Recently, reports on two enzyme-like substances increased our interest in enzymes. One is “ribozyme,” which is composed of RNA, and catalyzes the making and breaking of phosphodiester bonds (1). The other is the catalytic antibody, which catalyzes a given chemical reaction. The catalytic antibody is also termed “abzyme” (2), which was derived from antibody enzyme, and this term will be mainly used in this review for simplicity. Since the first report on the abzymes, over 80 papers, many reviews and comments, and news have been published. As there have been many reviews, I will briefly describe the background of abzyme studies and the present knowledge of the enzymic mechanism as an introduction. Then, I will focus on examples of abzymes from viewpoints that the previous reviews did not cover well. As only a limited number of references can be cited, some important papers may be missed in the references, but can be found in the review articles (3-5).

**Background**

**History:** A detailed description of the historical background is given in Green’s review (2). The basis for the production of catalytic antibodies is in Pauling’s lecture on the action of enzymes. He stated that enzymes are complementary to the structures of the transition states of the reactions that they catalyze. This led Jencks to propose that an enzyme can be produced by making an antibody against the transition state analog of the reaction concerned. The first abzymes produced accelerated the hydrolysis of esters about $10^3$-fold (5, 6). Since then abzymes catalyzing various types of reactions have been reported, but the activities of not many abzymes approach those enzymes.

**Antibodies:** The prominent characteristics of antibodies are their diversity and specificity. The most representative antibody is IgG, which is composed of two heavy (H) and two light (L) chains. If B lymphocytes of the immune system are stimulated by an antigen, then an antibody will be generated. At least $10^5$ different antibody molecules may be generated by an antigen, and the number will increase with the somatic mutations (7, 8). The antibody binds strongly with the antigen and the dissociation constants of the ligand are around $10^{-5}-10^{-8}$M (9), and the highest dissociation constant was reported to be $10^{-14}$M (10). The binding of the ligand to the antibody is highly specific and small structural changes of the ligand prevent its binding. This nature of an antibody makes us to anticipate that a highly specific antibody can be generated against almost every substance. Usually, it is very hard to get antibodies against substances of less than $M_r$ 1,000, but using a hapten-carrier protein we can generate antibodies against smaller substances (11). These facts allow us to generate “tailor-made catalysts” using the immune system.

**Catalytic Mechanism of the Enzymic Reaction**

It is worth looking over the present knowledge of the catalytic mechanism of enzymes. Enzymic reactions are characterized by two principal properties, high catalytic efficiency and selectivity. Before discussing the mechanism, I would like to present some examples of how much natural enzymes can accelerate enzymic reactions. There are not so many examples (Table I), since the rates of uncatalyzed reactions are not easy to measure. The acceleration ranges from $10^6-10^{16}$, so “enzyme-like” catalysts should cause acceleration of around $10^6$.

Over the years numerous hypotheses and explanations have been put forward to account for enzymic catalysis. Page listed no fewer than 21 published theories (23). Recently, the “transition state stabilization” theory for enzymic reactions has seemed popular. The idea was originally proposed by Pauling. In this theory, the active site of an enzyme is precisely complementary to the reactants in their activated transition state geometry.
TABLE I. Rate acceleration by enzymes.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>$k_{\text{cat}}$ (s$^{-1}$)</th>
<th>$k_{\text{uncat}}$ (s$^{-1}$)</th>
<th>Accelerated ($k_{\text{cat}}/k_{\text{uncat}}$)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine deaminase</td>
<td>$1.8 \times 10^{-14}$</td>
<td>372</td>
<td>$2.1 \times 10^{12}$</td>
<td>12, 13</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>$10^{-15}$</td>
<td>$16^{6}$</td>
<td>$1.6 \times 10^{10}$</td>
<td>14, 15</td>
</tr>
<tr>
<td>Chorismate mutase</td>
<td>$2.6 \times 10^{-5}$</td>
<td>50.4</td>
<td>$1.9 \times 10^{6}$</td>
<td>16</td>
</tr>
<tr>
<td>Peptidase</td>
<td>$3.0 \times 10^{-5}$</td>
<td>44</td>
<td>$5.1 \times 10^{6}$</td>
<td>17, 18</td>
</tr>
<tr>
<td>Triose phosphate</td>
<td>$2.1 \times 10^{-7}$</td>
<td>$1.3 \times 10^{3}$</td>
<td>$6.2 \times 10^{10}$</td>
<td>19, 20</td>
</tr>
<tr>
<td>Isomerase</td>
<td>$1.6 \times 10^{-46}$</td>
<td>$3.7 \times 10^{4}$</td>
<td>$2.3 \times 10^{10}$</td>
<td>21, 22</td>
</tr>
</tbody>
</table>

*The values at 85°C extrapolated to 37°C. *Calculated by assuming a subunit $M$ of 45,000. *Calculated by using the Arrhenius eq. in Ref. 21.

Thus, an enzyme strongly binds to the transition state and greatly increases its concentration, and thereby accelerates the reaction proportionately. The theory emphasizes the binding of the enzyme with the transition state, but not that with the substrate. The importance of substrate to enzyme binding was mentioned by several authors. Here I would like to refer to the papers by Hackney (24) and Menger (25). The idea is that two principal properties of enzymes are ultimately derived from the binding of the substrate molecule at the active site and the subsequent stabilization of the transition state. Figure 1 presents a reaction coordinate diagram for the change in free energy on conversion of a hypothetical substrate, S, to product, P, via a high-energy transition-state species, TS. The nonenzymatic reaction proceeds along the solid line in Fig. 1. The transition state energy barrier is given by $\Delta G^*$. The same energy barrier results if the transition state for the uncatalyzed reaction must be generated before it can bind to the enzyme and be stabilized [solid line from (E+S) to (E+TS), then broken line to (E-TS)]. This case will cause no acceleration. So the binding of the substrate must occur at some stage before the complete conversion to the transition state (dotted line in Fig. 1). The energy barrier is $\Delta G^*$. Menger demonstrated schematically that the transition state stabilization is indeed a source of catalysis, but not the only one, and that the destabilization of the substrate via enforced distances and desolvation at the reactive site are also a direct repository of catalytic potential (25).

Advances regarding triosephosphate isomerase may be helpful for abzyme studies. This enzyme is an almost perfectly evolved one, catalyzes the interconversion of R-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, and possesses a 10 residue mobile "loop" that interacts directly with the phosphate of a substrate. But the loop is not directly involved in the actual enolization. Knowles proposed that the loop closes over a substrate upon binding to it, and impedes wasteful liberation of an unstable reaction intermediate, cis-enediol (26). Other enzymes contain a mobile segment (loop), and substrate-induced loop closure has been demonstrated for enzymes such as hexokinase (27), lactate dehydrogenase (28), and phosphoglycerate kinase (29). There is no doubt that these excellent features of enzymes were obtained through an evolutionary effect that has so far been spent on their improvement as catalysts. How can we generate abzymes having these characteristics?

**Monoclonal Catalytic Antibodies**

General procedures for obtaining monoclonal abzymes and examples of them were well documented in recent reviews and references cited therein (3, 4). So some of the problems encountered in obtaining abzymes and some examples of abzymes are mentioned briefly.

To generate abzymes, one must first design and synthesize the transition state analog for a desired reaction. The problem is to synthesize the transition state analog, but this may be solved through the cooperation with organic chemists.

Hybridoma technology is generally used for the generation of monoclonal antibodies (30). The direct screening of culture supernatants of such hybridomas for antibody catalysis hitherto was not possible due to mainly the low activity of the abzymes, so the catalytic activity can be examined only after the monoclonal antibodies has been obtained. The low abundance is also a problem. It is not a rare case not to obtain even one monoclonal abzyme after preparing a number of monoclonal antibodies. This costs a lot. To overcome these problems, various modifications have been made (3, 4). One is the non-hybridoma methodologies, such as combinatorial variable-region cloning in phages, by which we can select abzymes from among millions of possibilities (4). In this case too, a method for detecting catalytic activity in the early stage of cloning has been required. Green's group (31) invented a simple and sensitive screening method which can be applied to various types of reaction, substrates, or catalysts (enzymes, abzymes, and chemical catalysts). The method is termed catELISA. Substrates attached to bovine serum albumin are immobilized on ELISA plates, and then the plates are incubated with anti-hapten (transition state analog) antibodies, which may contain abzymes to catalyze the immobilized substrates to the products via the transition states. Then the products on the plates are incubated with rabbit serum containing anti-product antibodies. The rabbit antibodies bound to the immobilized products can be detected using the enzyme-labeled anti-rabbit IgG antibodies. The feasibility of the method was demonstrated by the generation of potent ester-hydrlyzing antibodies by direct screening of hybridoma supernatants (31). The
following are some reactions catalyzed by monoclonal abzymes reported so far (Table II).

a. Hydrolysis of esters: Hydrolytic abzymes for esters were first generated by mimicking the transition state for the hydrolysis of methyl p-nitrophenyl carbonate with the phosphonate ester (32). Using the same phosphonate as the antigen, Ono et al. (33) generated abzymes that catalyze the hydrolysis of carbonic esters. Interestingly, one of the abzymes catalyzed the hydrolysis of [S]2,2-dimethyl-1,3-dioxalane p-nitrophenyl carbonate with an unexpectedly high rate of acceleration ($6.4 \times 10^4$). The acceleration was explained as being due to the stabilization of the transition state on binding of the dioxalane part of the substrate to a site apart from that of chemical transformation. Chemical modification studies indicated the presence of a subite in both the H and L chains of the abzyme (Ref. 33 and our unpublished observation).

One of the common drug dependencies, addiction to cocaine, has proved to be very difficult to treat. Hydrolysis of cocaine’s benzoyl ester yields eegonine methyl ester and benzoic acid, fragments that retain none of cocaine’s stimulant or reinforcing activities. The transition state of the benzoyl ester cleavage reaction resembles the tetrahedral intermediate of second-order ester hydrolysis. Landry et al. (34) generated monoclonal antibodies by immunizing mice with a phosphonate monoester transition state analog of cocaine’s benzoyl ester cleavage reaction. They obtained two abzymes out of 29 monoclonal antibodies against the transition state analog. One abzyme had comparable activity to the enzyme, butyryl choline esterasse. This work is the exciting first movement of the potential use of abzymes for the treatment of human problems.

b. Amide hydrolysis: One of the major goals of abzyme studies is to generate a sequence-specific protease, since there are a few restriction peptidases (proteinases) including the residue-specific proteases so far reported. The first and maybe only restriction proteinase is coagulation factor Xa. The human (bovine) factor Xa is a Ser protease which recognizes the amino acid sequence of -Ile-Asp(Glu)-Gly-Arg- and cleaves the peptide bond on the C-terminal side of the Arg residue (35). Like the factor being used in gene technology, sequence-specific proteases may have applications in various fields.

The hydrolysis of peptide bonds has been referred to as an energy-demanding reaction. But even the activation energy of the reaction is not available to the best of my knowledge. Here the activation energy of peptide bond hydrolysis was roughly estimated by using the rate of hydrolysis of glycinamide (36) and that of Phe-Oly bonds (17). The value was approximately 32.5 kcal/mol, which is actually higher than the activation energy of the hydrolysis of carbonic acid (21 kcal/mol).

It was established that a zwitterionic tetrahedral intermediate is involved in the hydrolysis of amide bonds. A phosphonamidate analog could be a true transition state analog for the hydrolysis of an amide. Antibodies against a phosphonamidate hapten were generated, and one of the antibodies showed efficient catalytic activity. The rate enhancement was unexpectedly high, being $1.5 \times 10^9$ over the spontaneous hydrolysis at pH 9 and 37°C (37). Though the abzyme was generated against a single transition state analog, the abzyme promotes the hydrolysis of an anilide ester using a multistep kinetic sequence similar to that found in the serine protease-catalyzed reaction (4, 37). It is interesting how these enzyme-like characteristics of the abzymes were obtained during the short immunization period.

Many enzymes require nonamino acid cofactors for catalysis. The antibodies elicited against a hapten, Co(III)-triien-peptide complex, catalyzed the cleavage of a peptide bond sequence—specifically with Zn(II)-triien as a cofactor (38). This may be the first examples of the successful construction of a cofactor binding site in an abzyme, so studies on the antigen-catalytic activity relationship of the abzyme may lead to the production of tailor-made restriction proteasizes. To extend the catalytic activity of abzymes, some works were reported on the construction of cofactor binding sites in abzymes. But the catalytic activities so far reported were far below those of natural enzymes (3, 4, 40).

c. Cyclization: Through simple transition state stabilization, we cannot anticipate the generation of abzymes exhibiting rate acceleration higher than $10^6$–$10^4$ (18). So, we should rather attempt to generate abzymes for reactions not known to be catalyzed by enzymes. One such example is the antibody-catalyzed Diels-Alder reaction. This is the reaction between a diene and alkene giving rise to a cyclohexene product. A hapten that contained a bridge which locks the cyclohexene ring into a conformation resembling the transition state was synthesized (40). An antibody generated to this hapten enhanced the Diels-Alder reaction relative to the background level. This is one of the examples in which antibodies were used to lower the translational and rotational activation entropy of a reaction, and others in this category are those catalyzing Claiser rearrangement (41) and transacylation reactions (42, 43).

The chemically favored cyclization of an epoxyalcohol is the formation of tetrahydrofuran, but not tetrahydropyran, though the energy barriers for the favored and disfavored processes were only a few kcal/mol. Janda et al. (44) generated abzymes to catalyze this highly disfavored reaction (Table II). This may be the first example of the successful use of an antibody as a true transition state catalyst for accelerating a chemically disfavored transformation. The principles demonstrated in this study may be applicable to other disfavored chemical processes.

d. Decarboxylation: One mechanism of enzymic catalysis is the destabilization of charged substrates by extraction from an aqueous solution into the low-dielectric environment of the protein binding pocket. But it has been difficult to measure the extent to which desolvation contributes to the catalytic efficiency of enzymes. Using a model system of decarboxylation and an abzyme which catalyzes the decarboxylation, the effect of desolvation on decarboxylation was estimated (45). A model system that is sensitive to solvation in organic solvents is the decarboxylation of substituted carboxy benzisoxazoles. This reaction proceeds through a concerted, intermediateless process. Monoclonal antibodies against the transition state analog of the reaction were generated and an abzyme that catalyzes the decarboxylation of 5-nitro-3-carboxybenzisoxazole with $1.9 \times 10^4$-fold acceleration over the rate in aqueous buffer was obtained. As judged from the carbon kinetic isotope effects on the spontaneous and abzyme-catalyzed decarboxylation of the compound (45), the structure of the transition state does not undergo a significant change caused by organic solvents or by a protein binding pocket. Therefore, the
**TABLE II. Examples of abzymes.**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Hapten&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Substrate</th>
<th>Products</th>
<th>Comments&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Ref.</th>
</tr>
</thead>
</table>
| Ester hydrolysis             | ![Ester hydrolysis](image) | ![Ester hydrolysis](image) | ![Ester hydrolysis](image) | A = 29 min<sup>-1</sup>  
                              |                    |                       |                   | B = 1.6 × 10<sup>4</sup>  | 32  |
| Amide hydrolysis             | ![Amide hydrolysis](image) | ![Amide hydrolysis](image) | ![Amide hydrolysis](image) | A = 0.11 min<sup>-1</sup>  
                              |                    |                       |                   | B = 540                | 34  |
| Cyclization (Diels-Alder)    | ![Cyclization (Diels-Alder)](image) | ![Cyclization (Diels-Alder)](image) | ![Cyclization (Diels-Alder)](image) | A = 4.3 min<sup>-1</sup>  
                              |                    |                       |                   | B = 110                | 39  |
| (Disfavored)                 | ![Disfavored](image) | ![Disfavored](image) | ![Disfavored](image) | A = 0.91 min<sup>-1</sup>  
                              |                    |                       |                   | 44  |
| (Lactonization)              | ![Lactonization](image) | ![Lactonization](image) | ![Lactonization](image) | A = 0.50 min<sup>-1</sup>  
                              |                    |                       |                   | B = 167                | 42  |
| Amide bond formation         | ![Amide bond formation](image) | ![Amide bond formation](image) | ![Amide bond formation](image) | A = 0.066 min<sup>-1</sup>  
                              |                    |                       |                   | B = 1.6 × 10<sup>4</sup> | 43  |
| Claizen rearrangement        | ![Claizen rearrangement](image) | ![Claizen rearrangement](image) | ![Claizen rearrangement](image) | A = 2.7 min<sup>-1</sup>  
                              |                    |                       |                   | B = 1 × 10<sup>6</sup>  | 41  |
| Decarboxylation              | ![Decarboxylation](image) | ![Decarboxylation](image) | ![Decarboxylation](image) | A = 17 min<sup>-1</sup>  
                              |                    |                       |                   | B = 1.9 × 10<sup>4</sup> | 45  |
| Trityl hydrolysis            | ![Trityl hydrolysis](image) | ![Trityl hydrolysis](image) | ![Trityl hydrolysis](image) | A = 0.1 min<sup>-1</sup>  
                              |                    |                       |                   | B = 270                | 46  |
| Peroxidation                 | ![Peroxidation](image) | ![Peroxidation](image) | ![Peroxidation](image) | A = 394 min<sup>-1</sup>  
                              |                    |                       |                   | 40  |

<sup>a</sup>R = tether carrier.  
<sup>b</sup>A = k<sub>cat</sub>; B = k<sub>cat</sub>/k<sub>uncat</sub>.  
<sup>c</sup>Values obtained for monoclonal abzymes, see Ref. 54 for details.
great acceleration ($10^4$) by the abzyme was ascribed almost entirely to the desolvation.

**Polyclonal Catalytic Antibodies**

The abzymes first generated were monoclonal antibodies and early attempts to produce polyclonal catalytic antibodies failed (2). Shokot and Schultz suggested that the early lack of success in producing abzymes may have been due to the use of polyclonal rather than monoclonal antibodies (46). These reports may have discouraged investigation of the possibility of producing polyclonal abzymes. However, reports of the production of polyclonal abzymes which catalyze various reactions have been accumulating. Moreover, human autoantibodies catalyzing hydrolytic reactions have been reported. The advantages of polyclonal abzymes may be as follows: (i) the relative simplicity, rapidity, and low cost of producing polyclonal as against monoclonal antibodies, (ii) their use as catalysts for technological applications, (iii) their use for the assessment of potential therapeutic strategies involving catalytic activity induced in the serum, and (iv) for hapten design by analyzing sets of structurally related haptenes and comparing catalytic activities of the resulting immune response, as measured with polyclonal antibodies. The following are some polyclonal abzymes reported so far.

a. **Human polyclonal abzymes**: The first polyclonal abzymes described were for the hydrolysis of the human neuropeptide, vasoactive intestinal peptide (VIP) (47). VIP is a 28-amino acid peptide, and known as a neuropeptide widely distributed in the central and peripheral nervous systems. In the human airways, VIP is believed to be a mediator of relaxation of smooth muscle. Paul et al. (48) prepared autoantibodies from asthma patients with high VIP-binding affinity. Two out of six immune IgG preparations showed hydrolytic activity. One of the hydrolytic IgG preparations cleaved the Gln$^{16}$-Met$^{17}$ bond. The activity was shown not to be derived from contamination by peptidases according to several criteria, including that the Fab fragments showed hydrolytic activity. Hydrolysis at positions different from the Gln$^{16}$-Met$^{17}$ bond was observed for IgG from a patient with obstructive airway disease and a healthy individual (48). One problem in explaining the pathophysiology of asthma is that an unknown inhibitor is believed to be tightly bound to the abzymes under physiological conditions. The antigen for the abzymes is unknown. But the possibility of VIP being the antigen was shown by the fact that monoclonal antibodies capable of hydrolysing VIP were generated by using VIP as an antigen (49).

Structural information on the VIP hydrolyzing antibodies and the antigen–antibody relationship of the abzymes will help us to produce sequence-specific proteinases.

DNA-nicking activity in the sera of patients with a systemic lupus erythematosus (SLE) was detected (50). The activity corresponded to the size of IgM and IgG. The DNA hydrolyzing activity was stable as to acid shock, and the DNA degradation pattern was different from those of DNAase I and blood DNAase. The correlation of the enzymic activity to the physiology of SLE remains to be established.

b. **Hydrolitic abzymes**: The first polyclonal abzymes generated were obtained by immunizing sheep with 4-nitrophenyl acetate. The polyclonal antibodies produced catalyzed the hydrolysis of aryl carbonate (51). The hydrolytic activity of the abzymes was shown to be substantially better in most respects than those of analogous reactions of two other carbonic esters catalyzed by monoclonal abzymes. Gallacher et al. (52) generated amide-hydrolytic polyclonal abzymes using the same antigen as used for the esterolytic abzymes. The enhancement of the amide hydrolysis by the abzymes was over 102. To show that the catalytic activity in the polyclonal antibody preparations produced was not due to contaminating hydrolytic enzymes, Gallacher et al. (53) generated anti-4-nitrobenzyl, 4-‘(4-aminobutoxy)benzyl sulphone and anti-4-nitrophenyl-4‘-(carboxymethyl) phenyl hydrogen phosphate IgG, and found that the catalytic activity was only observed for the anti-phosphonate, but not for the anti-sulphone IgG. This confirms that the polyclonal abzymes were not contaminating enzymes.

Polyclonal abzymes for hydrolysing 4,4‘,4’‘-trimethoxytrityl ether by immunizing a rabbit with tris(4-methoxyphenyl)-(6-carboxyhexyl) phosphonium bromide hapten were generated (54). Monoclonal abzymes catalyzing the hydrolysis of triyl esters had already been generated. The apparent catalytic rate enhancement was 125. It was observed for the first time that the catalytic activity appeared later as compared with the hapten binding. An approach like this would provide a facile method for studying immunological aspects of the catalytic immune response.

c. **Anti-idiotypic abzymes**: Joron et al. (55) reported anti-idiotypic antibodies with catalytic activity. The monoclonal antibody, AE-2, raised against human erythrocyte acetylcholine esterase (AchE) has been shown to inhibit the enzymic activity. They used the property of AE-2 that the epitope is putatively the anionic active site. Antisera were obtained by immunizing rabbits with monoclonal antibody AE-2, and anti-idiotypic antibodies were purified from the serum. The antibodies showed nearly two orders of lower catalytic activity than erythrocyte AchE. This type of abzyme could not be a tailor-made abzyme, since abzymes could only be generated for enzymes already known.

**Concluding Remarks**

It is best to use a single catalytic species for the kinetic, mechanistic, and structural characterization of abzymes. But, there are many problems in producing monoclonal abzymes, though technical inventions have been made. On the other hand, there are several advantages to using polyclonal abzymes, as described in the above sections. I hope that the image of "risky work" of abzyme studies may become much easier to perform. It is recommended to test the feasibility of the design and synthesis of haptenes by generating polyclonal abzymes before trying to obtain monoclonal ones.

At the present stage, I feel that we had better put our energy into studies on the already known abzymes from the aspects of structure–function and antigen–catalytic activity relationships. It may be better to study abzymes that catalyze reactions unexpectedly, as to rate acceleration and substrate specificity.

Several important fields such as recombinant techniques in abzyme production, and abzyme-catalyzed stereospecific and enantioselective transformations were mentioned a little in this review. Reports on these can be found in...
reviews (3, 4) and the references therein.

I would like to express my thanks to my colleagues for their cooperation in the abzyme studies, Dr. Y. Kawamura-Konishi, Ms. E.B. Mukouyama, Dr. S. Sugano (School of Nursing), and Dr. C. Wada. I am also indebted to Dr. T. Kitazume, Tokyo Institute of Technology, for the comments on the manuscript, especially Table II.

REFERENCES

J. Biochem.