Probing the B-to-Z-DNA duplex transition using terminally stacking ethynyl pyrene-modified adenosine and uridine bases†

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Pyrene-modified adenosine and uridine bases located in the dangling positions of G,C-alternating oligodeoxynucleotides undergo π-stacking in their B-DNA duplexes, but not in their Z-DNA duplexes; fluorescence quenching in the former, through photoinduced electron transfer, but not in the latter, allows the state of the B-to-Z-DNA transition to be characterized visually.

Constructing and understanding the functions of biomaterials—DNA, RNA, and proteins—are becoming hot research topics in contemporary science. Noncovalent interactions between aromatic molecules are often important for stabilizing organized structures formed from biological molecules.[1] In particular, the presence of unpaired terminal nonpolar aromatic units appended to DNA strands (so-called "dangling ends") can increase the thermal stability of their duplexes.[2] Although these units' end stacking abilities have been studied extensively, they have not been applied to studies of the B-to-Z-DNA transition.

In this study, we sought to use such end stacking interactions in an attempt to monitor the states of B-to-Z-DNA transitions. Z-DNA, a left-handed helical conformer of double-stranded DNA (dsDNA), is formed during the replication period of gene expression;[3] in addition, it is formed when dsDNA comprising alternating dG and dC moieties encounters high concentrations of salt.[4] The recent discovery of a Z-DNA-specific binding protein has received much attention.[5]

Our goal was to study the effects of end stacking and to improve the method of detection of the B-to-Z transition. We based our approach on the quenching phenomenon, which occurs through photoinduced electron transfer (PET) in B-DNA, of nonpolar aromatic fluorophores stacked at the terminus of a dangle position.[6] The aromatic fluorophores we chose were the previously reported[7] pyrene-modified nucleotides $\mathbf{A}^{\mathbf{PY}}$ and $\mathbf{U}^{\mathbf{PY}}$ (Fig. 1).

We believed that they would stack readily at the termini of DNA strands because of their high quantum yielding, large, nonpolar, hydrophobic, and planar structures. We synthesized a series of oligodeoxynucleotides (ODNs) through standard phosphoramidite methods using a DNA synthesizer (Fig. 2).[8] The ODNs containing the $\mathbf{A}^{\mathbf{PY}}$ and $\mathbf{U}^{\mathbf{PY}}$ units were obtained efficiently after purification using reverse-phase HPLC. We confirmed the compositions of the ODNs through MALDI-TOF mass spectrometric analysis.

We believed that the dangle end stacking ability of $\mathbf{A}^{\mathbf{PY}}$ would change upon the transition from B- to Z-DNA because of their conformational difference. The helicities of B and Z are opposite to each other, and the diameter of Z-DNA (18 Å) is much narrower than B-DNA (20 Å). To separate the effects of the stacking interactions from those of the pairing (hydrogen bonding) interactions in the duplex DNA, we placed the modified nonpolar aromatic $\mathbf{A}^{\mathbf{PY}}$ and $\mathbf{U}^{\mathbf{PY}}$ moieties of interest at "dangling" positions (*i.e.*, in the absence of a pairing partner) at the ends of otherwise-base-matched duplexes (Fig. 2).

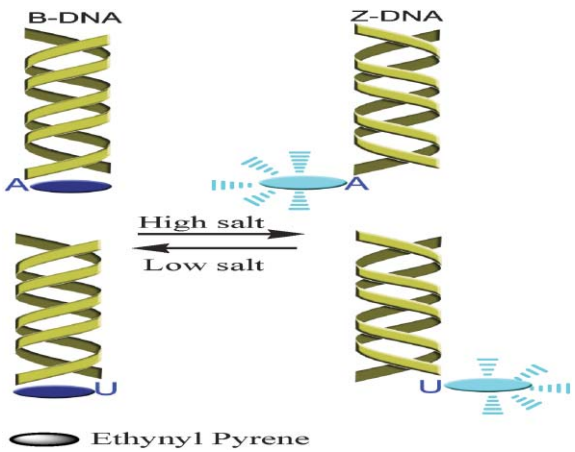
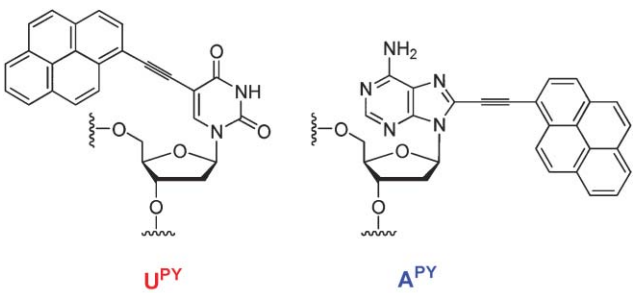


Fig. 1 Design of a probe for monitoring the B-to-Z-DNA transition.



A1 5'- A^{PY}GC GCG CGC G
U1 5'- CG CGC GCG CU^{PY}
N1 5'- GC GCG CGC G
N2 5'- CG CGC GCG C

Fig. 2 Fluorescent nucleosides and corresponding oligonucleotides.

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We then used circular dichroism spectroscopy to monitor the B-to-Z-DNA transitions of the ODNs. Fig. 3 displays the dramatic change observed in the CD spectrum—arising as a consequence of a conformational change from B-DNA to Z-DNA—that occurred upon changing the salt concentration. In 0.1 M NaCl, the **A1**–**N2** duplex exists mainly in the B-DNA conformation, but in 5 M NaCl it exists in the Z-DNA form. **U1**–**N1** also underwent such B-to-Z-DNA transfomation (see supporting information†).

We measured the melting temperatures to determine the strengths of the stacking interactions in the various forms. These data indicate how the stacking properties differ remarkably in the different B-DNA and Z-DNA conformations. In comparison with the melting temperatures of the unmodified ODNs **N1** and **N2**, the DNA duplexes containing the nonpolar aromatic termini were considerably more stable than their natural counterparts. The melting temperature for the **A1**–**N2** duplex in a high salt concentration (5 M NaCl; Z-DNA form) was 60 °C; at a low salt concentration (0.1 M; B-DNA form) it was much higher (70 °C). The B-DNA form of the **U1**–**N1** duplex melted at 68 °C; the Z-DNA form , at 61 °C. In contrast, the melting temperatures of the B- and Z-DNA forms of the unmodified duplex **N1**–**N2** were 64 and 59 °C, respectively (Table 1).

We observed remarkable differences between the stabilities of the unmodified duplex **N1**–**N2** and the dangling end π-stacking duplexes **A1**–**N2** and **U1**–**N1**. We found that the B-DNA form was more stable than the Z-DNA state not only for the unmodified duplex **N1**–**N2** but also for the fluorophore-modified duplexes **A1**–**N2** and **U1**–**N1**. In the Z-DNA form, the differences between the values of T_m of the **N1**–**N2** duplex and the **A1**–**N2** and **U1**–**N1** duplexes were only 1–2 °C, whereas in the B-forms of these duplexes, the differences were higher (*ca.* 4–6 °C). These observations suggest that aromatic stacking at the dangling end of the duplex in the Z-DNA form does not have an effect on the stability, but in the B-DNA form, such stacking of the nonpolar aromatic fluorophore stabilizes the conformation. Our results demonstrate clearly that these $\mathbf{A}^{\mathbf{PY}}$ and $\mathbf{U}^{\mathbf{PY}}$ units undergo π-stacking in the B-DNA form, which stabilizes the duplexes, but not in the Z-DNA form. It seems likely that the hydrophobicity of the two pyrene-modified bases ($\mathbf{A}^{\mathbf{PY}}$ and $\mathbf{U}^{\mathbf{PY}}$) contributes favorably to their enhanced stacking ability in the B-DNA.

We investigated the fluorescence emission properties of each B- and Z-DNA duplex state of the pyrene-modified ODNs (Fig. 4). In general, their Z-DNA forms exhibited a marked increase in fluorescence relative to those of the B-DNA forms. We observed dramatic fluorescence changes for the **A1**–**N2** and **U1**–**N1** duplexes.

We are not in a position presently to explain the exact mechanism of this phenomenon, but we think that the changes in fluorescence emission that occur during the B-to-Z-DNA conformational change must arise through changes in the electrostatic interactions (through PET and terminal π -stacking) between the $\mathbf{A}^{\mathbf{PY}}$ and $\mathbf{U}^{\mathbf{PY}}$ moieties and their neighboring nucleobases. A strongly donating or accepting terminal π-stacking moiety is usually critical for fluorescence quenching[9] in the B-DNA duplex state, while in Z-DNA, end stacking is probably very difficult to achieve because it possesses a relatively narrow duplex conformation and opposite helicity. We believe that this property allows our system to discriminate between the Z- and B-DNA forms.

In conclusion, we chose $\mathbf{A}^{\mathbf{PY}}$ and $\mathbf{U}^{\mathbf{PY}}$ for use as nonpolar aromatic moieties that undergo π-stacking at the dangling ends of DNA duplexes. Such π-stacking stabilizes the B-DNA forms of these duplexes, but not their Z-DNA forms. Terminal π -stacking induces fluorescence quenching in the B-DNA forms, but not in the Z-DNA forms; this phenomenon allows us to monitor the two states visually. Such pyrene-labeled C,G-alternating ODNs have potential for use as new types of optical DNA sensors.

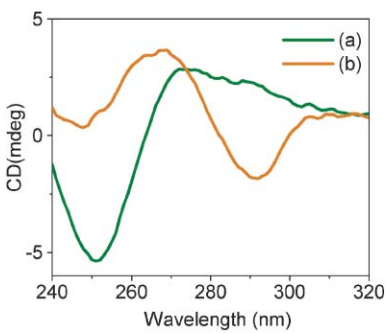

Fig. 3 Circular dichroism spectra recorded for the **A1**–**N2** duplex in the presence of (a) 0.1 M NaCl and (b) 5 M NaCl. The spectra were recorded at 20 °C in a buffer of 100 mM Tris–HCl (pH 7.2). Each concentration was 1.5 μM and the absorption wavelength was 260 nm.

Table 1 Thermal melting temperatures (T_m) of B- and Z-DNA

	Sample	T_m/°C	Sample	T_m/°C	Sample	T_m/°C
B-DNA	**A1**–**N2**	70	**U1**–**N1**	68	**N1**–**N2**	64
Z-DNA	**A1**–**N2**	60	**U1**–**N1**	61	**N2**–**N2**	59

[a] Measured at 260 nm in 100 mM Tris–HCl buffer (pH 7.2) containing 0.1 M NaCl (B-DNA) and 5 M NaCl (Z-DNA).

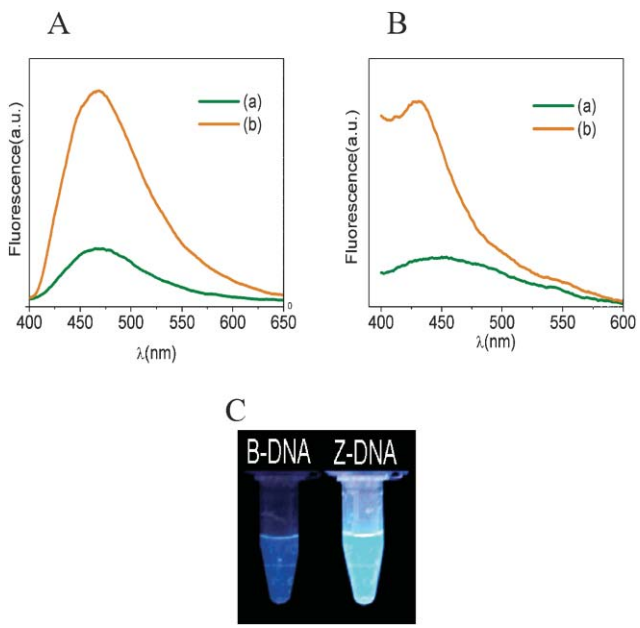

Fig. 4 Fluorescence spectra of the (A) **A1**–**N2** and (B) **U1**–**N1** duplexes [(a) B- and (b) Z-DNA forms]. These spectra were recorded at 20 °C in a buffer of 100 mM Tris–HCl (pH 7.2). Each concentration was 1.5 μM and the excitation wavelength was 386 nm. (C) Photo images of B- and Z-forms of **A1**–**N2**.

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