Catalytic Acyl Transfer by a Cyclic Porphyrin Trimer: Efficient Turnover without Product Inhibition

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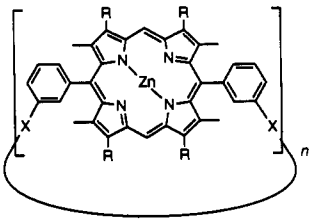
A key feature of enzymes is their ability to catalyze reactions by binding transition states and intermediates more strongly than substrates or products.[1] Biomimetic approaches to catalysis, including catalytic antibodies[2] and completely synthetic molecules,[3] often fail to reproduce this ability fully due to inhibition by strongly-bound products.[4] Our approach to biomimetic catalysis has been to create synthetic systems in which convergent binding sites are positioned in such a way that substrate molecules can be held in close proximity.[5] In principle such hosts would catalyze reactions simply by virtue of their binding properties: the unfavorable entropy of activation would be reduced or compensated for by the favorable enthalpy of binding. A transfer reaction of the type A + BC → AB + C (Figure 1) is 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 that the reactants. Furthermore, the intermediate or transition state is stabilized because it is doubly-bound to the host. We report here that the acyl-transfer reaction shown in Scheme 1 is indeed catalyzed by porphyrin trimers **1** and **2**, and we present evidence that the reaction proceeds through a tightly-bound intermediate as shown in Figure 2.



1 $n = 3$ X—X = C≡C—C≡C
2 $n = 3$ X—X = $CH_2CH_2CH_2CH_2$
5 $n = 1$ X—X = C≡CH HC≡C
6 $n = 2$ X—X = C≡C—C≡C
R = CH_2CH_2COY
Y = OMe or Y = O

Substrates **3** and **4** were chosen because model building suggested that, when they are bound inside the same trimer cavity, the nucleophilic hydroxy group of **3** can reach the carbonyl group of **4**. In toluene at 70 °C the apparent second-order rate constant for the uncatalyzed reaction is 3.4×10^{-5} L mol^{-1} s^{-1}. Standard reaction conditions were 9 mM concentrations of each substrate and 0.45 mM (5 mol %) porphyrin trimer or 1.35 mM (15 mol %) porphyrin monomer, **5**. Under these conditions, the butadiyne-linked trimer[6] **1** increased the initial rate of the transacylation some 16-fold and the more flexible tetramethylene-linked trimer **2** increased it 5-fold, while the same concentration of porphyrin units in monomeric form gave only a 50% increase (Figure 3).

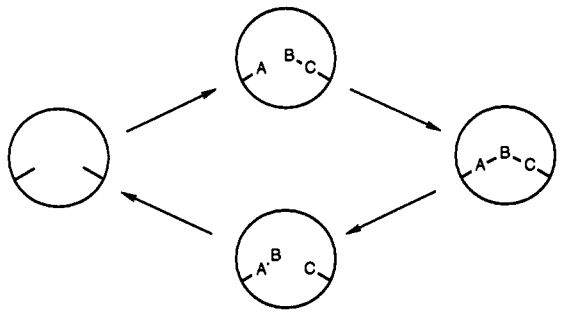


Figure 1. Schematic view of a proximity-catalyzed transfer reaction.

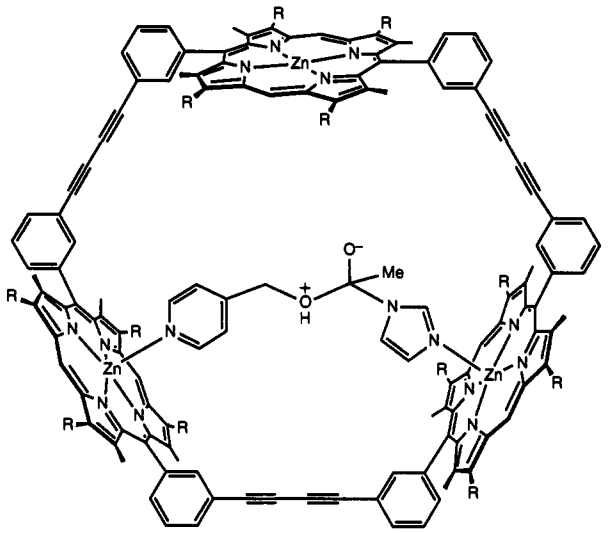
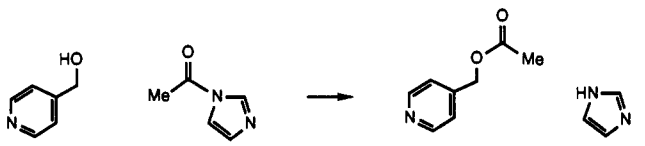


Figure 2. Tetrahedral intermediate doubly-bound inside cavity of trimer **1**. The state and position of protonation are unknown.

Scheme 1



3 **4**

It thus appears that the porphyrin monomer provides a small degree of catalysis, presumably via coordination to the acyl-imidazole, but also that the simultaneous binding of both substrates within a single cavity leads to additional acceleration. The reaction must involve nucleophilic attack to give a tetrahedral intermediate, proton transfer from the attacking oxygen, and expulsion of imidazole, although the timing of the proton-transfer reaction within the overall sequence is not known. The tetrahedral intermediate, whatever its exact structure, should be doubly-bound as shown in Figure 2; the singly-bound products are labile and have binding affinities comparable to those of the substrates. In the presence of excess substrates the products should dissociate rapidly and not lead to substantial product inhibition. Several control experiments support this picture:

(i) Increasing the concentration of trimer **1** to 9 mM leads to a relatively small increase in the observed initial reaction rate, presumably because the proportion of productive trimer molecules containing both substrates within a single cavity decreases when too many binding sites become available.

(1) (a) Pauling, L. *Am. Sci.* **1948**, *36*, 51. (b) Fersht, A. R. *Enzyme Structure and Mechanism*, 2nd ed.; Freeman: New York, 1985.

(2) For reviews, see the special issue of *Acc. Chem. Res.*, August 1993.

(3) For synthetic systems which accelerate the reaction of two bound substrate molecules, see: (a) Mock, W. L.; Irra, T. A.; Wepsiec, J. P.; Adhya, M. *J. Org. Chem.* **1989**, *54*, 5032. (b) Kelly, T. R.; Bridger, G. J.; Zhao, C. *J. Am. Chem. Soc.* **1990**, *112*, 8024. (c) Nowick, J. S.; Feng, Q.; Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 8831. (d) Schneider, H.-J.; Kramer, R.; Rammo, J. *J. Am. Chem. Soc.* **1993**, *115*, 8980.

(4) See, however: Johnsson, K.; Alleman, R. K.; Widmer, H.; Benner, S. A. *Nature* **1993**, *365*, 530.

(5) (a) Walter, C. J.; Anderson, H. L.; Sanders, J. K. M. *J. Chem. Soc., Chem. Commun.* **1993**, 458. (b) Bonar-Law, R. P.; Mackay, L. G.; Walter, C. J.; Marvaud, V.; Sanders, J. K. M. *Pure Appl. Chem.*, in press. (c) Anderson, H. L.; Bashall, A.; Henrick, K.; McPartlin, M.; Sanders, J. K. M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 429.

(6) Anderson, H. L.; Sanders, J. K. M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1400. To improve solubility, methyl ester trimer was converted to the isodecyl ester by transesterification (Anderson, H. L. *Inorg. Chem.*, in press); there is no significant difference in the binding properties of methyl and isodecyl esters. Trimer **2** was prepared by hydrogenation of **1** (Marvaud, V., and Sanders, J. K. M., unpublished).

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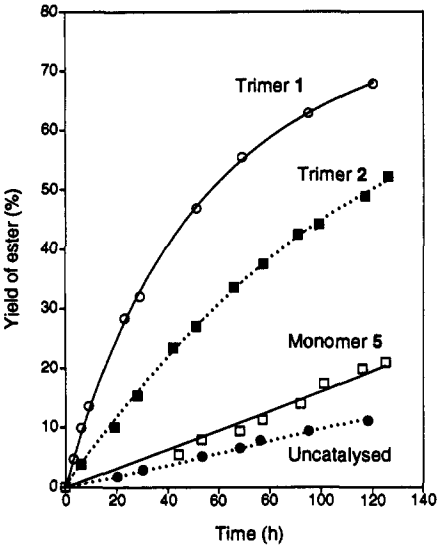
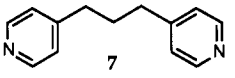


Figure 3. Progress curves for acyl transfer. Standard reaction conditions were 9 mM in each substrate in dry toluene solution, 70 °C, under argon. The reaction was monitored by GC on a fused silica capillary column at 100 °C using 5 mM biphenyl as an internal concentration standard: ●, uncatalyzed; □, 1.35 mM monomer **5**; ■, 0.45 mM trimer **2**; ○, 0.45 mM trimer **1**. Curves are drawn for visual guidance only.

(ii) Cyclic dimer **6** has a cavity which is too small to accommodate both substrates in a reactive configuration, and it is no more effective a catalyst than monomer; the corresponding linear dimer[7] is flexible but can take up the correct conformation to bring both substrates into proximity, and it is twice as effective as cyclic dimer.

(iii) Bis(pyridyl)propane **7** binds strongly to trimer **1** ($K = 2.3 \times 10^7$ M^{-1})[8] and acts as an effective competitive inhibitor; in order to bind this strongly it must be doubly-bound within the cavity and is therefore a crude transition-state analogue.



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(iv) A 4.5 mM concentration of imidazole ($K = 2.4 \times 10^3$ M^{-1}) has no significant effect on the trimer-catalyzed rate under these conditions.

(v) Addition of 9 mM fresh substrates after 100 h leads to renewed transacylation at essentially the same initial rate. This demonstrates that under these experimental conditions there is neither significant inhibition nor catalysis by products. The average turnover number per trimer after 160 h is around 25, with no detectable decomposition or loss of activity.

Calculation of pseudo-unimolecular rates for the reaction within the cavity is difficult as the binding equilibria are complex and the reactivities of the various species cannot be assessed easily. In general, the three binding sites of trimer **1** bind monodentate pyridine ligands independently; since the binding constants of monodentate pyridines to monomer **5** are normally equal to the corresponding microscopic binding constants with trimer **1**, it is reasonable to assume a statistical distribution of species with no net preference for binding either inside or outside the cavity. The microscopic binding constant for hydroxy pyridine **3** to trimer **1** is about 1.8×10^3 M^{-1} while that of acetylimidazole **4** is 350 M^{-1}: in the presence of 20-fold excess substrates, most trimers will be fully bound but only a small fraction will contain at least one of each substrate within a single cavity. Computer simulations using a simple binding model[9] give a rate constant for the reaction within trimer **1** of at least 6×10^{-5} s^{-1}, corresponding to an effective molarity of 2 M.

Cyclic oligoporphyrins such as trimer **1** have the potential to be versatile catalysts for many other processes because their major role is in recognition and orientation events which are decoupled from the reaction itself. One should be able to bring together a wide range of different reactive pairs by changing the substituents on the amine ligand, and the concept can be extended by changing the geometry of the host or the nature of the recognition event. A way is also now open to systematic dissection of the importance in supramolecular catalysis of such factors as host flexibility and quality of fit.

Acknowledgment. We thank the Association of Commonwealth Universities, the New Zealand Vice-Chancellors' Committee (L.G.M.), and NSERC Canada (R.S.W.) for financial support, Dr. H. L. Anderson for valuable advice, Dr. S. Anderson for porphyrin dimers, and Dr. V. Marvaud for the flexible trimer **2**.

(7) Anderson, S.; Anderson, H. L.; Sanders, J. K. M. *Acc. Chem. Res.*, **1993**, *26*, 469.

(8) Binding constants were measured in toluene solution between 20 and 55 °C by spectrophotometric titration. The values quoted for 70 °C were obtained by extrapolation of van't Hoff plots.

(9) Our simulation of the ligand binding assumes that the microscopic binding constants of **3** and **4** are equal, avoiding the complication of competition between the ligands for host binding sites. If $[L]_T$ represents the sum of the total concentrations of the two ligands and $[H]_T$ is the total host concentration, then the free ligand concentration [L] is obtained by solving

$$[L]^2 + \left(3[H]_T - [L]_T + \frac{1}{K}\right)[L] - \frac{[L]_T}{K} = 0$$

where K is the microscopic binding constant. The concentrations of the other species are calculated by substitution of [L] into

$$[HL_i] = \frac{3!}{i!(3-i)!}K^i[L]^i[H]_T / \sum_{i=0}^{3}\frac{3!}{i!(3-i)!}K^i[L]^i$$

Note that $[HL_0]$ is the concentration of free host, [H]. If the ligand concentrations are equal, then only one-half of HL_2 complexes will include both **3** and **4**, and only one-quarter of those will be catalytically active by virtue of having at least one molecule of each ligand within the cavity. Similar considerations for the triply-bound species lead to the following equation:

$$[HL_x]_{reactive} = [HL]_2/8 + 9[HL_3]/32$$

An approximate value for the intramolecular rate constant can then be calculated from the observed initial reaction rate. This model is crude, probably overestimating the concentration of active species and therefore underestimating the true catalytic effectiveness. Nevertheless it reproduces qualitatively the observation that increasing the trimer concentration to 9 mM leads to only a small increase in concentration of productive complex. More sophisticated computer models are currently being explored.