

The Potential of Catalytic Antibodies[†]

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The idea that antibodies raised against transition state analogues should show specific catalytic activity is beautiful and seductive. In the tenth year since the idea became an experimental reality it is possible to make at least a preliminary assessment of their potential. It is concluded that their high stereoselectivity makes abzymes excellent prospects for asymmetric synthesis, though their practical usefulness is currently limited by their catalytic efficiency.

In principle catalytic antibodies are ideal catalysts for doing asymmetric synthesis. They are naturally chiral; their potential for precise molecular recognition of a user-specified structure is unrivalled, so that they are reliably regio- and chemo-selective and protecting group chemistry becomes redundant: and – as other contributors to this Symposium have shown – they catalyse such a wide range of reactions that no conversion involving reasonably stable species is ruled out. Furthermore, the technology for actually doing the chemistry on a preparative scale is available – and its limitations understood – from work using enzymes in synthesis;¹ and single or multi-step conversions are feasible. However, a catalytic antibody is not at this stage the catalyst of choice for even the most avant-garde synthetic chemist wanting to do asymmetric synthesis. So an important question is – is this only a matter of time? This paper considers the problems and the promise of catalytic antibodies as practical reagents for organic synthesis, and for asymmetric synthesis in particular.

Availability, expense and reliability are all relevant, but the question crucial to any practical application is efficiency: will the catalyst do the job in a reasonable time, in acceptable yield and with good enantiomeric or diastereoisomeric excess? There is already enough information in the literature to answer at least the last question with some confidence. Catalytic efficiency is a more complicated problem, and is identified as a major limitation on the use of catalytic antibodies in the short term.

Stereospecificity

The typical catalytic antibody is raised against a synthetic antigen (the hapten) that is an analogue of the transition state for the target reaction. If a product is chiral the transition state leading to it will be also, so the binding interaction with a naturally chiral antibody will be different for its two enantiomers, and enantioselectivity is to be expected whether or not the hapten is chiral. It is thus not essential for haptens to be single enantiomers. In practice catalytic antibodies of interest are almost always selected on the basis of a binding assay: they have been screened directly for catalysis only occasionally, and not so far for chiral recognition in catalysis, though it is known that antigen-binding by ordinary (non-catalytic) antibodies is efficiently stereoselective.² So we need to be sure at the onset that a racemic, or even achiral, transition state analogue hapten will elicit antibodies which can catalyse reactions with good enantioselectivity. There is by now enough information in the literature to be confident that this is the case.

Catalytic antibodies have been raised against achiral, racemic and single enantiomer haptens, and against mixtures of diastereoisomers. Fig. 1 is a bar graph which summarises the published data (to August 1, 1995), for reactions involving or producing a chiral centre, where a reasonable measure of enantioselectivity (diastereoselectivity where a second chiral centre is present) is available. Of over sixty cases, selected as good hapten binders which also catalyse the reaction of a test substrate, rather than for their chiral discrimination, over half shown an enantiomeric excess of 98% or better, with 79% showing an ee of better than 90%. This is confirmation – if confirmation were needed – that catalytic antibodies show excellent potential for asymmetric catalysis.

In Fig. 2 the same data are broken down in terms of the chiral status of the hapten used. Results involving

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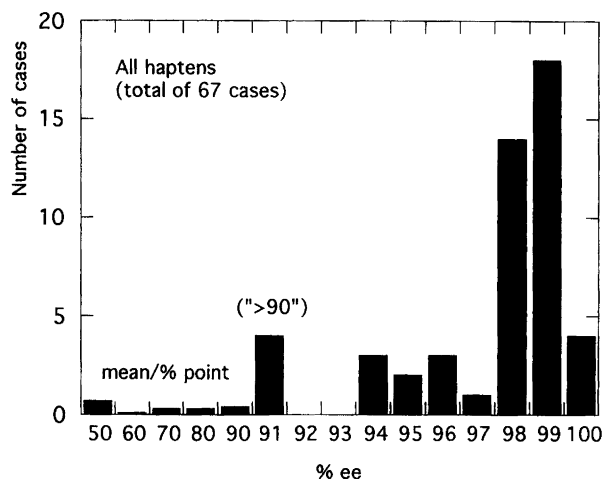


Fig. 1. Bar graph of published data for reactions involving or producing a chiral centre, where a reasonable measure of enantioselectivity (diastereoselectivity where a second chiral centre is present) is available. Data for ee < 90% represent mean values for the range of 10 percentage points up to the figure (50%, etc.) given.

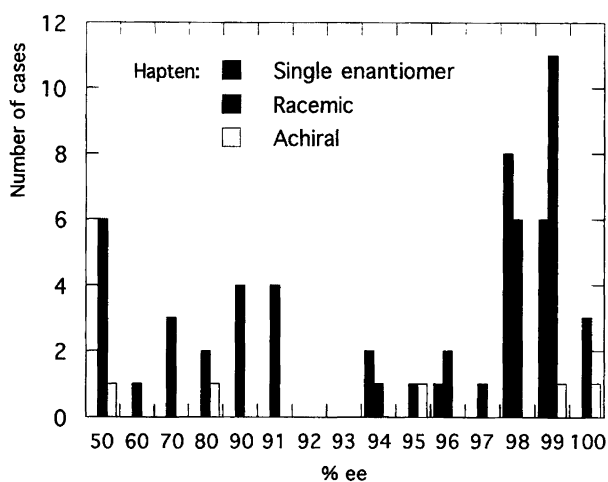
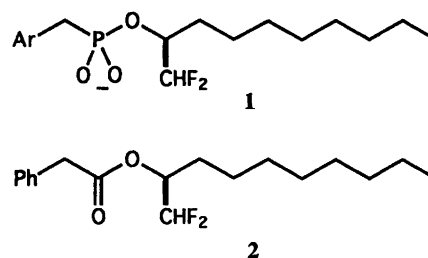


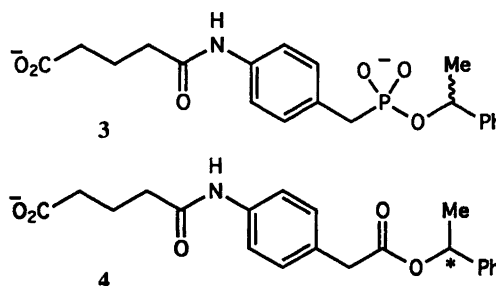
Fig. 2. Bar graph showing the data of Fig. 1 broken down in terms of the chiral status of the hapten used.

achiral (prochiral) haptens as transition state analogues are too few to be statistically meaningful,³ but at least show that an achiral hapten can elicit efficient asymmetric catalysts. Not surprisingly, in the great majority of cases racemic haptens have been used.⁴ The enantiomeric excess in the antibody-catalysed reaction is consistently high: 90% or better in 33 cases out of 45, and 98% or better in 20 of these. Finally, a small group of esterase antibodies raised against single-enantiomer haptens (e.g., **1**)⁵ are clearly more enantioselective than antibodies raised against their racemic modifications; e.g., the kinetic resolutions of fluorinated alcohols by way of their phenylacetate esters (e.g. **2**) investigated by Kitazume.^{5b,c}

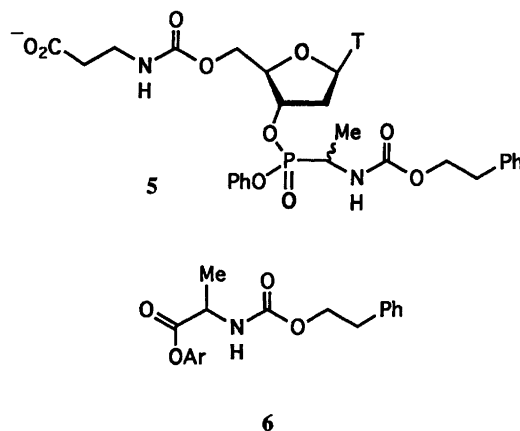
An advantage of using a racemic hapten is that antibodies specific for either substrate enantiomer (or both) may be available from the same experiment. Thus Janda



et al.^{4j} raised eleven different antibodies against hapten **3**, nine of which were capable of catalysing the hydrolysis of the *R* enantiomer of substrate ester **4**, while two were specific for *S*-**4**. In two cases (one *R*-specific, one *S*) investigated in detail, 'complete stereospecificity' was observed.



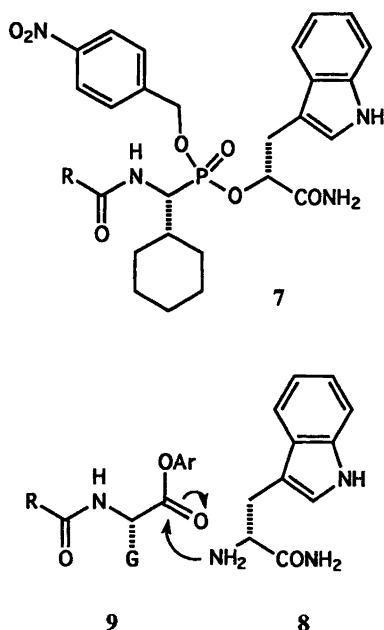
Similar results were obtained by the Schultz group for an ester aminolysis reaction mimicking the aminoacyl t-RNA synthetase reaction.^{4w} Here the phosphonate diester hapten (**5**) was a mixture of diastereoisomers, and the target reaction the acylation of the 3'-OH group of thymidine (5'-ethyl carbamate) by an amino-acid active ester. Of eight catalytic antibodies four were specific for the L- and three for the D-alanine active ester (**6**), while one showed no selectivity.



These results, for reactions differing only in the placement of a methyl group at the recognition centre of in-

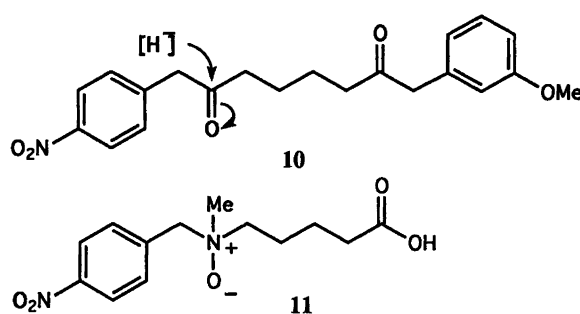
terest, show that at least the stereoselectivity of reactions catalysed by antibodies rivals that of enzyme reactions: a conclusion reached in a specific comparison of abzyme- and lipase-catalysed esterifications reported by Fernholtz *et al.*^{4h} [Note that a problem with the *formation* of an ester (or of any active acyl derivative) is that an antibody (or enzyme) that catalyses its formation may also catalyse its hydrolysis, with equal stereospecificity.]

An attempt to broaden substrate specificity in ester aminolysis, in the hope of generating an antibody catalyst for peptide bond formation, shows how stereospecificity falls off if the fit is not optimal. Hirschmann *et al.*^{4u} used the hapten **7**, with two of the three stereogenic centres defined (the third, at phosphorus, is not). The cyclohexyl group was designed to elicit a hydrophobic binding pocket spacious enough to accommodate a range of hydrophobic groups (G). The antibodies produced would catalyse the acylation of D-tryptophan amide **8** by active esters derived from various α -amino acids **9**. In the event the most effective antibody found showed little stereoselectivity, and even a slight preference for L-tryptophan amide. (It may be that the hydrophobic binding pocket is large enough to accommodate also the indole ring of the tryptophan amide: it is not unknown for enzymes to bind synthetic substrates the 'wrong way round'.)



These results, and many others described in other contributions to this Symposium, leave no doubt that good stereospecificity is available in reactions catalysed by antibodies derived from properly designed haptens. This is as expected. The binding specificity for a substrate methyl group vs. hydrogen can be over 2 kcal (8 kJ) mol⁻¹ in an enzyme-catalysed reaction,^{6a} enough in itself for an ee of 99%, if twice this factor is available for chiral discrimination between transition states. Energy differences

of this magnitude between competing pathways afford sufficient selectivity for most purposes in synthesis. Thus – in principle at least – antibody catalysis offers a potential solution to almost any problem of chemo- or regioselectivity: as witnessed by the remarkably selective cyanoborohydride reduction of the 1,6-diketone **10** reported by the Schultz group.⁷ An antibody raised against the simple hapten **11** catalysed the reduction of the *p*-nitrobenzyl ketone group (arrowed in **10**) to give the monohydroxy ketone with greater than 75:1 regioselectivity (and 96.3% ee). In the absence of antibody the other C = O group is more reactive.



Problems

If catalytic antibodies control reactivity as efficiently as this, why are they not already in general use in synthesis? The problems fall into the usual three classes: economic, technical and fundamental. Economic problems will disappear if the technical problems can be solved, and such is the pace of molecular biology that I have no doubt that any technical problems involved in producing sufficient quantities of a desired protein, whatever its origin, will be temporary. First though it is necessary to create and identify the desired protein. Here the problems are both technical and fundamental. Hapten design in particular is still more of an art than a science: although useful generalisations are emerging, the most useful is still the simplest – if you can't synthesise it easily, change the design. Hapten design is the major preoccupation of the final section of this paper.

The problems at the next chemical stage – identifying a promising catalytic antibody – are more practical than fundamental. Here the great attraction of harnessing the vast resources of the immune system meets technical reality. Current methods allow reasonably efficient screening of only a fraction of the enormous variety of antibodies potentially available, and specifically for hapten-binding rather than for catalysis (the best hapten binder is not necessarily the best catalyst for the reaction).[†] However, it seems entirely reasonable to be confident that these technical problems too can be solved – certainly if the catalysts produced prove worthwhile.

[†] New methods such as catELISA²¹ are being developed to address this problem.

A problem that has no simple solution arises directly from the exquisite specificity of antibody catalysis that is one of its principal attractions. Just as for enzyme catalysis, high specificity can be a disadvantage from an economic viewpoint: since a different catalyst may be necessary for each substrate, as well as for each reaction. A welcome suggestion that this conclusion may be too pessimistic comes from work on the versatile catalytic antibody 14D9, developed at Scripps, which has been shown to catalyse the hydrolysis not only of enol ethers^{4a,e,8} but also both the formation^{4d} and the ring-opening^{4b} of epoxides, as well as the hydrolysis of tetrahydropyranyl acetals for which it was originally designed.⁹ It is not unreasonable to expect that other antibodies will appear that recognise the similar transition states of related reactions, and have relaxed substrate-specificity in some positions.

A related recognition problem is product inhibition. The two stable structures closest to the transition state which the antibody is designed to bind are the starting material and the product. Binding the ground state too tightly makes catalysis less efficient by raising the activation energy for reaction; binding the product too tightly inhibits turnover. This latter problem is particularly severe for bond-forming processes: the formation of a bond between two strongly bound structures will reinforce the binding of both (the chelate effect); as observed, for example, in the aminoacylation reaction discussed above.^{4w} Here the product has all the recognition sites of the hapten (5), and differs only in the replacement of the PhOP=O group by C=O. The best solution (applied successfully, for example, in antibody-catalysed Diels–Alder reactions)^{3c,4t,10} is to build in significant structural differences between hapten and product.

I believe that none of these problems is insurmountable – as long as catalysis is sufficiently efficient. Jacobsen and Finney¹¹ have made the point explicitly that comparable gram-amounts of antibody catalyst are currently required to produce a given weight of product. Protein and other macromolecular catalysts will always suffer in comparisons of this sort, but the point is a valid one, solved in the case of enzyme catalysis by their extraordinary efficiency. So the key question for antibody catalysis is, what are the prospects for increasing efficiency? For some applications even an order of magnitude might be enough to make antibody catalysis the method of choice, but for general synthetic purposes increases of two or preferably at least three orders of magnitude are needed.

Efficiency of catalysis

There are very good practical reasons for wanting to improve the efficiency of antibody catalysis. The first is to reduce the amount of catalyst needed to carry out a given transformation in a reasonable length of time. A second is to ensure that the catalysed reaction is many (ideally

> 100) times faster than any competing background reaction producing racemic product. Third, and not least, is to extend the range of reactions that antibodies can catalyse.

Accelerations $k_{\text{cat}}/k_{\text{uncat}}$ of the order of 10^{3-4} are achieved in most (published) examples of antibody catalysis, and figures of 10^{6-7} have been obtained in specially favourable cases. Given enough catalyst such figures may be good enough to favour the desired over the background reaction sufficiently to give an acceptable enantiomeric or diastereomeric excess: or to favour what is normally a minor (disfavoured) pathway. [Note, however, that the ratio of rate constants $k_{\text{cat}}/k_{\text{uncat}}$ does not translate directly into a numerically comparable rate acceleration. For example, the actual enhancement of rates obtained under typical conditions of catalyst and substrate concentration (1 μM and 100 μM , respectively, with K_{M} equal to the substrate concentration), with a reasonable $k_{\text{cat}}/k_{\text{uncat}}$ of 10^3 , is only five.¹²] But even this is possible at a reasonable rate only if the reactions concerned are already relatively rapid. This is the case for many reactions of interest to the synthetic chemist, making catalytic antibodies particularly interesting for processes where control of stereochemistry is crucial but difficult. However, the best accelerations so far obtained are modest compared with those achieved by enzymes.

As a result reactions that are intrinsically very slow at pH 7 are not catalysed at a useful rate. For example, the sequence-specific cleavage of peptides or DNA is currently beyond the capabilities of man-made catalytic antibodies. Whether or not abzymes will become competitive with enzymes for catalysing very slow reactions of natural substrates is an open question: abzymes are particularly interesting because they are complementary to enzymes, and capable of doing chemistry with unnatural substrates. But it is to enzymes we look, and especially to enzymes catalysing such very slow reactions, to learn to improve antibody catalysis.

The most instructive measure of catalytic efficiency in both abzymes and enzymes is the binding of the transition state. Figure 3 represents the energy profile for a reaction catalysed by an abzyme or a (very simple) enzyme. Transition state binding ($\Delta G_{\text{TS}}^\ddagger$) is given by the expression:

$$-\Delta G_{\text{TS}}^\ddagger = \Delta G_{\text{cat}}^\ddagger - (\Delta G_{\text{un}}^\ddagger + \Delta G_{\text{ES}}^\circ)$$

which can be conveniently written in terms of rate and equilibrium constants as:

$$1/K_{\text{TS}} = (k_{\text{cat}}/k_{\text{uncat}})/K_{\text{M}}$$

where $1/K_{\text{TS}}$ is the association constant for the transition state to the abzyme or enzyme. For a perfectly efficient transition state analogue K_{TS} should be equal to K_{i} , which measures the strength of binding of the hapten, and logarithmic plots of $k_{\text{cat}}/k_{\text{uncat}}$ vs. $K_{\text{M}}/K_{\text{i}}$ do show reasonable

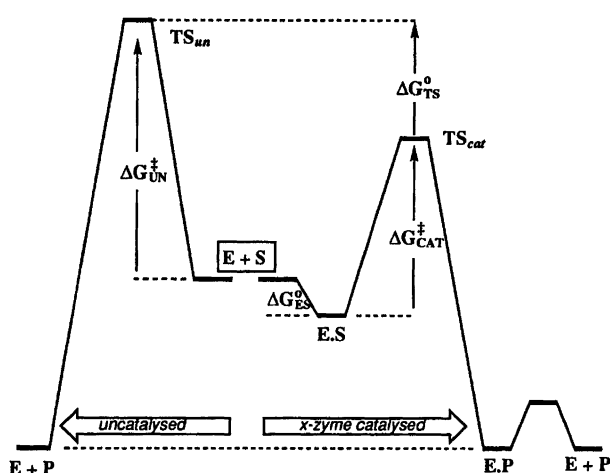


Fig. 3. Energy profiles comparing abzyme- or enzyme-catalysed (left to right) and uncatyalsed (right to left) reactions of a substrate S.

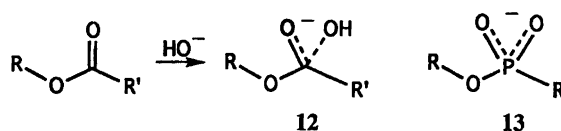
correlations, with points clustered about a line of unit slope.^{13,14}

These correlations are useful, enabling us to identify particularly efficient (or inefficient) abzymes; but the main conclusion is that K_i/K_{TS} does give a remarkably accurate indication of k_{cat}/k_{uncat} in many cases, and it is difficult to draw constructive conclusions at this stage.

Table 1 shows how the strength of transition state binding for a group of reasonably efficient abzyme-catalysed reactions compares with the binding of the substrate. Selective binding of up to 40 kJ mol^{-1} is achieved, though $20\text{--}30 \text{ kJ mol}^{-1}$ is more usual: it is this figure that must be improved. Substrate binding (K_M) varies very little, at least for this group of reactions, so we are justified in concentrating on transition state binding. Just how far we have to go is indicated by the similar set of data for enzyme reactions in Table 2. Here the binding selectivity goes up to almost 100 kJ mol^{-1} . For enzymes too the variation in K_M is relatively small, as might be expected for catalysts that have to work with comparable physiological concentrations of substrates. As a result the data from Tables 1 and 2 can be compared graphically by a plot of k_{cat}/k_{uncat} against the binding energy of the transition state. This plot shows very clearly the current dis-

parity in efficiency between enzymes and catalytic antibodies.

To understand the reasons for this disparity we need to think about the fundamentals of catalysis. The basic premise is that enzymes work by binding and thus stabilising selectively the transition states for the reactions they catalyse: to produce an antibody to do the same we use an antigen which mimics the transition state concerned as closely as possible. We know that antibodies are capable of binding antigens very strongly, so the first suspect is hapten design: how closely does a typical analogue really mimic a transition state? The answer in many cases is, not very well, at the most important site. Consider the phosphonate anion **13**, which has proved the most successful type of analogue, for the transition state **12** for the hydroxide-catalysed hydrolysis of an ester.



The phosphonate has the right tetrahedral shape, but its key electronic 'profile' at the phosphorus centre – which alone distinguishes the transition state from the ground state – is barely half right, while most of the apparently efficient binding is likely to come from recognition of the groups R and R', one at least of which needs to be fairly substantial to make the hapten immunogenic. But, as I argue below, it is the binding of the reaction centre that is generally the single most important factor in catalysis by enzymes.

It is helpful to distinguish two sorts of binding relevant to transition states: *passive*, referring to ordinary molecular recognition – hydrogen-bonding, hydrophobic interactions and the like – and *dynamic*.¹⁵ Dynamic binding refers to specific interactions between catalyst and substrate at the reaction centre. These interactions are of crucial importance for catalysis because they represent the most obvious difference in binding between substrate and transition state. They differ from those involved in passive binding, because they change progressively and substantially as the reaction proceeds, and bonds are

Table 1. Reactivity and transition state binding by catalytic antibodies.

Reaction	k_{cat}/s^{-1}	k_{cat}/k_{un}	$-\Delta G_{ES}^{\circ}/\text{kJ mol}^{-1}$	$-\Delta G_{TS}^{\circ}/\text{kJ mol}^{-1}$
Carbonate ester hydrolysis ²³	7×10^{-3}	770	21	37
Phenyl ester hydrolysis ⁴⁰	3.72	2.2×10^4	20	45
Phenyl acetate hydrolysis ²⁴	8.2×10^{-2}	2.5×10^5	19	50
<i>p</i> -Nitrobenzyl ester hydrolysis ²¹	0.12	2.6×10^5	20	51
Aryl arylacetate ester hydrolysis ²⁵	20	6.2×10^6	16	55
Ester exchange ^{4w}	0.24		18, 20	47
Unactivated amide hydrolysis ²⁶	1.7×10^{-7}	132	19	34
Activated amide hydrolysis ²²	0.46	2.5×10^5	19	50
Proton transfer from carbon ¹⁸	0.66	2.1×10^4	22	47

Table 2. Reactivity and transition state binding data for enzyme reactions.²⁷

Enzyme	$k_{\text{cat}}/\text{s}^{-1}$	$k_{\text{cat}}/k_{\text{un}}$	$-\Delta g_{\text{ES}}^{\circ}/\text{kJ mol}^{-1}$	$-\Delta g_{\text{TS}}^{\circ}/\text{kJ mol}^{-1}$
OMP decarboxylase	39	1.4×10^{17}	35	133
Staphylococcal nuclease	95	5.6×10^{14}	29	113
Adenosine deaminase	370	2.1×10^{12}	26	96
Carboxypeptidase A	578	1.9×10^{11}	23	88
Triose phosphate isomerase	4300	10^9	27	78
Carbonic anhydrase	10^6	7.7×10^6	12	51
Chorismate mutase	50	1.9×10^6	25	61

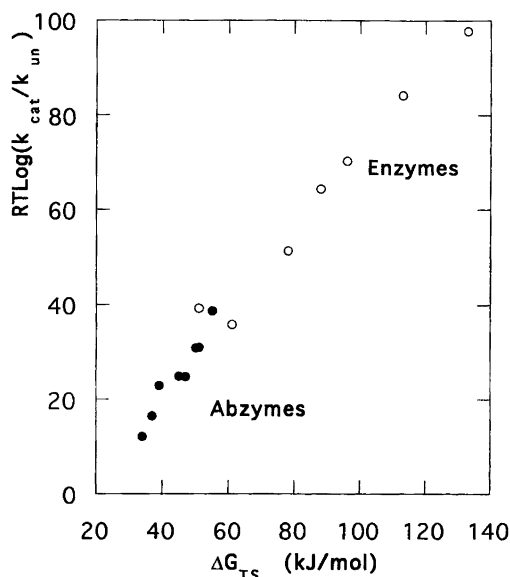


Fig. 4. Efficiency of abzyme and enzyme catalysis compared. Note that the two variables are not independent: this is simply a convenient graphical comparison of the data of Tables 1 and 2; linear because the variation in K_M is relatively small, and similar in both cases.

made and broken; and because they are generally stronger than the interactions involved in ordinary molecular recognition.

To illustrate dynamic binding[†] take the familiar reaction catalysed by a serine protease such as chymotrypsin [Fig. 5(a)],^{6b} in which a general base B (the imidazole of His-57 in the case of α -chymotrypsin) assists the nucleophilic addition of a serine OH group to the carbonyl carbon centre of the amide substrate; with further assistance from a backbone amide NH, acting in effect as a general acid (HA). In the transition state for this step [Fig. 5(b)] at least six bonds are being made and broken, probably simultaneously, and it is not obvious where transition state binding starts and finishes, since in this case the nucleophile is part of the enzyme. But the important point is that the 'binding' of the transition state by the enzyme involves more than ordinary molecular recognition: these

[†] It should be stressed that there is no clear-cut distinction between dynamic and passive binding. Even where the reaction centre is clearly defined binding interactions outside it may change significantly during the course of the reaction.

dynamic interactions at the reaction centre involve partially formed or broken covalent bonds [arrows in Fig. 5(b)].

These interactions – this 'dynamic binding' – can be substantially stronger than the individual interactions involved in ordinary molecular recognition, and so make the major contribution to catalytic efficiency. To oversimplify, the target transition state is not something like an egg, for which we must design a perfect egg-shaped cavity of just the right size (though that approach works pretty well for some pericyclic reactions),^{3c,4t,10} but a fugitive thing which depends on a pattern of specific dynamic interactions with functional groups. These inter-

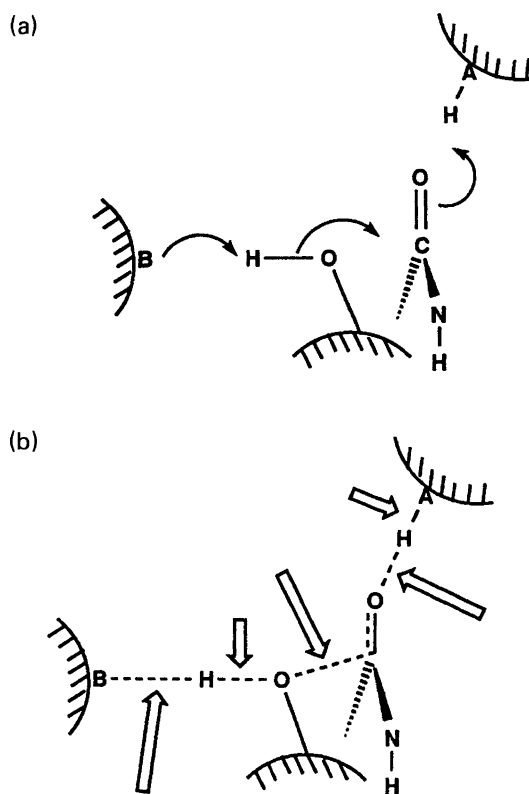
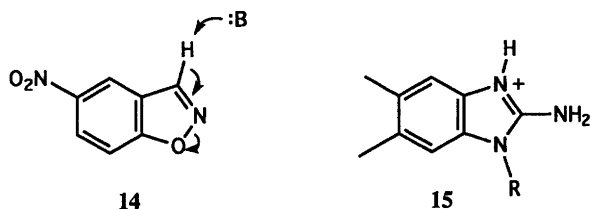


Fig. 5. (a) Schematic mechanism of the first, acylation step in a serine protease reaction. For clarity only the amide group of the substrate is shown. (b) The transition state for the reaction in (a). The arrows indicate bonds being formed and broken, corresponding to dynamic binding interactions between the enzyme and the transition state.

actions are largely missing in most abzyme-substrate reactions. So what can we do to introduce more 'dynamic binding' – which means participating active-site functional groups – into antibody-catalysed reactions?

The lesson from model systems is that nucleophilic catalysis – involving the formation of bonds between heavy-atom centres – is by far the most efficient; general acid-base catalysis is typically far less so, but we are learning how very accurate placement of general acids and bases can raise effective molarities (EM)¹⁶ from the customary single figures at least to the order of 10^{5-6} .¹⁷ At first sight the accuracy required would not suggest that this efficiency would be easy to reproduce in an abzyme-catalysed reaction, but Hilvert's antibodies¹⁸ which catalyse the elimination-isomerisation of the benzisoxazole **14** shows EMs of better than 10^4 M. The hapten used was the benzimidazolium cation **15**, with a charged and acidic NH^+ in precisely the right position to elicit a hydrogen-bonding anionic group in the binding site, thought to be carboxylate in the most successful catalyst.



This is a particularly encouraging result, because a single extra hydrogen-bonding interaction (absent in any case in the isomerised product from reaction **14**) is the least likely to lead to serious product inhibition. A similar instance of dynamic binding of a transition state involving proton transfer to carbon has been suggested to account for the enantioselectivity of enol ether hydrolysis catalysed by the versatile antibody 14D9 discussed above.¹⁹ But the approach cannot be simply extended to nucleophilic catalysis, because this would involve a new covalent bond between antibody and reactant, thus impeding turnover.[†] Enzymes like the serine proteases have evolved a sophisticated follow-up reaction to hydrolyse the covalent acyl-enzyme intermediate efficiently, but this is not a practical proposition for a simple catalytic antibody.

A pragmatic solution using nucleophilic catalysis may be more difficult to achieve. Making a new covalent bond between two bound substrates can be made very efficient – probably fast enough to be synthetically useful – with the right combination of functional group interactions at the active site: though the need to avoid product inhibition means compromises in hapten design. But such compromises should not be necessary for the right group

[†] Potential intermediates in nucleophilic catalysis are almost all too stable to be hydrolysed spontaneously at a useful rate, though an acylimidazole²² is a possible exception.

transfer reaction, where the nucleophile is a second bound substrate molecule, and covalent bond formation can be matched by bond breaking.

Although functional group interactions are likely to be the most effective, it is also possible that more passive selective transition state binding may be improved by screening directly for catalysis: though there are suggestions that the binding affinities of ordinary hydridoma-made antibodies is limited to association constants of the order of 10^{10} M^{-1} , corresponding to a free energy of binding of some 60 kJ mol^{-1} .²⁰ Any limit of this sort would mean that only reactions with very selective transition state binding could be synthetically useful. My general conclusion is that catalytic antibodies need to become more efficient before they are likely to be useful for general synthetic purposes, but that the increases in efficiency needed are not enormous; especially in the area of asymmetric catalysis.

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