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Molecular Recognition of Transition States

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Phil. Trans. R. Soc. Lond. A 1993 **345**, 67-76
doi: 10.1098/rsta.1993.0118

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Molecular recognition of transition states

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Enzymes work by binding and thus selectively stabilising the transition states for their reactions. Binding at the reacting centres of substrates, where bonds are made and broken, is a dynamic process. The recognition processes involved are studied most directly by kinetic techniques, and most simply in model systems.

1. Introduction

We are interested in catalysis, especially catalytic efficiency in enzyme reactions. The fundamental question is therefore how enzymes lower the free energies of the transition states for the reactions they catalyse. At one level the problem is a simple one – Alan Fersht's book (Fersht 1985) teaches in effect that 'binding is catalysis' – so the answer lies in the mode and efficiency of binding of transition states. In other words, molecular recognition of transition states.

It is convenient for the analysis – and only moderately misleading – to divide this recognition into two parts. Most of the binding interactions for a reasonably sized substrate molecule will be no different, in kind or in strength, from the interactions involved in recognition of the ground state. We might call this passive binding; though we must bear in mind that everything we have learned about the ways large structures, from proteins to crystal lattices (Kirby 1993), accommodate small structural variations, tells us that perturbations are not tightly localized, but may involve the dispersion of interaction energies over the whole system. It is therefore to be expected that in a fully evolved enzyme provision has been made for the geometrical changes involved in bond making and breaking, in terms of fine-tuning of binding of non-reacting parts of substrates.

Some 'normal' binding interactions are involved more directly in catalysis. These occur for example when substrate groups not directly involved in the reaction are nevertheless bound specifically in the transition state, and not in the Michaelis complex. These interactions are of great interest and importance for the catalytic mechanism. They can be involved in particular in setting up the optimum geometry for the reaction, as in the binding of the terminal phosphate group of ATP in the phosphate transfer step of the tyrosyl tRNA-synthetase reaction (Fersht 1987). Important interactions of this group with hydrogen-bonding side-chains of Thr-40 and His-45, which are absent in the Enz.Tyr.ATP complex, allow the stereoelectronic preference for in-line geometry for the displacement at the phosphorylating centre to be satisfied, and thus lower the barrier to reaction. Nevertheless, we will still count these as normal recognition processes, even though it would no longer be entirely accurate to classify them as passive, because they are probably in place before bond making and breaking starts.

Phil. Trans. R. Soc. Lond. A (1993) **345**, 67–76
Printed in Great Britain
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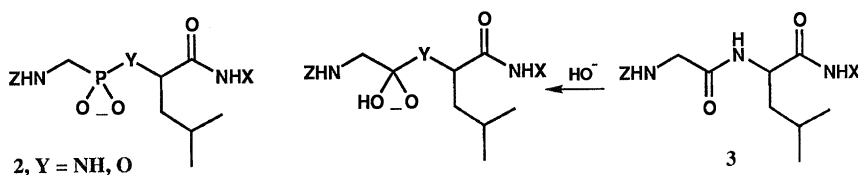
Our primary interest is in the key binding interactions at the reacting centre, where bonds are being made and broken. Here the recognition process has to deal with the partial bonds and charges of a system which has no significant lifetime. The forces involved are of course the same as in 'passive' recognition, but some at least will be stronger, bridging the gap between covalent and non-covalent bonds. Furthermore, the timescale involved imposes strict limitations on the chemistry, so that all the groups interacting with the reaction centre must be more or less precisely in place before a particular bond can be made or broken. Recognition at the reacting centre is therefore *dynamic*: interactions of the substrate with the catalytic apparatus of the active site must be strongest for the transition state structure, but they will be important – and changing – throughout the course of bond making and breaking.

Because of the timescale involved it is not possible, in principle, to get direct structural information about transition state binding. The most valuable indirect information comes from crystal structures of enzymes complexed to transition-state analogues (Lolis & Petsko 1990). (Though even this evidence can be misleading; tight binding to what appears to be a convincing transition state analogue can nevertheless result from coincidence, and not be directly relevant to catalysis (Williams *et al.* 1979).) For the most relevant of these the passive binding of parts of a substrate structure not directly involved in the reaction is supplemented by specific interactions with a new, stable structural unit which is designed to model the geometry and the charge distribution of the unstable transition state. A familiar example is the use of phosphonic acid derivatives† to model the transition states for the hydrolysis of carboxylic acid amides and esters (Bartlett & Lamden 1986: Bartlett & Marlowe 1987*a*; Hanson *et al.* 1989; Yuan & Gelb 1988). Tight binding of the analogues can be achieved, and rational structure-activity correlations observed in suitable cases. Thus Bartlett & Marlowe (1987*a*) found that the phosphonamidate analogues **2** (Y = NH) of the tetrahedral intermediates involved in the hydrolysis of ZGly-Leu-X substrates (**3**) bound uniformly more strongly to thermolysin, by 4 kcal mol^{-1}, than the corresponding phosphonate esters **2** (Y = O); and that K_i for the phosphonamidates showed a strong correlation with K_M/k_{cat} (Bartlett & Marlowe 1987*b*) as expected for true transition state analogues.



This figure of 4 kcal mol^{-1} was reasonably taken as a measure of the strength of hydrogen-bonding to the phosphoramidate NH, and there is indeed good evidence that such a hydrogen-bonding interaction is involved in the reaction of the peptide substrate (Tronrud *et al.* 1987). However, 4 kcal mol^{-1} seems too large in the light of other results, some of which have been discussed in this meeting. The difficulties of interpretation of such data are further underlined by the observation that a phosphonamidate analogue (corresponding to **2**, Y = NH) of the tetrahedral

† In most cases accessible transition state analogues are actually analogues of high energy intermediates (Schloss & Cleland 1982).

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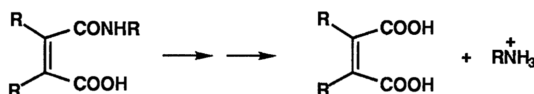
intermediate involved in the hydrolysis of a model tripeptide is bound *less* strongly than the corresponding phosphonate ester to carboxypeptidase (Hanson *et al.* 1989).

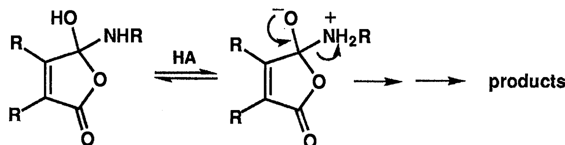
Our approach to identifying and quantifying the interactions which stabilize transition states starts not from a particular structure but from the reaction. We ask the question, what – in the context of what we know about enzyme active sites – is the most efficient way of doing the particular reaction? A clear-cut answer to this question will define the stereoelectronic requirements that dynamic binding of the transition state must satisfy, providing basic information necessary for designing analogues or receptors.

We start from a minimal system consisting of a substrate group and one catalytic group, chosen from the five available on amino-acid side-chains, and we choose to work in water. We bring these two groups together in various ways, thus modelling the formation of the corresponding enzyme–substrate complex, and in each situation measure the rate and identify the mechanism of the reaction between them. We add further catalytic groups as and where the chemistry demands them. The overall aim is to maximize catalytic efficiency. That is, to make the reaction go as fast as possible: the implicit assumption being that (at least any family of) enzymes doing the same reaction under the same conditions will have evolved to do it with maximum efficiency; so that if we can do the same we will arrive at the same solution.

The first thing we have to do, so far in every case, is to make the reaction intramolecular. Otherwise it is simply not possible to see reactions of normal substrates – amides, esters, acetals and so on – going at measurable rates under close-to physiological conditions. An early example of the power of the approach involved amide hydrolysis catalysed by the carboxyl group (Kirby & Lancaster 1972; Kirby *et al.* 1974). The amide group in this, our most reactive system, has a half-life of less than a second, compared with many years in the absence of the neighbouring COOH group. This simple system is doing the reaction about as fast as pepsin hydrolysing a good dipeptide substrate.



For this most reactive system the rate determining step is a proton transfer, as shown by the appearance of general acid catalysis (Kirby *et al.* 1974). The proton transfer concerned is the one that converts the first-formed tetrahedral intermediate to the zwitterion, which can then break down to products with the loss of the neutral amine.



This general acid catalysed reaction is diffusion-controlled (Kirby *et al.* 1974). It is thermodynamically favourable – and thus goes at the maximum rate – only for general acids RCOOH. The intrinsic chemistry of the system therefore defines specific requirements for efficient catalysis. Our interpretation is that the proton transfer goes by the concerted mechanism shown (**4**), and we can readily quantify the advantage of this mechanism over the slower reaction involving transfer of the proton through the solvent.

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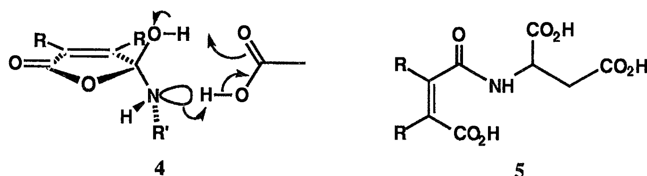

We could also quantify the advantage of having the acid group built in to the system, by making the proton transfer step intramolecular also; because in a suitable derivative (5) of a β-amino-acid, the requirement for an external general acid disappears (Kirby *et al.* 1974).

In this system, and in others like it, the specific non-covalent interactions which do most to stabilize the transition state relative to the ground state (which is itself a model for the enzyme–substrate complex) are with the protons and lone pair electrons of the catalytic general acid and general base. If we can optimize these interactions (by what has been called 'substrate engineering') we can quantify the potential stabilization energy available in terms of free energies of activation, in systems which are simple enough to understand in detail. There is inevitably a price to pay for the simplicity of the system in that specific solvation interactions cannot be identified: and for the introduction of one or more covalent bonds between the substrate and catalytic groups. But the approach allows relatively easy access to fundamental information about interactions at reacting centres, as well as guidelines to more general relations between structure and catalytic efficiency. I discuss four cases which illustrate different facets of our work. The first involves a fairly conventional, rather loosely planned application of the principles of molecular recognition to transition state stabilization.

2. Glycosidase transition states

We are all familiar with at least the outline of the lysozyme mechanism, which represents a general solution to the problem of glycoside hydrolysis and formation. Details may differ from author to author, but there is general agreement that the reaction involves Glu-35 acting as a general acid to assist the departure of the leaving group, with assistance from Asp-52. This is present as the carboxylate anion on the opposite face of the reacting sugar residue, and stabilizes the developing oxocarbocation either electrostatically, or more likely by the formation of a partial covalent bond, as sketched in **6** (Kirby 1987). The oxocarbonium ion is a very high energy intermediate, which will not exist in the active site as the free species, but requires stabilization, usually by at least partial bonding to a nucleophile (Kirby *et al.* 1978; Sinnott & Jencks 1980). In the lysozyme reaction it must be stabilized efficiently not only as it is formed, but also long enough for the leaving group to be replaced by a molecule of water, which adds on from the site originally occupied by ROH to give the product with retention of configuration.

An attractive explanation for this stabilization is that the cation is formed in a sort of electrostatic sandwich (**6**), between the permanent anion of Asp-52 and the temporary one formed by Glu-35. We have tried to test this model, in the following way.

There is good evidence that *myo*-inositol hexaphosphate (**7**), which has one axial and five equatorial phosphate groups, is converted at high pH to the inverted chair

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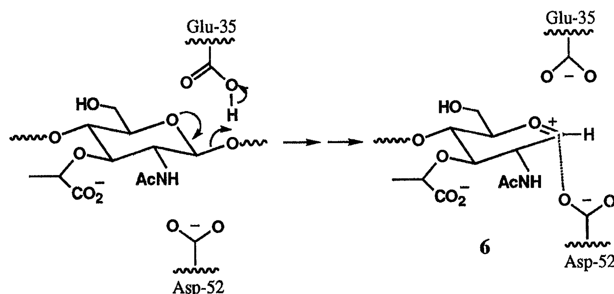
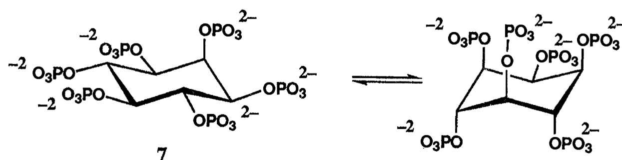
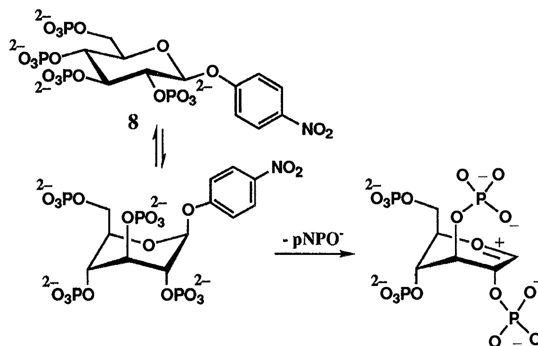


structure (Isbrandt & Oertel 1980), which would have five axial phosphate groups, all presumably in the dianion form.



We wondered whether the same inversion might occur, at least to some extent, with a glycoside tetraphosphate. If this happens a loose electrostatic sandwich structure, of the sort discussed for the lysozyme transition state would be generated. As a simple test of this idea we made the α- and β-anomers of *p*-nitrophenyl glucoside tetraphosphate (P. Camilleri, R. C. F. Jones, A. J. Kirby & R. Strömberg, unpublished work).



The system is more complicated than appears at first glance. But the β-glucoside (**8**) is hydrolysed at high pH in a pH-independent reaction which is 100 times faster than the pH-independent hydrolysis of the 2-methoxy derivative (**9**, below) of the parent *p*-nitrophenyl-β-D-glucoside, which is the simplest control reaction we could devise. (*p*-Nitrophenyl-β-D-glucoside itself undergoes a base-catalysed reaction above pH 7 which involves the ionization of the 2′-OH group of the sugar.)

3. Efficiency of proton transfer catalysis

A major aim of our research for many years has been to identify the factors controlling the efficiency of general acid–base catalysis. This is the most common of the reactions catalysed by enzymes, yet its efficiency in intramolecular reactions is conspicuously low. We measure efficiency using the *effective molarity* (EM; Kirby

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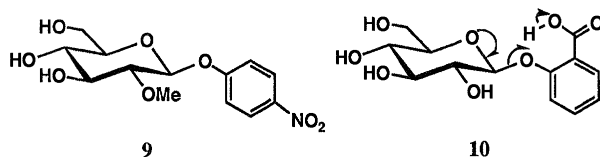
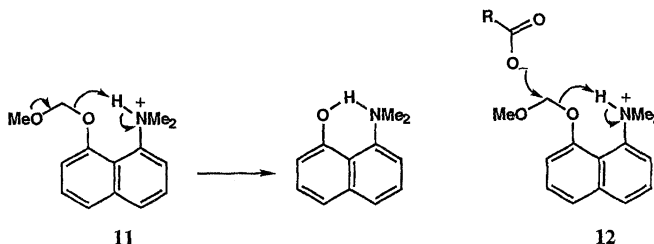
1980). This is defined as the molarity of the catalytic group needed in a separate molecule, to make the intermolecular reaction go at the rate of the intramolecular process of interest. Effective molarities as high as 10^9 M are attainable in (cyclization) reactions involving nucleophilic catalysis, but EMs for general acid–base catalysis are typically less than 10 M. The single exception when this work began was the hydrolysis of acetals derived from salicylic acid, for example Capon's model **10** (Capon 1963) for the general acid catalysis part of the lysozyme mechanism:



In some systems based on salicylic acid EMs of greater than 10^4 were estimated (Buffet & Lamaty 1976), and our first aim was to identify the factor or factors involved.

We are now confident that the key factor for efficient general acid–base catalysis is the strength of the bonding to the proton in the transition state: what might be called molecular recognition of the in-flight proton. This is worth about 4 kcal mol^{-1} in the reactions of salicylic acid derivatives, and we have ruled out alternative explanations by designing alternative systems with strong intramolecular hydrogen bonds. (An important criterion for our work is that the effects should be observed in water: it is easy to find strong intramolecular H-bonding in organic solvents, but until recently salicylate was the only case known where a strong hydrogen bond involving the OH group as the donor persists in water.) One of our systems, which we are still developing, involves the protonated dimethylamino-group, placed in the *peri*-position of naphthalene to set up the correct geometry (**11**). General acid catalysis in this system is the most efficient known, with EM $> 10^5$ M for proton transfer to oxygen (Kirby & Percy 1989). Also, uniquely in this system, we can see both parts of the putative lysozyme mechanism: efficient general acid catalysis by the NH^+ allows nucleophilic displacement of the otherwise poor leaving group by an external carboxylate anion (**12**).



The clear conclusion from this work is that the geometrical and electrostatic requirements for efficient binding of the in-flight proton are those for strong hydrogen-bonding. These are undoubtedly readily available in enzyme active sites.

We are currently trying to extend this work, to develop efficient systems involving ordinary inactivated, rather then phenolic leaving groups. For example, we have recently applied both the naphthalene and the salicylate systems to reactions involving proton transfer to carbon. The hydrolysis of the enol ether **13** shows highly efficient general acid catalysis by the COOH group, with the EM 300 M for the E isomer shown, and 2000 M for the Z isomer (Kirby & Williams 1991). For the

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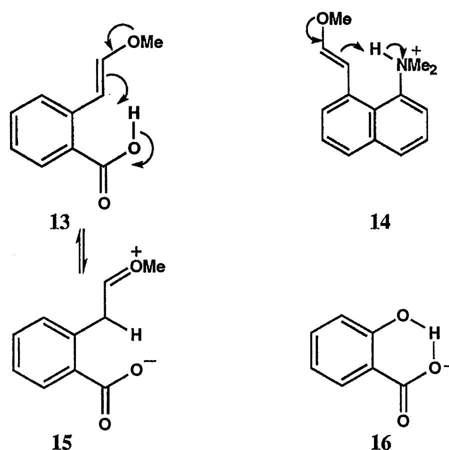
naphthylamine derivative **14** the EM is about 10^5 (A. J. Kirby & F. O'Carroll, unpublished work).

Proton transfer to and from carbon is the key step in many important reactions which involve enol or dienol formation, and the idea of a strong hydrogen-bond to a C–H protein was initially difficult to accept. However, a simple calculation (Kirby & Williams 1991), shows that a strong hydrogen-bond to a C–H proton in the transition state can be entirely reasonable. The point is that a C–H next to a *protonated* carbonyl group becomes a relatively strong acid, especially in our (phenylacetaldehyde) system, where we estimate its pK_a as -5 or less (Kirby & Williams 1991), and quite different from the C–H of the neutral compound. (A similar conclusion has been reached independently by Gerlt *et al.* (1991).) So it is in fact entirely reasonable that there should be strong intramolecular hydrogen bonding to the C–H of the cation **15**, which is designed to have the same geometry as the salicylate system (**16**).



4. Reactions of phosphate diesters

In our most recent work on transition states for proton transfer catalysis the intrinsic chemistry is once more sending us messages relevant to molecular recognition. The reaction of interest is attack by the hydroxyl group at the phosphorus centre of phosphate diesters. This is the reaction catalysed by nucleases of various sorts, but also by a vast range of enzymes involved in more subtle nucleic acid processing reactions. The nucleophile can be water, or the hydroxyl group of serine or tyrosine, and we are looking at a number of systems designed to bring hydroxyl groups into efficient intramolecular contact with phosphate diester phosphorus. The most interesting so far is the ester **17**, which cyclizes as the dianion (**18**), losing methoxide in the rate determining step with a half-life of 2 min at 50 °C. A phosphate diester anion is probably the most stable of all the functional groups in living systems, and this represents an enormous acceleration, and an EM of at least 10^{10} M (Dalby, *et al.* 1992, 1993).

An alkoxide is a very poor leaving group, and the P–O bond breaking step will need to be general acid catalysed in any enzyme reaction. We observe general acid catalysis of the loss of methoxide in the cyclization of **17**, and have characterized this reaction in some detail for the first time (Dalby *et al.* 1992). Its efficiency depends

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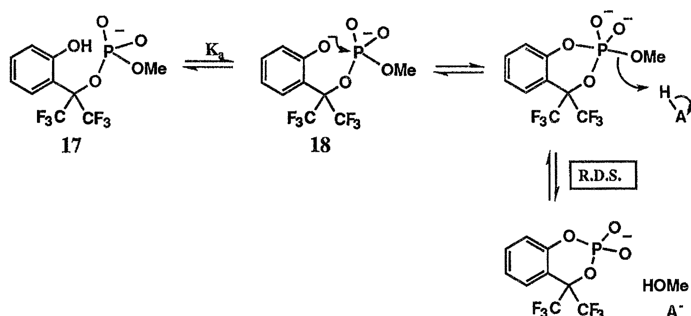
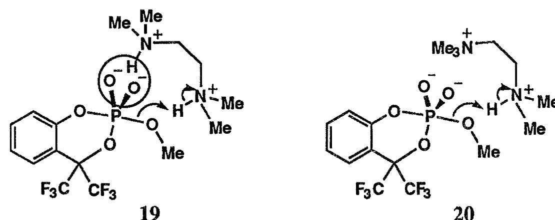


not only on the strength of the general acid but also on its charge type, so that alkylammonium ions are good but oxyanion acids relatively poor catalysts. This in water, so that any electrostatic effects are strongly damped by the solvent. Most interesting is the effect of a second positive charge on the general acid. Diammonium dications are more efficient than monocations, and the effect is strongly dependent on the structure of the general acid: it falls off and eventually disappears as the distance from the catalytic general acid of the second cationic group increases. But the effect is worth up to 2.75 kcal mol^{-1} for simple derivatives of ethylene diamine. The system is not set up to form strong hydrogen bonds, but the proton at the second centre appears to play a role (**19**), because the effect mostly disappears if it is replaced by a methyl group, as in **20** (Dalby *et al.* 1992). The system that is emerging from our exploration of the intrinsic chemistry of the system is strikingly similar to the arrangements in many nuclease active sites.



Here again we appear to be seeing potentially efficient binding of at least one, and possibly two protons in the transition state. The effect is substantial even in water. And what is especially interesting is that we have yet to apply the lessons we have learned about how to make particularly strong hydrogen bonds. We have a number of systems in mind which we hope and expect will deliver more efficient catalysis still of this important reaction.

5. Catalytic antibodies

This last topic is mentioned only briefly, since we have only very preliminary results. Nowhere is the molecular recognition of transition states more relevant than in the field of catalytic antibodies. The whole field is based on the idea that an antibody raised against a transition state will bind it, and thus be a catalyst for the reaction involved. For the usual reasons it is necessary to use transition state analogues as haptens, but the experiment works, and there are enough results available to be able to make some useful generalizations. Most interesting for this meeting is the closeness of the correlation between the structure of the transition

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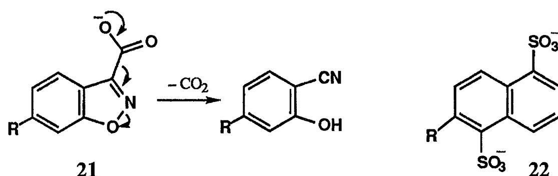
state analogue used and the efficiency of catalysis obtainable. The first generalization seems to be that the correlation is not a very strong one: if there is a conflict between design and ease of synthesis workers in the field will most often compromise on design; in the fairly confident expectation that this is unlikely to reduce the prospects for success significantly.

A reaction we are interested in is the eliminative decarboxylation of benzisoxazole-3-carboxylates (**21**). This reaction is known to be extraordinarily sensitive to medium effects, and we are using haptens of different charge types to explore the microenvironment elicited in the antibody binding site. Hilvert has characterised antibodies which catalyse the decarboxylation reaction, but these were raised not against a transition state or even a product or substrate analogue, but against the very remotely related disulphonate **22** (Lewis *et al.* 1991).



Other workers have had more success using conventional transition state analogues as haptens, and it is certain that the technique will be refined, and possible that the technique will produce some useful catalysts. But the body of the evidence does not suggest that it provides a reliable way of assessing molecular recognition of transition states.

6. Conclusion

Recognition and binding of transition states by enzymes involves a complex mixture of dynamic and passive interactions, which are not readily analysed using transition state analogues. Interactions at centres where bonds are made and broken are studied most directly by kinetic techniques, and most simply in model systems. Results are complementary to those from binding studies using stable molecules, and provide the essential framework for discussions of catalytic efficiency.

References

Bartlett, P. A. & Lamden, L. A. 1986 Inhibition of chymotrypsin by phosphonate and phosphonamidate analogues. *Bioorg. Chem.* **14**, 356–377.

Barlett, P. A. & Marlowe, C. K. 1987*a* Evaluation of intrinsic binding energy from a hydrogen bonding group in an enzyme inhibitor. *Science, Wash.* **235**, 569–571.

Barlett, P. A. & Marlowe, C. K. 1987*b* Phosphonamidates as transition-state analogue inhibitors for thermolysin. *Biochemistry* **22**, 4618–4624.

Buffet, C. & Lamaty, G. 1976 Catalyse acide intramoléculaire dans l'hydrolyase des acétals. *Rec. Trav. Chim.* **95**, 1–10.

Capon, B. 1963 Intramolecular catalysis in glucoside hydrolysis. *Tetrahedron Lett.* 911–913.

Dalby, K. N., Hollfelder, F. & Kirby, A. J. 1992 Electrostatic catalysis of the hydrolysis of a phosphate diester in water. *J. chem. Soc. chem. Commun.*, 1770–1771.

Dalby, K. N. Hollfelder, F. & Kirby, A. J. 1993 Models for nuclease catalysis: mechanisms for general acid catalysis of the rapid intramolecular displacement of methoxide from a phosphate diester. *J. chem. Soc. Perkin Trans.* II, 1269–1281.

Fersht, A. R. 1985 *Enzyme structure and mechanism*, 2nd edn. New York: W. H. Freeman.

Fersht, A. R. 1987 Dissection of the structure and activity of the tyrosyl-tRNA synthetase by site-directed mutagenesis. *Biochemistry* **26**, 8031–8037.

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Gelt, J. A., Kozarich, J. W., Kenyon, G. L. & Gassman, P. G. 1991 Electrophilic catalysis can explain the unexpected acidity of carbon acids in enzyme-catalysed reactions. *J. Am. chem. Soc.* **113**, 9667–9669.

Hanson, J. E., Kaplan, A. P. & Bartlett, P. A. 1989 Phosphonate analogues of carboxypeptidase A substrates are potent transition state inhibitors. *Biochemistry* **28**, 6294–6305.

Isbrandt, L. R. & Oertel, R. P. 1980 Conformational states of *myo*-inositol hexakis (phosphate) in aqueous solution. *J. Am. chem. Soc.* **102**, 3144–3148.

Kirby, A. J. 1980 Effective molarities for intramolecular reactions. *Adv. phys. org. Chem.* **17**, 183–278.

Kirby, A. J. 1987 Mechanism and stereoelectronic effects on the lysozyme reaction. *Crit. Rev. Biochem.* **22**, 283–315.

Kirby, A. J. 1993 Crystallographic approaches to transition state structures. *Adv. phys. org. Chem.* (In the press.)

Kirby, A. J., Craze, G.-A. & Osborne, R. 1978 Bimolecular nucleophilic substitution on an acetal. *J. chem. Soc. Perkin Trans.* II, 357–368.

Kirby, A. J. & Lancaster, P. W. 1972 Structure and efficiency in intramolecular and enzymic catalysis. Catalysis of amide hydrolysis by the carboxyl group of substituted maleamic acids. *J. chem. Soc. Perkin Trans.* II, 1206–1214.

Kirby, A. J., McDonald, R. S. & Smith, C. R. 1974 Intramolecular catalysis of amide hydrolysis by two carboxyl groups. *J. chem. Soc. Perkin Trans.* II, 1495–1504

Kirby, A. J. & Percy, J. M. 1989 Intramolecular proton transfer catalysis of acetal hydrolysis. The hydrolysis of 8-dimethylamino-1-methoxymethoxynaphthalene. *J. chem. Soc. Perkin Trans.* II, 907–912.

Kirby, A. J. & Williams, N. H. 1991 Efficient intramolecular general acid catalysis of vinyl ether hydrolysis by the neighbouring carboxylic acid group. *J. Chem. Soc. chem. Commun.*, 1643–1644.

Lewis, C. T., Krämer, T., Robinson, S. & Hilvert D. 1991 Medium effects in antibody catalysed reactions. *Science, Wash.* **253**, 1019–1022.

Lolis, E. & Petsko, G. A. 1990 Transition state analogues in protein crystallography: probes of the structural source of enzyme catalysis. *A. Rev. Biochem.* **59**, 597–630.

Schloss, J. V. & Cleland, W. W. 1982 *Biochemistry* **21**, 4420.

Sinnott, M. L. & Jencks, W. P., 1980 Solvolysis of D-glucopyranosyl derivatives in mixtures of ethanol and 2,2,2,-trifluorethanol. *J. Am. chem. Soc.* **102**, 2026–2032.

Tronrud, D. E., Holden, H. M. & Matthews, B. W. 1987 Structures of two thermolysin-inhibitor complexes that differ by a single hydrogen bond. *Science, Wash.* **235**, 571–574.

Williams, J. W., Morrison, J. F. & Duggleby, R. 1979 Methotrexate, a high affinity pseudo-substrate of dihydrofolate reductase. *Biochemistry* **18**, 2567–2573.

Yuan, W. & Gelb, M. H. 1988 Phosphonate containing phospholipid analogues as tight-binding inhibitors of phospholipase A_2. *J. Am. chem. Soc.* **110**, 2665–2667.

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