*6–10*). For example, the planar phenanthrene transition-state analog **44** has been used to screen nucleic acid libraries for RNAs that catalyze the isomerization of the substituted biphenyl **45** (Fig. 14) (*74*). In vitro mechanism-based selections using large RNA libraries have yielded catalytic RNAs for a variety of reactions. Such experiments are extending RNA catalysis from phosphoryl transfer reactions to new classes of chemical transformations. The success of chemistry in exploiting the molecular diversity of the immune system to generate selective catalysts points to the tremendous potential of combinatorial libraries. Indeed the lessons learned from the catalytic antibodies have recently found their way from the biology and chemistry communities to the physics community with the report that libraries encompassing virtually the entire periodic table can now be screened for interesting new physical properties such as superconductivity and magnetism (*10*).

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