ARTICLES

Antibody Catalysis of Difficult Chemical Transformations

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Received April 15, 1993

Since the original reports of antibody catalysts in 1986,[1,2] many chemical transformations have been catalyzed by antibodies.[3] The specificity and, in some cases, the rates of antibody-catalyzed reactions rival those of enzymatic reactions. In each study, the binding energy of the antibody molecule was used in a highly specific way to control the outcome of a chemical reaction. In the earliest examples, simple transformations with well-studied mechanisms were chosen. For example, antibodies were used to stabilize the tetrahedral, negatively-charged transition state in ester hydrolysis.[1,2] These reactions were followed by more sophisticated experiments involving general acid, general base catalysis, nucleophilic catalysis, and catalysis by approximation.[3] More recently, the field has begun to focus on selective chemical transformations that are difficult to achieve via existing chemical methods. These include "disfavored" chemical reactions, reactions along one of many nearly equivalent reaction coordinates, and reactions in which the inherent reactivity or molarity of the reactants is reversed. Recent advances related to this aspect of antibody catalysis are reviewed below.

Disfavored Chemical Reactions

For reactions under kinetic control in which a number of reaction products are possible, the product distribution reflects the relative free energies of each transition state. In many reactions, such as Diels–Alder and intramolecular cyclization reactions, the product ratios can be understood in terms of the stereoelectronic features of a favored and a disfavored transition state (e.g., endo vs exo cycloaddition, 5-exo vs 6-endo nucleophilic substitution (Figure 1)). In practice, it has proven difficult to chemically discriminate and control the relative energies of the two transition states, e.g., form the exo adduct of a Diels–Alder reaction or the anti-Baldwin product of a ring closure reaction. Recently, an antibody has been generated that overcomes these constraints and catalyzes a highly disfavored reaction, the 6-*endo-tet* ring closure of an epoxy–alcohol to form a tetrahydropyran.[4] This antibody-catalyzed reaction is formally a violation of Baldwin's "rules" for ring closure reactions which state that, in the case of an intramolecular nucleophilic substitution reaction, the product arising from the preferred 180° transition-state geometry is the 5-*exo-tet* product (Figure 2).

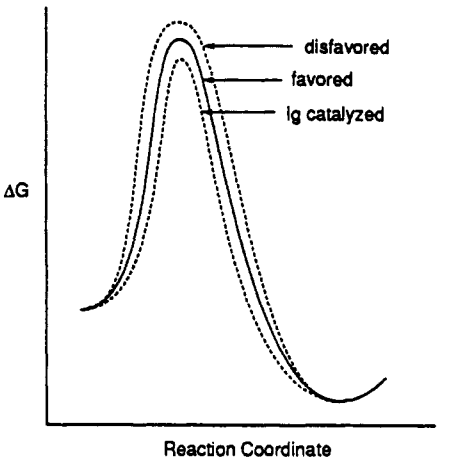

Figure 1.

In order to catalyze this reaction, 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) vs five-membered (favored) ring

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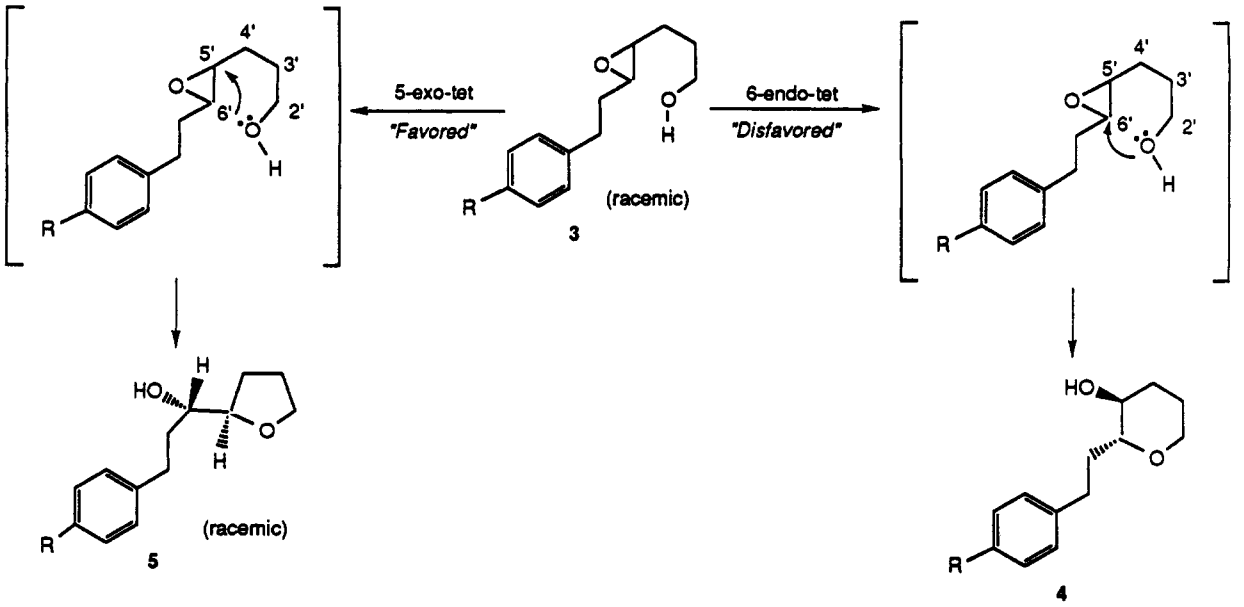

Figure 2.

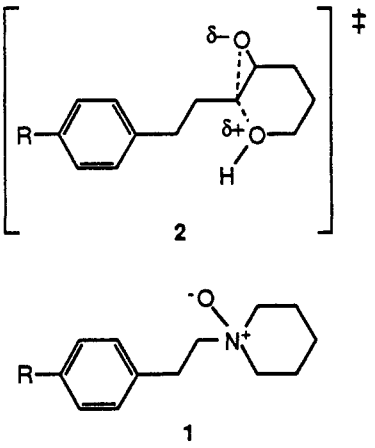

Figure 3.

transition state. This requires that several mechanistic criteria be accommodated in the structure of the hapten used to "induce" the combining site. In this case it was anticipated that *N*-oxide 1 would generate a combining state that would stabilize both the developing charge in the breaking C–O bond and the six-membered-ring transition state 2 required to give product 4 (Figure 3). Moreover, the difference in dipole of the hapten and reaction product was expected to minimize product inhibition, a key aspect in the design of any antibody-catalyzed reaction.

Two antibodies generated against *N*-oxide 1 were found to catalyze the regioselective ring opening of epoxide 3 to form the six membered-ring product 4. The initial rate of ring closure catalyzed by antibody 26D9 followed Michaelis–Menten kinetics with a k_{cat} value of 0.91 min^{-1} and a K_m of 356 μM. Comparison of the k_{cat}/k_{uncat} with the uncatalyzed reaction was not possible since in the absence of antibody only the five-membered Baldwin ring closure product was formed. In addition, only the *S,S* epoxide was a substrate for antibody-catalyzed pyran ring formation. Thus the antibody controls both the regio- and stereochemistry of this reaction. This antibody-catalyzed reaction underscores the degree to which the relative energies of transition states can be controlled (and reversed) using the selective binding energy of the immune system.

Controlling the Regio- and Enantioselectivity of Reactions

Several catalytic methods have been developed in recent years for the regio- and stereoselective synthesis of enantiomerically pure compounds.[6] These include chiral epoxidations,[7] enantioselective hydrogenations[8] and hydride transfers,[9] and asymmetric dihydroxylations.[10] However, the rational design of such catalysts is still in its infancy, and high stereoselection is usually contingent upon neighboring ligands or restricted sets of substituents.[6] Moreover, the ability to discriminate between chemically similar functional groups in the same molecule can often be achieved only by the application of extensive protecting group strategies. Given the extraordinary specificity of the immune system, it should be possible to generate antibodies that distinguish enantiomeric transition states regardless of chemical environment and substrate complexity. In fact, a number of such reactions have been previously reported including enantioselective ester hydrolysis[11-14]

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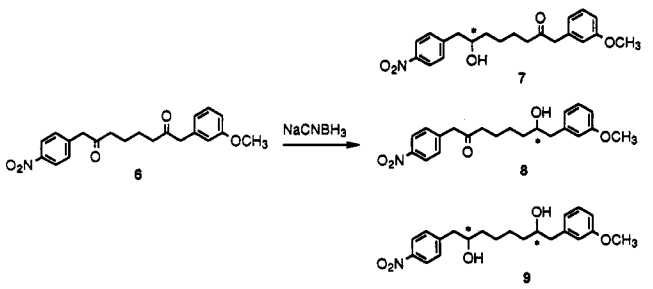

Figure 4.

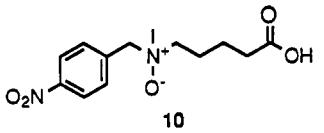

Figure 5.

and transesterification reactions,[15,16] lactonization reactions,[17] Schiff base formation,[18] and Claisen rearrangements.[19,20]

Even more challenging to the synthetic chemist are reactions which involve both regio- and stereoselective control, for example, the selective reduction of diketone **6** to a single enantiomer of hydroxy ketone **7**[21] (Figure 4). The similar chemical environments of the two carbonyl moieties in the substrate (distinguishable only by methoxy and nitro groups five and six atoms away) render this transformation extremely difficult to achieve by known chemical methods. In order to catalyze the regio- and stereoselective reduction of **6**, antibodies were raised against *N*-oxide hapten **10** (Figure 5). These antibodies should not only stabilize the tetrahedral transition state resulting from nucleophilic attack of hydride on the carbonyl group but also direct regioselective addition of hydride to the nitrobenzyl-substituted carbonyl group of substrate **6**. Moreover, the chiral environment of an antibody combining site induced by one of the two enantiomers of hapten **10** should discriminate between the enantiotopic faces of a prochiral substrate, affording a highly stereoselective reduction. In order to avoid the need for cofactor recycling, the inexpensive reductant sodium cyanoborohydride ($NaBH_3CN$) was chosen as the hydride donor for the antibody-catalyzed reaction.

A number of antibodies generated to *N*-oxide **10** catalyzed the $NaBH_3CN$ dependent reduction of ketone **6**. One antibody, 37B39.3, catalyzed the reduction regioselectively with greater than 75:1 selectivity for one of the two nearly equivalent ketone moieties. Moreover, the reaction was highly stereoselective, affording the *S* enantiomer of the hydroxy ketone in 96.3% enantiomeric excess (96.3% ee). In contrast, the nitrobenzyl carbonyl group was reduced more slowly than the methoxybenzyl carbonyl group in the uncatalyzed reaction (V_{rel} = 0.74). The overall yield of hydroxy ketone (*S*)-**7** was 94%, which is significant in light of the fact that the background reaction produces eight products.

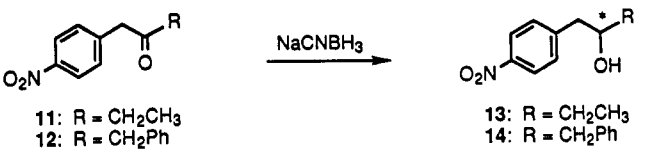

Figure 6.

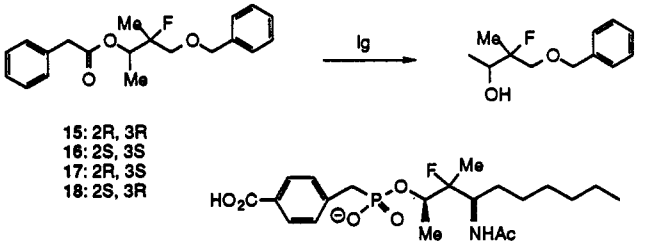

Figure 7.

Table I

	hapten abs config	k_{cat}, min^{-1}	K_m, μM	hc, %	op, % ee
15	2*R*,3*R* (+)	0.88 ± 0.3	390 ± 70	23	99
16	2*S*,3*S* (−)	0.91 ± 0.2	400 ± 70	23.5	>98.5
17	2*R*,3*S* (+)	0.94 ± 0.2	410 ± 90	23	98.5
18	2*S*,3*R* (−)	0.86 ± 0.3	380 ± 50	23	98

Antibody 37B39.3 also catalyzed the stereoselective reduction of a number of substituted benzyl ketones, including 1-(*p*-nitrophenyl)butan-2-one (**11**) (96% ee) and 1-(*p*-nitrophenyl)-3-phenylpropan-2-one (**12**) (87% ee), in which the carbonyl substituents are distinguishable only by the *p*-nitro group (Figure 6). The second-order rate constant, $k_{cat,app}/K_{m,app}$, for the antibody-catalyzed reduction of **11** was 1.9×10^3 min^{-1} M^{-1}, a considerable acceleration over the uncatalyzed reaction, $k_{uncat} = 1.1 \times 10^{-3}$ min^{-1} M^{-1}.

A second example in which antibodies were able to stabilize selectively one of a number of nearly equivalent transition states is a recently reported diastereoselective esterolytic reaction.[22] Antibodies were generated against each of four diastereomeric phosphonate analogues of the transition states for the hydrolysis of the corresponding 1-(benzyloxy)-2-fluoro-2-methyl-3-hydroxybutane esters **15**–**18** (Figure 7). Each of the four esters were hydrolyzed in ≥97% ee and in greater than 23% overall conversion (25% is theoretical) with the corresponding antibody (Table I). Given that at present there exist no general chemical methods for generating stereoselective esterolytic catalysts, esterolytic antibodies might find applications in the chiral resolution of synthetic intermediates containing either acid or alcohol functionality. These model studies again illustrate the degree to which reactions can be controlled via the "programmable" binding energy of antibodies. In a sense, the antibody combining site can be viewed as a heterogeneous solvent that can be fitted to the reaction pathway of interest.

Control of Proton Transfers

The enantioselective control of proton transfers has, to date, proven a difficult problem in organic chem-

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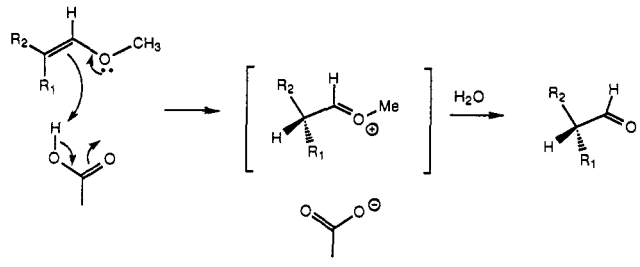

Figure 8.

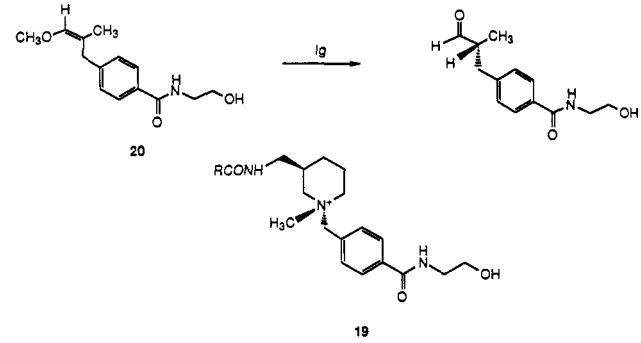

Figure 9.

istry.[23] In contrast, many enzyme-catalyzed reactions involve the selective protonation of one face of a prochiral substrate. In order to determine whether antibodies can catalyze this important class of chemical transformations, the enantioselective protonation of a prochiral enol ether to the corresponding chiral aldehyde was attempted in aqueous solution.[24] This reaction requires both stabilization of the charged oxocarbenium ion-like transition state and the positioning of an active site acid on one face of the substrate (Figure 8).

The *N*-methylpiperidinium cation **19** was used as the hapten to generate an antibody with the appropriate geometric disposition of catalytic groups. The positive charge on the nitrogen atom can be expected to induce a carboxyl group in the antibody combining site which can both donate a proton and, in the ionized form, stabilize the oxocarbenium ion. The tetrahedral ammonium ion can be expected to induce a geometry in the antibody molecule which favors the pyramidalization of the carbon undergoing protonation. Finally, the entire process takes place in the asymmetric antibody binding site, thereby leading to enantioselective delivery of the proton. Indeed, a number of antibodies induced to the piperidinium cation were found to catalyze the hydrolysis of **20** (Figure 9). One antibody, 14D9, which was studied in detail, catalyzed the reaction with a rate acceleration of 2500 over the uncatalyzed reaction. Importantly, analysis of the chirality of the aldehyde product revealed an enantiomeric excess of 96% in this reaction. Mechanistic studies have provided evidence for the participation of an ionizable side chain, probably a carboxylate in the active site.[25]

Transesterification Reactions

The product distributions of kinetically controlled bimolecular reactions depend on the relative concentrations and physical properties (acidity, polarizability, steric bulk) of the reactants. A high degree of chemical selectivity could be achieved if catalysts could be designed which can modulate the "effective molarity" or inherent reactivities of substrates, e.g., catalyze the transfer of an activated acyl group to 2-propanol in the presence of equimolar methanol. This challenging notion was recently addressed by both our groups with the antibody-catalyzed transesterification reactions illustrated in Figure 10.[15,16] In both reactions an acyl group is being transferred in the presence of 55 M H_2O to a more hindered secondary alcohol present at submillimolar concentrations. (The first reaction[16] is related to the aminoacylation of the terminal 2′,3′-diol of tRNAs with activated amino acids. This key step in protein biosynthesis represents one of the most important examples of chemical selectivity in nature.)

In order to generate catalytic antibodies for these reactions a number of mechanistic criteria were accommodated in the design of haptens **21** and **22**. Antibodies raised against these haptens were expected to bind both substrates in a favorable orientation, thereby significantly accelerating the reaction by acting as "entropy traps".[26] Moreover, the dipole of the P=O bond reflects the developing negative charge on the carbonyl oxygen in the transition state. Finally, antibodies complementary to the tetrahedral phosphonate haptens should have considerably lower affinity for the trigonal products, thereby preventing product inhibition.

Antibodies generated to both haptens were found to be remarkably efficient catalysts for the corresponding transesterification reactions. The kinetic constants for antibody-catalyzed transesterification of thymidine with the alanyl ester are $k_{cat} = 14.2\ min^{-1}$ and $K_M = 770\ \mu M$ and 260 μM for **24** and **23**, respectively. The calculated second-order rate constant k_{cat}/K_M is equal to $1.84 \times 10^4\ M^{-1}\ min^{-1}$ for **24** and $5.4 \times 10^4\ M^{-1}\ min^{-1}$ for **23**. For comparison the uncatalyzed transesterification reaction rate constant was measured to be $2.6 \times 10^{-4}\ M^{-1}\ min^{-1}$. The transesterification reaction of the vinyl ester **27** with 2-phenethyl alcohol **26** was calculated to have an effective molarity on the order of 10^5–10^6 M. The fact that this value is close to the predicted theoretical upper limits of 10^8–10^{11} for bimolecular reactions[26] emphasizes the importance of entropic considerations in catalyst design.

Neither antibody catalyzed acyl transfer to water to any appreciable extent. This observation is remarkable given that water is present in both reactions at a concentration some 10^5 times that of the more sterically hindered secondary alcohol. The ability to control the reactivity of competing reactions to this extent will become increasingly important as more efficient and environmentally acceptable chemical catalysts are required.

Surprisingly these two antibodies were found to function via different mechanisms. In the case of thymidine aminoacylation[16] the differential binding affinity of the antibody to the phosphonate diester relative to substrates appears to account for a large fraction of the catalytic advantage in this reaction,

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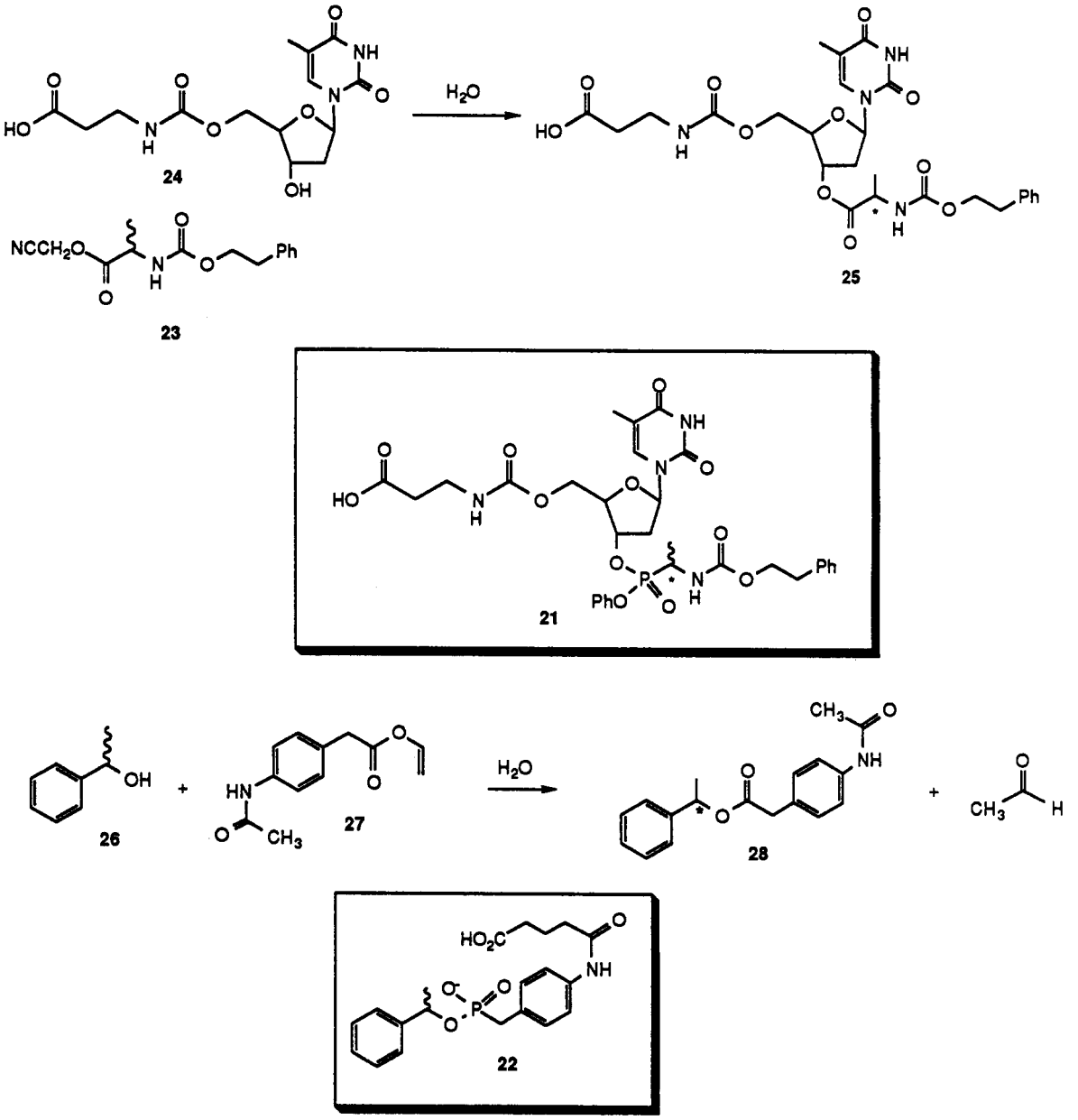

Figure 10.

consistent with the classic notion of transition-state complementarity of Haldane[27] and Pauling.[28] In contrast, the second transesterification reaction was found to proceed through a ping-pong mechanism involving a covalent antibody–substrate intermediate.[15] Thus, the tremendous diversity of the immune system has provided two mechanistic alternatives for similar acyl transfer reactions, in much the same way that enzymes have evolved to carry out similar reactions by different mechanisms (such as the acid, serine, and Zn^{2+} proteases).

Conclusion

Antibody catalysis has moved from simple transformations to reactions which require a high degree of control over transition-state energies in order to achieve chemical selectivity. The ability of antibodies to selectively catalyze such reactions stems from their high affinity and selectivities and from the ability of the experimenter to "program" the molecular structure of the antibody combining site via the powerful selective processes of the immune system. Catalytic antibodies may be expected to give the organic chemists access to many other chemical transformations which are otherwise unattainable.

The authors acknowledge the valuable contributions of their co-workers who made all of this work possible. P.G.S. acknowledges funding by the Office of Naval Research and the National Institutes of Health.

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