# Commercially valuable catalytic antibodies: the life to come

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Man-made catalytic antibodies have considerable commercial potential in many different applications. Anticipation of catalytic antibody-based products has been heightened by successful prototype experiments in therapeutic applications, such as prodrug activation and cocaine inactivation, and in nontherapeutic applications, such as biosensors and organic synthesis. The aim of this review is to locate catalytic antibodies on the road from discovery to commercialization through discussion of past successes, current barriers and keys to future development.

Nearly a decade ago, the first literature reports of catalytic antibodies[1,2] sparked excitement and imaginative speculation among both academic scientists and enterprising companies[3]. It was realized that by thoughtful design, monoclonal antibodies could be generated with combining sites that would efficiently catalyze unique and commercially valuable chemical reactions. To date, most catalytic antibodies have been elicited to transition state analog (TSA) haptens[4,5] – stable, low-molecular weight compounds designed to mimic the structures of the energetically unstable transition state species that briefly (approximate half-life $10^{-13}$ s) appear along reaction pathways between reactants and products. Anti-TSA antibodies, like natural enzymes[6,7], are thought to selectively bind and stabilize transition states, thereby easing the passage of reactants to products.

Although truly amazing technological and intellectual advances have been made in the field[8], no catalytic antibodies have yet been commercialized. The lack of catalytic antibody products stems more from technological immaturity than from a lack of need or effort. Catalytic antibodies, and indeed other designer catalysts, have potentially extremely diverse and important economic importance. As artificial enzymes, they could carry out reactions for which no known natural enzymes or nonbiological catalysts are available. Therapeutic applications have been most commonly suggested and have been the objects of several prototype studies (Table 1). Catalytic antibodies are theoretically more attractive than noncatalytic antibodies as therapeutic agents because, being catalytic, they may be used in lower doses, and also because their effects are usually irreversible (for example, peptide bond cleavage rather than binding)[22]. In therapy, purified catalytic antibodies could be directly administered to a patient, or alternatively the patient's own catalytic antibody response could be elicited by immunization with an appropriate hapten[23,24]. Perhaps most often, catalytic antibodies have been proposed as sequence-specific protein-cleaving agents that could target and hydrolytically destroy critical proteins or peptides associated with tumor cells or virus particles, but not found on normal cells.

One particularly fascinating potential application of therapeutic catalytic antibodies is in the area of prodrug activation in cancer treatment[25,26]. One drawback of conventional cancer chemotherapy is the nonspecificity of the drugs; although they kill cancer cells, they are also cytotoxic to normal cells. In prodrug therapy, nontoxic derivatives of established

*Man's mind stretched to a new idea never goes back to its original dimensions*
Oliver Wendell Holmes

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**Table 1. Some suggested practical applications of catalytic antibodies[a]**

| Therapeutic applications | Nontherapeutic applications |
|---|---|
| Amide[9–12] and glycosidic[13] bond cleavage: | Organic synthesis: |
| tumors[14], | stereo/regio/chemoselection[27–30], |
| viruses, | disfavored reactions[31–33], |
| arterial plaques, | concerted reactions[34–36], |
| blood clots | redox reactions[37–39] |
| Cocaine inactivation[15] | Biosensors[40] |
| Vaccines | Research reagents |
| Nerve gas detoxification[16–18] | Immunoassays |
| Prodrug activation[19,20] | Environmental detoxification |
| Endotoxin inactivation | |
| Cyanide inactivation[21] | |

[a]The noted citations are non-comprehensive references to prototype studies

antitumor agents (prodrugs) are converted to the highly toxic parent drug only at the site of the tumor.

Conceptually, prodrug therapy involves at least three steps. The first step involves administration of a bifunctional biomolecule consisting of an antitumor antibody conjugated to a prodrug-activating molecule, such as an enzyme or catalytic antibody. Conjugation can be carried out by chemical coupling of the two molecules or by expression of a single, bifunctional protein from a fused gene construct. The antitumor antibody portion of the circulating conjugate specifically binds to its cognate antigen on tumor cells (Figure 1a). The second step involves a waiting period to allow clearance of any nonbound conjugate from the bloodstream (Figure 1b). In the third step, a suitably derivatized less toxic version of an established anticancer drug is administered. Following circulation to the tumor site, the prodrug is converted to the active parent drug by the catalytic portion (enzyme or catalytic antibody) of the conjugate and attacks not only antigen-bearing tumor cells but also antigen negative (bystander) tumor cells (Figure 1c). There are two major advantages that catalytic antibodies have over natural enzymes in prodrug activation. First, reactions can be designed to avoid any incidental activation by circulating endogenous enzymes, and second, pro-forms of drugs can be designed and altered to maximize toxicity ratios between the drug and its pro-form.

Several nontherapeutic applications of catalytic antibodies have also been envisioned (Table 1). Catalytic antibodies could be used as clinical diagnostic tools or as regioselective or stereoselective catalysts in the synthesis of fine chemicals (cost of antibody production would preclude catalytic antibody use in preparation of less expensive chemicals). An advantage of nontherapeutic over therapeutic uses of catalytic antibodies is that they are not constrained to function under physiological conditions. Catalytic antibodies often operate by mechanisms that require nonphysiological conditions, such as alkaline pH, to work effectively. Such characteristics favor applications in diagnostics or industry where conditions (pH, salt concentration or temperature) can be varied. A second advantage of nontherapeutic over therapeutic catalytic antibodies is that there may be more flexibility in the choice of the reaction to be catalyzed. In therapeutic applications, treatment of a given disease may require efficient catalysis of a technically intractable reaction, such as hydrolysis of a specific amino acid sequence in the coat protein of a virus, or the target substrate may be present in extremely low concentrations (nanomolar or picomolar) or inaccessibly localized (e.g. within cells).

It is clear that there are many potential commercial applications for catalytic antibodies. The purpose of this review is to describe in general terms the progress and unpredicted hurdles that the field has encountered, the current state of the art, and the technological innovations that have been proposed in the literature that should continue to drive catalytic antibodies towards commercialization.

## Progress to date

Catalytic antibodies typify the interdisciplinary trend of modern science. Extremely powerful discoveries are increasingly being made at the interfaces of previously isolated scientific disciplines. In common with other fields requiring cooperation of disparate disciplines, catalytic antibody research is generally beyond the technical capabilities of small, independent academic laboratories. Generation and characterization of catalytic antibodies require expertise in organic chemistry, immunology and enzymology, and are greatly enhanced by skills in other fields, especially molecular biology. The situation is further complicated for commercial development, where expertise in additional areas is required (e.g. pharmacology or process engineering). Although the potential of catalytic antibodies is as widely

*New roads; new ruts*
G.K. Chesterton

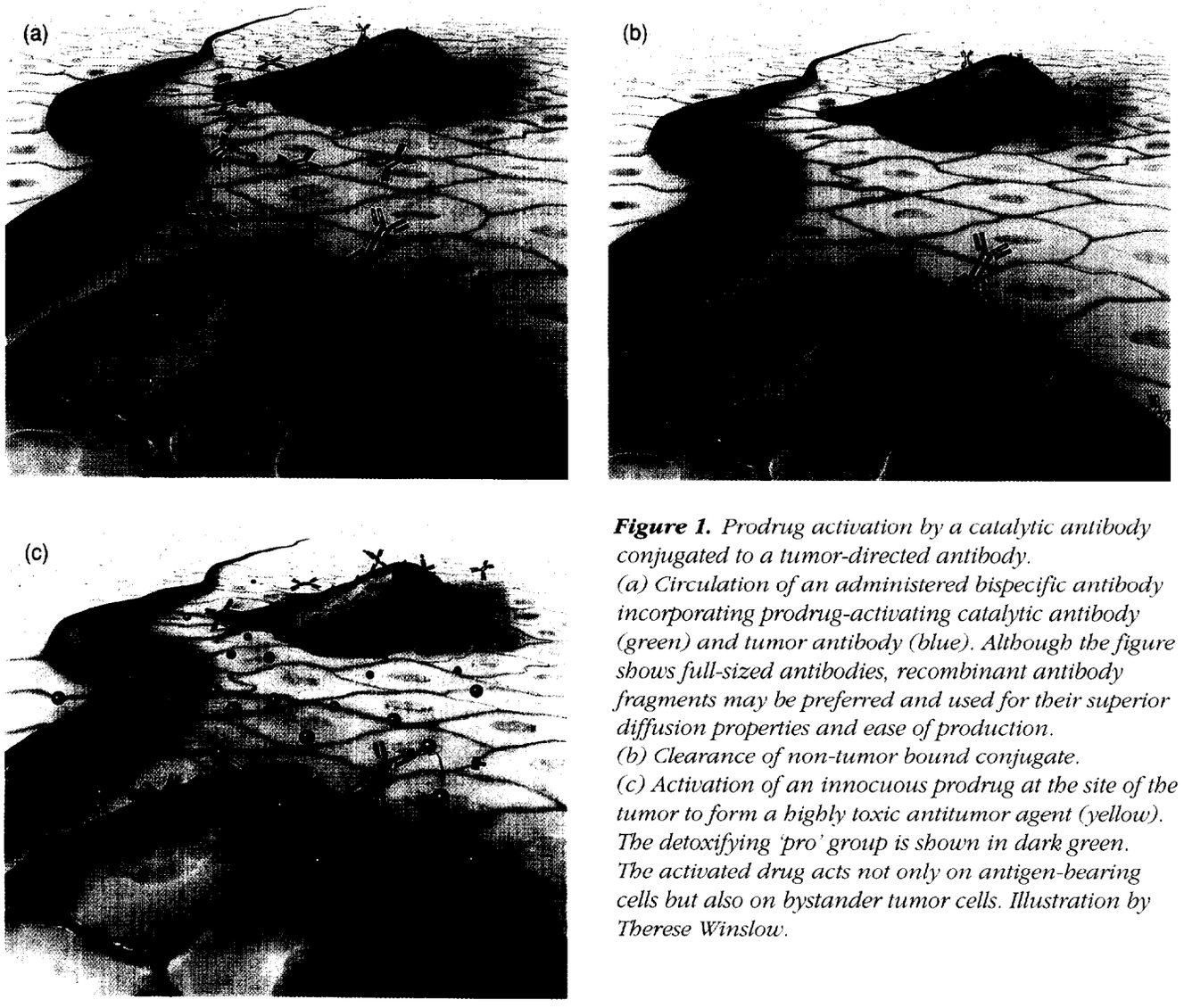


***Figure 1.*** *Prodrug activation by a catalytic antibody conjugated to a tumor-directed antibody.*
*(a) Circulation of an administered bispecific antibody incorporating prodrug-activating catalytic antibody (green) and tumor antibody (blue). Although the figure shows full-sized antibodies, recombinant antibody fragments may be preferred and used for their superior diffusion properties and ease of production.*
*(b) Clearance of non-tumor bound conjugate.*
*(c) Activation of an innocuous prodrug at the site of the tumor to form a highly toxic antitumor agent (yellow). The detoxifying 'pro' group is shown in dark green. The activated drug acts not only on antigen-bearing cells but also on bystander tumor cells. Illustration by Therese Winslow.*

recognized as it was 10 years ago, the complexity and hurdles of interdisciplinary research have contributed to the protraction of commercial development.

Nevertheless, astounding progress has been made in catalytic antibody research. Perhaps most impressive is the number of reactions that have been found to be amenable to antibody catalysis (Box 1)[4,5]. In some cases, types of reactions have been catalyzed by antibodies that are not catalyzed by any known enzymes (e.g. Diels–Alder reaction).

### *Modest rate accelerations*

Although many different types of reactions have been catalyzed, the rate accelerations of most catalytic antibodies are often modest compared to those of natural enzymes. For example, hydrolytic reactions catalyzed by antibodies tend to have rate accelerations ($k_{cat}/k_{uncat}$; the antibody catalyzed rate divided by the uncatalyzed rate) of the order of $10^4$ or less. For comparison, protease enzyme-catalyzed reactions often have rate accelerations of $10^{11}$ or greater. Moreover, many reported antibody-catalyzed reactions have been hydrolysis reactions of fairly kinetically-labile bonds, such as aromatic ester linkages.

There are different reasons for the facts that reported catalytic antibody-catalyzed rates have been fairly modest and that many substrates used in hydrolysis reactions have had labile scissile bonds. On one hand, the apparent $10^4$ rate enhancement 'barrier' is most likely to have resulted from small but significant differences between the hapten structures

**Box 1. Reactions amenable to antibody catalysis[4,5]**

Hydrolysis of esters, carbonates, amides, imides, phosphate esters, carbamates and enol ethers
Synthesis of esters, amides and imines
Thymine dimer photocleavage
β-elimination
Diels–Alder reactions
Claisen rearrangements
Porphyrin metallation
Peptide bond rearrangement
*Cis–trans* isomerizations
Decarboxylation
Cyclization reactions

and the structure of the actual hydrolytic transition states. On the other hand, the reason why antibody-catalyzed hydrolyses of labile substrates are common in the literature relates to the technical difficulty of measuring rather slow absolute rates expected in hydrolysis of kinetically-inert compounds. It is a common misconception that it is 'easier' for an antibody to hydrolyze, for example, an unstable *p*-nitrophenyl ester than a stable alkyl ester. As shown in the equations below, a given amount of antibody transition state binding energy results in the same rate acceleration, regardless of the reaction[14]. The rate of a reaction is exponentially related to the height of the Gibbs free energy barrier (ΔG‡) as follows:

$$k_{uncat} = (kT/h)\exp^{-\Delta G^{\ddagger}/RT} \quad (1)$$

where $k_{uncat}$ is the (uncatalyzed) reaction rate, k is the Boltzmann constant, T is temperature, h is the Planck constant, and R is the gas constant. Similarly, the rate of an antibody- (or enzyme-) catalyzed reaction ($k_{cat}$) is related to the height of the Gibbs free energy barrier minus the change in Gibbs free energy in the transition state brought about by antibody binding (ΔΔG‡):

$$k_{cat} = (kT/h)\exp^{-(\Delta G^{\ddagger} - \Delta\Delta G^{\ddagger})/RT} \quad (2)$$

Thus, the rate acceleration brought about by an antibody ($k_{cat}/k_{uncat}$) is equal to:

$$(k_{cat}/k_{uncat}) = [(kT/h)\exp^{-(\Delta G^{\ddagger} - \Delta\Delta G^{\ddagger})/RT} / (kT/h)\exp^{-\Delta G^{\ddagger}/RT}] \quad (3)$$

which reduces to;

$$(k_{cat}/k_{uncat}) = \exp^{\Delta\Delta G^{\ddagger}/RT} \quad (4)$$

Equation (4) demonstrates that the rate acceleration ($k_{cat}/k_{uncat}$) catalyzed by an antibody is independent of the height of the Gibbs free energy barrier of the uncatalyzed reaction (ΔG‡). Thus, it is equally 'easy' [i.e. it requires the same amount of antibody transition state binding energy (ΔΔG‡)] for a given rate acceleration, regardless of the reaction.

So why are there more reported reactions of labile substrates, such as aromatic esters, than more inert substrates, such as alkyl esters? The main reason probably relates to the difficulties of screening for and routinely assaying low levels of activity. Especially for hydrolysis reactions, trace concentrations of adventitious enzymes can give artifactual results. Moreover, assays for low levels of activity can require long incubation times of a week or more, during which time the buffer can become contaminated or the antibody can denature. Clearly, caution and sensitive activity detection methods are critical to studies of transformations that have slow uncatalyzed rates.

### *Mechanisms of action*

A range of studies have explored the structures and mechanisms of catalytic antibodies. This work has been valuable in gaining an understanding of how catalytic antibodies work, thus providing information for improving future designs of antibodies. The mechanism of the phenyl acetate-hydrolyzing catalytic antibody 20G9 is particularly well-characterized[41]. This antibody was raised to a typical hapten (a phenyl phosphonate designed to resemble a negatively charged tetrahedral transition state of ester hydrolysis) and hydrolyzes phenyl acetate to the products phenol and acetate. Used in some of the first prototype studies of commercial applications, 20G9 was found to be active in reverse micelles[41] and was successfully used as a recognition element in a biosensor[40].

Mechanistic work with 20G9 showed that catalytic antibodies do not always operate by expected kinetic or chemical mechanisms[42,43]. Kinetic studies showed complicated regulation by the product, phenol (reminiscent of feedback regulation in metabolic enzymes). Instead of an expected linear progress curve, an extremely unusual three-stage progress curve was seen (Figure 2). At the beginning of a typical assay, 20G9 shows a burst of activity in hydrolyzing phenyl acetate, followed by a slower linear 'steady state' rate (see dashed line in Figure 2), and finally a rate increase to a rate similar to that seen at the beginning of the burst. Detailed characterization showed that the activity decline in the burst was caused by partial inhibition by the product, phenol, and the reactivation stage resulted from binding of a second, activating molecule

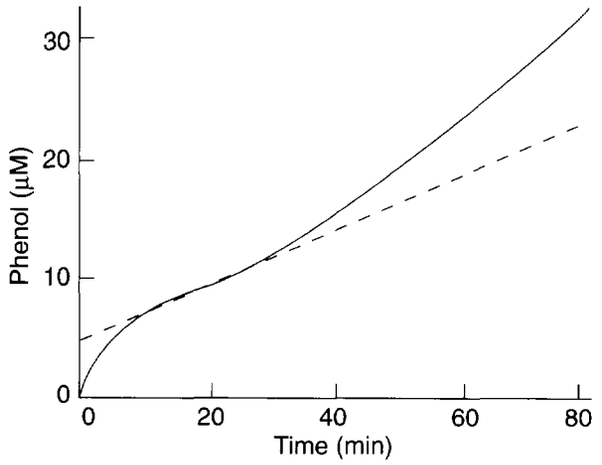


***Figure 2.*** *Unexpected sophisticated product regulation during antibody-catalysis. Under the conditions used here for 20G9-catalyzed phenyl acetate hydrolysis, a linear progress curve would be expected if no regulation were occurring. The activity decline during the first 10 min of the reaction results from partial product inhibition by the formed product, phenol. Following a brief linear 'steady state' period (dashed line), a second phenol binds to 20G9, which restores activity to the antibody by acting as a deinhibitor, preventing binding of the first, partially inhibitory, phenol (M. Martin, unpublished work).*

of phenol (Martin, M.T., unpublished work). Although this high degree of regulation by product is uncommon for catalytic antibodies, it illustrates the much more prevalent problem of product inhibition (catalytic antibodies do not 'know' to let go of the products) and the unpredictable enzyme-like regulatory properties sometimes seen in well-characterized catalytic antibodies.

In addition to unexpected kinetic regulatory properties, the chemical mechanism of 20G9 is also remarkable. Phenyl acetate hydrolysis by 20G9 occurs via an acyl-tyrosyl covalent intermediate (Figure 3)[42,43]. Similar unexpected acyl mechanisms have been found in other hydrolytic antibodies where, instead of a tyrosine residue, the antibodies have been shown to be transiently acylated on histidine[44] and serine[45] residues. Clearly, the natural chemical reactivities of antibody-combining site amino acids can facilitate reactions by unexpected chemical mechanisms. Because tyrosines are common in antibody combining sites, acyl-tyrosyl mechanisms may be frequently encountered (by chance or design) in future hydrolytic antibodies. In addition to an acyl-tyrosyl intermediate, the mechanism shown in Figure 3 has the feature of transition state stabilization (shown in brackets) during both formation and breakdown of the acyl intermediate. Studies of a series of different catalytic antibodies that were raised in the same immunization as 20G9 demonstrated that all five hydrolyzed phenyl acetate by variations of the same acyl-tyrosyl mechanism[43]. Moreover, their deduced amino acid sequences were also found to be very similar. A comparison of the kinetic and hapten binding properties of these 'isoabzymes' provided evidence of a mechanism that combined both hapten-induced complementarity in the transition state and an acyl intermediate. Thus, the mechanism of 20G9 and its isoabzymes appears to include a mixture of the expected (designed) and the unexpected (serendipitous).

Recent studies of catalytic antibody structures have also provided a wealth of information that may be useful in designing future antibody catalysts. Because of recurring three-dimensional structural themes in antibody combining sites, antibodies can be computer-modeled with good reliability[46]. Molecular modeling has supported and helped to elucidate mechanistic work on catalytic antibodies[47]. X-ray crystal structures of catalytic antibodies have recently become available that give welcome insight into the mechanisms of antibodies with esterolytic[45,48] and chorismate mutase-like[49] antibodies. Future structural work will surely provide valuable insight into the mechanisms and regulation of catalytic antibodies.

### *Antibody engineering*

The robust field of antibody engineering has also made a distinctive mark on the field of catalytic antibody research. Antibody engineering in its broadest sense consists of rational chemical modification, genetic engineering, or a combination of the two. Chemical derivatization has been used to introduce reactive moieties such as imidazole[50] and heme[51] to antibody combining sites. Analogously, site-directed mutagenesis has been used to make a noncatalytic antibody catalytic[52]. Besides engineering antibody combining sites, the overall structure of antibodies has been modified. Engineering of antibodies is useful for therapeutic applications in order to 'humanize' murine antibodies to reduce their immunogenicity or to reduce their size for better solid tumor penetration in cancer treatment (e.g. prodrug activation, see above). Antibodies are also engineered for practical reasons in order to facilitate expression and purification in bacteria[53] or use in antibody combinatorial libraries[54–56]. One form of engineered antibody is the single-chain antibody (sFv), which is a monovalent binding molecule

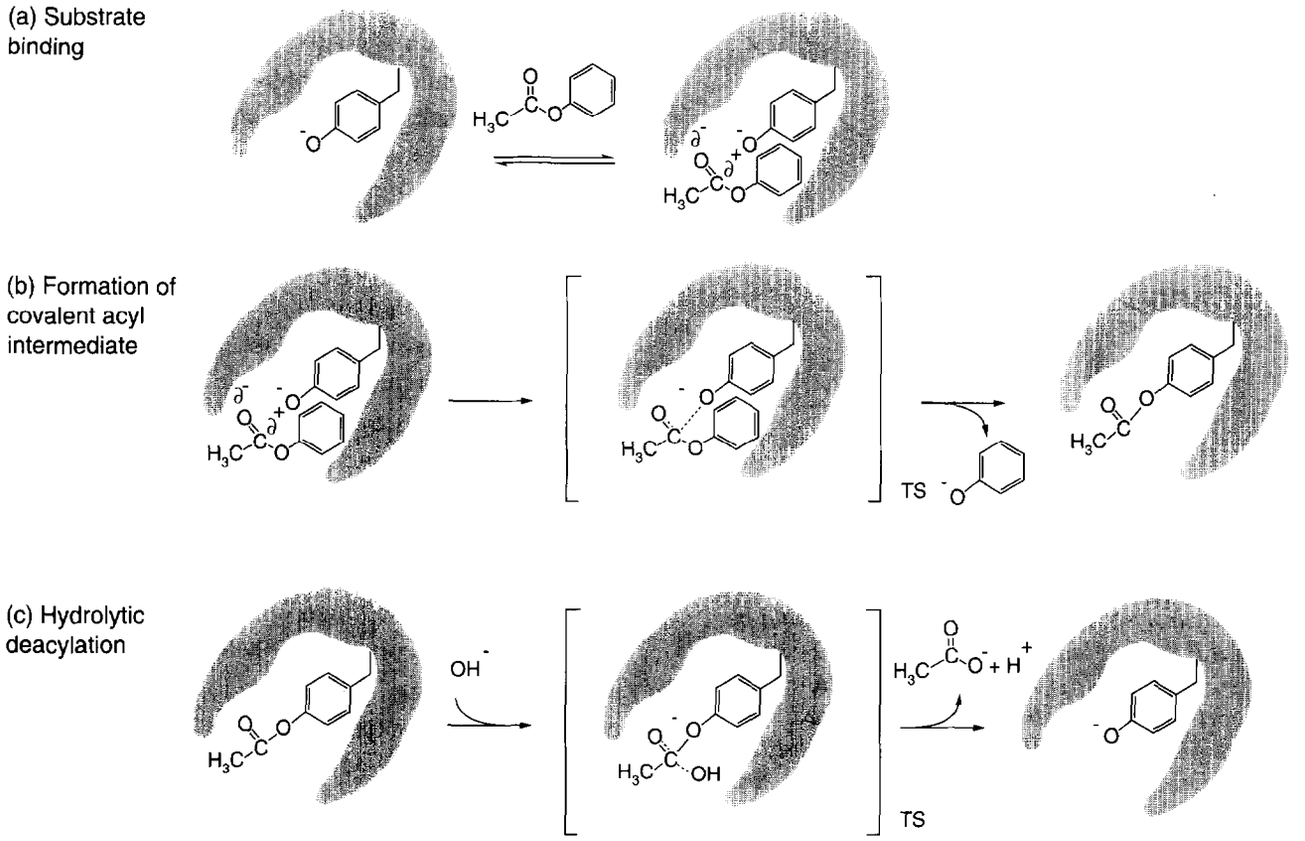


***Figure 3.*** *Mechanism of 20G9-catalyzed phenyl acetate hydrolysis. Phenyl acetate hydrolysis is catalyzed by a combination of unexpected[42] (acyl-tyrosyl intermediate) and designed[43] (hapten-induced transition state [TS] complementarity shown in brackets) features.*

consisting of one heavy and one light chain variable region joined *via* a designed flexible peptide linker[53,57]. sFv proteins (25 kDa) are smaller than natural-occurring IgG molecules (150 kDa) from which they are usually derived. Because sFv molecules are relatively low molecular weight plasmid-encoded proteins that are devoid of disulfide bonds, they can be conveniently further manipulated and expressed from *E. coli*. Additionally, if antibodies are raised in mice, the sFv versions should be less immunogenic than natural antibodies because they do not have a murine Fc constant region. In spite of the rather drastic size reduction brought about by converting an IgG to an sFv, the specific activities of catalytic sFv molecules are similar to their parent antibodies. Figure 4 shows an activity comparison of 20G9 IgG and the sFv derived from 20G9 (sFv activity measured with and without inhibitory hapten)[53].

An advantage of sFv molecules or other similarly engineered antibody fragments over natural antibodies is that they are amenable to display on the surfaces of filamentous bacteriophages[54,56]. Proteins such as sFv molecules can be displayed on the surface of a phage while their genes are encoded on a plasmid inside the phage. Thus, a simple system contains both the functional activity of the protein displayed on the outside and the protein-encoding genetic material on the inside. Because they are bacteriophages, once a desired phage is selected using the functional properties of the recombinant surface protein, it can be expressed or amplified and resecreted in *E. coli*. Combinatorial libraries consisting of more than $10^{10}$ different antibody fragments displayed on different phages can be tested for binding to an immobilized hapten or substrate[54].

### *Selection of catalytic antibodies*

In practice, most anti-TSA hapten monoclonal antibodies are not catalytic, and much effort has gone into labor-intensive screening of monoclonal antibodies for catalysis[12].

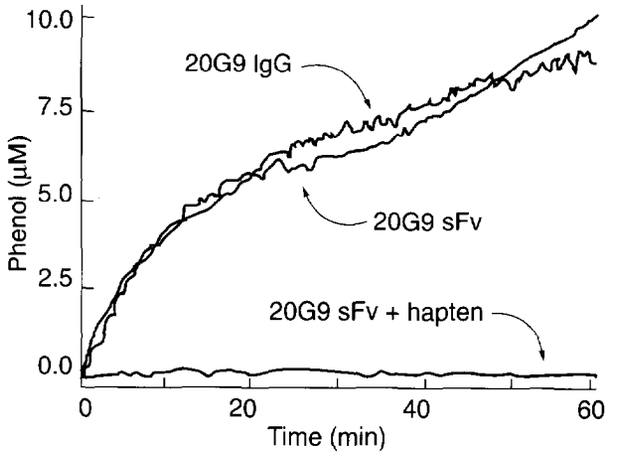


***Figure 4.*** *Comparison of the hydrolytic activity of 20G9 with its engineered single-chain Fv fragment. Activities and regulatory properties of 20G9 and its engineered sFv fragment were similar*[53]*. As a control, the sFv was shown to be specifically inhibited by the hapten (lower curve). The reactivation stage seen in the 20G9 progress curve in Figure 2 is absent here because of differences in conditions used that resulted in the production of a lower concentration of (reactivating) phenol in this experiment.*

An alternative to screening is selection, whereby catalytic antibodies are separated from a pool of noncatalytic antibodies according to whether or not they possess catalysis-related physical or chemical properties. One such selection method is to use auxotrophic bacteria as antibody fragment expressing hosts. If the antibody-derived molecule has the appropriate level of catalytic activity to allow the host bacterium to live, it will be selected[58–60]. A drawback to this powerful technique is that only certain catalytic activities can be selected and these are limited by biologically-relevant reactions and the availability of appropriate auxotrophic bacteria. A second selection technique employs mechanism-based inhibitors ('suicide substrates') to pick out catalytic antibodies from a pool of largely noncatalytic antibodies. Mechanism-based inhibitors are small substrate-like molecules that the enzyme or catalytic antibody initially recognizes as a substrate. However, during the reaction, the inhibitor diverges from normal substrate behavior by forming an irreversible covalent linkage to the catalyst, thus terminating the reaction and permanently rendering the catalyst inactive[61].

The IGEN group has demonstrated the feasibility of this concept using isoabzymes of 20G9[62]. The mechanism-based inhibitor, CBZ-Gly-O-Phe, was turned over an average of 3.6 times (Figure 5, right branch) as a normal substrate before being inactivated by a covalent nonhydrolyzed acyl–tyrosyl complex (Figure 5, lower branch). In practice, such mechanism-based inhibitors could be immobilized on a solid surface, such that catalytic antibodies (or sFv proteins displayed on phages) could be selected by being covalently immobilized on that surface. This method is attractive because it can be used to select for catalysis rather than binding.

A third method, which is simpler but less powerful than using a mechanism-based inhibitor, is to select, not for catalysis, but for a given desired amino acid that could be present in a catalytic antibody. For example, a sulfhydryl-reacting reagent has been used to select rare cysteine-containing antibodies[63].

Lastly and most recently, a clever variation of these reaction-based selection methods called reactive immunization has been introduced, in which the hapten used for immunization is itself reactive[64].

Although the focus of this review is on advances in designed catalytic antibodies, it should also be noted that research on naturally-occurring human catalytic antibodies has been carried out in a small number of laboratories[65,66]. Such antibodies may be associated with certain autoimmune disease states. For example, vasoactive intestinal peptide (VIP) hydrolyzing antibodies have been linked to asthma[65] and DNA hydrolyzing antibodies to systemic lupus erythematosus[66]. The therapeutic potential of natural catalytic antibodies or their inhibitors can be envisioned.

## Potential keys to commercialization

Since their discovery, catalytic antibodies have been shown to be capable of catalyzing several types of commercially valuable reactions. In particular, specific hydrolysis of amides or esters has been commonly mentioned (Table 1). For practical reasons, the substrates usually used in prototype catalytic antibody research have not been the actual commercially-valuable target but compounds that structurally resemble the target. In order to be commercialized as products, catalytic antibodies must be able to catalyze

*Still round the corner there may wait,*
*a new road, or a secret gate*
J.R.R. Tolkien

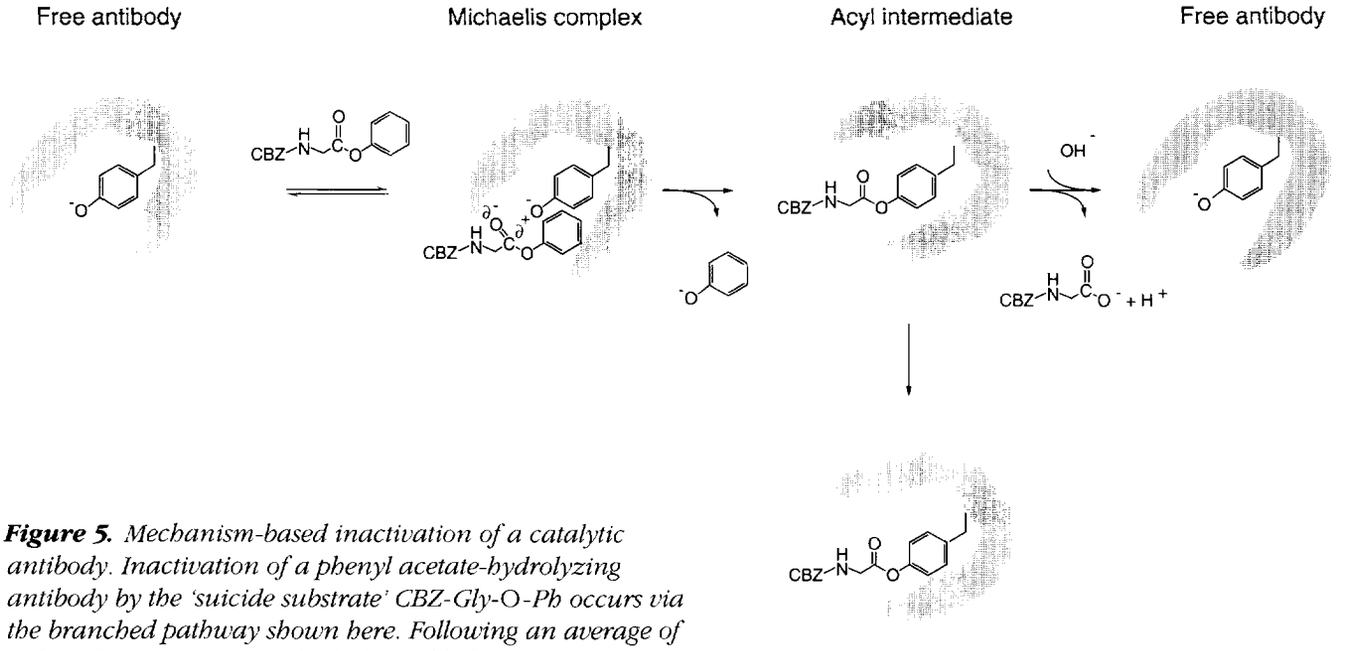


***Figure 5.*** *Mechanism-based inactivation of a catalytic antibody. Inactivation of a phenyl acetate-hydrolyzing antibody by the 'suicide substrate' CBZ-Gly-O-Ph occurs via the branched pathway shown here. Following an average of 3.6 productive turnovers (right branch) the antibody, 18H4, became specifically acylated on an essential combining site tyrosine resulting in covalent inactivation (lower branch)*[61].

valuable reactions with acceptable efficiencies. The most profound accomplishment of the field to date has been antibody catalysis of many different types of chemical reactions. The major remaining barrier to commercialization is the isolation of catalytic antibodies with enzyme-like efficiencies.

There are perhaps three major ways in which the potential of catalytic antibodies will develop in future. First, novel and ingenious haptens are being designed that are more sophisticated than earlier simple TSA haptens[36,67–69]. Haptens with two or more functional groups will be designed to elicit multiple antibody amino acid residues with different mechanistic roles, similar to the essential amino acids of enzyme active sites. Second, selection methods for picking out rare efficient catalytic antibodies will be developed[62–64]. This may involve the design and preparation of mechanism-based inhibitors that could be used to select antibody fragments displayed on phages[62]. Third, antibody engineering will be extremely valuable. The efficiencies of catalytic antibodies can be improved through combinatorial libraries[54–56] and through random or site-directed mutagenesis[52]. Combining site modifications will be facilitated by mechanistic[42,43] and structural studies[47–49] and with the use of phage display technology[54,56]. Antibody engineering will also have the benefit of decreasing the cost of producing catalytic antibody products, which will in turn increase the number of applications that are economically feasible[53,57]. These three key areas to catalytic antibody development are not mutually exclusive and may synergistically accelerate progress if used together.

Finally, it should be noted that in a field as prolifically innovative as catalytic antibody research, there is always the possibility that new and unforeseen breakthroughs will suddenly propel catalytic antibodies into the marketplace.

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