Communications to the Editor

H_2D3: A Cationic Porphyrin Designed to Intercalate into B-Form DNA (H_2D3 = *trans*-Di(*N*-methylpyridium-3-yl)porphyrin)

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This study describes a new, water-soluble, dicationic porphyrin *trans*-di(*N*-methylpyridinium-3-yl)porphyrin (H_2D3 in Scheme 1) with novel DNA-binding properties. Interest in cationic porphyrins, particularly derivatives of *meso*-tetra(*N*-methylpyridinium-4-yl)porphyrin (H_2T4 in Scheme 1),[1,2,3] abounds because they are versatile DNA-binding agents that could find application in photodynamic therapy[4,5] or as antiviral agents.[6] Coulombic, hydrophobic, and steric forces influence the mode of binding which varies with the structure of the porphyrin as well as the composition of the DNA.[2,3,7]

The peripheral pyridinium groups of the porphyrin provide a Coulombic attraction to DNA, but at a cost of introducing steric contraints. Early concerns focused on the idea that out-of-plane substituents would interfere with insertion between base pairs.[7] Later, molecular dynamics calculations also suggested that clashes with thymine methyl substituents prevent intercalation of H_2T4 in a 5′-TpA-3′ step.[8] Finally, a crystal structure revealed that severe steric clashes occur in the minor groove region whenever a bulky porphyrin like Cu(T4), the copper(II)-containing derivative of H_2T4, intercalates into DNA.[9] The upshot is that intercalative binding is only feasible if the DNA has a robust hydrogen-bonding framework, i.e., is rich in guanine/cytosine (G≡C) base pairs.[10] In contrast, strictly external binders, like five-coordinate Zn(T4), preferentially bind to DNA that is rich in adenine/thymine (A═T) base pairs, i.e., flexible runs of DNA that deform easily and permit evolution of a suitable binding pocket.[12] With only two meso substituents, the H_2D3 system will pose minimal steric problems and should be a valuable addition to the existing library of DNA-seeking porphyrins.

A MacDonald-type method[13] of coupling dipyrromethane with pyridine-3-carboxaldehyde in acetic acid at 90 °C in the presence of air first yielded the neutral precursor to H_2D3 along with other products. Step elution from a florisil column with increasing amounts of methanol (1−5 vol %) in dichloromethane permitted the isolation of the *trans*-5,15-di(3-pyridyl)porphyrin. With an overall yield of about 5%, the latter proved to be the principal porphyrin species present, other products being the monopyridyl derivative and chlorins. For identification purposes the ^{1}H NMR spectrum of *trans*-5,15-di(3-pyridyl)porphyrin exhibited a characteristic methine resonance at 10.4 ppm in $CDCl_3$, and the absorption spectrum showed that the Soret band occurs at 405 nm. A crystal structure confirmed the identity of the compound.[14] Subsequent treatment with excess methyl 4-toluenesulfonate in refluxing DMF yielded the dication H_2D3 as the hemihydrated, di(4-toluenesulfonate) salt. (Calcd for $C_{46}H_{40}N_6S_2O_6 \cdot {}^1/_2H_2O$: 65.31C, 4.88 H, 9.93 N. Found: 65.09 C, 4.75 H, 9.96 N.) In water H_2D3 exhibits a Soret band at 403 nm with a molar extinction coefficient of 200 000 M^{-1} cm^{-1}, while the maxima in the uncorrected fluorescence spectrum occur at 620 and 680 nm.

Spectral titrations reveal that H_2D3 binds to B-form DNA with a similar affinity as the well-studied H_2T4 system but in a much more uniform fashion. Thus, interaction of H_2D3 with either [poly(dG-dC)]$_2$ or [poly(dA-dT)]$_2$ induces a similar, large spectral shift (16−20 nm) and significant hypochromism (24−36%) in the Soret transition (Figure 1). The magnitudes of the spectral perturbations are prima facie evidence for intercalative binding because they establish that the π systems of H_2D3 and the DNA bases are in

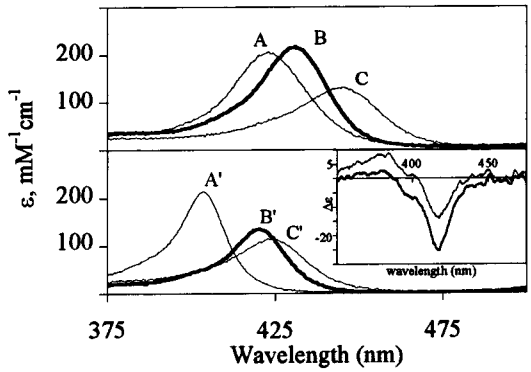
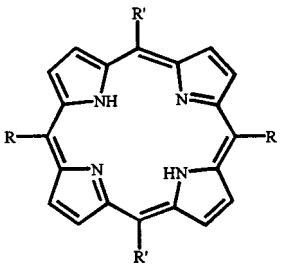


Figure 1. Absorbance data in 0.1 M pH 6.8 phosphate buffer at 80:1 DNA-base/porphyrin ratios except as noted. H_2T4 with (A) no DNA, (B) (thick) [poly(dA-dT)]$_2$, (C) [poly(dG-dC)]$_2$. H_2D3 with (A′) no DNA (B′) (thick) [poly(dA-dT)]$_2$, (C′) [poly(dG-dC)]$_2$. Inset: CD spectra of B′ (thick) and C′ (thin) samples.

Scheme 1



H_2T4: R=R′=N-methylpyridinium-4-yl
H_2D3: R=H, R′=N-methylpyridinium-3-yl

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Table 1. Spectral Data for Adducts in μ = 0.1, pH 6.8 Phosphate[a]

porphyrin	DNA	abs. $\Delta\lambda$,[b] nm (% H)[c]	CD λ_{max} ($\Delta\epsilon$, $M^{-1}\,cm^{-1}$)
H_2T4	dA-dT[d]	9 (−4)	430 (+26)
H_2D3		16 (24)	417 (−25)
H_2T4	dG-dC[e]	23 (34)	443 (−12)
H_2D3		20 (36)	420 br (−3)
	{TT}	17 (29)	417 (−18)
	{CG}	19 (44)	417 (−10)
Zn(T4)[f]	dA-dT	2 (−6)	424 (+5)
			438 (+6)
Zn(D3)		9 (26)	415 (−15)
Zn(T4)[f]	dG-dC	0 (4)	~445 (+4)
Zn(D3)		12 (26)	416 (−3)

[a] Except as noted the DNA-base/porphyrin ratio is 80:1. [b] Shift induced in Soret maximum. [c] Percent drop in abs. intensity. [d] [poly(dA-dT)]$_2$. [e] [poly(dG-dC)]$_2$. [f] Reference 15.

intimate contact. For comparison, note that external binding of H_2T4 to [poly(dA-dT)]$_2$ induces a relatively small shift in the Soret band of the porphyrin and a weak hypochromic response (Figure 1 and refs 2, 3, and 15). Both H_2D3 adducts also exhibit a *negative* signal in the Soret region of the circular dichroism (CD) spectrum (Table 1 and Figure 1, inset). These findings are telling because Pasternack and others have demonstrated that induction of a negative CD signal is diagnostic of intercalataive binding.[2,15]

Experiments with DNA hairpins indicate the same trend. The hairpin-forming 16-mers used in this study each contain mutually complementary base sequences at the 5′ and 3′ ends and an interior run of four thymines that forms the turn in the stem-loop structure.[16] The shorthand names for the oligos are {CG} = 5′-GACGACTTTTGTCGTC-3′ and {TT} = 5′-GATTACTTTTG-TAATC-3′. In line with intercalation, the data in Table 1 indicate that adduct formation between H_2D3 and either hairpin induces pronounced hypochromism and a negative CD signal in the Soret region. In contrast, previous studies have established that H_2T4 and Cu(T4) both bind externally to the stem of the {TT} hairpin, which has a preponderance of A═T base pairs.[17]

Experiments with the Zn(D3) derivative provide striking evidence of the reduced steric demands of the dipyridyl porphyrin. As evidenced by a shift of the Soret band to 412 nm, it was easy to prepare a stock solution of Zn(D3) by addition of a slight excess of aqueous $ZnCl_2$ to a buffer solution containing H_2D3. Data in Table 1 show that additional spectral evolution occurs upon combining Zn(D3) with either [poly(dG-dC)]$_2$ or [poly(dA-dT)]$_2$ and leave no doubt but that the chromophore loses its axial ligand and binds as an intercalator. Viscometry measurements[18] confirm this interpretation because uptake of Zn(D3) *enhances* the specific viscosity of salmon testes by around 80%. Contrasting results reported in Table 1 and elsewhere[15,18] reveal that the bulkiness of the Zn(T4) system relegates it to external binding.

In summary, cationic porphyrins are now available that bind to DNA by intercalation, irrespective of the base composition. Access to less sterically demanding porphyrins, like H_2D3, opens the way to a variety of new and important DNA-binding studies. More specifically, it will be possible to investigate effects like charge on the porphyrin, substituent orientation, metal content, and DNA composition have on the binding interaction in the absence of destabilizing steric forces. In addition, dipyridyl porphyrins could be useful cofactors in bifunctional reagents designed to target or modify DNA.[19,20]

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