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Porphyrin intercalation and non-specific 'edge on' outside binding to natural DNA

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A theoretical two-mode binding model for porphyrin binding to natural DNA is presented. One of the binding modes is assumed to be base sequence specific with binding sites n base-pairs long. The other binding mode has binding sites which consist of only one base-pair and can involve cooperativity. The model fits satisfactorily to data for H_2TMPyP-4, Cu(II)TMPyP-3 and Cu(II)TMPyP-4 binding to calf thymus DNA in both a high ($\mu \simeq 1.0$ M) and a low ($\mu \simeq 0.2$ M) ionic strength buffer. The results show that the fraction of porphyrin bound in the non-specific mode reaches a maximum at certain input DNA to porphyrin concentration ratios. The value of this maximum decreased, and its position shifted to higher DNA to porphyrin concentration ratios for binding in the high ionic strength buffer. The value of the cooperativity parameter obtained through the fitting process suggests that the non-specific binding is positively cooperative. The results are compared with the data analysed using other techniques.

1. Introduction

At least two types of interactions, or binding modes, have been identified for certain porphyrin molecules, such as meso-tetra(4-*N*-methylpyridyl)-porphine (H_2TMPyP-4) and its copper derivatives Cu(II)TMPyP-4 and Cu(II)TMPyP-3, binding to natural DNA [1–7]. One is intercalation, which has a CG preference [1,3,5,7–10], while the other is an external, or 'outside' binding mode which is largely electrostatic [1–3,5–7].

The experimental data for two-mode binding of porphyrin to DNA cannot be fitted easily using existing theoretical models. The models that have been used so far are either a straight line [1] or the McGhee and Von Hippel model [3,5]. The latter model does not fit the data well when obvious multi-mode binding and/or base sequence specificity is involved, since it was developed for single-mode binding to a homogeneous lattice [11]. The discrepancy between the prediction of the model and the experimental data is more conspicuous in the region of high bound porphyrin to DNA concentration ratios. Here the Scatchard plots, the form in which most binding data are published, run almost parallel to the horizontal axis. But the McGhee and Von Hippel model predicts that the plots should intersect the horizontal axis, and that the intercept is equal to the number of base-pairs 'covered' by the porphyrin [11]. For binding in low ionic strength buffers, the two-mode nature of the binding becomes so significant that the model cannot fit the data at all [2,5].

Many other theoretical models have been developed to tackle multi-mode binding. They apply to

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various situations such as binding to alternating binding sites [12], to two types of completely independent binding sites [13,14], to sites of identical sizes [13,15,16] or to non-specific sites with neighbour exclusivity and cooperativity [14,17–19]. None of the existing models is entirely suitable for the current problem, which involves two-mode binding with one of the binding modes being base sequence specific.

A base sequence specific interaction implies exclusion of certain parts of DNA as potential binding sites. It is not appropriate, therefore, to use a model in which it is assumed that every base-pair in the DNA molecule is capable of being bound by a ligand, in our case a porphyrin, or part of a ligand in any one of several binding modes, i.e., non-base sequence specific binding. It is also unlikely that the binding sites in these two binding modes are of the same sizes or completely independent. A new model is needed to take into account the two-mode binding as well as the base sequence specific nature of the binding in one case. Such a model is proposed in the present work. Pertinent binding data have been fitted or refitted and results are discussed in relation to other experimental observations.

2. Theory

In our model, a porphyrin molecule can bind to DNA in two binding modes. One mode is base sequence specific and the other non-base sequence specific. These two binding modes are mutually exclusive and a porphyrin molecule bound in the base sequence specific binding mode will block n binding sites for the non-base sequence specific binding. Porphyrins bound in the non-base sequence specific binding mode may also interact with their nearest neighbours. In the context of this paper, the base sequence specific mode is referred as intercalation and the non-base sequence specific mode designated as outside binding.

The base sequence specific nature of intercalation means it can only occur at a site with the right type and sequence of base-pairs. This is a rather strict requirement on the binding. It implies that only a small proportion of total DNA base-pairs can form intercalating sites, most of which are separated from each other. If the base sequence of a DNA molecule is random, then the probability q_n of finding a specific site consisting of n base-pairs is [20]:

$$q_n = \begin{cases} \dfrac{1}{2^{2n-1}} & \text{for } n \text{ odd} \\ \dfrac{1}{(2^{2n-1} + 2^{n-1})} & \text{for } n \text{ even} \end{cases} \quad (1)$$

e.g., the probabilities for finding a specific site consisting of two, three or five base-pairs along the DNA molecule are about 10, 3 and 0.2%, respectively. Naturally, the probability of finding two adjacent specific sites is even smaller. This is supported by a footprinting experiment which showed well-separated regions of DNA being protected by H_2TMPyP-4 [21]. An intercalated porphyrin, therefore, will have little chance of interacting with other intercalated porphyrins.

In contrast to intercalation, outside binding, being a largely electrostatic interaction between porphyrin cations and charged groups of DNA phosphate backbone, can occur at any base-pair, including those forming potential intercalating sites. These two binding modes are mutually exclusive: once a site is occupied by a porphyrin in a certain mode, it cannot be occupied by another porphyrin in any other binding mode. We assume that each outside binding site consists of only one base-pair, as in the case of porphyrin 'edge on' outside binding. Since the total number of base-pairs is usually fixed in practice, the number of potential binding sites is constrained by the relation

$$(N_i \times n) + N_0 = N, \quad (2)$$

where N_i is the number of intercalated porphyrins, each of them occupying n base-pairs, and N_0 and N denote the number of outside binding sites and the total number of base-pairs, respectively. Thus, the number of available binding sites in either mode depends on the number of bound porphyrin molecules in both modes.

Let us consider a linear DNA molecule consisting of N base-pairs with g intercalating sites each of size n. For every base-pair of the DNA molecule, there are two possible states: unoccupied (denoted by 0) or occupied by an outside bound porphyrin (denoted by 1). For a base-pair which forms part of an intercalating site, there is an additional possible state: occupied by part of an intercalated porphyrin (denoted by 2). Thus, the overall state, or configuration, of the DNA molecule can be represented as a sequence of numbers 0, 1 and 2:

$$011001\overbrace{2\cdots2}^{n}10\cdots\overbrace{2\cdots2}^{n}\cdots101. \tag{3}$$

The first number denotes the state of the first base-pair from, say, the 3′-end of the DNA molecule. Note that 2's may only appear at certain fixed locations of the sequence because of the base specificity, and when they do so, they are always in groups of n consecutive 2's.

The sequence, eq. 3, can be represented concisely by replacing each group of n consecutive 2's with a $\{2\}_n$:

$$011001\{2\}_n10\cdots\{2\}_n\cdots101. \tag{4}$$

The number of all possible configurations of the DNA molecule with r_i intercalated porphyrins is obviously

$$\frac{g!}{r_i!(g-r_i)!}2^{N-nr_i},\quad r_i\le g \tag{5}$$

and the total number of possible configurations of the DNA molecule is

$$\sum_{r_i=0}^{g}\frac{g!}{r_i!(g-r_i)!}2^{N-nr_i}. \tag{6}$$

The partition function for the binding system is a summation over all these configurations, each configuration being assigned a statistical weight determined by the energy state of the DNA, and hence by the numbers and sometimes locations of the bound porphyrins in both binding modes. Once the partition function has been calculated, the thermodynamic properties, such as the number of the porphyrins bound in a particular binding mode, can be derived.

Conventional matrix methods cannot be used at this point to calculate the partition function since the $\{2\}_n$ only appear at certain locations randomly scattered along the sequence, eq. 4. In order to represent the partition function as a matrix multiplication, one must divide the sequence into units and each unit, no matter how it is defined and how many base-pairs it includes, must be represented at least once by each element of the matrix during the calculation of the partition function. It is very hard to divide the sequence of eq. 4 into a set of units to construct such a matrix when no information is available about the exact locations of the $\{2\}_n$ in the sequence.

If, however, the bound porphyrins do not interact with each other, the task of finding the partition function is greatly simplified, because then the statistical weight no longer depends on the location of the $\{2\}_n$. The two binding modes then become virtually independent except that the number of binding sites in each binding mode has to satisfy eq. 2. If we now rearrange eq. 4 by shifting all of the $\{2\}_n$ to the beginning of the sequence,

$$\overbrace{\{2\}_n\{2\}_n\cdots\{2\}_n}^{r_i}01100110\cdots101 \tag{7}$$

we can use the standard techniques to calculate the partition function [22],

$$\begin{aligned}Q&=\sum_{r_i=0}^{g}\frac{g!}{r_i!(g-r_i)!}s_i^{r_i}\\&\quad\times\sum_{j_1=0}^{1}\sum_{j_2=0}^{1}\cdots\sum_{j_{N-nr_i}=0}^{1}w_{j_1,j_2}w_{j_2,j_3}\cdots w_{j_{N-nr_i},1}\\&=\sum_{r_i=0}^{g}\frac{g!}{r_i!(g-r_i)!}s_i^{r_i}\vec{u}^{\dagger}\boldsymbol{W}^{N-nr_i}\vec{v}\end{aligned} \tag{8}$$

where

$$\vec{u}^{\dagger}=(1,1);\ \vec{v}=\begin{pmatrix}1\\0\end{pmatrix};\ \boldsymbol{W}=\begin{pmatrix}w_{00}&w_{01}\\w_{10}&w_{11}\end{pmatrix}=\begin{pmatrix}1&1\\s&s\end{pmatrix}, \tag{9}$$

and

$$w_{m,n}=e^{-G_m/RT}\quad m,\ n=1,0 \tag{10}$$

where G_0 and G_1 are the Gibbs free energies for an empty site and a site being occupied by an outside bound porphyrin, respectively, and $s = w_{11}/w_{00} = e^{-\Delta G_0/RT}$ is the statistical weight for a site occupied by an outside bound porphyrin molecule and $s_i = e^{-\Delta G_i/RT}$ that for a site occupied by an intercalated porphyrin molecule. ΔG_0 and ΔG_i are the changes of Gibbs free energy due to a site being occupied by an outside bound porphyrin and an intercalated porphyrin molecule, respectively.

It is the interaction, or cooperativity, therefore, among the bound porphyrins which makes it difficult to calculate the partition function using matrix methods. When cooperative binding occurs, rearranging eq. 4 into eq. 7 changes the statistical weight of the configuration, but this change can be compensated for by properly redefining the statistical weight for sites occupied by intercalated porphyrins.

Every $\{2\}_n$ in eq. 4 has four possible nearest-neighbour configurations: $1\{2\}_n1$, $0\{2\}_n1$, $1\{2\}_n0$ and $0\{2\}_n0$. If we denote the interaction energy between an intercalated porphyrin and an adjacent outside bound porphyrin as E_{12}, and that between two adjacent outside bound porphyrins as E_{11}, then the changes in the interaction energy caused by shifting a $\{2\}_n$ to the beginning of the sequence are:

Before change	After change	Energy change
$\cdots 0\{2\}_n0\cdots$	$\{2\}_n\cdots 00$	$0-0$
$\cdots 1\{2\}_n0\cdots$	$\{2\}_n\cdots 10$	$0-E_{12}$
$\cdots 0\{2\}_n1\cdots$	$\{2\}_n\cdots 01$	$0-E_{12}$
$\cdots 1\{2\}_n1\cdots$	$\{2\}_n\cdots 11$	$E_{11}-2E_{12}$.

During the calculation of the partition function, all four configurations listed above will occur the same number of times. To keep the mathematics tractable, the individual energy changes are approximated by

$$E_{av} = \frac{E_{11} - 4E_{12}}{4} \tag{11}$$

The partition function can then be approximated by

$$Q' \simeq \sum_{r_i=0}^{g} \frac{g!}{r_i!(g-r_i)!} s_i^{r_i} e^{-r_i(E_{av}/RT)} \sum_{j_1=0}^{1} \sum_{j_2=0}^{1} \times \cdots \sum_{j_{N-nr_i}=0}^{1} w_{j_1,j_2} w_{j_2,j_3} \cdots w_{j_{N-nr_i},1}$$

$$= \sum_{r_i=0}^{g} \frac{g!}{r_i!(g-r_i)!} s_i'^{r_i} \vec{u}^{\dagger} W'^{N-nr_i} \vec{v} \tag{12}$$

where $s_i' = s_i e^{-E_{av}/RT}$ and

$$W' = \begin{pmatrix} 1 & 1 \\ \sigma s & s \end{pmatrix}, \tag{13}$$

where σ is a cooperativity parameter for outside binding. It is defined by the equation

$$K^{\sharp} = \sigma K \tag{14}$$

where $K^{\sharp}$ is the binding constant for an initial nucleation of the outside binding, in which a porphyrin binds to a site without any adjacent bound porphyrin, and K is the binding constant for a porphyrin binding to a site adjacent to an already bound porphyrin [22]. It is easy to see that $\sigma < 1$ indicates positive cooperativity, $\sigma > 1$ corresponds to negative cooperativity and $\sigma = 1$ non-cooperativity.

The matrix W' can be diagonalized with standard techniques and the partition function then becomes

$$Q' = \sum_{r_i=0}^{g} \frac{g!}{r_i!(g-r_i)!} s_i'^{r_i} \vec{u}^{\dagger} M \begin{pmatrix} \lambda_0^{N-nr_i} & 0 \\ 0 & \lambda_1^{N-nr_i} \end{pmatrix} M^{-1} \vec{v}$$

$$= \vec{u}^{\dagger} M \begin{pmatrix} \sum_{r_i=0}^{g} \frac{g!}{r_i!(g-r_i)!} s_i'^{r_i} \lambda_0^{N-nr_i} & 0 \\ 0 & \sum_{r_i=0}^{g} \frac{g!}{r_i!(g-r_i)!} s_i'^{r_i} \lambda_1^{N-nr_i} \end{pmatrix} \times M^{-1} \vec{v}$$

$$= \vec{u}^{\dagger} M \begin{pmatrix} \lambda_0^N \left(1 + \frac{s_i'}{\lambda_0^n}\right)^g & 0 \\ 0 & \lambda_1^N \left(1 + \frac{s_i'}{\lambda_1^n}\right)^g \end{pmatrix} M^{-1} \vec{v} \tag{15}$$

where

$$\lambda_0, \lambda_1 = \tfrac{1}{2}\left[1 + s \pm \sqrt{(1-s)^2 + 4\sigma s}\right] \tag{16}$$

are the eigenvalues of the matrix W' and M is the matrix used to diagonalize W'.

The partition function finally reduces to

$$Q' \simeq \frac{1}{\lambda_1 - \lambda_0}\left[(\lambda_1 - 1)\lambda_0^{N+1}\xi_0 - (\lambda_0 - 1)\lambda_0^{N+1}\xi_1\right], \tag{17}$$

with

$$\xi_0 = \left(1 + \frac{s_i'}{\lambda_0^n}\right)^g; \ \xi_1 = \left(1 + \frac{s_i'}{\lambda_1^n}\right)^g. \tag{18}$$

3. Fitting experimental data

The average fraction of total binding sites occupied by intercalated porphyrins at equilibrium can easily be calculated from eq. 17

$$\bar{\theta}_i = \frac{\bar{r}_i}{N} = \frac{1}{N}\frac{\partial(\ln Q')}{\partial(\ln s_i')}, \tag{19}$$

where the bar above the corresponding variables indicates the equilibrium value. Since it is easy to obtain DNA molecules containing more than 10^4 base-pairs, N is usually taken to be a large number and the approximation $N \to \infty$ may be used. Then,

$$\bar{\theta}_{i,\infty} = \lim_{N\to\infty} \bar{\theta}_i \simeq \frac{g}{N}\frac{s_i'}{s_i' + \lambda_0^n} = \frac{g}{N}\frac{\beta s}{\beta s + \lambda_0^n}, \tag{20}$$

where $\beta = s_i'/s$.

The average fraction of outside binding sites occupied at equilibrium, $\bar{\theta}_o$, can be derived by first calculating the average fraction of occupied outside binding sites when there are r_i intercalated porphyrin molecules [22],

$$\bar{r}_o(r_i) = \frac{1}{Q'}\sum_{r=1}^{N-nr_i} (\vec{u}W'^{r-1})_1 (W'^{N-nr_i+1-r}\vec{V})_1, \tag{21}$$

and then adding $\bar{r}_0(r_i)$ from $r_i = 0$ to $r_i = g$ with proper statistical weights.

$$\bar{\theta}_o = \sum_{r_i=0}^{g} \frac{g!}{r_i!(g-r_i)!} s_i'^{r_i} \bar{r}_o(r_i). \tag{22}$$

It is easy to show that, as $N \to \infty$,

$$\lim_{N\to\infty} \bar{\theta}_o = \bar{\theta}_{o,\infty} \simeq \frac{\lambda_0 - 1}{\lambda_0 - \lambda_1}(1 - n\bar{\theta}_{i,\infty})$$

$$= \tfrac{1}{2}\left(1 - \frac{1-s}{\sqrt{(1-s)^2 + 4\sigma s}}\right)(1 - n\bar{\theta}_{i,\infty}) \tag{23}$$

where $\bar{\theta}_{i,\infty}$ is given by eq. 20. In what follows, θ_i and θ_0 will be used instead of $\bar{\theta}_{i,\infty}$ and $\bar{\theta}_{o,\infty}$ to represent the average fractions of binding sites occupied by intercalated and outside bound porphyrin molecules, respectively.

Instead of using Scatchard plots or binding isotherms to represent experimental data and the results of theoretical calculations, we have chosen to use a plot in which the fraction of the bound porphyrins is represented as a function of input DNA base-pair to porphyrin concentration ratio. This choice not only makes fitting data easier, but also makes interpreting results more straightforward. Data published in the form of Scatchard plots or binding isotherms can be transformed easily into the form used in the present work and vice versa (see the appendix).

The fraction of bound porphyrin, γ, is obtained by adding the fractions of intercalated and outside bound porphyrin,

$$\gamma = \frac{\bar{n}}{L_0} = \frac{\bar{n}_i}{L_0} + \frac{\bar{n}_o}{L_0} = \gamma_i + \gamma_o \tag{24}$$

where L_0 is the total number of porphyrin molecules, $\bar{n}$ the average total number of bound porphyrin molecules and γ_i and γ_o the fraction of intercalated and outside bound porphyrin molecules, respectively.

If we write

$$s = s_0(1 - \gamma), \tag{25}$$

where s_0 is the value of s when no porphyrin molecule has been bound, and note that

$$\theta_j = \frac{N_j}{N} = \frac{N_j}{L_0\frac{N}{L_0}} = \frac{\gamma_j}{p}, \ j = \mathrm{i}, \mathrm{o}. \tag{26}$$

where $p = [\text{DNA}]/[\text{porphrin}]$ is the input DNA base-pair to porphyrin concentration ratio, then substituting eq. 25 into eqs 20 and 23 will result in the following equation for γ

$$\gamma = \gamma_i + \gamma_o = p[\theta_i(\gamma) + \theta_o(\gamma)] \quad (27)$$

This equation can be solved numerically and gives γ as a function of p with s_0, n, g/N, β and σ as parameters.

Two approaches are adopted to fit the calculated values of γ to the experimental data. In the first approach, both n and g/N are kept constant and the data are fitted by changing s_0, β and σ. Values of $n = 2$ and $n = 3$ were used and the g/N values were calculated according to eq. 1. In the second, only n is kept constant and g/N is allowed to vary as an independent parameter.

The fitting is carried out using a general fitting package GFIT based on the Harwell library subroutine VA05A which gives optimum binding parameters as the χ^2 value of the fit converges. Standard deviations of the optimum parameters are calculated with a subroutine published by Clifford [24]. The data obtained for Cu(II)TMPyP-4 and Cu(II)TMPyP-3 binding in the low ionic strength buffer are weighted with their experimental uncertainties. No weights are applied to other binding data, since the information regarding their experimental uncertainties is not available. Several initial guesses for the binding parameters are used in order to reach the global minimum of the χ^2. The experimental data are plotted with their best fits together with the calculated fractions of intercalated and outside bound porphyrins as functions of p.

4. Experimental

Calf thymus DNA (Sigma, type 1) was deproteinised by phenolization, and dialysed extensively against a buffer consisting of 2 mM Hepes, 10 μM EDTA and 0.2 M NaCl (pH 7.0). Milli-Q water and reagent grade chemicals were used throughout. The metalloporphyrins Cu(II)TMPyP-3 and Cu(II)TMPyP-4 were obtained as described by Dougherty et al. [5].

Ultraviolet-visible spectra were recorded on a Varian DMS 100 spectrometer connected to an LSI 11/23 laboratory computer. The concentrations of bound and free porphyrin were calculated with the method described in ref. 5. The difference between the total porphyrin concentration and the sum of calculated free and bound porphyrin concentrations was taken as the uncertainty for the bound porphyrin concentration. These uncertainties were used later as the weights in fitting the binding data.

5. Results

Data obtained by Dougherty et al. in this laboratory [5], by Fiel et al. [1] and Pasternack et al. [3] for H_2TMPyP-4 binding to calf thymus DNA were fitted and the results given in table 1. Pasternack et al. had treated their results using two different values for the molar absorptivity, ϵ_b, for porphyrin-DNA complexes and thus produced two sets of binding data [3]. Both sets of data due to Pasternack et al. were fitted using the new model. The results for Cu(II)TMPyP-4 and Cu(II)TMPyP-3 binding to calf thymus DNA are listed in table 2 and 3, respectively. For each porphyrin, data obtained from binding in a high ionic strength buffer ($\mu \simeq 1.0$ M) and a low ionic strength buffer ($\mu \simeq 0.2$ M) have been fitted. The standard deviations of the optimum binding parameters g/N, s_0, β and σ are included in parentheses following the uncertain digit of the corresponding parameter (e.g., 0.29 (1) means 0.29 ± 0.01). Those listed without stated errors are parameters which were kept constant during the fitting.

The proposed model fits the existing experimental data well. In most cases, $\chi^2 \leq 10^{-3}$ even when only three parameters are allowed to vary. In figs 1, 2 and 3, the data due to Fiel et al. and their best fit are presented as the fraction of bound porphyrin vs input DNA to porphyrin concentration ratio, the Scatchard plot and the binding isotherm, respectively. In fig. 2, the best fit obtained using the McGhee and Von Hippel model [11] is also presented. It can be seen from fig. 2 that the current model fits the data well throughout

Table 1

Optimum binding parameters and their uncertainty for H_2TMPyP-4 binding to calf thymus DNA

Numbers in parentheses are the parameter uncertainties for the last digit of the corresponding parameter. Parameters listed without uncertainty were kept constant. $K^{\#}$ denotes the binding constant for isolated outside binding.

μ (M)	n	g/N	s_0	β	σ	$K^{\#}$ ($\times 10^5$) (M^{-1})	$\beta K^{\#}$ ($\times 10^6$) (M^{-1})	χ^2 ($\times 10^{-4}$)
0.196 [a]	2	0.1	1.6675 (2)	62.176 (4)	0.2207 (5)	0.8	5.0	0.27
	2	0.1272 (5)	1.7916 (2)	26.754 (3)	0.141 (1)	0.5	1.4	0.20
	3	0.130 (2)	1.9801 (3)	23.392 (3)	0.1380 (1)	0.6	1.4	0.20
0.2 [b]	2	0.1	0.617 (2)	46.82 (1)	0.286 (4)	0.6	2.8	1.37
	2	0.085 (8)	0.541 (8)	82.57 (3)	0.42 (2)	0.7	5.8	1.49
	3	0.0313	0.17 (1)	76.52 (1)	0.427 (8)	0.8	6.1	1.48
0.2 [c]	2	0.1	0.29 (1)	10.433 (4)	0.99 (1)	0.9	9.0	1.69
	2	0.147 (4)	0.46 (2)	8.14 (4)	0.34 (7)	0.5	4.0	2.35
	3	0.14 (1)	0.38 (4)	7.16 (6)	0.7 (1)	0.8	5.6	2.02
1.0 [d]	2	0.1	0.076 (6)	277.8 (2)	1.025 (7)	0.8	22	0.44
	2	0.1077 (5)	0.186 (6)	92.72 (6)	0.295 (8)	0.6	59	0.48
	3	0.1073 (5)	0.200 (6)	92.73 (5)	0.318 (8)	0.6	56	0.48

[a] Data from ref. 1.
[b] Data from ref. 3 when $\epsilon_b = 1.2 \times 10^5\ M^{-1}\,cm^{-1}$.
[c] Data from ref. 3 when $\epsilon_b = 1.1 \times 10^5\ M^{-1}\,cm^{-1}$.
[d] Data used in ref. 5.

the whole data range, but that of McGhee and Von Hippel only gives a good fit of the data at low values of r. Similar results were obtained with the data of Pasternack et al. (fig. 5). The current model also satisfactorily fits the data for Cu(II)TMPyP-4 and Cu(II)TMPyP-3 binding in the low ionic strength buffer (figs 7 and 9). The previous attempts at fitting binding data obtained in buffers of similar ionic strength ($\mu = 0.1$ M) using the McGhee and Von Hippel model failed [2,5]. At least one group of optimum binding parameters with reasonably small uncertainties can be obtained for every set of data. Consistent optimum binding parameters have been obtained by fitting two independent sets of data, one from Fiel et al. [1] and the other from Pasternack et al. [3],

Table 2

Optimum binding parameters and their uncertainty for Cu(II)TMPyP-4 binding to calf thymus DNA

Data obtained in $\mu = 0.2$ M buffer are weighted by their relative experimental uncertainty in the fitting. For additional details see legend to table 1.

μ (M)	n	g/N	s_0	β	σ	$K^{\#}$ ($\times 10^5$) (M^{-1})	$\beta K^{\#}$ ($\times 10^6$) (M^{-1})	χ^2 ($\times 10^{-4}$)
0.2	2	0.1	1.035 (1)	59.95 (1)	0.052 (2)	1.2	7.2	0.2
	2	0.127 (5)	0.59 (1)	60.0 (1)	0.51 (2)	6.6	40	0.4
	3	0.1319 (3)	0.543 (2)	65.13 (1)	0.147 (3)	1.9	12	0.04
1.0 [d]	2	0.02508 (5)	0.1249 (3)	178.399 (1)	0.0331 (2)	0.1	1.8	0.05
	3	0.02501 (5)	0.1180 (5)	191.545 (9)	0.0396 (5)	0.1	1.9	0.05

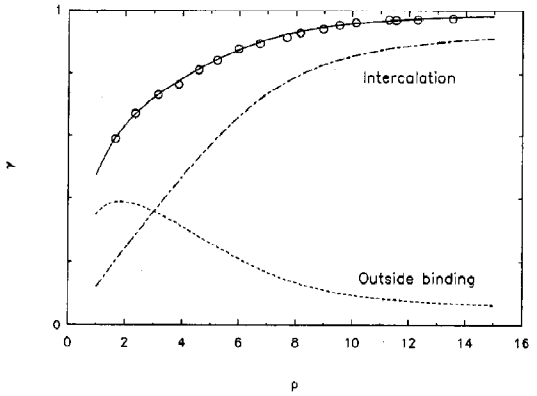

Fig. 1. Results for H_2TMPyP-4 binding in a $\mu = 0.196$ M buffer. Open circles are the experimental points converted from the data given by Fiel et al. [1] in the form of a Scatchard plot. Solid line is the best fit calculated using the model proposed in this paper. Optimum parameters: n, 2; g/N, 0.13; s_0, 1.79; β, 26.7; σ, 0.14. Total porphyrin concentration: 4.7 μM.

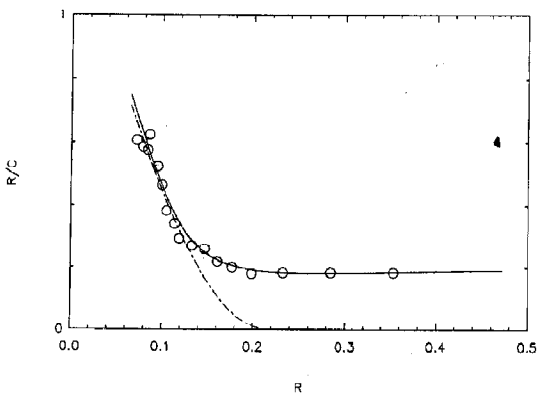

Fig. 2. Scatchard plot for H_2TMPyP-4 binding in a $\mu = 0.196$ M buffer [1]. Solid line represents the best fit using the model presented in this article. Dash-dotted line represents the best fit using the McGhee and Von Hippel model [11]. Optimum binding parameters used to produce the best fit with the present model are the same as those used in fig. 1. Optimum binding parameters from the McGhee and Von Hippel model: $n = 4.3$, $K_{app} = 1.3 \times 10^6$ M^{-1}.

for H_2TMPyP-4 binding in buffers of similar ionic strengths ($\mu \sim 0.2$ M).

Only small n values, 2 and 3, have been used to fit the experimental data. The choice is based upon an analysis of the results obtained by Ford et al. [21] for H_2TMPyP-4 footprinting. Two DNA segments, of 166 and 102 base-pairs, respectively, were used in their experiment and each produced a differential cleavage plot. Each base-pair within the DNA segments has its corresponding value on the plot. The values run from -2 to 2, and positive values indicate relative enhancement while negative values represent blockage of DNA cleavage at corresponding base-pairs by DNase I. By assuming that a base-pair is protected when its value on the differential cleavage plot falls below -1, we have determined the sizes and the frequencies of occurrence of the protected sites. The results are shown in table 4. It can be seen from table 4 that more than 70% of protected sites consist of two or three base-pairs. Larger sites

Table 3

Optimum binding parameters and their uncertainty for Cu(II)TMPyP-3 binding to calf thymus DNA

Data obtained in $\mu = 0.2$ M buffer are weighted by their relative experimental uncertainty in the fitting. The model does not fit satisfactorily the data obtained in the buffer of high ionic strength with g/N values calculated according to eq. 1. For additional details see legend to table 1.

μ (M)	n	g/N	s_0	β	σ	$K^{\#}$ ($\times 10^5$) (M^{-1})	$\beta K^{\#}$ ($\times 10^6$) (M^{-1})	χ^2 ($\times 10^{-4}$)
0.2	2	0.1	0.07 (4)	80.4 (3)	0.60 (4)	8.4	67	1.8
	2	0.031 (9)	0.26 (2)	78.9 (1)	0.68 (2)	3.6	28	2.5
	3	0.0313	0.354 (5)	81.51 (3)	0.32 (1)	2.3	12	1.2
	3	0.029 (8)	0.35 (1)	85.1 (2)	0.34 (2)	2.4	20	1.2
1.0 [d]	2	0.01315 (2)	0.2005 (7)	340.117 (8)	0.0167 (9)	0.02	0.68	0.4
	2	0.01312 (1)	0.1995 (2)	352.479 (4)	0.0172 (2)	0.02	0.70	0.4

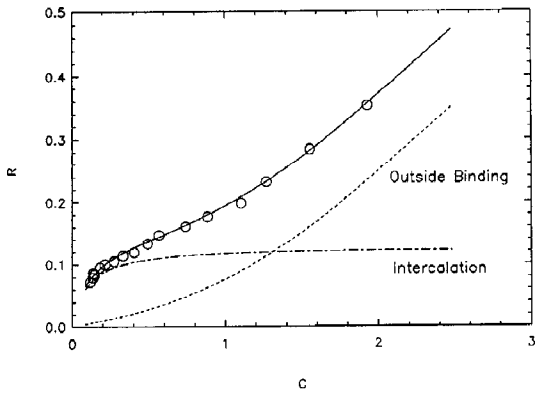

Fig. 3. Binding isotherm for H_2TMPyP-4 binding in a $\mu = 0.196$ M buffer [1]. Optimum binding parameters used to produce the best fit are the same as those used in fig. 1.

might also result from adjacent smaller sites. Although the base sequences of the larger sites cannot be matched exactly by putting smaller sites together, one could argue that since short DNA segments were used, there could be yet other possible sequences for the smaller sites but which are not present in the DNA segments used.

When g/N is fixed, the quality of fitting using $n = 2$ is consistently better than that using $n = 3$ for H_2TMPyP-4. When $n = 3$ and $g/N = 0.0313$, χ^2 for the best fit ranges from 2 to about 50 times

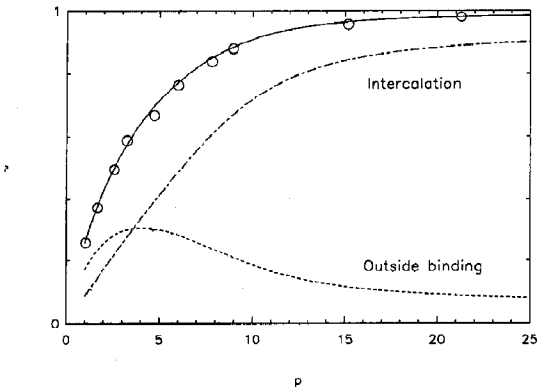

Fig. 4. Results for H_2TMPyP-4 binding in a $\mu = 0.2$ M buffer. Open circles are the experimental points converted from the data given by Pasternack et al. [3] in the form of a Scatchard plot. Solid line is the best fit calculated using the model proposed in this paper. Optimum parameters: n, 2; g/N, 0.09; s_0, 0.54; β, 82.6; σ, 0.42. Total porphyrin concentration: 3.3 μM. $\epsilon_b = 1.2 \times 10^5$ $M^{-1}cm^{-1}$.

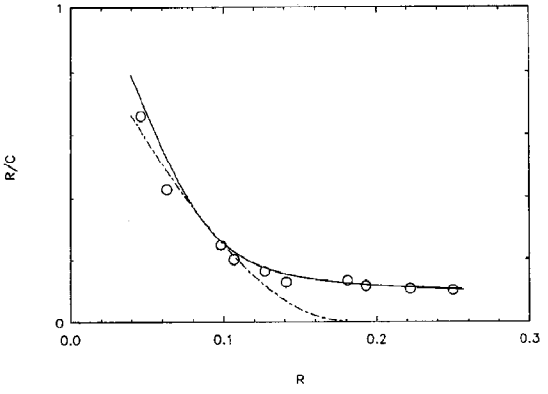

Fig. 5. Results for H_2TMPyP-4 binding in a $\mu = 0.2$ M buffer [3]. Solid line represents the best fit using the model in this article. Dash-dotted line represents the best fit using the McGhee and Von Hippel model [11]. Optimum binding parameters used to produce the best fit with the present model are the same as those used in fig. 4. Optimum binding parameters for McGhee and Von Hippel model: $n = 4.9$, $K_{app} = 1.0 \times 10^6$ M^{-1}.

as large as those obtained using $n = 2$ and $g/N = 0.1$ and the visual fit of calculated fraction of bound porphyrin to the experimental data is poor (results not shown). This observation is consistent with the result obtained by fitting data for H_2TMPyP-4 binding to poly(dG · dC) with the McGhee and Von Hippel model, in which case

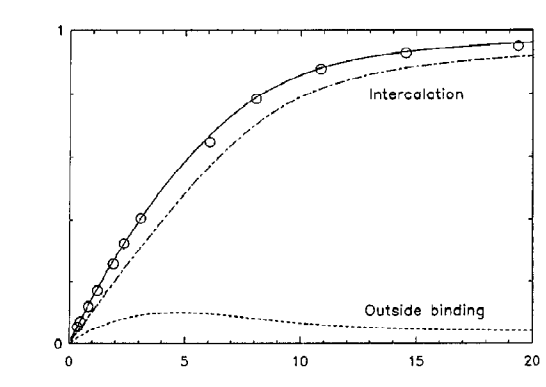

Fig. 6. Results for H_2TMPyP-4 binding in a $\mu = 1.0$ M buffer. Open circles are the experimental points used by Dougherty et al. [5]. Solid line is the best fit calculated using the model proposed in this paper. Optimum parameters: n, 2; g/N, 0.11; s_0, 0.19; β, 97.7; σ, 0.29. Total porphyrin concentration: 1.0 μM.

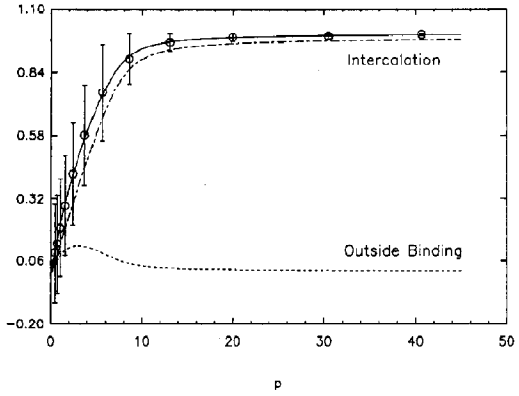

Fig. 7. Results for Cu(II)TMPyP-4 binding in a $\mu = 0.2$ M buffer. Open circles are the experimental points. Solid line is the best fit calculated using the proposed model. Optimum parameters: n, 3; g/N, 0.13; s_0, 0.54; β, 65; σ, 0.15. Total porphyrin concentration: 0.46 μM.

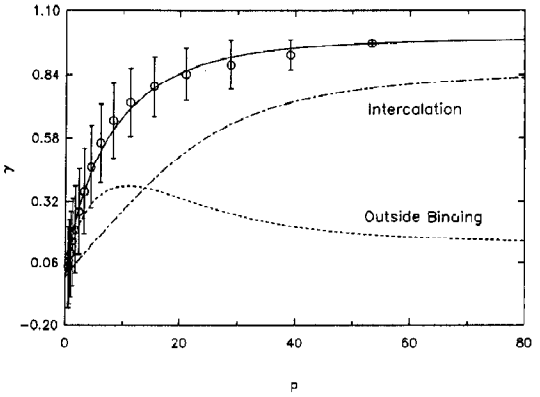

Fig. 9. Results for Cu(II)TMPyP-3 binding in a $\mu = 0.2$ M buffer. Open circles are the experimental points. Solid line is the best fit calculated using the proposed model. Optimum parameters: n, 3; g/N, 0.03; s_0, 0.35; β, 82; σ, 0.32. Total porphyrin concentration: 0.49 μM.

only intercalation occurs and a good fit is obtained when $n = 2$ [3]. The same conclusion is reached for Cu(II)TMPyP-4 binding in the low ionic strength buffer. However, for Cu(II)TMPyP-4 binding in the high ionic strength buffer and for Cu(II)TMPyP-3 in both the high and low ionic strength buffers, the trend is not so obvious. The model cannot satisfactorily fit data for Cu(II)TMPyP-3 and Cu(II)TMPyP-4 binding in the high ionic strength buffer using g/N values calculated according to eq. 1.

When g/N is varied as an independent parameter, its optimum value is close to 0.1 for H_2TMPyP-4 binding in both the high and low ionic strength buffers, and for Cu(II)TMPyP-4 binding in the low ionic strength buffer. The optimum values of g/N for Cu(II)TMPyP-4 binding in the high ionic strength buffer and for Cu(II)TMPyP-3 are far smaller. Similar optimum g/N values are obtained for both $n = 2$ and $n = 3$,

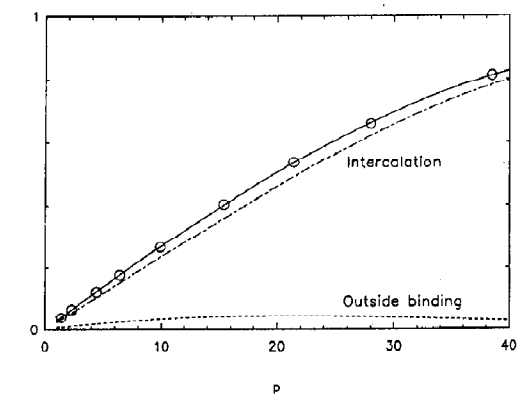

Fig. 8. Results for Cu(II)TMPyP-4 binding in a $\mu = 1.0$ M buffer. Open circles are the experimental points used by Dougherty et al. [5]. Solid line is the best fit calculated using the proposed model. Optimum parameters: n, 2; g/N, 0.03; s_0, 0.12; β, 178; σ, 0.03. Total porphyrin concentration: 1.0 μM.

Table 4

Numbers and sizes of protected sites in H_2TMPyP-4 footprinting

A base-pair is considered as being protected when its corresponding value on the differential cleavage plot is less than −1. The original differential cleavage plot is published in ref. 21.

166 bp *ptyr*2 DNA fragment (total no. of protected sites, 11; total no. of protected bp, 43)			102 bp *Aat*II-*Hind*III DNA fragment (total no. of protected sites, 10; total no. of protected bp, 35)		
Size	Number	Frequency (%)	Size	Number	Frequency (%)
2	5	45	2	4	40
3	3	27	3	4	40
6	2	18	4	1	10
12	1	9	11	1	10

suggesting that the model is more sensitive to the changes in g/N than to those in n.

The values $K^{\sharp} = \sigma s_0/L_0$ listed in the tables is the binding constant for an isolated outside bound porphyrin [22]. No error for $K^{\sharp}$ is given because no information is available on the uncertainties in L_0. It is obvious that $K^{\sharp}$ is smaller for Cu(II) TMPyP-4 and Cu(II)TMPyP-3 binding in the high ionic strength buffer. It does not change significantly for H_2TMPyP-4 binding in buffers of different ionic strengths.

The values of σ, except for the less reliable ones such as those obtained when $n = 3$ and $g/N = 0.0313$ for H_2TMPyP-4, are all less than or close to unity, indicating that the outside binding is positively cooperative.

Of particular interest are the results shown by plotting fractions of intercalated and outside bound porphyrins as functions of p (the input DNA base-pair to porphyrin concentration ratio). In many cases, the plots show an initial increase in γ_0 (the fraction of outside bound porphyrin) as p increases. The value of γ_0 reaches a maximum at certain value of p and then decreases as p increases further.

This change in the fraction of outside bound porphyrins can be explained as an interplay between the different binding strengths and availability of sites for the two binding modes. Compared to outside binding, intercalation has a larger binding energy but fewer binding sites. In the limit of small p, binding sites for both modes are limited. An increase in p will lead to an increase in the overall number of binding sites and, therefore, to an increase in the fractions of both intercalated and outside bound porphyrin. Further increase in p will provide additional binding sites and lead to further binding of porphyrin, until a stage is reached when sufficient binding sites are available, mostly for outside binding that they no longer place a limit on the binding. It is then that the maximum outside binding occurs. Any further increase in p from this point will make more intercalating sites available and lead to more intercalated porphyrin molecules. Since the total number of porphyrins is fixed, this increase in the number of intercalated porphyrin molecules will inevitably result in a decrease in the number of outside bound porphyrin molecules. At the limit of very large p, when sufficient sites for both binding modes are available, most porphyrins will be intercalated, since intercalation has a greater binding strength than outside binding.

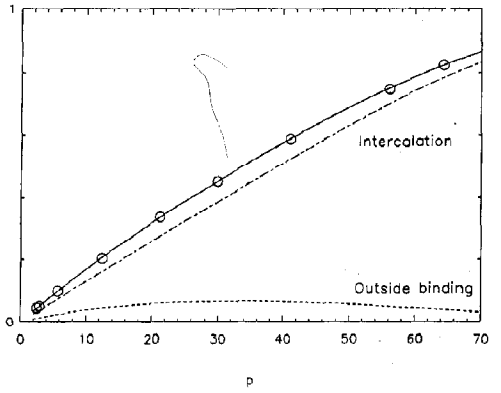

Fig. 10. Results for Cu(II)TMPyP-3 binding in a $\mu = 1.0$ M buffer. Open circles are the experimental points used by Dougherty et al. [5]. Solid line is the best fit calculated using the proposed model. Optimum parameters: n, 2; g/N, 0.01; s_0, 0.20; β, 340; σ, 0.02. Total porphyrin concentration: 1.0 μM.

It is noteworthy that for the binding of all three porphyrins in the higher ionic strength buffer, the maximum fraction of the outside bound porphyrins is not only smaller, but also occurs at larger p (figs 6, 8 and 10), compared with binding in the low ionic strength buffer (figs 1, 4, 7 and 9). This observation corroborates the expectation that high ionic strength buffers reduce the strength of outside binding. The reduced binding strength will decrease the number of outside bound porphyrin molecules as well as shift the equilibrium towards the free porphyrin. To reach the maximum, more binding sites are needed to compensate for the shifted equilibrium, which results in a maximum occurring at a higher p value.

The argument above is supported by the observation that the value of β, which is a function of the relative strength of intercalation to outside binding, increases for all three porphyrins binding in the high ionic strength buffer. This can be interpreted as resulting from weaker outside binding in such a buffer.

6. Discussion

For two-mode binding between porphyrin and natural DNA, knowledge of the strength of binding, the number of binding sites and the degree of the interaction between bound porphyrin molecules is essential in determining the number of porphyrin molecules bound in each binding mode. Since a constraint is imposed on the total number of potential binding sites by the total number of available base-pairs, four independent parameters are needed to characterize the system, such as s_0, β, g/N and σ used in this paper. The number of base-pairs 'covered' by a bound porphyrin, or the size of a binding site, affects the equilibrium binding only through its effect on the number of potential binding sites 'seen' by a free porphyrin. If the number of available binding sites is estimated correctly, a small change in the size of binding sites would produce little change in the final bound porphyrin distribution between the two binding modes. This is why the model is more sensitive to the change in g/N than that in n. The number of parameters involved in the proposed model is actually the minimum that is needed to characterize this two-mode binding system.

Strictly speaking, the assumption that an outside binding site consists of only one base-pair restricts the application of the proposed model to 'edge on' binding of porphyrins. Such a binding geometry has been suggested by Banville et al. [25]. Alternative geometries, for example a 'face on' binding, call for corresponding modifications of the model such as to take into account the neighbour exclusive effect, which results from the fact one face on binding site requires more than one base-pair. This modification can be achieved by following the same line of argument as used to derive the current model, but, as mentioned earlier, this modification will only change the number of potential binding sites for outside binding and have less impact on the qualitative predictions of the model. A further complication would be introduced if one has to consider the possible change of edge on to face on binding. In this case, the model will no longer be a two-mode but a three-mode binding model, leading inevitably to the introduction of more parameters and to less definitive results.

Another assumption of the model is that all sites occupied by intercalated porphyrins have the same statistical weight. This assumption will hold only if the intercalation is exclusively specific to a unique base sequence. Marzilli et al. [10] have concluded that H_2TMPyP-4 intercalation does require such a unique site, 5'CG3', based on NMR studies on H_2TMPyP-4 binding to poly[d(G-C)$_2$] and five other oligonucleotides. However, a footprinting experiment on H_2TMPyP-4 binding to natural DNA failed to reach same conclusion [21], although the result is not conclusive since footprinting experiments cannot really distinguish between binding in different modes. A molecular modelling study shows two partly intercalated positions for H_2TMPyP-4 in a TA site [7]. One such position has a total interaction energy only 6% (~ 7 kcal/mol^{-1}) higher than that of a conventional fully intercalated position in a CG site. If porphyrins do not have unique intercalating sites, but the total interaction energy does not differ greatly for a porphyrin intercalating into different sites, then the complexes formed by porphyrin molecules at those sites should have similar statistical weights. For such cases, the model will still be valid except that s_i' will be an average statistical weight over all different types of intercalating sites, and g/N will be the frequency of occurrence for such intercalating sites to occur in a DNA molecule possessing N base-pairs. It is noteworthy that when g/N is allowed to vary independently, the optimum value obtained for H_2TMPyP-4 binding is fairly close to 10%. H_2TMPyP-4 footprinting shows that protected sites occur at a similar frequency (see table 4). Kinetic studies of a different CG preferring intercalator, actinomycin, also show that the preferred sites constitute about 10% of the total sites [26].

Compared with intercalation, outside binding of porphyrins to DNA is less well-defined. Fiel et al. in their early work [1] suggested there might be three binding modes: one due to intercalation and the other two due to outside binding. Later studies suggest that the major outside binding, which pro-

duces a positive visible band in the CD spectra, occurs preferentially in AT-rich regions of DNA [2,3]. This evidence, combined with the facts that molecular modelling with H_2TMPyP-4 and an AT dinucleotide showed two partly intercalated low-energy positions and that a footprinting experiment with H_2TMPyP-4 showed both protected CG-rich and AT-rich regions, makes one wonder whether this partly intercalated outside binding should be classified as another intercalating mode, as opposed to the non-specific electrostatic binding or outside binding as used in this paper.

The possible reclassification of the major outside binding mode, which prefers AT-rich regions, into a partial intercalation might also solve the dilemma regarding the dependence of outside binding on the ionic strength of the buffer. A purely electrostatic interaction should be weakened in a buffer of high ionic strength, because the presence of a large amount of Na^+ screens the electrostatic interaction in a manner like the Debye-Hückel effect and thus reduces the strength of the interaction. Comparison of figs 1, 3, 5 and 7 with figs 4, 6 and 8 in this paper leads to the same conclusion. EPR experiments also showed evidence of suppressed outside binding in high ionic strength buffers [5]. Other experiments have shown that this major outside binding mode is actually enhanced in a high ionic strength buffer [2,6], but this is hard to explain if electrostatic attraction is still considered as the predominant binding mechanism.

The conclusion that outside binding is positively cooperative is drawn from observations that all reliable values of σ are less than or close to unity. One set of H_2TMPyP-4 binding data due to Pasternack et al. showed a humped Scatchard plot (ref. 3; fig. 5). Humped Scatchard plots have been interpreted in the past as a sign of positive cooperativity for several drugs, such as adriamycin and daunorubin [27], DHAQ [28] and *m*-AMSA [29]. The interpretation is based on a two-mode binding model proposed by Rosenberg et al. [28], which assumes that there are two independent types of binding sites. Rosenberg et al. used eq. 15 in McGhee and Von Hippel's model [11] for each type of sites and fitted experimental data using a sum of two such equations. They obtained satisfactory fitting in the large p regions and drew their conclusions from the binding parameters thus obtained. There is, however, a major flaw in their treatment: that eq. 10 and subsequently eq. 15 in McGhee and Von Hippel's paper [11] are derived under the assumption of single-mode binding to a homogeneous lattice. If this assumption had been lifted, neither equation would retain its current form. The sum of two McGhee and Von Hippel equations actually gives a result for two independent types of lattices rather than for that of binding sites as suggested by fig. 2 in the article of Rosenberg et al.

The introduction of E_{av} into calculating the partition function is an approximation made to include the nearest-neighbour interaction of intercalated porphyrins within the framework of the model and to keep the results in the simplest possible form. The approximation is justified when the E_{av} is small in comparison with the ΔG_i, the change of Gibbs free energy due to intercalation. The consequences of the approximation are that the probability is slightly overestimated for intercalated porphyrins adjacent to no more than one outside bound porphyrin, and it is slightly underestimated for intercalated porphyrins flanked by two outside bound porphyrins. When the interaction energy between the adjacent bound porphyrins is negligible compared with ΔG_i and ΔG_o, the partition function will reduce to that given by eq. 8 and the predictions become accurate.

A comparison between the binding constants obtained using the present model and that of McGhee and Von Hippel, although not entirely appropriate given the assumptions and approximations involved in these two models, is still capable of shedding some light on the binding problem. It can be seen from tables 1–3 that the value obtained for $\beta K^{\sharp}$, the binding constant for the process in which porphyrins intercalate in DNA and interact with their nearest neighbours with an average energy E_{av}, is generally greater than the value of K_{app}, interpreted in the McGhee and Von Hippel model [11] as the binding constant for the process in which porphyrins intercalate into DNA without any neighbour interaction ($K_{app} = 1.5 \times 10^6$ and 1.45×10^5 M^{-1} for

H_2TMPyP-4 binding in 0.2 and 1.0 M ionic strength buffers, respectively [3,5]; $K_{app} = 8.0 \times 10^5$ and 5.2×10^5 M^{-1} for Cu(II)TMPyP-4 binding in 0.2 and 1.0 M ionic strength buffers, respectively [3,5]; $K_{app} = 2.8 \times 10^5$ M^{-1} for Cu(II) TMPyP-3 binding in a 1.0 M ionic strength buffer [5]). The values of $K^{\ddagger}$, the binding constant for isolated outside bound porphyrins, are generally smaller than those of K_{app}. These results are qualitatively consistent with the conclusion that intercalation is stronger than the outside binding, but, to what extent the interaction between the intercalated porphyrins and their nearest neighbours contributes to the value of β, and how accurate these values of binding constants are, remain to be tackled by techniques independent of binding models.

Unfortunately, not much independent information is available about outside binding, its geometry and the extent and degree of cooperativity. Even fewer studies have been carried out on interactions between porphyrins bound in different binding modes. This lack of experimental data restricts us from developing a fully quantitative understanding of parameters such as σ and β.

The standard deviations of optimum binding parameters given in tables 1–3 should be interpreted as indications for the quality of fitting. Their values are proportional to the value of χ^2 of the best fit [24]. One could, therefore, obtain very small uncertainties for the optimum binding parameter if the theoretical model fits the experimental data well. However, the uncertainties obtained this way are not 'real' because the accuracy of the original binding data has not been taken into account. Although a detailed investigation cannot be carried out due to lack of detailed information, it is unlikely that the accuracy of the optimum binding parameters obtained from fitting binding data is better than two significant figures, since that usually is the accuracy achieved when determining the total concentration of DNA and porphyrins.

7. Conclusion

Naturally, any theoretical model with its inevitable simplifications cannot fully characterize a complicated real binding system. There is also a limit to the amount of information one can extract from a single type of experiment, namely, the measurement of overall concentration of bound porphyrin under various conditions. Nevertheless, the proposed model does seem to reflect certain essential features of two-mode binding: it describes the influences that the number of binding sites and the strength of binding have upon the equilibrium distribution of the bound porphyrin between the two binding modes. The base sequence specific feature of intercalation is stressed and nearest-neighbour interactions between bound porphyrins is allowed for. The model fits the existing binding data satisfactorily, including some data which had not been adequately fitted previously. It also raises questions concerning the geometry and energetics of outside binding. To clarify these matters requires further work.

Appendix

A Scatchard plot is a plot of r/C vs r, where

$r =$ [bound ligand]/[DNA]

$r/C = r/$[free ligand],

and where square brackets denote concentration. It is very easy to obtain γ and p as functions of r and r/C:

$$\gamma = 1 - \frac{C}{L_0} = 1 - \frac{r}{(r/C)L_0}$$
$$p = \frac{L_0 - C}{rL_0} = \frac{\gamma}{r} = \frac{1}{r}\left(1 - \frac{r}{(r/C)L_0}\right), \qquad \text{(A1)}$$

where L_0 is the total concentration of porphyrin, which is usually fixed in experiments. Eq. A1 provides the basis for transforming data in the form of Scatchard plot and binding isotherm, which is a plot of r vs C, to the form used in the present work and vice versa.

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