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Fluorescence properties of 2-aminopurine–cytidine–7-deazaguanine (5′-ApC^{dz}G-3′) trimer in B- and Z-DNA

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The electron transfer quenching of 2-aminopurine by guanine and 7-deazaguanine was investigated in B- and Z-DNA, and an increase in the fluorescence intensity of 2-aminopurine upon B- to Z-DNA transition was demonstrated.

In addition to the canonical right-handed B-form structure, DNA can adopt a variety of structures including the A-form, triplex, quadruplex, and left-handed Z-form DNA.[1–3] Such polymorphism of the DNA structure is thought to play various important biological roles in processes such as gene expression by altering the DNA–protein interactions.[4] The recent discovery of a Z-DNA specific binding protein further supported the existence of Z-DNA *in vivo*, and the biological role of Z-DNA has received much attention as a topical subject.[5–7] To elucidate the biological role of Z-DNA, the development of a probe that can detect Z-DNA *in vivo* is desired. Sugiyama *et al.* demonstrated the potential of the 5-halouracil photoreaction as a probe for Z-DNA, in which a Z-conformation specific photoproduct offers the detection of Z-DNA *in vivo*.[8,9] Rich *et al.* reported the construction of a Z-DNA specific restriction endonuclease as a probe for Z-DNA.[10] However, these methods are irreversible and it is difficult to detect Z-DNA in real time. In the present study, we selected 2-aminopurine (Ap) as the fluorescent probe of Z-DNA, and an increase in the fluorescence intensity of Ap upon B- to Z-DNA transition was demonstrated.

Ap is a fluorescent adenine isomer able to form a Watson–Crick base-pair with thymine.[11,12] The fluorescence properties of Ap in DNA are strongly influenced by the electron transfer quenching process from guanine (G).[13–17] Since the electron transfer efficiency in DNA is sensitive to the change in the base-stacking interaction, Ap has been widely employed as a fluorescence probe of protein-induced local conformational changes in DNA.[18–20] The structural characteristic of Z-DNA is an alternating *syn–anti* conformation of the nucleoside unit, which results in a disorder of base stacking. Thus, the conformational transition from B- to Z-DNA is expected to strongly affect the electron transfer from G to the excited Ap (^{1}Ap*), or the fluorescence properties of Ap.[21] Furthermore, the incorporation of 7-deazaguanine (^{dz}G), which has a much lower oxidation potential than G, is expected to amplify the effects of electron transfer on the Ap fluorescence quenching. Here, to test the possibility of using Ap as a probe for Z-DNA, the effect of the B- to Z-DNA transition on the electron transfer quenching of ^{1}Ap* by G and ^{dz}G was investigated.

Changing one C–G base-pair to the A–T base-pair causes a significant destabilization of Z-DNA relative to B-DNA. Incorporation of the Ap–T base pair in C–G repeats did not affect the mid-point NaCl concentration in the B- to Z-DNA transition (*ca.* NaCl 2.6 M), hence the hydrogen bond between the N2 amino group of purine and the phosphate oxygen either directly or through bridging water molecules is crucial for the stabilization of Z-DNA.[22] Therefore, Ap was incorporated into DNA without altering the equilibrium between B-DNA and Z-DNA. Conversion of the B-form d(CG)$_n$ oligodeoxynucleotides (ODNs) to the Z-form requires a high salt concentration. The substitution of 8-bromoguanine (^{br}G) for G lowers the NaCl concentration to induce the B–Z transition at the physiological salt concentration.[23] In ODNs **Z1**–**Z4**, the Ap strand was hybridized with the ^{br}G-containing strand. At a low salt concentration they are in the B-form, and are converted to the Z-form by increasing the salt concentration, with a mid-point at about 500 mM. Thus, ODNs **Z1**–**Z4** form a B-conformation at a low salt concentration (NaCl 0.1 M), whereas under high salt conditions (NaCl 1 M), they adopt a typical Z-conformation. Interestingly, for all **Z1**–**Z4** ODNs, a higher fluorescence intensity was observed in the Z-form compared with the B-form (Table 1). The relative increase in the fluorescence intensity of Ap upon the B- to Z-DNA transition (F_Z/F_B) was amplified in the presence of ^{dz}G, and F_Z/F_B was the highest for ODN **Z4** with the 5′-ApC^{dz}G-3′ sequence, which demonstrated a 10-fold increase in the Ap fluorescence intensity. Figure 1 shows the fluorescence spectra of **Z1** and **Z4** in their B- and Z-conformations. The incorporation of ^{dz}G at the 3′ site of Ap significantly decreased the fluorescence intensity of Ap in the B-form due to the efficient electron transfer from ^{dz}G to ^{1}Ap*. On the other hand, ^{dz}G at the 3′ site of Ap only slightly decreased the fluorescence intensity of Ap in the Z-conformation. Thus, the higher fluorescence intensity of Ap in the Z-conformation is explained by the effective electron transfer from G and ^{dz}G to ^{1}Ap* in the B-form DNA compared to that in the Z-form DNA where the base-stacking is interrupted.

Barton *et al.* have previously demonstrated that the electron transfer rate in B-DNA depends on the direction and is faster in the 3′-5′ direction than in the 5′-3′direction.[17] In contrast, it is suggested that the electron transfer in Z-DNA is faster in the 5′-3′

Table 1 Steady-state fluorescence intensities at 370 nm, F^a, for Ap in DNA

	DNA sequences	$F_B{}^b$	$F_Z{}^c$	F_Z/F_B
Z1^d	5′-CGCGCApCGCG-3′	0.329	1.96	6.0
Z2	5′-C^{dz}GCGCApCGCG-3′	0.252	1.80	7.1
Z3	5′-CGC^{dz}GCApCGCG-3′	0.159	1.30	8.2
Z4	5′-CGCGCApC^{dz}GCG-3′	0.161	1.92	12
B1^e	5′-CGC^{dz}GCApCGTG-3′	0.221	0.235	1.1

^{a} Estimated error of the reported value of fluorescence intensities F was ± 3%. ^{b} In the presence of 0.1 M NaCl. ^{c} In the presence of 1 M NaCl. ^{d} **Z1**, **Z2**, **Z3**, and **Z4** are hybridized with the ^{br}G containing complementary strand (5′-C^{br}GCGTGC^{br}GCG-3′). The NaCl concentrations at the mid-point of the B–Z transition on **Z1**–**Z4** duplexes are around 0.5 M. ^{e} **B1** is hybridized with complementary strand (5′-CACGTGCGCG-3′). **B1** duplex is still in the B-form in the presence of 1 M NaCl.

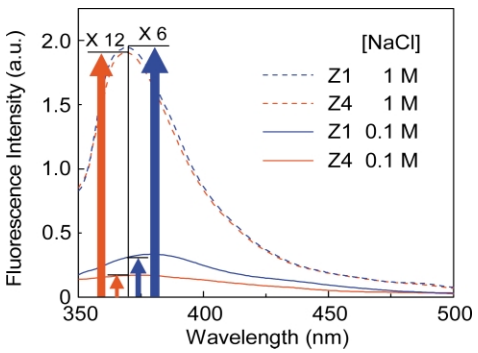

Fig. 1 Fluorescence spectra (λ_{ex} = 340 nm) of **Z1** and **Z4** in their B- (solid line) and Z- (dashed line) conformations. Arrows show the fluorescence intensities at 370 nm. Sample concentration: 40 μM (base conc.) ODN, 5 mM sodium phosphate buffer, pH 7.0, at 5 °C.

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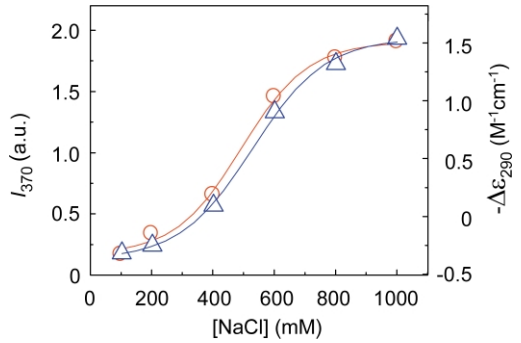

Fig. 2 The fluorescence intensity of **Z4** excited at 340 nm (circles) and the CD at 290 nm (triangles) as a function of NaCl concentration. (The measurement conditions are similar to Fig. 1.)

direction than in the 3′-5′direction, as the fluorescence quenching of Ap by ^{dz}G in the Z-conformation was more efficient in **Z3** than in **Z4**. This opposite directional preference of the electron transfer in the B- and Z-conformations also contributes to the increase in the fluorescence intensity of 5′-ApC^{dz}G-3′ upon the B- to Z-DNA transition.[24]

In the case of ODN **B1** which does not adopt the Z-conformation, the fluorescence intensity of Ap was unaffected by the addition of NaCl. Figure 2 shows the fluorescence intensity of Ap and the CD at 290 nm as a function of NaCl for ODN **Z4**. The fluorescence intensity of Ap proportionally increased with the increasing Z-DNA ratio. Thus, the results clearly demonstrate that the increase in the fluorescence intensity of Ap is due to the B- to Z-conformational transition of the ODNs.

Our results showed that the unit of 5′-ApC^{dz}G-3′ is sensitive to the B- to Z-DNA transition. The Z-DNA sequences are known to be located near the transcription initiation site and Z-DNA is suggested to play a role in the regulation of the transcription. This 5′-ApC^{dz}G-3′ fluorescent unit may be a useful tool for the study of such a biological role of Z-DNA.

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