0273-2289/94/4702-03—0257$04.80

Immune Recognition, Antigen Design, and Catalytic Antibody Production

ALFONSO TRAMONTANO

IGEN Research Institute, Seattle, WA 98109

ABSTRACT

Catalytic antibodies have been developed by experimental approaches exploiting the analogy between antibody-antigen and enzyme-substrate interaction. Haptens have been prepared to model the electrostatic or geometric attributes of a reaction's transition state and to induce combining sites having appropriate catalytic residues. The relative merits of these design strategies may be gleaned from the apparent activities and efficiencies of the respective catalysts. The implications of screening strategies on the kinetic characteristics of the resulting abzymes are also considered. Combining-site hypermutation provides the variation in the antibody repertoire from which high-affinity clones are selected. The same mechanism can also lead to a subset of antibodies with reduced hapten affinity, but improved catalytic activity. This possibility has not been adequately characterized, but is suggested by a number of considerations. These include the unexplained efficiency and diversity of mechanisms utilized by various antibody catalysts, and the observed catalytic activity of antibodies found in autoimmune serum. This article attempts to assess critically the evidence for rational design of catalytic activity in antibodies. Correlations among abzymes and their relevant models could lead to revised or novel strategies for producing better catalysts.

Index Entries: Catalytic antibodies; hapten design; enzyme mimics; molecular recognition.

INTRODUCTION

The study of antibodies as catalysts represents a promising approach to the design of new enzyme mimics. The characteristic binding specificity of antibodies offers the potential for unique substrate selectivity by catalytic antibodies (abzymes). Strategic approaches to the development of abzymes are guided by insights into chemical mechanisms and by intuition into the details of molecular recognition by antibodies. The use of transition-state analogs (TSA) as haptens for obtaining catalytic antibodies also provides support for the idea that enzymes operate by stabilizing the reaction's transition state, as initially proposed by Pauling (*1*). This primary paradigm has been applied in further examples of catalytic antibody production in recent years. Other notions from the bioorganic field have been used to elaborate additional approaches to eliciting abzymes for specific reactions. These achievements raise expectations for the future development of truly practical, tailor-made catalysts and for fundamental insights that could lead to greater understanding and control of biological catalysis.

As examples of antibody-catalyzed reactions accumulate, their general characteristics begin to emerge. Comparisons to enzymes show both intriguing similarities and contrasts. The field also encompasses the use of abzymes for nonbiological processes that demand regiochemical or stereochemical specificity. For these applications, the comparison to the respective uncatalyzed process is invoked for demonstrating advantages other than simple rate acceleration. In each case, the mechanisms of catalysis and the strategies involved in eliciting the antibodies are of interest. It is useful to address these issues within the context of a broad comparison among relevant catalysts. The ultimate potential or fundamental limitations of these new species are not yet obvious. A perspective is needed from which the present territory can be mapped and a future direction could be charted.

The status of catalytic antibody research can be assessed by examining the kinetic features of various examples within the context of the approaches used to derive them. This overview takes the form of a series of questions: What types of reactions are amenable to antibody catalysis? What strategies are used to obtain diverse abzymes? What are the characteristic rates of reactions catalyzed by antibodies? How efficient are the specific abzymes as compared with other catalysts? To what extent do the current strategies for eliciting catalytic antibodies exploit affinity maturation? Are there natural limits or untapped potential for antibodies as catalysts?

The application of abzymes to diverse reactions suggests that the methodology could indeed lead to a general approach for design of custom catalysts. Meanwhile, these examples provide a growing data set within which meaningful generalities may be found. Catalytic antibody

function can be evaluated by the same criteria as other enzymes. In addition, it can be assessed by the degree to which activities conform to the rational basis on which their design is based.

CLASSES OF REACTIONS CATALYZED

Recent examples of abzymes are applied to reactions with biochemical analogy and to those with only remote biological relevance. Some categories include olefin isomerization, reduction-oxidations, electrocyclic reactions, and addition-eliminations (*2*). A common feature of these examples is the relatively low activation energies and, therefore, readily measured rates of the uncatalyzed reactions. This should not be considered a criterion for reactions suitable for catalysis, but merely a convenience for establishing a reference frame for the low to moderate activities that could be expected under specific conditions. With incremental improvements in abzyme activity, the uncatalyzed rate becomes less important.

Simple unimolecular processes, such as lactonization (*3*), decarboxylation (*4*), olefin isomerization (*5*), and the Claisen rearrangement (*6,7*), have attracted attention because of the relatively modest requirements for catalysis. These reactions could presumably benefit from the shape recognition provided by the antibody-binding site in guiding the substrate toward a productive conformation or straining it in favor of the transition-state geometry. Other considerations in the choice of reactions for targeting abzymes include processes that introduce a stereochemical center in the product, thus utilizing the potential for the antibody to act as a stereospecific catalyst (*8*), and processes that offer potential synthetic utility, such as the Diels-Alder reaction (*9*). Though turnover numbers and limited substrate conversion may restrict practical application, in each case the example demonstrates a basic approach and describes the appreciable specificities and rate accelerations that are attainable. The broad range of reactions and substrate structures amenable to the technique encourages the view that these catalysts may find a niche for practical use.

Antigenicity is a key feature in generating antihapten antibodies. Most substrates and haptens designed for abzyme research are typically hydrophobic, aromatic compounds that also make good antigens. Although antibodies can be elicited against a variety of small molecules, certain very polar or hydrophilic compounds, such as saccharides, tend to be poor haptens. The consequence of this for catalytic antibody production is likely to be in the designation of substrates. This could indirectly influence the potential for catalysis of certain types of reactions. For instance, poor antigenicity could deter the targeting of certain transformations in which polar groups surround the site of chemical action. Thus, glycosidase activities and endonuclease activities represent highly desirable targets, which have thus far eluded successful demonstration of antibody catalysis.

HAPTEN DESIGN STRATEGIES FOR ELICITING ABZYMES

Three fundamental insights into enzyme mechanisms have contributed to the strategies for hapten design. These include:

1. Recognition of topological changes, or shape, in the transition state;
2. Recognition of developing charge (electrostatics); and
3. The participation of chemical groups in catalysis.

The application of these structure-based strategies assumes that antigen binding can be a reliable means of identifying antibodies with the desired function. Selection of antibodies by screening for hapten binding represents an indirect strategy for identifying abzymes. The potential consequences and implications of this procedure shall be treated separately from the discussion of hapten design strategies.

Shape Recognition

Reactions that proceed through highly structured transition states can presumably be accelerated by providing a highly specific binding site that distinguishes the molecular "shape" of the transition state. Because of this, these processes are considered excellent candidates for antibody catalysts (*6,7,9*). Classical examples of such reactions are the Claisen rearrangement, the Diels-Alder process, and other "no mechanism" (sigmatropic rearrangements, cycloadditions, and pericyclic) reactions. The entropic factor is a principal component to the activation energy, suggesting that a highly ordered transition state is involved. Molecular recognition by the antibody-combining site can be tailored to the presumed steric character of the reaction's transition state. The structure of this entity is simply deduced from the rigid form that the substrate must assume in order to undergo "concerted" bond reorganization. Thus the shape of the complex formed in a sigmatropic process, like the Claisen rearrangement or a Diels-Alder cycloaddition, between a diene and a dienophile is likely to have a rigid cyclic framework that supports the breaking and making of carbon–carbon and carbon–oxygen bonds.

Other reactions that may be catalyzed by the shape recognition mechanism include any process that has an apparent entropic requirement. Antibody catalysis of intramolecular acyl transfer processes has been investigated on the basis that the shape recognition component is likely to reduce the rotational entropy loss for the reaction (*3,10*). Bimolecular processes depend on the probability of the encounter (translational entropy) of two molecules to form a transient complex, and so also come under consideration. Multisubstrate analogs can be devised as mimics for the molecular shape of the reactive complex (*11–13*).

Electrostatic Complementarity

In any chemical reaction, the making or breaking of bonds is accompanied by the development of charged intermediates or charge separation in the transition state. The stabilization of this transient charge character is thought to be a principal feature of enzyme catalysts. Antibodies are also equipped to exploit electrostatics in molecular complexation through ion pairing between antibody and antigen. The relationship between antigenicity and enzymatic activity should therefore be particularly evident in examples where charged intermediates or transition states are modeled by ionic analogs as haptens. The majority of antibodies produced for catalysis of ester (*14–17*) and amide bond hydrolysis (*18*) were elicited against negatively charged haptens. The anionic character of phosphonates and phosphoamidates describes the oxyanionic intermediate or transition state in acyl transfer reactions. These abzymes generally show optimal activity in alkaline pH. This suggests a mechanism where hydroxide ion, or its equivalent, reacts with the ester or amide substrate generating a full negative charge on the transition state. The mechanism for hydrolysis at neutral pH, in which neutral nucleophiles or water participate in the reaction, is best described by charge separation in the transition state. In that case, a zwitterionic hapten would be a more appropriate mimic for eliciting a catalyst that employs such a mechanism.

Since most simple reactions can be described by charge separation or polarization of reactive bonds, this mechanism of catalysis by proteins is likely to be universal. Though catalysis by antibodies can be analyzed in these terms, it is difficult to separate the contribution of the electrostatic component from other possible effects on the mechanism. In any event, the importance of ion pair or electrostatic interactions in both antibody–antigen complexation and in enzyme catalysis points to an important consideration for the design of abzymes.

Acid-Base Complementarily

Molecular recognition in biological systems takes advantage of ion pairing, hydrogen bonding, and hydrophobic interactions between ligands. In enzymes, polar groups and hydrogen bond donors or acceptors can also participate in the chemical mechanism at the active site. Therefore, it could be expected that antigens presenting polar or ionized groups elicit antibodies whose combining-site residues can potentially act as chemical groups in catalysis. Thus, the hapten could be designed so as to lure the specific antibodies in which key residues are properly oriented with respect to a bound substrate. This strategy, alternatively known as a "bait and switch" approach (*19,20*), assumes that the hapten has discrete structural components that represent either substrate

features or the feature required to recruit a complementary chemical group. Since the latter feature is typically an electrostatic functional group, one can debate whether such a strategy is fundamentally different from that described above as electrostatic complementarity. Support for the "bait and switch" mechanism comes from evidence suggesting the presence of the expected catalytic group in the examples of antibody catalysts derived by this strategy. This evidence generally amounts to a pH dependence and protein chemical group modification of the abzyme that are consistent with the predicted mechanism involving general acid or base catalysis. An additional argument for a fundamental difference between this and the previous approach is the apparent lack of analogy between the hapten and the expected transition-state structure.

It is worth noting that the distinctions in the approaches are theoretical, and in practice, there can be contributions from each of these elementary models to any hapten design. For instance, charged groups in the hapten have been used as baits for catalytic groups and to simulate electrostatic properties of reactive intermediates. In both approaches, antibodies have been found that show pH dependence and chemical modification sensitivity implicating the participation of a chemical group in the catalytic mechanism. Perhaps more relevant is the fact that both types of antibodies are selected on the basis of their high affinity for a hapten presenting an ionic group.

CATALYTIC ACTIVITY AND AFFINITY MATURATION

The overt assumption in experimental approaches at obtaining monoclonal antibodies with catalytic activity is that the selection of high-affinity antibodies guides the selection of the best catalysts. The frequent success of the approach supports that premise. Yet it remains unclear whether the limitations to catalytic activity are the result of the upper limit on antibody-hapten affinity or whether the assumption has limited use in further improvement of catalytic activity. Reported kinetic values of various catalytic antibodies could shed light on this question. Simple transition-state theory predicts a correlation between the kinetic ratio k_{cat}/k_{uncat}, as an indicator of the differential free energy of activation, and the thermodynamic ratio K_s/K_{TS}, the differential binding free energy of substrate and transition state (Fig. 1). If the measurable quantities K_m, the Michaelis constant, and K_i, the hapten (TSA) inhibition constant, are reasonable approximations for K_s and K_{TS}, respectively, the experimental values provide a means to test that prediction (*21*).

Examples are noted, in particular where the hapten design emphasizes intramolecular geometric constraints in the transition state, that show reasonable agreement between the kinetic and thermodynamic ratios.

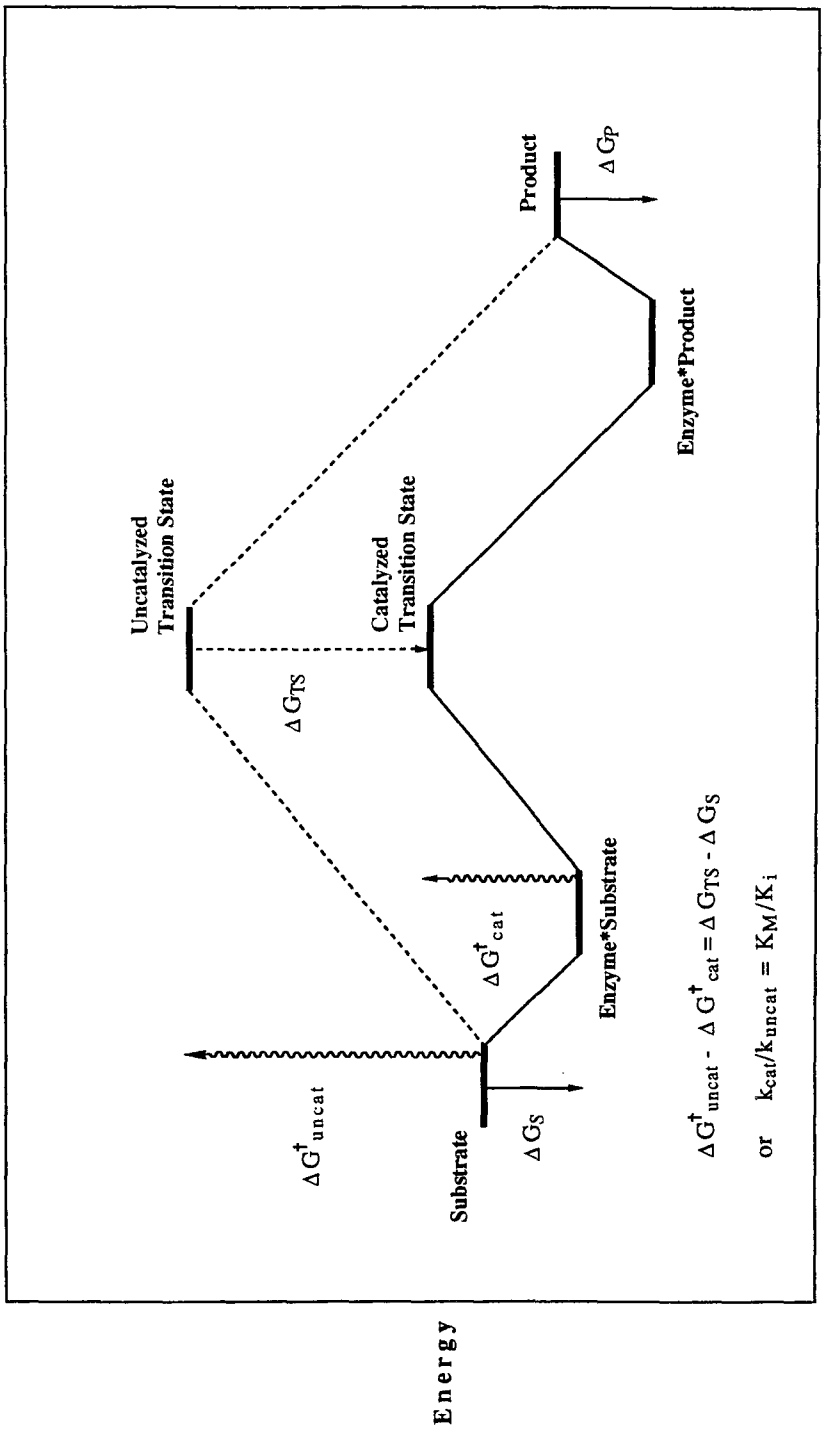


Fig. 1. Free energy diagram for catalyzed and uncatalyzed reactions proceeding through a single (related) transition state The arrows illustrate free energy changes for activation and for binding to the catalyst. In the relationship described, K_i and K_m approximate the values from ΔG_{TS} and ΔG_S, respectively.

Table 1
Correlation of Rate Accelerations
and Differential Stabilization of Substrate and TSA[a,c]

Abzyme[b]	k_{cat}/k_{uncat}	K_m/K_i
Amidase (*19*)	2.5×10^5	37–56
Claisen (*6*)	190	83
(*7*)	1×10^4	28
Esterase (*16*)	7×10^6	10^4
Lactonizing (*3*)	790	10^3
Olefin isomerase (*5*)	1.5×10^4	31
Decarboxylase (*4*)	1.9×10^4	2.6×10^4

[a]Examples are given for relevant unimolecular reactions for which kinetic data are available.
[b]Literature reference cited in text.
[c]$k_{cat}/k_{uncat} = K_m/K_i$.

These defend the premise that the entropic component to the reaction's activation energy is likely to be responsive to the shape complementarity provided by an antibody-binding site. However, a variety of examples (Table 1) show little or no correlation between the quantities. The discordance is particularly obvious between individual examples that target identical reactions and substrates and use the same TSA structure. Thus, it can be maintained that chemical catalysis by combining-site residues is involved in the mechanism of catalysis by abzymes whose kinetic characteristics do not satisfy the relationship. Specifically, the magnitude of k_{cat} and k_{uncat} cannot be related to simple thermodynamic quantities when the transition states for catalyzed and uncatalyzed reactions may differ significantly.

The unexplained efficiency of many catalytic antibodies exposes inconsistencies in the transition-state affinity argument. Although the hapten design strategies have been useful in eliciting antibodies with catalytic activity, it is not clear that high affinity for the hapten has a direct correlation to catalytic activity. For antiphosphonate antibodies, there appears to be a discrepancy between the pH optimum for catalysis of ester and amide hydrolysis and the conditions for binding of the antigen. Since affinity maturation is presumed to take place at physiological pH, it is not clear why catalytic activity should require alkaline conditions for simple catalysis by oxyanion stabilization. Antibodies may present various opportunities for chemical catalysis by nature of the chemical heterogeneity of the combining site. Mechanisms involving protein residues and cofactors as chemical groups, molecular recognition, and macromolecular dynamics describe the unique qualities of proteins as templates for chemical reactions. Molecular recognition alone should be seen as a single dimension in the complexity of structure–activity relationships.

SCREENING STRATEGIES

Methods for obtaining catalytic antibodies from monoclonal hybridomas rely as much on the screening strategy as on the hapten design strategy. The current methods have focused almost exclusively on the preliminary screening of hybridoma supernants by immunoassay to detect binding to the haptens (*22*). Presently, this is recognized as a compromise of convenience. The large number of clones and the limited amount of antibody obtained in cell culture discourage the application of simple catalytic activity assays in direct screening. The difference in rates between the antibody-mediated process and the background process become diminishingly small, in particular when the reaction has a significant uncatalyzed rate or is accelerated by other components of the assay media (*22*).

Prescreening for hapten-binding activity is consistent with the notion that binding free energy is utilized by an enzyme or abzyme to accelerate a chemical process. Thus, the antibodies that exhibit the best affinity for the designer hapten are presumably the best candidates for catalytic activity. If the hapten design includes transition-state analogy, the potential for catalysis by transition-state stabilization is theoretically represented in the set of high-affinity antihapten antibodies. The findings appear to support this assumption since catalytic activity is a frequent attribute of the small number of antihapten antibodies. Control studies showing correlation of activity with the hapten design feature are not routinely done in parallel with the successful production of abzymes. However, in at least one case, a control was included to support the hapten design and screening method (*20*).

There are equally compelling reasons to avoid antibodies with very high affinity for the immunizing hapten. Since the haptens will also present substrate features, the antihapten antibodies are as likely to bind substrates nonproductively. Even though antibodies are found that catalyze the reaction, substrate or product binding may limit the turnover rate and overall efficiency of catalysis. The ability to discriminate between transition state and substrates could be compromised in a screening method dependent on ligand-binding interactions. One modification to screening for catalytic antibodies includes sequential screening to the hapten and to "short TSA" ligands that should identify likely abzymes (*23*). However, binding is not simply described by a single recognition feature. Rather, it depends on a number of interactions that are dispersed over the structure. Substrates or products will most likely also present structural features important for recognition and affinity. Furthermore, if there is any advantage to be obtained from the chemical variability of the antibody-combining site, the potential could be diminished as affinity maturation proceeds to restrict variability. This is suggested by the limited sequence variability found among a set of high-affinity antibodies derived against the same hapten (*24,25*).

The implications of the screening procedure cannot be fully defined until alternative methods are devised. Most recently, the development of a sensitive direct screening of antibodies for catalysis of ester hydrolysis (cat ELISA) has helped to identify abzymes with efficient activity (*26*). This assay retains some of the features of an immunoassay since the substrate and product are processed on a heterogeneous support. Additional formats would be useful for identification of antibodies that are active in dilute solution. As this methodology is more fully elaborated, the range of activities and efficiencies of abzymes and the degree of correlation with antigen-binding activities should become apparent.

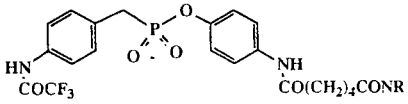
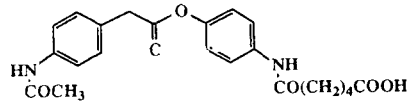
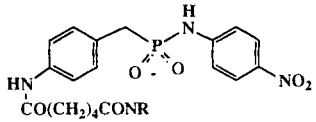
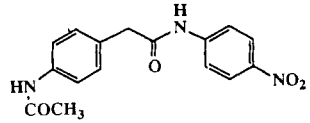
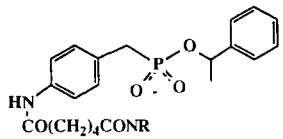
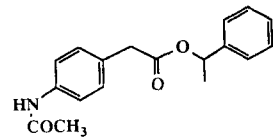
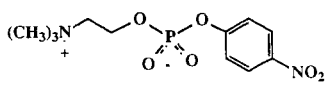
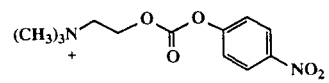
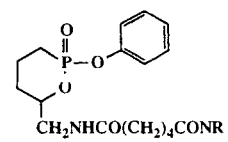
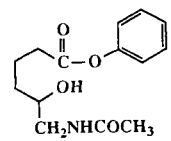
COMPARISONS OF ABYZME CATALYTIC EFFICIENCY

Relative to Uncatalyzed Rates

The efficiency of catalytic antibodies has been expressed in various ways. The most common comparison is the rate acceleration relative to the uncatalyzed reaction as measured under similar conditions. The quantity, defined as the ratio of rate constants k_{cat}/k_{uncat}, demonstrates impressive activity that can reach several million-fold (*16*). As a guide to understanding function, however, the comparison can be misleading if the uncatalyzed and catalyzed reactions proceed through different mechanisms. Often a correction can be made by using the respective general acid or general base-catalyzed reaction deduced from the likely antibody-catalyzed mechanism as a reference reaction. Even so, the comparison is fraught with assumptions. Moreover, this analysis reveals a wide range of rate enhancements among abzymes related through their design strategies. Acyl transfer activities of antiphosphonate antibodies vary by more than four orders of magnitude as expressed by the rate factor (Table 2). However, the rate factor has a practical implication for approaches to a next generation of abzymes. Small rate accelerations are most easily detected when the uncatalyzed reaction has a well-defined rate. Similar accelerations of more difficult reactions will be difficult to detect if a spontaneous reaction cannot be accurately measured. Therefore, it is not possible to say whether the probability of success will be better or worse than found for catalysis of facile reactions.

The relationship of k_{cat} to k_{uncat} has already been considered in defining the role for affinity selection in catalyst design. The factor provides an estimate of the free energy difference between the antibody-bound and the solution transition state, which may relate to thermodynamic quantities measured independently. The assumption of similarity in mechanism between catalyzed and uncatalyzed reactions must hold for this comparison to be meaningful.

Table 2
Rate Enhancements of Acyl Transfer by Antiphosphonate Antibodies

Hapten	Substrate	k_{cat}/k_{uncat}[a]
		6×10^6 (*16*)
		2×10^5 (*18*)
		8×10^4 (*17*)
		800 (*15*)
		790 (*3,21*)

[a] Data from literature reference cited in text.

Table 3
Relative Rates of Abyzmes and Enzymes with Similar Function

Activity	Abzyme[a]	Enzyme	$k_{cat}(Ab)/k_{cat}(Enz)$
Esterase	(17)	Acetylcholinesterase	10^{-3}
		Pig liver esterase	10^{-2}
		α-Chymotrypsin	10^{2}-10^{4}
Claisen Rearrangement	(6)	Chorismate mutase	$3\ 10^{-5}$
	(7)	Chorismate mutase	3×10^{-3}

[a] Example from literature reference cited in text.

Relative to Similar Enzymes

The catalytic efficiency of antibodies, as estimated by the turnover constant k_{cat}, can be directly compared to the k_{cat} of enzymes that are functionally similar. This analysis subordinates the question of mechanistic similarity to the practical utility of the catalyst of interest. Thus, esterase activities of abzymes can be compared to the known lipases or esterases. Enzymes like acetylcholinesterase are very efficient at their task, thus providing a very demanding benchmark for abzyme efficiency. Other enzymes that have been used for ester hydrolysis are significantly less efficient, and some esterolytic abzymes compare favorably in their specific activities (Table 3).

A direct comparison of antibody catalysis to an enzymatic counterpart may have added significance in the case of catalysis of chorismic acid to prephenic acid owing to the presumably uncomplicated mechanism of the Claisen reaction. Two independent examples of catalytic antibodies for the same reaction were derived by essentially the same strategy, utilizing identical haptens (*6,7*). The two antibodies are less effective than the enzyme, but also quite different in their relative efficiencies. One approaches about one percent of the enzyme's activity, whereas the other has only one-ten thousandth of that rate. Numerous experimental parameters could account for the differences in activity, and perhaps this discourages any attempt to draw quantitative correlations based solely on hapten design considerations. The comparison draws attention to the great variability that may exist in the catalytic antibody repertoire, even when the experimental parameters are well defined.

Relative to Diffusion-Limited Enzymes

A universal way to assess the efficiency of a catalyst is on a scale of theoretical perfection. The limit to catalysis by enzymes is set by the maximum rate for diffusion of molecules that must come together to form a complex (*27,28*). This upper limit is generally estimated at about $10^{8}M^{-1}$

Table 4
Comparison of Abzymes to Diffusion-Limited Enzymes

Antibody activity[a]	k_{cat}/K_m $s^{-1}M^{-1}$	Enzyme[b]	k_{cat}/K_m $s^{-1}M^{-1}$
Esterase (*17*)	10^4	Acetylcholinesterase	10^8
Decarboxylase (4)	10^3	Carbonic anhydrase	10^8
Redox (*13*)	$10^4/10^c$	Catalase	10^7
Amidase (*18*)	6.7	β-lactamase	10^8
Olefin isomerase (5)	364	Fumarase	10^7–10^8

[a]Data from literature reference cited in text.
[b]Reference (*27*) was source for these data.
[c]Values calculated with respect to each of two substrates.

s^{-1}. An enzyme's specificity constant k_{cat}/K_m approximates a second-order rate constant that indicates the catalyst's relative efficiency on this scale. Although many efficient enzymes have values that approach the theoretical limit, abzymes appear to be limited at $10^4 M^{-1}$ s^{-1}. Typical antibody catalysts have much smaller values, with most examples falling in the broad range of < 1–$10^4 M^{-1}$ s^{-1} (Table 4).

It is not clear whether the activities, as evaluated in this manner, suggest a fundamental limitation for antibody catalysis. Other factors should be considered, such as potential rate-limiting desorption of products from the active site. Since antibodies are selected for high affinity to haptens and affinity maturation is characterized by the decreasing off-rate for the binding interactions, a catalytic antibody may also be limited by this process, particularly when the product has significant hapten analogy.

Relative to Other Abzymes

The previously discussed structural models for design of haptens have been used with varying degrees of success in generating abzymes. Abzymes obtained by different strategies could reflect the relative merit of each design. However, the limited number of examples and the disparate activities observed among related examples offer little confidence in this type of analysis. Ideally, the diverse schemes emphasizing shape complementarity, electrostatic complementarity, or chemical group "baiting" could be compared independently of mechanistics considerations since these express fundamental aspects of catalysis available to enzymes (Table 5).

Although the comparison should be qualified by the uncertainities mentioned earlier, this analysis invites speculation to assess the relative contributions to enzymatic catalysis from these various structural hypotheses. If, as suggested by the values in Table 5, electrostatic interactions account for a great part of the efficiency of abzymes, this would reinforce a

Table 5
Relative Efficiency of Abzymes Obtained by Hapten Design Strategies

Strategy/activity	Hapten feature	Substrate	k_{cat}/K_m ($s^{-1}M^{-1}$)[a]	
Charge complementarity				
Esterase	Phosphonate	Aryl ester	10^4	(*16*)
	Phosphonate	Alkyl ester	10	(*17*)
Amidase	Phosphonamidate	Aryl amide	7	(*18*)
Shape complementarity				
Claisen rearrangement	Ether (cyclic)	Ether (acyclic)	23	(*6*)
			173	(*7*)
Lactonization	Phosphonoester (cyclic)	Ester (acyclic)	520	(*3,21*)
Peptide bond rearrangement	Phosphinate (cyclic)	Peptides (acyclic)	0.63	(*10*)
Chemical group baiting				
β-Elimination	Aminium	Fluoroketone	23	(*19*)
Esterase	Pyridinium	Ester	0.08	(*20*)

[a]Data from literature reference cited in text.

theoretical model for the origin of enzymatic catalysis (*29*). In practical terms, the analysis could be useful in elaborating strategic designs for improvement of catalytic antibodies. Hapten designs that incorporate two or more useful features might be devised. Notwithstanding the apparently modest potential of the approach, it is interesting to speculate on the possibility for a further advantage from a baiting strategy in which two or more groups act cooperatively to provide a more efficient catalytic activity. The equivalent to this hypothesis is the concept of multifunctional catalysis by enzymes (*27,28*). The enzyme-active site is typically composed of several polar or ionizable groups that are precisely oriented with respect to the bound substrate. In order to elicit antibodies with such a complex combining site, the hapten design will require careful consideration for presenting the appropriate groups in combination. It is not clear that structural designs alone are sufficient to address the problem.

ABZYMES AS BIOCATALYSTS

Enzymes and antibodies, both being proteins, are naturally related. As such, antibodies should be better candidates for enzyme mimics than other types of macromolecules or bioorganic models. Antibodies are adapted to function in biological systems. In structural terms, enzymes

are assembled into compact globular forms with a large variety of folding motifs and tertiary structure. Antibodies, on the other hand, use only one basic tertiary structure described as an antiparallel greek-key β-barrel (*30*). The variable domain framework supports the combining site that is comprised of loop regions (CDRs) interconnecting the strands of β-sheet. The motif is among the most common found in protein structure and appears to be adaptable to several different functional purposes. In particular, the proteolytic enzymes of the trypsin family possess the analogous motif. It might be deduced from this that common ancestral genes were utilized for evolution of antibodies and enzymes like trypsin (*27*).

A probable mechanism of protein evolution is through internal gene duplication. Within closely related sequences, the occurrence of duplication events can be surmised with confidence. However, the sequences of proteins that diverged after ancient gene duplication may have drifted beyond recognition, though it is intriguing that homologies have been noted between the CDR sequence of light-chain-variable regions and the active-site region of serine proteases (*31*). The observation gives rise to the interesting speculation that immunoglobulins may possess intrinsic proteolytic activities. That notion has recently received experimental support with the report that specific antipeptide antibodies appear to catalyze breakdown of the peptide ligand. Antibodies against vasoactive intestinal peptide isolated from human serum provided the first evidence for peptidase activity by naturally occurring immunoglobulins (*32*). Subsequently, in collaborative studies with Sudhir Paul's group, we have begun to characterize the peptidase activity of murine monoclonal antibody obtained against VIP (*33*).

Another possible example of naturally occurring catalytic antibodies comes from the report of DNA-cleaving activity by immunoglobulin fractions from serum of human subjects with autoimmune pathologies (*34*). Here too the activity was observed by chance, though there was precedent for the anti-DNA autoimmune response in certain disease states, such as systemic lupus erythematosus. Prior to such reports, it was commonly thought that catalysis by antibodies could arise only through manipulation of the immune response with synthetic haptens. The activities now being identified suggest that abzymes are occasionally encountered in the immune response to various natural antigens. The immune system is regulated through complex mechanisms that include MHC restriction or self-tolerance. The occurrence of antibodies with catalytic activities expressed in vivo might be detrimental and so may also be subject to negative selection. The clonal selection theory of antibody generation would also predict that unconventional or catalytic antibodies that do not fulfill the functional requirement may be suppressed in further selection driven by affinity maturation. From this viewpoint, it is intuitively reasonable to look for natural catalytic antibodies in subjects with immune disorders.

In conclusion, the developments of catalytic antibody research have opened possibilities for production of efficient enzyme mimics for a variety

of processes. Catalyst engineering holds great promise for biotechnology, pharmaceutical chemistry, and therapeutic applications. Further development of antigen design strategies and methodology for screening and selection of monoclonal antibodies with efficient catalytic activities will explore the limits of abzyme technology. Immediate goals include the demonstration that abzymes can efficiently catalyze reactions with high activation energies (low spontaneous rates) and under physiological conditions. On another front, a path has cleared for the premise that antibodies may have natural properties as biocatalysts. Intriguing preliminary reports should encourage advances through protein chemistry, molecular biology, and structure-function predictions to explore this new biochemical premise further. Ultimately, the chemical and biological approaches may converge to provide a unified view of the phenomenon. Such a merging of fundamental and technological developments in catalysis by antibodies may also illuminate related areas of biocatalyst research.

ACKNOWLEDGMENT

I wish to thank the conference organizers for the invitation to contribute. I am grateful to Dr. Richard Massey and Professor Sudhir Paul for valued discussions and continued support.

REFERENCES

1. Pauling, L. (1948), *Am. Scient.* **36,** 51-58.
2. Lerner, R. A., Benkovic, S. J., and Schultz, P. G. (1991), *Science* **252,** 659-667.
3. Napper, A. D., Benkovic, S. J., Tramontano, A., and Lerner, R. A. (1987), *Science* **237,** 1041-1043.
4. Lewis, C., Kramer, T., Robinson, S., and Hilvert, D. (1991), *Science* **253,** 1010-1022.
5. Jackson, D. Y. and Schultz, P. G. (1991), *JACS* **113,** 2319-2321.
6. Hilvert, D., Carpenter, S. H., Nared, K. D., and Auditor, M. T. M. (1988), *PNAS. USA* **85,** 4953-5955.
7. Jackson, D. Y., Jacobs, J. W., Sugasawara, R., Reich, S. H., Bartlett, P. A., and Schultz, P. G. (1988), *J. Am. Chem. Soc.* **110,** 4841,4842.
8. Hsieh, L. C., Yonkovich, S., Kochersperger, L., and Schultz, P. G. (1993), *Science* **260,** 337-339.
9. Hilvert, D., Hill, K. W., Nared, K. D., and Auditor, M. T. M. (1989), *J. Am. Chem. Soc.* **111,** 9261,9262.
10. Gibbs, R. A., Taylor, S., and Benkovic, S. J. (1992), *Science* **258,** 803-805.
11. Janjic, N., Schloeder, D., and Tramontano, A. (1989), *JACS* **111,** 6374-6377.
12. Janjic, N. and Tramontano, A. (1990), *Biochemistry* **29,** 8867-8872.
13. Janjic, N. and Tramontano, A. (1989), *JACS* **111,** 9109,9110.

14. Benkovic, S. J., Adams, J. A., Borders, C. C., Jr., Janda, K. D., and Lerner, R. A. (1990), *Science* **250,** 1135.
15. Pollack, S. J., Jacobs, J. W., and Schultz, P. G. (1986), *Science* **234,** 1570–1573.
16. Tramontano, A., Ammann, A. A., and Lerner, R. A. (1988), *J. Am. Chem. Soc.* **110,** 2282–2286.
17. Janda, K. D., Benkovic, S. J., and Lerner, R. A. (1989), *Science* **244,** 437–440.
18. Janda, K. D., Schloeder, D., Benkovic, S. J., and Lerner, R. A. (1988), *Science* **241,** 1188–1191.
19. Shokat, K. M., Leumann, C. J., Sugasawara, R., and Schultz, P. G. (1989), *Nature* **338,** 269–271.
20. Janda, K. D., Weinhouse, M. I., Schloeder, D. M., and Lerner, R. A. (1990), *J. Am. Chem. Soc.* **112,** 1274,1275.
21. Benkovic, S. J., Napper, A. D., and Lerner, R. A. (1988), *Proc. Natl. Acad. Sci. USA* **85,** 5355–5358.
22. Tramontano, A. and Schloeder, D. (1989), *Methods Enzymol.* **178,** 531–550.
23. Tawfik, D. S., Zemel, R. R., Yellin, R. A., Green, B. S., and Eshhaz, Z. (1990), *Biochemistry* **29,** 9916–9921.
24. Berek, C., Griffiths, G. M., and Milstein, C. (1985), *Nature* **316,** 412–418.
25. Foote, J. and Milstein, C. (1991), *Nature* **352,** 530–532.
26. Tawfik, D. S., Green, B. S., Chap, R., Sela, M., and Eshhar, Z. (1993), *PNAS* **90,** 373–377.
27. Fersht, A. (1985), *Enzyme Structure and Mechanism,* W. H. Freeman, New York.
28. Jencks, W. P. (1975), *Adv. Enzymol.* **43,** 219–410.
29. Warshel, A., Naray-Szabo, G., Sussman, F., and Hwang, J. K. (1989), *Biochemistry* **28,** 3629.
30. Richardson, J. S. (1981), *Adv. Pro. Chem.* **34,** 168–339.
31. Erhan, S. and Greller, L. D. (1974), *Nature* **251,** 353–355.
32. Paul, S., Volle, D. J., Beach, C. M., Johnson, D. R., Powell, M. J., and Massey, R. J. (1989), *Science* **244,** 1158–1162.
33. Paul, S., Sun, M., Mody, R., Tewary, H. K., Stemmer, P., Massey, R. J., Gianferrara, T., Mehrotra, S., Dreyer, T., Meldal, H., and Tramontano, A. (1992), *J. Biol. Chem.* **267,** 13,142–13,145.
34. Shuster, A. M., Gololobov, G. V., Kvashuk, O. A., Bogomolova, A. E., Smirnov, I. V., and Gabibov, A. G. (1992), *Science* **256,** 665–667.

DISCUSSION

A. Tramontano

Gavibov: From the binding to catalysis is only one step. The simplicity of this statement was shown in your slide showing overlapping frequency distributions of antibodies that only bind antigen and those that catalyze its chemical transformation. Have you considered algorithms that may permit calculation of the degree of overlap between the two types of antibodies?

Tramontano: Yes, but we do not yet have sufficient basic information or rules to derive a mathematical solution.

Paul: I am often surprised by the reluctance of investigators in this field to compare the properties and kinetic constants of naturally occurring antibodies with those raised against transition-state analogs. Do you see a problem in making this comparison?

Tramontano: Concerning the naturally occurring antibodies, the only examples are provided by your work and Dr. Gabibov's work. Your observation of much higher k_{cat}/K_m values compared to antitransition-state analog antibodies would suggest that you have found a different class of abzymes, but we have to know more about these entities, the naturally occurring antibodies.

Paul: The same can be said for antibodies to transition-state analogs, given the difficulties and unpredictability associated with these antibodies. Comparisons of kinetic constants are valuable because they provide clues about mechanism.

Tramontano: I tried to present in my talk a *prima facie* comparison between a set of catalytic antibody molecules derived by rational design of the immunogen. We should make comparisons with other catalysts, including conventional enzymes, to get an idea of where we stand, but we also need to understand the paradigms and strategies used to get the catalyst. We still do not know how the natural antibodies evolve or how they are elicited.

Gabibov: It is very difficult to compare the k_{cat} and K_m of naturally occurring antibodies with monoclonal antibodies because we are not sure that the catalyst is homogeneous in the former case. We have to be very cautious. You mention the K_i of the immunizing hapten. I think it is good to find catalytic antibodies by screening for the hapten binding, but the values of K_i and K_m will depend on the mechanism of action of abzyme and do not provide a fundamental insight unless the immunizing hapten is really very similar in structure to the transition state. The K_i and K_m are approximations for K_{TSA} and K_s, and reflect ΔG for transition state and substrate binding only in an idealized case.

Tramontano: Let me just say that type of analysis is widely done. It helps assess the theory that has been used to generate the catalyst. If the results do not agree with the theory, obviously we must rethink the theory.

Zouali: You have made a number of speculations during your talk. One of them was that enzymes and abzymes originate from a common ancestor. It seems to me that few people will accept this speculation, because if you look at the genetic organization of enzymes and antibodies, they are very different.

Tramontano: The overall structure of enzymes and antibodies is certainly different. The suggestion I am making is based on observations of shared domains on enzymes and antibodies. It is useful to think that this domain can be utilized for catalysis or for binding.

Zouali: Usually, when there is selection at the protein level, this is also reflected at the genomic level.

Tramontano: I think my analogy was strictly based on topology of the variable domain and not on more detailed comparisons. The variable region gene may have evolved from a primordial sequence that gave rise to other proteins, like trypsin, or other enzymes that have the β-barrel architecture. The higher-level genetic organization of antibodies or enzymes is not relevant to this argument.