Catalytic Antibodies: Concept and Promise

Desirazu N Rao and Bharath Wootla


(left) Desirazu N Rao is a Professor in the Department of Biochemistry, Indian Institute of Science, Bangalore, India. His research interests are in the area of DNA-Protein Interactions using restriction-modification enzymes and DNA mismatch repair proteins as model systems.

(right) Bharath Wootla is a doctoral student in the 'Team-16' of UMR S 872, at the Centre de Recherche des Cordeliers, 75006 Paris - France and at the Université de Technologie de Compiègne, 60203 Compiègne - France. His research interests are in the field of antibody catalysis and immune response to coagulation protein 'Factor VIII'.

Keywords
Catalytic antibodies, abzymes, hybridome technology, Diels–Alder reaction, Michaelis–Menten kinetics, Factor VIII.

While chemistry provides the framework for understanding the structure and function of biomolecules, the immune system provides a highly evolved natural process to generate one class of complex biomolecules – the antibodies. A combination of the two could be exploited to generate new classes of molecules with novel functions. Indeed, one example of this productive interplay is the generation of catalytic antibodies or abzymes. A catalytic antibody is a sort of natural artificial enzyme – it is a natural protein synthesized by the usual biological processes and is intended to catalyze a reaction for which no real enzyme is available. The essential idea is to raise antibodies to a molecule considered to mimic the transition state intermediate of a reaction that is to be catalyzed i.e., a molecule resembling a strained structure intermediate between the substrate and product, believed to occur in the reaction pathway. The hope is that some of the antibodies produced will happen to possess groups capable of promoting the reaction.

In order to enhance the rates of chemical reactions, enzymes, which are biocatalysts, use their binding energy to overcome unfavourable entropy (see *Box* 1) and to position the substrates in proximity to each other as well as to reactive amino acid side chains. Proper orientation and the nearness of the substrate with respect to the catalytic groups, which has been referred to as the 'proximity effect', contribute 10^3 to10^5– fold to the rate enhancement observed with enzymes. Thus, the geometry of the enzyme binding pocket is carved by evolutionary pressure to the extent that some enzymes operate at diffusional rates; i.e. encounter of the enzyme and its substrate is controlled by the rate of diffusion (usually between 10^8–10^9 s^{-1} M^{-1}).

The immune system is capable of synthesizing large folded

polypeptides (immunoglobulins or antibodies) that can bind virtually any natural or synthetic molecule with high affinity and exquisite selectivity. B-lymphocytes, the cells of the immune system that produce antibody molecules, make use of genetic recombination to generate a pool of antibody molecules, each possessing a unique combining site amino acid sequence. Typically, a mammalian cell is able to mount a primary immune response consisting of some 10^8 different antibody molecules. Antibodies bind ligands with association constants that range from 10^4 to 10^{14} M^{-1}. The specificity of antibodies for their ligands can exceed that of enzymes for substrates. As first postulated by Linus Pauling, the basic processes that determine binding of substrates to enzymes and antigens to antibodies are the same. Both achieve binding by using forces such as hydrogen bonds and van der Waals force, which manifests when small molecules come within a few angstroms of each other.

The ability of the highly evolved machinery of immune system to produce structurally and functionally complex molecules like antibodies offers tremendous opportunities for chemists to design biomolecules with novel properties such as catalytic antibodies.

The idea of using antibodies to catalyze chemical reactions can be traced back to the catalytic concept of Linus Pauling. According to Pauling, if the structure of the antigen binding site of antibodies were to be produced in a random manner, the antigen binding site of some of the antibodies may resemble the active site of enzymes and such molecules could have the ability to transform substrates to products. As Pauling first pointed out more than 50 years ago, the fundamental difference between enzymes and antibodies is that the former selectively bind transition states and the latter bind ground states of substrates. The other major distinction is that antibody specificities evolve on a time scale of weeks, whereas enzyme specificity evolves over a million years.

William Jencks, on the other hand, suggested that stable molecules resembling the transition state of a reaction might be used as haptens[1] to elicit antibodies with engineered catalytic activities

Box 1.

Entropy is a thermodynamic term, which defines the extent of disorder in a system. At equilibrium, entropy is maximal. For example, in solution two reactants A and B exist in many different orientations. The chances of A and B coming together with the correct geometric orientation and with enough energy to react is small at 37°C and in dilute solution. However, if an enzyme with two high-affinity binding sites for A and B is introduced into a dilute solution of these reactants, A and B will be bound to the enzyme in the correct orientation for the reaction to occur. They will be bound with the correct stoichiometry, and the effective concentration of the reactants will be increased on the enzyme surface.

[1]The term hapten is derived from the Greek word *haptein*, meaning, 'to fasten'. A hapten is a small molecule, which can elicit an immune response only when attached to a large carrier such as a protein; the carrier itself may not elicit an antibody response.

and selectivities. Implementation of this ingenious concept was made possible by the development of monoclonal antibodies[2], viable transition state analogs, and unique screening assays. Since then, antibodies have been generated that catalyze a wide variety of chemical reactions.

[2] Antibodies produced by a single clone of B cells that recognize a single antigenic determinant or epitope are termed monoclonal antibodies. It is possible to produce monoclonal antibodies that can bind specifically to (almost) any molecule. The monoclonal antibody technology (Hybridoma technology) has provided an easy and efficient way to detect or purify the substance of interest. The technology has now become an important tool in biochemistry, molecular biology and medicine.

Different Strategies for Generating Catalytic Antibodies

A number of approaches have been used to generate catalytic antibodies including (a) the generation of antibodies with catalytic groups and cofactors in their combining sites; (b) the use of antibodies to stabilize negatively and positively charged transition states; (c) the use of antibodies as entropic traps, and (d) the use of cofactors by catalytic antibodies.

a) *The Generation of Antibodies with Catalytic Groups in their Combining Sites*

The complementarity of an antibody to its corresponding hapten (the ligand against which the antibody is elicited) has been exploited to generate combining sites. These sites are complementary to the rate-determining transition state, that act to overcome the entropy requirements involved in orienting reaction partners, or that contain an appropriately positioned catalytic amino-acid chain or cofactor. Catalytic groups have also been introduced directly into the combining site of an antibody by site-directed mutagenesis of the corresponding gene. These strategies have led to the generation of antibodies that catalyze a wide array of chemical and biological reactions.

Many enzyme active sites contain nucleophilic, electrophilic, basic, or acidic amino acid side chains that are precisely positioned to react with a bound substrate. For instance, serine 195 in chymotrypsin is acylated by the substrate. The introduction of such an amino acid side chain into an antibody combining site should be an effective method of catalyzing a hydrolytic reaction. The high effective concentration of the catalytic group in the antibody combining site as well as favorable orbital alignment of reactants should lower considerably the entropy and enthalpy of

activation for reaction. Electrostatic interactions play an important role in the recognition of charged haptens by antibodies. For example, while the combining sites of antibodies raised toward p-azobenzene-trimethylammonium cation had negatively charged aspartate or glutamate residues, the combining sites of antibodies raised toward negatively charged p-azobenzoate had positively charged arginine and lysine residues (*Figure* 1). George Schultz and his group used the electrostatic complementarity idea to generate a carboxylate in an antibody combining site. The carboxylate was appropriately aligned to catalyze the elimination of hydrogen fluoride from a β-fluoroketone by abstraction of an α-carbon proton (*Figure* 1).

Another approach for generating antibodies that contain active site catalytic groups is by the method of site-directed mutagenesis. By this technique one could introduce catalytic histidines into the active sites of dinitrophenyl and phosphorylcholine antibodies. The resulting antibodies have significant esterolytic activity, upto 10^5 times that of the corresponding imidazole-catalysed reaction.

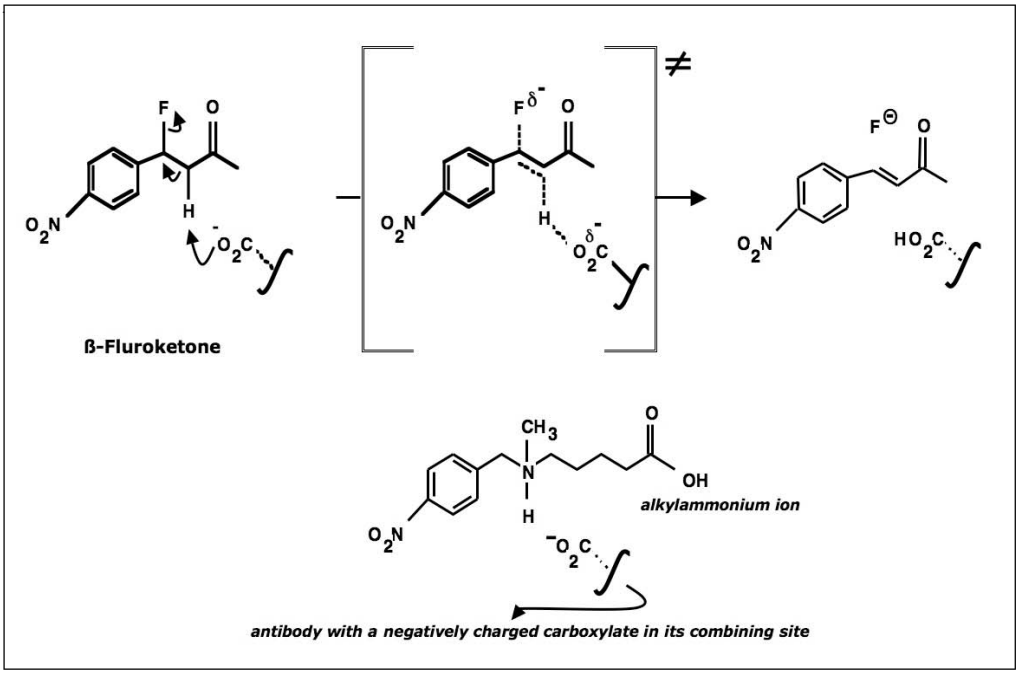


Figure 1. Elimination of HF from a β-fluoroketone by a catalytic antibody with a negatively charged carboxylate in its combining site.

Box 2.

A chemical reaction of reactants A and B to form products P + Q goes through a transition state that has a higher free energy than does either substrates or products. As a reacting system passes along a notional 'reaction coordinate', it must pass through a continuum of energy states and at one stage it must surpass a state of maximum energy. This maximum energy state is the transition state. The difference in free energy between the transition state and the substrate is called the activation energy, E_A. Much of the catalytic power of enzymes comes from their bringing substrates together in favourable orientations to promote the formation of transition state complexes (see *Figure* 2).

b) *The Use of Transition-state Analogs*

Yet another approach for the generation of catalytic antibodies exploits the notion of transition-state stabilization (*Box* 2). As stated earlier, enzymes have evolved to selectively bind the transition state of a reaction, and antibodies have evolved to specifically bind molecules in ground state. It follows then, that an antibody that selectively binds a stable analog of a transition state will have enzyme activity. About 40 years ago, William Jencks first proposed an experiment to test this possibility.

Transition-state analogs, originally synthesized as enzyme inhibitors, thus play an important role as haptens for the generation of catalytic antibodies and indeed have been critical for the development of this technology. The suggestion that proteins which bind transition-state analogs strongly should be able to catalyze chemical reactions is a logical consequence of the argument that such analogs should bind tightly as enzyme inhibitors. By generating an antibody to a transition-state analog, a protein may be created that can bind the transition state more tightly than the substrate or products and thus accelerate their interconversion. The first experimental demonstration of this idea involved acyl transfer reactions, specifically simple hydrolytic reactions (*Figure* 3). These reactions are among the most thoroughly understood organic reactions and the steric and electronic nature

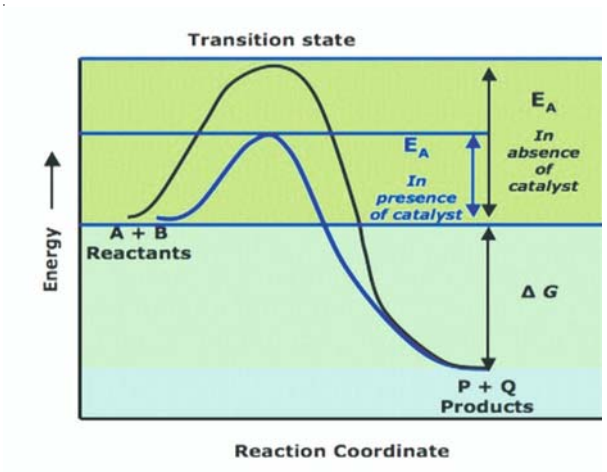


Figure 2. Reaction profile according to transition-state theory.

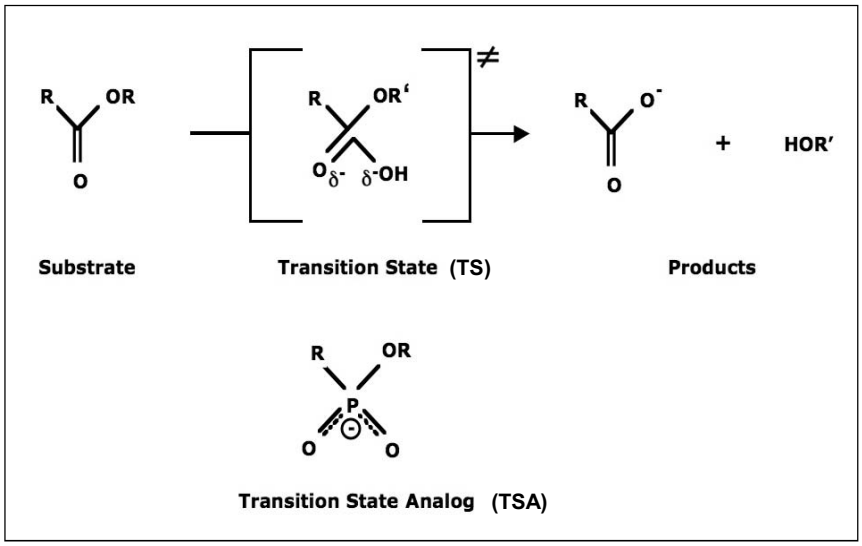


Figure 3. The hydrolysis of an ester involving the formation of a tetrahedral, negatively charged transition state. The corresponding phosphonate is a stable transition-state analog.

of the transition state for these reactions (tetrahedral and negatively charged) is sufficiently different from that of the substrate (planar and neutral).The characteristics of these species are mimicked by stable phosphates, phosphonates, and phosphonamidates, which are known to be potent inhibitors of several acyl-transfer enzymes and were thus presumed to be good transition state analogs.

Research groups of Richard Lerner and George Schultz in 1986 characterized antibodies that catalyzed the hydrolysis of esters and carbonates. The rate limiting step in these reactions is the formation of the negatively charged tetrahedral transition state. A stable analog of such a structure is formed by substitution of a tetrahedral phosphorus atom for a tetrahedral carbon. These phosphonate transition-state analogs are somewhat similar to the negatively charged tetrahedral transition state for ester hydrolysis. Antibodies specific for transition state analogs selectively accelerate the hydrolysis of their respective substrates. These antibodies have higher binding affinities for the transition state analogs than for the reaction substrates suggesting that they function by stabilization of the transition state. These initial successes clearly proved that hapten-antibody complementarity could be used as a general strategy for generating catalytic

The demonstrations that antibodies can function as catalysts in organic solvents and immobilized antibodies, retaining activity and specificity in organic solvents are just two examples which bear on commercial applications of catalytic antibodies.

antibodies. Since these early experiments, more than 20 acyl transfer reactions have been catalyzed with rate accelerations approaching 10^8 M^{-1}over the uncatalyzed reactions.

Antibodies that catalyze the hydrolysis of unactivated alkyl esters have been generated by the groups of Stephen Benkovic and George Schultz. These catalytic antibodies hydrolyze substrates in a stereospecific manner i.e., some of the antibodies elicited to the phosphonate analog of the transition state for the hydrolysis of benzyl ester, catalyzed the hydrolysis of (R)-benzyl ester while others catalyzed the hydrolysis of the (S)-ester. These results are significant because at present no general chemical method exists for generating stereospecific esterolytic catalysts. The specificity of such antibodies has already been exploited in the production of a biosensor. The demonstrations that antibodies can function as catalysts in organic solvents and immobilized antibodies, retaining activity and specificity in organic solvents are just two examples which bear on commercial applications of catalytic antibodies. The versatility of the use of transition-state analogs to generate catalytic antibody is clearly seen in the case of catalytic antibodies that catalyze the addition of a metal ion to a porphyrin. Transition-state stabilization should be applicable to the generation of antibodies that catalyse a wide variety of other reactions, including the hydrolysis of glycosidic bonds and the hydrolysis of phosphodiester bonds.

Cocaine can be effectively degraded by hydrolysis of its benzoyl ester and the products of this hydrolysis are neither reinforcing nor toxic. A high-activity catalytic antibody was elicited using a transition-state analog for the hydrolysis of cocaine to nontoxic products. This artificial cocaine esterase is thus a rationally designed cocaine antagonist and thus this catalytic antibody has the potential for medicinal use. A small molecule analog of cocaine, if administered as a blocker of cocaine, would be short-lived *in vivo*. At the same time, catalytic antibodies if administered would have plasma life of about 21 days. A humanized monoclonal antibody with a half-life sufficient for a dosing interval of several weeks would thus provide an appropriate

treatment. An anticocaine catalytic antibody could be a useful emergency room therapeutic for patients who present with serious complications.

An anticocaine catalytic antibody could be a useful emergency room therapeutic for patients who present with serious complications.

Marijuana is one of the most commonly abused illicit drugs in the world. Currently there is no clinical treatment for marijuana abuse, but a promising approach for treatment would be to bind the target drug before it can reach its cognate receptor. An antibody- catalyzed oxidation of Δ^9-tetrahydrocannabinol (Δ^9-THC, a major psychoactive component of marijuana) has been recently reported, which degrades Δ^9-THC to cannabitriol. Cannabitriol being more polar in nature will not be able to pass through the blood-brain barrier and thus, the drug can be eliminated from the system.

Another version of transition-state analogue approach is the anti-idiotypic approach of Alan Friboulet and his co-workers. Mice were immunized using an enzyme in order to produce a monoclonal antibody (referred to as Ab1), the antigen-binding site of which is structurally complementary to the active site of the enzyme. Monoclonal antibodies (Ab2) specific for the antigen-binding site of the Ab1 are then subsequently generated. Some of these Ab2 will have a structural image of the active site of the enzyme and will mimic the catalytic function of the enzyme. This original approach has allowed the production of antibodies endowed with esterase, amidase and serine protease activities, by using acetylcholine esterase, β-lactamase and subtilisin as immunogens, respectively.

c) ***The Use of Antibodies as Entropic Traps***

The relation between proximity effects and rate accelerations in biological catalysts is a well established fact. These entropic effects can account for effective molarities of up to 10^{-8} M in enzyme-catalyzed reactions. Therefore, antibodies should be capable of efficiently catalyzing reactions with unfavourable entropies of activation by acting as 'entropy traps'. Antibodies that catalyze unimolecular reactions as well as bimolecular

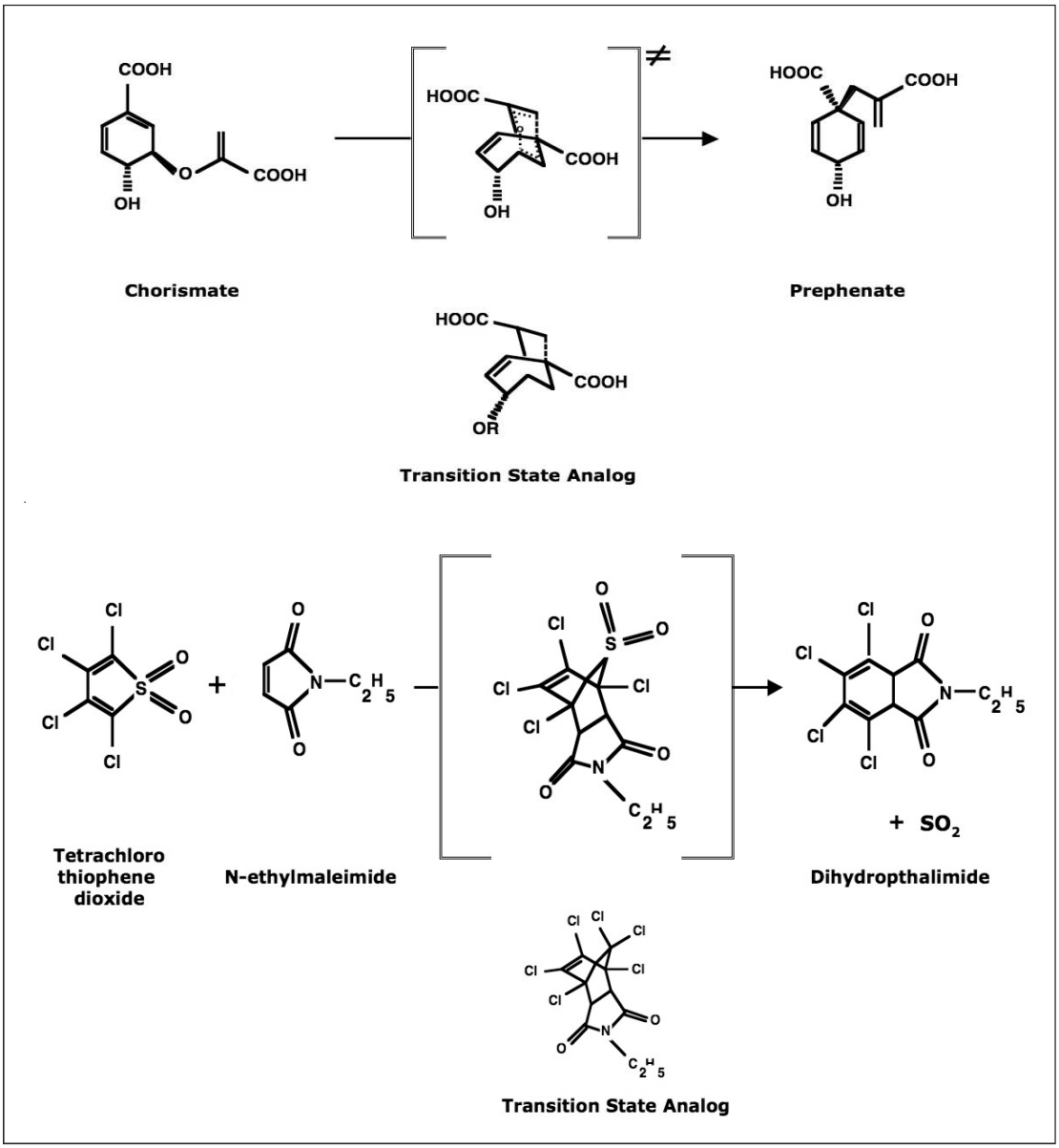


Figure 4. Classical organic chemistry reactions catalyzed by antibodies raised against stable transition-state analogs.

reactions and transacylation reactions have been generated using the notion of entropy trapping. The conversion of chorismate to prephenate (Claisen rearrangement) is an essential step in the biological synthesis of aromatic amino acids in bacteria. Antibodies that catalyze the Claisen rearrangement have been generated (*Figure* 4). These antibodies accelerate the rearrangement

10^4-fold over the uncatalyzed rate although the enzyme, Chorismate mutase which catalyses this reaction in *E.coli* accelerates the reaction approximately 3×10^6 fold over the background reaction. Another example of the use of catalytic antibodies to act as entropic traps involves an antibody-catalysed Diels-Alder reaction. This is a reaction between a diene and an alkene giving rise to a cyclohexene product. In *Figure* 4, the addition of N-ethyl maleimide to tetrachlorothiophene dioxide to give rise to dihydrophthalimide and sulfur dioxide is shown. There is no documented example of an enzyme-catalyzed Diels-Alder reaction until recently.

d) ***The Use of Cofactors by Catalytic Antibodies***

Nature makes use of a powerful set of nonpeptidyl catalytic groups termed cofactors to catalyse a huge number of reactions. These include metal ions, hemes, thiamine, flavins and pyridoxal. By incorporating these cofactors into antibody combining sites, one should be able to expand the scope of antibody catalysis. Sequence specific cleavage of a peptide bond with Zn(II) as a cofactor is one of the many examples of antibody-catalyzed reactions. Another example of the use of cofactors in antibody catalysis is the demonstration that light could be used by an antibody to break two carbon-carbon bonds of a cis-syn thymine dimer. Cochran, Schultz and co-workers have shown that antibodies generated to a thymine-dimer derivative accelerate the conversion of thymine dimer to thymine in the presence of 300 nm light. The catalytic constant (k_{cat}) or turnover number of the antibody was 1.2 per minute, close to the k_{cat} of 3.4 per minute for thymine dimer cleavage by the repair enzyme, *E. coli* DNA photolyase.

Box 3.

Turnover Number (k_{cat}), is the number of substrate molecules converted into product by an enzyme molecule in unit time when the enzyme is fully saturated with the substrate. For example, carbonic anhydrase catalyzes the formation of carbonic acid from carbon dioxide and water. The turnover number of carbonic anhydrase is 600,000/second. This means that one molecule of carbonic anhydrase would convert 600,000 carbon dioxide and water molecules to 600,000 carbonic acid molecules in one second. This turnover number is one of the largest known. The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 100,000 per second.

Kinetics of Catalytic Antibodies

Catalytic antibodies are characterized by high substrate specificity and share many mechanistic features with enzymes, including catalysis by proximity, transition-state stabilization and covalent catalysis. In some cases their rates are comparable to that of

enzymes. The specificity of these antibody-catalyzed reactions rivals or exceeds that of enzymatic reactions. In some cases, rates approaching those of enzymes have been achieved, but typically the antibody-catalyzed reactions proceed with rates 10^3 to 10^6 – fold faster than the uncatalyzed reactions. In general, antibody catalyzed reactions, just as those catalyzed by enzymes, involve noncovalent complexation of substrate by antibody, followed by conversion of substrate to product. Most catalytic antibodies have been shown to follow traditional Michaelis-Menten kinetics for conversion of substrate, S to product, P. These reactions demonstrate saturation kinetics and in several cases characterized by the following kinetic scheme:

$$\mathrm{Ab} + \mathrm{S} \longleftrightarrow \mathrm{Ab.S} \xrightarrow{k_{cat}} \mathrm{Ab} + \mathrm{P}$$

where Ab is the catalytic antibody, S is the substrate and P is the product. It must be noted here that the assumptions made in deriving the Michaelis-Menten equation for enzymes are appropriate for their application to catalytic antibodies.

It is imperative to demonstrate that the transition-state analog used to elicit antibody inhibits catalysis in a competitive fashion, and moreover, binds with a higher affinity than substrate.

The kinetic constants derived from a representative set of antibody-catalysed reactions are shown in *Table* 1. As can be seen, both antibodies and enzymes exhibit Michaelis-Menten constant[3], K_m values from the micromolar to millimolar ranges. However, many catalytic antibodies differ from their enzymatic counterparts in their k_{cat} values. These data clearly indicate that catalytic antibodies are limited in their ability to accelerate reactions. While enzymatic k_{cat}/K_m values are typically in the range of 10^5 to 10^8 M^{-1} s^{-1}, this value for a majo-rity of catalytic antibodies is in the range of 1 to 10^3 M^{-1} s^{-1}. For example, amino-acylation of the 3′-hydroxyl group of thymidine with an alanyl ester can be catalysed by an antibody raised against a phosphonate diester with a k_{cat}/K_m value of only about 10^3 M^{-1} s^{-1}.

[3] Michaelis–Menten constant (K_m) is the substrate concentration at which the reaction rate is half its maximal value. In other words, it is the concentration of substrate at which half the active sites are filled. It provides a measure of the substrate concentration required for significant catalysis to occur.

Antibody (Function)	$k_{un}(s^{-1})$	$k_{cat}(s^{-1})$	$K_m(M)$	$k_{cat}/K_m(M^{-1}S^{-1})$
38E1 (phosphatase)	2.5×10^{-9}	0.00002	1.6×10^{-4}	0.13
13D11 (amide hydrolase)	1.3×10^{-9}	1.7×10^{-7}	4.3×10^{-4}	3.9×10^{-4}
43D4 – 3D12 (elimination)	3.5×10^{-8}	0.0031	0.18	1.7×10^{-2}
2H12E4 (esterase)	1.2×10^{-6}	0.00031	1.5×10^{-5}	21
11F1 – 2E11 (mutase)	4.5×10^{-6}	0.045	2.6×10^{-4}	1.7×10^{2}
19F3.1 (thiol S – transferase)	1.2×10^{-6}	0.0022	1.3×10^{-2}	0.17
3B9 (cocaine hydrolase)	3.3×10^{-6}	0.0018	4.9×10^{-4}	3.7
34E4 (decarboxylase)	3.1×10^{-5}	0.66	1.2×10^{-4}	5.5×10^{3}
18C10 (peptide ligase)	1.1×10^{-3}	0.21	4.0×10^{-3} (RCOX)	52 (RCOX)
7D4 (Diels – Alderase)	2.9×10^{-6}	2×10^{-5}	2.7×10^{-3} (diene)	7.4×10^{-3} (diene)

Table 1. Kinetic constants for some antibody-catalyzed reactions.

One reason for the poor rates shown by catalytic antibodies could be due to the slow release of product from the binding site. But a more critical reason for the poor performance of catalytic antibodies could be the poor design of the hapten that can induce the required active-site geometry and functionality. A major criticism of catalytic antibodies is that their turnover (k_{cat}) is lower than that of conventional enzymes. Only antibodies that stabilize the antigen transition state (TS) more than the ground state (GS) can display catalysis and the turnover is proportional to the difference between the free energy obtained from TS and GS binding. As Cornish–Bowden comments, "One wonders whether achievements by catalytic antibodies to date have justified the excitement".

Patho-physiological Occurrence of Catalytic Antibodies

Antibodies with proteolytic and other catalytic activities have been characterized in the blood and mucosal secretions of humans. These findings were naturally met with some skepticism. However, it is now realized that specific catalytic antibodies are possible if one combines the natural reactivity and non-covalent recognition of epitope regions remote from the reaction center. Catalytic antibodies may be generated spontaneously by

Box 4.

Factor VIII is a glycoprotein pro-cofactor that circulates in the bloodstream after synthesis. In circulation, it is mainly bound to von Willebrand Factor (vWF) to form a stable complex. Upon activation by thrombin or factor Xa, it dissociates from the complex to interact with factor IXa of the coagulation cascade. It is a cofactor to factor IXa in the activation of factor X, which, in turn, with its cofactor factor Va, activates more thrombin. Thrombin cleaves fibrinogen into fibrin, which polymerizes and cross-links (using factor XIII) to form a blood clot at the site of injury. The lack of factor VIII may lead to severe bleeding complications.

the immune system, without prior immunization. Catalysts formed by natural immune mechanisms have been identified by several groups. Patients with several autoimmune diseases have been described to be positive for catalytic auto-antibodies. Sudhir Paul and coworkers isolated from patients with autoimmune diseases polyclonal antibodies that are capable of catalyzing the hydrolysis of the glutamate-methionine peptide bond in the physiologically occurring vasoactive intestinal peptide (VIP). VIP-specific catalytic autoantibodies have been observed only in individuals with disease even though healthy humans also produce VIP-binding antibodies.

LaCroix-Desmazes and coworkers observed that a subpopulation of Haemophilia A patients receiving Factor VIII therapy as replacement for the deficient endogeneous Factor VIII developed some IgG class anti-Factor VIII antibodies which hydrolyze Factor VIII (see *Box* 4). This is the first example of catalytic antibodies with a possible role in the etiology of a disease. Proteolytic antibodies specific for thyroglobulin and prothrombin have been reported in patients with Hashimoto's thyroditis and multiple myeloma respectively. Nucleic acid hydrolyzing antibodies have been isolated from the serum of patients with systemic lupus erythematosus, multiple sclerosis and rheumatoid arthritis.

The importance of natural immunological mechanisms in producing artificial catalysts is exemplified by the reports describing increased synthesis of esterase antibodies in autoimmune mice compared to normal mice in response to transition-state analogue immunizations. It has been suggested that catalytic antibodies may participate in the (a) maintenance of homeostasis of the organism and (b) in the defense of the organism against infections. Wentworth and coworkers have shown that all antibodies have bactericidal activity in that they are able to promote the production of hydrogen peroxide and ozone. Antibodies harbouring protein kinase and DNase activity have been described in normal human milk. In general, it has been observed that when the homeostasis of the immune system is perturbed, during

pregnancy or during autoimmune or inflammatory disorders, the amount of catalytic antibodies increase.

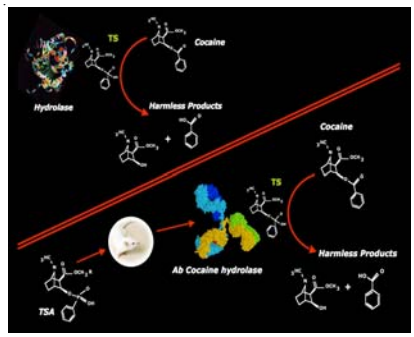
Since the first reports that antibodies can be programmed to perform 'enzyme-like' biocatalytic processes, the therapeutic potential of catalytic antibodies has been a source of considerable speculation. Antibodies that have the ability to recognize foreign antigens *in vivo*, such as in HIV infection or septicaemia and destroy them, could revolutionize the field of immunology. On the other hand, antibodies that can perform the function of the complement system and phagocytes offers exciting possibilities in effector disease state where the immune system of the body is destructive, as in autoimmune disorders or after organ transplant. In such cases, catalytic antibodies could be targeted to the body's own immune system components and destroy them, thus causing an amelioration of the disorder. Considerable progress has already been made in the application of antibody catalysts to a number of biomedically relevant areas. The inherent short plasma half-lives and associated peripheral and non-specific toxicity of reactive therapeutics, particularly in the area of cancer and viral chemotherapies, is a serious limitation to their application and results in numerous undesirable side effects. There are now intensive efforts underway to improve the pharmacodynamic and toxicological profiles of such agents. Antibody-directed enzyme prodrug therapy (ADEPT) is a two component strategy that comprises of an antibody-enzyme conjugate and an anticancer prodrug. The antibody binds to a tumor-associated antigen and thus shuttles the enzyme to the tumor surface. A non-toxic prodrug of an anticancer agent is administered. The enzyme then hydrolyses the inactive prodrug into its active form at the tumor surface. This therapy can be limited by the immunogenicity of the enzyme, usually of non-human origin, which triggers the destruction of the ADEPT conjugate by the host's immune system. However, prodrug activation by a humanizable catalytic antibody could be a viable solution. A number of prodrug/ antibody catalyst systems have been designed to improve the profile of a range of anticancer drugs.

Since the first reports that antibodies can be programmed to perform 'enzyme-like' biocatalytic processes, the therapeutic potential of catalytic antibodies has been a source of considerable speculation.

Summary and Future Perspectives

Since 1986 when catalytic antibodies were discovered, the number and diversity of antibody-catalyzed reactions has been growing. While greater than seventy chemical reactions have been catalysed by antibodies, this is still a fraction of the plethora of accessible transformations. A considerable number of reactions have been catalyzed using antibodies ranging from amide bond cleavage to carbon-carbon bond forming and breaking reactions. Characterization of catalytic antibodies is providing insights into fundamental notions of enzymatic catalysis. More than 150 antibodies have been characterized that catalyze a wide variety of chemical reactions, including difficult transformations that are kinetically unfavourable in the absence of the antibody catalyst. There has been an increase in research activity aimed at understanding the structure-function relations of antibody catalysis. One of the goals of future studies is to acquire adequate insight into the mechanisms of abzymes such that improvements in hapten design can be made. The next few years should likely see an emphasis on increasing the catalytic efficiency of antibodies by generating active sites in which catalytic groups, cofactors and entropic effects work in concert. Clearly, the demonstration of the generality of catalytic antibodies and their role in understanding fundamental aspects of catalysis has generated excitement and opened new horizons of scientific and industrial opportunity that bridge enzymology, immunology and chemistry.

Figure 5. Catalytic antibodies are capable of performing almost any type of reaction with high selectivity and stereospecificity. They can even perform reactions that are not catalyzed by endogenous enzymes. For instance, catalytic antibodies have been generated that cleave the cocaine molecule at specific bonds, thereby eliminating the toxic effect of the drug. In fact, catalytic antibodies are peerless designer catalysts because of their programmability and ability to catalyze an amazing diversity of reactions.



There are a number of chemical transformations which have energetic or steric requirements which make them particularly amenable to antibody catalysis. The ability to generate stereospecific and regiospecific catalysts could be important to the pharmaceutical and fine chemical industries for the synthesis and manufacture of new and purer drugs. At present there are only a few examples of antibodies catalyzing reactions for which there are no known biocatalysts and it is arguably this ability that offers the greatest immediate potential for their exploitation. Antibodies that catalyze efficient aminoacylation reaction could be used

for facilitating aminoacylation of tRNAs with novel amino acids for incorporation into proteins and studies of protein biosynthesis. Unlike highly evolved enzymes, catalytic antibodies may reveal design features about primitive forms of protein-substrate interactions which will help track enzyme evolution.

Although catalytic antibodies have the advantages of permanent antigen destruction and turnover of multiple antigen molecules per antibody molecule, no proteolytic antibodies have entered any clinical trials. Although the exploitation of catalytic antibodies for therapeutic purposes is still in its infancy, there does exist the exciting possibility of developing some of these proteolytic antibodies to treat diseases like HIV infection and Alzheimer's disease.

The ability to use catalytic antibodies in organic synthesis, medicine, and industry raises the same issues as with other enzymes, such as turnover, accessibility and cost.

The boundaries of chemistry and biology are without doubt going to be pushed back rapidly and in the next ten years, this exciting and challenging research area of chemical biology i.e., the potential of abzymes in immunotherapy, will become more clearly defined.

Address for Correspondence
Desirazu N Rao and
Bharath Wootla*
Department of Biochemistry
Indian Institute of Science
Bangalore 560 012, India.
Email:
dnrao@biochem.iisc.ernet.in

* Centre de Recherche des Cordeliers, Université Pierre et Marie Curie – Paris 6, Équipe 16, UMR S 872, Paris, F-75006 France
Email: Bharath.Wootla@crc.jussieu.fr

Suggested Reading

[1] L Pauling, Chemical Achievement and Hope for the Future, *American Scinetist*, Vol.36, pp.51, 1948.

[2] P G Schultz and R A Lerner, From Molecular Diversity to Catalysis: Lessons from the Immune System, *Science*, Vol.269, pp.1835–1842, 1995.

[3] R A Lerner and S J Benko-vic, Principles of Antibody Catalysis, *Bioessays*, Vol.9, pp.107–112, 1988.

[4] S Lacroix-Desmazes, B Wootla, S Delignat, S Dasgupta, V Nagaraja, M D Kazatchkine and S Kaveri, Pathophysiology of catalytic antibodies, *Immunol. Letts*., Vol.103, pp.3-7, 2006.

[5] L H Jones and P Wentworth, The therapeutic potential for catalytic antibodies: From a concept to a promise, *Mini Reviews in Medicinal Chemistry*, Vol.1, pp.125–132, 2001.

[6] S Paul, Y Nishiyama, S Planque and H Taguchi, Theory of proteolytic antibody occurrence, *Immunol. Letts*., Vol.103, pp.8–16, 2006.

[7] D Hilvert, Critical analysis of antibody catalysis, *Annu. Rev. Biochem*., Vol.69, pp.751–793, 2000.

[8] R A Lerner, S J Benkovic and P G Schultz, At the Crossroads of Chemistry and Immunology: Catalytic Antibodies, *Science*, Vol.252, pp.659–667, 1991.

[9] H Surya Prakash Rao, Capping Drugs: Development of Prodrugs, *Resonance*, Vol.6, No.2, pp.19–27, 2003.