Comparison of the Binding Geometry of Free-Base and Hexacoordinated Cationic Porphyrins to A- and B-Form DNA

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Supporting Information

ABSTRACT: Although the transition from B-DNA to the A-form is essential for many biological concerns, the properties of this transition have not been resolved. The B to A equilibrium can be analyzed conveniently because of the significant changes in circular dichroism (CD) and absorption spectrum, CD and linear dichroism (LD) methods were used to examine the binding of water-soluble meso-tetrakis(N-methylpyridinium-4-yl)porphyrin (TMPyP) and its derivatives, Co-TMPyP, with B- and A-calf thymus DNA. B- to A-transitions occurred when the physiological buffer was replaced with a water-ethanol mixture (~80 v/v %), and the fluorescence emission spectra of TMPyP bound to DNA showed a different pattern under ethanol—water conditions and water alone. The featureless broad emission bands of TMPyP were split into two peaks near at 658 and 715 nm in the presence of DNA under an aqueous solution. In the case of an ethanol—water system, however, the emission bands are split near at 648 and 708 nm and 658 and 715 nm with and without DNA, respectively. This may be due to a change in the solution polarity. On the basis of the CD and LD data, TMPyP interacts with B-DNA via intercalation at a low ratio under a low ionic strength, 1 mM sodium phosphate. On the other hand, the interaction with A-DNA (80 v/v % ethanol—water system) occurs in a nonintercalating manner. This difference might be because the structural conformations, such as the groove of A-DNA, are not as deep as in B-DNA and the bases are much more tilted. In the case of Co-TMPyP, porphyrin binds preferably via an outside self-stacking mode with B- and A-DNA.

INTRODUCTION

The structure of DNA can be described by a number of parameters, such as the diameter of the helix, the tilt of the base pairs, the nature of the grooves of the helix, and the twist of the base pairs.1,2 Most of the DNA is in the classic Watson–Crick model, simply called B-form DNA. Under certain conditions, different forms of DNAs appear like A (originally identified by X-ray diffraction of analysis of DNA fibers at 75% relative humidity),3,4 Z (left-handed double-helical structure winds to the left in a zig-zag pattern),5 C (formed at 66% relative humidity and in the presence of Li+ and Mg2+ ions),6 D (rare variant with eight base pairs per helical turn, form in structure devoid of guanine), and E (extended or eccentric)-DNA (Vinitha Unnikrishnan, 2015, unpublished results). This deviation is based on their structural diversity. Ethanol at high salt concentrations will induce the aggregation of DNA.7 Synthetic polynucleotides such as poly[d(A-T)2] and poly[d-(G-C)2] also undergo a B–A transition under suitable conditions. Structural transitions were examined in various synthetic polynucleotides.8,9 Some studies have shown that a significant activation barrier exists in the B to A transition and that the helical states are clearly separated from each other.10 Recently, new molecular dynamics simulations from A-DNA to B-DNA in solution were reported.11,12 The B to A transition of DNA in water—ethanol solutions has been studied by circular dichroism (referred to as CD) since the beginning of 1974. Valery et al. reported that the B–A equilibrium is not influenced by temperature. This transition may have only a slight dependence (if any) on the GC content because the transition width is the same for the heterogeneous calf thymus DNA (referred to as DNA).5 Some research found that water–water interaction energy correlates with the entropy change, thereby indicating a role of water in the entropy reduction in the B to A transition.13 The B to A transition, in which low-humidity conditions locally change the base-stacking arrangement and globally induce DNA condensation, may eventually stabilize the molecular contour-length reduction.14 It was reported that the conductance of DNA duplexes increases by approximately 1 order of magnitude when its conformation is changed from the B-form to the A-form.15 Hole transport in A-form DNA/RNA hybrid duplexes has been studied. On the basis of the results, there is no directional preference of hole migration toward 5′ or 3′ end for both the B-form and A-form duplexes.16 The Z-conformation favors alternating purine–pyrimidine repeats, particularly alternated G–C base-pairs, even though the Z-form is known in other mixed sequences.17

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Cationic porphyrins have been known to bind DNA. They display a wide variety of binding modes, including intercalation,\(^{18,19}\) monomeric minor groove binding,\(^{20−25}\) and moderate and extensive stacking to B-form DNA.\(^{26−29}\) Avestisyan et al. examined the interaction of \(\text{meso-tetra-(4N-oxoethylpyridyl)}\)-porphyrin (TOEPyP4) with the A-form of DNA. They explored that porphyrin interacts with B-DNA via an intercalation at low relative concentrations and external binding with increasing porphyrin to the DNA ratio, whereas the interaction of TOEPyP4 with A-DNA occurs via the outside binding only.\(^\text{Proceedings of the YSU, Physics & Mathematics, 2014, 3, 43−48, unpublished result.}\) In the case of Co-TMPyP, porphyrin interacts with B- and A-DNA only through external groove binding. To better understand the binding properties between DNA and porphyrin derivatives under ethanol−water conditions, herein, we have studied the binding properties of TMPyP and its Co-containing derivative (Co-TMPyP) bound to the B- and A-forms of DNA.

### RESULTS AND DISCUSSION

The concentration of DNA for this series of measurements was fixed at 100 \(\mu\text{M}\), whereas that of TMPyP was varied from 1 to 5 \(\mu\text{M}\) in 1 \(\mu\text{M}\) increments. The resulting absorption spectra were normalized to the highest TMPyP concentration for the ease of comparison. Fluorescence emission spectra measured with a 10-fold diluted DNA−drug solution to minimize the inner-filter effects.

**Absorption Spectroscopic Properties of TMPyP and Co-TMPyP with DNA in 80% Relative Humidity.** The absorption spectrum of TMPyP associated with DNA in an aqueous solution may be classified into two categories. TMPyP that is intercalated to DNA or a GC-rich synthetic polynucleotide produced large hypochromism and a red shift because of the increasing \(\pi−\pi\) stacking and changes in the porphyrin environment.\(^{30,31}\) The extent of changes in the absorption spectrum is less pronounced when it binds to the minor groove or stacked along the DNA stem. The absorption spectra of TMPyP complexed with DNA under 80% ethanol and aqueous solutions are depicted in Figure 1A-B. In the case of ethanol presence, the absorption maxima appeared at ca. 425 nm. Upon binding to DNA, a 23% hypochromism and a 3 nm red shift were observed (from 424 nm of free TMPyP to 433 nm in the presence of DNA). Under an aqueous buffer solution, the absorption maxima appeared at ca. 421 nm. The hypochromism and red shift were 47.3% and 18 nm (from 421 to 439 nm), respectively. The binding environment may reflect the change in the binding property.

**Figure S1** depicts the absorption spectrum of Co-TMPyP bound to DNA under 80% ethanol (A) and aqueous solution (B). Under the ethanol condition, hypochromicity was 18% with no wavelength shift, and a 21% hyperchromism and a 6 nm red shift were observed in an aqueous buffer solution. The absorption results suggest that the interactions or binding characteristics of TMPyP or Co-TMPyP with DNA against those reaction buffer systems (e.g., with different water activities at the same ionic strength). Herein, the term “water activity” describes the amount of water available for hydration of materials) are different from each other.

**CD Spectroscopic Properties.** Circular dichroism is a useful tool for tracing the transition of the B-form to A-form, which is the available one to detect the shift curve under ethanol/trifluoroethanol conditions.\(^{32,33}\) Figures 2 and S2 show the CD spectra that were recorded at an R-ratio of 0.05, when associated with DNA in an 80% ethanol solution, TMPyP produced a complex CD spectrum with one positive maxima at 433 nm and a negative band at 451 nm (Figure 2A). The insertion in Figure 2A is the CD spectrum of the B-form (blue line) and A-form (red line) DNA in the DNA region. The magnitude of the A-form DNA is much larger than that of the classical B-form. Under the aqueous buffer, however, a negative CD spectrum was observed. In general, a negative CD signal is the main indicator of the intercalative binding of TMPyP to DNA. On the other hand, the CD spectrum of the DNA and Co-TMPyP complex under ethanol−water conditions was characterized by the apparent positive band at 454 nm (Figure S2A), which almost coincides with that observed in the aqueous solution (Figure S2B). This type of positive CD band for the porphyrin family is considered diagnostic for the outside binding mode. A clear bisignate CD spectrum produced by the DNA−TMPyP complex, which is usually considered to be the result of a \(\pi−\pi\) interaction between DNA-bound porphyrin (stacking interaction), was also against the intercalation binding mode.

**Fluorescence Emission Spectroscopic Properties.** Fluorescence spectroscopy is useful for examining the association of the dyes with proteins, particularly regarding the binding mechanism in terms of the binding site and structural and conformational alternation under various micro-environments.\(^{34}\) The intercalative binding geometry of the DNA intercalator, such as 9-aminoacridin to DNA, which is
independent of the ionic strength, was confirmed to be different compared to that of the classical intercalation mode.35

Figure 3A,B shows the fluorescence emission spectra of TMPyP bound to DNA under different buffer systems: ethanol-containing conditions and aqueous buffer solution, respectively. Under a less polar environment, which is simulated by 80% ethanol, the observations of DNA were different from those with the aqueous buffer system. The emission bands of free TMPyP were split into two peaks near 658 and 715 nm, and the TMPyP—DNA complex was split at 648 and 708 nm.

In the case of TMPyP in an aqueous solution, the featureless broad emission bands of the free TMPyP were split into two peaks near 656 and 715 nm in the presence of DNA. As expected, the observations were different under the ethanol—water system and under aqueous buffer system, respectively. These results are probably due to the different solution polarity or local environment.

Reduced Linear Dichroism Spectroscopic Properties. Figure 4 shows the reduced linear dichroism (LD') spectrum obtained by division of the measured LD by the absorption spectrum. In the DNA—TMPyP complex in an aqueous solution, a wavelength-dependent signal with a comparable or larger magnitude than that of the DNA absorption region was apparent,32 suggesting an intercalative binding mode. On the other hand, a large tilt between the $B_x$ and $B_y$ transition moments of porphyrin in the intercalation pocket can be observed from the wavelength-dependent LD' signal (Figure 4B). In the presence of 80% ethanol, an apparent positive magnitude was observed in the Soret region (Figure 4A). In general, this pattern reflects that the angle between the $B_x$ and $B_y$ transition moments of porphyrin and the local DNA helix axis did not match with the intercalation binding mode. In other words, the positive contributions in the LD' spectrum may be understood as a strong tilt of the transition moment of TMPyP with respect to the DNA helix axis because of a conformational change by 80% ethanol. The porphyrin moieties under low water activity conditions tend to self-stack at the outside of DNA. In the DNA and Co-TMPyP complex in an aqueous solution and ethanol—water conditions, a wavelength-dependent signal with a much smaller magnitude than that of the DNA absorption region was observed (Figure S3), suggesting outside stacking binding mode or that a part of the porphyrin is conceivably bound at the groove.

Analysis of the LD' Spectrum of TMPyP Bound to A- and B-Form DNA. If there is a single electric transition moment for a DNA-bound molecule and the binding mode is homogeneous, a wavelength-independent LD' is expected in the molecule absorption region. The appearance of wavelength-dependent LD' in the absorption region of the DNA-bound molecule suggests that at least two electric transition moments with different angles relative to the local DNA helix axis are involved in a given absorption band. In particular, the wavelength-dependent LD' in the Soret region of the DNA-
bound TMPyP suggests that the degeneracy of the $B_x$ and $B_y$ transitions of TMPyP is removed partially. In this case, the LD spectrum may be analyzed by noticing that the absorption and LD spectra are the sum of the contributions of the two electric transition moments

$$\lambda = t_1 T_1(\lambda) + t_2 T_2(\lambda)$$  
$$\text{LD}(\lambda) = t_3 T_3(\lambda) + t_4 T_4(\lambda)$$

where $T_1$ and $T_2$ are the contributions of the absorption profile of $B_x$ and $B_y$ to the absorption spectrum, and $T_3$ and $T_4$ are those that contribute to the LD spectrum. The $t_i$ values are coefficients. The pure contribution of $T_1$ can be obtained by a step-wise subtraction of the properly tuned LD spectrum, $\kappa \text{LD}(\lambda)$, multiplied by a weighing factor ($\kappa$), from the measured absorption spectrum because the contribution of the two transition moments to the absorption and LD spectra are different.

$$T_1(\lambda) = A(\lambda) - \kappa \text{LD}(\lambda)$$

This method of analysis of the LD spectrum has been applied occasionally to the DNA–porphyrin complexes.\textsuperscript{36,37} Figure 5A shows the $A(\lambda) - \kappa \text{LD}(\lambda)$ spectrum of the DNA–TMPyP complex in an aqueous solution, with $\kappa$ ranging from −0.5 to −1.2 with a −0.1 increment. The measured absorption spectrum is shown at the top of panel A. The absorption profile with $\kappa = −0.9$ (curve b, solid curve) was chosen to be the most representative of the short-wavelength transition, $T_1(\lambda)$. The subtraction of $T_1(\lambda)$ from the measured absorption spectrum results in $T_2(\lambda)$, the long-wavelength transition moment. Figure 5B presents the two absorption spectra corresponding to $B_x$ and $B_y$. Using a similar method, the contributions from two transition moments for the LD spectrum were analyzed, and the resulting LD spectra are shown in Figure 5C.

Once the absorption and LD spectra are separated, LD$^\parallel$ can be calculated from the ratio of the corresponding absorption and LD spectra; consequently, the angle, $\alpha$, was calculated using eq 4. The angles were 45.8° for one transition, indicating that the $B_x$ or $B_y$ transitions are strongly tilted from the DNA base plane or the local DNA helix axis. These results are in contrast with the published data in an aqueous environment (that is, angle for intercalation binding mode at a low mixing ratio).\textsuperscript{38} In this case, even in an aqueous solution, TMPyP is not fully intercalated to DNA. According to the angle, porphyrin might be able to partially intercalate through the
of ethanol. Difference between the TMPyP− and Co-TMPyP−
DNA complexes in the LD spectrum is the LD magnitude in
the DNA absorption region. In the case of both TMPyP−
and Co-TMPyP−DNA complexes in an aqueous solution, the
magnitude of the LD signal at 260 nm remained with increasing
TMPyP and Co-TMPyP concentration, but it decreased
significantly in the ethanol–water system. When both

porphyrins bound to the A-form DNA, their conformation
changed gradually with the increasing concentration of
porphyrins. This may be due to local changes in the base-
stacking arrangement in the ethanol environment. On the other
hand, once the B-form DNA complex formed with Co-TMPyP
and TMPyP, there was no further structural change with increasing porphyrin concentration.

■ SUMMARY

This study was performed under low ionic strength commonly
used for studies of the B to A transition. The results suggest
that TMPyP interacts with B-DNA via intercalation at low
ratios under low salt conditions. In contrast, the interaction
with A-DNA occurs in an outside binding manner. This
difference may be because the structural conformations, such as
the groove of A-DNA, are not as deep as in B-DNA and the
bases are much more tilted. In the case of Co-TMPyP,
porphyrin binds preferably with the B- and A-DNA via outside
self-stacking mode. These results may be useful in developing a
rational design of porphyrin-based ligands with predictable
affinity and specificity.

■ EXPERIMENTAL SECTION

Materials. Calf thymus DNA (DNA) was purchased from
Sigma-Aldrich. DNA was dissolved in 1 mM sodium phosphate
buffer (Na2HPO4 + NaH2PO4, pH 7) by exhaustive shaking at
4 °C. The concentrations of DNA and TMPyP were
determined spectrophotometrically in aqueous solution using the
extinction coefficients, ε260nm = 6700 cm−1·M−1 (DNA base
or phosphate) and ε434nm = 226 000 cm−1·M−1 and ε434nm =
215 000 cm−1·M−1 for TMPyP and Co-TMPyP, respectively.
All measurements were taken at ambient temperature.

Absorption Spectroscopy and Fluorescence Spectroscopy. Absorption spectra were recorded on a Cary 100
instrument. Fluorescence spectroscopy was used to examine the
binding characteristics between DNA and TMPyP with a
cacodylate buffer or 80% ethanol solution. Steady state
fluorescence measurements were taken using Jasco FP-8300.
The detailed experimental conditions are depicted in the figure
legend.

Polarized Light Spectroscopy. The shape of the CD
signal in the DNA absorption region represents the
conformation of the DNA. It is a useful tool for tracing the
transition of the B- to A-form or vice versa. In general, the
origin of the induced CD upon binding of an achiral drug to
DNA is believed to be the interaction between the electric
transition moments of the drugs and chirally arranged electric
transition moments of the DNA bases.22−24 The CD spectra
were obtained using a Jasco J-715 spectropolarimeter (Tokyo,
Japan). LD is defined as the difference in absorbance between
radiation polarized parallel and perpendicular and is a sensitive
tool for examining the binding properties of small molecules to
native DNA and to synthetic polynucleotides. The division of the
measured LD spectrum by the isotropic absorption
spectrum results in a dimensionless quantity called reduced
LD (LD′), which is related to the orientation factor (S), optical
factor (O), and the angle (α) between the electric transition
moment of the ligand and the DNA helix axis (orientation axis)
through eq 4.

\[
LD'(\lambda) = \frac{LD(\lambda)}{A_{iso}(\lambda)} = 1.5S(3 \cos^2 \alpha - 1)
\]  

(4)
Polarized light spectra were averaged over several scans when necessary.

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**Notes**

The authors declare no competing financial interest.

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