29 Catalytic Antibodies: New Characters in the Protein Repertoire

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INTRODUCTION

Classically, antibodies have been characterized as proteins produced by the immune system that have the sole function of binding other molecules, called antigens, with the goal of eliciting an immune response. Most natural antigens for antibodies are proteins, and the interactions of antigens with antibodies are very strong, with reported K_d values between 0.01 and 10 nM (Karush 1978). In this classic conception, antibody function is similar to that of enzymes in specifically binding other molecules; however, antibodies are distinct from enzymes in that they do not have the ability to catalyze chemical conversions of their bound partners. For the vast majority of antibodies, this observation is correct. However, in a 1946 consideration of the catalytic function of enzymes, Linus Pauling first hypothesized that the active center of an enzyme is closely juxtaposed to a "strained configuration" of its substrate (that is, targeted against the structure of the transition state) rather than to the native conformation of the substrate molecule (Pauling 1946). On the basis of this hypothesis, it logically followed that the binding energy between enzyme and substrate could play a significant role in lowering activation energy, thereby catalyzing a chemical

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change. This idea led Jencks in 1969 to propose that antibodies generated in an anti-hapten immune response against chemically stable analogs of the transition state of a reaction of interest could potentially display an enzymatic activity (Jencks 1969). Indeed, this prediction was confirmed in 1986 when the first anti-hapten monoclonal catalytic antibodies were obtained (see below) and termed "abzymes" (derived from *a*nti*b*ody en*zyme*). At present, artificial monoclonal abzymes catalyzing more than 100 distinct chemical reactions have been obtained. Moreover, many naturally occurring abzymes have been detected in the sera of patients with several pathologies, as well as in the milk of healthy human mothers.

From the perspective of control of protein–protein interactions, and in particular, of the study of extracellular signaling, the development of abzymes holds a unique promise: the ability selectively to modify or eliminate defined interacting proteins. For example, to date, a relatively limited set of proteases has been defined that recognize specific sequences on target proteins, and subsequently induce specific cleavages. These "restriction endoproteases" include the coagulation factor Xa (Nagai and Thogersen 1987), the caspases, and calpain (for a discussion, see Wang 2000). These proteases recognize and cleave short peptide sequences that are found on one or multiple cellular targets. The ability to create antibody-based enzymes that target different, or more selective, sequences would allow the specific cleavage and modification of essentially any surface-displayed protein to which the antibody has access. In theory, given the appropriate starting hapten, abzymes could potentially be developed that target any protein sequence of interest, allowing exact control of cell signaling processes. In practice, the first amide-catalyzing antibody was generated in 1988 and shown to enhance the rate of catalysis of its substrate 250,000 times over the uncatalyzed reaction (Janda et al. 1988). Since that time, the work of a number of groups has begun to explore the ability of abzymes as small-molecule or protein-modulatory agents. This chapter reviews some of the main themes of this work and provides a description of the important issues in identifying naturally occurring polyclonal abzymes.

DESIGN AND POTENTIALITIES OF "ARTIFICIAL" ABZYMES

In 1985, a general method for generating catalytic monoclonal antibodies (mAbs) against transition-state analogs, and a way to use those antibodies to accelerate chemical reactions, was first described (Schochetman and Massey 1985). One year later, two groups were able to produce the first mAbs with catalytic properties that were generated against hapten analogs of the transition states for *p*-nitrophenylphosphorylcholine (Pollack et al. 1986) or for monoaryl phosphonate esters (Tramontano et al. 1986a,b). Since that time, abzymes catalyzing the hydrolysis of amides and esters, as well as reactions of cyclization (see, e.g., Janda et al. 1993; Li et al. 1994, 1996; Wentworth et al. 1998), decarboxylation (see, e.g., Smiley and Benkovic 1994; Barbas et al. 1997; Hotta et al. 2000), lactonization (Napper et al. 1987), peroxidation (Ding et al. 1998), photochemical thymine dimer cleavage, bimolecular amide-bond formation, and other reactions not known to be catalyzed by known enzymes have been described. A number of papers have described abzymes that perform other specific functions directed against proteins, including formation of cyclic peptides (Smithrud et al. 2000), catalysis of peptidyl-prolyl *cis-trans* isomerization in protein folding (Ma et al. 1998), and development of a novel enzymatic activity cleaving the bacterial protein HPr (Liu et al. 1998). Some abzymes have been described that require cofactors for activity, similar to standard enzymes (Iverson and Lerner 1989). The field of artificial abzymes has been amply reviewed recently (see Lerner and Tramontano 1987; Stewart and Benkovic 1993; Suzuki 1994; Martin and Schultz 1999, and references therein, for a more detailed description of the relevant reactions).

The evolution of the technology of artificial abzymes during the last 15 years has led not only to the rapid development of direct approaches for the generation of antibodies with specified properties, but also to the creation of strategies to revise the targeting specificity of individual

abzymes. Such modifications of antigen-binding specificity can be achieved genetically in vitro by application of site-directed mutagenesis, genetic selection, or screening (using approaches such as phage display detailed in Chapter 8). Alternatively, modification can be induced directly on purified antibody via selective chemical modification involving direct introduction of catalytic groups into the antibody combining site. Some studies describing these approaches include Ersoy et al. (1999), Gao and Paul (1995), Miller et al. (1997), Roberts et al. (1994), Stewart et al. (1994), and references therein. As a result of application of these approaches, the substrate specificity (and/or the specific activity) of some artificial abzymes is comparable to or even higher than that of enzymes with the same catalytic activity (Gouverneur et al. 1993; Barbas et al. 1997; Janda et al. 1997). The mechanistic basis for the activity of such abzymes is becoming well understood (see, e.g., Thayer et al. 1999 and discussion below).

NATURAL CATALYTIC ANTIBODIES

In contrast to the artificially designed transition-state-directed antibodies described in the preceding section, an alternative means of developing catalytic antibodies is through study and purification of autoantibodies produced naturally in human sera. As noted by Suzuki, naturally occurring antibodies can be quickly and easily purified at low cost, possess novel catalytic activities of interest, can be informative for development of therapeutic strategies involving antibodies administered in serum, and can be useful for design of haptens for production of artificial antibodies (Suzuki 1994). Other relevant issues in comparison of artificial versus natural, and in vitro versus in vivo, abzyme generation approaches are discussed in Fastrez (1997). The remainder of this section collates rigid criteria indicating that efficient naturally occurring catalytic antibodies do exist, and describes methods of natural catalytic antibody purification.

The idea that catalytic antibodies might be involved in natural immunity first arose after the discovery by Paul and coworkers of natural catalytic antibodies specifically hydrolyzing vasoactive intestinal peptide (VIP), a 28-amino-acid neurotransmitter, in the serum immunoglobulin G (IgG) fractions of ~50% of asthmatic patients (Paul et al. 1989). As with the overwhelming majority of antibodies, these antibodies exhibit very strong binding affinity for their cognate antigen, with an affinity for VIP (K_d ~0.76 nM). Seven antibody-sensitive peptide bonds were identified, of which six were clustered between residues 14 and 22 (Paul et al. 1991). In functional studies, separated light chains of these antibodies were found to be active in the hydrolysis of VIP (Sun et al. 1994, 1997). Compared to reversible antigen binding, it was found that antigen cleavage by catalytic antibodies was a particularly potent means to achieve the neutralization of targeted antigens (Paul 1998). Significantly, the structure and function of these antibodies were inherited via a germ-line variable light (V_L) gene, providing evidence that certain antibodies possessed an innate catalytic function of certain antibodies and arguing against the idea that abzymes required artificial haptens mimicking transition states for their generation (Gololobov et al. 1999). Subsequently, monoclonal anti-VIP abzymes were obtained and analyzed (see below), yielding further insight into their function.

Following the initial discovery of anti-VIP abzymes, a number of natural catalytic antibodies with diverse activities were detected in sera of patients with different immune pathologies. Proteolytic antibodies directed at thyroglobulin (Tg), a precursor of thyroid hormone, were found in the serum IgG of patients with Hashimoto's thyroiditis and systemic lupus erythematosus (SLE) (Li et al. 1995; Paul et al. 1997). Light chains isolated from patients with multiple myeloma possessed prothrombinase activity (Thiagarajan et al. 2000). Others have reported factor VIII-cleaving alloantibodies in the sera of patients with severe hemophilia (Lacroix-Desmazes et al. 1999). The identified proteolytic antibodies cleaved their protein substrates at a small number of fixed positions, indicating specificity of action. The K_M values of Tg- and prothrombin-directed antibodies for Tg and a peptide 268–271 of prothrombin were 39 nM and 103 μM, respectively, indi-

cating high-affinity substrate recognition. In a study of the development of catalytic specificity by abzymes, the polyreactive (nonspecific) peptidase activity of serum IgGs from healthy individuals and patients with autoimmune disease was compared, based on the extent to which these sera cleaved a synthetic protease substrate, Pro-Phe-Arg-methylcoumarinamide (Paul et al. 1997). A transition from a polyreactive proteolytic activity to autoantigen-directed activity in autoimmune disease was suggested (Paul et al. 1997). Finally, the identification of a human immunodeficiency virus gp120-cleaving antibody light chain from multiple myeloma patients has recently demonstrated that natural catalytic immunity is not restricted to autoantigenic substrates (Paul et al. 2000).

In addition to abzymes directed against proteins, DNA- and RNA-hydrolyzing antibodies have also been found in the sera of patients with SLE and studied in detail (for examples, see Shuster et al. 1992; Buneva et al. 1994; Gololobov et al. 1995; Vlassov et al. 1998a,b; Andrievskaya et al. 2000, and references therein). Subsequently, RNA- and/or DNA-hydrolyzing IgM and/or IgG were detected in the sera of patients with several autoimmune, viral, and lymphoproliferative diseases. These sources include Hashimoto's thyroiditis, polyarthritis (Vlassov et al. 1998a,b), multiple sclerosis (MS) (Baranovskii et al. 1998), B-cell lymphomas (Kozyr et al. 1998), viral hepatitis (Baranovsky et al. 1997), and acquired immunodeficiency syndrome (AIDS) (Gabibov et al. 1994). Such abzymes were not detected in sera from normal controls or patients with other disorders, including influenza, pneumonia, tuberculosis, tonsillitis, duodenal ulcer, and some types of cancer (Baranovsky et al. 1997), suggesting specificity of generation. Last, and intriguingly, in addition to IgG nucleic acid-hydrolyzing abzymes (Kanyshkova et al. 1997; Buneva et al. 1998; Semenov et al. 1998; Nevinsky et al. 2000), amylolitic abzymes (Savel'ev et al. 1999) and protein-phosphorylating sIgA- (or IgA) abzymes (Kit et al. 1995; Nevinsky et al. 1998) were found in the milk of healthy human mothers. Of these, the "kinase" abzymes were active against a number of proteins including the major milk components α-casein and β-casein, and they represent a first example of natural abzymes catalyzing a bisubstrate synthetic reaction.

Quality Control Criteria for Intrinsic Catalytic Activities of Antibodies

Because antibodies can form complexes with other proteins, and antibody-mediated catalysis is sometimes characterized by relatively low reaction rates, it is important to prove that a catalytic activity of Ig fractions is not due to a contamination with "classic" enzymes with the same specificity. For example, if the turnover number, k_{cat}, for the antibody is 1 min^{-1}, it may be a result of 0.002% admixture of antibody with an enzyme with k_{cat} of 5×10^4 min^{-1}. Application of a set of rigid criteria worked out by Paul et al. (1989) in the first articles concerning natural abzymes (for review, see Paul 1998) allowed the authors of the initial study to conclude that the observed VIP-hydrolyzing activity is an intrinsic property of IgGs from the sera of patients with asthma. We note the consideration of these criteria is also useful in evaluating the properties of artificially generated abzymes, as described above. The most important criteria used in this process by Paul and coworkers were:

1. Electrophoretic homogeneity of the IgG (based on silver staining of an SDS-PAGE gel).
2. Retention of VIP-hydrolyzing activity by Fab fragments of the purified antibodies.
3. Complete adsorption of the catalytic activity by anti-IgG Sepharose and its elution from the adsorbent with low-pH buffer.
4. Immunoprecipitation of abzymes by anti-IgG antibodies and disappearance of catalytic activity from the solution.
5. Demonstration of the VIP-hydrolyzing activity in the antibodies of only 50% of asthma patients but in no healthy controls.

6. Demonstration that gel filtration of IgG at pH 2.7, in conditions providing dissociation of any non-covalent protein complex ("acidic shock"), did not lead to the disappearance of abzyme activity, whereas the peak of activity tracked exactly with a 150-kD IgG.
7. Characterization of a low K_m value of the abzymes for VIP (38 nM) hydrolysis, which testified to a high affinity to the substrate and was comparable with the K_d values of many antibody complexes with their antigens.
8. Determination that the substrate specificity of IgG-dependent hydrolysis of VIP was different from that of any known protease.

These rigorous checks for intrinsic catalytic activities of abzymes have been used by different groups, with some modifications, as the basis for detection of natural catalytic antibodies. As the field has developed, other controls that have been added into the characterization regimen have been developed by our groups and others. For example:

1. Preservation of the catalytic activity of antibodies is demonstrated after gel filtration not only under conditions of extremely low pH, but also in other severe conditions that effectively dissociate noncovalent complexes. Examples of such conditions include use of buffers with strongly alkaline pH (pH 10–11) or neutral buffers containing 5 M thiocyanate or 6 M guanidine chloride (Kit et al. 1995; Nevinsky et al. 1998).
2. Model experiments have been used to analyze the effectiveness of separation of mixtures containing homogeneous abzymes and human enzymes of similar specificities. After chromatography on protein A–Sepharose and gel filtration in severe conditions, an effective separation of abzymes from the added enzymes should be clearly achieved (see Andrievskaya et al. 2000 and references therein; Vlassov et al. 1998a,b).
3. Detection of a catalytic activity associated with separated L- and/or H-subunits of Ig isolated by affinity chromatography after their dissociation in mild conditions (1 mM mercaptoethanol and 4 M urea) provides good evidence of intrinsic catalytic properties of antibodies (Kanyshkova et al. 1997; Buneva et al. 1998; Nevinsky et al. 1998, 2000).
4. Direct evidence that antibodies possess enzymatic activities can also be obtained by using an affinity modification method. In this approach, covalent binding of a reactive analog of the substrate to the abzyme but not to possible contaminating proteins is demonstrated (Buneva et al. 1994; Kit et al. 1995; Kanyshkova et al. 1997; Nevinsky et al. 1998, 2000; Semenov et al. 1998).
5. A further very strong criterion of the existence of antibodies as catalysts is the in situ detection of catalytic activities of antibodies versus their Fab fragments in a gel containing the corresponding substrate (Kanyshkova et al. 1997; Baranovskii et al. 1998; Buneva et al. 1998; Semenov et al. 1998; Andrievskaya et al. 2000). For example, when IgG from the milk of healthy mothers and the Fab fragments derived from this IgG are separated by SDS-PAGE in nonreducing conditions in a DNA-containing gel, a DNA-hydrolyzing activity is revealed in the bands corresponding to both IgG and Fab fragments (Fig. 1) (Buneva et al. 1998). The same results are obtained for RNA-hydrolyzing activity (Andrievskaya et al. 2000). These criteria can in some cases be supplemented by the in situ detection of catalytic activities of separated Ig chains after SDS-PAGE using reducing conditions (in the presence of 2-mercaptoethanol). For some abzymes, a DNA-hydrolyzing activity is revealed in a DNA-containing gel for individual antibody chains (Kanyshkova et al. 1997; Baranovskii et al. 1998; Andrievskaya et al. 2000). However, this criterion is not universal: After reduction of some other Igs, the in-gel assay showed the absence of DNA-hydrolyzing activity in the separated L and H chains (Semenov et al. 1998; Nevinsky et al. 2000).

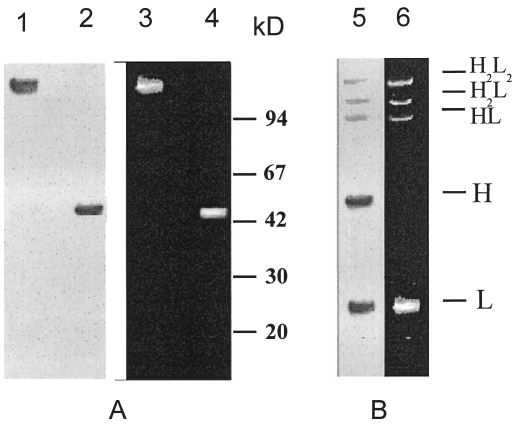

FIGURE 1. In situ gel assay of DNase activity of milk IgG (lanes *1*, *3*, *5*, *6*) and its Fab fragments (lanes *2*, *4*) by SDS-PAGE in a gel containing DNA. Before electrophoresis the samples were incubated in nonreducing (*A*) or reducing conditions (in the presence of 2-mercapthoethanol) (*B*). DNase activity was revealed as a sharp dark band on the fluorescent background after ethidium bromide staining (lanes *3*, *4*, and *6*) (the negatives of the films are shown). Gels stained with Coomassie Blue show positions of IgG (lane *1*), its Fab fragments (lane *2*), and separated heavy (H) and light (L) chains (lane *5*). Arrows indicate the positions of molecular mass markers (Buneva et al. 1998).

In conjunction with Paul's original list, these tests provide strong evidence supporting the catalysis of various reactions by natural abzymes. Some of these tests, and particularly the in situ observation of catalytic activity induced by an antibody, its Fab fragment, and one of its individual separated chains, may be considered as the most rigid argument unambiguously assigning observed catalytic activity to antibodies versus contaminating proteins, while also providing the basis for subsequent structural and functional analysis of abzyme activity.

Issues in Isolation of Natural Abzymes Possessing a High Catalytic Activity

Abzymes constitute a subset of normal antibodies, and are purified and processed using similar experimental protocols (Harlow and Lane 1988). The standard scheme of antibody isolation includes as a first step a separation of a total antibody fraction consisting of many different Igs with differing affinity for various antigens, including proteins, nucleic acids, and other species, which may be tightly complexed with these antibodies. Next, monospecific preparations of antibodies against the single natural antigen are isolated. These antibodies are polyclonal in origin and may contain an extremely diverse set of Ig molecules, which include abzymes along with common antigen-binding antibodies without catalytic activity. The repertoire of antibodies differs between individual patients, and the affinity of distinct antibody fractions for an immobilized ligand may differ by several orders of magnitude (Kit et al. 1995; Nevinsky et al. 1998). To simplify problems of purification, generally only the fraction of antibodies with the highest affinity for the substrate of interest is used.

Affinity chromatography on adsorbents bearing anti-IgG, anti-IgM, and anti-IgA antibodies on protein-A Sepharose in harsh conditions is used as the first step of purification for natural abzymes. As a rule, this step yields homogeneous preparations of antibodies according to SDS-PAGE (with silver staining) and western blot with immunoprobes, and ensures the effective removal of proteins nonspecifically bound with antibodies. The next steps are chromatography on DEAE-cellulose followed by incubation of antibody preparations for 1 hour at pH 2.6 to dissociate any non-covalent complexes, followed by gel filtration on Toyopearl HW-60 or other

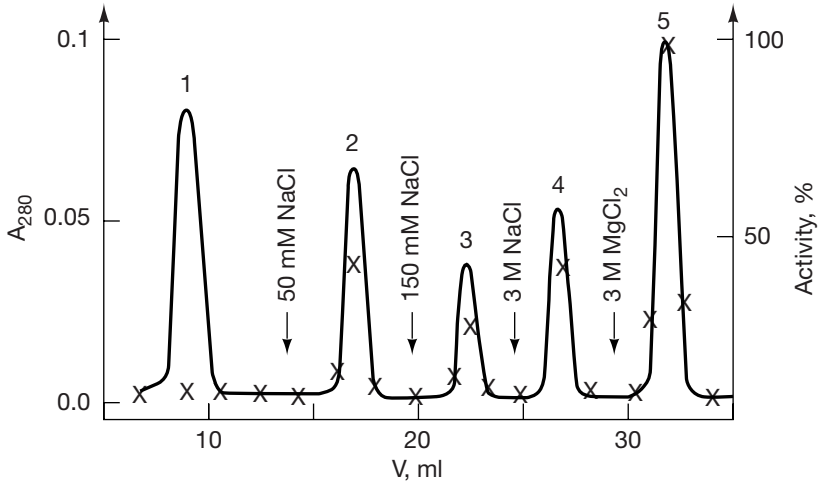

FIGURE 2. Affinity chromatography of milk sIgA with casein kinase activity on ATP-Sepharose. (–) Absorption at 280 nm; (x), relative casein kinase activity of sIgA (in % to the fraction with highest activity). Fraction 1 was eluted by 50 mM Tris-HCl (pH 6.8). (Nevinsky et al 1998).

adsorbents equilibrated with the same acidic buffer (Kit et al. 1995; Nevinsky et al. 1998 and references therein). These steps greatly reduce the likelihood that preparations of abzymes will contain any contaminating proteins: However, we note that one risk of this purification regimen is that incubation at low pH may disrupt the catalytic activity of some abzymes. The affinity chromatography of catalytic antibody preparations on an adsorbent bearing immobilized substrates (proteins, nucleic acids, nucleotides, etc.) is used as a last step and usually leads to their resolution into a set of distinct fractions demonstrating an extremely pronounced heterogeneity of monospecific catalytic antibodies, which differ from individual to individual. For example, affinity chromatography of human milk sIgA preparations on ATP-Sepharose (Fig. 2) demonstrated that these sIgA possessed a heterogeneous affinity for ATP. Usually, some fractions can only be eluted under conditions required for disruption of very stable immune complexes (3 M NaCl, 3 M $MgCl_2$) (Nevinsky et al. 1998). This step permits enrichment of antibodies with strong substrate binding.

Ideally, the final step of purification of abzymes should be their separation from noncatalytic antibodies of the same specificity. Unfortunately, at this time, no convenient method for this has been described. As a result, all described polyclonal abzyme preparations represent a mixture of catalytic and noncatalytic antibodies of similar specificity. Because of this, the relative specific activities of polyclonal enzymes may be essentially underestimated, especially as the proportion of abzymes in the high-affinity antibody fraction isolated by affinity chromatography for a given substrate may be in the order of 1–5% of the total population (G.A.Nevinsky et al., unpubl.).

MECHANISTIC STUDIES OF ABZYMES

To develop abzymes as tools for biological manipulations, it is necessary to understand the structural basis of their activity in detail. Although study of polyclonal abzymes provides important information about the potential catalytic activities of antibodies, structural studies require pure, uniform, material concentrated to high levels. Hence, these studies rely on monoclonal or recombinant sources of antibodies. A number of different catalytic antibodies have been analyzed as models for understanding the basis of catalysis. Some of this work is briefly summarized below.

The VIP-hydrolyzing abzyme, which was the first polyclonal abzyme described (Paul et al. 1989), was adapted for mechanistic analysis. The light chain of the VIP abzyme was expressed in bacteria, purified, and found to possess independent catalytic activity (Gao and Paul 1995; Tyutyulkova et al. 1996). Subsequently, single-chain Fv constructs containing the V_L domain of the anti-VIP light chain linked via a 14-residue peptide to its natural V_H domain partner were developed that possessed an increased affinity for the substrate ground state. From these and other data, a model of catalysis by the anti-VIP antibodies was proposed according to which the essential catalytic residues are located in the V_L domain and additional residues from the V_H domain are involved in high-affinity binding of the substrate (Sun et al. 1997).

Molecular modeling suggested the presence of a serine protease-like site in the light chain of VIP-hydrolyzing abzyme. This assumption was supported by inhibition of the hydrolytic activity of recombinant L chain by serine protease inhibitors, but not by inhibitors of other classes of protease. The serine protease mechanism was further supported by the observation that catalytic activity was lost following site-directed mutagenesis at a framework region residue, Asp1 (Gololobov et al. 1999), and at two complementarity determining region (CDR) residues, Ser-27a and His-93 (Gao and Paul 1995), residues forming a catalytic triad modeled to be similar to that found in serine proteases. The effect of these mutations was specific to catalysis rather than binding, and the affinity of the light chain for the substrate ground state was nearly unaffected by mutations at Ser-27a or His-93. In contrast, a Ser-26 single mutant and His-27d/Asp-28 double mutant displayed increased K_M (by about tenfold) and increased turnover (by about tenfold). Thus, two types of light-chain amino acid residues participating in catalysis were suggested: those essential for catalysis and those participating in VIP binding and indirectly limiting abzyme/substrate turnover. Of note, all three critical catalytic residues (Ser-27a, His-93, Asp-1) were present in the germ-line counterpart of the mature V_L, whereas the mature and germ-line sequences differed only by four amino acids remote from the catalytic site (Gololobov et al. 1999). Differences between the kinetic constants of the mature and germ-line light chains were marginal. These data show that catalytic activity of VIP-hydrolyzing antibodies is encoded by a germ-line VL gene, but can potentially be improved by somatic sequence diversification and pairing of the L chain with the appropriate heavy chain (for reviews, see Paul 1998; Gololobov et al. 2000).

Another intensively studied system has been catalytic antibodies with esterase-like activity. Analysis of the crystal structure of the abzyme Fab fragment has shown that the ligand *p*-nitrophenyl ester interacts with amino acid residues of both light and heavy chains of the abzyme (Golinelli-Pimpaneau et al. 1994). Two tyrosines were found to mimic the oxyanion-binding hole of serine proteases, at the catalytic core of the antibody. In a series of studies, the changes in binding of abzyme for a nitrophenyl phosphonate substrate were compared in germ-line versus affinity-matured Fab fragment (Patten et al. 1996; Wedemayer et al. 1997a,b). Intriguingly, the binding properties of the two species differed, with the germ-line abzyme showing significant changes in conformation subsequent to binding of hapten, whereas the affinity-matured Fab bound by a lock-and-key mechanism. These changes were accompanied by an increased affinity of abzyme for substrate by a factor of 10^4, reflecting a decrease in complex dissociation rate. The implication of these studies is that in vitro mutational approaches guided by detailed structural knowledge may be a useful means of generating improved novel abzyme catalytic activities targeted against the cleavage of proteins of biological interest.

SUMMARY

A growing body of data suggests that catalytic abzymes may be important mediators of immunological defense, regulation, and autoimmune dysfunction. The degree to which abzymes contribute to these biological phenomena will require continuing studies of antibody-mediated catalysis fol-

lowing experimental immunization and autoimmune disease, as well as mechanistic investigation of catalysis by antibodies and their subunits. From a technological point of view, the further study of both artificial and natural abzymes with the goal of understanding of structure–function relationships, to enable production of tailor-made catalysts of potential therapeutic application, is of high importance. Because the catalytic activity of certain antibodies is an innate function (Gololobov et al. 1999), catalysts with specificity for virtually any target polypeptide could potentially be developed, then improved by in vitro or in vivo affinity selection. The increasing number of available X-ray structures of catalytic antibodies shows the multiplicity of solutions to the question of how an antibody can catalyze an enzyme-like reaction. These strategies include amino acid arrangements analogous to those in enzymes (Zhou et al. 1994), but also arrangements completely different from those selected in enzymes by natural evolution (Charbonnier et al. 1995). Anti-idiotype approaches may enable the mimicry by abzymes of many useful enzymes (Kolesnikov et al. 2000), enhancing their possible functions. The phenomenon of abzyme catalysis can potentially be applied to isolate efficient catalysts suitable for passive immunotherapy of major diseases. For example, cocaine-hydrolyzing abzymes have been developed and may provide a novel approach to the problems of drug addiction (De Prada et al. 2000). Abzymes that cleave the gp120 protein of human immunodeficiency virus (HIV) may be of use in the treatment of AIDS (Paul et al. 2000). Through the rational design of abzymes with specified and novel catalytic functions allowing the selective cleavage of surface proteins, it is hoped that an unprecedented level of control over in vivo protein–protein associations may be achieved.

REFERENCES

Andrievskaya O.A., Buneva V.N., Naumov V.A., and Nevinsky G.A. 2000. Catalytic heterogenity of polyclonal RNA-hydrolyzing IgM from sera of patients with lupus erythematosus. *Med. Sci. Monit.* **6:** 460–470.

Baranovskii A.G., Kanyshkova T.G., Mogelnitskii A.S. Naumov V.A., Buneva V.N., Gusev E.I., Boiko A.N., Zargarova T.A., Favorova O.O., and Nevinsky G.A. 1998. Polyclonal antibodies from blood and cerebrospinal fluid of patients with multiple sclerosis effectively hydrolyze DNA and RNA. *Biochemistry* **63:** 1239–1248.

Baranovsky A.G., Matushin V.G., Vlassov A.V., Zabara V.G., Naumov V.A., Giege R., Buneva V.N., and Nevinsky G.A. 1997. DNA- and RNA-hydrolyzing antibodies from the blood of patients with various forms of viral hepatitis. *Biochemistry* **62:** 1358–1366.

Barbas III, C.F., Heine A., Zhong G., Hoffmann T., Gramatikova S., Bjornestedt R., List B., Anderson J., Stura E.A., Wilson I.A., and Lerner R.A. 1997. Immune versus natural selection: Antibody aldolases with enzymic rates but broader scope. *Science* **278:** 2085–2092.

Buneva V.N., Andrievskaia O.A., Romannikova I.V., Gololobov G.V., Iadav R.P., Iamkovoi V.I., and Nevinskii G.A. 1994. Interaction of catalytically active antibodies with oligoribonucleotides. *Mol. Biol.* **28:** 738–743.

Buneva V.N., Kanyshkova T.G., Vlassov A.V., Semenov D.V., Khlimankov D., Breusova L.R., and Nevinsky G.A. 1998. Catalytic DNA- and RNA-hydrolyzing antibodies from milk of healthy human mothers. *Appl. Biochem. Biotechnol.* **75:** 63–76.

Charbonnier J.B., Carpenter E., Gigant B., Golinelli-Pimpaneau B., Eshhar Z., Green B.S., and Knossow M. 1995. Crystal structure of the complex of a catalytic antibody Fab fragment with a transition state analog: Structural similarities in esterase-like catalytic antibodies. *Proc. Natl. Acad. Sci.* **92:** 11721–11725.

De Prada P., Winger G., and Landry D.W. 2000. Application of artifical enzymes to the problem of cocaine. *Ann. N.Y. Acad. Sci.* **909:** 159–169.

Ding L., Liu Z., Zhu Z., Luo G., Zhao D., and Ni J. 1998. Biochemical characterization of selenium-containing catalytic antibody as a cytosolic glutathione peroxidase mimic. *Biochem. J.* **332:** 251–255.

Ersoy O., Fleck R., Blanco M.J., and Masamune S. 1999. Design and syntheses of three haptens to generate catalytic antibodies that cleave amide bonds with nucleophilic catalysis. *Bioorg. Med. Chem.* **7:** 279–286.

Fastrez J. 1997. In vivo versus in vitro screening or selection for catalytic activity in enzymes and abzymes. *Mol. Biotechnol.* **7:** 37–55.

Gabibov A.G., Gololobov G.V., Makarevich O.I., Schourov D.V., Chernova E.A., and Yadav R.P. 1994. DNA-hydrolyzing autoantibodies. *Appl. Biochem. Biotechnol.* **47:** 293–303.

Gao Q.S. and Paul S. 1995. Site-directed mutagenesis of antibody-variable regions. *Methods Mol. Biol.* **51:** 319–327.

Golinelli-Pimpaneau B., Gigant B., Bizebard T., Navaza J., Saludjian P., Zemel R., Tawfik D.S., Eshhar Z., Green B.S., and Knossow M. 1994. Crystal structure of a catalytic antibody Fab with esterase-like activity. *Structure* **2:** 175–183.

Gololobov G., Sun M., and Paul S. 1999. Innate antibody catalysis. *Mol. Immunol.* **36:** 1215–1222.

Gololobov G., Tramontano A., and Paul S. 2000. Nucleophilic proteolytic antibodies. *Appl. Biochem. Biotechnol.* **83:** 221–232; 297–313.

Gololobov G.V., Chernova E.A., Schourov D.V., Smirnov I.V., Kudelina I.A., and Gabibov A.G. 1995. Cleavage of supercoiled plasmid DNA by autoantibody Fab fragment: Application of the flow linear dichroism technique. *Proc. Natl. Acad. Sci.* **92:** 254–257.

Gouverneur V.E., Houk K.N., de Pascual-Teresa B., Beno B., Janda K.D., and Lerner R.A. 1993. Control of the exo and endo pathways of the Diels-Alder reaction by antibody catalysis. *Science* **262:** 204–208.

Harlow E. and Lane D. 1988. *Antibodies: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Hotta K., Lange H., Tantillo D.J., Houk K.N., Hilvert D., and Wilson I.A. 2000. Catalysis of decarboxylation by a preorganized heterogeneous microenvironment: Crystal structures of abzyme 21D8. *J. Mol. Biol.* **302:** 1213–1225.

Iverson B.L. and Lerner R.A. 1989. Sequence-specific peptide cleavage catalyzed by an antibody. *Science* **243:** 1184–1188.

Janda K.D., Shevlin C.G., and Lerner R.A. 1993. Antibody catalysis of a disfavored chemical transformation. *Science* **259:** 490–493.

Janda K.D., Schloeder D., Benkovic S.J., and Lerner R.A. 1988. Induction of an antibody that catalyzes the hydrolysis of an amide bond. *Science* **241:** 1188–1191.

Janda K.D., Lo L.C., Lo C.H., Sim M.M., Wang R., Wong C.H., and Lerner R.A. 1997. Chemical selection for catalysis in combinatorial antibody libraries. *Science* **275:** 945–948.

Jencks W. 1969. *Catalysis in chemistry and enzymology*. McGraw-Hill, New York.

Kanyshkova T.G., Semenov D.V., Vlasov A.V., Khlimankov D.I., Baranovskii A.G., Shipitsyn M.V., Iamkovoi V.I., Buneva B.N., and Nevinskii G.A. 1997. DNA- and RNA- hydrolysed antibodies from human milk and its biological role. *Mol. Biol.* **31:** 1082–1091.

Karush F. 1978. In *Immunoglobulins* (ed. G.V. Litman and R.A. Good), p. 3. Plenum Press, New York.

Kit I.I., Semenov D.V., and Nevinskii G.A. 1995. Do catalytically active antibodies exist in healthy people? (Protein kinase activity of sIgA antibodies from human milk.) *Mol. Biol.* **29:** 893–906.

Kolesnikov A.V., Kozyr A.V., Alexandrova E.S., Koralewski F., Demin A.V., Titov M.I., Avalle B., Tramontano A., Paul S., Thomas D., Gabibov A.G., and Friboulet A. 2000. Enzyme mimicry by the antiidiotypic antibody approach. *Proc. Natl. Acad. Sci.* **97:** 13526–13531.

Kozyr A.V., Kolesnikov A.V., Aleksandrova E.S., Sashchenko L.P., Gnuchev N.V., Favorov P.V., Kotelnikov M.A., Iakhnina E.I., Astsaturov I.A., Prokaeva T.B., Alekberova Z.S., Suchkov S.V., and Gabibov A.G. 1998. Novel functional activities of anti-DNA autoantibodies from sera of patients with lymphoproliferative and autoimmune diseases. *Appl. Biochem. Biotechnol.* **75:** 45–61.

Lacroix-Desmazes S., Moreau A., Sooryanarayana, Bonnemain C., Stieltjes N., Pashov A., Sultan Y., Hoebeke J., Kazatchkine M.D., and Kaveri S.V. 1999. Catalytic activity of antibodies against factor VIII in patients with hemophilia A. *Nat. Med.* **5:** 1044–1047.

Lerner R.A. and Tramontano A. 1987. Antibodies as enzymes. *Trends Biochem. Sci.* **12:** 427–438.

Li L., Paul S., Tyutyulkova S., Kazatchkine M.D., and Kaveri S. 1995. Catalytic activity of anti-thyroglobulin antibodies. *J. Immunol.* **154:** 3328–3332.

Li T., Janda K.D., and Lerner R.A. 1996. Cationic cyclopropanation by antibody catalysis. *Nature* **379:** 326–327.

Li T., Janda K.D., Ashley J.A., and Lerner R.A. 1994. Antibody catalyzed cationic cyclization. *Science* **264:** 1289–1293.

Liu E., Prasad L., Delbaere L.T., Waygood E.B., and Lee J.S. 1998. Conversion of an antibody into an enzyme which cleaves the protein HPr. *Mol. Immunol.* **35:** 1069–1077.

Ma L., Hsieh-Wilson L.C., and Schultz P.G. 1998. Antibody catalysis of peptidyl-prolyl cis-trans isomerization in the folding of RNase T1. *Proc. Natl. Acad. Sci.* **95:** 7251–7256.

Martin A.B. and Schultz P.G. 1999. Opportunities at the interface of chemistry and biology. *Trends Cell Biol.* **9:** 24–28.

Miller G.P., Posner B.A., and Benkovic S.J. 1997. Expanding the 43C9 class of catalytic antibodies using a chain- shuffling approach. *Bioorg. Med. Chem.* **5:** 581–590.

Nagai K. and Thogersen H.C. 1987. Synthesis and sequence-specific proteolysis of hybrid proteins produced in *Escherichia coli*. *Methods Enzymol.* **153:** 461–481.

Napper A.D., Benkovic S.J., Tramontano A., and Lerner R.A. 1987. A stereospecific cyclization catalyzed by an antibody. *Science* **237:** 1041–1043.

Nevinsky G.A., Kit Y., Semenov D.V., Khlimankov D., and Buneva V.N. 1998. Secretory immunoglobulin A from human milk catalyzes milk protein phosphorylation. *Appl. Biochem. Biotechnol.* **75:** 77–91.

Nevinsky G.A., Kanyshkova T.G., Semenov D.V., Vlassov A.V., Gal'vita A.V., and Buneva V.N. 2000. Secretory immunoglobulin A from healthy human mothers' milk catalyzes nucleic acid hydrolysis. *Appl. Biochem. Biotechnol.* **83:** 115–130; 145–153.

Patten P.A., Gray N.S., Yang P.L., Marks C.B., Wedemayer G.J., Boniface J.J., Stevens R.C., and Schultz P.G. 1996. The immunological evolution of catalysis. *Science* **271:** 1086–1091.

Paul S. 1998. Mechanism and functional role of antibody catalysis. *Appl. Biochem. Biotechnol.* **75:** 13–24.

Paul S., Johnson D.R., and Massey R. 1991. Binding and multiple hydrolytic sites in epitopes recognized by catalytic anti-peptide antibodies. *Ciba Found. Symp.* **159:** 156–167.

Paul S., Kalaga R.S., Gololobov G., and Brenneman D. 2000. Natural catalytic immunity is not restricted to autoantigenic substrates: Identification of a human immunodeficiency virus gp 120- cleaving antibody light chain. *Appl. Biochem. Biotechnol.* **83:** 71–84; 145–153.

Paul S., Volle D.J., Beach C.M., Johnson D.R., Powell M.J., and Massey R.J. 1989. Catalytic hydrolysis of vasoactive intestinal peptide by human autoantibody. *Science* **244:** 1158–1162.

Paul S., Li L., Kalaga R., O'Dell J., Dannenbring Jr., R.E., Swindells S., Hinrichs S., Caturegli P., and Rose N.R. 1997. Characterization of thyroglobulin-directed and polyreactive catalytic antibodies in autoimmune disease. *J. Immunol.* **159:** 1530–1566.

Pauling L. 1946. Molecular basis of biological specificity. *Chem. Eng. News* **24:** 1375–1377.

Pollack S.J., Jacobs J.W., and Schultz P.G. 1986. Selective chemical catalysis by an antibody. *Science* **234:** 1570–1573.

Roberts V.A., Stewart J., Benkovic S.J., and Getzoff E.D. 1994. Catalytic antibody model and mutagenesis implicate arginine in transition-state stabilization. *J. Mol. Biol.* **235:** 1098–1116.

Savel'ev A.N., Eneyskaya E.V., Shabalin K.A., Filatov M.V., and Neustroev K.N. 1999. Antibodies with amilolytic activity. *Protein Peptide Lett.* **6:** 179–184.

Schochetman G. and Massey R. 1985. International Patent no. W085/02414.

Semenov D.V., Kanyshkova T.G., Kit Y.Y., Khlimankov D.Y., Akimzhanov A.M., Gorbunov D.A., Buneva V.N., and Nevinsky G.A. 1998. Human breast milk immunoglobulins G hydrolyze nucleotides. *Biochemistry* **63:** 935–943.

Shuster A.M., Gololobov G.V., Kvashuk O.A., Bogomolova A.E., Smirnov I.V., and Gabibov A.G. 1992. DNA hydrolyzing autoantibodies. *Science* **256:** 665–667.

Smiley J.A. and Benkovic S.J. 1994. Selection of catalytic antibodies for a biosynthetic reaction from a combinatorial cDNA library by complementation of an auxotrophic *Escherichia coli:* Antibodies for orotate decarboxylation. *Proc. Natl. Acad. Sci.* **91:** 8319–8323.

Smithrud D.B., Benkovic P.A., Benkovic S.J., Roberts V., Liu J., Neagu I., Iwama S., Phillips B.W., Smith III, A.B. and Hirschmann R. 2000. Cyclic peptide formation catalyzed by an antibody ligase. *Proc. Natl. Acad. Sci.* **97:** 1953–1958.

Stewart J.D. and Benkovic S.J. 1993. Recent developments in catalytic antibodies. *Int. Rev. Immunol.* **10:** 229–240.

Stewart J.D., Krebs J.F., Siuzdak G., Berdis A.J., Smithrud D.B., and Benkovic S.J. 1994. Dissection of an antibody-catalyzed reaction. *Proc. Natl. Acad. Sci.* **91:** 7404–7409.

Sun M., Li L., Gao Q.S., and Paul S. 1994. Antigen recognition by an antibody light chain. *J. Biol. Chem.* **269:** 734–738.

Sun M., Gao Q.S., Kirnarskiy L., Rees A., and Paul S. 1997. Cleavage specificity of a proteolytic antibody light chain and effects of the heavy chain variable domain. *J. Mol. Biol.* **271:** 374–385.

Suzuki H. 1994. Recent advances in abzyme studies. *J. Biochem.* **115:** 623–628.

Thayer M.M., Olender E.H., Arvai A.S., Koike C.K., Canestrelli I.L., Stewart J.D., Benkovic S.J., Getzoff E.D., and Roberts V.A. 1999. Structural basis for amide hydrolysis catalyzed by the 43C9 antibody. *J. Mol. Biol.* **291:** 329–345.

Thiagarajan P., Dannenbring R., Matsuura K., Tramontano A., Gololobov G., and Paul S. 2000. Monoclonal antibody light chain with prothrombinase activity. *Biochemistry* **39:** 6459–6465.

Tramontano A., Janda K.D., and Lerner R.A. 1986a. Catalytic antibodies. *Science* **234:** 1566–1570.

———. 1986b. Chemical reactivity at an antibody binding site elicited by mechanistic design of a synthetic antigen. *Proc. Natl. Acad. Sci.* **83:** 6736–6740.

Tyutyulkova S., Gao Q.S., Thompson A., Rennard S., and Paul S. 1996. Efficient vasoactive intestinal polypeptide hydrolyzing autoantibody light chains selected by phage display. *Biochim. Biophys. Acta* **1316:** 217–223.

Vlassov A., Florentz C., Helm M., Naumov V., Buneva V., Nevinsky G., and Giege R. 1998a. Characterization

and selectivity of catalytic antibodies from human serum with RNase activity. *Nucleic Acids Res.* **26:** 5243–5250.

Vlassov A.V., Baranovskii A.G., Kanyshkova T.G., Prints A.V., Zabara V.G., Naumov V., Breusov A.A., Giege R., Buneva V.N., and Nevinsky G. 1998b. Substrate specificity of DNA- and RNA-hydrolyzing antibodies from blood of patients with polyarthritis and Hashimoto's thyroiditis. *Mol. Biol* **32:** 559–569.

Wang K.K. 2000. Calpain and caspase: Can you tell the difference? *Trends Neurosci.* **23:** 20–26.

Wedemayer G.J., Patten P.A., Wang L.H., Schultz P.G., and Stevens R.C. 1997a. Structural insights into the evolution of an antibody combining site. *Science* **276:** 1665–1669.

Wedemayer G.J., Wang L.H., Patten P.A., Schultz P.G., and Stevens R.C. 1997b. Crystal structures of the free and liganded form of an esterolytic catalytic antibody. *J. Mol. Biol.* **268:** 390–400.

Wentworth Jr., P., Liu Y., Wentworth A.D., Fan P., Foley M.J., and Janda K.D. 1998. A bait and switch hapten strategy generates catalytic antibodies for phosphodiester hydrolysis. *Proc. Natl. Acad. Sci.* **95:** 5971–5975.

Zhou G.W., Guo J., Huang W., Fletterick R.J., and Scanlan T.S. 1994. Crystal structure of a catalytic antibody with a serine protease active site. *Science* **265:** 1059–1064.