Enzyme catalysis

Antibodies with some bite

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THE spectacular specificities and rate enhancements of enzymes are without equal among all known catalysts. It is no wonder then that the design of new enzymes has long been a goal of biochemists. Now two independent groups, one led by Alfonso Tramontano and Richard Lerner of the Scripps Clinic and Research Foundation[1], and the other by Peter Schultz of the University of California at Berkeley[2], have taken steps towards this goal by demonstrating that antibodies can have catalytic activity.

These accomplishments stem from almost a century of research on enzyme catalysis that began with the legendary chemist Emil Fischer. In 1902, Fischer[3] stated in his Nobel Prize lecture

> The examination of synthetic glucosides has shown that the action of the enzymes depends to a large extent on the geometrical structure of the molecule to be attacked, that the two must match like a lock and key . . . To equal Nature here, the same means have to be applied, and I therefore foresee the day when physiological chemistry will not only make extensive use of natural enzymes as agents, but when it will also prepare synthetic [enzymes] for its purposes.

Since then there have been revolutionary advances in our understanding of enzyme catalysis and of protein structure, but it is a measure of the immense complexity of enzymes that a completely synthetic system has yet to be prepared. Present thinking on the basis of enzyme catalysis began in the 1940s when Linus Pauling suggested that Fischer's Lock and key analogy must be modified: an enzyme active site should not match the substrate, it should rather be complementary to the activated complex (or transition state) of the reaction being catalysed. Much recent work on enzyme mechanisms has supported Pauling's view, and one may convincingly argue that most of the principles of enzyme catalysis are now well understood (even if we do not necessarily see how these principles are manifested).

Although a concomitant understanding of protein folding and structure has not so far been achieved, a strategy for generating new enzymes that depends on an understanding of catalysis, but not on the ability to design polypeptides that will fold properly, was first alluded to by Pauling[4] in 1948

> An enzyme has a structure closely similar to that found for antibodies, but with one important difference. Namely that the surface configuration of the enzyme is not so closely complementary to its specific substrate as is that of an antibody to its antigen, but instead is complementary to the activated complex.

William Jencks[5], more directly, stated in 1969

> If complementarity between active site and transition state contributes significantly to enzyme catalysis, it should be possible to synthesize an enzyme by constructing a binding site. One way to do this is to prepare an antibody to a haptenic group which resembles the transition state of a given reaction. The combining sites of such antibodies should be complementary to the transition state and should cause an acceleration by forcing bound substrate to resemble transition state.

With the potential for more than 10^{12} different antibody molecules, one may speculate that every antibody catalyst imaginable is obtainable from the existing repertoire of antibodies (just as every antibody needed to bind all antigens seems to be accessible). The challenge is therefore in selecting an antibody with the desired catalytic function from this huge repertoire. However, as pointed out by Jencks, if in a stable molecule one could model the transition state for a reaction, such a selection process might be at hand.

Although several early attempts to isolate antibodies with catalytic activity failed[6-8], the transition state analogue strategy has now succeeded. Both the Scripps group and the Berkeley group used tetrahedral phosphorus compounds to mimic the presumed transition state in the hydrolysis of a carboxylate ester. Tramontano *et al.* generated monoclonal antibodies against four phosphonate esters and demonstrated that some of these antibodies have catalytic activity (these workers distressingly call these catalytic antibodies 'abzymes'). The values of the first-order rate constant k_{cat} at *p*H 8 for two ester substrates with the monoclonal antibody 6D4 are 1.62 min^{-1} and 0.48 min^{-1}. The corresponding K_m values, the concentrations of substrate required for half-maximal velocity, are 1.90 μM and 0.62 μM.

Schultz *et al.* tested the mouse monoclonal antibody MOPC167, which is known to bind nitrophenyl phosphorylcholine, as a catalyst for the hydrolysis of an analogous carbamate and measured a k_{cat} of 0.4 min^{-1} and a K_m of 208 μM at *p*H 7. These rates correspond to accelerations of approximately 1,000-fold over the uncatalysed rates, but are approximately 5,000-fold slower than, for example, the rate for a good ester substrate of α-chymotrypsin.

Both research groups note that their antibodies have many of the same characteristics as enzymes: each shows good substrate specificity, exhibits saturation kinetics and is subject to competitive inhibition. Although the detailed chemical mechanisms of these catalytic antibodies have yet to be determined, it is reasonable to suppose that the ester or carbamate is strained towards a tetrahedral geometry on binding, thus facilitating the attack of the hydroxide ion. Furthermore, the Scripps group has already found that their antibody can act via both nucleophilic and general base catalysis, depending on the substrate used.

The fact that this approach succeeded at all gives great hope to those attempting to isolate even more efficient antibody catalysts by this and by other strategies. In addition, it may be possible to modify existing catalytic antibodies. Because the crystal structures of monoclonal antibodies containing their bound haptens can be determined — and in fact such a crystal structure had already been solved for the antibody used by the Berkeley group — site-directed mutagenesis can be intelligently used to change particular amino acids so as to generate enhanced specificity or catalytic function. In addition, both groups are planning to attach cofactors and metal ions to the antibodies in attempts to increase catalytic efficiency further. One immediate goal is the creation of sequence-specific proteases, which would have many important biochemical applications. Overall, the possibilities appear almost limitless, and as Schultz's group concludes, "these approaches may enable us to tailor-make catalysts for use as tools in biology, chemical synthesis and medicine".

Given the fact that antibody molecules can act as catalysts, it will now be possible to address several theoretical questions about enzyme catalysis. For example, enzymes are flexible molecules that may assume different conformations during a catalytic cycle, but the contribution of these motions to catalysis remains uncertain. Antibodies, on the other hand, although not static molecules, seem to have only one important functional conformation. It will be of great interest to see if antibodies with catalytic efficiencies equal to those of enzymes can be obtained, or if, ultimately, fundamental differences between antibodies and enzymes will limit this approach. □

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