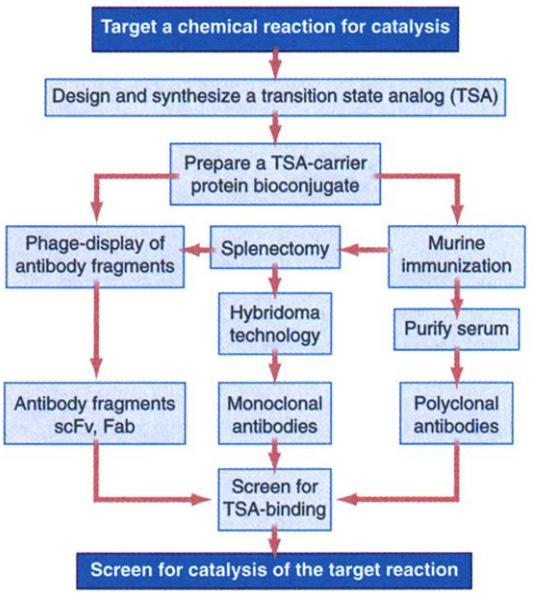
Antibody Design by Man and Nature

Paul Wentworth, Jr.

Throughout evolutionary history, nature has in effect screened large libraries of proteins to solve key problems of molecular recognition and catalysis, resulting in the present-day functional proteome. Sixteen years ago, Lerner (*1*) and Schultz (*2*) independently reported that probing the natural repertoire of antibodies with transition state analogs enables new catalysts to be identified on the evolutionary time scale of the immune response (2 to 3 weeks), and they demonstrated that the antibody molecule can mediate chemistry more complex than simple binding.

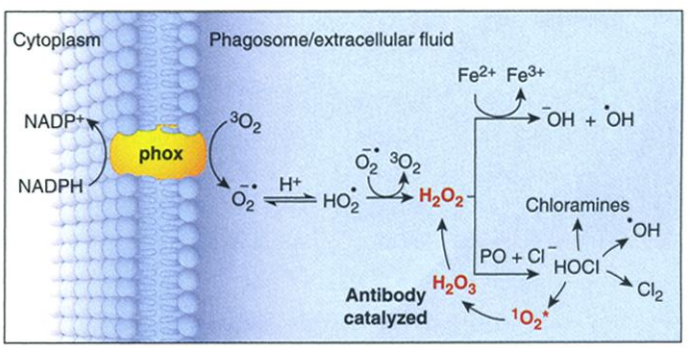
The original reports of catalytic antibodies focused on well-characterized transformations, such as acyl transfer. Scientists have since shown, however, that antibodies can be programmed to catalyze many different classes of chemical reactions with typical rate enhancements ranging between 10^3 and 10^6 (*3*). These processes have included "disfavored" reactions that are difficult to carry out with existing chemical methods, such as an anti-Baldwin cyclization (*4*) and $B_{AC}2$ carbamate ester hydrolysis (*5*). In addition, catalytic antibodies can perform reactions that are not catalyzed by endogenous enzymes. These have been used, for example, to activate anticancer prodrugs (*6*). Antibodies can also catalyze processes with highly reactive chemical intermediates, such as carbocations (*7*), 1,3-dipoles (*8*), and triplet biradicals (*9*). They generally perform well in experiments that require the control of chirality, typically giving high enantio- and diastereoselectivities (*10*). In fact, antibodies are peerless designer catalysts because of their programmability and ability to catalyze an amazing diversity of reactions (*3*).

The increasingly sophisticated strategies for generating catalytic antibodies parallel the complex chemical reactions that antibodies can catalyze. In the primary method for eliciting antibody catalysts, an animal (typically, a mouse) is immunized with a stable analog of the transition state for a given reaction (see figure, above). The researcher then harvests and immortalizes the antibody-generating cells (B cells) with Köhler and Milstein's hybridoma technology (*11*). Thus, in principle, each monoclonal antibody in the immune repertoire library that was elicited to the hapten is isolated, expressed in milligram to gram levels in high purity, and screened for catalytic activity. On the basis of the principles of transition state theory, antibodies that bind tightly and stabilize the transition state for a reaction should catalyze that reaction (*12*). This successful approach (called transition state stabilization) has been expanded to incorporate more sophisticated catalytic mechanisms, such as general acid or general base and covalent catalysis, into antibody catalysts. Haptens that contain a positively charged species (such as a quaternary ammonium group) tend to elicit negatively charged amino acid residues (such as glutamic or aspartic acid) in the antibody combining site and vice versa. These charged residues function as either general acid or general base residues in a number of different antibody-catalyzed reactions (*13*). The strategy whereby a charged hapten elicits such catalytic machinery has been dubbed bait-and-switch (*14*). Reactive immunization immunogens are haptens that elicit a direct covalent reaction with residues within the antibody's combining site during the immune response (*15*). These immunogens generate catalytic antibodies containing nucleophilic amino acid residues (such as lysine or serine) within their combining sites that can partake in reactions that proceed by covalent catalysis. This reactive immunization approach has led to the production of antibodies with high-catalytic proficiencies for aryl ester hydrolysis (*16*).



Key stages of catalytic antibody production. TSA (transition state analog); scFv (single chain–variable domain fragment); Fab (antigen-binding fragment).

Molecular biologists and biotechnologists often use phage display of peptide libraries, in which a DNA library that encodes a protein library is cloned into a bacteriophage that then expresses individual members of the protein library on its surface. They use this approach to generate antibody Fab (antigen-binding fragment) or scFv (single chain–variable domain fragment) libraries and probe them for catalytic activity for a given reaction. There are a number of advantages of this method over the previously described hybridoma technique. First, this technique can be used to clone the human immune repertoire into phage, allowing rapid entry into the generation of human catalytic antibodies for therapeutic applications. Second, by increasing the size of the DNA library, one can theoretically expand the immune



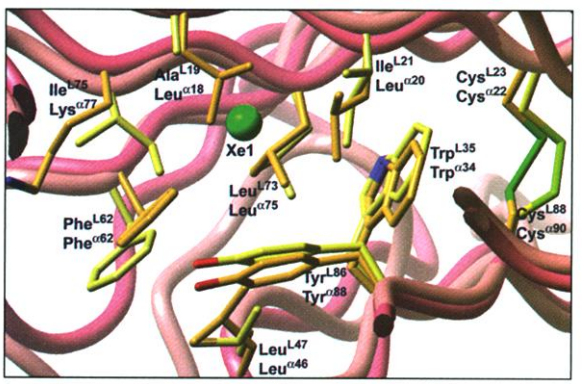
Phagosomal oxygen-dependent microbicidal mechanisms. Phox (phagocyte oxidase); PO (myeloperoxidase); $^3O_2$ (triplet or ground state dioxygen).

The author is in the Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA 92037, USA. E-mail: paulw@scripps.edu

repertoire beyond that which exists in nature. Third, this method does not require animal immunization and is, therefore, faster than the hybridoma approach. Janda and coworkers have exploited the phage display of the catalytic antibody Fabs method to generate catalytic antibodies with glycosidase activity (*17*).

The successful use of catalytic antibodies in organic synthesis, therapeutics, and diagnostics will rely on the satisfaction of common features such as turnover, accessibility, and cost. Current efforts focus on improving the catalytic rates of antibodies, which occasionally rival those of enzymes. Many chemically significant reactions, however, still lack a known antibody catalyst. Therefore, scientists are working to design haptens that will allow production of catalysts for these hitherto unexplored reactions. Antibody catalysts have a very high substrate specificity because they each evolved to bind a single molecule. This high selectivity is a tremendous asset for medical applications in vivo, but for certain applications, such as organic synthesis, a catalyst must perform a given reaction regardless of the structural composition of the substrate. Recently, Barbas and Lerner (*19*) used a reactive immunization hapten to generate a catalytic antibody with broad substrate tolerance. They created an antibody that catalyzes an enantioselective aldol reaction between a range of ketones and aldehydes and made it commercially available as aldolase monoclonal antibody 38C2.

Use of hybridoma technology and phage display techniques have made monoclonal antibodies akin to chemical reagents in that they can be produced in gram quantities, transferred between laboratories, and stored and sold as required. The high cost of monoclonal antibody production, however, causes scientists to seek more economical approaches, such as large-scale production of catalytic antibodies by cloning and expression in plants (*18*). The most viable of these involves the production of polyclonal antibodies, in which scientists immunize rabbits and sheep with a suitable hapten and then isolate and purify the immunoglobulin fraction from serum, thus producing polyclonal antibodies on the multi-gram scale. This approach should be acceptable for catalytic antibodies used in diagnostics and organic synthesis; however, the inherently unknown composition of the antibody mixture may prove problematic for approval by regulatory authorities such as the Food and Drug Administration for therapeutic application.



Active site. Overlay of Fab 4C6 and the 2C αβ T cell receptor (1TCR) around a conserved Xe binding site (Xe1). The backbone Cα trace of V_L (pink) and side chains (yellow) and the corresponding Vα and side chain residues of the 2C αβ T cell receptor are superimposed. From (*23*).

In the immune system, antibodies are thought to mark foreign substances for removal by the complement cascade and/or phagocytosis (*20*). Now, the advent of antibody catalysis has demonstrated that antibodies can be programmed to perform complex chem-

istry. A logical question now arises: Does the potential for catalysis relate to antibody function? Recently, we reported that the immune system appears to have a chemical activity intrinsic to the catalytic potential of the antibody molecule itself. All antibodies, regardless of source or antigenic specificity, catalyze the generation of hydrogen peroxide (H_2O_2) from singlet dioxygen ($^1O_2$*) (*21*).

The microbicidal action of polymorphonuclear leukocytes (PMNs) requires the generation of reactive oxygen species. Superoxide anion ($O_2^{\Sigma-}$), H_2O_2, $^1O_2$*, hydroxyl radical (HO•), and other strongly oxidizing species such as hypochlorous acid (HOCl) (*22*) are generated during the oxidative burst, in response to internalization of antibody-coated xenobiotics within phagosomes in the PMNs (see figure, bottom of p. 2247). Therefore, it is reasonable to speculate that this hitherto unknown and intrinsic catalytic ability of all antibodies may have evolved to modulate H_2O_2 formation in the phagosome and, hence, to play a role in pathogen destruction.

Antibodies are a unique class of proteins that can catalyze the production of up to 500 mole equivalents of H_2O_2 from $^1O_2$* without a reduction in rate. Other proteins generate from 0 to 10 mole equivalents before oxidative destruction renders them inactive (*23*). For all antibodies to catalyze this reaction, there must be regions conserved to facilitate the reaction. X-ray crystallographic studies that localize xenon binding to antibody Fabs point to conserved oxygen-binding sites within the antibody fold (and in other immunoglobulin fold proteins that catalyze this reaction, such as the 2C αβ T cell receptor), where this chemistry could be initiated (see figure, previous page).

Throughout evolution, organisms have defended themselves by production of relatively simple chemicals. With the appearance of the immune system in mammals, this simple defense mechanism was thought to be largely abandoned because a sophisticated targeting mechanism had evolved. The ability of antibodies to generate H_2O_2 from $^1O_2$* realigns recognition with the potential for killing within the same molecule and suggests that the evolution of catalytic antibodies significantly predates the original reports of their rational design.

References

1. A. Tramontano, K. D. Janda, R. A. Lerner, *Science* **234**, 1566 (1986).
2. S. J. Pollack, J. W. Jacobs, P. G. Schultz, *Science* **234**, 1570 (1986).
3. P. Wentworth Jr., K. D. Janda, *Curr. Opin. Chem. Biol.* **2**, 138 (1998); G. M. Blackburn, A. Datta, H. Denham, P. Wentworth Jr., *Adv. Phys. Org. Chem.* **31**, 249 (1998).
4. K. D. Janda, C. G. Shevlin, R. A. Lerner, *Science* **259**, 490 (1993).
5. P. Wentworth Jr. *et al.*, *J. Am. Chem. Soc.* **119**, 2315 (1997).
6. P. Wentworth Jr. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 799 (1996).
7. T. Li, K. D. Janda, J. A. Ashley, R. A. Lerner, *Science* **264**, 1289 (1994).
8. J. D. Toker *et al.*, *J. Am. Chem. Soc.* **122**, 3244 (2000).
9. L. H. Jones *et al.*, *J. Am. Chem. Soc.* **123** (2001).
10. P. Wentworth Jr., K. D. Janda, in *Comprehensive Asymmetric Catalysis*, E. N. Jacobsen, A. Pfaltz, H. Yamamoto, Eds. (Springer-Verlag, New York, 1999), vol. I to III, pp. 1403.
11. G. Köhler, C. Milstein, *Nature* **256**, 495 (1975).
12. W. P. Jencks, in *Catalysis in Chemistry and Enzymology*, (McGraw Hill, New York, 1969), p. 3.
13. P. Wentworth Jr. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 5971 (1998).
14. K. D. Janda *et al.*, *J. Am. Chem. Soc.* **112**, 1274 (1990).
15. P. Wirsching *et al.*, *Science* **270**, 1775 (1995).
16. C.-H. L. Lo *et al.*, *J. Am. Chem. Soc.* **119**, 10251 (1997).
17. C. Gao *et al.*, *J. Am. Chem. Soc.* **120**, 2211 (1998).
18. M. B. Hein *et al.*, *Biotechnol. Prog.* **7**, 455 (1991).
19. J. Wagner, R. A. Lerner, C. F. Barbas III, *Science* **270**, 1797 (1995).
20. G. J. Arlaud, M. G. Colomb, J. Gagnon, *Immunol. Today* **8**, 1076 (1987); R. B. Sim, K. B. Reid, *Immunol. Today* **12**, 307 (1991).
21. A. D. Wentworth *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 10930 (2000).
22. S. Klebanoff, *Encycl. Immunol.* **3**, 1713 (1998).
23. P. Wentworth Jr. *et al.*, *Science* **293**, 1806 (2001).