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Binding Studies of G-Quadruplex DNA and Porphyrins: Cu(T4) vs Sterically Friendly Cu(*t*D4)

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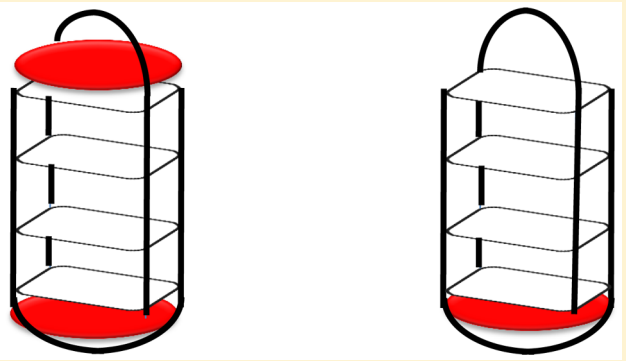
Supporting Information

ABSTRACT: This investigation explores binding interactions involving G-quadruplex DNA and two copper(II)-containing porphyrins (5,10,15,20-tetra(*N*-methylpyridinium-4-yl)-porphyrinato)copper(II) and the sterically friendlier analogue (*trans*-5,15-di(*N*-methylpyridinium-4-yl)porphyrinato)copper-(II), or Cu(T4) and Cu(*t*D4), respectively. The study incorporates five different DNA sequences that support the formation of unimolecular and bimolecular G-quadruplex hosts capable of exhibiting at least nine different structures in toto. Absorbance and emission results establish that G-quadruplex DNA is more adept at sequestering Cu(*t*D4) compared with the bulkier Cu(T4) ligand, even though the predominant mode of uptake is by end-capping, irrespective of the porphyrin or DNA sequence employed. One of the more impressive observations is that the emission intensities exhibited by Cu(*t*D4) bound to G-quadruplex DNA are many-fold higher than corresponding signals obtained with single- or double-stranded DNA hosts. With human sequence DNA the Cu(*t*D4) system is also unusual in that it preferentially binds to structures containing antiparallel strands. Refining the binding properties of porphyrin ligands is of interest because work from many laboratories has established that stabilizing G-quadruplex structure is an effective way to inhibit telomerase, a key enzyme involved in the immortalization of most types of cancer cells.

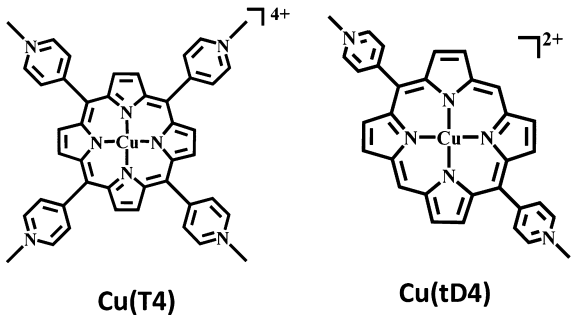


INTRODUCTION

This investigation reveals that DNA quadruplex hosts preferentially internalize cationic porphyrins in solution, as opposed to the solid state, where a crystallographic study has established that external binding occurs instead.[1] Ligands that bind to G-quadruplex DNA structures are intriguing because stabilizing quadruplex structure is an effective way of inhibiting telomerase, an enzyme that plays a key role in the immortalization of a variety of tumor cells.[2−10] G-Quadruplex structures may also have regulatory roles, as they occur in gene promoter regions of DNA.[11−14] Early reports showed that cationic porphyrins are effective ligands and inhibitors of telomerase,[2,11,15] and interest in the binding of porphyrins has continued apace.[8] However, the selectivity is not always high for quadruplex vis-à-vis duplex structures.[16] In light of the complexity of the systems it is probably not surprising that investigators have hypothesized at least three distinct motifs for porphyin binding to quadruplex DNA: intercalation between adjacent G-leaflets,[15,17] end-capping an outer leaflet,[4,11] and external or groove binding along the stack.[1] The conventional wisdom is that the high surface area π system of the porphyrin ring is advantageous for G-leaflet recognition.[11,17] Bulky substituents on the periphery can hinder stacking;[1] however, they promote solubility and electrostatic attraction.

One of the aims of this investigation has been to determine how steric requirements of the porphyrin influence binding to DNA G-quadruplexes. The porphyrins investigated are the copper(II)-containing forms of the much-studied H_2T4, or 5,10,15,20-tetra(*N*-methylpyridinium-4-yl)porphyrin, and the sterically friendlier analogue H_2*t*D4, or *trans*-5,15-di(*N*-methylpyridinium-4-yl)porphyrin. See Chart 1 for structures and abbreviations. The Cu(*t*D4) system is less sterically demanding because it has half as many *N*-methylpyridinium-4-yl substituents twisted out of the plane of the porphyrin ring.[18−20] Another aim has been to compare binding to different G-quadruplexes structures. The hosts chosen include

Chart 1



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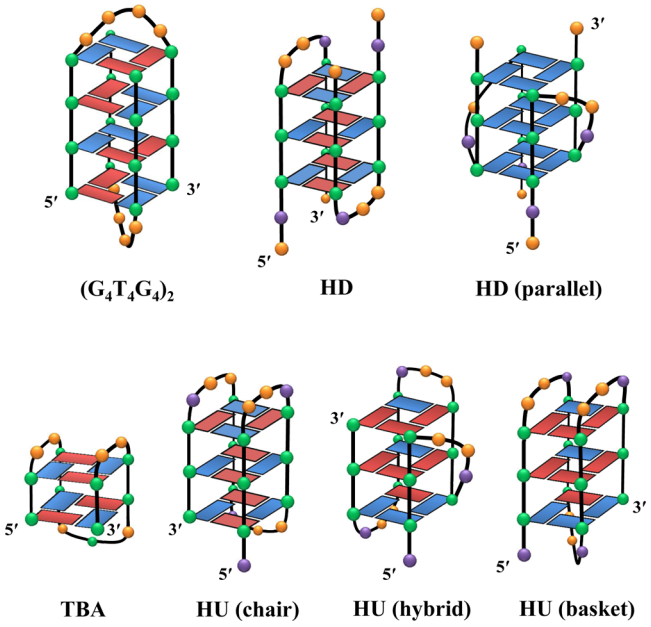
two bimolecular and three unimolecular DNA quadruplexes. The core of any G-quadruplex is a stack of G-leaflets, each composed of a quartet of guanine bases hydrogen-bonded together in Hoogsteen fashion.[6,21] As one example, the bimolecular $(G_4T_4G_4)_2$ system has a four-deep stack of G-leaflets within a basket-shaped structure in which adjacent sugar−phosphate chains run in opposite 5′-to-3′ directions.[22] In such an antiparallel structure, the base orientation characteristically alternates up and down a stack as depicted in Chart 2. The other bimolecular quadruplex, HD, actually

Chart 2



exists as a mixture of antiparallel and parallel forms.[23] Here the loop sequences are shorter, and in the antiparallel form lateral loops arch over an edge of the G-quartet, as opposed to traversing a diagonal (Chart 2). The structure that results is, in effect, a face-to-face adduct of two DNA hairpins. The parallel form of HD has entirely different loops that cross over from one end of the stack to the other and have a propeller-like shape. The upshot is that the sugar−phosphate chains all run in one direction, and the bases eclipse each other within a stack (Chart 2). TBA is the acronym for the thrombin-binding aptamer,[24] and it represents the smallest unimolecular quadruplex employed in this study. TBA adopts a chair shape, defined by two G-leaflets and three lateral loops that once again produce antiparallel chains. The c-MYC-like sequence employed herein supports short propeller loops, a three-deep stack of G-leaflets, and all-parallel chains. The sequence used is a variation of two that form structurally characterized G-quadruplexes: PU[25] and myc-2345,[26] which differ in having slightly shorter and longer 5′- and 3′-overhangs, respectively. Finally, there is HU, or human sequence DNA, which is capable of adopting at least four fundamentally different structures.[8,27,28] The simplest of these has only propeller loops and an all-parallel structure like c-MYC. Complementary structures include a strictly antiparallel, chair-like structure and a basket-like structure depicted in Chart 2. Lastly, there are "mixed" or hybrid structures exhibiting a propeller loop and two lateral loops. Chart 2 displays one of the hybrid forms; in the variant the two lateral loops precede the propeller loop in the course of the overall 5′−3′ run.[29−31]

EXPERIMENTAL SECTION

Materials. The supplier for all DNA sequences listed in Table 1 was Integrated DNA Technologies (IDT). Values for the molar

Table 1. DNA Sequences and Molar Absorptivity Data

abbreviation	strand or porphyrin	ε (260 nm, M^{-1} cm^{-1})
	Quadruplexes	
	$(G_4T_4G_4)_2$	115 200
HD	$(TAG_3T_2AG_3T)_2$	122 800
c-MYC	$TAG_3TG_3GAG_3TG_3GA$	220 600
TBA	$G_2T_2G_2TGTG_2T_2G_2$	143 300
HU	$AG_3(T_2AG_3)_3$	228 500
	Hairpins	
TT[t_4]	5′-GATTACttttGTAATC-3′	17 750[32]
CG[t_4]	5′-GACGACttttGTCGTC-3′	18 260[32]
	Porphyrins	
Cu(T4)		231 000[33]
Cu(*t*D4)		137 000[18]

absorptivities came from the vendor except as noted. The porphyrin salts $[Cu(T4)](NO_3)_4$ and $[Cu(tD4)](NO_3)_2$ were available from a previous study.[18] Sigma supplied the silanization solution (5% dichlorodimethylsilanes in *n*-heptane) as well as Trizma HCl and Trizma base. Potassium chloride (KCl), methanol (MeOH), and hexanes came from Mallinckrodt, while KOPTEC was the source for ethanol.

Methods. Treatment with a standard silane minimized the adsorption of the cationic porphyins by the glass walls of cuvettes and volumetric flasks.[34] The procedure for a spectrophotometric titration was the same as that employed in previous studies.[18,35] Each step in a titration involved the addition of a series of aliquots taken from previously prepared stock solutions. In this way the porphyrin concentration remained constant at 3.0 μM in the case of Cu(T4) and 3.5 μM for Cu(*t*D4). Likewise, serial additions from another stock solution kept the KCl or NaCl concentration constant at 150 mM. The DNA concentration, on the other hand, always increased from one addition to the next until the spectrum stopped responding and the limiting spectrum appeared. Calibration of a DNA or porphyrin stock solution was possible using absorbance data, Beer's law, and the appropriate extinction coefficient from Table 1. The DNA stock solution contained DNA as received from IDT in 1000 μL of 0.05 M Tris HCl buffer at pH 7.5. Day to day storage involved freezing the solution in a plastic vial. The $[Cu(T4)](NO_3)_2$ reagent was also in Tris HCl buffer and kept in the dark at room temperature or at 5 °C. However, the $[Cu(tD4)](NO_3)_2$ stock solution was in 50/50 MeOH/Tris HCl buffer. The solution was filtered into a plastic vial through a 0.2 μm PTFE syringe filter. The filter was rinsed with the 50:50 MeOH/Tris HCl buffer solution as before.[18] During a titration the only MeOH present came from the porphyrin stock solution. However, the medium used for measuring the reference spectrum contained 50% MeOH to ensure the porphyrin was in a monomeric form. The addition of DNA generally produced a hypochromic response. The calculation of the percent hypochromism, %H, involved the use of eq 1, where $A(\lambda_0)$ is the absorbance at the Soret maximum in the reference spectrum and $A(\lambda')$ is the corresponding absorbance value in the DNA-containing solution.

$$\%H = \frac{A(\lambda_0) - A(\lambda')}{A(\lambda_0)} \times 100 \quad (1)$$

The other physical methods used are emission spectroscopy and circular dichroism (CD). Application of eq 2 to the emission data facilitates intensity comparisons by correcting for absorbance changes during a titration or from host to host. In eq 2 $I(\lambda)$ is the adjusted

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emission intensity at wavelength λ, $I_R(\lambda)$ is the raw emission intensity, and $A(\lambda_{abs})$ is the absorbance at the wavelength of excitation.[36]

$$I(\lambda) = \frac{I_R(\lambda)}{1 - 10^{-A(\lambda_{abs})}} \quad (2)$$

The raw intensity data collected from the CD spectrometer are in millidegrees, but eq 3 converts the data to $\Delta\varepsilon$ values, where θ is the readout in millidegrees, $Q = 32\,980$ is a conversion factor, l is the path length in centimeters, and c is the concentration of the absorbing species.

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

The path length was either 1.00 cm or 2.00 mm as appropriate to keep the total absorbance below 2.0. In the Soret region the porphyrin is the absorbing species, but for the UV wavelengths c is the concentration of DNA.

Instumentation. The absorbance data came from a Varian Cary 300 Bio or a Varian Cary 100 spectrophotometer. The spectrofluorometer was a Varian Cary Eclipse equipped with an R3896 phototube detector. The pH meter was a Fisher Accumet Basic AB15 unit. Finally, a JASCO-J180 spectropolarimeter yielded CD spectra.

RESULTS

Quadruplex Structures. CD spectra obtained in the UV region provide useful information about DNA structure (Figure 1). In three out of five cases the CD spectrum is in keeping with the host adopting a unique structure. Thus, the CD spectrum of $(G_4T_4G_4)_2$ exhibits a negative band at ~265 nm and a higher amplitude positive band at ~295 nm, consistent with adjacent strands of the structure running in opposite directions, antiparallel to each other.[37] As shown in Chart 2, the relatively long TTTT runs of the two 5′-$G_4T_4G_4$-3′ sequences form diagonal loops at opposite ends of a stack of four G-tetrads.[22] Although TBA has very different loops, the strands still run strictly antiparallel to each other and give rise to a very similar CD spectrum. In contrast, the CD spectrum of the c-MYC exhibits a maximum at around 264 nm bracketed by one minimum centered around ~244 nm and another around 290 nm (Figure 1). Here, the strands of the quadruplex all run *parallel* to each other due to the utilization of propeller loops, vide supra. Because the bimolecular HD system exists as a mixture of two structures, it gives a composite CD spectrum. The parallel-stranded form is responsible for the maximum that occurs at 265 nm in the CD spectrum, and the component with antiparallel strands gives rise to a second maximum at 290 nm.[23] Finally, the spectrum of the unimolecular HU system confirms that it, too, presents a mix of parallel and antiparallel structure. Conformations in which the strands are either all parallel or all antiparallel may make some contribution, but the hybrid structures probably dominate.[28]

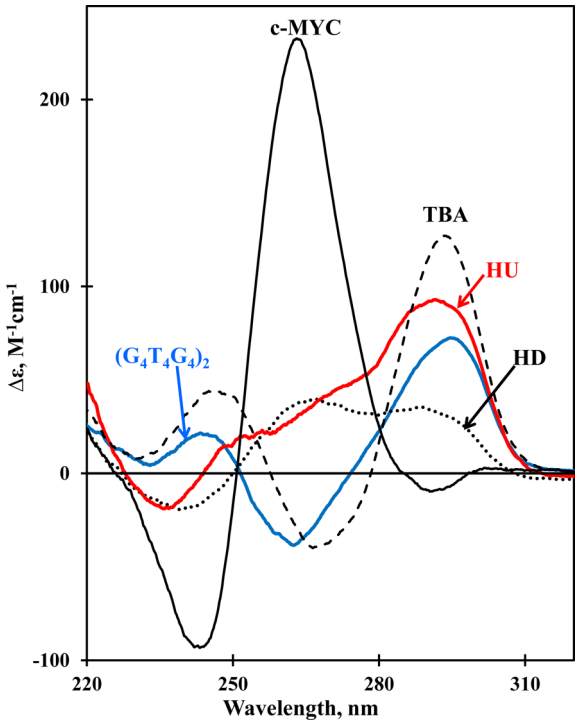

Figure 1. UV CD spectra of the quadruplex hosts c-MYC, TBA, HU, and HD in 0.05 M pH 7.5 Tris buffer containing 150 mM KCl and $(G_4T_4G_4)_2$ in the same buffer except with 150 mM NaCl.

Titrations of Cu(T4). Spectroscopic studies reveal that titrating G-quadruplex into a solution of Cu(T4) produces qualitatively similar results for four of the hosts. Except with TBA, interaction with the DNA induces a limiting bathochromic shift of $\Delta\lambda \gtrsim 7$ nm, a hypochromic response of %H $\gtrsim 30$, and an absorbance-corrected emission intensity of 5−6 at the wavelength of the emission maximum (Table 2). Figure 2 presents the absorbance and emission data obtained with c-MYC as host. These results are impressive because the data in Table 2 show that the interaction with c-MYC produces 50% *greater* emission intensity from Cu(T4) compared with intercalation into the double-stranded host CG[t_4]. The limiting $\Delta\lambda$ and %H values also exceed those found for binding to CG[t_4]. Interactions with HU, HD, and $(G_4T_4G_4)_2$ yield qualitatively similar results. In contrast, the limiting $\Delta\lambda$ and I values are only 3 nm and 1, respectively, in the case of TBA. Another difference is that the interaction with TBA induces a strongly positive iCD signal in the Soret region, whereas all of the other hosts induce a signal with a negative amplitude.[38] At the same time the UV CD spectra suggest that the uptake of

Table 2. Spectral Data from DNA Titrations with ds[a] and Quadruplex DNA

		absorbance		emission	iCD	
porphyrin	DNA host	$\Delta\lambda$, nm	%H	I	λ, nm	$\Delta\varepsilon$, M^{-1} cm^{-1}
Cu(T4)	$(G_4T_4G_4)_2$	7	27	5.0	432	−6
	HD	8	27	6.0	439	−7
	TBA	3	13	1.0	433	24
	HU	8	26	5.0	445	−7
	c-MYC	18	43	6.0	442	−34
	TT[t_4][18]	5	2	<0.5	420	15
	CG[t_4][18]	10	34	3.9	436	−29
Cu(*t*D4)	$(G_4T_4G_4)_2$	15	32	22.0	420	15
	HD	18	38	42.0	433	9
	TBA	11	42	14.0	434	−36
	HU	17	39	29.0	417	5
					440	−12
	c-MYC	23	34	18.0	442	−17
	TT[t_4][18]	16	24	3.7	415	−20
	CG[t_4][18]	16	28	4.8	414	−10

[a]Double-stranded hosts TT[t_4] and CG[t_4] are hairpin-forming DNA sequences.

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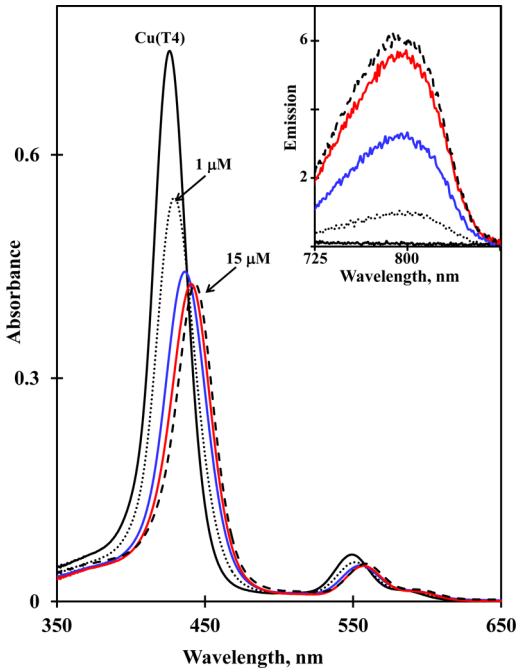

Figure 2. Absorbance titration of c-MYC into 3.0 μM Cu(T4) in Tris buffer containing 150 mM KCl. The Soret band shifts to longer wavelength as the DNA strand concentration increases in the order 1.0 (dotted), 3.0 (blue), 6.0 (red), and 15.0 μM (dashed). Inset: Corresponding emission spectra.

Cu(T4) has no significant impact on the conformation of TBA (Figure 3).

Titrations of Cu(*t*D4). The results obtained with TBA interacting with Cu(*t*D4) likewise stand apart from those obtained with other quadruplex hosts. More specifically, results in Table 2 show that interaction with TBA induces the weakest bathochromic response and the weakest emission signal from Cu(*t*D4). Every other host produces a much larger limiting $\Delta\lambda$ value, generally 2-fold larger than that realized with Cu(T4). The differential increase is not as great with c-MYC, but the $\Delta\lambda$ value is the largest measured in this study. See Figure 4 for absorbance titration data. The fact that the Soret band of Cu(*t*D4) assumes an asymmetric shape when involved with adduct formation to DNA may account in part for the magnitude of the spectral shift.[18] The emission intensities observed with Cu(*t*D4) are also impressive and are as much as 700% stronger than corresponding signals from Cu(T4). The emission signal is weakest when Cu(*t*D4) binds to TBA, but even then it exceeds the best signal from Cu(T4) by at least a factor of 2 (Figure 5).

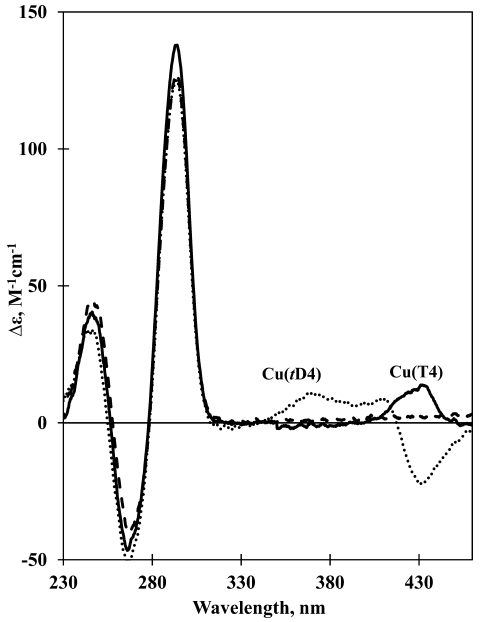

Figure 3. CD spectra of Cu(T4) and Cu(*t*D4) in the presence of excess TBA compared with the spectrum of TBA (dashed) in the absence of ligand.

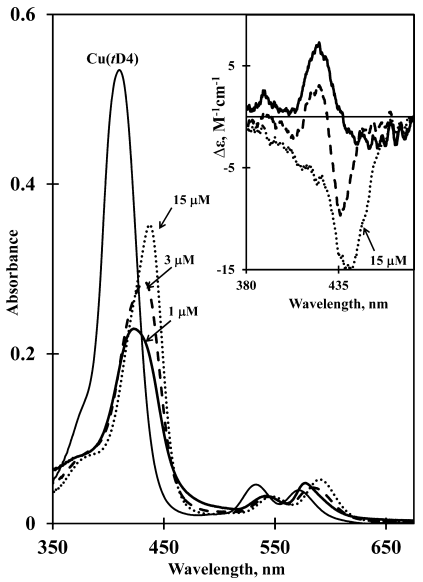

Figure 4. Absorbance of 3.5 μM Cu(*t*D4) in the presence of 0.0 (thin trace), 1.0 (thick trace), 3.0 (dashed trace), and 15.0 μM (dotted trace) c-MYC DNA. Inset: Corresponding iCD spectra.

Data obtained prior to the achievement of the limiting spectrum sometimes offer additional insights. One of the most interesting effects becomes apparent in the CD spectrum during the titration of Cu(*t*D4) with HD. More specifically, the uptake of a stoichiometric amount of Cu(*t*D4) results in the loss of a band that appears at 265 nm in the host spectrum (Figure 6). Later in the titration, however, the band reappears. Figure 6 also reveals that the CD spectrum is comparatively insensitive to the presence of Cu(T4). Introducing stoichiometric amounts of Cu(*t*D4) also leads to a conformational change in the HU system, as shown in the inset to Figure 6. More specifically, the uptake of Cu(*t*D4) results in the appearance of a negative CD band at 260 nm and the loss of the shoulder at ~278 nm. A completely different phenomenon becomes apparent in Figure 7, which depicts the absorbance changes that occur during the titration of $(G_4T_4G_4)_2$ into a solution containing Cu(*t*D4). Here the hypochromic response is much greater when approximately stoichiometric levels of host are present as compared with an excess of host. The same effect occurs in the titration with c-MYC as well (Figure 4). This pattern of absorbance changes is consonant with formation of a 2:1 adduct when excess ligand is present and excitonic coupling between the transition dipole moments of the bound porphyrins. The bisignate iCD signal that occurs in the inset of Figure 4 is a further indication of excitonic

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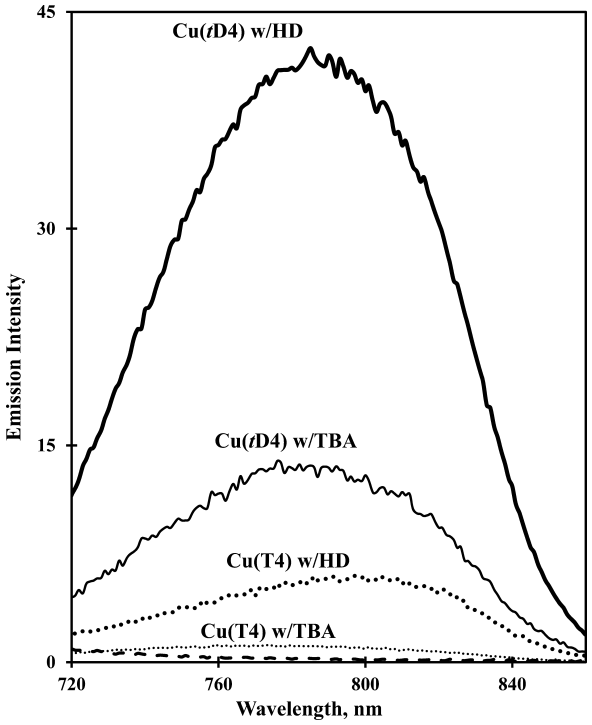

Figure 5. Limiting emission spectra of 3.5 μM Cu(*t*D4) with 20 μM HD dimer (thick) and 15 μM TBA (thin); 3.0 μM Cu(T4) with 20 μM HD dimer (thick dotted) and 15 μM TBA (thin dotted). The buffer is 0.05 M pH 7.5 Tris buffer containing 150 mM KCl except for the baseline (dashed) measured in 50/50 buffer/methanol.

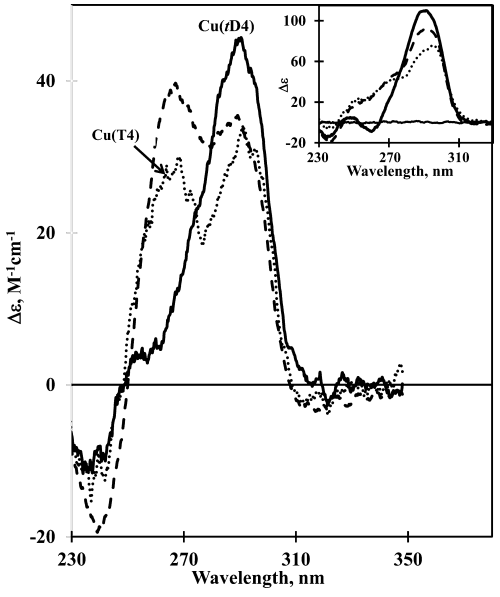

Figure 6. CD spectrum of the HD dimer in buffer (dashed line) compared with spectra obtained when a stoichiometric amount of Cu(T4) or Cu(*t*D4) is present in solution. The buffer is always 0.05 M pH 7.5 Tris buffer containing 150 mM KCl. Inset: Corresponding spectra obtained with HU as the G-quadruplex.

coupling. Similar effects occur during the uptake of porphyins by double-stranded DNA hosts.[39]

The porphyrin-based iCD signals obtained with Cu(*t*D4) are otherwise hard to interpret. The limiting iCD signal obtained with c-MYC is negative, as is the case with TBA as well. However, when Cu(*t*D4) interacts with either bimolecular host,

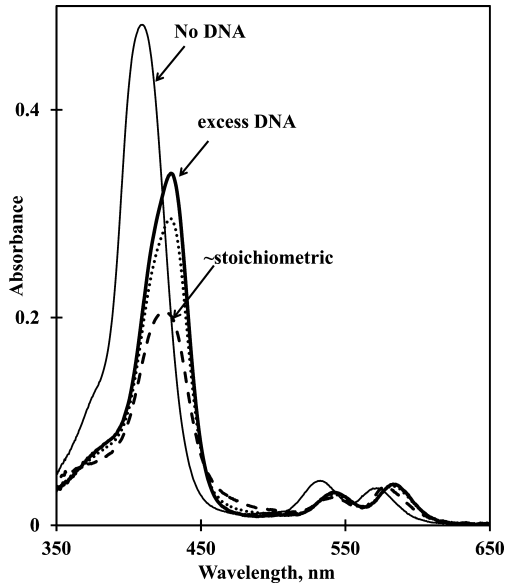

Figure 7. Absorbance of 3.5 μM Cu(*t*D4) in the presence of 0.0 (thin trace), 4.0 (dashed trace), 6.0 (dotted trace), and 12.0 μM (thick trace) $(G_4T_4G_4)_2$ in Tris buffer containing 150 mM NaCl. The DNA concentrations are in units of quadruplexes, i.e., half the strand concentrations.

the iCD signal is positive (Table 2). Finally, the HU system is unique in yielding a limiting iCD signal that is bisignate. The titration with HU is also peculiar in that the iCD signal changes from positive to bisignate before becoming predominantly negative in the approach to the limiting spectrum.

DISCUSSION

Porphyrin Binding Motifs. The first ideas about how DNA quadruplexes take up porphyrins probably drew upon the results of binding studies involving double-stranded DNA hosts. Thus, on the basis of calorimentry and absorption measurements, Hak et al. proposed that H_2T4 intercalates between adjacent G-leaflets.[15] Later, using the results of a series of photoassays, Hurley and co-workers concluded that H_2T4 *preferentially* binds by end-capping the G-tetrads, although secondary intercalation was also a possibility.[11] A nearly simultaneous paper by Shi et al. addressed end-capping in more detail.[4] In support of that motif, they pointed out that H_2T4 or Cu(T4) binds to quadruplex hosts more readily than axially ligated iron- or manganese-containing analogues. They went on to posit that a base or bases emanating from a lateral or diagonal loop can complement the G-tetrad by stacking on the opposite side of the bound porphyrin. That type of end-capping would also be consistent with the findings of McGuire and McMillin, who studied the interactions of copper-containing porphyrins with a series of tetramolecular G-quadruplexes.[40] However, the picture became more cloudy when Parkinson et al. solved a crystal structure and identified a different mode of binding.[1] The structure involved H_2T4 complexed with a bimolecular quadruplex derived from a truncated version of the sequence found in HD. The structure showed that the porphyrin actually *avoids* the exposed G-tetrad and sandwiches between bases supplied by adjacent hosts. In rationalizing the result the authors noted that the sheer expanse of a G-tetrad makes it ill-suited to stacking with the H_2T4 ligand because of the canted nature of its pyridiniumyl substituents. More recently, another reported structure revealed

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that *N*-methyl mesoporphyrin-IX (NMM) binds to the parallel-stranded form of HU strictly by end-capping.[41] The latter structure does not contradict the conclusions of Parkinson et al. because the NMM ligand is devoid of bulky aryl substituents. Taken together, however, the results suggest that reducing the number of bulky ligand substituents may well alter the nature of the adduct that forms.

Binding Interactions of Cu(T4). In line with the reports of the Hurley group,[4,11] the results described herein are most consistent with end-capping as the predominant binding motif adopted by Cu(T4) interacting with G-quadruplex hosts in solution. The emission results are most telling, because of the unique sensitivity copper(II) porphyrins have to solvent-induced quenching.[20,42] The reason is a copper(II) porphyrin emits from a ligand-based $\pi-\pi^*$ excited state in the absence of axial ligands.[43] However, if a fifth ligand is present, or attacks the metal center during the lifetime of the excited state, the excitation shifts to a *nonemitting* metal-centered excited state.[44,45] As a result Cu(T4) exhibits no appreciable emission signal in aqueous solution because the exposed metal center is subject to immediate attack by water molecules. The same is true when the copper porphyrin binds externally to a DNA duplex. In contrast, intercalation into a DNA duplex blocks the approach of potentially coordinating ligands, and Cu(T4) becomes emissive.[18,20,44] Here the remarkable finding is that quadruplex hosts are even more effective at internalizing the bulky Cu(T4) ligand. Thus, the emission signal is 25% *stronger* when Cu(T4) binds to G-quadruplex DNA as opposed to CG[t_4], a DNA hairpin that supports intercalative binding.[18,32] External binding to a loop domain of a G-quadruplex cannot explain this result because Cu(T4) exhibits a very weak signal when it binds to single-stranded DNA in solution.[46] The other important observation is that the uptake of Cu(T4) by a G-quadruplex typically induces a hypochromic response in excess of 25%. Such a pronounced response is an indication of stacking interactions with DNA bases and efficient coupling among the transition dipole moments of all of the participating π systems.[20,47,48] Simple end-capping of a G-leaflet might account for the hypochromism alone, but the intensity of the emission signal suggests there is more to the story. As argued by Shi et al. on computational grounds,[4] it is also likely that additional stacking occurs on the opposite face of the porphyrin. This interaction, which involves other bases extending from the host, further protects the metal center from ligand attack.

Ligand uptake is obviously a multifaceted process, and multiple investigators have attempted to measure binding constants for H_2T4. However, the task is difficult because the constants are large, of the order of 10^6 M^{-1},[49,50] and cooperative effects are frequently prevalent.[17] Even the stoichiometry of adduct formation remains quite uncertain. Thus, Wei and co-workers report a ratio of 4:1 for the binding of H_2T4 with HU,[17] while Wheelhouse et al. quote a value of 2:1.[2] Employing a variation of HU with an extra thymine residue at each end of the sequence, Zhang et al. report a stoichiometry as high as 3:1 depending on the technique employed.[49] In deference to those studies and the inherent uncertainties, the current investigation has focused on conditions involving an excess of host, encouraging 1:1 binding, and ascertaining the nature of the primary binding motif.

Binding Interactions of Cu(*t*D4). The evidence for the internalization of Cu(*t*D4) by DNA quadruplexes is even more dramatic. Figure 8 shows how the emission intensity from

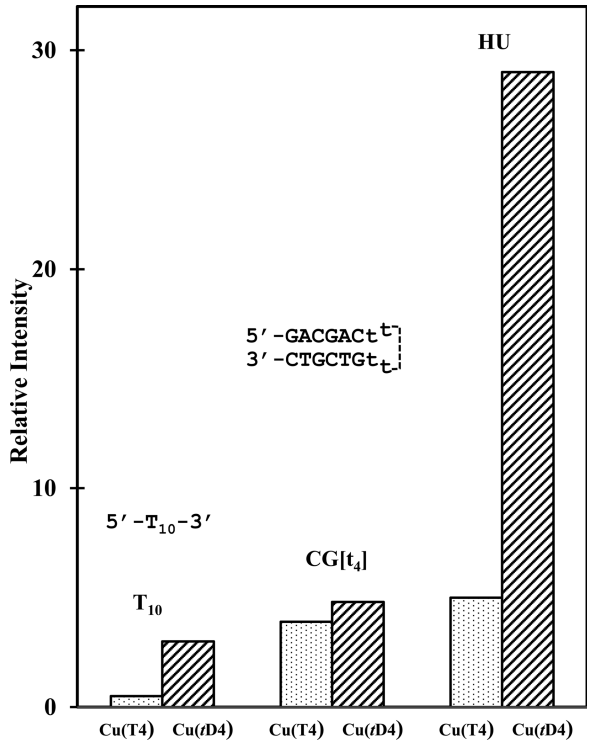

Figure 8. Bar graph showing in relative terms how the limiting emission intensity achieved with Cu(T4) (dotted) as well as Cu(*t*D4) (striped) changes as the DNA host ranges from single-stranded 5′-T_{10}-3′, to CG[t_4] with its double-stranded stem, to HU quadruplex DNA.

Cu(*t*D4) increases as the host changes from single-stranded DNA, to double-stranded DNA, to a G-quadruplex. Previous studies have shown that the porphyrin sandwiches between DNA bases of both the single- and double-stranded forms.[18,46] Hence it is impressive that DNA quadruplexes are capable of generating even stronger emission signals. The hypochromic responses are also strong, reaching levels of 35−40% and greatly exceeding those observed for Cu(T4) (Table 2). The explanation is that Cu(T4) suffers from the presence of four out-of-plane pyridiniumyl substituents, whereas the sterically less encumbered form Cu(*t*D4) is capable of achieving a closer approach to DNA residues.

With quadruplex hosts, the induced emission intensities are so large that stacking interactions must occur on both faces of the porphyrin ligand as well. When $(G_4T_4G_4)_2$ is the host, the extra base or bases needed to sandwich the porphyrin must extend from the overarching diagonal loop. On the other hand, due to the host's chair structure, one or more of the lateral loops of TBA must supply the additional covering bases. The c-MYC host is different because its connecting loops all adopt a propeller conformation and are too short to extend a base up over the ligand. Hence the added shielding must derive from overhanging TA residues at the 5′-end of the quadruplex or GA residues at the 3′-end. Finally, the more versatile HU and HD hosts generate the strongest emission signals from Cu(*t*D4). At each end of the stack of G-tetrads, these hosts have the option of stacking a base from an overhanging end *and/or* a lateral loop. The added flexibility presumably explains why they are superior from the standpoint of protecting the copper(II) center from external attack.

Conformational Effects. The HU and HD hosts are also unique in another way. Recall that HD exists as a mixture of

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two conformations, the parallel and antiparallel forms, giving rise to CD maxima at 265 and 290 nm, respectively.[23,37] As revealed in the spectra in Figure 6, however, the uptake of a stoichiometric amount of Cu(*t*D4) preferentially stabilizes the antiparallel form. In contrast, the uptake of Cu(T4) produces no significant change in the relative intensities of the CD maxima, consistent with no preference for one conformation or the other. That result is the more interesting because the published crystal structure has the parent porphyrin H_2T4 complexing exclusively with the *parallel* form of the HD dimer.[1] Results obtained during the titrations with the HU host are largely similar. Literature results show that hybrid forms of HU are dominant and each contains a mixture of parallel and antiparallel strands.[29,30] Logically, the parallel structure is responsible for the shoulder that appears at 270 nm in the CD spectrum. With this system the uptake of Cu(*t*D4) induces a loss of the shoulder in favor of a weak negative band at 260 nm, again consistent with an enhancement in the amount of antiparallel strand structure. Comparisons are difficult because no clear pattern has emerged from previous studies. On one hand, Han et al. have found that the *N*-methylpyridinium-3-yl-containing analogue of H_2T4 preferentially binds to the parallel form of an HU-like quadruplex.[11] On the other, Zhang et al. have reported that the uptake of H_2T4 preferentially induces the antiparallel form of the quadruplex in the absence of potassium ions in solution.[49]

OVERVIEW

Absorbance and emission results reveal that G-quadruplex DNA more effectively sequesters a porphyrin bearing fewer bulky aryl substituents. This finding accords with steric expectations.[51] Even for Cu(*t*D4), however, intercalative binding between G-leaflets is incompatible with the wide variations observed in emission intensities and iCD signals. Rather, the uptake of Cu(T4) or Cu(*t*D4) preferentially occurs by end-capping, virtually independent of the quadruplex structure employed. There is, indeed, growing recognition that G-quadruplex DNA reliably provides fused-ring aromatic ligands up to two binding sites at either end of a stack of G-leaflets.[12] Secondarily, appropriately positioned flanking residues and/or bases extending from one or more loops of the structure also influence adduct formation.[12,50,52−54] Certainly the observed emission intensities indicate that the superstructure and the terminal G-leaflet condition the binding interactions of Cu(T4) as well as Cu(*t*D4). It may also account for the differences in the observed iCD responses. Yet another possibility is that the same auxiliary interactions are responsible for the preferential binding of Cu(*t*D4) to the antiparallel structure in the HU and HD hosts. Recognition of such effects may influence the choice of hosts in future investigations. It is, for example, hard to compare binding interactions with the parallel forms of HD and HU, because the former presents so many more overhanging bases. Any reorganization of the host itself attending the uptake of a ligand provides for an "induced fit", while a complementary reorganization of the ligand leads to mutual accommodation.[48] Differences in the accommodation achieved could, for example, explain why the ostensibly similar ligands H_2T4, Cu(T4), and Pd(T4) inhibit telomerase to very different degrees.[4] One documented example reveals that cratering of the exposed G-leaflet favors the binding of a dome-shaped ligand.[41] If cratering of the outer G-leaflet proves to be a more general phenomenon, a side benefit could be a suppression of the rate of base-induced quenching of a copper(II)-containing porphyrin. As a reminder in closing, the current study has focused on conditions involving an excess of host, so as to favor 1:1 binding. One reason is that exogenous ligands are apt to be in short supply in intracellular compartments as well. Also, when complications due to higher loading and ligand−ligand coupling are absent, it is easier to establish how interaction with the host shapes the iCD signal. Findings may well differ in a crystalline environment where binding sites between quadruplexes become available.[1] Finally, molecular crowding poses yet another complication, since it may affect the quadruplex conformation.[55]

ASSOCIATED CONTENT

Supporting Information

Figures showing limiting emission spectra exhibited by Cu(T4) and Cu(*t*D4) in the presence of the full range of G-quadruplex hosts. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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