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The influence of the loop sequence in binding studies involving cationic porphyrins and DNA hairpins

Stephanie A. Bejune and David R. McMillin*

Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, Indiana, USA 47907-2084. E-mail: mcmillin@purdue.edu

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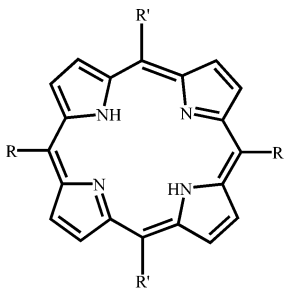
DNA hairpins are extremely versatile hosts for investigating DNA-binding interactions, but studies with a dicationic zinc(II) porphyrin reveal that the choice of loop sequence is critical when the aim is to understand adduct formation with very much longer, naturally occurring sequences.

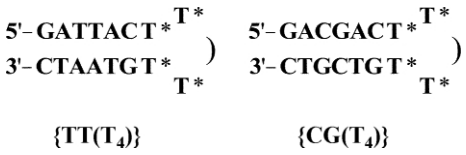
A stem-loop, or hairpin structure forms naturally in any strand of RNA or DNA that incorporates properly spaced and oriented, self-complementary runs of bases. *In vivo*, the formation of a pre-programmed hairpin structure is beneficial when it allows for the recognition of a DNA-binding protein.[1] On the other hand, the onset of some diseases probably traces back to inopportune hairpin formation during the transcription process.[2] For the elucidation of base-dependent DNA binding interactions involving cationic porphyrins, the McMillin group first employed a library of hairpin-forming oligos as convenient B-form DNA substrates.[3] Initial studies revolved around the binding of Cu(T4), the copper-containing derivative of the tetracation, 5,10,15,20-tetra(*N*-methylpyridinium-4-yl)porphyrin, or H_2T4 for short. Subsequent base-replacement strategies provided convincing evidence that the binding occurs in the stem region and that hydrogen-bonding within the double helix plays a critical role in determining the mode of interaction.[4,5] Later studies with H_2T4 provided relative binding constants for different DNA sequences.[6] For high throughput survey studies of new binding agents, Boger and co-workers have demonstrated how to use arrays of hairpins to identify high-affinity sequences.[7]



H_2T4: R = R' = N-methylpyridinium-4-yl

H_2D4: R = N-methylpyridinium-4-yl; R' = H

The flexibility of the DNA substrate is a critical element for uptake studies involving bulky agents like tetra-substituted porphyrins.[6,8,9] Thus, a variety of evidence reveals that H_2T4 intercalates into the relatively rigid polymer [poly(dG-dC)]$_2$, whereas the more flexible polymer [poly(dA-dT)]$_2$ is able to contort enough that external binding becomes a higher affinity process.[8–10] Binding of Cu(T4) shows the same base dependence. In contrast, Bejune *et al*. have shown that the less bulky di-cationic porphyrin 5,15-di(*N*-methylpyridinium-4-yl)porphyrin, designated herein as H_2D4, binds strictly as an intercalator with either [poly(dG-dC)]$_2$ or [poly(dA-dT)]$_2$.[9] The same is true of the zinc(II) derivative Zn(D4). Bejune *et al*. also found that H_2D4 intercalates into the DNA hairpins {TT(T_4)} and {CG(T_4)}. Studies with the hairpins serve as useful comparisons because {TT(T_4)} has a stem that contains mostly A=T base pairs, while that of {CG(T_4)} has more G≡C base pairs. Both hairpins derive from 16-mer strands that fold to give a 6-base-pair stem and a 4-base loop, 5′-TTTT-3′, the latter depicted with asterisks in the structures provided. For convenience the shorthand names simply designate bases that vary, in positions 3 and 4 of the oligonucleotide as well as those in the loop domain. Striking new results described herein reveal that Zn(D4) binds externally to those same hairpins but that it intercalates into the analogous hairpins with a 5′-GAAA-3′ loop sequence. Thus, simple loop replacement yields hairpins with binding properties that more faithfully mimic long-chain DNA polymers.



As noted earlier, Zn(D4) binds to [poly(dA-dT)]$_2$ or [poly(dG-dC)]$_2$ exclusively by intercalation.[9] In the Soret region, the telltale spectroscopic signs of intercalative binding include a strong (≥20%) hypochromic response and induction of a negative CD signal.[10,11] In sharp contrast, however, the adduct of Zn(D4) with either {TT(T_4)} or {CG(T_4)} exhibits relatively weak hypochromism (Table 1). In each case, the amplitude of the induced CD signal is also *positive* in the Soret region. From the very earliest work with cationic porphyrins, a positive induced CD signal has been recognized as the hallmark of external binding.[12] On the other hand, the adducts formed with the {TT(GA_3)} and {CG(GA3)} hairpins exhibit strictly *negative* CD signals and strong hypochromism, in keeping with results involving DNA polymers. A negative CD signal is only consistent with intercalative binding; see Fig. 1 for a comparison of the signals obtained for Zn(D4) binding with the {TT(T_4)} and {TT(GA_3)} systems. Loop replacement also has a pronounced effect on the progression of spectrophotometric titrations involving Zn(D4). More specifically, adduct formation is complete at a 5 : 1 hairpin : porphyrin ratio with {TT(T_4)}, but spectral changes continue to occur with the addition of {TT(GA_3)} until the hairpin : porphyrin ratio is about 15 : 1.

The above findings reflect the contrasting requirements of the two modes of binding. Recall that high-affinity external binding of a cationic porphyrin like H_2T4 is most compatible with [poly(dA-dT)]$_2$ because of its relatively weak internal hydrogen bonding

Table 1 Spectral data for Zn(D4) interacting with DNA

	Soret absorbance		Circular dichroism	
DNA host	$\Delta\lambda$/nm	%Hypo-chromism	λ_{max}/nm	$\Delta\varepsilon$/ $M^{-1}cm^{-1}$
Polymer controls				
[poly(dG-dC)]$_2$[a]	15	39	436	−3
[poly(dA-dT)]$_2$[a]	12	26	431	−16
DNA hairpins				
{TT(T_4)}	10	5	431	+10
{CG(T_4)}	9	18	430	+6
{TT(GA_3)}	13	23	430[b]	−5
{CG(GA_3)}	12	34	425	−5
{CCGG(T_4)}	10	15	436	−33

[a] Data from ref. 9. [b] Mean band position.

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scheme. From that point of view external binding should be a more favorable process with the {TT(T_4)} and {CG(T_4)} substrates due to the relatively low melting temperatures.[4] The difference in melting temperatures arises because hydrogen bonding is inherently weaker within a (5′-T_4-3′) loop as compared with a (5′-GA_3-3′) loop.[13] However, that cannot be the whole story; otherwise, H_2D4 would bind externally as well. The other piece to the puzzle is that the intercalation of Zn(D4) requires dissociation of the axially bound ligand. As a consequence the binding constant for intercalation is lower for Zn(D4) than for H_2D4.[9] Thus, a combination of factors explains why external binding is the preferred mode of interaction of Zn(D4) with {TT(T_4)} or {CG(T_4)}.

That there is no diminution in the affinity for external binding of Zn(D4) is, on the other hand, a strong indication that the metal center *remains five coordinate*. In line with this reasoning, Kelly and co-workers have reported that Zn(T4) and H_2T4 both bind externally to [poly(dA-dT)]$_2$ with virtually the same affinity.[14] Kelly and co-workers also reported that the adduct of Zn(T4) with [poly(dA-dT)]$_2$ fluoresces at somewhat shorter wavelengths than the free zinc porphyrin even though the adduct absorbs at longer wavelengths.[14] The difference in Stokes shift is indicative of some type of structural change, and substitution of the axial ligand is a likely possibility. If so, the DNA substrate may supply the fifth ligand for externally bound zinc porphyrin, and in the case of {TT(T_4)} or {CG(T_4)}, the fifth ligand could come from the (5′-T_4-3′) loop. That point not withstanding, a control study confirms that the flexibility of the DNA is the overriding issue that controls binding. Increasing the stem length provides an alternative means of bolstering hydrogen bonding within the DNA framework, and the hairpin selected for the control study was the 20-mer {CCGG(T_4)}. It differs from {CG(T_4)} by the addition of another C≡G base pair on either side of the 5′-CpG-3′ step. While the adduct of {CCGG(T_4)} with Zn(D4) exhibits modest hypochromism (H = 15%), the induced CD signal is by far the most negative ($\Delta\varepsilon$ = −33 M^{-1} cm^{-1}) in Table 1. Thus, stem extension promotes the intercalation of Zn(D4), even in the presence of a 5′-TTTT-3′ loop.

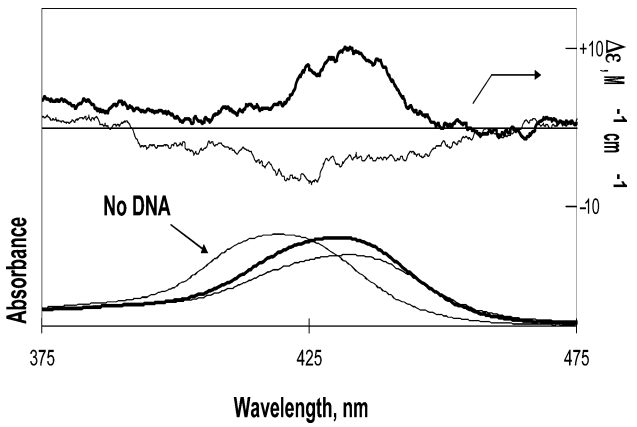

Fig. 1 Top: CD spectra of the adducts of Zn(D4) with {TT(T_4)} (thick trace) and {TT(GA_3)}. Bottom: Absorbance

The results of this investigation point to the remarkable versatility that DNA hairpins offer as hosts for binding studies. Relatively rigid constructs with short stem lengths are ideal for competitive binding studies involving reagents that have 5–10 Å footprints. One obvious advantage of the hairpin substrate is that base variations are easy to introduce in the arms of the double helix. At the same time, the short double-stranded binding domain insures that there is minimal opportunity for the binding agent to slip along the sequence. However, the results with Zn(D4) reveal that the composition of the loop is no less an important consideration. Loops that give rise to relatively fluid structures will prove useful for ascertaining steric problems and/or reorganizational requirements that particular binding agents may present. The likelihood is that loop-replacement studies will become an integral part of future investigations involving hairpin substrates.

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