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Length and sequence effect on the B-Z transition of $[d(A\text{-}T)_n]_2$ oligonucleotide induced by a cationic porphyrin

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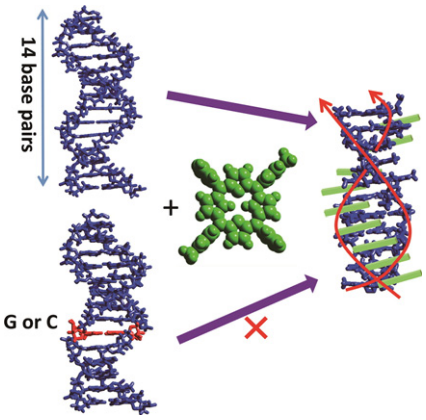
HIGHLIGHTS

- Formation of the *Z*-form requires at least 14 alternated AT base-pairs.
- The B-Z transition of the oligonucleotide couples with the formation of extensive porphyrin stacking.
- The time-dependent B-Z transition and porphyrin stacking can be elucidated by the sum of two exponential curves.
- The presence of even one GC base-pair in the middle of alternated AT sequence prevents the B-Z transition.

GRAPHICAL ABSTRACT



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ABSTRACT

trans-BMPyP induced the B-Z transition for alternated AT oligonucleotides as it was evident by inversed CD spectrum. The transition occurred simultaneously with appearance of the extensive stacking of porphyrin. Complete B-Z transition required at least 14 base-pairs long. Insertion of one or two GC base pairs prevented the B-Z transition.

1. Introduction

Z-form DNA, which is an extended, left handed double helical form of DNA, was first detected by circular dichroism (CD) and absorption spectroscopy [1], and its structure was resolved at the atomic level few year later [2]. The biological importance of *Z*-DNA was highlighted by the discovery of a range of *Z*-DNA specific proteins [3–7]. The Z-conformation favours alternated GC base-pairs under relatively extreme conditions, such as a high ionic strength, dehydration, and chemical modification [8–10]. On the other hand, it has been reported that some DNA binding ligands, such as cationic porphyrin, spermine porphyrin conjugates and Ru(II) complexes, induces the *Z*-conformation for an AT-rich sequence and native DNAs under mild

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conditions [11–14]. For example, one of the cationic porphyrins, *trans*-bis(*N*-methylpyrodinium-4-yl)diphenyl porphyrin (referred to as *trans*-BMPyP, Fig. 1) induces the *Z*-form for poly[d(A-T)$_2$], as evident by the characteristic CD spectrum that corresponds to the Z-conformation and two distinguishable peaks in the ^{31}P NMR spectrum at a low salt concentration [14]. The stacking of the cationic porphyrin was proposed to be the reason for the B-Z transition. The spermine conjugates of the cationic porphyrin also induced a B-Z transition for [d(A-T)$_n$]$_2$ sequence [13]. In both porphyrin derivatives related cases, B-Z transition was specific for alternated AT sequences. The alternated GC sequences were not transformed to *Z*-conformation. This report shows that cationic porphyrin induced B-Z transition of alternated AT sequence is related to the length of oligonucleotide and is dependent on the sequence.

2. Materials and methods

The *trans*-BMPyP was purchased from Frontier Scientific, Inc. (Utah, USA) and used as received. The concentrations of the porphyrins were measured spectrophotometrically using the extinction coefficients of $\varepsilon_{419nm} = 2.4 \times 10^5\ cm^{-1}\ M^{-1}$. All oligonucleotides were purchased from Bionia Inc. (Daejon, Korea) and used without further purification. Formation of double helix was checked by their Uv melting curves. Buffer solution used was 5 mM cacodylate buffer, pH 7.0. Absorption and CD spectra were recorded at 4 °C, on a Cary 100 Bio spectrophotometer (Australia) and on a Jasco J810 spectro-polarimeter (Tokyo, Japan), respectively.

3. Results and discussion

3.1. trans-BMPyP induced the B-Z transition for alternated AT oligonucleotides

Fig. 2(A) shows change in CD spectrum of [d(A-T)$_7$]$_2$ both in DNA absorption region and the Soret region with increasing porphyrin concentration. In the absence of *trans*-BMPyP, CD spectrum in DNA absorption region consists of a positive peak at 269 nm and negative peak at 248 nm, representing a right-handed B-form. An increase in porphyrin concentration resulted in a decrease in the magnitude of the both CD peaks and new negative band at 277 nm and positive band at 260 nm

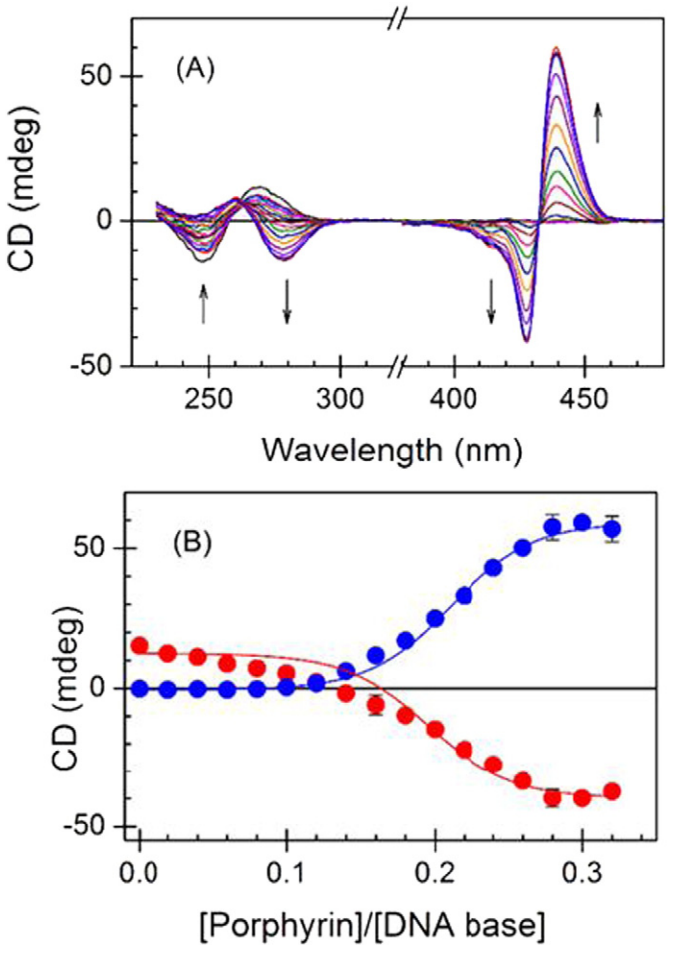
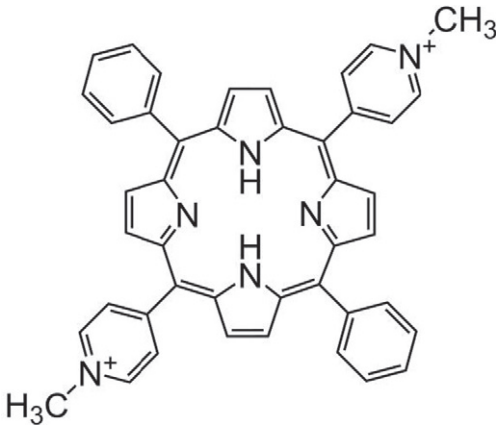


Fig. 2. (A) CD spectrum of [d(A-T)$_2$]$_7$ with increasing porphyrin concentration. [[d(A-T)$_2$]$_7$] = 100 μM in base and [porphyrin] = 0 to 32 μM to arrow direction with increment of 2 μM. Porphyrin concentration increases to the arrow direction. (B) Changes in CD intensity at 278 nm (red circles) multiplied by 3 and 440 nm (blue). The solid curves are to guide the eyes and do not have any meaning.



[d(A-T)$_n$]$_2$, n=4,5,6,7,8,9

[d(A-T)$_3$]$_2$C[d(A-T)$_4$]$_2$ [d(A-T)$_5$]$_2$C[d(A-T)$_2$]$_2$

[d(A-T)$_7$]$_2$C$_m$, m=1,2,3 [d(A-T)$_7$]$_2$C[d(A-T)$_3$]$_2$

Fig. 1. Chemical structure of *trans*-BMPyP and 5′ → 3′ sequence of oligonucleotide duplexes tested in this work.

appeared. No further change was observed when [porphyrin]/[DNA base] ratio reached 0.28, (Fig. 2(B)). At this mixing ratio, the shape of the CD spectrum in the DNA absorption region coincides with that of the *Z*-form. The [porphyrin]/[DNA base] ratio of 0.28 at which all oligonucleotide transformed to the Z-conformation is slightly higher than that of 0.24 observed for poly[d(A-T)$_2$] [14]. No other polynucleotides including poly[d(G-C)$_2$], poly(dA)·poly(dT), and poly(dG)·poly(dC), underwent a B-Z transition under similar conditions [14]. In the Soret region, the CD spectrum consisting of a negative band at 428 nm and positive one at 439 nm appeared as the porphyrin concentration increased. This type of bisignate CD spectrum is considered to be diagnostic for the porphyrins stacked extensively along the DNA stem [15–17].

Porphyrins have a well-extended conjugated π-system and are expected to stack in aqueous solutions. As an early example, the ^{1}H NMR study showed that *trans*-BMPyP stack on top of each other with two different possibilities, i.e., "slipped face-to-face" dimer or "completely overlapped face-to-face dimer" [18]. In the latter dimer, *trans*-BMPyP on the top rotate 45° anti-clockwise. Recently, an interesting helical form of J-aggregates with well-defined chirality was reported for *meso*-tetrakis(4-sulfonatophenyl)porphyrin (H_2TPPS4) [19–20]. In the aggregates, negatively charged sulfonatophenyl moieties of H_2TPPS4 in the upper layer locate at the centre of porphyrin in the lower layer. The stacking extends to form either the left- (Δ J-aggregates) or right-handed (Λ J-aggregates) helix. The Δ J-aggregates produced a strong