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Coordination Chemistry Reviews 249 (2005) 1451–1459
COORDINATION CHEMISTRY REVIEWS
www.elsevier.com/locate/ccr

Review

Understanding binding interactions of cationic porphyrins with B-form DNA

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Received 11 February 2004; accepted 11 November 2004
Available online 5 January 2005

Contents

Abstract

The DNA-binding interactions of 5,10,15,20-tetra(*N*-methylpyridinium-4-yl)porphyrin, herein denoted H_2T4, and related cationic porphyrins have long been of interest because of the potential for therapeutic applications and the novel binding interactions observed with DNA. A brief review of available physical studies reveals that the monomeric porphyrin can bind either externally, or by intercalation, depending on the nature of the DNA substrate. Coulombic interactions, van der Waals' forces, and hydrophobic effects provide for stability of the adduct, while the pyridiniumyl substituents and axial ligands on metalated forms pose important steric constraints. Competitive binding studies involving DNA hairpin substrates reveal that the base composition, not the sequence, dictates the mode of binding. An overarching principle is that relatively rigid stretches of DNA, i.e., runs containing 50% or more G≡C base pairs, do not support high-affinity external binding. Instead, H_2T4 binds by intercalation despite unfavorable steric contacts that arise within the minor groove. The same pattern holds true for Cu(T4) and other metalated forms lacking axial ligands. New results presented include structures of a pair of less bulky, disubstituted porphyrins, H_2D3*n* (5,15-di(3-pyridyl)porphyrin) and H_2D4 (5,15-di(*N*-methylpyridinium-4-yl)porphyrin), both of which intercalate into

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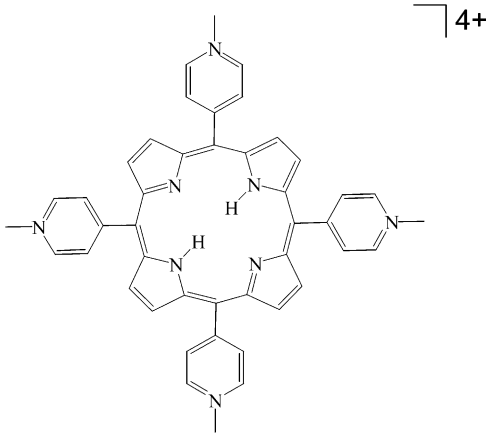
doi:10.1016/j.ccr.2004.11.016

B-form DNA regardless of the base composition. Even the zinc(II) derivatives prove to be good intercalators. A stepwise energy breakdown provides a simple, but effective way to illustrate competing effects that influence the binding.

Keywords: DNA-binding; Cationic porphyrin; Intercalation; External binding; Structure; Emission

1. Introduction

One of the driving forces for the exploration of porphyrins is applicability to photodynamic therapy, a promising non-invasive modality for targeting diseased cells or viruses. The selectivity derives, in part, from the fact that the cytotoxic response extends only to the irradiated cell mass. An added benefit in the case of tumor cells is that they tend to take up the photosensitizer more avidly than normal cells [1–3]. Porphyrins are attractive sensitizers because they absorb well into the red end of the visible spectrum, i.e., at wavelengths that penetrate tissue relatively efficiently [1]. Fiel and co-workers initiated studies of cationic porphyrins, such as H_2T4, and demonstrated that these amphiphilic, water-soluble systems have a natural affinity for a potentially important intracellular target, namely the DNA [4,5]. That early work motivated studies by many other investigators as recounted in several reviews available in the literature [6–9]. Subsequent observations of physiological interest include the fact that cationic porphyrins tend to accumulate in mitochondria [10]. These same porphyrins also act as inhibitors of telomerase, an enzyme that has an important influence on tumor longevity [11], and there are reports of antiviral activity as well [12,13].



H_2T4

2. Outline of DNA binding studies

2.1. Modes of binding

In vitro studies of Fiel and co-workers showed that cationic porphyrins like H_2T4 bind to DNA in multiple ways. For example, high loading of the porphyrin can lead to aggregation/stacking along the surface of the DNA substrate [14]. At lower loading, especially in a region that is rich in adenine–thymine (A=T) base pairs, an individual porphyrin molecule may bind externally to DNA [15]. Intercalation, the third binding motif, is more common in G≡C (guanine–cytosine) rich sequences [5]. Subsequent work has confirmed these ideas [16–18]. Introduction of a metal ion like Ru(II), Co(II), or Mn(III) can change the mode of binding because derivatives with axial ligands are incapable of intercalating between base pairs [18,19]. Early NMR studies of Marzilli and co-workers suggested that the *base sequence* is another important factor determining binding. In particular, the suggestion was that H_2T4 only intercalates in one-step type, namely 5′-CpG-3′ [20], but see below.

Various physical methods are useful for investigating adduct formation, but UV–Vis techniques provide unique insights into the effect that uptake has on the π system of the porphyrin. In the case of H_2T4, external binding has a modest effect on π–π^* absorption in the Soret region such that the absorption maximum exhibits a bathochromic shift of $\Delta\lambda \leq 8$ nm and weak hypochromism ($H \leq 10\%$). External binding also induces a circular dichroism (CD) signal with a positive amplitude [8,18]. Due to more intimate contact with the π system, intercalative binding results in larger spectroscopic perturbations. The Soret band not only shows a larger bathochromic shift ($\Delta\lambda \geq 15$ nm), but stronger hypochromism ($H \geq 35\%$) as well. Another important distinction is that the induced CD signal has a negative amplitude [8,18]. Binding interactions induce smaller, less distinctive shifts in the emission spectra, but the effects on the emission intensities are quite interesting [21]. In studies with H_2T4, Kelly and co-workers noted that external binding to A=T rich sequences induced sharpening of the vibronic structure and an increase in emission intensity by comparison with the unbound form [22]. In contrast, they found that intercalation into a G≡C rich sequence produced weaker emission possibly due to electron-transfer involving guanine, the most reducing of the four bases. With the paramagnetic Cu(T4) system, the results are quite different in that only the intercalated form is emissive [23]. In the presence of copper, the emission stems from a lower-energy, less oxidizing $^3\pi$–π^* excited state, and quenching occurs because of a novel type of exciplex quenching that is normally extremely efficient in coordinating solvents such as water [9,23,24]. The intercalated form of Cu(T4) is emissive because the DNA framework shields the axial coordination sites from attack by Lewis bases. Exciplex quenching remains efficient for externally bound porphyrin, and time-resolved resonance Raman and

excited-state absorption studies have provided valuable insight into the structure of the exciplex(es) formed as well as the reaction dynamics [24–27]. As this brief overview suggests, spectral investigations generally provide a reliable basis for assessing the mode of binding. However, viscometry studies provide corroborative evidence for intercalative binding [28,29].

2.2. Steric considerations

Over the years multiple steric issues have come to light in the quest to understand the DNA-binding interactions of H_2T4 and related porphyrins. Periplanar interactions that keep the pyridiniumyl substituents out of the plane of the porphryin core drew attention first [4]. However, it is now clear that transient structural fluctuations allow B-form DNA to take up various types of so-called threading intercalators that have bulky end groups [30,31]. The uptake may be slow, but the binding constant need not suffer. Later, Neidle and co-workers carried out molecular dynamics calculations which suggested that steric interactions keep H_2T4 from intercalating into a 5′-TpA-3′ step [32]. In particular, they predicted that the problem arises in the major groove where methyl groups on the thymines clash with pyridiniumyl groups of the porphyrin. Later, Williams and co-workers identified what may be the critical steric issue for an intercalated form of the porphyrin. Their structural studies of a Cu(T4) adduct indicated that clashes occur in the minor groove where pyridiniumyl groups have to wedge between the relatively closely spaced sugar-phosphate chains [33].

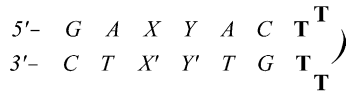
2.3. DNA-binding affinities

Binding constants for cationic porphyrins interacting with DNA are difficult to measure. The major problem is that the binding constants are large, $K \approx 10^6$ to $10^7\ M^{-1}$ if the DNA concentration is in units of base pairs (bp) [17,34]. Measurements involving near stoichiometric levels of porphyrin are therefore critically important, but this is precisely the concentration regime where cooperative binding effects confuse the issue [18,35]. Studies with short oligonucleotides could, in principle, provide insight into the effect the local sequence has on binding; however, the equilibrium concentration of single-stranded forms would be relatively high, and they also bind the porphyrin [36].

McMillin and co-workers have shown, DNA hairpins, or stem-loop structures, provide useful comparisons. With hairpins, formation of a double-stranded stem is extremely favorable, even for stems as short as six base pairs, and the sequence is easy to vary. Base-replacement studies are also straightforward and permit hypothesis testing [21,37]. Binding in the loop region is a potential complication, but this domain is comparatively rigid [38], and base-replacement studies are consistent with the assumption that cationic porphyrins bind almost exclusively in the stem region [39]. Of special note is the observation that hairpin substrates provide a means of determining *relative* binding constants for different sequences [21]. Working with large excesses of the competing hairpins avoids complications associated with cooperative binding effects. The only requirement is that the individual adducts have distinguishable absorption, CD, or emission spectra.

2.4. Base dependence of binding within hairpin stems

Results obtained with a series of hairpin-forming, 16-mer DNA oligonucleotides have been quite informative. In the hairpin depicted, the sequence is 5′-GAXYACTTTTGTY′X′TC-3′, where G, A, T, and C respectively designate fixed



guanine, adenine, thymine, and cytosine residues, where X and Y, and their complements (X′ and Y′) represent variable bases, and where the loop residues appear in bold. The short-hand name $\{XY(T_4)\}$ identifies the bases that reside in positions 3 and 4, as well as the loop sequence, 5′-TTTT-3′ in the case at hand. Thus, $\{CG(T_4)\}$ has X = C, Y = G and X′ = G, Y′ = C in the structure shown, while $\{GC(T_4)\}$has the same 5′-TTTT-3′ loop with X = G, Y = C and X′ = C, Y′ = G in the stem. Previous work demonstrated that the copper porphyrin Cu(T4) intercalates into $\{CG(T_4)\}$, $\{GC(T_4)\}$, as well as $\{AG(T_4)\}$ [39,40], and the same is true for the free porphyrin H_2T4 [21]. In general, tetra-cationic porphyrins of this ilk intercalate into the B-form part of the hairpin as long as the stem contains 50% or more G≡C base pairs, irrespective of the sequence. For each hairpin listed in Table 1, K_{rel} is

Table 1
Binding data for H_2T4 with DNA hairpins in 0.1 M pH 6.8 phosphate buffer[a]

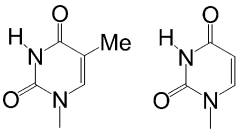
Hairpin[b]	Stem	K_{rel} (M^{-1})
Externally bound H_2T4		
$\{TA(T_4)\}$	5′-GATAAC 3′-CTATTG	1.1[c]
$\{TT(T_4)\}$	5′-GATTAC 3′-CTAATG	0.8
Intercalated H_2T4		
$\{CG(T_4)\}$	5′-GACGAC 3′-CTGCTG	1.0
$\{GC(T_4)\}$	5′-GAGCAC 3′-CTCGTG	1.0
$\{AG(T_4)\}$	5′-GAAGAC 3′-CTTCTG	0.5
12-U-$\{AG(T_4)\}$	5′-GAAGAC 3′-CTTCUG	0.5
14-U-$\{AG(T_4)\}$	5′-GAAGAC 3′-CTUCTG	0.5

[a] Ref. [21].
[b] Each hairpin with a 5′-TTTT-3′ loop sequence.
[c] Constants all relative to that for $\{CG(T_4)\}$.

the binding constant of H_2T4 *relative* to that for binding to {CG(T_4)}. The first thing to note is that, within experimental error, H_2T4 intercalates into {CG(T_4)} and {GC(T_4)} with the *same* affinity. Intercalation does not even require contiguous G≡C base pairs because H_2T4 intercalates into the {AG(T_4)} hairpin, albeit with a reduced binding constant. So far, there are no definitive data establishing the exact locus of binding within the stem. However, absorbance, CD, and emission data are all consistent with interaction involving the center of the stem, i.e., in the vicinity of positions 3, 4, 13, and 14 in the hairpins utilized [21].

2.5. Base replacement studies

Uridine-for-thymine replacement studies allow for a test of the prediction that steric clashes involving the methyl groups on the thymine residues disfavor intercalation between A=T base pairs. In fact, competition studies show that H_2T4 binds

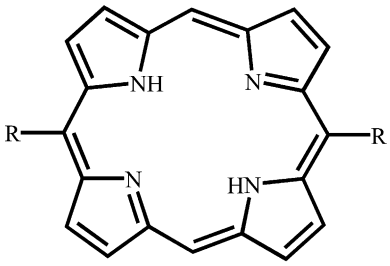


externally to the {TA(T_4)} hairpin *as well as the analogue with U in place of T in each position of the stem* [21]. Clearly, the predicted steric interactions with the thymine methyls are not solely responsible for the mode of binding, because H_2T4 binds externally even with uridine residues in the stem. Nevertheless, the steric interactions could still be destabilizing for the intercalated form. On the assumption that H_2T4 intercalates next to the guanine base in position 4 of the hairpin {AG(T_4)}, further tests of the steric effects are possible. The experiment involves the base-replacement derivatives 12-U-{AG(T_4)} and 14-U-{AG(T_4)} which contain a uridine in place of the thymine in positions 12 and 14, respectively, on either side of the central G≡C base pair. Competition studies show that there is, in fact, no measurable difference in the binding constant for intercalation of H_2T4 into {AG(T_4)}, 12-U-{AG(T_4)}, or 14-U-{AG(T_4)}, so there is just no indication of a significant steric interaction involving the thymine methyl group [21]. As reported by Williams and co-workers, the steric problems associated with intercalation most likely arise in the minor groove [33]. The studies described next address this problem by utilizing porphyrins bearing fewer bulky aryl substituents.

3. 5,15-Dipyridiniumylporphyrins

3.1. Synthesis

The synthesis of di-substituted porphyrins is difficult by comparison with the more symmetric tetra-substituted analogues discussed to this point. For simple diaryl derivatives,



H_2D3n: R = 3-pyridyl
H_2D3: R = N-methylpyridinium-3-yl
H_2D4: R = N-methylpyridinium-4-yl

such as 5,15-diphenylporphyrin, acid-catalyzed condensation of dipyrromethane with benzaldehyde in dry dichloromethane yields the porphyrinogen, which converts to porphyrin upon oxidation by a quinone [41]. However, this procedure has not proved to be practical for syntheses involving pyridine carboxaldehydes, even with pyrroles [42,43]. Under Adler-like conditions [44], the synthesis goes better, but by ordinary standards the yield is still very poor, typically less than 4% [45]. Nevertheless, the yields are workable, and treatments with standard alkylating agents produce the derivatives of interest: H_2D3 = 5,15-di(*N*-methylpyridinium-3-yl)porphyrin and H_2D4 = 5,15-di(*N*-methylpyridinium-4-yl)porphyrin. Structures described below establish the authenticity of the new porphyrins. Finally, incubation in a Tris buffer containing a slight excess of a soluble zinc(II) salt gives the corresponding zinc derivatives Zn(D3) and Zn(D4).

3.2. Structural studies

The diffractometer was a Nonius KappaCCD, and the X-ray source was a rotating anode that yields Mo Kα radiation (λ = 0.71073 Å). Direct methods implemented with the SIR 97 program [46] located most atoms, and others appeared in succeeding difference Fourier maps. The refinement, by the SHELX-97 package [47], included hydrogen atoms but with the constraint that they remain bonded to pre-assigned neighbor atoms.

Blue needles of the neutral porphyrin H_2D3n ($C_{30}H_{20}N_6$ = 5,15-di(3-pyridyl)porphyrin) deposited from a 15:85 (v/v) mixture of dichloromethane and cyclohexane on cooling. The crystal chosen for structure determination had approximate dimensions 0.35 mm × 0.17 mm × 0.17 mm. For the other X-ray study, slow evaporation of a 15:10 (v/v mixture of methanol and *n*-propanol produced purple plates of $C_{47}H_{44}N_6O_7S_2$ ($[H_2D4](PTS)_2{\cdot}CH_3OH$, where PTS denotes the *p*-tolyl-sulfonate anion). The crystal selected for X-ray studies had approximate dimensions 0.35 mm × 0.30 mm × 0.05 mm.

All unit cell parameters appear in Tables 2 and 3 reports mean bond distances.

In the lattice, the neutral H_2D3n molecule sits on a center of inversion. See Fig. 1 for an ORTEP drawing of the

Table 2
Crystallographic data

	H_2D3n	$[H_2D4](PTS)_2{\cdot}CH_3OH$
Formula	$C_{30}H_{20}N_6$	$C_{47}H_{44}N_6O_7S_2$
fw	464.53	869.04
Crystal system	Triclinic	Monoclinic
Space group	$P\bar{1}$	$P2_1/c$
a (Å)	6.719(1)	27.9104(13)
b (Å)	9.082(1)	8.8451(4)
c (Å)	9.393(1)	17.3176(6)
α (°)	84.61(1)	
β (°)	77.88(1)	105.3291(112)
γ (°)	78.67(0)	
V (Å)^3	548.6	4123.1(3)
Z	1	4
T (K)	150(1)	150(1)
$\lambda_{Mo\ K\alpha}$ (Å)	0.71073	0.71073
ρ_{calcd} (g cm^{-3})	1.41	1.40
μ (mm^{-1}) (average)	0.940	0.932
Reflns colld	4609	21798
Unique reflns	2376	3806
$R1$ [$I > 2\sigma(I)$][a]	0.054	0.100
$wR2$ [$I > 2\sigma(I)$][b]	0.129	0.188

[a] $R1 = \sum |F_o - F_c| / \sum F_o$.

[b] $wR2 = \left(\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2 \right)^{1/2}$.

Table 3
Average bond distances[a] (±0.01 Å) in selected porphyrins

Bond	H_2D3n	$[H_2D4](PTS)_2{\cdot}CH_3OH$	$[H_2T4](PTS)_2$[b]
C_β—C_β	1.35	1.34	1.35
N—C_α	1.37	1.37	1.37
C_α—C_m	1.40	1.39	1.39
C_α—C_β	1.44	1.44	1.44

[a] C_α connects to N and C_β within a pyrrole ring and a C_m atom without.
[b] Ref. [32].

H_2D3n molecule, the porphyrin core of which is approximately planar. The four nitrogen atoms deviate most as they alternate above and below the mean plane of the ligand. More specifically, the imine nitrogen atoms fall ±0.060 Å out of the plane, while the corresponding displacements are ±0.089 Å for the NH nitrogen atoms. The mean plane of each pyridine substituent meets the mean plane the porphyrin at a dihedral angle of 60.5°.

In the $[H_2D4](PTS)_2{\cdot}CH_3OH$ crystal, the porphyrin sits on a general position, and the ORTEP representation of the porphyrin part of the asymmetric unit appears in Fig. 2. Again the porphyrin is essentially planar, but there is a hint of a ruffling distortion in that alternating pyrrole rings cant in opposite directions relative to the mean plane of the porphyrin. Thus, C5 and C24 rise above the mean plane while C6 and

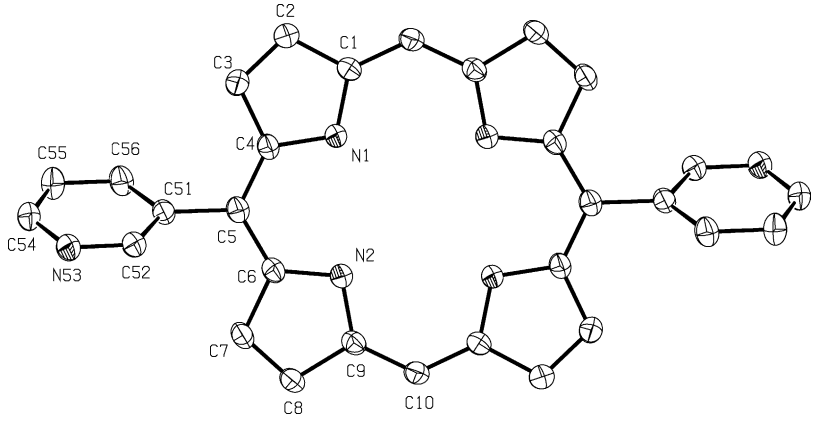


Fig. 1. ORTEP drawing of the neutral porphyrin H_2D3n with 50% probability ellipsoids and the crystallographic numbering scheme.

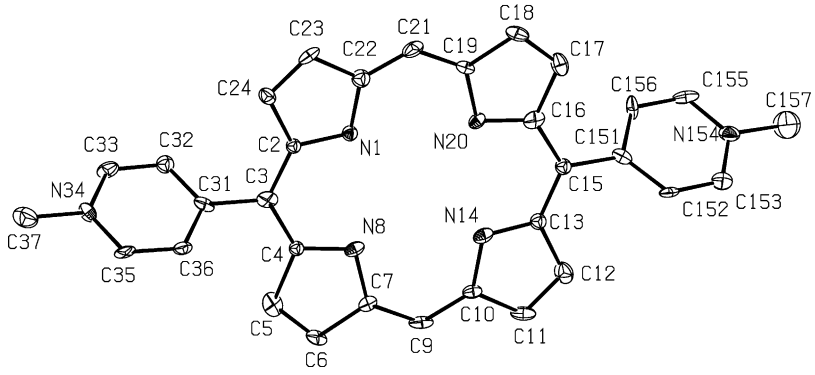


Fig. 2. ORTEP drawing of dicationic H_2D4 with 50% probability ellipsoids and the crystallographic atom-numbering scheme.

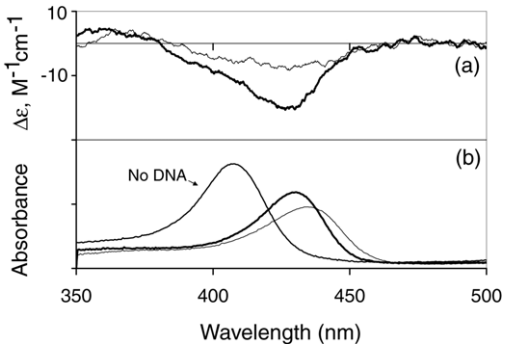


Fig. 3. (a) Absorption spectrum of H_2D4 in buffer, bound to $[poly(dA\text{-}dT)]_2$ (thick), or bound to $[poly(dG\text{-}dC)]_2$. (b) CD spectrum of H_2D4 bound to $[poly(dA\text{-}dT)]_2$ (thick) or $[poly(dG\text{-}dC)]_2$.

C23 fall below it. The N34 and N154 pyridine rings intersect the porphyrin at dihedral angles of 53.6° and 50.3°, respectively.

3.3. DNA-binding studies with di-substituted porphyrins

Fig. 3 shows absorption and CD data obtained upon exposing H_2D4 to excess $[poly(dG\text{-}dC)]_2$ or $[poly(dA\text{-}dT)]_2$ [45]. In each case, binding induces a substantial shift in the Soret absorption toward longer wavelength, and the induced CD signal is negative. Thus, the spectroscopic data strongly suggest that the dipyridiniumyl porphyrin H_2D4 intercalates into both DNA hosts. Viscometry studies have confirmed this interpretation [45]. As shown in Table 4, the experimental results are completely analogous for the H_2D3 porphyrin as well [48]. More surprisingly, data compiled in the Table 4 show zinc(II) derivatives Zn(D3) and Zn(D4) also intercalate into $[poly(dG\text{-}dC)]_2$ or $[poly(dA\text{-}dT)]_2$ [45,48]. These results are striking because external binding is the norm for the analogous Zn(T4) system, partly because intercalative binding requires the loss of an axial ligand [49]. Although there are structures in which the Zn(II) center is four coordinate and in the plane of a porphyrin [50], the free energy is lower with an axial ligand in place in a coordinating solvent like water. A multi-stage analysis of the interaction energy presented below suggests a possible basis for intercalative binding of the Zn(D3) and Zn(D4) systems.

Table 4
Spectral data for cationic porphyrins binding with DNA in $\mu = 0.1$ M pH 6.8 phosphate buffer (Ref. [45])

DNA	Porphyrin	Soret[a]		CD[b]	
		Δλ (nm)	H (%)	λ (nm)	Δϵ (M^{-1} cm^{-1})
$[poly(dG\text{-}dC)]_2$	H_2T4	23	34	443	−12
	Zn(T4)	0	4	445	4
	H_2D4	27	42	428	−3
	H_2D3	20	36	420br	−3
	Zn(D4)	15	39	436	−3
	Zn(D3)	12	26	416	−3
$[poly(dA\text{-}dT)]_2$	H_2T4	9	−4	430	26
	Zn(T4)	2	−6	424	5
	H_2D4	22	28	427	−20
	H_2D3	16	24	417	−25
	Zn(D4)	12	26	431	−16
	Zn(D3)	4	26	415	−15
$\{CG(T_4)\}$	H_2D4	23	37	422	−10
	H_2D3	19	44	417	−10
$\{TT(T_4)\}$	H_2D4	21	27	423	−17
	H_2D3	17	29	417	−18
$\{TA(T_4)\}$	H_2D4	21	23	426	−17

[a] Δλ is the shift to longer wavelength of the absorption by the adduct, and H (%) the percent decrease in intensity at the absorption maximum.
[b] Circular dichroism induced by adduct formation.

4. Stepwise energy analysis

4.1. Model requirements

Any model that purports to explain the fundamental binding interactions must account for key observations, including those that follow:

(1) The base dependence observed for the binding of H_2T4 as well as metalated forms that do not incorporate axial ligands [16–18,40].
(2) The fact that Zn(T4) almost always adopts an external binding motif and has a comparatively weak affinity for binding to $[poly(dG\text{-}dC)]_2$ host [16].
(3) The contrasting behavior of the dipyridiumyl derivative Zn(D4) which strictly intercalates into $[poly(dG\text{-}dC)]_2$ or $[poly(dA\text{-}dT)]_2$ [45,48].

In concrete terms, Eq. (1) provides perhaps the simplest framework capable of rationalizing all of the observations noted. In the equation, ΔG denotes the net interaction energy between the porphyrin and the DNA host,

$$\Delta G = \Delta G_R + \Delta G_B \quad (1)$$

ΔG_R is the combined reorganization energy required for the host and the ligand, and ΔG_B the free energy for the binding of preorganized components [21,45].

4.2. Reorganization energy ΔG_R

For intercalative binding the energy costs include the fact that the DNA molecule has to unwind to some degree in order to create a binding cavity [51]. In addition, the pyridiniumyl substituents undergo some reorientation, and there is the desolvation of the porphyrin. With Zn(T4), the reorganizational energy is still higher because intercalation requires dissociation of an inner-sphere ligand, namely an axially coordinated water molecule. In the absence of information about structure, the reorganizational energy requirements for external binding are much less certain. There is a crystal structure available in the literature [52], but the relevance to solution is doubtful

because the porphyrin acts as a bridge between two duplexes in the crystal lattice. The fact that Zn(T4) has a low affinity for external binding to [poly(dG-dC)]$_2$ is probably the most telling observation [16], and the implication is that [poly(dG-dC)]$_2$ actually *resists* external binding [45]. This hypothesis is consistent with suggestions that external binding of porphyrins like H_2T4 entails partial melting and/or contortion of the canonical DNA double helical structure [35,39]. Significant deformation of the double helix is much more difficult when large numbers of G≡C base pairs are present because of the more extensive hydrogen bonding.

4.3. Binding energy ΔG_B

Favorable interactions between the porphyrin and the DNA host ultimately drive the uptake of the ligand, in spite of the reorganizational energy requirements and repulsive effects that moderate the overall free energy change [21,45]. Optimization of the coulombic contacts with the DNA sugar-phosphate backbone presumably provides a major part of the driving force for external binding. For intercalation of an amphiphilic porphyrin like H_2T4, the hydrophobic effect, van der Waals' interactions with the DNA bases, and coulombic interactions involving the positively charged pyridiniumyl substituents are all factors promoting uptake. However, there are indications that steric strain associated with introducing two bulky substituents into the minor groove detracts from ΔG_B for intercalative binding [33].

4.4. Comparison of H_2T4 and Zn(T4)

Fig. 4 provides a pictorial representation of the key factors that influence the binding of H_2T4 to [poly(dA-dT)]$_2$. In the figure, P + DNA designates the unbound state, and the P^i or P^e (DNA^i or DNA^e) symbols designate porphyrin (DNA) preorganized for formation of either the intercalated adduct P^i/DNA^i or the externally bound form P^e/DNA^e. The reorganization energy is much greater for external binding due to the need for partial melting of the DNA double helix. However, the deformation provides a very favorable binding pocket so that the ΔG^e_B term is exoergic enough for external binding with [poly(dA-dT)]$_2$ to end up being the preferred motif. With [poly(dG-dC)]$_2$, on the other hand, the reorganization energy required for producing a comparable binding pocket is much higher, and the energy balance favors intercalative binding in spite of strain generated in the minor groove. For the interaction of Zn(T4) with [poly(dG-dC)]$_2$, the situation is more complicated. Intercalation is a less attractive option because the need to dissociate the axial water ligand raises the ΔG^i_R term. In this case, the reorganizational energy poses a major penalty for either mode of uptake, and the affinity for [poly(dG-dC)]$_2$ is low. Under most conditions Zn(T4) binds externally, but there is evidence that intercalative binding can occur at relatively low ionic strengths [53].

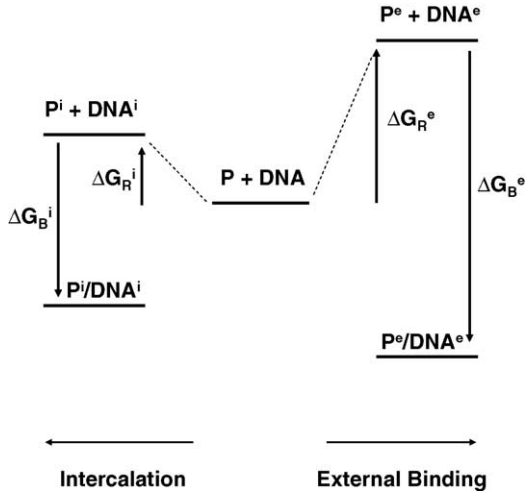


Fig. 4. Qualitative energy diagram for the competitive binding of H_2T4 to [poly(dA-dT)]$_2$. $\Delta G^e_R(\Delta G^i_R)$ denotes the reorganization energy for external (intercalative) binding, while $\Delta G^e_B(\Delta G^i_B)$ represents the binding energy for preorganized species. P + DNA designates the free components, and a slash symbol denotes adduct formation.

4.5. Contrasting cases of H_2D4 and Zn(D4)

The same framework is useful for assessing why the dipyridiniumyl porphyrin H_2D4 binds into B-form DNA as an intercalator, regardless of the base content. The most obvious explanation is the absence of steric interactions that adversely influence the ΔG_B term for intercalative binding. The more striking effect is that intercalation also becomes the favored binding motif for the zinc(II) derivative, Zn(D4), despite the need for dissociation of an axially bound water. External binding may suffer from the fact that the dipyridiniumyl systems have only half the charge of the H_2T4 system, since coulombic interactions with the sugar-phosphate backbone probably drive external binding. In the case of the H_2D4 system, hydrophobic forces probably play a more prominent role in the binding, and they favor intercalation. Therefore, a 5,15-disubstitution pattern may predispose a porphyrin to intercalative binding for both steric and electronic reasons. Would Zn(D4) bind externally to a sufficiently flexible host? Recent experiments with low-melting hairpin substrates suggest that is the case [54].

5. Vista

Eq. (1) describes a simple model capable of qualitatively rationalizing all results obtained to date. In the model the total binding energy represents a balance between multiple attractive and repulsive factors. The latter includes the resistance to structural reorganization of the DNA double helix, as well as any steric strain associated with the uptake of bulky porphyrin ligands. However, the impact of the steric strain remains somewhat of an open question. In a pioneering X-ray crystallographic study, Williams and co-workers found

that intercalation of Cu(T4) entails the insertion of two *N*-methylpyridinium-4-yl substituents into the minor groove of the double helix and that the substituents experience significant steric interactions with the sugar-phosphate backbone [33]. They proposed that intercalation of the bulky porphyrin only occurs with the extrusion of a base. However, subsequent DNA-binding studies with different hairpin substrates cast doubt on that hypothesis [37]. Later, Galiev and Leontis determined another structure in fluid solution via NMR methods and concluded that the intercalation of H_2T4 had no significant influence on the hydrogen bonding within the binding site [55]. Clearly, questions remain regarding the forces acting between cationic porphyrins and B-form DNA.

As described in this report, one way to approach the problem is to broaden the ligand set and to investigate new types of binding interactions. With the aim of eliminating strain, we developed a series of new 5,15-disubstituted porphyrins. In fact, the H_2D3 and H_2D4 porphyrins turn out to be universal intercalators; even the zinc(II) derivatives intercalate. The results are consistent with the idea that steric strain inhibits the intercalation of bulkier systems like H_2T4. Other disubstituted systems with different steric requirements include H_2D2, the di-(*N*-methylpyridinium-2-yl) analogue [45], as well as ligands involving cationic substituents based on five-membered ring systems [56,57]. More experiments with new porphyrins will help complete the picture, though the balance of electrostatic and hydrophobic interactions will differ depending on the ligand. The charge distribution is yet another variable. For example, a di-substituted derivative can have the charges in adjacent or opposite *meso* positions of the porphyrin ring. The charge distribution influences the trafficking of cationic porphyrins in vivo [58], and it could affect the DNA-binding preferences, too.

Another worthwhile aim would be to determine the energy terms in Eq. (1). For ΔG itself, the most obvious approach would be to measure equilibrium constants as a function of temperature. However, obtaining reliable equilibrium constants can be a challenge. At least two complications occur in the concentration regime which involves comparable amounts of ligand and DNA base pairs in solution. One is the likelihood that cooperative effects will come into play when two or more ligands bind in the same small stretch of DNA base pairs. In addition, the porphyrin is apt to aggregate on the DNA host, in which case the speciation becomes hard to define.

Acknowledgment

The National Science Foundation helped fund this research through grant number CHE 01-08902.

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