Catalytic Antibodies: Mechanistic and Practical Considerations

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1 Introduction

The field of catalytic antibodies has been amply reviewed recently.[1-3] Its origins, key underlying tenets, and the range of reaction types presently known to be susceptible to antibody catalysis have all been described. Initial efforts at defining the mechanism of action of these agents have also been summarized.[2] In order not to be merely repetitious, we will focus primarily on two issues: first, what are we learning about catalysis in general from a study of these agents; and secondly, how do these agents rank relative to other biological catalysts in terms of their present catalytic efficiency and in their potential for improvement? We have chosen to address these issues by examining, in turn, the characteristics of abzymes that catalyse the hydrolysis of various esters and amides and the properties of selected catalytic antibodies in facilitating stereospecific transformations.

2 Hydrolytic Reactions

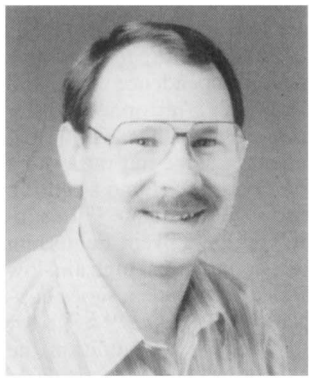
The induction of antibodies capable of facilitating the hydrolysis of amide and ester bonds has been based on transition-state analogues that mimic the tetrahedral intermediates anticipated to be involved in these processes.[1,2,4] Since enzymes appear to have active sites that are electronically and geometrically complementary to the transition states involved in the rate-limiting step of the substrate to product conversion,[5,6] potent inhibitors of many hydrolytic enzymes have been created through the synthesis of stable tetrahedral intermediate analogues.[7] Foremost among these inhibitors for hydrolytic enzymes have been compounds containing either charged tetrahedral phosphorus or secondary alcohols to substitute for the carbonyl of the scissile amide or ester bond.[8,9] These compounds in turn have been used to elicit antibodies capable of catalysing various reactions.

Examples of several catalytic antibodies generated to phosphonate, phosphonamidate, and secondary alcohol transition-state analogues are listed in Table 1.[4,10-16] In all cases the reported data were obtained from a single monoclonal antibody characterized from a panel of *ca.* 25—50 monoclonal antibodies that were selected owing to their high affinity for the corresponding hapten in an ELISA (enzyme-linked immuno-assay) assay. High affinity, however, is no guarantee of catalytic power since, for example, only one out of forty antibodies generated against hapten (4) showed activity against the anilide substrate. This behaviour is another manifestation of the diversity of the immunological response and the myriad ways of tightly binding a particular hapten. Given the presence of aromatic elements which provide strong binding determinants in all the hapten structures in Table 1, we can imagine hapten–antibody complexes where the tetrahedral mimic is either excluded from the binding pocket or so protected that the carbonyl centre of the corresponding substrate might not be accessible to water.

There are two general indices based on steady-state kinetic analyses that are used to assess the catalytic efficiency of an antibody: k_{cat}/k_{uncat} and k_{cat}/K_M. The steady-state kinetics for all abzymes obey the Michaelis–Menten rate expression for both K_M (the concentration of substrate that produces one-half the maximal catalytic rate) and k_{cat} (the rate constant for product formation under conditions when the antibody is saturated with substrate). Note that the K_M parameter also represents an approximate measure for the dissociation of the abzyme–substrate complex. The meaning of the first index – k_{cat}/k_{uncat}, where k_{uncat} is the rate constant for the same chemical process in the absence of antibody – is obvious and for enzymes may exceed 10^{10}. (For hydrolytic reactions both k_{cat} and k_{uncat} have the same units since the activity of water is set equal to unity.) The second index of efficiency, the ratio k_{cat}/K_M, represents a measure of the kinetic barrier encountered commencing with the combination of antibody and substrate and proceeding along the reaction coordinate to the transition state of highest energy. This ratio has a limit of approximately 10^7 M^{-1} s^{-1} when the reaction is limited by diffusion together of the substrate and antibody.[17]

The values of k_{cat}/k_{uncat} in Table 1 span a range from 70 to 10^6. This spread also encompasses all the antibody-catalysed chemical transformations reported to date. If one accepts our initial premise that antibodies owe their catalytic activity to their

Jon D. Stewart, born in Elmira, N.Y. in 1964, received his B.S. and M.S. degrees in chemistry from Bucknell University in 1986 and his Ph.D. from Cornell University in 1991. He is currently a post-doctoral fellow of the Helen Hay Whitney Foundation in Professor Benkovic's laboratory where he has used protein engineering techniques to investigate catalytic antibody mechanisms. He has accepted a position as an assistant professor in the Chemistry Department at the University of Florida.



Stephen J. Benkovic was born in Orange, New Jersey in 1938 and received his undergraduate degree at Lehigh and his Ph.D. at Cornell. After a period as postdoctoral research associate at U.C.-Santa Barbara, he joined the faculty at Penn State University in 1965. He now holds the rank of Evan Pugh Professor, and holds the Eberly Chair in Chemistry. He has been recognized by numerous national and international awards, and was elected a member of the National Academy of Science in 1985.

Benkovic's research has emphasized the study of the mechanisms of enzyme-catalysed reactions either through modelling of the reaction in physical organic studies or experiments on the enzymes themselves.

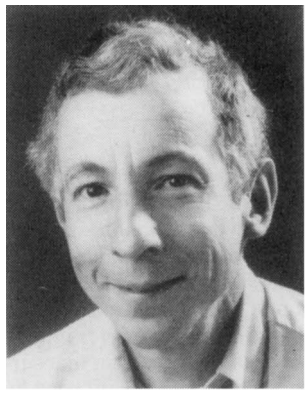
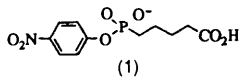
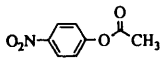
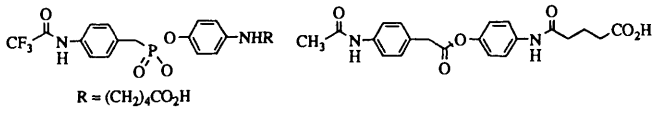
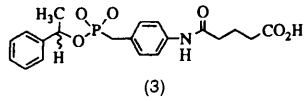
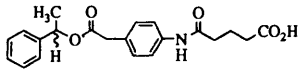
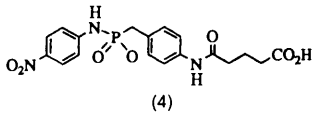
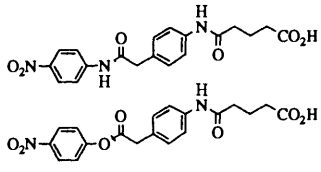
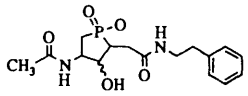
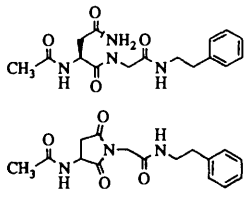
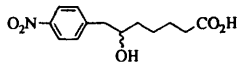
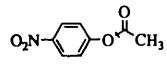
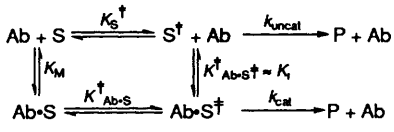


Table 1

Hapten	Substrate	k_{cat} (s $^{-1}$)	k_{cat}/k_{uncat}	K_M/K_i	k_{cat}/K_M (μM $^{-1}$s $^{-1}$)	Ref
 (1)		0 12 (pH 8 5)	1600	1 3 × 10^{3}	2 8 × 10 ^{4}	*a b*
 R = $(CH_2)_4CO_2H$ (2)		20 (pH 8 0)	6 25 × 10^{6}	3 0 × 10^{4}	1 3 × 10 ^{2}	*c*
 (3)		7 7 × 10 ^{2} (*R*) 1 5 × 10 ^{3} (*S*) (pH 9 0)	8 2 × 10^{4} 1600	2000 2000	3 8 × 10 ^{6} (*R*) 1 9 × 10 ^{5} (*S*)	*d*
 (4)		8 3 × 10 ^{4} (pH 9 0) 25 (pH 9 3)	1 5 × 10^{5} 2 7 × 10^{4}	3 7 × 10^{5} 5 3 × 10^{4}	2 2 × 10 ^{6} 0 47	*e f*
 (5)		1 2 × 10 ^{4} (pH 8 5) D isomer	70	N D	6 3 × 10 ^{7}	*g*
		8 7 × 10 ^{3} (*iso* Asp) 9 3 × 10 ^{3} (Asp) (pH 8 5) D succinimide	70 290	N D N D	3 6 × 10 ^{2} 3 9 × 10 ^{2}	*h*
 (6)		1 2 × 10 ^{2} (pH 7 5)	2250	26	3 3 × 10 ^{6}	*a*

[a] K M Shokat M K Ko T S Scanlan L Hochersperger S Yankovic S Thaisrivongs and P G Schultz *Angew Chem Int Ed Engl* 1990 **29** 1296 [b] J W Jacobs P G Schultz R Sugsawara and M Powell *J Am Chem Soc* 1987 **109** 2174 A Tramontano A A Ammann and R A Lerner *J Am Chem Soc* 1988 **110** 2282 [d] K D Janda S J Benkovic and R A Lerner *Science* 1989 **244** 437 [e] K D Janda D Schloeder S J Benkovic and R A Lerner *Science* 1988 **241** 1188 [f] R A Gibbs P A Benkovic K D Janda R A Lerner and S J Benkovic *J Am Chem Soc* 1992 **114** 3528 [g] R A Gibbs S Taylor and S J Benkovic *Science* 1992 **258** 803 [h] L J Liotta P A Benkovic G P Miller and S J Benkovic *J Am Chem Soc* 1993 **115** 350

ability to stabilize the transition state for their respective reaction, k_{cat} and k_{uncat} can be inter-related through a thermodynamic cycle to the K_M for the substrate and K_i (the dissociation constant for the hapten from the antibody–hapten complex), Scheme 1 [18] The K_i term is presumed to be an approximation of the hypothetical and unmeasurable disocciation constant, $K^{\ddagger}_{Ab\,S\ddagger}$, describing binding of the transition state, S‡, to the antibody The relationship is thus $(k_{cat}/k_{uncat}) = (K_M/K_i)$



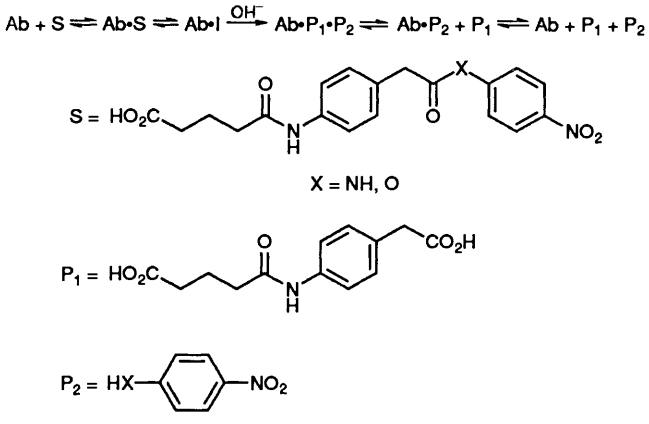
Scheme 1

For hydrolytic antibodies raised against haptens (1), (2), (3*S*), and (4) this equation is largely satisfied, in addition some eighteen other anti-phosphonate catalytic antibodies exhibit kinetic parameters that are also within an order of magnitude of the K_M/K_i ratio [19] On the other hand, for haptens (3*R*) and (6), the values of K_M/K_i is smaller than k_{cat}/k_{uncat}, but only by an order of magnitude [the K_i value for (5) is not yet determined] These discrepancies may be more a consequence of an inaccuracy in the K_i values, for example, the K_i for antibody induced by (4) has been revised from 10 $^{-6}$ to 10 $^{-9}$ after direct titration by hapten Thus, despite the uncertainties inherent in presuming that K_i for the hapten accurately reflects transition-state binding, the relationship of the k_{cat}/k_{uncat} to K_M/K_i for a given catalytic antibody is fairly well satisfied Nevertheless, this agreement may mask a greater complexity in the antibody's catalytic mechanism This is indeed the case for an antibody induced by hapten (4) described below, whose mechanism has been studied in detail

Examination of the values for k_{cat}/k_{uncat} and k_{cat}/K_M for the hydrolytic reactions listed in Table 1 reveals that antibody catalysis generally is less than optimal Antibody 43C9 [induced to hapten (4)] is one of the most reactive antibodies, and it has been the object of extensive experimental scrutiny to discern its mechanism of action Studies of the pH-dependence of k_{cat} and k_{cat}/K_M for both the *p*-nitroanilide and *p*-nitrophenyl ester substrates exhibited an apparent p$K_a \approx 9$, which was not attributed to the ionization of a binding site residue but rather to a change in the rate-limiting step of the process around a central, transient antibody–substrate-generated intermediate [20] In support of that postulate were the differential solvent deuterium isotope effects and electronic effects encountered at high and low pH when the substrates were expanded to include a series of *p*-substituted phenyl esters [14 21] Specifically, $k^{H_2O}/k^{D_2O} \approx 3\,8$ for amide hydrolysis at pH < 9 but ~ 1 at pH > 9 0 In addition, the ρ value of + 2 3 at pH > 9 0 (against Hammett σ) is particularly diagnostic of an acylation reaction involving a neutral nitrogen

nucleophile contributed by the antibody binding site but is inconsistent with a general base mechanism, such as one involving formation of a bound tetrahedral intermediate from base-assisted attack by water

The recent cloning and sequencing of the heavy and light chains of 43C9 revealed the presence of two histidines, one in the heavy and one in the light chain [22] Computer modelling of this sequence with the hapten (4) docked in the antibody binding site suggests that the light chain histidine is proximal to the phosphonamidate phosphorus atom (within 3 Å) and by implication the substrate carbonyl [23] The imidazole of this histidine is then a likely candidate for the nucleophile that is acylated and deacylated in the course of the hydrolysis reaction A kinetic scheme consistent with these collective observations is presented in Scheme 2



Scheme 2

The individual steps in the kinetic sequence of Scheme 2 were evaluated from a combination of steady- and pre-steady-state methods and the data transformed into a free-energy reaction coordinate diagram (Figure 1) There are several salient features which are instructive with respect to our opening inquiry It is clear that for both the ester and anilide substrates at pH 7 0, the deacylation step [Ab·I → AbP$_1$·P$_2$] is primarily rate-limiting At higher pH (owing to the rate of deacylation being first order in hydroxide ion) the barrier between Ab·I → AbP$_1$·P$_2$ decreases so that product desorption (ester substrate) and/or acylation (anilide substrate) become rate-limiting At no pH value does Ab·I accumulate since the slow loss of P$_2$ favours the return of Ab·I to Ab·S It is highly probable that this profile will be typical for most catalytic antibodies, neither the chemical or product release steps should be optimized since the immunological response evolves simply to maximize binding and then stops A more optimal situation would balance the differences in ΔG between the external and internal ground states and their respective transition states, so that no single ΔG barrier would be egregious [24]

Perhaps we should be more astonished by the richness of the kinetic sequence that pertains to the catalytic activity of antibody 43C9 The phosphonamidate transition state analogue has

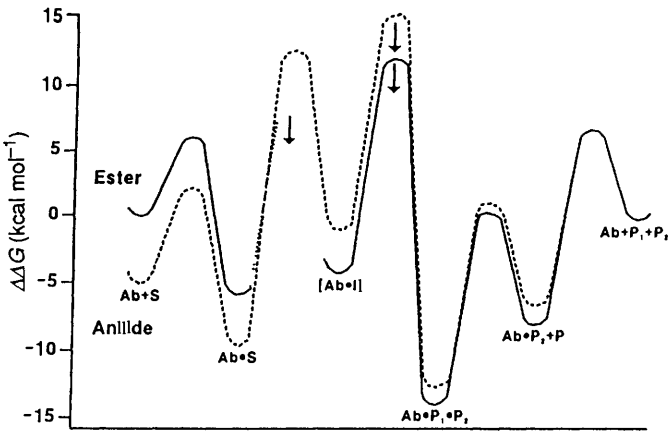


Figure 1 Reaction ΔG profile for antibody-catalysed ester (——) and anilide (----) hydrolysis at pH 7 The ground-state energy of the anilide was set 5 kcal mole $^{-1}$ lower than the arbitrarily fixed value for the ester The free-product ground-state energies for protonated acid and neutral *p*-nitrophenol and *p*-nitroaniline were fixed at 0 kcal mole $^{-1}$ and were not corrected to pH 7 The standard states of all substrate and products are 1 M The uncertainty in the forward rate constant for Ab·I formation from the ester is highlighted by the dotted line The arrows indicate that transition states set at their maximum free energy values

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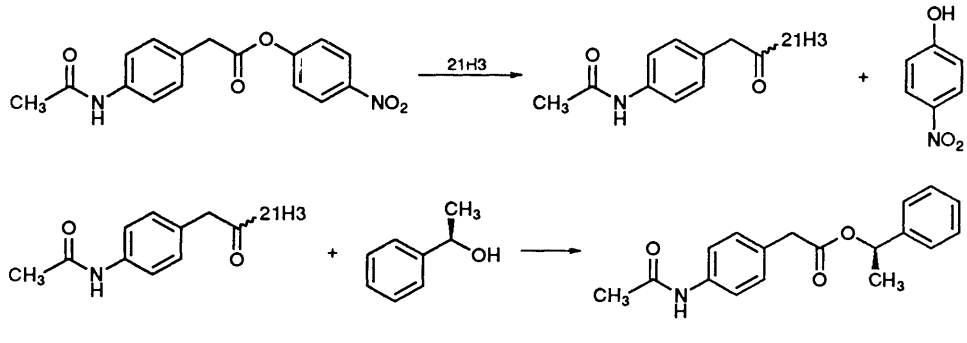
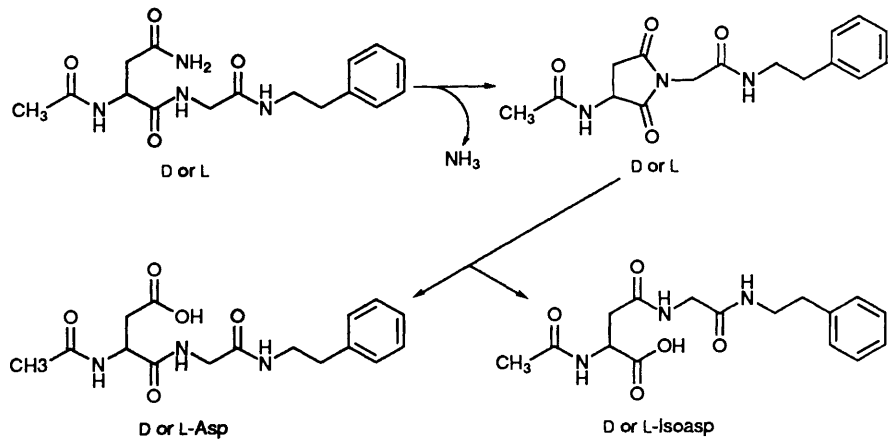
induced an active-site pocket that contains at least one nitrogen nucleophile proximal to the substrate carbonyl which is capable of reversible acylation In this case, the acylimidazole is hydrolysed to complete the cycle, unlike many macrocyclic systems that react stoichiometrically However, as a consequence, the transition-state analogue is no longer an ideal mimic of the tetrahedral intermediates anticipated in the hydrolytic reaction, suggesting that the catalytic activity of 43C9 may be further improved

The generation of a covalent antibody–substrate intermediate is not an isolated example There is strong kinetic evidence[25] to implicate an acyl–antibody intermediate in the transesterification reaction of 21H3 (Scheme 3), *viz* a pre-steady-state burst of *p*-nitrophenol formation equivalent to the antibody concentration and steady-state ping-pong kinetics This antibody also exhibits an induced fit by the second substrate to activate the acyl intermediate for subsequent chemical reaction Although 21H3 is unusually reactive as a transesterification agent (k_{cat}/k_{uncat} is estimated as $\geqslant 10^6$), it is a poorer catalyst for hydrolysis, presumably owing to a rate-limiting deacylation Consequently nucleophilic catalysis *per se* is insufficient to provide a k_{cat}/k_{uncat} ratio greater than K_M/K_i

On the other hand, the induction of antibodies using a reaction pathway involving an acyl–antibody intermediate is not a general immunological response to the tetrahedral phosphorus-containing haptens For example, hapten (1) induces a catalytic antibody that does not function *via* such an acyl intermediate, as its pH rate profile for the hydrolysis of *p*-



Scheme 3



Scheme 4

nitrophenyl acetate is simply first-order in hydroxide ion throughout.

Hapten (5) represents the first successful attempt to generate abzymes which catalyse a succeessive two-step process (Scheme 4).[15,16] The deamidation of an Asn-Gly peptide is known to proceed through a succinimide and this intermediate can be opened by the attack of water at either carbonyl. The two hydrolytic pathways yield either the Asp-Gly or the Isoasp-Gly peptide as the final products. Furthermore, electronic effects favour formation of the Isoasp product over the Asp product by a factor of 3.6.

Three classes of catalytic antibodies which hydrolysed the Asn-Gly substrate were isolated: those selective for the D-isomer, those selective for the L-isomer, and those which catalysed the hydrolysis of both isomers. This latter property was demonstrated by a change in the Isoasp/Asp product ratio to values either greater (16) or lower (1) than the normal background (3.6) reaction, and was dependent on the given antibody as well as the stereoisomer being processed.

To further investigate this reaction, kinetic measurements were made for the antibody-catalysed hydrolysis of both D- and L-isomers of synthetic succinimide. These studies indicated that both tetrahedral mimics of hapten (5) – the phosphinate as well as the secondary alcohol – induced complementary binding pockets within the antibodies which were capable of catalysing reaction at both carbonyls of the succinimide intermediate. Note that either the D- or L-isomers of the succinimide could conceivably occupy the binding site of a single antibody since the only difference is the location of the *N*-acetyl group (Figure 2). A direct comparison of the rate constants for partitioning by a given antibody of the succinimide to the two products suggests that the charged phosphinate moiety is some 6—8-fold more effective in inducing a catalytic active site than a secondary alcohol. Similarly the antibodies generated in response to the secondary hydroxy hapten (6) are less reactive, coincidentally by an order of magnitude, than those to the phosphinate (1).

The above study illustrates that the structure of the transition-state mimic is crucial for producing antibodies with high catalytic activity. Although existing transition-state mimics have provided antibodies with fair turnover relative to the spontaneous reaction, the full power of the technology may not be realized until other structural mimics are explored.

3 Stereospecific Transformations

Several recent examples of reactions catalysed by antibodies that are attractive in a synthetic sense are listed in Table 2. The first

Figure 2 Schematic illustration of D- and L-succinimide binding to the active site of an antibody generated against phosphinate (5).

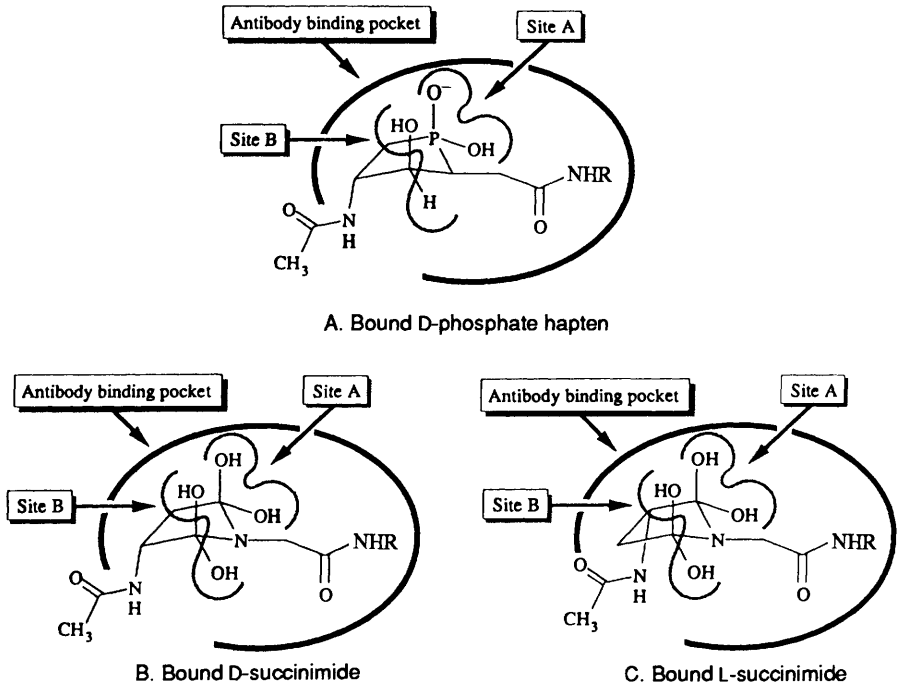
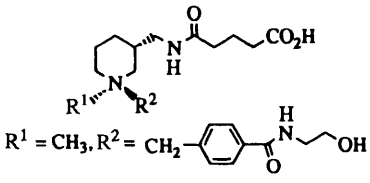
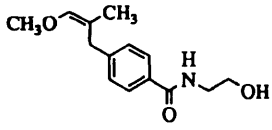
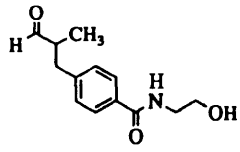
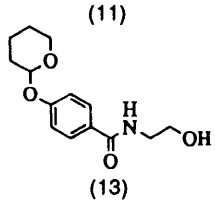
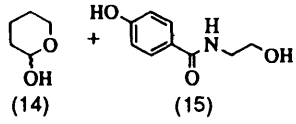
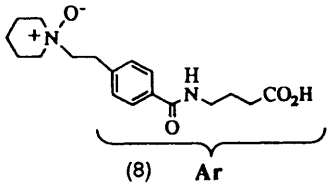
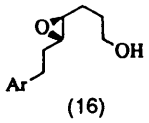
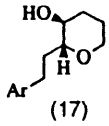
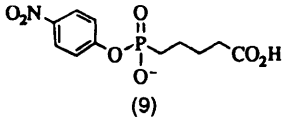
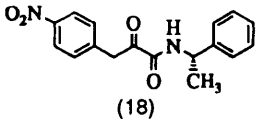
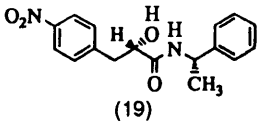
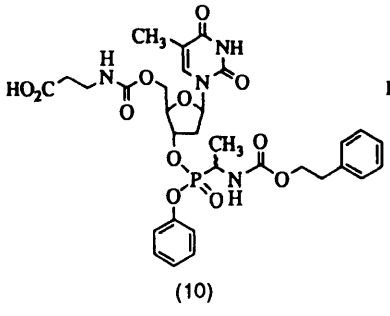
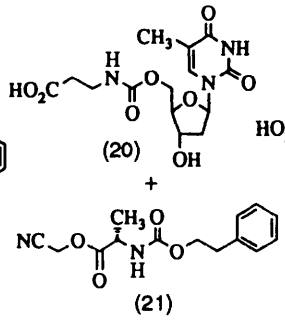
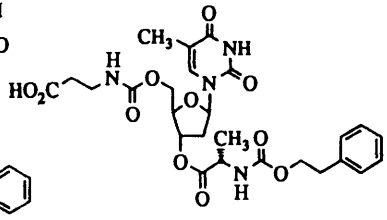


Table 2

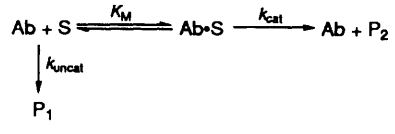
Hapten	Substrate(s)	Product(s)	K_M (μM)	k_{cat}/k_{uncat}	Ref.
 (7) (as racemate)	 (11)	 (12)	300	1.1×10^3	*a*
	 (13)	 (14) (15)	100	70	*b*
 (8) Ar	 (16)	 (17)	400	—	*c*
 (9)	 (18)	 (19)	1200	290 (1 mM $NaBH_4$)	*d*
 (10)	 (20) OH + (21)	 (22)	800 (19) 300 (20)	5.3×10^4	*e*

[a] J.-L. Reymond, K. D. Janda, and R. A. Lerner, *J. Am. Chem. Soc.*, 1992, **114**, 2257. [b] J.-L. Reymond, K. D. Janda, and R. A. Lerner, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1711. [c] K. D. Janda, C. G. Shevlin, and R. A. Lerner, *Science*, 1993, **259**, 490. [d] G. R. Nakayama and P. G. Schultz, *J. Am. Chem. Soc.*, 1992, **114**, 780. [e] R. Jacobsen, J. R. Prudent, L. Kochensperger, S. Yonkovich, P. G. Schultz, *Science*, 1992, **256**, 365.

entry features an enantioselective protonation; the same antibody also catalyses the hydrolysis of the aryloxytetrahydropyran acetal (13).[26,27] Another antibody catalyses the cyclization of the hydroxy epoxide (16) preferentially to a 6-*endo* product, a disfavoured reaction.[28] A third, induced by hapten (9), facilitates the stereospecific reduction by $NaCNBH_3$ of an α-ketoamide, (18).[29] Finally, an antibody elicited by hapten (10) promotes formation of a 3′-ester linkage in the 2′-deoxy sugar (19).[30]

All five reactions exhibit the high reaction stereospecificity and substrate diastereo- or enantioselectivity anticipated for antibody-catalysed processes. For example, hydrolysis of enol ether (11) to aldehyde (12) proceeds in 96% enantiomeric excess; the reduction of α-ketoamide (18) to alcohol (19) by $NaCNBH_3$ in the presence of antibody yields the *S*-isomer in 99% diastereomeric excess. Another attractive use for catalytic antibodies is to divert reaction pathways away from those favoured in solution to those desired in synthesis. In the first such example, Janda and co-workers reported the conversion of epoxy-alcohol (16) into the 6-*endo* (17) rather than the normally-favoured 5-*exo* product. Note that the latter is the only product found in the absence of antibody. Since the abzyme-catalysed and uncatalysed reactions yield different products, a k_{cat}/k_{uncat} ratio cannot be evaluated for this case.

It is instructive to predict the reaction courses based on the kinetic parameters assigned to these antibodies, since we anticipate that such modelling will be generally useful in judging whether the efficiency of a given catalytic antibody is sufficient for it to determine the outcome of the reaction. Using a kinetic simulation program, we first generated the expected extent of product formation for the simple, single substrate reaction time course described in Scheme 5 where P_2 is the desired product formed by an antibody-catalysed pathway and P_1 is an undesired product resulting from a spontaneous, uncatalysed reaction of the substrate. The critical parameter in these simulations is $[P_2]/[S]$, the fraction of substrate converted into the desired product when the reaction is run to completion. The time course



Scheme 5

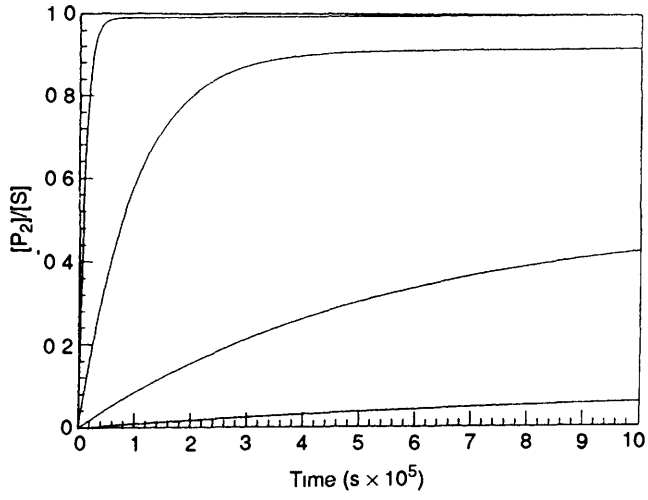


Figure 3 Kinetic simulation of the mechanism shown in Scheme 5 with a K_M value for substrate of 1 mM The on rate for substrate binding to the antibody was assumed to be diffusion controlled (1×10^8 $M^{-1}s^{-1}$) and k_{uncat} was fixed at $1 \times 10^{-6}s^{-1}$ The substrate concentration was 100 μM and the antibody concentration was 1 μM The time courses shown utilized (starting from the bottom) k_{cat}/k_{uncat} ratios of 10^2, 10^3, 10^4, and 10^5

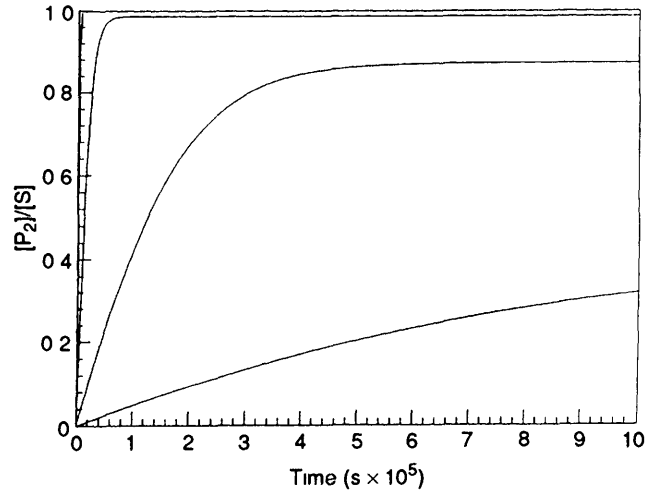
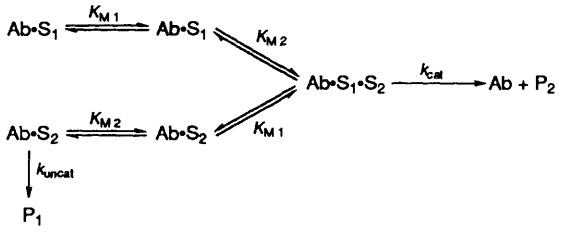


Figure 4 As in Figure 3 except that K_M for substrate was set at 0 1 mM

was run at a typical concentration of [S] (100 μM) and of [Ab] (1 μM) and at varying ratios of k_{cat}/k_{uncat} The result is shown in Figure 3 for a K_M value of 1 mM Figure 4 repeats this exercise at a ten-fold lower K_M value (100 μM) We then extended our simulations to the two substrate situation shown in Scheme 6, where the binding of S_1 and S_2 was presumed to be a random process [The antibody induced by hapten (10) follows a random sequential process] The time courses generated from the above k_{cat}/k_{uncat} ratios at two different K_{M1} and K_{M2} values are given in Figures 5 and 6

The simulations were indexed to antibody concentrations of 1 μM, since this represents a reasonably obtainable concentration



Scheme 6

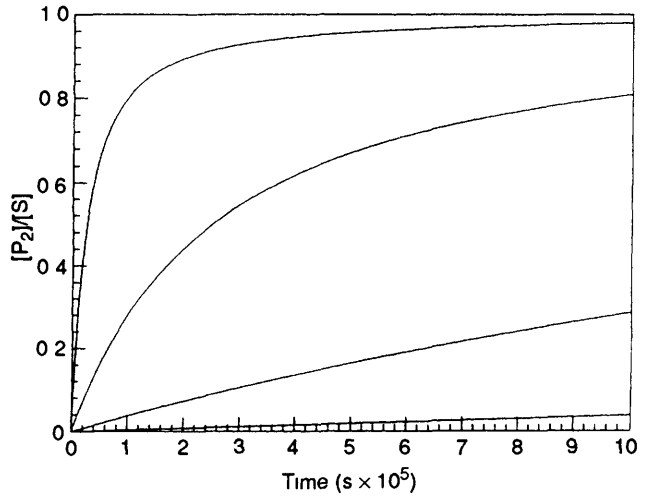


Figure 5 Kinetic simulation of the mechanism shown in Scheme 6 with K_M values for both substrates set at 1 mM The on rates for substrate binding to the antibody were assumed to be diffusion controlled (1×10^8 $M^{-1}s^{-1}$) and k_{uncat} was fixed at $1 \times 10^6s^{-1}$ The concentrations of both substrates were 100 μM and the antibody concentration was set at 1 μM The time courses shown utilized (starting from the bottom) k_{cat}/k_{uncat} ratios of 10^2, 10^3, 10^4, and 10^5

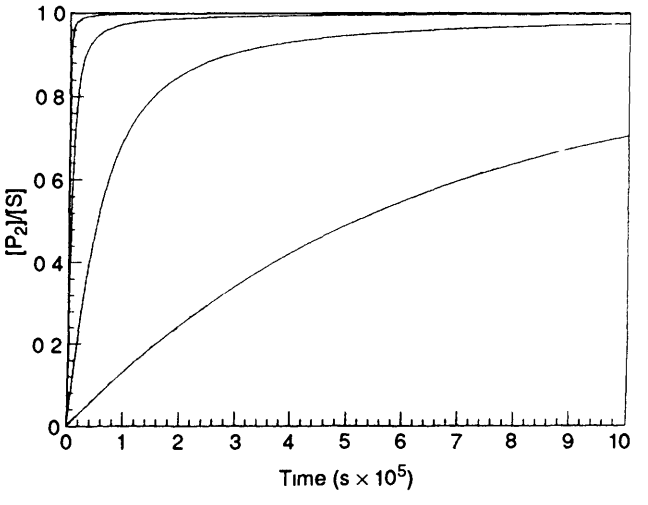


Figure 6 As in Figure 5 except that K_M values for both substrates were set at 0 1 mM

of catalyst given the technology for producing antibodies Decreasing the level of antibody would reduce proportionately the P_2/S ratio A base-line of $k_{uncat} = 10^{-6}$ s^{-1} was chosen, which approximates the values for the reactions listed in Table 2 Obviously, increasing the value of k_{uncat} (and keeping the same k_{cat}/k_{uncat} ratio) would shorten the time axis accordingly, but would not change the final P_2/S ratio Inspection of Figures 3 and 4 reveals that the extent of conversion into P_2 (the desired product) is strongly dependent on the k_{cat}/k_{uncat} ratio and is also quite sensitive to the value of K_M Thus, k_{cat}/k_{uncat} ratios of $> 10^4$ and $K_M < 0$ 1 mM are clearly desirable for antibodies to have practical utility as catalysts The same ratios are necessary in the bimolecular case (Figures 5 and 6) as well In addition, bimolecular reactions should be more subject to product inhibition because the transition-state mimic generally contains fragments of both S_1 and S_2 in P_2

The antibody-catalysed reactions in Table 2 exhibit K_M values between 0 1—1 mM and k_{cat}/k_{uncat} ratios of 70—1000 and therefore should follow product (P_2/S) time courses in the intermediate ranges of our simulations While the antibody catalysing the bimolecular aminoacylation reaction (20) + (21) → (22) exhibits particularly desirable kinetic parameters, its effectiveness is compromised by strong product

inhibition by (22) Expansion of Scheme 6 to include the observed product inhibition by (22) (K_i = 0 2 μM) followed by simulation illustrates its effect on the yield of the product (Figure 7) Likewise, a simulation of the antibody-catalysed epoxide opening (16) → (17) using the steady-state kinetic parameters reported by Janda and co-workers shows that 1 μM antibody is sufficient to direct approximately 95% of the substrate to the desired 6-*endo* product [17] We used a value of 2 2 × 10^{-6} s^{-1} for the conversion of (16) into the five-membered ring product for these simulations [31] Unfortunately, the impact of product inhibition on this simulation could not be evaluated since the affinity of the abzyme for the reaction-products was not reported

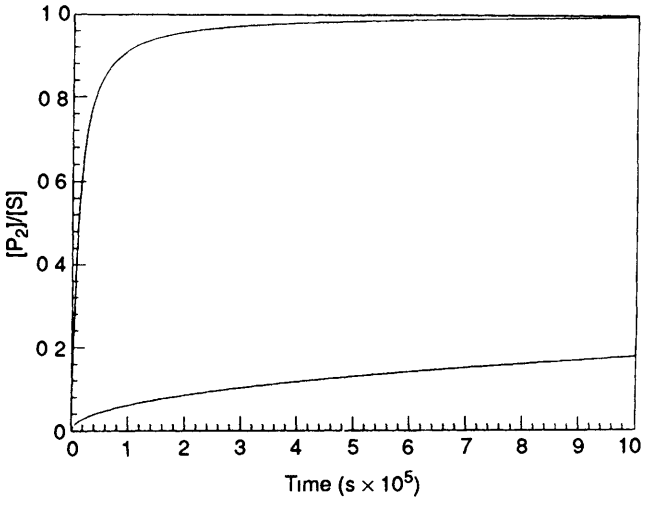


Figure 7 Kinetic simulation of the aminoacylation reaction (19) + (20) → (21) using the reported steady-state kinetic parameters with an antibody concentration of 1 μM and both substrate concentrations set at 100 μM (a) Top curve reaction time course in the absence of product inhibition by (21) (b) Bottom curve reaction time course incorporating the observed product inhibition by (21) (K_i = 0 2 μM)

4 Conclusion

It would appear that to further increase the catalytic efficacy of antibodies, it will be necessary to improve the opportunities for general acid–base chemistry at the substrate binding site of the antibody Antibodies exhibiting favourable kinetic parameters appear to operate through complex kinetic sequences caused by such active-site–substrate chemistry Appropriate side-chain residues can be introduced *via* site-directed mutagenesis of specific amino acids, by replacing whole chains,[32] or by constructing binding sites for metal ions or other cofactors [33] Obviously, the objective would be to increase the k_{cat}/k_{uncat} ratio consistently to values > 10^4 and also to maintain a low K_M value for substrate This increased substrate binding, however, is a two-edged sword, since tighter binding of substrate most likely will be associated with tighter binding of product Inhibition by product may be overcome by changes in transition state analogue design or through the use of substrates that are not spatially congruent with the analogue at a distance from the reaction centre—a more general version of bait and switch catalysis [34] We are optimistic that by building upon unique antibody frameworks, sculptured by nature to complement closely our transition analogues, the desired increase in catalytic efficacies will be achieved

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