0273-2289/98/7501—0003$10.50

Antibody Catalysis Based on Functional Mimicry

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Received December 10, 1997; Accepted April 13, 1998

ABSTRACT

Approaches aiming at eliciting antibodies (Abs) that catalyze specific chemical transformations are numerous. Most of the developed methods are based on the chemical steps of the reaction catalyzed rather than on the structure of known enzyme active sites. The authors have developed an approach that rests on the mimicry properties of the idiotypic network of immune regulation. Recent results, together with the existence of natural catalytic Abs in autoimmune diseases, indicate the need to better understand the regulation properties of immune response, in order to improve the efficiency of tailor-made catalytic Abs.

Index Entries: Catalytic antibodies; abzymes; anti-idiotypes; internal image.

INTRODUCTION

Protein engineering has recorded an extensive technological development in recent years. This trend has had great impact on elucidation of structure–function relationships and in vitro generation of catalysts with new properties *(1–4)*. Catalysis by antibodies (Abs) has witnessed an increasing interest over the past decade. Since the pioneer reports by Tramontano et al. *(5)* and Pollack et al. *(6)*, more sophisticated transformations have been obtained, including bimolecular reactions, pericyclic rearrangements, redox reactions, elimination reactions, or even reactions

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not catalyzed by natural enzyme *(7–10)*. These results have a critical importance for fundamental understanding of biocatalysis and for potential biotechnological applications, but also bring new fundamental insights *(11,12)*. Most catalytic Abs were obtained by a ligand-based approach, using a transition-state analog (TSA) *(5,6,13–15)* or molecules mimicking electrostatic features of reaction intermediates *(16,17)*. Protein engineering has also allowed the generation of Abs able to catalyze reactions by introducing catalytic groups acting directly on, or contributing to, the binding of essential cofactors *(18–20)*.

The authors have proposed an alternative strategy to this ligand-based approach to elicit catalytic Abs *(21–23)*. The basic idea of this approach rests on the idiotypic network theory, which states that an Ab raised against the combining site of a first Ab may contain information regarding the three-dimensional structure of the original antigen (Ag). If the active site of an enzyme is used as the Ag, the properties of internal imagery of anti-idiotypic Abs may be exploited to design catalytic Abs.

All these approaches, together with the isolation of natural catalytic Abs in autoimmune diseases, are now faced with the same fundamental problems concerning complementarity, specificity, and efficiency.

COMPLEMENTARITY AND SPECIFICITY

One of the most remarkable features of the immune system is its ability to respond to an apparently limitless array of Ags. The tremendous diversity coupled to the particular Ag-binding specificity of each Ab is achieved by the somatic organization and mutation of gene segments encoding the variable (V) and constant (C) domains of its heavy (H) and light (L) chains *(24)*. This property confers to Abs the unique capacity among proteins to recognize almost any foreign molecule. Although Abs share the common structural features of bivalent chains linked to each other by disulfide bonds, their specificity is defined by the tremendous variability of their complementarity determining regions (CDR) within the V domains *(25,26)*. The more conserved regions of the V domains form β-sheets and the hypervariable regions are loops that can arrange in various structures, from rather flat surfaces to deep cavities. All available data deny the conclusion that the Ab-binding site is a simple pocket in which the Ag lies *(27,28)*. The extent of the Ab–Ag interaction depends on the sequence of amino acids of the CDRs, interactions between side chains of these amino acids and the Ag, and the size of the loops.

As a pioneer of the modern concept of molecular complementarity, Pauling proposed that an enzyme is a flexible molecular template that is complementary to the reactants in their activated transition-state geometry *(29)*. Its efficiency is achieved in part as the result of transition-state stabilization, strain, acid-base catalysis, and proximity.

ANTIBODIES AS CATALYSTS

Recognition through complementarity features is a phenomenon shared by many proteins, notably by Abs and enzymes. From this observation arose the proposal by Jencks *(30)* that Ab could be used to selectively stabilize the rate-determining transition state of a chemical reaction by eliciting these Abs against a stable structure resembling the transition state. A break through in the field came from the development of the monoclonal antibody (MAb) technique by Köhler and Milstein in 1975 *(31)*, which has provided the means for producing Abs with a single antigenic defined specificity. This was of critical importance for characterizing catalytic activities. Since the first reports by Tramontano et al. *(5)* and Pollack et al. *(6)*, catalytic Abs generated following this ligand-based approach have been obtained for a large range of chemical reactions, including for the transformation of substrates that are not recognized by natural enzymes.

According to now-available molecular three-dimensional structures *(32–34)*, the level of complementarity influences the extent of catalysis by Abs. The engineering of the combining site is a consequence of the immune response to the haptenic structure. This response is an indication of the diversity of the solutions foreseen by the immune system to optimize the molecular interactions between the side chain of Abs and Ags.

Despite these observations, catalytic Abs elicited against TSAs generally displayed a very high degree of specificity, but weak activities when compared to their enzymatic counterparts; rate-enhancement factors are generally 1000-fold smaller than that measured with enzymes *(35,36)*. However, a recent paper by Barbas et al. *(37)* demonstrates that catalytic Abs that have efficiency of natural enzymes can be obtained by reactive immunization. This approach results from the observation that enzyme selection during evolution proceeds presumably by catalytic selection, but effective clonal selection in the immune system is based on improved binding. The use of a reactive Ag, designed so that a covalent bond occurs in the binding pocket of induced Abs, allows the immune system to change the selection criteria from simple binding to chemical reactivity. It is interesting to note that although efficiency is improved, specificity is lowered.

As alternatives, molecular biology techniques and chemical engineering of Abs can be exploited to improve the catalytic efficiency of Abs.

ANTI-IDIOTYPES AND CATALYTIC FUNCTIONS

Considerable attention has been paid to the potential applications of molecular mimicry properties of anti-idiotypic Abs in curative medicine and synthesis of new vaccines for various infectious diseases *(39,40)*. These properties of internal imagery have also been proposed as a possible explanation for the amazing presence of natural catalytic Abs in the sera of patients with

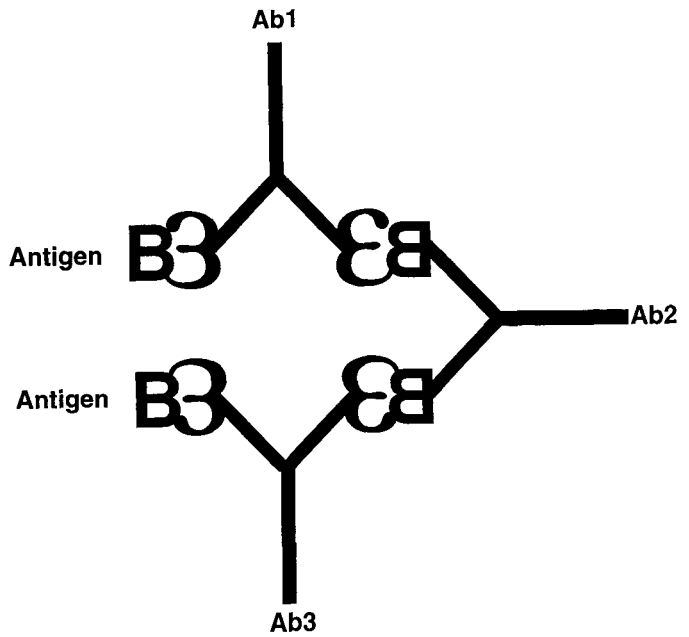

Fig. 1. Schematic representation of idiotypic interactions: B-type elements interact with ϵ-type Abs.

autoimmune diseases *(41–44)*. According to the Jerne's proposal of idiotypic regulation of the immune system for each immunoglobulin (Fig. 1, Ab1, 3-type) generated against an antigenic determinant (Fig. 1, B-type), there exists a complementary Ab (Ab2) directed against the idiotypic determinants of Ab1. By inference, the idiotypes of Ab2 could cause the production of Ab3s, and then, in turn, the production of Ab4, and so on. Among the Ab2 (or anti-idiotypic) molecules, some of them mimic the Ag's determinants and are designated as internal images of the original Ag (Fig. 1, Ab2, B-type).

The validation of the idiotypic network theory has come from structural and theoretical studies about complementarity of contact surfaces *(45–49)*. According to these data, the author proposed to exploit these remarkable properties of internal imagery of anti-idiotypic Abs to design catalytic Abs that mimick the activities of known enzymes. A first Ab (idiotypic Ab, Ab1) is raised that recognizes the active site of an enzyme, so that the combining site of Ab1 has structural features complementary to those of the enzyme. This first Ab, which is characterized by its inhibitory potency against the enzymatic reaction, is a mirror image of the target site, both in a spatial and electronic sense. After screening, production, and purification of this idiotypic Ab, a second set of Abs (Ab2) is produced against the Ab1 combining site. Among these second-generation, or anti-idiotypic, Abs, some may not only present the binding function for the substrates of the enzyme used as Ag, but may also be able to catalyze their transformation.

Table 1
Catalytic Parameters of Acetylthiocholine Hydrolysis by Human Erythrocyte Acetylcholinesterase (HE-AChE), Rabbit Polyclonal Anti-idiotypic Abs (Anti-idAb2), and Mouse Monoclonal Anti-idiotypic IgM 9A8[a]

Source	K_m(mM)	K_{ss}(mM)	k_{cat}/s	k_{cat}/k_{uncat}	Ref.
HE-AChE	0.13	22	8000	$410 \cdot 10^8$	*(21)*
Anti-idAb2	65	–	17.5	$0.9 \cdot 10^8$	*(21)*
IgM 9A8	0.6	–	81	$4.15 \cdot 10^8$	*(22)*

[a]The latter two Abs do not display substrate inhibition. K_m, Michaelis-Menten constant; K_{ss}, inhibition by excess substrate constant; k_{cat}, catalytic constant.

The first results were obtained with an esterolytic activity initially borne by human erythrocyte acetylcholinesterase (Table 1). Ab1 was a previously described IgG (AE-2) directed against the active site of the enzyme *(50)*. After immunization with AE-2, rabbit serum was submitted to sequential adsorption to immunosorbent columns (protein A-Sepharose, then AE-2 Sepharose) and subsequent elution of anti-idiotypic Abs. The polyclonal preparation exhibited a cholinesterase-like activity with respect to substrate and inhibitor specificity *(21)*. To further characterize abzymes produced in response to AE-2 immunization, MAbs were prepared. After fusion of myeloma cells with the spleen cells of Biozzi mice immunized by AE-2, 1 of 600 hybridoma cells was found to secrete a catalytic Ab, IgM 9A8 *(22)*. This MAb exhibits a cholinesterase activity whose catalytic properties are very close to those obtained with the polyclonal preparation. The first-order rate constant (about 100/s) differs by two orders from that of acetylcholinesterase, the K_m value is fivefold higher than the value measured with the natural enzyme, and the activity is not inhibited by excess substrate or by peripheral site-directed inhibitors of acetylcholinesterase. Although the efficiency appears very high when compared with most catalytic Abs elicited by TSAs the specificity for substrate and inhibitors looks like a relaxed specificity from the model enzyme.

The second attempt concerns β-lactamase, an enzyme involved in the hydrolysis of antibiotics containing a β-lactam ring *(51,52)*. This activity was chosen not only because of the high efficiency of β-lactamase for hydrolysis of penicillins and cephalosporins, but also because this enzymatic activity is absent in mammals. This allows ruling out the possibility of artifactual results linked to a contaminant enzyme, and also direct measurement of the appearance of catalytic Abs in the serum of mice during the immunization procedure. The first key step in this approach is the

Table 2
Experimental Assay Conditions for Screening of Monoclonal Antiactive-Site Ab (Inhibitory Ab1)[a]

Source	Treatment	Temperature	Incubation time	Buffer	Antibody/ Enzyme ratio
Culture supernatants	Precipitation dialysis	4, 30, and 37°C	1 h, 4 h, 2I h	NS or S	ND
Ascitic fluid	Protein A	4 or 37°C	From 1 to 24 h	S	From 1:1 to 20:1

[a]Experiments were performed both with Abs from culture supernatants after precipitation and dialysis, and Abs from ascitic fluids purified on protein A. The different conditions were tested in order to optimize the screening. NS, nonstabilizing buffer (phosphate buffer 0.1 *M* pH 7.4); S, Stabilizing buffer (NS containing 0.5% gelatin).

selection of the proper Ab1, complementary to the enzymatic active site. Mice were immunized with *Bacillus cereus* β-lactamase in order to produce hundreds of MAbs recognizing the enzyme. The inhibition of enzymatic activity appeared to be one of the most favorable criteria to screen an Ab complementary to the active site. Several inhibition experiments, summed up in Table 2, connected to statistical tests and to competition experiments with ligands known to bind the β-lactamase active site, resulted in the selection of one IgG 7AF9, displaying inhibitory characteristics (up to 60% of initial activity) and mixed inhibition features *(53)*. This Ab1 was used to elicit anti-idiotypic Abs. IgG 7AF9 was injected into Biozzi mice according to standard immunization procedures. Blood samples were collected after each step of the immunization. The detection of β-lactamase activity was directly performed with diluted antisera, using a β-lactam cephalosporin (7-[thienyl-2-acetamido]-3-[2-(4-N,N-dimethylaminophenylazo)-pyridinium methyl]-3-cephem-4 carboxylic acid PADAC) as the substrate. The detection of the hydrolytic activity of polyclonal Ab2 Abs was experimentally accompanied by the evaluation of specific anti-7AF9 Ab levels using an enzyme-linked immunosorbent assay (ELISA): Results are shown in Table 3. According to ELISA values, the ability of polyclonal Ab2 to hydrolyze the β-lactam substrate concomitantly increases with the appearance of Ab2 against 7AF9. This result argues for the possibility of eliciting a functional image of an enzymatic active site throughout a classical anti-idiotypic response. The characterization of MAbs bearing a β-lactamase-like activity is now in progress. Results obtained with polyclonal Abs clearly indicate that the elicited Ab2 can be directly screened for their catalytic activity.

Different catalytic activities are now investigated using the anti-idiotypic approach. For example, the generation of anti-idiotypic Abs with

Table 3
Evolution of Immunological and Catalytic Properties of Polyclonal Anti-Ab1 Abs Before and After Four Immunizations with Ab1 7AF9 at 2-wk Intervals (I1–I4)[a]

Collected mouse serum	IgG level OD at 405 nm	IgM level OD at 405 nm	Relative catalytic activity
Nonimmunized	0.075	0.05	1.7
I1	0.08	0.11	3
I2	0.21	0.165	2.8
I3	0.28	0.15	11.5
I4	0.31	0.12	15

[a]Sera were collected 9 d after each immunization, and anti-Ab1 IgG and IgM levels were evaluated by ELISA. Sera were diluted 200-fold in phosphate buffer saline and then incubated overnight in microtiter wells previously coated with Ab1 7AF9 $F(ab)'_2$ pepsin-digested fragments. Bound Abs were revealed using 2.5 μg/mL peroxidase conjugated anti-mouse IgM (γ-chain specific) or IgG (μ-chain specific) by measuring the hydrolysis of a peroxidase substrate, azinobis-ethylbenzothiazoline sulfonate (ABTS) at 405 nm. The β-lactamase activity was assayed with five-fold diluted antiserum by measuring at 570 nm the hydrolysis of PADAC. The relative activity was calculated as the ratio of catalytic activity of Ab2 vs noncatalyzed reaction.

protease activities would be of great interest for producing protein sequence-specific biocatalysts, for potential therapeutic applications, and maybe for the understanding of the appearance of natural Abs or Bence-Jones proteins in several diseases. The molecular complementarity concept may also be exploited to translate the change in specificity induced by chemical modifications of enzymes, or by introduction of a metal-binding site into purely proteic copies of the modified catalyst that have integrated the chemical information via the idiotypic cascade. This latter approach would allow not only mimicking of the activity of a known enzyme, but generation of abzymes possessing new specificities or catalyzing new chemical transformations.

CONCLUSION

The generation of catalytic Abs is of considerable fundamental and applied interest, for the understanding of structure–function relationships, the production of tailor-made catalysts, and for potential therapeutic applications.

Different strategies have been exploited to generate catalytic Abs. In most cases, these approaches rely on a ligand-based approach in which the

substrate to be transformed is the central point around which are elaborated the various strategies. A thorough knowledge of the relevant reaction and its molecular intermediates, as well as mechanism, is necessary in this approach.

As an alternative, the authors have proposed to derive benefits of the structural complementarity demonstrated for idiotype/anti-idiotype interactions, and have demonstrated the feasibility of this approach with two different enzymatic activities. On the other hand, the presence of natural catalytic Abs with DNase, amidase, and peptidase activities in several autoimmune diseases is now well documented.

All these approaches are now faced with fundamental questions related to the understanding of enzyme selection during evolution compared with Ab maturation during immune response, to the clonal selection in normal mice compared with clonal selection in mice with autoimmune mice, and to the understanding of critical molecular steps during catalysis, in order to develop the most efficient screening strategies. The increasing number of available X-ray structures of catalytic Abs clearly shows the multiplicity of solutions for an Ab to catalyze an enzyme-like reaction, from amino acid arrangements corresponding to that present in enzymes *(54)*, to solutions that greatly differ from those selected in enzymes by natural evolution *(55)*. As another example, the recent paper by Barbas et al. *(37)* clearly indicates that, as initially proposed by Emil Fisher in 1902 at his Nobel lecture, ". . . if we wish to catch up with Nature, we shall need to use the same methods as she does . . ." to screen antibodies with efficient properties. Moreover, it has been shown that immunization by a TSA of mice with autoimmune diseases gives rise to an unexpectedly high increase in the number of clones secreting catalytic Abs compared with normal mice *(56)*.

All these data clearly indicate that important progress in the field of catalytic Abs will be obtained by considering the results obtained in all of the different approaches, by improving screening methodologies, by improving strategies to elicit catalytic amino acid in the combining sites, and by taking into account the physiological regulation of the immune response and the clonal selection.

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