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Porphyrin Binding to Quadruplexed T_4G_4

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ABSTRACT: We have recently reported the cation-induced self-assembly of DNA oligomers of the general sequence $C_4T_4G_4T_{1-4}G_4$ into high-molecular weight multistranded structures [Marotta, S. P., Tamburri, P. A., and Sheardy, R. D. (1996) *Biochemistry 35*, 10484−10492]. The architecture of the proposed structure consists of a series of four leafed G_4 tetrads tethered together via one or two T_{1-4} strands and thus resembles a long four-sided hollow tube with periodic "pockets". These pockets possess electrostatic, hydrogen bonding, and hydrophobic contact points and should be ideal candidates for the binding of small molecules. To assess the potential of using porphyrins as probes for these structures, we have investigated the interaction of tetrakis(4-*N*-methylpyridyl)porphine (H_2TMPyP) with the simple quadruplex formed by T_4G_4 and with the duplex formed by CGCGATATCGCG. Visible absorption, circular dichroism, and fluorescent energy transfer studies indicate that H_2TMPyP binds to both the duplex and quadruplex via intercalation at low [porphyrin]/[DNA molecule] ratios, i.e., in the presence of excess potential DNA binding sites. Analyses of Scatchard plots show that H_2TMpyP binds with high affinity to both DNA secondary structures but binds to the quadruplex with an affinity 2 times greater than that of the duplex.

The formation of four-stranded DNA structures (i.e., quadruplexes) by guanine-rich DNA oligomers has received considerable interest in the recent literature. Such structures have been implicated in single-stranded telomeric DNA. Telomeric DNA is the noncoding DNA found at the ends of linear eukaryotic chromosomes with the general sequence of tandemly repeating segments of six to eight bases possessing guanine clusters, such as TTGGGG, found in *Tetrahymena*, and TTAGGG, found in humans (*1*−*7*). Most of the telomeric DNA is duplexed to its complement; however, at the 3′ terminus, a single-stranded segment containing the G-rich repeat is found.

The use of specifically designed DNA oligomers with sequences similar to those found in natural telomeric DNA affords a means for modeling the architecture and thermodynamics of these DNA structural motifs. Such studies have implicated G·G Hoogstein pairing involving N-7 of guanine in quadruplex formation to give rise to the G tetrad as shown in Figure 1A (*8*−*12*). The molecular architectures of the resultant four-stranded structures are highly dependent upon the sequence of the DNA and the conditions under which it has been prepared. Oligomers of the sequence T_4G_4 and T_2G_4 form simple parallel-stranded quadruplexes (*8*, *12*−*15*). It has been demonstrated that K^+ is more effective in inducing quadruplex formation than Na^+ for simple oligomers with single G_x ($x = 2-4$) repeats and may give rise to different conformations and/or stabilities (*13*, *15*−*19*). In addition, Mg^{2+} has not been shown to have a favorable influence on quadruplex formation (*19*).

Since the sequence of T_4G_4 used for this study is typical of other sequences found in naturally occurring telomeric DNA, its structure and ligand binding properties are of biological interest. The focus of this work was to investigate

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the ligand binding properties of the quadruplex formed from T_4G_4. The use of small molecules to probe quadruplexed DNA structures may give us additional information on the stability of quadruplexes and help us to understand better the role of telomeres in preserving the integrity of chromosomal replication.

To probe the quadruplex formed from T_4G_4, a molecule such as H_2TMpyP (shown in Figure 1B) was a proper choice for the following reasons. (1) The dimensions of H_2TMpyP are roughly similar to those of a G tetrad. (2) It has constituents that can hydrophobically and electostatically interact with a G tetrad. (3) It has been shown that H_2TMpyP intercalates into double-stranded DNA, and these interactions can be monitored conveniently by visible spectroscopy to calculate binding constants (*20*−*23*).

Here we present studies focusing on the specificity of interaction of H_2TMpyP with the quadruplex formed from T_4G_4 and with a 12-base pair DNA duplex for comparison. The results indicate that the secondary DNA structure plays a role in the binding of porphyrins.

MATERIALS AND METHODS

DNA Synthesis and Preparation. The DNA oligomers were synthesized on a 1.0 μm scale via the phosphoramidite method on an Applied Biosystems 380B DNA synthesizer with purification by trityl select reverse phase HPLC as previously reported (*24*, *25*). After the second HPLC purification, the oligomers were exhaustively dialyzed versus water and then lyophilized to dryness. Product purity was verified via analytical HPLC. The lyophilized DNA samples were reconstituted in standard TBE buffer (100 mM TRIS-borate and 0.01 mM EDTA at pH 8.0) containing 100 mM K^+ and 20 mM Mg^{2+}, heated to 90 °C for 2 min, allowed to slowly cool to room temperature, and then stored for 48 h at 4 °C before use. The presence of both K^+ and Mg^{2+} allows for the formation of the quadruplex for T_4G_4 (*25*). The concentrations of DNA were calculated spectrophotometrically using an ϵ_{260} of 6.5×10^3 M^{-1} cm^{-1} in DNA bases.

The porphyrin-free base H_2TMPyP [5,10,15,20-tetrakis-(1-methyl-4-pyridyl)-21*H*-porphine, tetra-*p*-tosylate salt] was purchased from Aldrich Chemical Co. and was used without further purification. Concentrations of the porphyrin in the absence of DNA were calculated spectrophotometrically using an ϵ_{424} of 2.26×10^5 M^{-1} cm^{-1} (*21*, *22*). Porphyrin solutions were prepared in the same buffer as the DNA oligomers for all experiments.

Visible Spectroscopy. The binding of H_2TMPyP with the two different DNAs was monitored by visible spectroscopy. For each oligomer, a solution of DNA ([DNA] = 0.25−0.40 mM in bases) and H_2TMPyP ([H_2TMPyP] = 3.0−5.0 mM) was titrated into a solution of H_2TMPyP at the same porphyrin concentration (i.e., at 3.0−5.0 mM) and the spectrum recorded from 400 to 500 nm after incubation for 5 min. The spectrum of the porphyrin before addition of the DNA/porphyrin solution was also recorded.

Circular Dichroism. Samples were prepared in TBE buffer with 100 mM K^+ and 20 mM Mg^{2+} containing the DNA at different concentrations and the porphyrin at a fixed concentration of 3.0 mM. For the DNAs, the concentrations of $(CG)_4ATAT(CG)_4$ and T_4G_4 were either 4.2 or 8.4 mM in the duplex and quadruplex, respectively. Hence, the porphyrin is fully bound under these conditions. The circular dichroism spectrum for each porphyrin/DNA complex at 25 °C was recorded on an AVIV 62A DS circular dichroism spectropolarimeter. Wavelengths of 220−320 nm were used to monitor the DNA region and 400−500 nm to monitor the porphyrin Soret band.

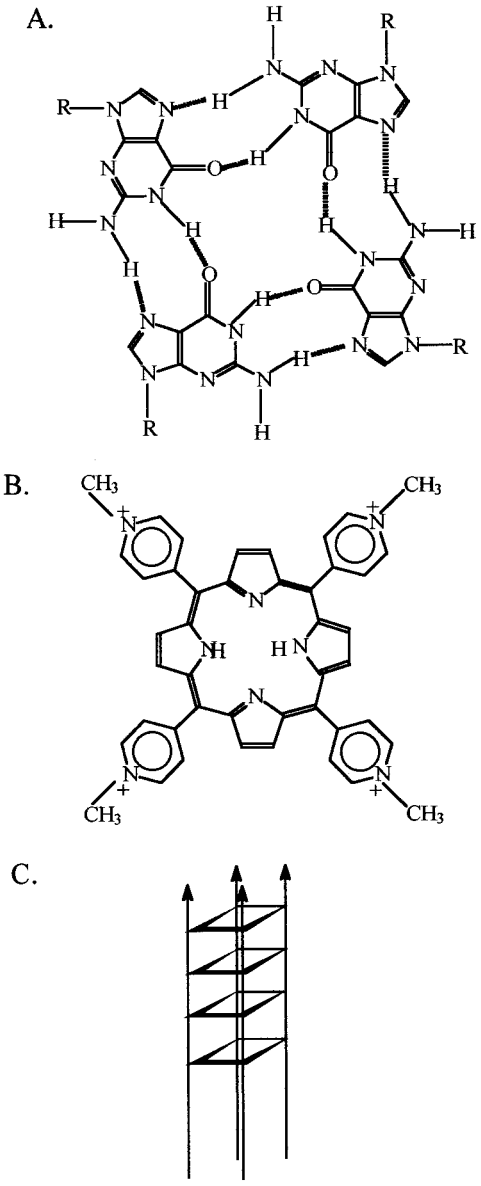

FIGURE 1: (A) Individual G tetrad showing the Hoogstein base pairing (dashed lines). The R groups represent the sugar−phosphate backbone. (B) Structure of the porphyrin tetrakis(4-*N*-methylpyridyl)porphine (H_2TMPyP) drawn to the same scale as the G quartet in panel A. (C) Schematic representation of the parallel-stranded quadruplex formed from T_4G_4. The arrows reprersent the sugar−phosphate backbones running in a 5′ to 3′ direction, and the leaflets are individual G tetrads as depicted in the upper panel.

Energy Transfer Experiments. Fluorescence energy transfer experiments were carried out with a SPEX Tau-2 fluorescence spectrofluorimeter using right angle geometry by a procedure described by Le Pecq and Paolett (*26*, *27*). The emission monochromator was set at 660 nm, and the excitation spectra were measured from 240 to 314 nm at 2 nm intervals. A yellow filter was used at the emission side to filter out the second-order spectra. A correction for the

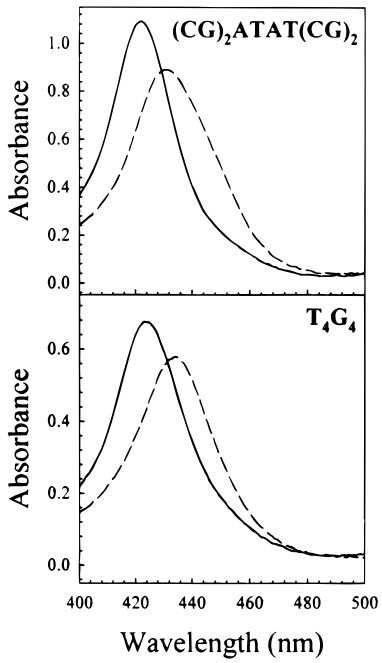

FIGURE 2: Visible spectra of H_2TMPyP in the absence (solid line) or presence (dashed line) of $(CG)_2ATAT(CG)_2$ at a [porphyrin]/[DNA duplex] ratio of 8.33 and of T_4G_4 at a [porphyrin]/[DNA quadruplex] ratio of 8.33. The concentration of H_2TMPyP is 5.0 mM in the absence or presence of duplex and 3.0 mM in the absence or presence of quadruplex. Notice the pronounced red shift and hypochromicity in the Soret band (i.e., 422 nm) of the porphyrin in the presence of either DNA. The spectral data are summarized in Table 1.

inner filter effect was applied by multiplying the measured fluorescent intensity by the decimal antilog of the absorbance of the solution (*27*). The concentration of DNA was 40 mM in bases, and the concentration of porphyrin was 4 mM for these determinations.

RESULTS

The oligomers used for this study were T_4G_4, which forms a simple parallel four-stranded quadruplex (*8*, *13*−*15*) and the self-complementary 12-mer, $(CG)_2ATAT(CG)_2$, which exists as a simple right-handed duplex under the experimental conditions. A schematic representation of the four-stranded structure formed from T_4G_4 is shown in Figure 1C. Both oligomers were greater than 99% pure as determined via analytical HPLC and denaturing polyacrylamide gel electrophoresis.

Visible Absorption Titration Studies. Figure 2 shows the visible spectra of the porphyrin in the absence or presence of excess T_4G_4 or 12-mer. For both DNA oligomers, changes in the Soret band (i.e., at 422 nm) of the porphyrin are observed in the presence of excess DNA; the spectra are characterized by red shifts and hypochromicities in this band. The magnitudes in these changes are quite different for two different DNAs; the quadruplex produces a larger shift in λ_s, the Soret band, and a greater degree in hypochromicity for this band. Table 1 summarizes these results.

The free porphyrin was titrated with a DNA/porphyrin solution (at the same porphyrin concentration) to construct Scatchard plots of r/C_f versus r (Figure 3). The binding ratio r is defined as C_b/[DNA], where the molar concentration of bound porphyrin, C_b, is equal to $\Delta A/\Delta\epsilon$ (ΔA is the difference

Table 1: Effect of DNA on the Spectral Properties of H_2TMPyP

	Soret band[a]		induced CD[b]	
oligomer	$\Delta\lambda_s$	% hypochromicity	λ_I	$\Delta\epsilon$
$(CG)_2ATAT(CG)_2$	10	34.4	450	−45.3
T_4G_4	12	40.6	428	−17.6

[a] $\Delta\lambda_s$ is calculated as $\lambda_b - \lambda_f$, where λ_f is the wavelength of maximum absorbance of the Soret band for the free porphyrin and λ_b is the wavelength of maximum absorbance of the Soret band for the porphyrin in the presence of excess DNA (i.e., under conditions where the porphyrin is fully bound). The % hypochromicity was calculated using the expression $\%H = [(\epsilon_f - \epsilon_b)/\epsilon_f] \times 100\%$, where ϵ_f and ϵ_b are, respectively, the extinction coefficients of free and fully bound porphyrin at λ_s, at the Soret band at 422 nm. [b] λ_I is the wavelength of maximum induced molar ellipticity. $\Delta\epsilon$ is the magnitude of the induced molar ellipticity at a [porphyrin]/[DNA duplex] or [porphyrin]/[DNA quadruplex] ratio of 0.73 for $(CG)_2ATAT(CG)_2$ or T_4G_4, respectively.

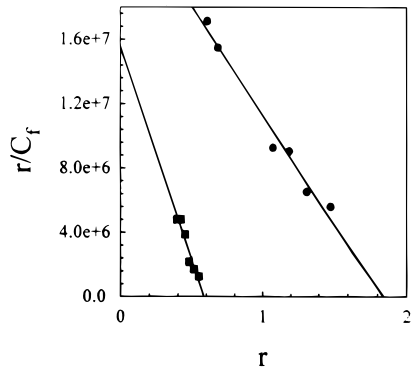

FIGURE 3: Scatchard plots for the titration of H_2TMPyP with $(CG)_2ATAT(CG)_2$ (circles) and T_4G_4 (squares). The value of r was calculated in terms of the concentration of DNA duplex for $(CG)_2ATAT(CG)_2$ and DNA quadruplex for T_4G_4. The data are represented by the points, and the fits, calculated with eq 1, are represented by the lines. For $(CG)_2ATAT(CG)_2$, the slope of the resultant fitted line is -1.3×10^7 and the y-intercept is 2.5×10^7 with an r^2 of 0.979; for T_4G_4, the slope of the resultant fitted line is -2.7×10^7 and the y-intercept is 1.6×10^6 with an r^2 of 0.924. The resultant calculated K and n values are listed in Table 2.

in absorption of the free porphyrin and porphyrin in the presence of DNA at 424 nm and $\Delta\epsilon$ is the difference in molar extinction coefficients between the free and bound porphyrin, $\epsilon_f - \epsilon_b$). The value of ϵ_b was calculated by dividing the absorbance of the porphyrin Soret band at 424 nm by the concentration of porphyrin (i.e., 5.0 mM) of a solution containing the porphyrin at that concentration with excess DNA (i.e., [porphyrin]/[DNA duplex] or [porphyrin]/[DNA qudruplex] = 8.33). The concentration of free porphyrin, C_f, was calculated using $C_t = C_b + C_f$, where C_t is the total concentration of porphyrin (i.e., 5 mM). The DNA concentration is expressed in terms of the molar concentration of quadruplex for T_4G_4 or molar duplex concentration for the 12-mer. Due to the simplicity of the DNA molecules, the data were fit via the simple Scatchard equation:

$$r/C_f = K(n - r) \quad (1)$$

where K is the equilibrium binding constant and n represents the number of ligands bound per DNA duplex for $(CG)_2ATAT(CG)_2$ or quadruplex for T_4G_4. The high degree of linearity of the fits indicates good correlation to the identical-and-independent sites model applied. The resultant binding parameters are listed in Table 2. Porphyrin binding to the

Table 2: Equilibrium Binding Properties of DNA Sequences for H_2TMPyP[a]

oligomer	K (mol^{-1})	n
$(CG)_2ATAT(CG)_2$	1.3×10^7	1.92
T_4G_4	2.7×10^7	0.59

[a] All measurements were carried out in 100 mM TBE buffer with 100 mM K^+ and 20 mM Mg^{2+}. The values of K and n were evaluated with eq 1. K represents the binding constant per mole of duplex or quadruplex, and n is the maximum number of porphyrin molecules bound by intercalation into DNA duplex for $(CG)_2ATAT(CG)_2$ or DNA quadruplex for T_4G_4.

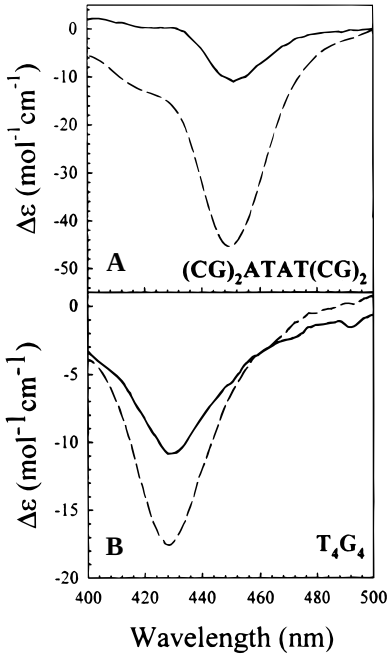

FIGURE 4: Induced CD in the Soret region of H_2TMPyP in the presence of DNA at [porphyrin]/[DNA] ratios of 0.37 (solid line) and 0.73 (dashed line) for $(CG)_2ATAT(CG)_2$ and T_4G_4. The [porphyrin]/[DNA] values were calculated in terms of the concentration of DNA duplex for $(CG)_2ATAT(CG)_2$ and DNA quadruplex for T_4G_4.

quadruplex is about 2-fold stronger than binding to the duplex. The value of n indicates a binding of only one porphyrin for every quadruplex, while two porphyrins bind per duplex (since it is not physically possible to have a fractional number of molecules bound to the DNA, the values of n have been rounded here to the nearest whole number).

Circular Dichroism Studies. In the absence of the porphyrin, the CD spectrum for T_4G_4 was typical of parallel four-stranded oligomers (*13*, *24*, *25*), characterized by a major peak at 259 nm and shallow trough at 238 nm. The 12-mer duplex had a CD spectrum typical of right-handed double-stranded DNA oligomers. The addition of the porphyrin to DNA causes a slight decrease in the molar ellipticity of the quadruplex at 259 nm and for the 275 nm peak of the 12-mer duplex (data not shown).

Figure 4A shows the CD spectra for H_2TMPyP in the presence of the DNAs under consideration at two different [porphyrin]/[DNA] ratios. A negative induced CD band is observed for both oligomers: at 428 nm in the presence of the quadruplex and at 450 in the presence of the duplex. Although the magnitudes of $\Delta\epsilon$ are identical for the two DNA structures when [porphyrin]/[DNA molecule] = 0.36

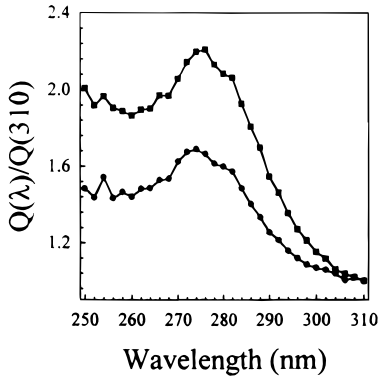

FIGURE 5: Energy transfer from DNA bases to H_2TMPyP for $(CG)_2ATAT(CG)_2$ (circles) at a [porphyrin]/[DNA] ratio of 1.2 and T_4G_4 (squares) at a [porphyrin]/[DNA] ratio of 1.6. The [porphyrin]/[DNA] values were calculated in terms of the concentration of DNA duplex for $(CG)_2ATAT(CG)_2$ and DNA quadruplex for T_4G_4. $Q(\lambda)/Q(310)$, the ratio of quantum efficiency at a given wavelength to that at 310 nm, was calculated according to eq 2.

(i.e., -10.9 M^{-1} cm^{-1} for both the duplex and quadruplex), the magnitudes are quite different at the higher ratios. Specifically, at a [porphyrin]/[DNA molecule] ratio of 0.73, the value of $\Delta\epsilon$ is -17.56 M^{-1} cm^{-1} for the quadruplex and -44.95 M^{-1} cm^{-1} for the duplex. These CD data are summarized in Table 1.

Fluorescence Energy Transfer Studies. If a ligand is in close contact with the DNA base pairs, fluorescent energy transfer from the base pairs to the ligand can occur (*26*, *27*). This technique has been used to ascertain intercalation for fluorescent probes such as porphyrins and ethidium. Other modes of binding such as groove binding or external binding give rise to a situation where the porphyrin is not in close contact with the bases and is in an unfavorable orientation for energy transfer. The relative quantum efficiency of energy transfer is calculated as shown below:

$$\phi(\lambda)/\phi(310) = \{[I(\lambda)/I(310)][\epsilon(310)/\epsilon(\lambda)]\}_b\{[I(310)/I(\lambda)][\epsilon(\lambda)/\epsilon(310)]\}_f \quad (2)$$

where ϕ is the quantum yield at either λ or 310 nm, I is the fluorescence intensity, and ϵ is the extinction coefficient at the indicated wavelengths. The subscripts b and f refer to bound and free ligand, respectively. A value for λ of 310 nm is chosen as the normalization wavelength as DNA has a very low absorbance in this region. A ratio of $\phi(\lambda)/\phi(310)$ of greater than unity indicates intercalation (*13*). Figure 5 indicates that $\phi(\lambda)/\phi(310) = 1.69$ for the duplex and 2.21 for the quadruplex.

DISCUSSION

A number of studies have investigated the interaction specificities of porphyrins with nucleic acids (*28* and references therein). Most of these studies have used either polymeric calf thymus DNA or alternating poly(dG-dC) and poly(dA-T). The primary modes of binding of the porphyrin to the DNA lattices are intercalation, outside stacking, or groove binding. The exact nature of the interaction depends on the DNA base content, the ionic strength and pH of the media, and whether the porphyrin is metalated or exists as

the free base (*28*, *29*). Intercalation is the preferred mode of binding for the unmetalated H_2TMPyP to DNA with GC sites, while outside binding without stacking is favored at AT sites at moderate concentrations of porphyrin and ionic strengths (*21*, *22*, *28*). Intercalation of the porphyrin between the base pairs of DNA is typically indicated by large red shifts of the Soret band, substantial hypochromism of the Soret band, and a negative induced CD band in the Soret region (*21*, *29*). On the other hand, outside binding without stacking is typically characterized by small red shifts of the Soret band, small hypochromicity or even hyperchromicity of the Soret band, and a positive induced CD in the Soret region (*21*, *29*). Outside binding with stacking is indicated by variable effects on the Soret band (*29*).

Recently, an X-ray structure of a porphyrin−DNA complex was presented. For the complex, the Cu(II) derivative of H_2TMPyP was bound to a DNA hexamer duplex of the sequence $[d(CGATCG)]_2$. The porphyrin binds by simple intercalation to the GC site of the 5′-TCG-3′ trinucleotide step with extruding the C of the 5′-CGA-3′ trinucleotide step (*30*).

In this study, we have demonstrated that H_2TMPyP interacts with both a DNA oligomeric duplex and DNA quadruplex. Upon binding to either DNA structure, the complex is characterized by a moderate red shift (10−12 nm) and substantial hypochromism (34−41%) in the Soret band of the porphyrin. Although the observed red shift is intermediate between what is observed for intercalation (≥15 nm) and that for outside binding (≤8 nm), the observed hypochromism is typical for an intercalated porphyrin (*21*).

The intercalation of H_2TMPyP into poly(dG-dC) results in induced CD bands in the Soret region of the porphyrin arising from the coupling of the transition moments to those of DNA bases. The observation of a negative induced CD band in the Soret region for both the duplex and the quadruplex indicates that the mode of interaction of the porphyrin with these DNAs is intercalative. The wavelength of the induced CD band for the 12-mer at 450 nm suggests that the porphyrin may be protonated in the complex (*29*, *31*). The observation of energy transfer from the DNA bases to the porphyrin lends further support to the possibility that H_2TMPyP intercalates into both DNA secondary structures. Taken as a whole, the spectroscopic and energy transfer experiments all indicate that H_2TMPyP interacts with the oligomer duplex and quadruplex via intercalation at low concentrations of porphyrin. It should be noted that, at higher concentrations of porphyrin, more complex behavior is observed, which suggests mixed modes of binding (N. V. Anantha and R. D. Sheardy, unpublished results).

A limited number of reports have investigated the interaction of other small molecules with quadruplexed DNA structures (*32*−*37*). Kallenbach followed the interaction of ethidium bromide with the quadruplexed forms of (dT_4G_4), which forms a four-stranded quadruplex, and $(dT_4G_4)_4$, which forms a one-stranded fold-back "aptamer" quadruplex, via optical and calorimetric methods. The results indicated that ethidium binds to the tetramolecular (dT_4G_4) with an affinity similar to that a DNA duplex but did not bind to the monomolecular $(dT_4G_4)_4$ (*32*). Actinomycin, ethidium, and chromomycin have also been shown by Chen to have low affinities for the monomolecular $(dT_4G_4)_4$ and $(dT_2G_4)_4$ quadruplexes (*33*). However, bleomycin binds to and cleaves the monomolecular $(dT_4G_4)_4$ but not the dimeric form of $(dG_4T_4G_4)$ (*34*). Monomolecular aptamers formed from G-rich oligonucleotides have also been shown to bind ATP and adenosine (*35*) as well as anionic porphyrins (*36*). Finally, a carbocyanine dye, 3,3′-diethyloxadicarbocyanine, will bind to the dimeric fold-back quadruplexes formed by DNA oligomers possessing two G_x ($x = 3$ or 4) sequences separated by linkers of 3−6 bases (*37*).

The purpose of this study was to investigate how porphyrins would interact with DNA quadruplexes. H_2TMPyP was chosen since its dimensions are quite similar to that of the G quartet and thus the stacking, in the event of intercalation, should be substantial. The G tetrad can be considered a square of 10−11 Å per side, while the pyridyl N to pyridyl N distance of the porphyrin is about 9 Å. The positive charges on the porphyrin can also interact with the phosphates of the tetrad. Hence, electrostatic interactions should also be significant, even at the ionic strength of the buffer used for these studies (i.e., 215 mM). The porphyrin system should thus be an ideal probe for quadruplex structure.

The visible and CD spectral as well as the energy transfer data strongly indicate that, at low [porphyrin]/[DNA] ratios, H_2TMPyP binds to the quadruplex formed by T_4G_4 or the duplex formed from $(CG)_2ATAT(CG)_2$ via intercalation with high affinity. Further, H_2TMPyP binds to the quadruplex 2 times more strongly than it does to the duplex. Kallenbach's group previously demonstrated that ethidium bromide binds to T_4G_4 with a binding constant K of 1.5×10^5 M^{-1} and an n of 1 at 200 mM Na^+ (*32*). Thus, under similar ionic strength conditions, H_2TMPyP binds to the same quadruplex with an affinity 180 times greater than that of ethidium. In addition, the values of the maximum number of porphyrins bound per DNA structure are quite reasonable. In the case of the 12-mer, n is close to 2. H_2TMPyP prefers to intercalate at CG sites (*21*, *22*), and the 12-mer has two such sites separated by an ATAT linker. For the quadruplex, n is close to 1 and the quadruplex has four G tetrads giving rise to three potential intercalation sites. The binding of only one porphyrin to the quadruplex can be interpreted in light of neighbor exclusion. Another possibility that cannot be ruled out at this time is the formation of a "sandwich" type complex involving one porphyrin stacked between the 5′ termini G tetrads of two quadruplexes. Further studies are underway to address this question. Finally, the application of the Scatchard analysis using the independent-and-noninteracting sites model is quite appropriate for the binding data.

The sequence of T_4G_4 used for the ligand binding studies presented here is typically that found at the ends of eukaryotic chromosomes. During DNA cell replication prior to cell division, some of the telomeric sequences are lost; as long as the lost fragments are telomeric and not coding sequences, the cell maintains its viability (*38*−*42*). It has been suggested that the length of the entire telomeric segment predetermines how many times a cell can normally reproduce (*38*, *39*). The enzyme telomerase rebuilds the chromosomal ends using an RNA fragment as the part of the primer (*40*). It has been shown that, in more than 85% of all types of cancer cells tested, both the telomeric DNA segment has been shortened and the telomerase activity is high (*41*). Hence, the enhanced telomerase activity is rebuilding the ends of the chromosomes and allowing the cell to reproduce more and faster than normal (*42*).

Although the structure associated with the single-stranded region of the telomeric DNA at the termini of chromosomes is not known, it has been suggested that this segment is quadruplexed in some fashion. If this is indeed the case, telomerase activity must be predicated by denaturation of the quadraplexed region into single strands. Compounds that could selectively recognize, and hence stabilize, quadraplexed structures would inhibit the mandated denaturation and thus inhibit telomerase activity. The development of such compounds would lead to a whole new class of anti-cancer drugs with high specificity for cancer cells. A basic understanding of how small ligands interact with quadraplexed structure would make a significant contribution to the development of such therapeutic agents. The results presented here suggest that porphyrin type molecules could be the starting point for the design of telomerase inhibitors. Appropriate modification of the basic porphyrin structure should give rise to compounds with affinities for quadruplexes much greater than those for duplexes. This condition must be met in order to specifcally target quadruplex structures in vivo.

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