Duplex hydrogen bonding promotes intercalation of Cu(T4) in DNA hairpins (Cu(T4) = *meso*-tetrakis(4-(*N*-methylpyridyl))porphyrincopper(II))

Denise K. Crites Tears and David R. McMillin*

Department of Chemistry, Purdue University, West Lafayette, IN 47907-1393, USA. E-mail: mcmillin@purdue.edu

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Inosine-for-guanine replacement in DNA hairpin hosts reveals that the intercalative binding of Cu(T4) depends upon strong hydrogen bonding within the stem.

Water-soluble porphyrin derivatives are of interest for photodynamic therapy,[1,2] as antiviral agents,[3,4] and, in the case of cationic systems, as versatile ligands for DNA-binding studies.[5,6] A wealth of physical data shows that metalated forms lacking axial ligands, such as Cu(T4), intercalate into DNA sequences that are rich in guanine–cytosine (G≡C) base pairs[5,6,7] (H_2(T4) = *meso*-tetrakis(4-(*N*-methylpyridyl))porphyrin). On the other hand, adenine–thymine (A=T) base pairs support external binding in groove regions. A recent report based on DNA hairpin substrates indicated that robust hydrogen bonding within the local B-form structure, not the specific base sequence, promotes intercalative binding.[8] The replacement of a guanine base by inosine provides a strict test of the hypothesis because it is a small perturbation that noticeably impacts the hydrogen bonding within DNA. The effect is to weaken the B-form structure because inosine lacks an NH_2 substituent at its C2 position and can form only two hydrogen bonds with cytosine. The following results show that even one such replacement in a six-base-pair run can dramatically alter the binding of Cu(T4).

The hexadecamers employed all came from the Macromolecular Structure Facility of Purdue University as custom syntheses. In all spectral runs the hairpin-to-copper ratio was 5 : 1. Quantification of the hairpin concentration was possible *via* the absorbance of a denatured sample at 80 °C in conjunction with the ε_{260} values (molar absorptivities at 260 nm) of the component mononucleotides. The extremum in the derivative of the absorbance *versus* temperature profile indicated the melting temperature, T_m. The sequence is normally constant except for residues 3 and 4 and their complements (positions 13 and 14). The two-letter abbreviation identifies the bases in the 3,4 positions:

$$\text{CI} = \begin{matrix}5'\text{-GA}\mathbf{CI}\text{AC}^T\text{T}\\ 3'\text{-CTICTG}_T\text{T}\end{matrix} \qquad \text{AI} = \begin{matrix}5'\text{-GA}\mathbf{AI}\text{AC}^T\text{T}\\ 3'\text{-CTTCTG}_T\text{T}\end{matrix}$$

The data in Table 1 show that replacement of a mid-stem guanine by inosine has a significant impact on the melting temperature and the reaction chemistry of the hairpin. Thus, each replacement reduces the melting temperature by about 12 °C. As a result, the CI, IC and AI hairpins all exhibit about the same T_m, presumably because they have the same number of hydrogen bonds in the stem. As previously reported, the spectral and physical data reveal that Cu(T4) intercalates into the CG hairpin.[8] In contrast, with the CI hairpin all indications point to a complete shift in the mode of binding. More specifically, the weak emission intensity from the latter adduct is a clear sign of an exposed copper center that is subject to axial attack by solvent or basic centers on the surface of the hairpin.[6] Furthermore, in the Soret region the CI adduct shows a small bathochromic shift in the absorption maximum, hyperchromism and an induced CD signal with a positive amplitude—all signs of groove binding.[5,6,9] Similar spectral changes occur in switching from the AG to the AI hairpin except for the inversion in the sign of the induced CD signal (Fig. 1). The remarkable aspect of the AG system is that the loss of just one of fifteen possible hydrogen bonds in the stem drastically alters the binding of Cu(T4).

Regardless of the mode of binding, the DNA must undergo a structural adjustment to accommodate the Cu(T4) ligand because there is no pre-organized binding site. In other words, the uptake of the porphyrin is an induced-fit process. For intercalation, the minimum necessary structural reorganization entails partial unwinding of the double helix and creation of a cavity to house Cu(T4). Even with those modifications, strain is evident,[10,11] and groove binding of Cu(T4) is a competitive phenomenon in DNA hosts containing a short run of A=T base pairs amidst long segments of G≡C steps.[6,12] Groove binding is also disruptive as Cu(T4) cannot conform to the natural contour of DNA. Consequently, uptake occurs with partial melting of the double helix and generation of an appropriate binding pocket.[6,13] Transient Raman studies of Cu(T4) by Nakamoto and co-workers reveal that a run of four consecutive adenine–thiamine (A=T) base pairs suffices to provide the surface area

Table 1 Physical data for Cu(T4) adducts with DNA hairpins[a]

		Absorption		CD		Emission	
Hairpin	T_m/°C	$\Delta\lambda$/nm[b]	%H[c]	λ/nm	$\Delta\varepsilon$/M^{-1} cm^{-1}	λ/nm[d]	I_{rel}[e]
CG	74	10	25	434	−27	434	0.9
CI	50	4	−9	424	16	429	< 0.1
GC[f]	75	8	20	433	−22	432	1.0
IC	50	6	9	432	−13	432	0.2
AG[f]	61	9	25	434	−17	433	0.7
AI	53	5	−5	428	−10	433	0.1
CA[f]	65	7	12	434	−17	433	0.5
CTA[g]	45	10	29	434	−20		0.8

[a] At a hairpin-to-copper ratio of 5 : 1. [b] Bathochromic shift of Soret band from 424 nm. [c] Percent decrease in absorbance at the Soret maximum. [d] Maximum in the excitation spectrum. [e] Relative intensity of the (uncorrected) emission signal. [f] Data from ref. 8. [g] Bulge derivative of CA with unmatched T after C3.

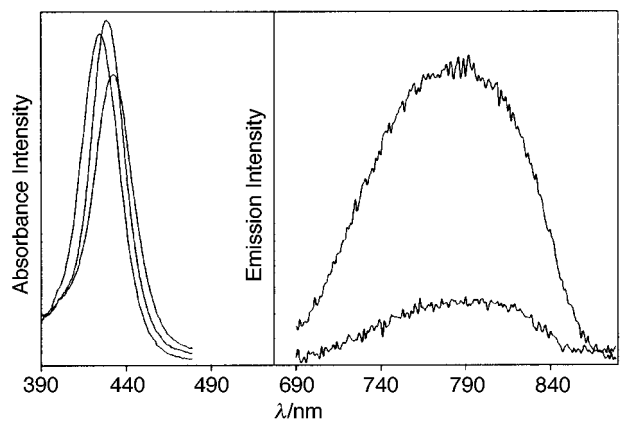

Fig. 1 Absorbance and emission data. Left: in order of decreasing intensity, Soret absorbance of adduct with AI, free Cu(T4), adduct with AG. Right: emissions from adducts with AG (upper) and AI (lower) hairpins.

and/or the flexibility necessary for groove binding.[12] From the standpoint of internalization of the ligand, intercalation and groove binding represent limiting cases of a continuum of possible interactions. Intermediate situations are certainly feasible; for example, an extruded base might stack with an externally bound porphyrin. The adduct with the IC system may, in fact, be some type of an intermediate case. To be sure, the modest hypochromism apparent in the Soret absorption is evidence for some degree of stacking with the DNA bases, and partial protection of the axial coordination positions at copper would explain the definite, albeit weak, emission signal of the adduct.[14] However, classical intercalation does not occur because the emission intensity pales in comparison with that of the GC adduct. More than one type of association may occur because the absorption and excitation maxima do not coincide for the IC or the AI adducts. Yet, the CD spectrum of the latter is clearly not the sum of the signals of a classical groove binder and a classical intercalator.

Though questions remain, the present results provide important insight into the forces influencing the binding of Cu(T4). The main conclusion is that for intercalation of Cu(T4) to occur, a robust hydrogen bonding network must exist within the B-form DNA to compensate for the steric problems posed by the bulky porphyrin. Two predictions follow. One is that the presence of a bulge in the stem may promote intercalative binding by reducing the strain and/or the energy requirement for cavity creation.[15] Indeed, comparisons of the hypochromism and the emission intensity show that Cu(T4) is a more avid intercalator for the CTA hairpin, with the bulge thymine, than the CA control (Table 1). The second prediction is that the mode of binding known as hemiintercalation, which has been observed in the solid,[11] is unlikely to occur in solution, at least in sequences rich in G≡C base pairs. The reason is that hemiintercalation would require base extrusion, but the preservation of hydrogen bonding between bases is, in reality, one of the most important factors that favors internalization over groove binding.

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