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Internal Versus External Binding of Cationic Porphyrins to Single-Stranded DNA

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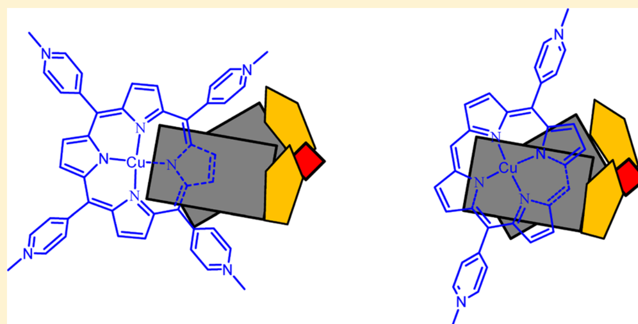
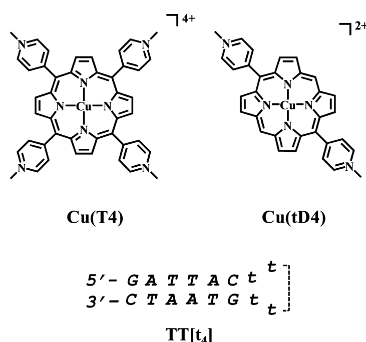
ABSTRACT: Absorbance, induced circular dichroism, and emission studies establish that the tetrasubstituted cationic porphyrin Cu(T4) preferentially binds externally to single-stranded (ss) DNA sequences, except in a purine-rich system like 5′-$(dA)_{10}$-3′ where a degree of internalization occurs. On the other hand, the sterically friendly, disubstituted Cu(*t*D4) system exclusively binds to ss DNA by internalization, that is, pseudointercalation. By and large the results show that double-stranded DNA hosts decisively outcompete more flexible ss hosts for the uptake of a porphyrin, regardless of the binding motif. The findings are relevant because ss domains of DNA appear during replication, in different types of DNA-secondary structure, and as products of the disassembly of multistranded forms.



■ INTRODUCTION

Peripheral substitution has a dramatic impact on the binding of cationic porphyrins to multistranded DNA hosts.[1] Possible applications of these systems in photodynamic therapy and in antibacterial and anticancer regimens help motivate the work.[2,3] Fiel and co-workers introduced the most commonly studied ligand, 5,10,15,20-tetra(*N*-methylpyridinium-4-yl)porphyrin,[4] or H_2T4, depicted in Chart 1 as the copper(II)-containing form Cu(T4). Depending on a number of factors, H_2T4 and its metal-containing forms engage in three different types of binding with double-stranded (ds) B-form DNA: intercalation between base pairs, external or groove binding, and/or outside stacking.[2,3,5] Retaining a bulky, H_2T4-like platform, Marzilli and co-workers have extended the aryl substituents so as to vary the number and location of cationic charge centers.[6] An alternative approach is to reduce the number of substituents and curtail steric demands, albeit at the cost of lowering the charge-derived affinity for DNA.[7−11]

Chart 1



Single-stranded (ss) DNA belongs in the library of hosts because it is present during replication as well as in various types of DNA secondary structure.[12] Binding interactions with ss DNA potentially also affect melting processes and/or encourage the disassembly of multistranded forms. In terms of DNA structures, the ss form is probably the most flexible of all and, as such, provides unrivaled access to individual bases. Previous binding studies with ss DNA have dealt with ligands ranging from acridines[13] and thiazole orange[14] to short peptides[15] and a cationic porphyrin.[5] The ss DNA-binding studies presented herein focus on copper(II)-containing forms of H_2T4 and *trans*-5,15-di(*N*-pyridinium-4-yl)porphyrin, or Cu(*t*D4) in Chart 1. The copper(II) porphyrins are useful because their unique emission properties help establish the binding motif.[7,8,16] More specifically, in order for the copper(II) porphyrin to exhibit luminescence, the host must internalize the ligand and protect the metal center from axial attack of Lewis bases, including water. Pseudointercalation between bases of ss DNA proves to be the preferred binding motif for the Cu(*t*D4) system but impractical for the bulkier form Cu(T4).

■ EXPERIMENTAL SECTION

Materials and Instrumentation. The DNA sequences were products of Integrated DNA Technologies (IDT). The single-base hosts included 5′-$(dA)_{10}$-3′, 5′-$(dC)_{10}$-3′, 5′- $(dT)_{10}$-3′, 5′-$(d\,T)_{16}$-3′, 5′-$(dU)_8$-3′, and 5′-d(AACCAACCAACCAACC)-3′, abbreviated A_{10}, C_{10}, T_{10}, T_{16}, U_8, and $[A_2C_2]_4$, respectively. The abbreviation $A_4C_4A_4$ denotes the mixed-base host 5′-d(AAAACCCCAAAA)-3′. The 16-mer, hairpin-forming sequences were 5′-d(GATTACttttGTAATC)-3′ and 5′-d(GACGACttttGTCGTC)-3′, where the lower-case letters

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designate an internal, loop-forming run of thymines, and the abbreviated names for the hosts are TT[t_4] and CG[t_4], respectively. Silanization solution (5% dichlorodimethylsilanes in *n*-heptane), Trizma HCl, and Trizma Base came from Sigma. Mallinckrodt was the source for potassium chloride (KCl), methanol (MeOH), and hexanes; Koptec supplied ethanol. [Cu(T4)](NO_3)$_4$ and [Cu(tD4)](NO_3)$_2$ were available from previous studies.[17] In terms of equipment, the absorbance spectrophotometer was a Varian Cary 100. A Varian Cary Eclipse with a R3896 phototube detector and a JASCO-J180 unit served as the fluorimeter and spectropolarimeter, respectively. The pH meter was a Fisher Accumet Basic AB15 model.

Methods. Equation 1 was useful for normalizing emission spectra to a common absorbance value,[18] where $I'(\lambda)$ is the adjusted emission intensity, $I_F(\lambda)$ is the measured emission intensity at wavelength λ, and $A(\lambda_{abs})$ is the absorbance at the exciting wavelength.

$$I'(\lambda) = \frac{I_F(\lambda)}{1 - 10^{-A(\lambda_{abs})}} \quad (1)$$

Equation 2 yielded the percent hypochromism, %*H*, where $A(\lambda_0)$ is the maximum absorbance of the free porphyrin and $A(\lambda')$ is the maximum absorbance of the bound form.

$$\%H = \frac{A(\lambda_0) - A(\lambda')}{A(\lambda_0)} \times 100 \quad (2)$$

Equation 3 allowed the conversion of circular dichroism (CD) data to a $\Delta\varepsilon(\lambda)$ representation, where $\theta(\lambda)$ is the recorded value in millidegrees, Q = 32 980 is a conversion factor, l is the path length in cm, and c is the concentration of the absorbing species, porphyrin, or DNA host.

$$\Delta\varepsilon(\lambda) = \frac{\theta(\lambda)}{Qlc} \quad (3)$$

A published method served for silanization of glassware.[19] The solvent used for the stock solution of [Cu(T4)](NO_3)$_4$ was 0.05 M tris(hydroxymethyl)aminomethane (Tris) HCl buffer, while the solvent for the stock solution of [Cu(*t*D4)](NO_3)$_2$ contains 50% by volume methanol. The abbreviations used for the ions in solution are Cu(T4) and Cu(*t*D4). The method used for carrying out spectrophotometric titrations was as before,[17] except here the KCl concentration was 150 mM. The concentration of Cu(T4) was 3 μM versus 2.5 μM for Cu(tD4). Competitive binding studies followed the method of Thomas et al.[20] By design in that experiment, the concentration of each host present is always high enough (5 strands/porphyrin) to take up all of the porphyrin. A comparison of the responses obtained with the mixed-host solution and the two controls, each containing only a single host, yields information about relative binding constants. The total porphyrin concentration was always the same. Overnight incubation at room temperature ensured complete equilibration. Extinction coefficients used for obtaining concentrations appear in Table 1.

Table 1. List of Molar Extinction Coefficients

species	ε(260 nm, M^{-1} cm^{-1})
A_{10}	123 400
C_{10}	72 200
T_{10}	81 600
U_8	57 600
$A_4C_4A_4$	148 600
T_{16}	130 200
$[A_2C_2]_4$	156 800
TT[t_4]	142 000
Cu(T4)[a]	2.31×10^5
Cu(tD4)[b]	1.37×10^5

[a]Wavelength of 424 nm. [b]Wavelength of 410 nm in 50% by volume methanol.

Equation 4 serves for the calculation of the binding-constant ratio, K_H/K_S, where K_H (K_S) is the formation constant for the 1:1 adduct of porphyrin with a ds (ss) host.[20] The definition of R appears in eq 5 where W_H represents the fraction of porphyrin bound to the ds host in a competition experiment. Finally, C_H (C_S) is the concentration of the ds (ss) host in strands per unit volume, and P_T is the total porphyrin concentration in solution.

$$\frac{K_H}{K_S} = R\left[\frac{C_S(1 + R) - P_T}{C_H(1 + R) - RP_T}\right] \quad (4)$$

$$R = \frac{W_H}{(1 - W_H)} \quad (5)$$

RESULTS

With each ss DNA host, uptake of Cu(T4) induces a modest bathochromic shift of 4−6 nm and a very weak emission signal from the porphyrin (Table 2). Results in Figure 1A and Table 2 also reveal the Soret band generally exhibits a *hyperchromic* response, much like one observes with external binding of Cu(T4) to [poly(dA-dT)]$_2$.[2,3,5] The exception involves the A_{10} host, which results in a *hypochromic* response. In all cases the induced circular dichroism (iCD) signals in the Soret region are negative and extremely weak (Figure 2), consistent with what Pasternack et al. reported for Pt(T4) interacting with poly(dA).[5] The results are very different for the uptake of Cu(*t*D4). First, hypochromic responses generated are much larger, ranging from 40 to 80% in the Soret region (Table 2 and Figure 1B), even though the bathochromic shifts remain modest at 6−8 nm. As is evident in Figure 1B, the hypochromic effect is particularly strong when the host is A_{10}. Another marked difference is that the bound forms of Cu(*t*D4) exhibit much stronger emission signals. Figure 3 reveals that binding to A_{10} induces an emission signal from Cu(*t*D4) that is 10 times stronger, per unit absorbance, than that obtained from Cu(T4) under similar conditions. Indeed, the absorbance-corrected emission signal obtained with A_{10} is comparable to those observed from Cu(*t*D4) intercalated into double-stranded DNA hosts (Table 2).[17] Other single-stranded hosts also protect the copper center from attack by Lewis bases but not as effectively. In particular, interaction with U_8 produces an emission signal that is about 50% weaker, while those obtained with C_{10} and T_{10} are of intermediate strength (Table 2). In terms of iCD signals, Figure 2 reveals the uptake of Cu(*t*D4) produces *positive* iCD signals in the Soret region. As recorded in Table 2, the highest amplitude iCD signals occur when the host is T_{10} or C_{10}, while A_{10} generates the weakest response. Another curious finding is that the iCD signal generally maximizes at a longer wavelength than the Soret absorption, by ca. 10 nm.

The data in Table 2 pertain to limiting spectra obtained for 1:1 binding in the presence of excess host, while Figure 4A shows what one finds in a titration of Cu(*t*D4) with the T_{10} host. This system is actually atypical; not surprisingly, the only other system that behaves similarly entails Cu(*t*D4) combining with the T_{16} host. Figure 4B illustrates the pattern of absorbance changes one normally finds when Cu(*t*D4) combines with a ss DNA host. Thus, upon addition of U_8, the system begins to exhibit limiting behavior by the point at which a stoichiometric number of strands is present in solution. In contrast, with T_{10} as the host, the absorption spectrum varies significantly as the DNA-host-to-porphyrin ratio changes. Figure 4A reveals that at low concentrations of host, the shift

Table 2. Physical Data[a] for Cu(T4) and Cu(*t*D4) Interacting with ss DNA Hosts, Except as Noted

porphyrin	DNA	absorbance $\Delta\lambda$, nm[b]	absorbance %H	emission λ_{em}, nm	emission int.	circular dichroism λ_{ext}, nm[c]	circular dichroism $\Delta\varepsilon$, M^{-1} cm^{-1}
Cu(T4)[d]	T_{10}	4	−8	795	0.5	427	−2
	U_8	5	−8	795	0.4	425	−2
	C_{10}	6	0	800	0.4		
	$A_4C_4A_4$	5	7	800	0.5	433	−10
	A_{10}	4	13	803	0.4	433	−6
	T_{16}	4	−18			422	−3
	$[A_2C_2]_4$	4	8	800	0.5	428	−9
	$TT[t_4]$[e]	5	2		<0.5	420	15
	$CG[t_4]$[e]	10	34		2.3	436	−29
Cu(*t*D4)[f]	T_{10}	8	40	795	3.	425	20
	U_8	6	65	810	2.	425	8
	C_{10}	6	55	795	3.	425	16
	$A_4C_4A_4$	11	40	795	3.	435	12
	A_{10}	9	85	820	4.	440	2
	T_{16}	4	13			425	23
	$TT[t_4]$[e]	16	24		3.7	415	−20
	$CG[t_4]$[e]	16	28		4.8	414	−10

[a]Data correspond to limiting spectra obtained at high strand-to-porphyrin ratios. [b]Bathochromic shift in Soret peak. [c]Wavelength where max or min occurs in iCD spectrum. [d]Soret maximum at 424 nm in buffer. [e]Results with DNA hairpin.[17] [f]Soret maximum at 410 nm in 50% MeOH.

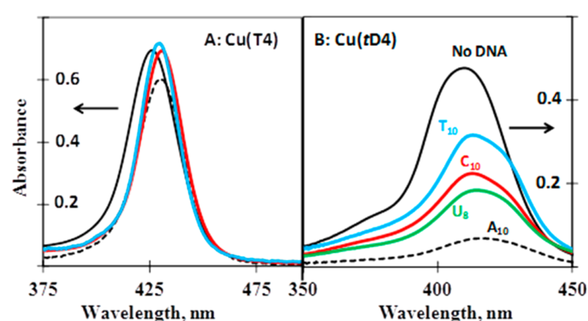

Figure 1. (A) Absorbance of 3.0 μM Cu(T4) without DNA (—, black), with 24 μM T_{10} (—, blue), with 24 μM C_{10} (—, red), and with 24 μM A_{10} (- - -), all in 0.05 M pH 7.5 M Tris buffer containing 150 mM KCl. (B) Absorbance of 2.5 μM Cu(*t*D4) with 24 μM T_{10} (—, blue), with 24 μM C_{10} (—, red), with 24 μM U_8 (—, green), and with 24 μM A_{10} (- - -), all in 0.05 M pH 7.5 M Tris buffer containing 150 mM KCl. However, the no-DNA reference solution (—, black) contains 50% MeOH. DNA host concentrations in moles strand per liter.

is hypsochromic rather than bathochromic, and the hypochromic effect is comparatively large. Only later in the titration, when excess T_{10} is present, does the shift become bathochromic as the system approaches the limiting absorption spectrum. Shelton et al. have observed similar behavior in binding studies involving double-stranded hosts.[9] There is no perceptible change in the CD spectrum in the UV region, but there are always many more DNA bases present than porphyrin in solution.

Finally, competitive binding studies reveal the relative affinities the porphyrins have for a ds as opposed to a ss DNA platform. The experiment involves allowing Cu(*t*D4) and Cu(T4) by turns to equilibrate in a solution containing both a large excess of a single-stranded host, generally T_{16}, and a DNA hairpin such as $TT[t_4]$. Hairpin-forming sequences make useful DNA hosts because the double-stranded stems readily takes up porphyrin ligands.[7,21] For the competition experiment a 16-mer like T_{16} is the ss substrate of interest so that both hosts contain

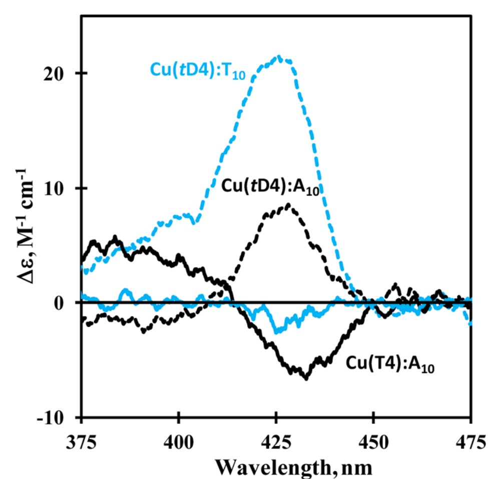

Figure 2. Induced circular dichroism of 3.0 μM Cu(T4) in the presence of T_{10} (—, blue) as well as A_{10} (—, black) at strand concentrations of 24 μM; and iCD spectra of 2.5 μM Cu(*t*D4) in the presence of T_{10} (- - -, blue) as well as A_{10} (- - -), again at 24 μM strand concentration.

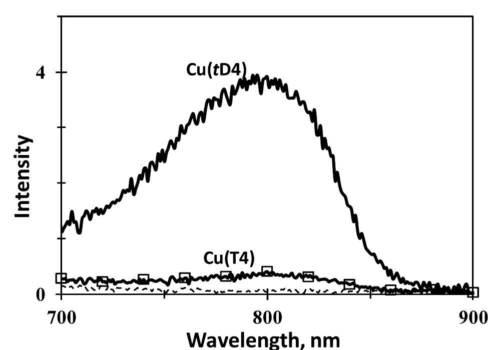

Figure 3. Relative emission spectra of 2.5 μM Cu(*t*D4) (—, black) and 3.0 μM Cu(T4) (—□—) in the presence of 24 μM A_{10}. The dashed trace connotes there is no signal from either porphyrin in the absence of DNA.

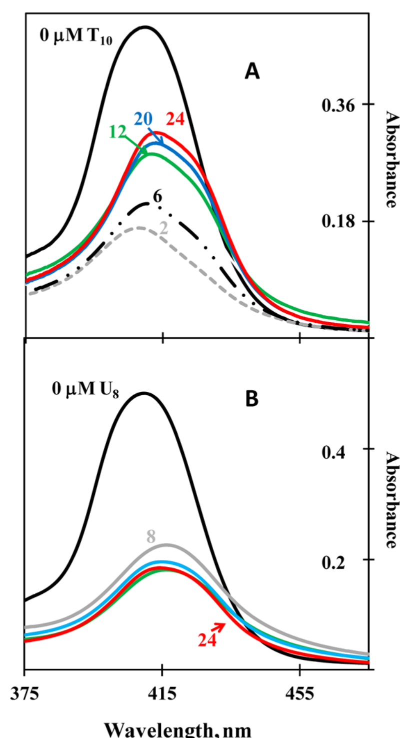

Figure 4. (A) Absorbance spectra of 2.5 μM Cu(tD4) with 2 μM (- - -), 6 μM (— · ·), 12 μM (—, green), 20 μM (—, blue), and 24 μM (—, red) T_{10} in 0.05 M pH 7.5 M Tris buffer containing 150 mM KCl. (B) Absorbance spectra of 2.5 μM Cu(tD4) with 8 μM (—, gray), 12 μM (—, green), 20 μM (—, blue), and 24 μM (—, red) U_8 in 0.05 M pH 7.5 M Tris buffer containing 150 mM KCl. The no-DNA reference solutions contain 50% by volume MeOH with buffer.

the same number of bases. The other hairpin used is CG[t_4]. The latter is an apt choice because Cu(T4) binds by intercalation due to the larger number of G≡C base pairs in the stem.[7,17]

The first finding of note is that TT[t_4] decisively outcompetes T_{16} for both copper-containing porphyrins. Figure 5 presents data from competition experiments involving Cu(tD4). Analysis of the CD results is convenient because both hosts internalize Cu(tD4) but induce iCD signals of opposite sign. Tellingly, for the mixed-host solution containing T_{16} and TT[t_4], the iCD signal is negative and reveals no hint of the adduct formed with T_{16}. The signal-to-noise ratio is inherently

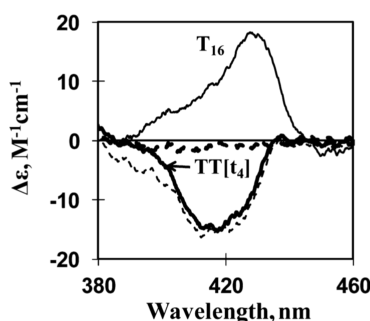

Figure 5. Competition study monitored by iCD: involving Cu(tD4) interacting with excess ss T_{16} (–, thin), excess TT[t_4] (—, thick), a mixture of both hosts (- - -, thin), and a control solution containing only the porphyrin (- - -, thick). The deviation of the latter from the zero line gives an idea of the inherent error in the measurement.

rather low because the measurement involves measuring the difference between two comparatively large absorbances. Nonetheless, within the error a least-squares analysis finds no hint of a signal from the T_{16} adduct, and a conservative estimation is that the binding constant is at least 10 times higher for the hairpin host. The bulkier Cu(T4) system is quite interesting because it binds *externally* to each host, and each once again induces iCD signals of the opposite sign. Binding to the ds host still dominates because the iCD signal is strictly positive if the solution contains either a mixture of T_{16} and TT[t_4] or TT[t_4] only.

In the competition involving Cu(T4) and CG[t_4] along with T_{16} as hosts, an analysis based on absorbance data is possible because of the shifts that occur when the porphyrin intercalates into ds DNA. Here binding to the ss host is more competitive, and the ratio of the apparent binding constants is only 1.9 ± 0.1 in favor of the CG[t_4] host. See Figure 6 for a presentation of

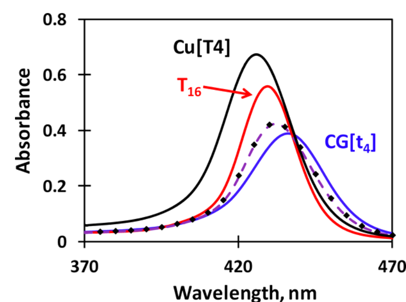

Figure 6. Competition study monitored by absorption spectroscopy: 3.0 μM Cu(T4) (—, black), interacting with 15 μM ss T_{16} (—, red), 15 μM CG[t_4] (—, blue), and a mixture 15 μM in both hosts (- - -). The diamond (♦) symbols designate calculated points from the least-squares fit.

the calculated and experimental spectra involved. In contrast, the sterically friendly Cu(tD4) system persists in showing a higher binding constant for the ds host CG[t_4] by a factor of at least 10 to 1. Thus, of the four systems studied binding to the ss host T_{16} is truly competitive only when the bulky Cu(T4) system has the opportunity to bind externally to T_{16} or intercalate into the CG[t_4] host. The CG[t_4] host fares much better in a competition for Cu(T4) with the 16-mer $[A_2C_2]_4$, as the binding constant ratio is at least 10 times greater for binding to the hairpin. Thus, incorporating bicyclic adenines into the sequence suppresses binding to the ss host.

■ DISCUSSION

Choices of Hosts. While poly(dT) and poly(dA) would both be serviceable single-stranded hosts,[1] shorter oligonucleotides with specified lengths, like T_{10} and A_{10}, are attractive alternatives. One reason is that physiologically important structures like a transcription bubble present ss DNA segments of limited length. The lengths of the hosts employed herein are admittedly arbitrary, but runs of 8−12 nucleotides are certainly reasonable choices in view of published estimates that intercalative or external binding of H_2T4 derivatives typically encompasses anywhere from 2−4 base pairs of ds DNA.[22−24] Including an oligonucleotide with uridine bases instead of thymine bases is worthwhile because the extra methyl group on thymine may impact the uptake of a bulky porphyrin for steric reasons.[25] Few of the oligonucleotides in Table 1 incorporate guanine as a base because the focus is on binding interactions

with ss DNA. With guanine-rich sequences self-association is common due to favorable stacking interactions and numerous options for base-to-base hydrogen bonding.[26] Electrophoresis shows, for example, that T_6 migrates as a monomer, but G_6 migrates as a higher molecular weight species.[27] In practice, poly(A) is also capable of self-association but normally only at low pH.[28]

In terms of ds DNA, the TT[t_4] and CG[t_4] systems are ideal for competition studies. The reason is comparisons are more straightforward when the two kinds of hosts involved have commensurate numbers of bases; however, the formation constants for duplexes with such short runs of nucleotides are quite low.[29] Fortunately, utilizing an appropriate hairpin-forming sequence solves the problem,[21,30] and a hairpin like TT[t_4], depicted schematically in Chart 1, is an apt choice for a number of reasons. One reason is that the presence of a tight 5′-t_4-3′ interior loop domain helps stabilize the hairpin structure and minimizes the opportunities for loop-based binding.[30] Closing C≡G base pairs at either end of the stem, and especially at the loop end, also limits fraying of the ds domain.[31] Multiple reports have, in fact, established the viability of hairpins as ds hosts, as DNA intercalators generally target the stem domains.[17,21,32,33] Beyond that, studies of the ligands H_2T4, Cu(T4), and Pd(T4) interacting with programmable hosts like TT[t_4] and CG[t_4] have established that the base composition of the stem domain completely determines whether the porphyrin binds by intercalation or externally.[8,17,34]

Binding Motif. Spectroscopic methods can be reliable indicators of the binding motif a cationic porphyrin adopts when interacting with a nucleic acid host.[3,6,35−38] In light of the extremely intense electronic spectrum, it is not surprising that absorption spectroscopy has been one of the most often applied methods. In the region of the Soret absorption, for example, sizable bathochromic and hypochromic effects occur when the porphyrin internalizes into a host and stacks amidst aromatic base residues.[3,11,35] In contrast, external binding induces a smaller bathochromic shift and little or no hypochromism, by virtue of weaker coupling with the bases. Luminescence studies of copper(II) porphyrins are also diagnostic. Although the porphyrin-based phosphorescence is weak, at best, by comparison with the fluorescence of the unmetalated porphyrin, the signal from the copper(II) form is uniquely sensitive to the local environment and indicative of internalization into the host. The unusual sensitivity comes about because interaction of the copper(II) center with any coordinating agent, including a solvent molecule, results in extremely efficient emission quenching.[3,7,9,16,17,39] As a consequence, only internally bound copper porphyrins are emissive, due to shielding by DNA or RNA bases. Externally bound forms are effectively nonemitting.[16,17,37]

Spectral comparisons clearly reveal that Cu(T4) and Cu(*t*D4) interact very differently with ss DNA hosts. In the case of Cu(T4), the binding-induced changes in absorbance and emission spectra are very modest and consistent with external binding. The lone exception is the interaction with A_{10}, which produces a significant hypochromic response. In keeping with results previously reported for poly(dA),[5] this host is one that could plausibly support internalization/pseudointercalation of Cu(T4) due to the presence of the large-surface-area, bicyclic, adenine bases. Even with A_{10}, however, the bound form of Cu(T4) does not exhibit a significant emission signal. In contrast, even all-pyrimidine hosts clearly internalize Cu(*t*D4) as evinced by the strength of the emission signals and the hypochromic responses. The magnitude of the hypochromism strongly suggests that bases of the host extend over both the top and bottom faces of the bound form of Cu(*t*D4). Hypochromic responses recorded in Table 2, in fact, exceed those observed with ds DNA hosts.[8,17] With an ss host, achieving an induced fit of the porphyrin[7,40] may be an easier proposition because there are no base-pairing constraints.[13]

Top-and-bottom stacking, or pseudointercalation, is clearly a plausible mode of binding for Cu(*t*D4) because base stacking within runs of ss DNA often gives rise to local helix formation.[5,41] However, as with B-form DNA, the bulky Cu(T4) system usually binds externally for steric reasons. Recognizing the steric issues posed by H_2T4-like porphyrins, early workers focused on the fact that all four *N*-methylpyridinium-4-yl substituents must twist out of the plane of the porphyrin core to avoid clashes involving ortho groups.[4,7,42] The analogous porphyrin with four *N*-methylpyridinium-2-yl substituents in place is so rigid that it is altogether incapable of intercalating into B-form DNA.[23] Clashes with sugar−phosphate residues can also be problematic as revealed by an X-ray crystallographic study of the adduct formed by Cu(T4) and a B-form host.[43] Finally, published molecular dynamics calculations suggest there are unfavorable steric contacts with methyl groups on thymine when H_2T4 intercalates amidst alternating A=T base pairs.[44]

Thomas and McMillin later compared the binding of H_2T4 with B-form hosts that had U=T instead of A=T base pairs, but they found no difference in binding motif.[20] They concluded that the steric influence of the methyl group at the C5 position of thymine could not be the reason the porphyrin opts for external binding as opposed to intercalation between A=T base pairs. However, the steric demands of the thymine methyl group originally recognized by Ford et al.[44] may actually influence binding to ss hosts by limiting the extent of stacking with the porphyrin ligand. The telling indicator is that the hypochromic effect observed with U_8 greatly exceeds that obtained with T_{10} or T_{16} (Table 2). As revealed in Figure 4, the hypochromic effect is actually much greater with T_{10} at low host concentration; however, the enhanced hypochromism cannot be due to interaction with DNA bases, because the Soret band experiences a hypsochromic as opposed to a bathochromic shift. The same phenomenon can occur during titrations involving B-form DNA hosts, especially with low-charge-bearing, sterically friendly porphyrins like Cu(*t*D4),[9,28] due to exciton coupling between near-neighbor porphyrins. Cooperative uptake facilitates near-neighbor binding and promotes porphyrin−porphyrin interactions. Cooperative binding is likely when the structural reorganization that attends the binding of one ligand facilitates the uptake of the next; Giri et al. has reported the same effect occurs with ligand binding to poly(rA).[45] Later in the titration, when excess T_{10} is present in solution, the hypochromism becomes less pronounced as entropy encourages ligand migration to separate domains (Figure 4). Ligand−ligand coupling can also give rise to a characteristically bisignate, or conservative iCD signal;[9,28] however, there is no indication of a bisignate iCD signal during the titration with T_{10}. The relatively flexible T_{10} system may simply be incapable of maintaining a chiral relationship between near-neighbor ligands in the same way a ds host can.

Competitive Binding and iCD Spectroscopy. In a competition study the iCD spectrum can be quite informative because the free porphyrins are achiral so that adduct formation is completely responsible for signal generation. In a simple

dipole-allowed electronic absorption, the ground and excited state wave functions must interfere with each other and generate, at least transiently, a net charge displacement along some axis of the molecule.[46] To observe circular dichroism, on the other hand, the charge flow must be somewhat helical.[47] A common method of inducing a CD signal is via exciton coupling to a chirally related chromophore of the host,[48,49] but it is also possible for binding to DNA to induce a chiral distortion in the porphyrin itself.[34,50] Predicting the response is not easy, and the contribution from excitonic coupling depends critically on the relative orientations of the transition moments involved. Indeed, reorientation of the chromophore relative to the bases of the host explains why the iCD response differs markedly when actinomycin D binds by intercalation into ds DNA as opposed to pseudointercalation into ss DNA.[51] With porphyrins, for which absorption is electron-dipole allowed in any in-plane direction, the analysis is almost always empirical.[52,53] One rule of thumb is that H_2T4 and its metalated forms exhibit negative iCD signals when they intercalate into ds DNA.[7,22,35] On the other hand, external binding frequently induces an iCD signal with a positive sign. The signal is sometimes bisignate, perhaps because the geometry of the externally bound adduct is more variable.[21,54] Since excitonic interactions with the DNA bases are apt to be weaker with external binding, it is also possible that a chiral distortion, imposed by the induced fit,[7] may be an important factor determining the response.

Even if the understanding of the induction mechanism(s) remains incomplete, the iCD signal can be useful for analyzing the results of a competitive binding experiment. As the signal-to-noise ratio is inherently greater in absorption spectroscopy, however, the latter technique offers better precision when the two types of adducts exhibit very distinctive absorption spectra. Regardless of the method used, however, analysis reveals that Cu(*t*D4) and Cu(T4) consistently show a preference for binding to a ds as opposed to a ss DNA host. The reason may simply be that ds DNA brings larger numbers of bases and phosphate groups to bear at the locus of binding. The case in which ss binding is most competitive occurs when CG[t_4] and T_{16} compete for Cu(T4). Here, however, well-recognized factors destabilize interactions with the ds host.[3,7] One originates in steric clashes, which occur at the periphery of the porphyrin and destabilize intercalative binding. An even more consequential weakening of external binding is due to the relatively high content of G≡C base pairs, which strengthen the double helical framework and inhibit the restructuring necessary for formation of a high-affinity binding pocket. Even here, the binding constant remains a factor of 1.9 smaller for binding to T_{16}, and that comparison has to be regarded as qualified. The reason is the familiar onset of 2:1 adduct formation with the ss host. To see that this happens, recall the results in Table 2, which show that the 1:1 adduct of Cu(T4) with T_{16} produces a strictly hyperchromic response. At the high host concentrations used in the competition experiments, however, a hypochromic effect clearly sets in and is attributable to 2:1 adduct formation (Figure 6).[20]

CONCLUSIONS

Previous studies involving 9-substituted acridines suggest that ss DNA hosts are better at internalizing a ligand encumbered by bulky substituents.[13] However, absorbance and emission studies establish that the bulky Cu(T4) porphyrin binds externally to ss DNA hosts, except in the case of a purine-rich host such as A_{10}. Even with A_{10}, internalization is modest as judged by the extent of emission quenching, which suggests the copper(II) center is readily accessible. On the other hand, pseudointercalation is the preferred binding motif for the less-substituted Cu(*t*D4) analogue, which also binds to ds DNA exclusively by intercalation.[17] Here, however, sterically active groups of the host, namely, the C5 methyl of thymine, affect the binding. This is clear from the fact that interaction with the ss U_8 host induces a much greater hypochromic effect on Cu(*t*D4) than does binding to the T_{10} analogue. With Cu(*t*D4), binding to an ss DNA host produces a greater hypochromic response, consistent with the notion that a ds DNA host is less adept at exposing a lipophilic surface.[15] The binding constant for Cu(*t*D4) is nevertheless at least 10-fold greater for a 16-mer that folds into a hairpin structure, and the same trend almost always holds for Cu(T4) as well. The one exception identified so far occurs when Cu(T4) distributes between the G≡C rich hairpin CG[t_4] and T_{16}. Even here, the binding constant for the ds host remains about two times higher, in spite of the fact that a second molecule of T_{16} begins to associate with the porphyrin at high host concentrations.

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Notes

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