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Enzymes and abzymes relationships

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Abstract

Antibodies and enzymes are proteins that bind macromolecules and small natural and synthetic ligands with high specificity and high affinity. While enzymes have been perfecting their skills during evolution to reach very efficient structures for catalysis, antibody-binding sites evolve toward efficient structures for binding in a few weeks. The generation of antibodies bearing catalytic activities (abzymes) brings new insights in the understanding on the evolution of the catalytic function, and also on functions that the immune system could play in metabolism. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The immune system is able to generate and screen tremendous numbers of antibodies that bind virtually any natural or synthetic molecule with high affinity and exquisite selectivity. Selective recognition is achieved through a large number of weak bonding interactions involving hydrogen bonds, van der Waals and electrostatic interactions, and solvent effects. The new field of catalytic antibodies (abzymes) has shown the possibility to create tailor-made antibodies that not only bind a molecule but also are able to act as enzymes. However, going from binding, a basic property of antibodies, to catalysis is not trivial and there is a need to achieve a step of critical importance. The usual low efficiency of abzymes when compared to enzymes clearly demonstrates the actual need to understand and to elucidate the molecular mechanisms involved in the critical step.

Compelling evidence for the catalytic activity of antibodies has been reported for three distinct approaches. The first one employs the ‘‘classical’’ strategy of immunization with compounds that mimic the transition state of a chemical reaction. Catalytic antibodies were also isolated from the sera of patients with several diseases. The third strategy using the properties of the idiotypic network of the immune system was

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proposed by our group to elicit catalytic antibodies. Abzymes obtained using these three approaches bring complementary information on structure-activity relationships and on evolution of the catalyst during the maturation of the immune response.

2. Complementarity and specificity

As a pioneer of the modern concept of molecular complementarity, Linus Pauling [1] proposed that an enzyme is a flexible molecular template that is complementary to the reactants in their activated transition-state geometry. Its efficiency is achieved in part as the result of transition state stabilization, strain, acid–base catalysis, and proximity. However, to explain the astonishing high specificity and the optimal rate acceleration exhibited by enzymes, it is necessary to take into account the summation of multiple interactions that have slowly evolved to reach optimal structures.

One of the most remarkable features of the immune system is its ability to respond to an apparently limitless array of antigens (Ags). The tremendous diversity coupled to the particular Ag-binding specificity 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 [2]. This property confers to antibodies the unique capacity among mammalian proteins to evolve toward very efficient structures in a few weeks. Although antibodies 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 [3,4]. The more conserved regions of the variable 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 to consider the antibody-binding site as a simple pocket into which lies the Ag [5,6]. The extent of the antibody–Ags interaction depends on the sequence of amino acids of the CDRs, interactions between lateral chains of these amino acids and Ags, and the size of the loops.

3. Antibodies as catalysts

Recognition through complementarity features is a phenomenon shared by many proteins, notably by antibodies and enzymes. From this observation arose the proposal by W. Jencks that antibodies could be used to selectively stabilize the rate-determining transition state of a chemical reaction by eliciting these antibodies against a stable structure resembling the transition state [7] (Fig. 1a). However, a breakdown in the field came from the development of the monoclonal antibody technique by Köhler and Milstein in 1975 [8] that has provided the means for producing antibodies with a single antigenic defined specificity. This was of critical importance for characterizing catalytic activities. Since the first reports by the groups of Lerner and Schultz [9,10], catalytic antibodies 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 [11–13], the thorough complementarity influences the proper catalysis by antibodies. 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 antibodies and Ags.

Despite these observations, catalytic antibodies elicited against transition state analogs 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 [14,15]. However, a

new strategy, reactive immunization, developed by Barbas et al. [16] allowed to elicit catalytic antibodies that have efficiency close to that of natural enzymes. This approach results from the observation that enzyme selection during evolution proceeds presumably by catalytic selection

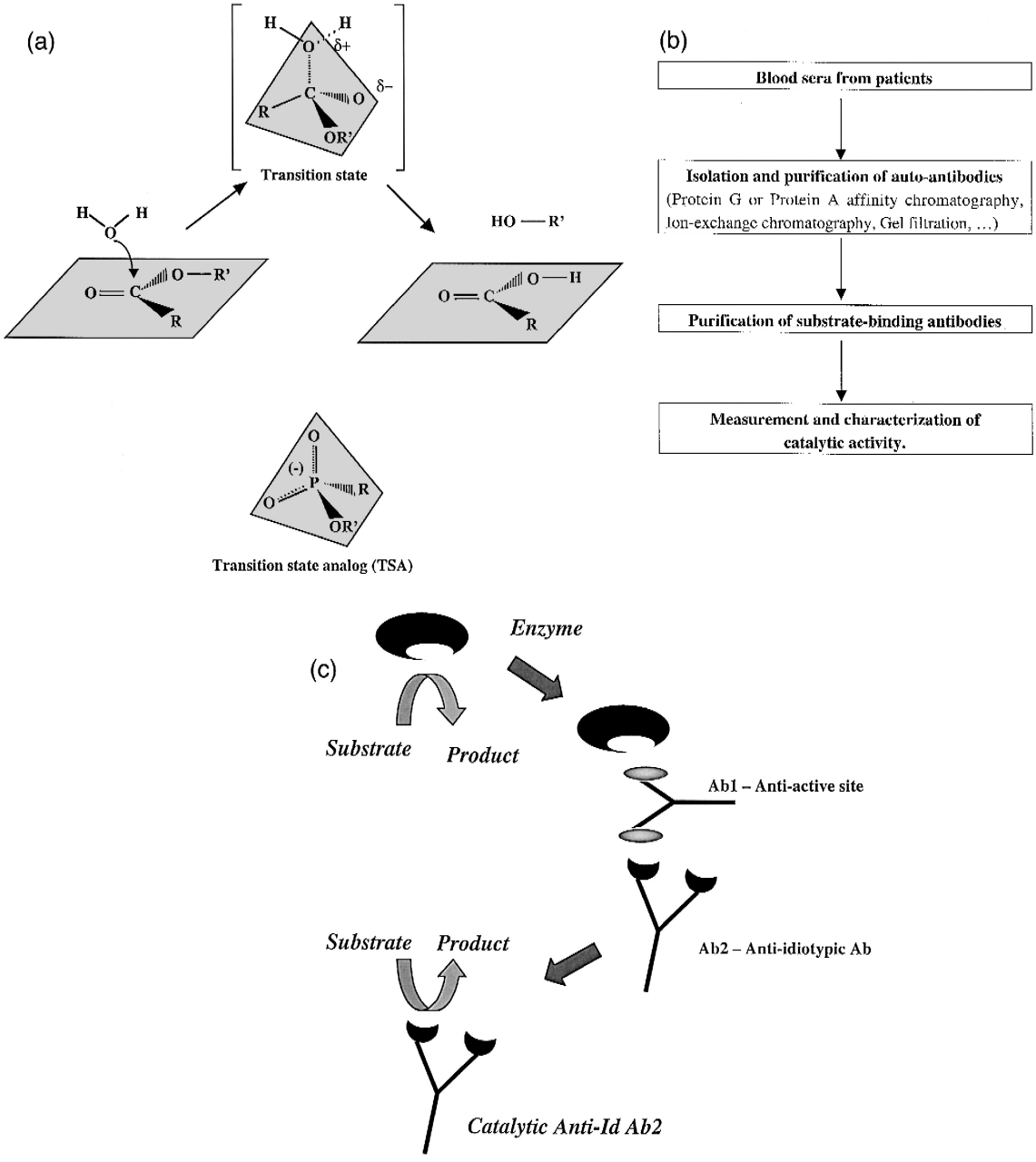

Fig. 1. Schematic representation of the strategies used to produce catalytic antibodies. a — The ligand-based approach: the structure of the transition state of an ester bond hydrolysis can be mimicked by a stable phosphonate analog that is used to elicit catalytic antibodies (anti-TSA Abs). b — Isolation of naturally occurring abzymes: the overall strategy consists in isolation of antibodies with catalytic activity from the sera of patients with autoimmune diseases. c — Anti-idiotypic catalytic antibodies (Anti-Id Abs): structural copies of an enzyme active site can be produced by using the properties of the idiotypic network.

while effective clonal selection in the immune system is based on improved binding. The use of reactive Ags designed so that a covalent bond occurs in the binding pocket of induced antibodies allow to change the selection criteria from simple binding to chemical reactivity. It is interesting to note that while efficiency is improved, specificity is lowered. On the other hand, autoimmunity can amplify the selection of clones with catalytic activity. Tawfik et al. [17] have shown that after immunization with a transition state analog, the number of clones expressing antibodies with catalytic activity increased more dramatically in autoimmune mouse strains, compared with normal mice. Taken together, these results strongly suggest that the regulatory mechanisms of the immune system for amplifying or inhibiting clones play a crucial role in selection of antibodies with catalytic activity.

4. Naturally occurring abzymes

Catalytic activity can arise by natural means in antibodies. Two different catalytic activities were first identified in the sera of patients with autoimmune diseases (Fig. 1b).

Antibodies with protease activity were first isolated in the serum of a patient with asthma [18,19]. These autoantibodies were found to hydrolyze vasoactive intestinal peptide (VIP) with good efficiency and specificity. Surprisingly, by immunizing mice with VIP in its ground state, it was possible to isolate a monoclonal antibody with a VIP-hydrolase activity. This allowed to further study the hydrolytic mechanism, and to demonstrate that at least a Ser and a His residue of the light chain are directly involved in catalysis, and that the isolated light chain of the antibody hydrolyzes VIP. Thyroglobulin-cleaving antibodies were also isolated from a patient with Hashimoto's thyroiditis [20]. Certain Bence Jones proteins, which are monoclonal human light chains found in the urine of approximately 60% of multiple myeloma patients, were also found to exhibit amidase and protease activities [21,22].

DNA-hydrolyzing autoantibodies were also isolated from the sera of patients with systemic lupus erythematosus or rheumatoid arthritis [23]. The DNA-hydrolyzing activity could be correlated to the presence of high levels of anti-topoisomerase I in the sera of patients [24]. This has led the authors to propose that these catalytic antibodies arose in serum as the result of an anti-idiotypic mechanism.

Another example has been recently described by studying the appearance of alloantibodies that inhibit the factor VIII (FVIII) procoagulant activity in hemophilia patients infused with homologous FVIII [25]. In 25% of patients, anti-FVIII antibodies were found not only to inhibit FVIII activity but also to exhibit a significant proteolytic activity against FVIII. The kinetic parameters of this catalytic activity are consistent with a possible functional role for the catalytic immune response in the inactivation of FVIII in vivo.

5. Anti-idiotypic and catalytic antibodies

In 1974, Niels Jerne [26] advanced the theory that the immune system could be regarded as a network of interacting idiotopes. A major postulate was that for each immunoglobulin (Ab1) generated against an antigenic determinant, there existed a complementary antibody (Ab2) directed against the idiotypic determinants of Ab1. When idiotopes are superimposed with the complementary regions of the binding sites, Ab2 (or anti-idiotypic) molecules can mimic the Ags structures and are designed as internal images of the original Ags. The most successful use of anti-idiotypic antibodies was the identification of cell-surface receptors for hormones, neurotransmitters, neuropeptides or growth factors. Our experimental approach to generate catalytic antibodies is schematized on Fig. 1c. A first antibody (idiotypic antibody, 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. A second set of antibodies (Ab2) is then produced against the Ab1 combining site. Among these second-generation, or anti-idiotypic antibodies, which can represent internal images of the target site, some antibodies may not only have the binding function for the substrate of the enzyme used as Ags but may also be able to catalyze its transformation. The first results were obtained with an esterolytic activity initially borne by acetylcholinesterase [27]. After immunization with an inhibitory antibody of the enzyme, both polyclonal and monoclonal catalytic antibodies were characterized [28]. The catalytic properties of a monoclonal Ab2, 9A8, were further studied and revealed a very high efficiency when compared with other esterase abzymes ($k_{cat} = 80$ s^{-1}, $K_M = 0.6$ mM). The second attempt concerned β-lactamase, an amidase enzyme involved in the hydrolysis of antibiotic containing a β-lactam ring, and which is absent in the serum of mammals. This activity allowed ruling out the possibility or artifactual results linked to a contaminant enzyme, and also direct measurement of the appearance of catalytic antibodies in the serum of mice during the immunization procedure. One IgG, 9G4H9 was selected for its capacity to hydrolyze penicillin substrates [29]. This antibody exhibits a moderate catalytic activity when compared with the natural enzyme (10^5-fold decrease in k_{cat} value), but this value is one of the highest when compared with antibodies previously generated to catalyze amide bond hydrolysis. Moreover, when 9G4H9 was used as Ag, immunized mice respond by producing Ab3 (anti-anti-idiotypic) that recognize the original Ag β-lactamase. This result clearly indicates the good conservation of structural features from Ab1 to Ab3 [29].

Other groups have used this approach to elicit catalytic antibodies. Recently, Du et al. [30] described the use of carboxypeptidase to elicit an anti-idiotypic monoclonal antibody IIF9D8 that exhibits an efficient esterase activity.

6. Abzymes versus enzymes active sites

The increasing number of available X-ray structures of catalytic antibodies clearly shows the multiplicity of solutions for an antibody to catalyze an enzyme-like reaction, from amino acid arrangements resembling those present in enzymes as catalytic dyads [31], to solutions that deeply differ that selected in enzymes by natural evolution [32], involving Tyr, Arg, Asn and His residues.

In the case of naturally occurring abzymes, the group of Paul used the catalytic light chain of a mouse monoclonal anti-VIP antibody to demonstrate by irreversible inhibition, site directed mutagenesis and molecular modeling that a Ser residue and a His residue are directly involved in the catalytic mechanism [33,34]. This result suggests that the solution selected for antibody protease activity resembles that usually found in enzymes using a catalytic triad.

Chemical modification experiments [28] and molecular modeling [35] of our cholinesterase-like anti-idiotypic antibody 9A8 clearly indicate the involvement of Ser (CDR3) and His (CDR1) residues in the catalytic mechanism. This suggests that the structural information borne by anti-idiotypic antibody has been sufficiently conserved from the enzyme to the abzyme to express a catalytic dyad able to cleave acetylcholine ester bond.

While it could be tempting to conclude that the most efficient catalytic antibodies are those were the molecular solutions selected for catalysis are those that are the closest to that observed in enzyme active sites, the number of resolved structures of abzymes is still insufficient to generalize these results.

7. Abzyme and metabolism

Catalytic antibodies were first developed as tools in chemistry. However, the demonstration for their presence in different pathologies together with the demonstration that the idiotypic

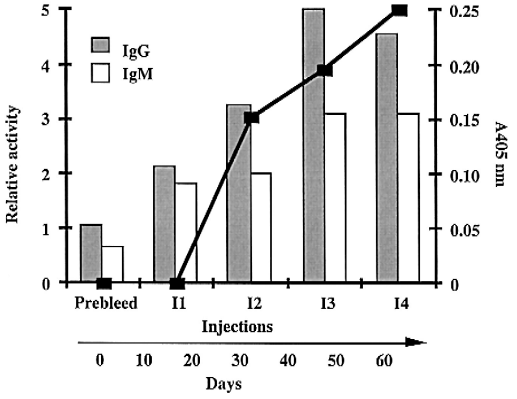

Fig. 2. Mice were injected with 150 μg of 7AF9, a monoclonal antibody directed against the active site of β-lactamase. One week after each immunization (from I1 to I4), the amount of IgG and IgM directed against 7AF9 Fab fragments in the serum was detected by ELISA, and the appearance of β-lactamase activity in 25-fold diluted antiserum was measured using PADAC, a chromogenic substrate.

network can generate efficient abzymes raise the question of their possible biological role.

While the first natural abzymes were isolated in the sera of patients, it was possible to elicit anti-VIP catalytic antibodies in mice by immunization with VIP in its ground state [36]. In the case of DNase abzymes isolated from patients with lymphoproliferative and autoimmune diseases, a crossreactivity of polyclonal DNA-abzymes to DNA-depleted nuclear matrix proteins suggests the possibility of abzyme production as antibodies to the energetically destabilized ground state of the Ags [37].

On the other hand, using the anti-idiotypic approach, it is possible to induce a measurable β-lactamase activity borne by antibody in the serum of mice immunized with an anti-β-lactamase antibody (Fig. 2). This result shows that non-classical catalytic activities can be induced in the serum of normal mice by the anti-idiotypic network.

Even in the ''classical'' approach using transition state analogs to elicit abzymes, Tawfik et al. [17] clearly demonstrated that the appearance of catalytic activities on antibodies is linked to the physiological condition of the animal. In mice with autoimmune disease, the level of antibodies with catalytic activity is significantly higher than in normal mice.

All these considerations have led us to propose that catalytic antibodies must not only be considered as laboratory oddness, but that they could play a role in metabolism. The idea that the immune system may interact with metabolism was first proposed by P. Grabar [38]. He hypothesized that antibodies may be indirectly involved in a fundamental physiological mechanism of transportation of catabolic and metabolic substances to facilitate their phagocytosis and degradation. Catalytic antibodies could be directly involved in a *CatAbolism* mechanism leading to the direct hydrolysis of these substances. According to P. Grabar, this leads us to consider the immune system not only as a mechanism for the defense of the organism against infection. Catalytic antibodies could be considered as one of the different functions that the immune system could play for metabolism or in different regulatory mechanism. All these functions of the immune system could be seen as an *Immunome*.

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