REVIEW ARTICLE

Catalytic antibodies

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INTRODUCTION

The secret of how enzymes work has tantalized research workers since the early part of this century – and will be certain to do so well into the next. The two most dramatic features of the activity of enzymes are molecular recognition and rate acceleration. Generally speaking, investigations on these two features have made independent and equally impressive progress.

Recognition is a phenomenon which enzymes share not only with other proteins, notably antibodies, but especially with nucleic acids. *Per contra*, biological catalysis has until recently appeared to be the exclusive prerogative of enzymes. Two discoveries have overturned that monopoly. The observation that ribonucleic acids can be responsible for their own maturation has been enshrined in the term ‘Ribozyme’ [1]. More recently, monoclonal antibodies raised against specific haptens have exhibited significant catalytic capacity for certain reactions which are characterized by transition states closely akin to the stable hapten species, both in molecular geometry and in electronic character [2–4].

Such ‘Abzymes’ have amply fulfilled the expectations of Linus Pauling and Bill Jencks. In 1948 the former wrote [5]: “I believe that enzymes are molecules that are complementary to the structure of the activated complexes of the reactions that they catalyse...” Jencks’ seminal 1969 volume on catalysis [6] set out the concept of how an antibody to a synthetic mimic of a transition state might provide proteins with the presumed binding characteristics of an enzyme.

Catalytic monoclonal antibodies ought, in principle, to be capable of showing greater substrate specificity than enzymes since typical hapten dissociation constants are as low as 10^{-11} M. Inevitably, stereospecificity will be an essential feature of such recognition. On the other hand, recognition of such accuracy seems likely to make product inhibition of the abzyme-catalysed reaction a major problem. The principle challenge, therefore, relates to the extent to which abzymes can manifest catalytic rate enhancement. Will they ever come close to achieving comparable performance to enzymes?

BASICS OF ENZYME CATALYSIS

The basic requirement for enzyme catalysis of a reaction is that the potential energy of the complex formation between enzyme and transition state should be greater than for complexation either with reactant(s) or with product(s) (Fig. 1).

Enzymes appear capable of lowering the energy barriers for the reactions they catalyse in several different

Fig. 1. An energy profile for a simplified reaction

ways. In order to answer the question of how much rate acceleration might we expect from a well-tuned abzyme, we must have some idea of the magnitude of the different components of catalysis which it might be expected to bring together.

In the last twenty years, physicochemical studies on enzymes and model systems have made it possible to estimate the magnitude of the various contributions of the general elements of catalysis. It has been suggested that bimolecular associative processes might benefit entropically [7] from assembly of the correctly orientated components of a transition state within an enzyme complex by a factor up to 10^8 . Acid–base catalysis appears unlikely to promote reactions by much more than 100-fold, though the absence of facilitated proton transfers would be likely to have a major retarding effect on reactions [8]. Covalent catalysis has produced remarkable rate acceleration in model systems (up to 10^9) but generally there will be a major entropy component in most such phenomena [9]. It is not easy to put solvation effects on a quantitative basis as long as the basic theory of short-range dipolar interactions is sufficiently imprecise to make even ‘guestimates’ of their influence rather uncertain [9]. Taken together, these effects may arguably explain enzyme rate enhancement of up to 10^{14} , while some enzymes work at the limit of diffusion control. This necessarily implies an extremely high degree of precision in the match between the complementary surfaces of the protein catalytic site and the transition state of the catalysed reaction. Whether ‘wild-type’ abzymes will achieve much above 10^8 acceleration over spontaneous processes, even for the tightest transition states, must hinge on the ability of scientists to reproduce in the laboratory the successes of the evolutionary process!

Abbreviations used: mAb, monoclonal antibody; TI, tetrahedral intermediate; KLH, keyhole limpet haemocyanin; BSA, bovine serum albumin.
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GENERAL FEATURES OF ABZYME-CATALYSED REACTIONS

How does such theorizing match with achievement? Not surprisingly, the earliest set of examples of reactions catalysed by antibodies are dissociative, hydrolytic processes. The relatively low energy barrier for hydrolysis of esters provided targets for the first phase of work. The rather more difficult hydrolysis of amides has also been accomplished, though rather active amides have been required. More recently, a β -elimination to generate a C=C bond has been described. In the second phase of work, attention turned to isomerization processes operating near equilibrium and involving C-C bond formation. Success in these areas has led to studies on 'anabolic' processes exemplified by lactone and amide syntheses which have been followed by more ambitious enterprises to elicit redox catalysis and the photolysis of a pyrimidine photodimer. Lastly, the modification of antibodies by chemical methods has been shown to lead to species with improved catalytic characteristics.

While these reactions have involved C-O, C-N, C-C, C-H and C-F bond formation processes at sp^2 and sp^3 hybridized centres, without doubt the major contributions have been achieved through the mimicry of transition states for associative reactions at C=O centres. The classical 'tetrahedral intermediates', TIs, for such reactions are neither particularly stable nor can they be described (for the most part) as genuine transition states.

Nonetheless, there is a long-established record of the successful design and synthesis of powerful 'transition state inhibitors' for enzyme-catalysed acyl transfer processes [10]. Many of the best of these are based on stable tetraco-ordinate phosphorus species [11,12] and progress in this area has amply justified their adaptation to the generation of catalytic antibodies.

We can illustrate this situation schematically by an acyl transfer reaction ($A \rightarrow B$), proceeding via a tetrahedral intermediate (1) which is well modelled by the phosphonate species (2) (Fig. 2). The question whether the real transition state for the reaction involves the formation of (1) or its breakdown appears, for the present, to be less important than the need for (1) to be relatively unstable and for (2) to be sufficiently stable for use as a hapten. In practice, (2) will be modified by the attachment of a 'linker' through which it is covalently attached to a carrier protein, usually keyhole limpet haemocyanin (KLH) or bovine serum albumin (BSA).

Fig. 2. An acyl transfer reaction $A \rightarrow B$ proceeding via a tetraco-ordinate intermediate (1), which is mimicked by the phosphonate species (2)

ABZYME TRANSACYLATION

The first report of acylation reactions involving monoclonal antibodies came from Kohen *et al.* who described the use of homologous haptens carrying ester appendages which were capable of adventitious reaction with nucleophilic site(s) in the antibody that had been raised against the parent hapten [13–15]. Since proteins such as BSA likewise are readily acylated by similar reagents, the observed acylation is not necessarily a property of the antibody. Similarly acyl transfer reactivity, though 100 times faster, was reported in 1986 for a monoclonal antibody to a mechanistically-designed hapten. Tramontano *et al.* [3,16] employed a mono-aryl ester of a benzylphosphonic acid [3; $R = -CO(CH_2)_4CON(COCH_2)_2$] to mimic the putative tetrahedral intermediate, TI^- (4), for the alkaline hydrolysis of an aryl phenylacetate (5) to the phenylacetate (6). The resulting mAbs were assayed for activity using a fluorometric substrate (7). No catalytic activity was detected. However, a related hapten with a dipicolinic acid appendage [10; $R = -CO(CH_2)_4CON(COCH_2)_2$] was conjugated to KLH and used to immunize mice from which hybridoma cultures were obtained. Three IgGs showed the ability to cleave the related substrate (7) by fluorescence assay of the released 7-hydroxycoumarin. The reaction seems to be similar to the acylation of serine proteases: it is stoichiometric, gives saturation kinetics, is inhibited by the hapten phosphonate (10; $R = -COCH_3$; $K_i = 160$ nM), and protein acylation can be reversed by hydroxylamine or by alkaline treatment.

ABZYMES CATALYSING DISSOCIATIVE REACTIONS

If man evolved in Kenya, catalytic antibodies were born in California. At the end of 1986, groups at Scripps Clinic, La Jolla, and at Berkeley simultaneously announced the discovery of catalysis of the hydrolysis of carboxylic esters by monoclonal antibodies. The Berkeley group took an existing antibody which binds *p*-nitrophenylphosphorylcholine (8), MOPC167, and used it to operate on the designed substrate *p*-nitrophenyloxy-carbonylcholine (9) [17]. The Scripps group found that selected antibodies raised against the phosphonate (10) conjugated to KLH could catalyse the hydrolysis of carboxylic ester (7) [18]. Genuine turnover was achieved in both cases and both processes showed typical enzyme kinetic behaviour (Table 1).

In neither case is a mechanism of hydrolysis known. However, the close homology of MOPC167 to McPC603-PC, whose complex with *p*-nitrophenylphosphorylcholine has been analysed by X-ray crystallography [19], has allowed Schultz [17] opportunity for speculation. Any of the conserved residues, Arg^{52H}, Tyr^{33H} and Lys^{54H}, may stabilize the transition state since they bind to the phosphate residue in the complex with (8). Additionally, because the antibody-catalysed hydrolysis of (9) is also hydroxide-dependent ($6 < pH < 8$), a two-step mechanism involving initial nucleophilic attack on the ester (9) by either Tyr^{33H} or Asp^{100H} could generate an acyl-enzyme intermediate to be hydrolysed in a second, rate-determining step by hydroxide attack. A similar duality of mechanism is offered by Tramontano *et al.* [18]. They suggest that a histidine residue in the active site of their anti-(10) antibody might act as a nucleophilic

Fig. 3. A monoaryl ester of benzylphosphinic acid (3) mimics the putative hydrolysis intermediate state (4) of the reaction (5) → (6); hydrolysis of the substrate (7) yields a fluorometric product

Fig. 4. Structure (8) is the stable tetraco-ordinate phosphoryl mimic of the putative transition intermediate of the hydrolysis of (9); likewise, (10) mimics the intermediate of the hydrolysis of (11)

Table 1. Kinetic and equilibrium data for the hydrolysis of (9) by MOPC 167 [17] and of (5) by mAb 6D4 [18]

	$V_{\text{max.}}$ ($\text{M}^{-1} \cdot \text{s}^{-1}$)	K_m (μM)	K_i (μM)	$k_{\text{cat.}}$ (s^{-1})	$k_{\text{uncat.}}$ (s^{-1})	Enhancement ratio†
MOPC 167*		208	5§	6.7×10^{-3}	8.6×10^{-6}	770
mAb 6D4†	2.2×10^{-9}	1.9	0.16	2.7×10^{-2}	2.8×10^{-5}	960

* Substrate (9) 100 μM ; [MOPC 167] 11.6 μM , (1.74 $\text{mg} \cdot \text{ml}^{-1}$); 30 °C, pH 7.0.

† Substrate (5) 5 μM ; [6D4] 15 $\mu\text{g} \cdot \text{ml}^{-1}$; 25 °C, pH 8.0.

‡ Enhancement ratio = $k_{\text{cat.}}/k_{\text{uncat.}}$.

§ Inhibition using (8).

|| Inhibition using (10, $R = -\text{COCH}_3$).

group towards substrates with good leaving groups, such as (7), but as a general acid-base catalyst for substrates with poorer leaving groups, such as (5).

It is clear that both abzymes exhibit good substrate specificity. MOPC167 shows significant increases in K_m for substrates having an extra CH_2 group in the choline moiety of (9) while the IgG 6D4 demands the trifluoroacetamide substituent on substrates (7) and (5) and does not hydrolyse the corresponding CH_3CONHAR compound!

Since the original publications, both teams have made further progress with esterolytic antibodies. Jacobs *et al.* [20] have moved into the production of antibodies against transition state analogues by mimicking the TI^- for hydrolysis of methyl *p*-nitrophenylcarbonate (12) with the phosphonate ester (13). The carboxylic acid function of (13) provides a spacer linked to lysine residues on KLH and BSA for the production of antibodies. The best IgG for hydrolysis of (12) had k_{cat} $2.3 \times 10^{-2} \pm 0.3 \times 10^{-2} \text{ s}^{-1}$ and K_m $660 \pm 120 \mu\text{M}$, with a K_i for methyl *p*-nitrophenylphosphate of $3.3 \mu\text{M}$. The reaction rate but not K_m is dependent on $[\text{OH}^-]$ and shows a clear selectivity for *p* $\gg$ *m* $>$ *o*-isomers of (12).

In a major refinement exercise, Tramontano *et al.* [21] examined a further 20 mAbs for their high affinity to carrier-coupled [3; $\text{R} = -\text{CO}(\text{CH}_2)_4\text{CON}(\text{COCH}_3)_2$] from which they successfully selected two efficient catalysts of the esterolysis, mAb 50D8 and 57G4. Both showed a 5-fold better hydrolysis of (5, $\text{R} = \text{COCH}_3$) than of the related carbonate (11). In particular, the activity of the esterolytic antibody 50D8 is in the range reported for known esterases. It has a turnover rate only 10–100 times slower than that of pig liver esterase for good substrates and some 10^3 times slower than acetylcholine esterase for similar substrates while it is 10^2 – 10^4

times faster than chymotrypsin for many substrates and almost equal to the best esterase performance of that enzyme. At the same time, it has superior substrate discrimination to most esterases, being able to accept (5) and reject its isomer which has the acyl groups reversed. Most impressively, it shows relatively weak binding of (5) as substrate, K_m 1.5 mM, and tight binding for the TI^- analogue (3, $\text{R} = \text{COCH}_3$), K_i 50 nM. With a k_{cat} of 20 s^{-1} , this abzyme exhibits a rate acceleration for the hydrolysis of (5) of several million-fold above the spontaneous rate of hydrolysis. Further attempts to improve this performance by site-directed mutagenesis will be followed with great interest.

The hydrolysis of an amide is a far tougher task than the esterolysis. A series of studies by Jencks has established [22–24] that a zwitterionic tetrahedral intermediate, $\text{TI}^\pm$, is involved and whose partitioning is pH-dependent. It follows that an analogue of such a $\text{TI}^\pm$ at neutral pH might well mimic both the transition state for its formation and that for its breakdown. Consequently, a phosphoramidate analogue could well be claimed to be a true transition state analogue for the hydrolysis of an amide. In order to gain advantage from the improved electrophilicity and the leaving group ability of amides of *p*-nitrophenylaniline (14), Janda *et al.* [25] selected its *N*-(4-acylamino phenylacetyl amino) derivative (15) as a target for amide hydrolysis. This is predicted to proceed via a TI^- whose breakdown to (14) must involve $\text{O} \rightarrow \text{N}$ proton transfer which implies a need for a general acid–base catalysis by the abzyme. This TI^- can be well mimicked by the phosphoramidate [16, $\text{R} = -\text{CO}(\text{CH}_2)_3\text{COON}(\text{COCH}_3)_2$] which was conjugated to a carrier protein, BSA, and used to raise antibodies in the usual manner. Out of 44 mAbs selected by an e.l.i.s.a. in the preliminary screen only IgG NPN43C9 was selected and processed to give a pure Fab fragment for further analysis.

This abzyme exhibited Michaelis–Menten kinetics for the hydrolysis of (15) with k_{cat} $8.3 \times 10^{-4} \text{ s}^{-1}$, K_m 0.37 mM (5% dimethylformamide in water). These data correspond to a rate enhancement of 1.5×10^5 compared with spontaneous hydrolysis at pH 9 and 37 °C. Since this is 50-fold greater than the enhancement ratio for the hydrolysis of a *p*-nitroanilide peptide by a subtilisin mutant in which the catalytic triad has been replaced by

Fig. 5. Structure (13) is the stable phosphonate mimic of the putative transition intermediate of the hydrolysis of (12)

Fig. 6. A phosphoramidate (16) mimics the transition intermediate (TI^-) for the hydrolysis of the amide (15) for the release of aniline (14)

three alanines [26], Janda and colleagues inferred that NPN43C9 must be doing more than just stabilizing the transition state for hydrolysis of (15). One possibility is that it uses general acid-base catalysis to facilitate the expulsion of the *p*-nitroaniline moiety (14).

This reaction represents the kinetically most difficult hydrolysis process yet catalysed by an antibody and opens the way to the development of abzymes for the hydrolysis of alkyl amides. The opportunities for sequence-specific hydrolysis of peptides and proteins beckon!

The remaining examples of a dissociative acyl transfer process concerns the cyclization of the phenyl ester (17) to form the δ -lactone (18). The putative transition state for this process (Fig. 7) is mimicked by a cyclic phenyl phosphonate ester whose chemical synthesis provided the racemic *trans*-diastereoisomer (19). A spacer to the carrier protein (KLH) was developed through the glutamide chain and from 24 antibodies, 24B11 was chosen for detailed analysis. Napper *et al.* [27] and Benkovic *et al.* [28] found that the lactonization process was accelerated by a factor of 780 relative to the spontaneous cyclization (Table 2).

When the chiral δ -lactone product (18) was analysed by n.m.r. using a chiral lanthanide shift reagent, it proved to have a 66% enantiomeric excess. Since the uncatalysed lactonization necessarily produces racemic (18), it can be calculated that the antibody-catalysed synthesis proceeds in an enantiomeric excess of $94 \pm 8\%$, which is probably nearly absolute within the limits of this experiment.

Fig. 7. A cyclic phosphonate (19) mimics the putative reaction intermediate of the lactonization of (17) to (18)

Three general points can be drawn from this elegant investigation. First, abzymes can be expected to exert stereochemical control of a very high order. Secondly, stereochemical resolution of the hapten is unnecessary since that task is precisely effected at the time of selection of the monoclonal line from which the abzyme is obtained. Thirdly, substrate-assisted catalysis, i.e. when the reactive nucleophile or catalytic function is within the substrate undergoing transformation, is a type of reaction system that should be particularly amenable to antibody-assisted catalysis.

Using a completely different approach, Shokat *et al.* [29] have described a strategy leading to the production of an abzyme for a β -elimination process. They argued that an antibody-combining site could be generated with a carboxylate group within bonding distance of an extractable substrate proton by the use of a hapten having an appropriately located positive charge. Monoclonal antibodies to hapten (21) were analysed for their ability to catalyse the elimination of HF from (22) to give (23). Four out of six mAbs proved to catalyse the elimination process and were inhibited by hapten (21). The reaction catalysed by IgG 43D4-3D3 shows Michaelis-Menten kinetics, with k_{cat} $3.2 \times 10^{-3} \text{ s}^{-1}$ and K_m 182 μM for (22) at pH 6.0, and racemic substrate leads to the formation of both *cis*- and *trans*-isomers of the enone product (23). The pH-dependence of the reaction and its inhibition by diazoacetamide, largely protected in the presence of a competitive inhibitor (22, F = H), suggest that an aspartate or glutamate residue is an essential component of the active site. A comparison with the general base-catalysed elimination of HF from (22) by acetate gives an effective enhancement ratio of 88000. Schultz suggests that an antibody which combines both general base catalysis and transition state stabilization might attain a much higher rate acceleration.

Fig. 8. Antibodies raised against (21) catalyse the β -elimination reaction of (22) to (23) with the release of HF

Table 2. Kinetic parameters for the cyclization of (17) catalysed by mAb 24B11 at 25 °C, pH 7.0 [27, 28]

V_{max} ($\text{M}^{-1} \cdot \text{s}^{-1}$)	K_m (μM)	K_i (μM)	k_{cat} (s^{-1})	k_{uncat} (s^{-1})	Enhancement ratio
1.6×10^{-2}	76	0.25*	$3.9 \times 10^{-2}\dagger$	5×10^{-5}	780

* Inhibition studies using the *N*-acetylphosphonate (20).

† Revised value from reference [28].

Table 3. Kinetic parameters for the reaction of lactone (18) with 1,4-phenylenediamine (24) catalysed by mAb 24B11 [28]

$V_{\max}$ ($\text{mM}^{-1} \cdot \text{s}^{-1}$)	$K_m^{(24)}$ (mM)	$K_m^{(18)}$ (mM)	K_i (nM)	k_{cat} (s^{-1})	$k_{\text{uncat.}}$ (s^{-1})	Enhancement ratio
7.67×10^{-6}	1.2 ± 0.8	4.9 ± 3.8	$75 \pm 30^*$	1.1×10^{-3}	1.35×10^{-4}	16

* Using phosphonate (20) and assuming activity of only one enantiomer.

Fig. 9. The reaction of lactone (18) with the aromatic amine (24) is thought to proceed via the putative transition intermediate (26) to the amide product (25)

ABZYMES FOR ASSOCIATIVE PROCESSES

Lateral thinking has plenty of scope in the search for abzyme-catalysed reactions. The mAb 24B11 described above for a lactonization process was later found to have the ability to catalyse the synthesis of an amide from the lactone (18) and 1,4-phenylenediamine (24) [28]. Benkovic's accurate analysis of the data (Table 3) showed that the abzyme is stereoselective for amide formation using only the (*R*)-enantiomer of the lactone (18) to give the amide (25) in a rapid equilibrium bireactant kinetic sequence. [It can also be inferred that the TI^- for the reaction is the diastereoisomer (26).]

As might be expected, this abzyme-catalysed amide synthesis is subject to strong inhibition by the product (25) so that reaction kinetics can only be monitored for the first 5–10 % of reaction. Nonetheless, detailed kinetic analysis suggests that the rate of the abzyme-catalysed reaction is limited by the chemical step rather than by the off rate for the product (25). The achievement of high enantioselectivity (> 90 %) for the (*R*) isomer of (18) remains a most impressive result. Moreover Benkovic *et al.* provided an analysis based on transition state theory that predicts from the ratio of binding constants for substrate and inhibitor the anticipated increase in rate provided by the antibody over the spontaneous reaction rate. This hypothesis correctly rationalized the behaviour of mAb 24B11 in catalysing the lactonization reaction and argues that rate enhancements above this predicted value might be due to the appearance of general acid-base catalysis (S. J. Benkovic, personal communication).

The Scripps group has also described an antibody-catalysed amide synthesis, using a mAb to the aryl phosphoramidate (27) [30]. While this hapten has the appearance of a transition state analogue for the hydrolysis of a 2-methyl-8-phenylacetylaminquinoline, antibodies to it failed to show hydrolytic activity towards aryl amides. When 13 out of 55 mAbs to (27) were found to have hydrolytic activity towards an aryl ester and good substrate binding for (28) was observed, the opportunity of binding an aromatic amine was explored. IgG 17G8 proved to augment the reaction of (28) with

Fig. 10. Antibodies against the phosphoramidate (27) catalyse the reaction of the phenyl ester (28) to form the aryl amide (29)

benzylamine though the effective turnover is only 4 and only some 20 % of (29) is produced. Nonetheless, the antibody shows selectivity for benzylamine over butylamine and can discriminate between the enantiomers of 1-phenylethylamine. Snatching success from the jaws of defeat is clearly an essential quality in the abzymes business!

ABZYMES FOR ISOMERIZATION PROCESSES

The isomerization of chorismate (30) into prephenate (31) is an ideal target for catalysis by an antibody. The spontaneous rearrangement proceeds at a finite rate ($\Delta H^\ddagger$ 86.65 $\text{kJ} \cdot \text{mol}^{-1}$, $\Delta S^\ddagger$ $-53.76 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$), the enzymic acceleration by chorismate mutase (EC 5.4.99.5) is 2×10^8 [31], the transition state (32) has been well-defined as chair-like [32], and powerful transition state inhibitors have been prepared, most notably the *endo*-bicyclic species (33), $K_i < 1.5 \times 10^{-7} \text{ M}$, made by Bartlett [33]. On the basis of this entropy of activation, one could expect an enhancement ratio of 10^3 for an antibody catalyst that functions exclusively as an 'entropy trap'.

Two research groups chose this inhibitor as a hapten, linked via its secondary hydroxyl group to carrier protein, and screened the mAbs produced for mutase-like catalytic

Table 4. Data for the isomerization of chorismate (30) into prephenate (31) catalysed by antibodies raised against (33)

Source	K_m (μM)	K_1^* (μM)	$k_{\text{cat.}}$ (s^{-1})	$k_{\text{uncat.}}$ (s^{-1})	Enhancement ratio
Jackson <i>et al.</i> [34]†	260	9.0	1.45×10^{-2}	1.45×10^{-6}	10 000
Hilvert <i>et al.</i> [35]‡	49	0.6	3.9×10^{-4}	1.56×10^{-6}	250

* Using the *endo*-diacid (33)

† 10 °C, pH 7.0.

‡ 14 °C, pH 8.0, using antibody 1F7.

Fig. 11. The Claisen 3,3-sigmatropic rearrangement of chorismate (30) to prephenate (31) proceeds via a chair-like intermediate (32); this intermediate is mimicked by the *endo*-bicyclic species (33)

activity. Jackson *et al.* [34] found one out of eight IgGs catalysed this Claisen, [3,3]-sigmatropic rearrangement with a 10^4 -fold acceleration relative to the spontaneous reaction (Table 4). This impressively puts the abzymes within a factor of 300 of chorismate mutase itself! This IgG was strongly inhibited by the transition state analogue (33), K_i 9 μM . Hilvert *et al.* [35] found one out of fifteen IgGs to catalyse the conversion of (30) into (31) and show saturation kinetics. They determined the activation parameters for the catalysed process, $\Delta H^\ddagger$ 63 $\text{kJ} \cdot \text{mol}^{-1}$ and $\Delta S^\ddagger$ $-92 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ (Table 4), and found that their IgG 1F7 effects the Claisen rearrangement by significantly lowering the enthalpy of activation, which could be attributed to binding energy being converted into strain in (30). In addition, they showed that only the (–)-enantiomer of (30) is converted into (31), thereby providing a further example of stereospecificity in the antibody catalysis.

A comparison between these two closely related studies clearly shows that neither tight binding of a transition state analogue by an mAb nor the best ratio for K_m/K_i is necessarily linked to optimum catalytic activity. Whether this is because the hapten employed, though a good inhibitor, does not adequately mimic the transition state (32) is a problem for future analysis. It is also somewhat surprising that IgG 1F7 does not have a beneficial effect on $\Delta S^\ddagger$ for the isomerization process [35].

ANTIBODIES AND PHOTOCHEMICAL REACTIONS

It is a small step from constraining species so that they react by processes with an inherently low activation energy (e.g. Claisen and Diels–Alder rearrangements) to holding them in correct orientation for a photochemical process. To date, one example of a photoaddition and one of a photolysis have employed antibodies for such purposes.

Balan *et al.* [36] raised an antibody to the *trans-syn-trans*-isomer of the dimethyl ester (34) and then found that ultraviolet irradiation of methyl *trans*-4-nitrocinnamate (35) in the presence of this antibody gave a 2.5-fold increase in the proportion of the *trans-syn-trans*-isomer of the cyclobutane photoproduct (34) compared with that obtained in the absence of antibody.

Working in the opposite sense, Cochran *et al.* [37] investigated the photolysis of the *cis-syn*-thymine photodimer (36), which is the stereoisomer responsible for intrachain linkage of adjacent thymines [38] and which can be cleaved by a bacterial photoreactivating enzyme which uses an active-site tryptophan and visible light ($> 300 \text{ nm}$) [39,40]. Five out of six antibodies were found to be active of which IgG 15F1-3B1 proved to be comparable in turnover number to the *Escherichia coli* enzyme, binding (36) with K_m 6.5 μM . The antibody-catalysed reaction is characterized by biphasic kinetics

Fig. 12. The photochemical reaction of the methyl *trans*-4-cinnamate (35) to the cyclobutane photoproduct (34) is stereospecifically enhanced by antibodies raised against the *trans-syn-trans* isomer; antibodies raised against the thymine dimer (36) enhance the photolysis of the dimer to the monomer

Fig. 13. Antibodies raised against the reduced form of (37) bind the oxidized form and enhance the reducing capacity

with a stoichiometric burst of product release. The slow, second phase shows Michaelis–Menten kinetics behaviour. It is thus suggested that the abzyme-catalysed photolysis involves an active tryptophan which uses the light quantum to generate a thymine dimer radical anion whose breakdown is partitioned by the antibody.

ANTIBODY-MEDIATED REDOX REACTION

The standard reduction potential between the oxidized and reduced flavin (37) in solution is -206 mV. Shokat *et al.* [41] argued that the significant difference between the planar oxidized form and the bent reduced form could be employed by an antibody raised to bind the oxidized form in order to increase the reduction potential of the bound reduced form. Specific binding features differentiating the reduced and oxidized forms include H-bonding, changed pK_a , dipole moment and non-planarity. In the event one mAb raised against (37 oxidized) showed a binding constant of 8 nM for (37 oxidized) and of 300 μ M for (37 reduced), and changed the reduction potential for the bound (37 reduced) to -342 mV. The enhanced reducing capacity of the complex IgG–(37 reduced) was demonstrated by using it to reduce Safranin T, a dye with a reduction potential -289 mV, which is not reduced by free (37 reduced). This experiment appears to open the way for the use of antibodies to mediate redox processes not thermodynamically accessible in free solution.

CHEMICALLY ENGINEERED ABZYMES

The majority of the above examples have used monoclonal antibodies generated against haptens to create binding sites which can act (i) to stabilize a transition state (or a related tetrahedral intermediate), (ii) as an entropic trap and (iii) to provide an active-site amino acid residue. This repertoire can be significantly expanded by chemically bonding any of a wide range of natural or synthetic catalytic functions directly into antibody combining sites. As a first example of this opportunity, Pollack and Schultz have created a semi-synthetic catalytic antibody by binding an imidazole residue into the combining site of MOPC 315, an antibody with a known high affinity for substituted 2,4-dinitrophenyl ligands.

Building on early work by Givol [42] who showed that bromoacetyl-*N*-(2,4-dinitrophenyl)-L-lysine and *N*-bromoacetyl-*N*-(2,4-dinitrophenyl)-ethylenediamine bind covalently in the combining site of MOPC315, Pollack *et al.* [43] treated this antibody with either the aldehyde (38a,b), followed by sodium cyanoborohydride, or with the bromomethylketones (39a–c). The former agents were shown to link to a lysine in the heavy chain, pre-

Fig. 14. Affinity labels, aldehydes (38a,b), and/or α -bromoketones (39a–c) were used to introduce thiol groups on the Fab fragment of MOPC 315; the abilities of the thiolated Fabs to hydrolyse substrates (40a–d) were determined

Fig. 15. The reaction scheme for the introduction of an imidazole functionality at the MOPC 315 binding site

Reagents: i (38b), NaBH_3CN ; ii, dithiothreitol; iii, 2-thiopyridyldisulphide; iv, 4-mercaptomethyl imidazole.

dominantly $\text{Lys}^{52\text{H}}$, and the later to a tyrosine in the light chain. After a free thiol group had been generated by means of reduction with dithiothreitol, the chemically-modified MOPC 315 conjugate with (38b) was found to accelerate the thiolysis of coumarin ester (40b) by 60000 (relative to 0.1 μ M-dithiothreitol), with a $k_{\text{cat.}}$ 1.45×10^{-2} s^{-1} and K_m 1.2 μ M.

In a sequel to this work [44], the free thiol group has been employed to generate a disulphide link to 4-mercaptomethylimidazole (Fig. 15). This derivatized Fab was then used as a catalyst for the hydrolysis of the coumarin esters (40a–d). Optimum activity was for ester (40b), which showed $k_{\text{cat.}}$ 8.7×10^{-4} s^{-1} and K_m 2.2 μ M. The activity of the protein was identified with a base of pK_a 7.5 and was destroyed by treatment with diethylpyrocarbonate (an imidazole-specific reagent). The chemically-modified antibody activity towards the homologous esters (40a,c and d) was markedly reduced and the hydrolysis reactions were completely inhibited by *N*-dinitrophenyl-glycine, K_i 4 μ M. There seems little doubt that the catalytic activity involves the added imidazole acting either as a general base or as a nucleophile in the hydrolysis of ester (40b) and the enhancement ratio relative to 4-methylimidazole was estimated to be 1100.

These experiments are clearly a major first step towards the creation of selective catalysts which bring together the high binding affinity characteristics of the immune system and the catalytic potential of a range of chemical functions.

FUTURE PROSPECTS FOR CATALYTIC ANTIBODIES

The above examples have demonstrated the effectiveness of two general methods for eliciting catalytic activity: antibodies to transition state mimics and chemically modified antibodies. Regarding the first of these, there are major problems in the accurate mimicry of transition states (the problem of a stable penta-coordinate mimic for a trigonal bipyramidal phosphorane is one example) and even more difficulty in defining transition states with the necessary precision. Paradoxically, research on this subject may stimulate fundamental chemical enquiry on the nature of transition states. In addition, we may also discover how limited is the repertoire of protein catalysts which are based on the relatively rigid, β -barrel structure which characterizes the Fab core.

By contrast, the combination of site-specific chemical functionalization and antibody recognition capability offers a fascinating prospect. For the present, this approach necessarily lacks the fine-tuning that will ultimately be demanded for specific catalysis of a designated process. Genetic engineering of antibodies to transition state analogues may offer greater precision in design in the short term. It also offers the possibility of splicing in flexible protein loops such as appears to be essential features of many kinases. Perhaps the future will really lie with an inspired combination of the best of all these opportunities!

NOTE ADDED IN PROOF

Two recent articles have appeared which describe the hydrolysis of peptides facilitated by an antibody. Iverson & Lerner outlined the strategy for the design of a peptide antigen incorporating a metal ligand complex to facilitate sequence-specific hydrolysis in the presence of the appropriate ligand metal complex [45]. The antibody generated could specifically cleave the Gly-Phe bond provided a metal cofactor was present. The investigation into the role of auto-antibodies against the neuropeptide vasoactive intestinal peptide (VIP) in man revealed the presence of an auto-antibody which facilitated the hydrolysis of a peptide bond (Gln¹⁶-Met¹⁷) in VIP [46]. Thus catalytic antibodies do occur in nature!

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