Molecular Devices

A Nanothermometer Based on the Different π Stackings of B- and Z-DNA

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The design and construction of molecular-scale devices are now challenging issues in nanotechnology. Molecular machines, with a two state on–off function, such as catenane,[1,2] rotaxane,[2,3] and ratchet[4] may be candidates for such devices. A nanomechanical device that uses DNA conformational change has recently been developed.[5,6] Double-helical DNA has a unique π-stack structure, and modulation of the π stackings through DNA, controlled by external stimuli coupled with an easily monitored output, is attractive for designing novel DNA-based nanodevices. It has been shown that the electronic properties largely depend on the orientation of the π stacking.[7–9] Because the overall base π stackings of B- and Z-DNA are different, the electronic properties of these two duplexes are also assumed to be different. Based on this hypothesis, a molecular thermometer that uses the different π stackings of B- and Z-DNA was designed. 2-Aminopurine,[10–12] which is a fluorescent probe for charge-transfer processes, was introduced into B- and Z-DNA and the emission monitored. Z-DNA showed a marked increase in fluorescence compared with B-DNA, with the fluorescence intensity correlating reproducibly with temperature.

Inspection of the X-ray structures of Z-form and B-form DNA suggests that there is significant difference in base π-stack orientation in both structures, that is, discrete clusters of interstrand four-base π stacks of G-C-C-G exist in the Z-form,[13] whereas there is a continuous π stack along each strand in B-DNA (Figure 1). We expected that the four-base π stacks would largely contribute to the electronic properties of Z-DNA, and that the different electronic properties between B- and Z-DNA could be monitored by 2-aminopurine (Ap), because the quenching of Ap fluorescence has been used as a probe for charge transfer within DNA duplexes, in which the purine bases (G and A) and pyrimidine bases (C and T) function as donors and acceptors, respectively.[8–11] Thus, the fluorescence intensity of the Ap-containing duplex decamer, 5′-d(CGCApCGCGCG)-3′ (ODN 1)/5′-d(CGCGCGTGCG)-3′ (ODN 2), was monitored under various conditions. The duplex decamer, 5′-d(CGCApC-

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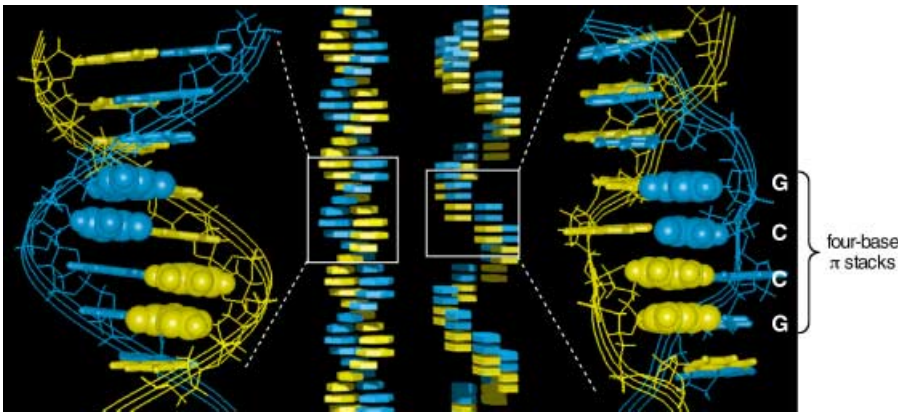

Figure 1. Molecular models of B-form (left) and Z-form DNA (right). The models were constructed by using the Insight II program (Accelrys) with standard B- and Z-form helical parameters. The two strands are in yellow and blue.

G*A*GCG)-3′ (ODN 3)/5′-d(CGC*T*CGTGCG)-3′ (ODN 4), which does not convert to the Z-conformation (even in 5 M NaCl because of the disordering of the preferred altenative purine–pyrimidine sequence of Z-DNA),[13] was used as a control. In 1 M NaCl, ODN 1–2, which mainly exists in the B-conformation (> 95 %), as assessed by using circular dichroism (CD) spectroscopy, showed very weak fluorescence, similar to ODN 3–4. The fluorescence of ODN 1–2 increased proportionally upon increasing the ratio of Z-conformation by increasing the NaCl concentration (1–5 M, Figure 2a), whereas no such evident increase of the fluorescence was observed in the control ODN 3–4. These results indicate that the electronic property of Z-DNA is different from that of B-DNA. The intense fluorescence of Ap in Z-DNA is in accord with the previous finding of almost no charge-transfer between the different four-base π stacks observed in the ^{Br}U photoreaction of Z-DNA.[14] The intense fluorescence of ODN 1–2 in the Z-conformation can be explained by the following reasons for disruption of charge-transfer. 1) Ap is located at the 5′-end of the four-base π stacks lacking a quenching base at the 5′-side. In fact, fluorescence of 5′-ApCCG-3′ is found to be twice as intense as 5′-CApCG-3′ (see Figure S3 in the Supporting Information). 2) Disruption of continuous π stacking[15] is due to the four-base π stacks in Z-DNA. Analogous slow interstrand charge transfer relative to the intrastrand process in B-DNA has been pointed out.[15,16]

We designed a molecular thermometer based on the change in π stacks upon converting from B- to Z-DNA. It is known that the equilibrium between the Z- and the B-conformation can be controlled by temperature.[17] At low temperature, the proportion of the Z-conformation is high due to its lower entropy. Therefore, an increase in temperature increases the proportion of the B-conformation. Thus, the fluorescence of ODN 1–2 was monitored at different temperatures in 3 M NaCl. Thermal denaturation studies revealed that ODN 1–2 and ODN 3–4 formed a duplex (> 95 %) below 32 °C.[18] As shown in Figure 3, it was found that the intensity of the fluorescence linearly correlated with the temperature. At $T = 32$ °C, at which 23 % of the ODN 1–2 was in the Z-conformation, the fluorescence of the ODN 1–2 was very weak, whereas the fluorescence of the ODN 1–2 dramatically increased at $T = 2$ °C (Figure 3a), when 53 % of the ODN 1–2 was in the Z-conformation. In clear contrast, such a response was not observed in the control ODN 3–4 sample under the same conditions (see Figure 3b). This function is fully reversible. Under repetitive temperature changes, a reproducible fluorescence of ODN 1–2 was observed according to the change in the proportion of B- and Z-conformations (Figure 3c).

These results clearly indicate that the B–Z transition induced by a change in temperature can be readily monitored by using the fluorescence intensity of Ap. Such a molecular thermometer can be applied in the investigation of localized temperature on a microscale. Because we previously reported that the pathway for electron transfer is different between B- and Z-DNA,[14] the B–Z transition could be useful

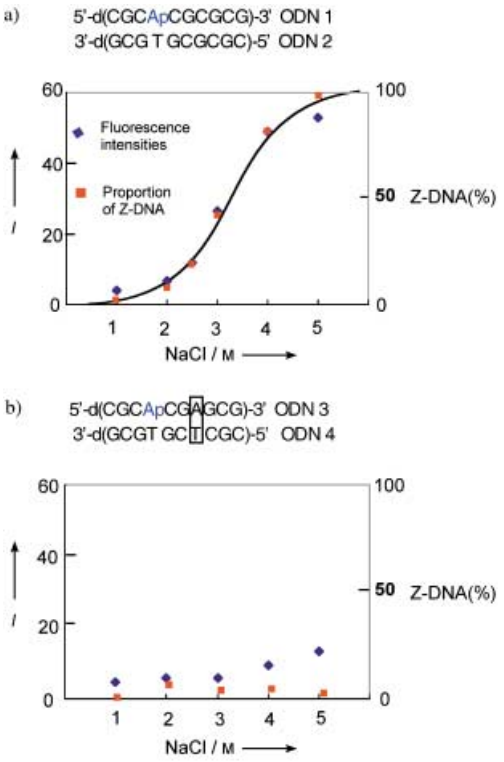

Figure 2. Steady-state fluorescence emission intensities and proportion of the Z-conformation of a) 5′-(CGCApCGCGCG)-3′/5′-(CGCGCGTGCG)-3′ (ODN 1–2), and b) 5′-(CGCApCGAGCG)-3′/5′-(CGCTCGTGCG)-3′ (ODN 3–4), as a function of NaCl concentration (1–5 M). Emission intensities for 30 μM (total base concentration) duplex samples in 20 mM phosphate buffer (pH 7.0) at 10 °C were measured. The samples were excited at 317 nm, and emission intensities were determined by monitoring emission at 360 nm. I = Intensity of fluorescence (arbitrary units).

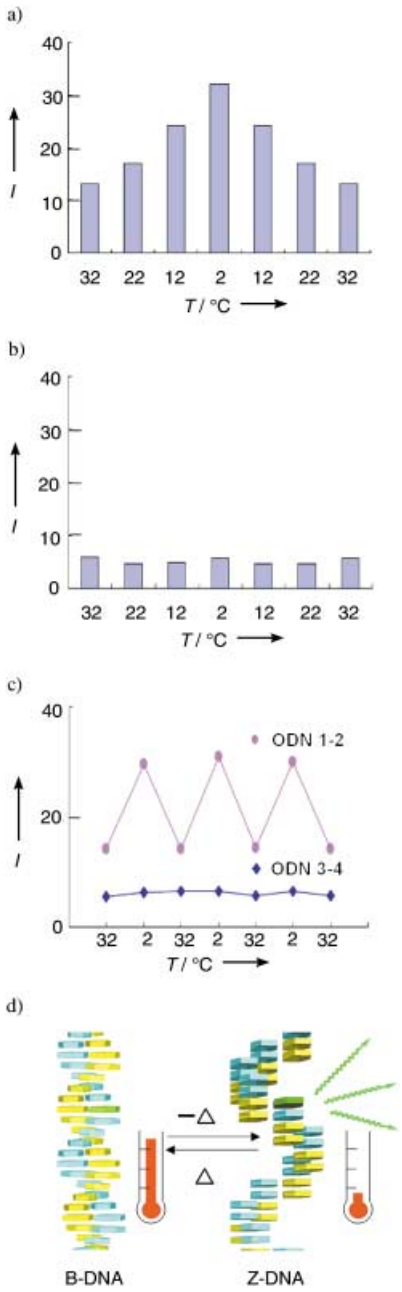

Figure 3. Steady-state fluorescence emission intensities of a) ODN 1–2, and b) ODN 3–4 at different temperatures (32–2 °C). Emission intensities of 30 μM (total base concentration) duplex oligomers in 20 mM phosphate buffer (pH 7.0) and 3 M NaCl were measured. c) Repeating experiments of fluorescence emission of ODN 1–2 and ODN 3–4 at 32 and 2 °C. d) Schematic representation of nanothermometer.

for new types of nanoelectronic devices, such as sensor, wire, memory, and molecular switches.

Experimental Section

CD spectra were measured with an AVIV MODEL 62 DS/202 CD spectrophotometer. Duplexes were hybridized by heating to 90 °C, followed by slow cooling to room temperature. CD spectra of ODN 1–2 and ODN 3–4 (30 μM base concentration in 50 mM sodium phosphate buffer, pH 7.0, at various NaCl concentrations) were recorded by using a 1 cm path-length cell. The proportions of Z, B and SS for these oligmers were estimated as previously reported.[19,20] To obtain the population of B- and Z-DNA (P_B and P_Z), we analyzed the CD spectra taking into account the three form (B, Z, SS) present in solution. Hence, at each temperature we made the following assumptions [Eq. (1) and (2)].

$$\Delta\varepsilon^{295} = \Delta\varepsilon_B^{295} P_B + \Delta\varepsilon_Z^{295} P_Z + \Delta\varepsilon_{SS}^{295} P_{SS} \quad (1)$$

$$1 = P_B + P_Z + P_{SS} \quad (2)$$

The P_B, P_Z, and P_{SS} are the molar fractions of the B, Z, and single strand components, respectively. The $\Delta\varepsilon$ values, relative to the limit forms, have been estimated from the CD signal at 295 nm. They were considered to be independent of the temperature and salt concentration. A UV melting experiment indicated that P_{SS} is negligible under the experimental conditions. Therefore, Equation (1) and (2), relative to each CD spectrum at a given temperature, were solved to provide estimates of P_B and P_Z.

Steady-state fluorescence measurements on Ap-containing DNA were conducted by using a JASCO FP-6300 spectrofluorometer. Measurements were performed by using fluorescence cells with a 1 cm path length. The duplex concentration for emission measurements was 30 μM (total base concentration). Steady-state fluorescence emission spectra and CD spectra of ODN 1–2 and 3–4 under different salt conditions are available in the Supporting Information.

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