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Review

Antibody–antigen pair probed by combinatorial approach and rational design: Bringing together structural insights, directed evolution, and novel functionality

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ABSTRACT

The unique hypervariability of the immunoglobulin (Ig) superfamily provides a means to create both binding and catalytic antibodies with almost any desired specificity and activity. The diversity of antigens and concept of adaptive response suggest that it is possible to find an antigen pair to any raised Ig. In the current review we discuss combinatorial approaches, which makes it possible to obtain an antibody with predefined properties, followed by 3D structure-based rational design to enhance or dramatically change its characteristics. A similar strategy, but applied to the second partner of the antibody–antigen pair, may result in selection of complementary substrates to the chosen Ig. Finally, 2D screening may be performed solving the "Chicken and Egg" problem when neither antibody nor antigen is known.

1. Introduction

Since the early 1970s, when IgG Fab and light chain dimer structures became available [1,2], it became obvious that detailed studies of antibody combining sites can provide extremely useful data concerning antibody–antigen interactions, thereby providing new insights for biomedical research and for diagnostic and therapeutic medicine. Moreover, it has become clear that knowledge of an exact antibody structure can enable the application of the unique property of efficient in vivo binding in arresting numerous pathological processes developing within an organism. These observations have uncovered the enormous therapeutic potential antibodies that has resulted in an exponential growth of antibody-based drugs [3]. On the other hand, the identification of natural antibodies specific for various targets in biological fluids now plays an important role as a biomarker of disease and variety of abnormalities [4].

Initially, antibody generation by scientists was based on the hybridoma technique [5]. This required researchers to use a somewhat unnatural system of adoptive response in living organisms. This approach was like a wormhole: it swallowed up the antigen and afterwards yielded a set of B cells, producing antibodies towards it. Indeed, the hybridoma technique is now one of the most important tools in modern immunology and biotechnology, although it has several significant restrictions. Today, dramatic breakthroughs in immunology, molecular biology, and crystallography have made it possible to create antibodies with new, predefined combining sites and rationally designed functionality. Selection of antibody repertoires from biological fluids, both by single-cell RT-PCR and phage-display libraries, has extraordinary potential for diagnostics, therapeutic, and biotechnology purposes. Moreover, not only antibodies but also antigens can be a target for combinatorial selection. The broad application of chemical synthetic libraries in the early 1980s, powered by combinatorial biology approaches, namely phage display [6,7], has enabled the use of 2D screening of chemical versus Ig libraries.

Immunization by low molecular weight haptens, that mimicked the transition state of chemical reactions, and by antibodies towards active sites of enzymes, the internal image approach, led to new artificial biocatalysts, christened abzymes [8–10]. Since then hundreds of different chemical transformations have been shown to be catalysed by abzymes [11,12]. The unique hypervariability of antibody structure offers the possibility to create a

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biocatalyst with almost any possible catalytic activity. This task may be accomplished by means of the above-mentioned screening technologies combined with rational design. As reported in our recent study, the screening of phage-display libraries using activated phosphonate anchors may be considered as a source of novel biocatalysts, or "reactibodies" [13].

Different classes of antibody-mediated reactions have been identified in autoimmunity [4,14–16]. Using natural mechanisms of antibody generation it is possible to force the immune system to produce a catalytic Ig with the required activity. Finally, regardless of the origin and selection procedure, each antibody can be subjected to rational tuning in order to enhance its properties or develop novel functionality. This review addresses the existing balance between rational design and high-throughout screening technologies to probe both antibody and antigen.

2. Combinatorial selection and directed evolution of antibodies. Reactibodies

Antibodies have a number of unique properties including good half-life in the blood stream, high specificity, developed protocols of expression, as well as a longstanding and successful application for the treatment of a variety of human diseases. The idea to bring even several catalytic turnovers to any adopted therapeutic antibody "binder" may be commercially acceptable [17]. Current progress in obtaining catalytic antibodies with various specificities gives us an exciting opportunity to make "catalytic vaccines" towards toxic low molecular weight molecules such as narcotics, pesticides, and drugs as well as against bacterial and viral proteins. As an impressive example, it is possible to mention the cocaine-hydrolysing antibody 15A10, which in contrast to simply binding Ig [18] significantly decreased mortality from cocaine and completely blocked its psycho-active effect *in vivo*.

More than 200 000 people die annually as a result of poisoning by organophosphorus (OP) pesticides according to the WHO. Moreover, existing chemical warfare agents are also mainly OPs. In order to find an abzyme towards OP compounds in our recent approach, we have used a combinatorial selection strategy to select biocatalysts from a human semi-synthetic antibody variable fragment library towards a reactive phosphonate. Phage display is a selection system whereby genetic information is directly linked to functional properties (such as enzyme or abzyme activity) of the coded polypeptide product. A number of such systems has been described recently [19,20]; some of them have been used in the selection of efficient catalytic antibodies [21]. Directed selection of a biocatalyst towards a certain reactive bait was demonstrated in a report [22]. Such in vitro selection technologies are extremely useful in the case of antigens potentially toxic for host animals and therefore not useable for immunization.

With this approach, we selected a diversified repertoire of human immunoglobulin variable light (V_L) and heavy (V_H) single chain fragments (scFv) displayed on phage particles [22] towards a biotinylated phosphonate ester. We identified a set of nucleophile-bearing scFv sites predisposed for covalent catalysis. Sequence analysis of the reactive clones revealed preferred pairing of lambda V_L and V_H chains with conserved CDR-H3 sequences. Basically, we observed two main structural motifs employing distinct tyrosine residues in V_L as a nucleophile. The majority of the clones (7/8) had a tyrosine (Y-L33) residue located in the CDR1 as a putative nucleophile. Nonetheless, A.17, the most reactive clone, used the tyrosine residue Y-L37 in a conserved framework also for this purpose. We suggested the term "reactibody" for these clones to emphasize that they were selected for a chemical reactivity rather than for ground state binding.

Further variable fragments of the A17 reactibody were integrated into the full-length human recombinant antibody and subsequently expressed in CHO cells. The resulted catalytic Ig demonstrated catalytic activity towards the original aryl phosphate ester, comparable to the activity of natural serine hydrolases, and also a weak esterase activity. High resolution crystallographic studies of phosphonylated and unmodified Fabs display a novel, 15 Å-deep, two-chamber cavity between V_H and V_L chains with nucleophilic Tyr37L at the base of the site [13]. Detailed examination of this crystal structure demonstrated that the Tyr37L residue of the active site can form hydrogen bonds with the N105 and the carbonyl oxygen atom of the main chain D106 located in the 3rd hypervariable region of the heavy chain. Thus, aspartic acid 106 and asparagine 105 of the heavy chain may be involved in "covalent catalysis". Further, we substituted two possible nucleophilic residues identified in scFv reactibodies Y-L33 and Y-L37 by phenylalanine. In line with previous studies and crystallographic data, only Y-L37F mutation led to loss of reactivity. Based on pre-steady state kinetic and X-ray data, we suggested that covalent modification of reactibody A.17 by the phosphonate could be described by an induced-fit model. The nucleophilic efficiency of A17 towards the phosphonate was more than an order of magnitude greater than the corresponding butyrylcholinesterase (BChE) activity and compared favourably with typical rates of serine protease covalent modification by phosphonates [23–25]. Importantly, this applied combinatorial selection towards a reactive phosphonate resulted in complementary matching of shape and chemical reactivity of the substrate to the selected reactibody, which exceeded the performance of an enzyme such as BChE with a classical phosphorylating agent. To test the ability of A17 to serve as a bioscavenger, we checked the reactivity for A17 of a number of toxic substances. Finally, we succeeded in demonstrating that the organophosphate pesticide paraoxon was hydrolysed by covalent catalysis with rate-limiting dephosphorylation. We observed accumulation of a covalent intermediate during interaction between A.17 and paraoxon, which supported the covalent catalysis mechanism for paraoxon hydrolysis by the A.17 reactibody. Thus, we may state that reactibody A17 is therefore a combinatorial-kinetically selected Ig template that has enzyme-like catalytic attributes.

Reactive selection demonstrates how architectures that support efficient chemistry can arise from relatively minor alterations of the protein interior. Collectively, our observations provide a compelling case of a symbiotic link between the dynamics of the chamber and the reactivity of the tyrosine side chain. Reactibodies have cavities that facilitate simple chemical processes and provide a useful model for how these templates may respond to various adaptations. Strategies to consider antibodies as enzymes have been criticized on the basis that the Ig template has not the ability to deploy residues suitable for covalent catalysis and that it lacks the flexibility required for dynamic mechanism [4]. Reactive immunization and kinetic selection have provided evidence to dispel these concerns.

Reactibodies thus described may be used as pre-matured templates to improve existing properties and/or bring novel functional activities (Fig. 1). The first step in rational design is the analysis of structural data from different sources (X-ray, NMR analysis), kinetic studies (both of steady- and pre-steady state kinetics), and thermodynamic data (ITC, DSC, etc.). All these data together suggest how the reaction is progressing in time and space and what may be improved rationally in order to enhance it. Precise analysis of high-resolution X-ray structures can define distinct amino acid residues or small clusters as candidates for rational mutagenesis. High-resolution 3D structures of the reactibody and its phosphonylated intermediated have validated this expectation [13]. Several examples of site-directed mutagenesis of antigen-binding site of