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Modelling the Influence of Porphyrin Supermacrocycles on the Kinetics of Bimolecular Reactions

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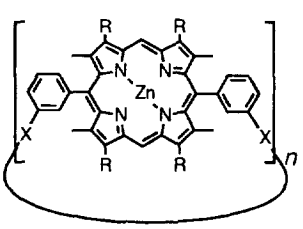
Abstract: Models of the binding of two or more substrates to a cyclic host with multiple binding sites are presented and evaluated. These equilibrium models are applied to the problem of simulating the kinetic influence of oligomeric zinc porphyrins on intermolecular reactions between functionalised amines. The approach is illustrated for the cases of initial rate and time-course kinetic studies.

INTRODUCTION

An essential feature of the catalytic activity of many enzymes is their ability to bind two substrate molecules in close proximity. The design of synthetic systems which can mimic this behaviour is an important problem in supramolecular chemistry. Artificial hosts can accelerate and even catalyse bimolecular reactions[1,2] but their efficiencies lag far behind those of enzymes; natural selection has optimised enzymatic transition state recognition[3] and minimized the extent of nonproductive binding within ternary complexes.[1b] With the present generation of enzyme mimics the effects of nonproductive binding and uncatalysed reactions cannot be ignored[1f] and consequently, a standard enzyme kinetic approach is often inappropriate. We present here a practical and straightforward approach to the modelling of binding equilibria and kinetic results from complex artificial host-guest systems, emphasising cases involving porphyrin oligomers developed within this laboratory.

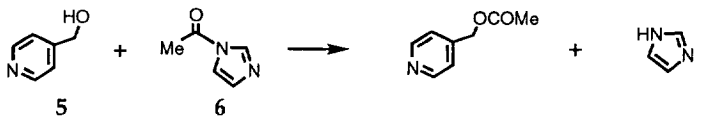
Bisacetylenic zinc porphyrins **1** are ideal building blocks for oligotopic co-receptor molecules with convergent binding sites. They are readily linked by means of a Glaser coupling reaction into cyclic oligomers **2-4** [4,5] and can coordinate a wide variety of aliphatic or heterocyclic amines, a feature which not only provides the desired substrate binding ability but can also be used to template cyclization.[5] The butadiyne linkages impose sufficient rigidity to pre-organise the binding sites without precluding the host distortion necessary to bind guests which do not fit the relaxed cavity.[4a]



1 $n = 1$ X—X = C≡CH HC≡C
2 $n = 3$ X—X = C≡C—C≡C
3 $n = 2$ X—X = C≡C—C≡C
4 $n = 3$ X—X = $CH_2CH_2CH_2CH_2$

R = CH_2CH_2COY
Y = OMe or Y = O

Cyclic oligomers based on zinc porphyrin subunits catalyse the transfer of an acyl group to 4-(hydroxymethyl)pyridine **5** from N-acetylimidazole **6** (Scheme 1)[2a] and accelerate stereoselectively the inter-



5 6

Scheme 1

molecular *exo*-Diels–Alder reaction between 4-(3-furyl)pyridine and 4-(maleimidomethyl)-pyridine (Scheme 2).[2b] The butadiyne-linked porphyrin trimers **2** are particularly effective in this respect, the corresponding

Scheme 2

monomers **1** exerting much less influence on the rate of reactions. These observations suggest that it is the oligomer-mediated positioning of the coordinated ligands in space which is the principal cause of the rate acceleration. It follows that a detailed interpretation of the kinetic results depends critically on the model adopted for the binding equilibria: at the very least such a model must account for the coordination of two reactants to a host with multiple binding sites. In the case of the cyclic oligomers currently under investigation, additional complications arise from the fact that the host cavity defines an interior and exterior face for each porphyrin subunit. For any ligand at a given binding site, one of two isomeric complexes may form: only a small fraction of the total number of host-ligand complexes that are present will be productive. The challenge of analysing these systems is to derive simple binding models that realistically describe substrate and product coordination and permit interpretation of the kinetic data. The approach set out below attempts to build models for reactions such as those in Schemes 1 and 2 which can effectively simulate the effects of ligand binding to porphyrin supermacrocycles for both initial rate and time course data. In doing so, we make explicit many of the assumptions which are commonly made implicitly, or are ignored in such analyses.

The general problem of modelling ligand-binding equilibria from experimental data has been thoroughly discussed.[6] The task of measuring the number of coordinated ligands and the corresponding equilibrium constants for hosts with multiple binding sites is especially difficult because as the number of potential binding sites increases, so do the uncertainties in the measured quantities. The situation is somewhat improved when host binding sites are distinct and when one or both of the components exhibit pronounced spectroscopic changes upon coordination. In this respect, the binding of amines by zinc porphyrin oligomers represents an almost ideal case.

Metalloporphyrins have intense absorption and emission bands in the visible region which shift dramatically upon axial ligand coordination,[7] while ligand ^{1}H NMR spectra change significantly due to the large diamagnetic ring-current of the porphyrin ring.[8] The coordination of an aliphatic or heterocyclic amine to a zinc porphyrin occurs through a predominantly electrostatic sigma-bonding interaction of the nitrogen lone pair with the zinc centre.[9] Although the strength of this bond may be significantly altered by ligand polarity, solvation, and porphyrin ring substituents, the overall geometry of the ligand-metalloporphyin complex is reasonably well-defined. Consequently, an analysis of the ligand binding of a zinc porphyrin oligomer begins with a good indication of the number of binding sites and an array of sensitive spectroscopic probes to confirm this. Equilibrium constants are conveniently measured by spectrophotometric titration: changes in the absorbance of the intense B (or Soret) band ($\varepsilon \approx 8 \times 10^5$ M^{-1}cm^{-1}) and the weaker Q bands ($\varepsilon \approx 3 \times 10^4$ M^{-1}cm^{-1}) are followed upon the addition of aliquots of an appropriate ligand solution and then fitted to the simplest binding model consistent with the known chemical structure and properties.

Ligand-binding models for porphyrin oligomers need to allow for a relatively limited set of molecular interactions. Self-association of ligands or porphyrins may occur in certain solvents, but can be tested by

examining the effects of varying the concentration of solutions containing only the component in question. Thus, porphyrin aggregation causes broadening and shifting of the B band due to exciton coupling and changes in the proton NMR spectrum due to interacting ring-currents.[7] Intramolecular association of porphyrin subunits may also occur when the linkages between them are sufficiently flexible;[7,8] the spectroscopic effects of this interaction are independent of the host concentration. In practice, the trimer **2** exhibits neither type of aggregation behaviour in the solvent systems currently used. Indeed, for ligand systems involving these particular hosts the only interactions that need be considered arise from the binding of ligand by the individual zinc porphyrin subunits. The success of modelling this behaviour will ultimately determine the reliability of the kinetic analysis.

THEORY

Modelling the Ligand Binding of Oligomeric Porphyrins

The Assumption of Independent Ligand Binding Sites. When a host has identical binding sites that behave independently, the distribution of bound species that form in the presence of a ligand will be determined solely by statistical factors.[10] This has the important consequence that an oligomeric host H at a total concentration of $[H]_t$ and with n binding sites will, upon titration with a monodentate ligand L, behave in a way that is indistinguishable from a monomeric host at a concentration of $n[H]_t$ with only one binding site. In other words, for systems characterised by independent ligand binding, the concentration of free L remains the same regardless of the number of species over which the bound ligand is partitioned, provided that the total concentration of binding sites remains the same. Thus if L is bound with an association constant of K_L by a binding *site* **H** ($[\mathbf{H}]_t = n[H]_t$) the free ligand concentration [L] in the presence of *host* H can be calculated analytically by solution of the following quadratic equation,

$$[L]^2 + \left(n[H]_t - [L]_t + \frac{1}{K_L}\right)[L] - \frac{[L]_t}{K_L} = 0 \qquad [1]$$

where $[L]_t$ is the total ligand concentration. The n overall formation constants for the species HL, HL_2,... HL_n are given by Eq. [2],[11]

$$\beta_i = \binom{n}{i} K_L^i \quad \text{where } i = 1 \ldots n \qquad [2]$$

The concentrations of these species can be calculated by substitution of [L] into Eq. [3],

$$[HL_i] = \frac{\binom{n}{i}(K_L[L])^i}{\sum_{p=0}^{n}\binom{n}{p}(K_L[L])^p}[H]_t \qquad [3]$$

Note that in this formula, $[HL_0]$ corresponds to [H], the free host concentration. Eqs. [1] to [3] represent a considerable simplification over the general case as can be seen by comparing them with Eqs. [4] and [5]. The latter must be solved to determine [L] when the ligand binds to a host H with two and three binding sites, respectively, that are interdependent or non-identical:

$$[L]^3 + \left(\frac{1}{K_2} - [L]_t + 2[H]_t\right)[L]^2 + \left(\frac{1}{K_1K_2} + \frac{[H]_t}{K_2} - \frac{[L]_t}{K_2}\right)[L] - \frac{[L]_t}{K_1K_2} = 0 \qquad [4]$$

$$[L]^4 + \left(\frac{1}{K_3} - [L]_t + 3[H]_t\right)[L]^3 + \left(\frac{1}{K_2K_3} + \frac{2[H]_t}{K_3} - \frac{[L]_t}{K_3}\right)[L]^2 + \left(\frac{1}{K_1K_2K_3} + \frac{[H]_t}{K_2K_3} - \frac{[L]_t}{K_2K_3}\right)[L] - \frac{[L]_t}{K_1K_2K_3} = 0 \qquad [5]$$

While a cubic equation such as [4] can in fact be solved analytically, application of the procedure is tedious; for a solution to Eq. [5] a numerical method must be employed.

The Independent Binding of More than one Ligand. The assumption that the binding sites of a host behave independently is especially useful if the competitive binding of two or more monodentate ligands is to be treated. If host H binds two ligands, A and B, and if the n binding sites are non-identical or interdependent then it is necessary to solve two related polynomial equations simultaneously in order to calculate the distribution of the various species. There are no good, general methods for solving systems of more than one such equation, although realistic estimates of [A], [B], and [H] may permit the successful application of the multidimensional Newton-Raphson algorithm (see below).[12] If, however, it can be assumed that the ligands bind independently with respect to the host and each other, then the problem simplifies dramatically. As in the case above involving only one ligand, the concentrations of free A and B remain the same, regardless of the number of species over which these ligands are partitioned.[13] If the binding site **H** binds ligand A with an association constant of $\boldsymbol{K_A}$ and ligand B with an association constant of $\boldsymbol{K_B}$ then the concentration of free **H** is given by the solution to Eq. [6],

$$[\mathbf{H}]^3 + \left(\frac{1}{\boldsymbol{K_A}} + \frac{1}{\boldsymbol{K_B}} + [\mathrm{A}]_t + [\mathrm{B}]_t - n\,[\mathrm{H}]_t\right)[\mathbf{H}]^2 + \left(\frac{1}{\boldsymbol{K_A K_B}} + \frac{[\mathrm{A}]_t}{\boldsymbol{K_B}} + \frac{[\mathrm{B}]_t}{\boldsymbol{K_A}} - n\frac{[\mathrm{H}]_t}{\boldsymbol{K_A}} - n\frac{[\mathrm{H}]_t}{\boldsymbol{K_B}}\right)[\mathbf{H}] - n\frac{[\mathrm{H}]_t}{\boldsymbol{K_A K_B}} = 0 \qquad [6]$$

This quantity [**H**] can be thought of as the concentration of unoccupied binding sites on host H. It allows the calculation of the actual concentrations of unbound A and B, by substitution into Eqs. [7] and [8],

$$[\mathrm{A}] = \left(\frac{1}{\boldsymbol{K_A}[\mathbf{H}] + 1}\right)[\mathrm{A}]_t \qquad [7]$$

$$[\mathrm{B}] = \left(\frac{1}{\boldsymbol{K_B}[\mathbf{H}] + 1}\right)[\mathrm{B}]_t \qquad [8]$$

There are N overall formation constants β_{ij} for the species HA_iB_j $(0 < i + j \leq n)$ where N is defined in Eq. [9] and β_{ij} in Eq. [10],

$$N = \binom{n+2}{2} - 1, \quad n \geq 2 \qquad [9]$$

$$\beta_{ij} = \binom{n}{i}\binom{n-i}{j}\boldsymbol{K_A}^i\,\boldsymbol{K_B}^j, \quad 0 < i + j \leq n \qquad [10]$$

The concentrations of the HA_iB_j can be calculated by substitution of [A] and [B] into Eq. [11],

$$[\mathrm{HA}_i\mathrm{B}_j] = \frac{\binom{n}{i}\binom{n-i}{j}(\boldsymbol{K_A}[\mathrm{A}])^i(\boldsymbol{K_B}[\mathrm{B}])^j}{\sum_{p=0}^{n}\sum_{q=0}^{n-p}\binom{n}{p}\binom{n-p}{q}(\boldsymbol{K_A}[\mathrm{A}])^p(\boldsymbol{K_B}[\mathrm{B}])^q}[\mathrm{H}]_t \qquad [11]$$

Note that in this equation, $[HA_0A_0]$ corresponds to [H], the free host concentration. Eq. [11] is a concise formulation of the concentration balance expression for $[H]_t$. The approach exemplified in Eqs. [6] to [11] can be readily extended to treat the binding of three and four different ligands to a host with n sites, as long as one is prepared to assume that the binding sites remain independent.

Ligand Binding at Non-independent Coordination Sites. The simple model of reactant binding presented above is based on two key assumptions: first that a reactant ligand is independently bound by host H in the absence of its co-reactant and second that the co-reactant modifies reactant binding only through competition for host binding sites. When one of these assumptions becomes untenable, the binding model must be made considerably more complicated and it is no longer possible to arrive at a single polynomial equation such as [6]. Instead, several polynomials containing mixed terms must be solved simultaneously for the free ligand and host concentrations; these polynomials are most conveniently formulated as the concentration balance expressions for the terms $[A]_t$, $[B]_t$ and $[H]_t$. When an approximate solution to these equations is available, the Newton-Raphson method for nonlinear systems of equations can be an effective technique for finding the exact solutions.[12]

Estimating Relative Reactivities of Cyclic Oligomer Ligand Complexes

The cyclic nature of the porphyrin oligomers **2** and **3** introduces a topological aspect to their ligand binding behaviour that is not present with monomer **1**. The central host cavity defines an interior and an exterior face for each zinc porphyrin subunit, greatly increasing the number of isomeric complexes which may form when two reactants are present. The convergent nature of the interior binding sites and the divergent nature of the exterior sites makes it probable that significant differences in reactivity exist between various complexes. Unfortunately, the extent to which coordinated reactants are partitioned between the interior and exterior environments is difficult to quantify because of the number of species which can form and the rapidity with which they can interconvert. In order even partially to characterise the kinetic influence of **2** on a bimolecular reaction, it is necessary to infer reactant distributions from fairly limited physical evidence.

Spatial Distributions of Coordinated Ligands. Consider the case for the independent binding of a monodentate ligand L to trimer **2**. There are three stepwise binding constants K_1, K_2 and K_3, for the formation of the species HL, HL_2 and HL_3, respectively. Since the binding sites are independent, K_1, K_2 and K_3 are in the ratio $3:1:{}^1/_3$ and can be characterised by the apparent 1:1 binding constant $\boldsymbol{K_L}$ numerically equivalent to K_2. The three species HL, HL_2 and HL_3 represent groups of isomeric complexes; for example HL_2 consists of HL_iL_i, HL_iL_o and HL_oL_o, where the subscripts i and o refer to inner and outer positions, respectively, of the coordinated ligand. The macroscopic binding constants K_1, K_2 and K_3 are the sums of the binding constants for each isomeric complex, thus $K_1 = K_{HLi} + K_{HLo}$ and so on.[14] If the ligands bind with no preference for either environment, the apparent 1:1 binding constant $\boldsymbol{K_L}$ for trimer **2** and ligand L should be equal to the actual 1:1 binding constant K_L for porphyrin monomer **1** and L.[15] If L is too large to fit inside the cavity of **2** but can still bind independently to the outer sites then $\boldsymbol{K_L}$ should be about half the magnitude of K_L. If L can bind at inner and outer sites but has a net preference for binding inside **2** (still independently) then $\boldsymbol{K_L}$ will be larger than K_L. Binding site preferences cannot be deduced solely from the magnitudes of these two constants because there is no indication of whether the inside or outside sites are favoured. In conjunction with the reasonable assumption that outer binding sites are similar to those of the monomer, however, the comparison of ligand binding constants for zinc porphyrin monomer and cyclic oligomers provides an indication of ligand preferences for exterior or interior coordination sites on the cyclic host.

The ligand binding preferences can be quantified by defining the coefficients f_i and f_o which represent the fractional extents of interior and exterior ligand coordination at a given binding site. They can be estimated from ligand binding constants for monomer **1** (K_L) and trimer **2** ($\boldsymbol{K_L}$) with Eqs. [12] and [13],

$$f_i = \frac{\boldsymbol{K_L} - {}^1/_2 K_L}{\boldsymbol{K_L}} \qquad [12]$$

$$f_o = 1 - f_i \qquad [13]$$

The overall concentrations of coordinated ligand in the two environments can be calculated from the statistically determined ligand distributions and are given in Eqs. [14] and [15],

$$[L]_i = f_i[HL] + 2(f_i f_o + f_i^2)[HL_2] + 3(f_i f_o^2 + 2f_i^2 f_o + f_i^3)[HL_3] \quad [14]$$

$$[L]_o = f_o[HL] + 2(f_i f_o + f_o^2)[HL_2] + 3(f_i^2 f_o + 2f_i f_o^2 + f_o^3)[HL_3]$$

or

$$[L]_o = [L]_t - ([L] + [L]_i) \quad [15]$$

This approach can be extended to the case in which two reactant ligands A and B bind independently with respect to the host **2** and each other. Fractional extents of interior and exterior binding must be calculated for both ligands by means of Eqs. [12] and [13].

Complexes of **2** in which both reactants A and B are bound inside the central host cavity are of particular interest because it is the intramolecular reactivity of these species which is presumed to accelerate the overall rate of the intermolecular reaction. These complexes can be divided into two classes, one in which there is a single ligand of both reactants bound inside the cavity (denoted X1) and one in which there is a single ligand of one reactant and two ligands of the other (denoted X2). The concentrations of X1 and X2 are determined by the statistical distributions of ligands A and B and are given in Eqs. [16] and [17],

$$[X1] = f^A_i f^B_i [HAB] + 2f^A_i f^B_i f^B_o[HAB_2] + 2f^B_i f^A_i f^A_o[HA_2B] \quad [16]$$

$$[X2] = f^A_i (f^B_i)^2[HAB_2] + f^B_i (f^A_i)^2[HA_2B] \quad [17]$$

The above equations ([12] - [17]) permit the partition of coordinated ligands into classes based on their spatial distribution with respect to the cavity of cyclic trimer **2**. The three binding sites of the host are treated as independent, an assumption which results in equations with relatively simple forms. If cooperative binding occurs, the fractions of ligand bound inside or outside the host may vary for each of the HL_n complexes; in this case the equations would become considerably more complex.

Reactivities of Coordinated Ligands. A simple calculation shows that cyclic trimer **2** can form 34 distinct complexes when present in a solution of two monodentate reactants. Obviously, simplifying assumptions about the reactivities of these species must be made before an analysis of the kinetic influence of **2** on a bimolecular reaction is feasible. Intrinsic changes in ligand reactivity due to coordination at a zinc centre can be detected in control reactions with monomer **1**. Eq. [18] shows a hypothetical rate law for the initial rate of a bimolecular reaction A + B → C in the presence of zinc porphyrin monomer,

$$\left|\frac{\delta[C]}{\delta t}\right|_{t=0} = k_0[A][B] + k_1([A]_t - [A])[B] + k_2[A]([B]_t - [B]) + k_3([A]_t - [A])([B]_t - [B]) \quad [18]$$

The terms $([A]_t - [A])$ and $(B_t - [B])$ are the concentrations of monomer-bound A and B, respectively. If the porphyrin monomer has no effect on the rate of reaction of either ligand (*ie.* $k_0 = k_1 = k_2 = k_3$) then Eq. [18] reduces to [19], the uncatalysed second-order reaction.

$$\left|\frac{\delta[C]}{\delta t}\right|_{t=0} = k_0[A]_t[B]_t \quad [19]$$

If only one ligand is affected, say A, then $k_0 = k_2$ and $k_1 = k_3$ and Eq. [18] simplifies to [20],

$$\left|\frac{\delta[C]}{\delta t}\right|_{t=0} = k_0[A][B]_t + k_1([A]_t - [A])[B]_t \quad [20]$$

Since a cyclic porphyrin oligomer is essentially a series of linked monomers, changes in intermolecular

ligand reactivity due to coordination by monomer will be duplicated with zinc porphyrin oligomers. Due the topological constraints imposed by the linkages and cyclisation, however, at least two additional effects may also be important. Firstly, host species containing more than one ligand of a given type in exterior positions, say HA_0A_0, may react intermolecularly with a given B species, say B, faster than a species with only one equivalent ligand, in this case HA_0. This would be an example of a statistical kinetic effect - species which have a greater number of reactive groups, but are otherwise similar, have higher reactivities.[16] If the ligands are distributed statistically over the host binding sites, however, the net reactivity should be essentially the same as that of the monomer complexes. Statistical kinetic factors could become important if ligand binding is non-independent but, even so, averaging across the ligand distributions would tend to keep the effect small. Secondly, the effect of intra-cavity ligand binding on intermolecular reactivity must be considered. Ligands bound at sites inside relatively rigid oligomers such as **2** or **3** should be somewhat shielded by the steric bulk of the host and hence less reactive. The magnitude of this effect is difficult to assess but is probably fairly small for the trimer since its central cavity is quite open.[17] The net effect of cyclic oligomer coordination on ligand intermolecular reactivity is likely to be small, especially when there are no intrinsic changes in reactivity due to coordination to the zinc centre. For the purposes of these numerical simulations we assume that these effects can be neglected and treat the influence of cyclic oligomer binding on intermolecular reactivity as being equivalent to that of the corresponding monomer.

The substantial increase in the initial rates of certain reactions when carried out in the presence of cyclic trimer **2** is probably due to the promotion of intramolecular reaction pathways.[2] CPK models suggest that only complexes in which both ligands are bound at inner sites can react intramolecularly. It is the reactivities of the species in classes X1 and X2 which are of greatest relevance in evaluating the effectiveness of the host as an enzyme mimic. In the simulations below we employ the reasonable but untested hypothesis that the X2 complexes are twice as reactive as the X1 complexes. The consequences of this assumption can be tested by comparing experimental and simulated results over a range of concentrations.

COMPUTATIONAL METHODS

Kinetics simulation programs were written in the Pascal programming language (TurboPascal 1.00A, Borland International) and were implemented on a Macintosh SE personal computer. All of the subroutines using the numerical methods described below were taken from *Numerical Recipes in Pascal* by Press *et al.*[12] Polynomials in one variable were solved with the function `rtsafe` which employs a combination of bisection and Newton-Raphson routines to find a root of an equation. Polynomials in two or more variables were solved using the multidimensional Newton-Raphson algorithm `mnewt` with initial estimates of the roots obtained by assuming independent binding and calculating as described above. Numerical integrations were carried out by the Bulirsch-Stoer method with the following subroutines and functions: `odeint` - a driver with adaptive stepsize control; `mmid` - modified midpoint calculation routine; `bsstep` - the Bulirsch-Stoer stepping routine; and `rzextr` - a diagonal rational function extrapolation routine.

RESULTS AND DISCUSSION

The assumption that cyclic zinc porphyrin oligomers have independent binding sites leads to simple polynomial equations that allow calculation of the concentration of each host-ligand species in solution. In conjunction with inferences about the spatial distributions of coordinated ligands and estimates of the relative reactivities of the various complexes, these equations permit the construction of kinetic models. We have carried out numerical simulations based on models for the reactions in Scheme 3 under a variety of initial conditions and reaction types; the results presented here illustrate the application of this approach to the analysis of both inital rate and time course kinetic studies.

The concentration of a host with multiple binding sites can have a dramatic effect on the initial rate of a reaction between two ligands. The simulations depicted in Fig. 1 were calculated for the system shown in Scheme 3 with initial rates according to Eq. [21] and rate enhancements from the quotient of the initial rate

of reaction in the presence of H and the initial rate of the uncatalysed reaction. In this system, the host is assumed to have an effective molarity (EM) of 20 M, a relatively large value for a catalytic artifical receptor but orders of magnitude less than the values typically reported for enzymes.

$$\left|\frac{\delta[C]}{\delta t}\right|_{t=0} = k_0[A]_t[B]_t + k_1([X1]+2[X2]) \qquad [21]$$

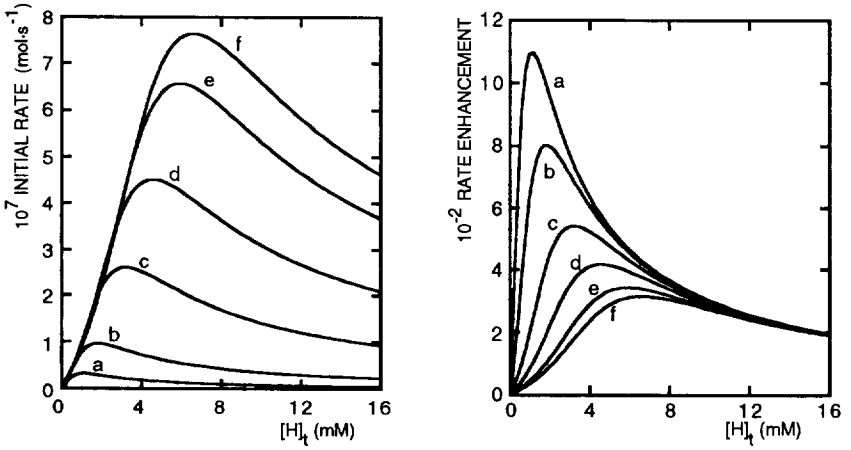


Fig. 1. Simulations of the effect of host H concentration on the initial rate of reaction of two monodentate ligands A and B (Scheme 3); $[A]_t = [B]_t$ = (a) 1 mM, (b) 2 mM, (c) 4 mM, (d) 6 mM, (e) 8 mM, (f) 9 mM.

$$A_t + B_t \xrightarrow{k_0 = 3.0 \times 10^{-5}\ M^{-1}s^{-1}} \text{products}$$

$$X1 \xrightarrow{k_1 = 6.0 \times 10^{-4}\ s^{-1}} \text{products} + H$$

$$X2 \xrightarrow{2k_1} \text{products} + H$$

H has 3 independent binding sites; $f^A_i = 0.5$, $f^B_i = 0.5$

$K_A = 3500\ M^{-1}$, $K_B = 350\ M^{-1}$

Scheme 3

Initial-rate kinetic studies are often the most feasible way to study complicated systems because effects such product inhibition can be neglected in the kinetic treatment. The simulations in Fig. 1 show distinctive profiles for the variation of initial rate with host H concentration. As the host concentration is increased from zero, the amounts of all the bound ligand species increase; the rising initial rates reflects the increasing importance of the intramolecular reaction pathways due to X1 and X2. The fractional distributions of the host-ligand species change as well however, and the host concentration cannot be increased indefinitely for further rate enhancement: eventually the concentrations of the HL_2 and HL_3 species begin to drop as HL species become more important. The corresponding drop in the initial rates at higher $[H]_t$ reflects this; the shift of the initial rate maxima towards $[H]_t$ for the systems with greater ligand concentrations occurs because HL_2 and HL_3 species are more favoured under these conditions.

Such simulations of kinetic results can help identify the experimental conditions most conducive to the measurement of accurate rate constants. The rate enhancements due to host catalysis (Fig. 1) increase as the concentration of the substrates is decreased, reflecting the increasing importance of first-order catalytic

pathways relative to the uncatalysed second-order reaction. At the same time the distribution of rate enhancements narrows because the concentrations of the bound substrate complexes become more sensistive to the overall host concentration as the total ligand concentration is reduced. From these considerations alone, it would appear that the best experiment would employ the substrates at low concentration relative to the host. Unfortunately, under these conditions the experimentally-measured initial rates vary within a very small range as the total host concentration is changed. Measurement errors would be likely to dominate a kinetic experiment carried out under these conditions, vitiating the benefits of the more defined rate enhancement curve. The simulations suggest that for this particular system, the most significant results are likely to be obtained with moderate substrate concentrations ($[A]_t = [B]_t = 4 - 6$ mM).

The assumption that two reactants bind independently and competitively remains useful even when not strictly valid. Consider the case in which one substrate binds non-independently to a host with three binding sites while the binding of the other is independent. This situation is relevant to the acyl-transfer reaction in Scheme 1; the binding of 4-(hydroxymethyl)pyridine (**5**) to trimer **2** exhibits cooperativity at 70 °C in toluene ($K_1 = 5.3 \times 10^3$ M^{-1}, $K_2 = 3.2 \times 10^3$ M^{-1}, $K_3 = 8.0 \times 10^2$ M^{-1}) while the binding of N-acetylimidazole (**6**) is independent (the apparent 1:1 binding constant K is 3.5×10^2 M^{-1})[2a]. In order to model a system like this accurately it is necessary to solve three equations simultaneously with the multidimensional Newton-Raphson algorithm. The successful application of this method cannot be guaranteed, but becomes likely if reasonable estimates of the roots of the equations can be made. If 4-(hydroxymethyl)pyridine binding is treated as if it is independent (with say $K = 3.5 \times 10^3$ M^{-1}) the unbound concentrations **[2]**, **[5]** and **[6]** can be calculated (Eqs. [6] - [10]) and used as initial estimates for the multidimensional polynomial solver. We have found that these estimates, although crude, are sufficient to permit convergence to the correct values. Table 1 compares values calculated by assuming independent ligand binding and more precise values from multidimensional Newton-Raphson calculations for several hypothetical binding equilibria involving ligands A and B and host H (3 binding sites). Note that even when the two methods give values of [A], [B] and [H] which are reasonably similar, the concentrations of other species such as HA_3 or HAB may differ substantially. It is the relative insensitivity of [A], [B] and [H] to the exact formulation of the binding equilibria which make independent binding assumptions useful in multidimensional equation solving.

Table 1 A Comparison of Ligand and Host Concentrations Calculated for Independent and Cooperative Binding Equilibria.[a]

Binding Constants (M^{-1})		Calculation Method[b]	[A] (M)	[B] (M)	[H] (M)
$K_A = 2000$	$K_B = 500$	I	3.557×10^{-4}	1.172×10^{-3}	4.122×10^{-4}
$K_{1A} = 6000$ $K_{2A} = 4000$ $K_{3A} = 600$	$K_B = 500$	NR	2.428×10^{-4}	2.036×10^{-3}	6.353×10^{-4}
$K_A = 500$	$K_B = 2000$	I	1.172×10^{-3}	3.557×10^{-4}	4.122×10^{-4}
$K_{1A} = 1500$ $K_{2A} = 1000$ $K_{3A} = 150$	$K_B = 2000$	NR	9.672×10^{-4}	3.659×10^{-4}	4.106×10^{-4}
$K_A = 16000$	$K_B = 5000$	I	5.890×10^{-5}	1.837×10^{-4}	2.135×10^{-4}
$K_A = 35000$	$K_B = 5000$	I	2.726×10^{-5}	1.848×10^{-4}	2.098×10^{-4}
$K_{1A} = 50000$ $K_{2A} = 35000$ $K_{3A} = 35000$	$K_B = 5000$	NR	3.121×10^{-5}	1.846×10^{-4}	2.774×10^{-4}

[a] $[A]_t = [B]_t = [H]_t = 5.0 \times 10^{-3}$M; host H has three binding sites.

[b] Calculation methods: I = independent binding (Eqs. [6] - [10]); NR = multidimensional Newton Raphson.

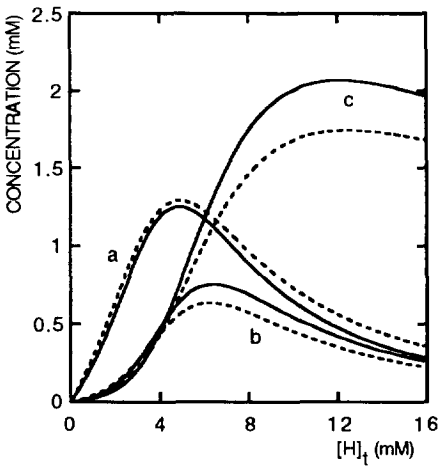


Fig. 2. Comparison of concentrations of host-substrate complexes predicted by independent (—) and non-independent (---) ligand-binding models for host **2** and ligands **5** (A) and **6** (B); (a) $[HA_2B]$, (b) $[HAB_2]$, (c) [HAB]; $[A]_t = [B]_t = 9.0$ mM.

Although the multidimensional Newton-Raphson algorithm is effective, it is somewhat tedious to implement because it requires evaluation of partial derivatives for the polynomial equations with respect to each variable. It is therefore of interest to consider the conditions under which independent binding approximations can be deemed to hold for systems with more complicated equilibria. Fig. 2 shows the effect of trimer **2** concentration on the concentrations of intramolecularly-reactive complexes as predicted by the two binding models for substrates **5** (A) and **6** (B). The evident disparity in the predictions indicates that the independent-binding assumption is not an acceptable approximation. An accurate kinetic analysis of the influence of **2** on the acyl-transfer reaction in Scheme 1 would require the more accurate treatment provided by the cooperative binding model. The discrepancies are especially significant at higher host concentrations, diminishing to about 10–15 % for species containing A when $[H]_t$ is 2 mM or less. Host species containing only B differ in concentration by about 50%; the concentrations of the unbound ligands are within 6 %. If host species which contain only B do not figure prominently in the kinetic model than the independent binding assumption could be used to provide an approximate treatment at low $[H]_t$. Note, however, that the spatial distributions of coordinated ligands predicted by the two models will differ significantly if the cooperativity of ligand binding reflects a preference for ligand placement within the host cavity. Eqs. [16] and [17] would have to be adjusted accordingly before the concentrations of the reactive fractions X1 and X2 could be calculated.

Simulations of reaction time courses can provide at least a qualitative indication of the effects of changing conditions on reaction behaviour and by comparison with experimental behaviour may reveal inadequacies in the kinetic model. Significant deviations from the predicted timecourse may indicate potentially interesting complications such as product catalysis. When there are competing catalytic mechansims, numerical simulations can help identify the experimental conditions which provide the most information about a particular reaction pathway. Implementation of timecourse simulations requires the evaluation of reactive species concentrations in the absence and presence of products, and a numerical integration of the differential equations. For the latter, we have used the Bulirsch-Stoer method, a powerful and efficient algorithm for obtaining high-accuracy solutions to ordinary differential equations.[12e] The time course of a reaction in which a host like trimer **2** catalyses the formation of two monodentate products from two monodentate substrates (Scheme 4) is depicted in Fig. 3. The binding constants of the ligands and the rate constant of the uncatalysed reaction resemble those of the acyl-transfer reaction between **5** and **6**.

$$A_t + B \xrightarrow{k_0 = 3.0 \times 10^{-5}\ M^{-1}s^{-1}} \text{products C + D}$$

$$A_t + \text{"HB"} \xrightarrow{k_1 = 3.0 \times 10^{-3}\ M^{-1}s^{-1}} \text{products C + D + H}$$

$$X1 \xrightarrow{k_2 = 6.0 \times 10^{-4}\ s^{-1}} \text{products + H}$$

$$X2 \xrightarrow{2k_2} \text{products + H}$$

H has 3 independent binding sites; $f^A_i = 0.5$, $f^B_i = 0.5$

$K_A = 3500\ M^{-1}$, $K_B = 350\ M^{-1}$, $K_C = 3500\ M^{-1}$, $K_D = 2000\ M^{-1}$

Scheme 4

The model and rate equation [22] is, however, entirely hypothetical and the substrate ligands are assumed to bind with no preference for either internal or external coordination sites ($f^A_i = f^B_i = 0.5$).

$$\frac{\delta[C]}{\delta t} = k_0[A]_t[B] + k_1[A]_t([B]_t - [B]) + k_2([X1] + 2[X2]) \qquad [22]$$

The rate constant k_1 reflects chemical catalysis of the reaction due to coordination of the weakly-binding ligand B; the reactivity of A is unaffected by coordination. It is somewhat unrealistic under these circumstances to make [X2] only twice as reactive as [X1] since this implies that $[HA_iB_iB_i]$ with two bound B ligands is no more reactive than $[HA_iA_iB_i]$. with one. Nevertheless, the simulations in Fig. 3 provide a qualitative indication of the behaviour expected from a system in which the catalytic activity of the host is not solely due to preorganisation of the substrates. Incorporation of [22] into a numerical integrator requires determination of the concentration of host-substrate complexes in the presence of competitively-binding product ligands. Eqs. [6]–[10] were readily extended to describe the independent binding of the four ligands during the progress of the reaction.

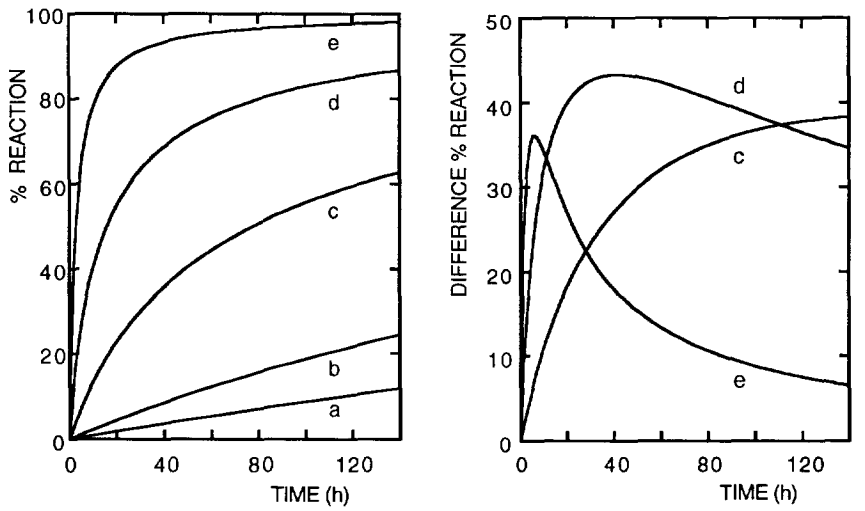


Fig. 3. Simulations of the effect of host concentration on the time course of a reaction between two monodentate ligands A and B (Scheme 5); $[A]_t = [B]_t$ = 9 mM; (a) uncatalysed reaction, (b) monomer, [H] = 1.35 mM, (c) trimer, [H] = 0.45 mM, (d) trimer, [H] = 1.8 mM, (e) trimer, [H] = 9 mM. Difference % reaction = % reaction due to trimer minus % reaction due to uncatalysed and intermolecularly-catalysed reaction pathways.

The effectiveness of a porphyrin supermacrocycle in promoting reactions between cavity-bound ligands can be assessed by comparing the kinetics of reaction between substrates in the presence of the *n*–site oligomer of concentration $[H]_t$ with the kinetics in the presence of *n* times the concentration of the monomeric subunit. The extent of product formation due to intramolecular pathways depends critically on the concentration of the oligomeric host. The difference curves obtained when simulated control reactions at the appropriate concentrations are subtracted from the reaction time courses (Fig. 3) indicate that the chemically-catalysed intermolecular pathway becomes increasingly significant as host concentration is raised. The changing form of these curves suggests that experimentally-obtained difference curves could provide a good test of this particular kinetic model.

In the reaction modelled above, catalytic turnover occurs because the products bind about as strongly as the substrates. In the Diels-Alder reaction of Scheme 2, the two monodentate substrates form a bidentate product which binds too strongly to the host to permit significant turnover under the experimental conditions.[2b] It is interesting to consider how efficient a synthetic system for a reaction like this would have to be in order to be truly catalytic. Figure 4 shows simulations based on the rate equation [23] and the model in Scheme 5.

$$\frac{\delta[B]}{\delta t} = k_0[A]_t^2 + k_1(f^A{}_i f^A{}_i [HA_2]) \qquad [23]$$

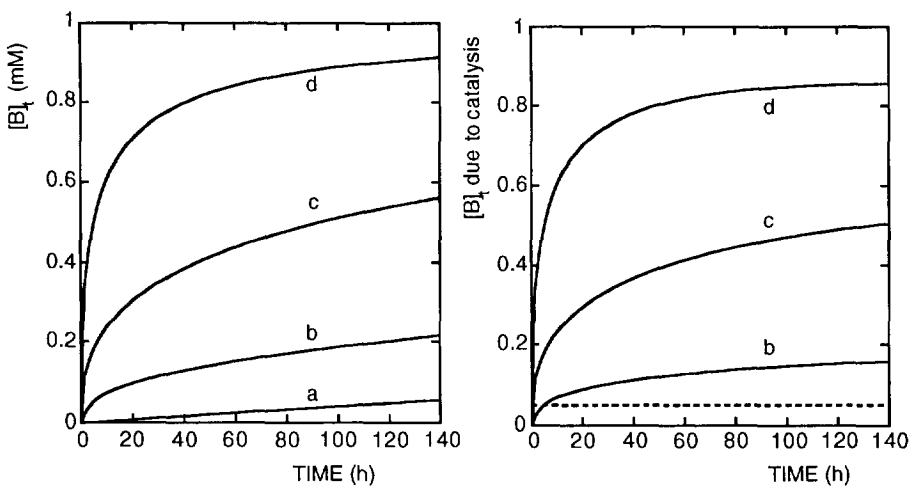


Fig 4. Simulations of the influence of a host-mediated intramolecular rate acceleration of bidentate product formation from dimerisation of a monodentate ligand A (Scheme 5); k_1 = (a) 0 s^{-1}, (b) 6.0×10^{-4} s^{-1} (EM = 20 M), (c) 1.5×10^{-2} s^{-1} (EM = 500 M) (d)3 s^{-1} (EM = 1.0×10^5 M). $[A]_t$ = 2 mM, $[H]_t$ = 0.05 mM (-----). $[B]_t$ due to catalysis is difference between reaction in presence and absence of host.

$$A_t + A_t \xrightarrow{k_0 = 3.0 \times 10^{-5}\ M^{-1}s^{-1}} B\ (\text{"A-A"})$$

$$X1 \xrightarrow{k_1} B + H$$

H has 2 independent binding sites; $f^A{}_i = 0.5$

$K_A = 2500\ M^{-1}$, $K_B = 2.5 \times 10^7\ M^{-1}$

Scheme 5

Because the product binding constant greatly exceeds that of the substrate, the host becomes quickly saturated; although only small amounts of the HA_2 complex can form, it will be converted to product at a significant rate if the host is efficient enough. Simulations were carried out for hosts with effective molarities ranging from 20 M to 100,000 M. Turnover occurs under these conditions for all of the hosts and is significant when the EM is ≥ 500 M. In a real system, the intramolecular rate constants k_1 for EMs > ≈ 10,000 could exceed the rate of dissociation of the host-bound product, causing product release to become the rate-determining step; this is the situation in Mock's cucurbituril-catalysed cycloaddition reaction.[1b]

Assessing the Validity of the Ligand-Binding Models

The mathematical formulae presented in the Theory section form the core of our approach to modelling the catalytic activity of porphyrin supermacrocycles. When implemented with the appropriate numerical methods they allow the straightforward simulation of complex kinetic behaviour. The simple model of reactant binding presented in Eqs. [6]-[11] is based on two key assumptions: first that a reactant ligand is independently bound by host H in the absence of its co-reactant and second that the co-reactant modifies reactant binding only through competition for host binding sites. The first assumption can be verified experimentally, since if a ligand is bound independently, the stepwise binding constants $K_1...K_n$ arise in a fixed ratio (Eq. [2]). The second assumption is difficult to test because the two ligands will react when they are present in the same solution, especially in the presence of a catalytic host. This tends to rule out equilibrium measurements based on competitive binding experiments. It is possible in principle to extract ligand binding constants from the effect of the host on reaction kinetics but the success of this strategy depends critically on the validity of the model proposed for the reactivities of the bound species.

Eqs. [12]-[17] provide a way of estimating reactant preferences for exterior or interior coordination sites on a cyclic host. Although plausible, they have the obvious defect of extrapolating complicated ligand interactions from rather limited physical evidence. Additional support for ligand binding patterns can be obtained from complexation-induced changes in the ligand NMR spectrum. Differences in the extent to which monomer **1** and trimer **2** cause ^1H chemical shift changes in ligand resonances largely reflect the effects of coordination at sites within the cavity of the cyclic trimer. By comparing the results for a series of ligands, it should be possible to correlate differences in limiting chemical shift changes with the calculated distribution coefficients f_i and f_o for the same systems. Kinetic data can also provide support, although the utility of this approach is tempered by the necessity to make additional assumptions about the reactivities of the various host-ligand complexes.

CONCLUSION

It is relatively easy to demonstrate experimentally the acceleration of a reaction by an enzyme mimic; it is much more difficult to identify precisely the source of that acceleration. That understanding requires a detailed kinetic mechanism which in turn depends on a complete description of the complex binding equilibria and an explicit treatment of the reactivities of all the relevant complexes. Such analyses are rarely attempted for enzyme mimics because of the number of unknowns involved, yet they are surely essential if the design rules governing their operation are to be properly understood. The approach described here provides a firm basis for more detailed kinetic investigations involving porphyrin supermacrocycles. Like any properly conducted kinetic simulation it may be used to predict initial rates and time courses for different initial conditions, allowing one to test a range of experimental protocols and so optimise the chance of seeing the desired catalysis. More importantly, by formulating explicit assumptions about the distributions of coordinated substrate and product ligands, it provides testable criteria which may be used to dissect the relative importance of binding and kinetic parameters in the overall scheme. This approach is currently being used to test and refine our understanding of the trimer-enhanced Diels-Alder and acyl transfer reactions.

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13. This treatment, although different in form, is consistent with that given in Connors, K. A. *op. cit.*, pp 54-55.

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