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Enzyme mimics

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Abstract. Chemists are trying to create synthetic molecules which mimic the recognition and catalytic properties of real enzymes. One target of interest is catalysis of reactions for which there are no known natural enzymes. Inspired by the examples of nature, approaches to the design of enzyme mimics for catalysis of Diels–Alder reaction are described. The design is based on porphyrin molecular boxes and zinc co-ordination. The potential of design of enzyme mimics employing cholic acid and other systems is also discussed.

Keywords. Enzyme mimics; Diels–Alder reaction; molecular recognition.

1. Introduction

Many chemists are trying to create synthetic molecules which mimic some of the recognition and catalytic properties of real enzymes. Enzymes catalyse chemical reactions with an efficiency that is awe-inspiring: they can bring together two unreactive substrate molecules, induce them to react, and then release the product at an astonishing speed. They are also subtle, usually forming just one of the many products that would result from a simple reaction carried out by a chemist. We know in general terms that enzymes achieve their catalysis by binding two substrate molecules in close proximity, by using binding energy to strain the substrate into a reactive conformation, and by using their own functional groups to intervene in the chemistry, but we do not know all the rules. In particular we do not understand the balance between structural rigidity leading to pre-organised binding sites, and flexibility which allows the site to recognise and respond to the shape and size of the bound substrate.

So why should a chemist want to build synthetic mimics which are bound to be inferior to the real thing? My group in Cambridge is not trying to mimic any particular enzyme but we are inspired by the example of nature to discover the principles which would allow chemists to design a catalyst for any reaction they wish to carry out. Our approach is to use large building blocks which can be assembled into macrocyclic host structures that enclose a cavity capable of binding two or more guest molecules. We use steroids and porphyrins as building blocks to create a variety of new molecular architectures, and then study the resulting binding and catalytic properties. Our synthetic systems possess convergent binding sites that are positioned in such a way that substrate molecules can be held in close proximity. Such hosts should catalyse reactions simply by virtue of their binding properties.

Our starting point is the butadiyne-linked porphyrin trimer schematically

illustrated in figure 1. When two or three pyridine ligands are bound within the cavity, their effective concentration is dramatically increased while at the same time their range of relative orientations is limited by the geometry of Zn-N coordination. The trimer should, therefore, act as an 'entropic trap' and accelerate any reaction whose transition-state geometry matches the orientation of bound ligands.

Recent progress in Cambridge has been exciting: Harry Anderson designed a porphyrin trimer with a large cavity, and developed an efficient templated synthesis based on its ligand-binding properties (Anderson *et al* 1993); Christopher Walter has shown that it acts as an efficient 'Diels–Alderase', accelerating the synthetically-important Diels–Alder reaction 10^4-fold and with high *exo*-selectivity (Walter *et al* 1993; Bonar-Law *et al* 1994; Walter and Sanders, unpublished results); Lindsey Mackay has shown that the same trimer catalyses an acyl transfer reaction (Mackay *et al* 1994) and experiments with a more flexible host show that these two reactions depend differently on the rigidity of the host for reasons we do not yet understand (Bonar-Law *et al* 1993; Mackay *et al* 1994). Meanwhile, Richard Bonar-Law has designed a 'molecular bowl' which selectively recognises the natural enantiomer of morphine (Bonar-Law *et al* 1993, 1994). The remainder of this article briefly reviews these latest catalysis and recognition results.

2. Accelerating the Diels–Alder Reaction (Walter *et al* 1993; Bonar-Law *et al* 1994; Walter and Sanders, unpublished results)

The arrangement of pyridines within the host as shown in figure 1 suggests that it should be easiest to accelerate a reaction which has a large entropy requirement by virtue of its geometrical demands. The Diels–Alder reaction attracted us because it has interesting stereo- and regiochemistry, a stringent geometrical requirement, no need for external reagents, and has been the subject of related studies using catalytic antibodies and cyclodextrins. It also offers the possibility that the stereo- and regiochemistry might be altered through geometry control within a cavity. We chose to use furan as a dienophile as its Diels–Alder reaction is reversible: this feature offers the opportunity of studying the kinetics (and therefore the approach to the transition state) in both the forward and reverse direction. The precise reaction studied is shown in figure 2. Model building suggested that the *endo*-adduct would fit less well into the trimer cavity than the *exo*-adduct.

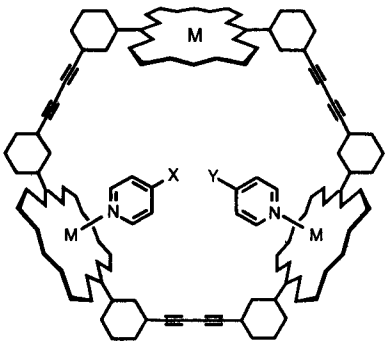

Figure 1. Schematic view of a porphyrin trimer with two ligands bound within the cavity.

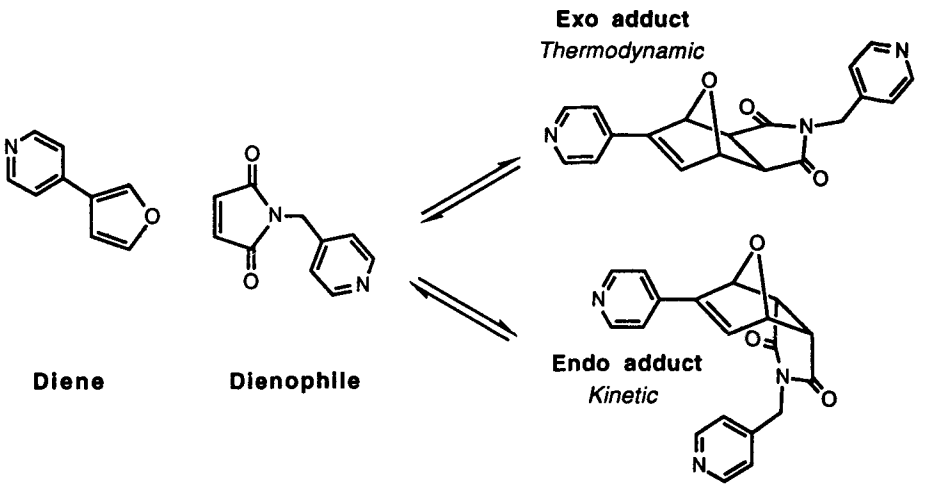

Figure 2. Reversible Diels–Alder reaction between a diene and dienophile which are designed to react within the trimer cavity.

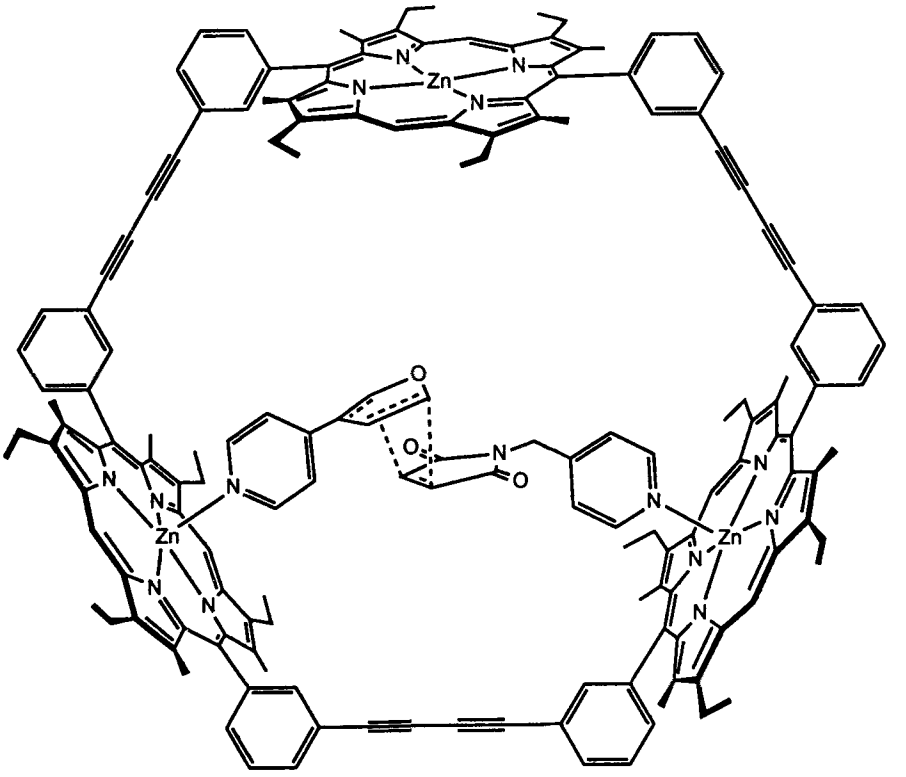

Figure 3. Proposed *exo*-transition state for the trimer-accelerated Diels–Alder reaction.

In the absence of added porphyrin trimer, the kinetic *endo*-adduct dominates at low temperature while the thermodynamic *exo*-adduct is the only product at high temperatures. Addition of one equivalent of trimer to a dilute solution of the two reactants (0·9 mM each in tetrachloroethane) accelerates the forward Diels–Alder

reaction around 1000-fold and yields the *exo*-adduct as the only detectable product. Stoichiometric amounts of trimer are required because the products bind strongly and so inhibit the trimer from further reaction. The initial reaction rate in the presence of trimer is almost temperature independent under the experimental conditions because as the temperature is raised the binding of diene and dienophile to the trimer decreases; this almost exactly offsets the intrinsic increase in rate of the reaction within the porphyrin cavity. Many control experiments involving other oligomers or competitive inhibitors indicate that the reaction is indeed occurring within the cavity and that the *exo*-transition state is well recognised as shown in figure 3. Detailed kinetic and binding studies also indicate that the *endo*-transition state is even less well bound than the *endo*-adduct, improving the *exo*-selectivity, and that the actual acceleration between bound millimolar ligands exceeds 10^4-fold.

Current studies are focussing on the importance of guest and host molecular design in this reaction: thus the isomeric dienophile with pyridine nitrogen meta to the maleimide is, as predicted, a much worse substrate for the trimer, while a more flexible trimer with methylene linkers instead of butadiyne is completely ineffective in the reaction. In the future we would like to change the traditional regiochemistry of the reaction by suitable ligand design.

3. **Catalysis of an acyl transfer reaction** (Mackay *et al* 1994)

The effect of our trimer on the Diels–Alder reaction is not catalytic because the product is strongly bound. However, a transfer reaction of the type A + BC → AB + C (figure 4) should be ideal for demonstrating catalysis and turnover: it should be accelerated by substrate proximity and should show efficient turnover because the products are no more strongly bound than the reactants. Furthermore, the intermediate or transition state is stabilised because it is doubly-bound to the host. The acyl transfer reaction shown in figure 5 is indeed catalysed by the same porphyrin trimer, and control experiments indicate that the trimer cavity is a crucial component. In accordance with expectation, the trimer is effective catalytically rather than stoichiometrically. We would like to believe that the tetrahedral intermediate shown in figure 5 is strongly bound within the trimer cavity, but do not yet have any direct evidence for it. Interestingly, the flexible methylene-linked trimer is still a catalyst for

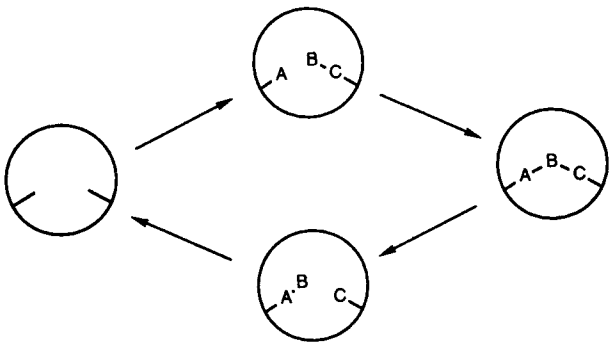

Figure 4. Schematic view of a proximity-catalysed transfer reaction.

this reaction, even though it is ineffective in the Diels–Alder reaction; we do not yet understand this difference in behaviour but are looking in more detail into the mechanism and scope of the catalysis.

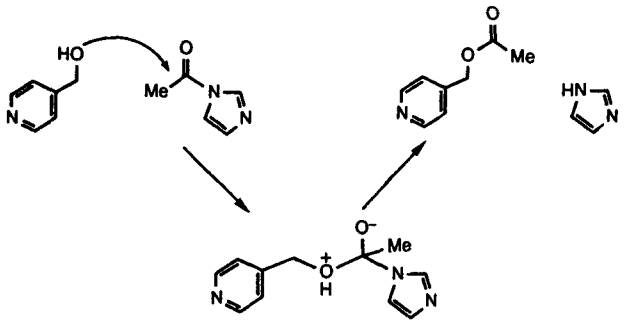

Figure 5. Acyl transfer reaction catalysed by porphyrin trimer. The precise nature of the tetrahedral intermediate, and the timing of the proton transfer to imidazole, are not known.

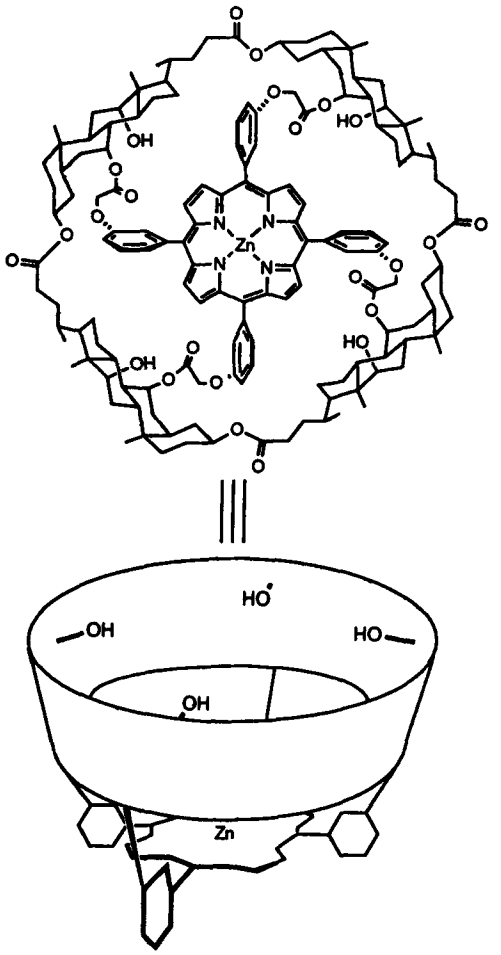

Figure 6. A molecular bowl.

4. Recognition by receptors based on cholic acid (Bonar-Law *et al* 1990, 1993, 1994; Bonar-Law and Sanders 1991)

Natural enzymes are of course single enantiomers, and their binding and chemistry are enantioselective, so this is a feature that enzyme mimics must also ultimately possess. There are many possibilities for chiral building blocks including amino acids and carbohydrates but we and others (Bonar-Law *et al* 1990) have made some progress in this area using cholic acid. This has the attraction of being cheap and naturally-occurring, with a rigid concave surface and several well-spaced functional groups. These can be connected together to create cyclic oligomers and also provide useful binding sites within the cavity.

One recent system of interest is the 'molecular bowl' shown in figure 6: this contains a porphyrin floor and four cholate walls. Each cholate possesses a hydroxyl group which faces into the cavity and is a potential binding or catalytic site. This molecular bowl selectively recognises morphine alkaloids by a combination of metal–nitrogen binding and hydrogen bonding, and even shows a 43-fold preference for the natural enantiomer of morphine.

5. Conclusion

Our approach is only one of many that are currently being explored; other groups are using catalytic antibodies (for reviews see the special issue of *Accounts of Chemical Research* 1993), designed peptides (Johnsson *et al* 1993) or other fully synthetic molecules (Mock *et al* 1989; Kelly *et al* 1990; Nowick *et al* 1991; Schneider *et al* 1993). Each approach will have its own advocates, merits and drawbacks, but the field is at an early stage of development and it is too soon to predict how it will turn out. We have a long way to go before we have synthetic enzymes worthy of the name, but we do at least know that it can be done.

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