Probing the microenvironments in the grooves of Z-DNA using dan-modified oligonucleotides†

Takumi Kimura, Kiyohiko Kawai and Tetsuro Majima*

Received (in Cambridge, UK) 3rd January 2006, Accepted 14th February 2006
First published as an Advance Article on the web 28th February 2006
DOI: 10.1039/b600026f

The environment-sensitive fluorophore dan (6-dimethylamino-2-acyl-naphthalene)- modified dC or dG bases were introduced into the Z-DNA forming sequence. It was demonstrated that both grooves of Z-DNA are more hydrated than those of B-DNA. Dan will be useful for probing the microenvironments in the grooves among the DNA polymorphs.

DNA is a polymorphic polymer that can adopt a variety of secondary structures ranging from the canonical right-handed B form to the left-handed Z conformation.[1,2] The latter conformation is less common than the right-handed B-DNA because, under physiological conditions, Z-DNA has a higher energy.[3] The discovery of a Z-DNA specific binding protein further supported the existence of Z-DNA *in vivo*.[4] More recently, the structure of the B–Z junction was revealed.[5] However, the biological role of Z-DNA still remains to be clarified.

In this study, we investigated the microenvironments in the grooves of B- and Z-DNA. We selected dan fluorophore as a probe for the DNA structures. It is known that the fluorophore dan undergoes a large charge redistribution upon excitation and has nearly ideal environment sensor properties.[6–8] Hence, the polarity sensitive dan fluorophore has been used to investigate the environment of the binding site in the protein or DNA.[9,10] We synthesized the groove environment-sensitive fluorescence probes, and demonstrated the ability to probe the microenvironment in the major and minor grooves of DNA.

It is known that the non-base-paired substituent on the N4- or N2-exocyclic amino groups of dC and dG in B-DNA extend into the center of the major and minor grooves, respectively.[11,12] Therefore, the dan fluoroprobe modifications to the amino group of dC or dG are expected to show only a modest steric effect during the duplex formation (Fig. 1).

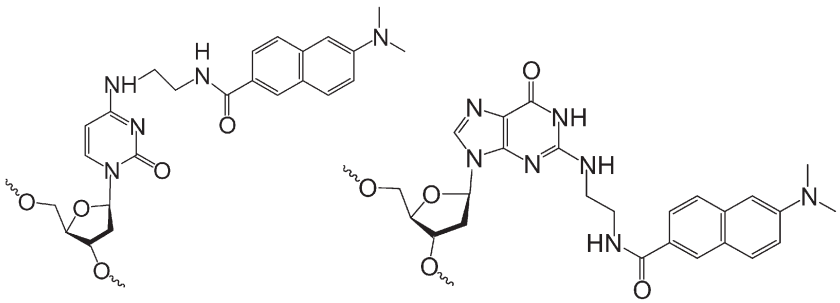
We synthesized a series of the Z-DNA forming dan-modified oligodeoxynucleotides (ODNs) (Table 1, **ODNs 1–3**). To avoid the self-assembly of the dan-modified ODNs, the AT base-pair was involved. Conversion of the B-form $d(CG)_n$ to the Z-form requires a high salt concentration.[13] The substitution of 8-bromo-2′-deoxyguanosine (^{Br}G) for G lowers the NaCl concentration that induces the B–Z transition at physiological salt concentration.[14] **ODNs 1–3** were hybridized with the ^{Br}G-containing complementary strand, so that the Z-form was stable under typical physiological conditions. 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 40 mM (see electronic supplementary information†). We then used circular dichroism spectroscopy to monitor the conformation of the ODNs. Fig. 2A shows that **ODNs 1–3** adopt a typical Z-DNA at a low salt concentration (sodium chloride 0.1 M). These results suggest that the dan fluorophore did not affect the Z-DNA formation.

We investigated the effect of the fluorescence properties of the dan-modified ODNs upon the Z-DNA (Fig. 2B). A remarkable difference between the ^{dan}C and ^{dan}G modified ODNs was observed. The **ODN3** showed a 9 nm blue shift of the dan emission compared to that of **ODN2**. Moreover, the fluorescence intensity of **ODN3** was about 4-fold higher. In **ODN3**, the dan moiety is located in the minor groove of Z-DNA. Therefore, the microenvironment in the minor groove of the Z-DNA has a higher hydrophobicity. We also addressed whether ^{Br}G affects dan fluorescence properties. However, no quenching was observed with an excess of ^{Br}G (up to 1 mM). Therefore, the fluorescence enhancement of **ODN3** was caused by the difference of hydrophobicities in both grooves.

In order to estimate the dielectric constants (ε) in both grooves of Z-DNA, the fluorescence parameters (λ_{ex}, λ_{em}) of the ^{dan}C monomer were measured in media with different dielectric constants generated by varying the ratios of dioxane–water (Table 1). We also followed the earlier method of measuring the Stoke's shift ($1/\lambda_{ex} - 1/\lambda_{em}$) of a related polarity-sensitive fluorophore in various media of known ε values.[15] The Stoke's shift ($\Delta\nu$) value of the major groove modified **ODN2** is 9316 cm^{-1}. This value corresponds to a dielectric constant of 74. On the other hand, the $\Delta\nu$ of the minor groove modified **ODN3** was 8597 cm^{-1}, which corresponds to the ε value of 43. Therefore, Z-DNA has different hydration conditions in its two grooves. However, the ε values of the major and minor grooves of B-form were 62 and 32, respectively, when Z-DNA forming sequences hybridized with the unmodified complementary strand (**ODNs 4** and **5**). According to our previous study, the ε values of the major and minor grooves of AT rich B-DNA were 61 and 30, respectively (**ODNs 6** and **7**).[16] In the B-form, the ε values of GC rich sequences (**ODNs 4** and **5**) were slightly more polar than the AT rich. The increases in the ε values of the major and minor grooves upon the B- to Z-DNA transition (**ODN4→2**, **ODN5→3**) were 19% and 34%, respectively. On the bases of these results, the microenvironments in both grooves of Z-DNA are more polar than those in B-DNA. The hydration (ε = 74) in the major groove of Z-DNA was very similar

The Institute of Scientific and Industrial Research (SANKEN), Osaka University Mihogaoka 8-1, Ibaraki, Osaka 567-0047, Japan. E-mail: majima@sanken.osaka-u.ac.jp; Fax: (+81)6-6879-8499; Tel: (+81)6-6879-8495

† Electronic supplementary information (ESI) available: UV titration of the Z-DNA forming sequence (**ODN1**). The excitation spectra of **ODNs 2** and **3** were monitored at 460 nm. The CD spectra of **ODNs 2–5** in the presence of NaCl. See DOI: 10.1039/b600026f



^{dan}C ^{dan}G

Fig. 1 Dan-modified dC (^{dan}**C**) and dG (^{dan}**G**).

Table 1 Stoke's shift ($\Delta\nu$)[a], dielectric constants (ε)[b] and solvent polarity $E_T(30)$[c] of dan-modified ODNs

No.	Dioxane (%)[d]	ε	$E_T(30)$/kcal mol^{-1}	Sequences	λ_{ex}/nm	λ_{em}/nm	$\Delta\nu$/cm^{-1}
	0	78.5	63.0	^{dan}C monomer	321	459	9366
	15	63.3	60.5	^{dan}C monomer	322	457	9168
	30	50.5	56.5	^{dan}C monomer	325	454	8743
	45	37.3	54.5	^{dan}C monomer	326	450	8452
	60	24.0	51.9	^{dan}C monomer	328	444	7965
1				$^{5'}$CACGCGCG$^{3'}$/$^{3'}$GT^{Br}GCGC^{Br}GC$^{5'}$ [e]			
2		74	62	$^{5'}$CACG^{dan}CGCG$^{3'}$/$^{3'}$GT^{Br}GCGC^{Br}GC$^{5'}$ [e]	322	460	9316
3		43	55	$^{5'}$CAC^{dan}GCGCG$^{3'}$/$^{3'}$GT^{Br}GCGC^{Br}GC$^{5'}$ [e]	325	451	8597
4		62	60	$^{5'}$CACG^{dan}CGCG$^{3'}$/$^{3'}$GTGCGCGC$^{5'}$ [f]	323	458	9125
5		32	54	$^{5'}$CAC^{dan}GCGCG$^{3'}$/$^{3'}$GTGCGCGC$^{5'}$ [f]	325	445	8298
6		61	60	$^{5'}$CGCTTTT^{dan}CAAAACGC$^{3'}$/$^{3'}$GCGAAAAGTTTTGCG$^{5'}$	323	457	9078
7		30	53	$^{5'}$CGCTTTT^{dan}GAAAACGC$^{3'}$/$^{3'}$GCGAAAACTTTTGCG$^{5'}$	325	443	8196

[a] All samples were excited at 330 nm and the emission was monitored at 460 nm using a spectral bandwidth of 2.5 nm. [b] The ε· values were calculated from ref. 15. [c] Empirical parameters of solvent polarity $E_T(30)$ derived from the long wavelength Vis/Near-IR absorption band of the Reichardt's dye. [d] Mixed solvents were prepared by stirring distilled water with appropriate volume percent of 1,4-dioxane (spectroscopic grade). [e] Duplex forms Z-form in the presence of 0.1 M NaCl. [f] Duplex is still in B-form in the presence of 0.1 M NaCl.

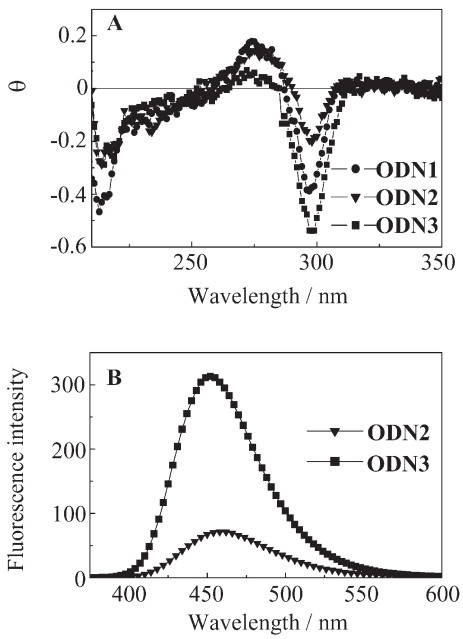


Fig. 2 **A**. CD spectra of unmodified and dan-modified Z-DNA forming sequences. Samples are DNA concentration of 100 μM (base conc.), sodium chloride of 100 mM, and pH 7, buffered by 5 mM sodium phosphate solution, at 7 °C. **B**. Fluorescence spectra of **ODNs 2** and **3**.

to that ($\varepsilon \approx 79$) in bulk water. On the other hand, the hydration ($\varepsilon = 43$) of the minor groove of Z-DNA was observed between the major and minor grooves of B-DNA.

We also estimated the $E_T(30)$ values of the grooves of B- and Z-DNA using the negatively solvatochromic pyridinium *N*-phenolate betaine (Reichardt's dye). It is known that the $E_T(30)$ values are also used to estimate solvent polarity.[17] In the results, the estimated $E_T(30)$ values of the major and minor grooves of Z-DNA were 62 and 55, respectively. The solvent polarities of major and minor grooves of Z-DNA are similar to those of water (63.1) and methanol (55.4), respectively.

It is known that the grooves of Z-DNA are significantly different from those of B-DNA. Z-DNA has no major groove and a very deep and nearly inaccessible minor groove. Therefore, the dan moiety in the major groove of Z-DNA can make contact with bulk water (Fig. 3). The CD spectrum of **ODN2** showed a weaker signal compared to that of the unmodified Z-DNA forming sequence. It is suggested that the fluorophore modification of the major groove of Z-DNA produces an unstable structure. However, the stabilization of Z-DNA was observed in the minor groove modification (**ODN3**).

In conclusion, we investigated the microenvironments in the grooves of Z-DNA using the dan-modified nucleobases (^{dan}C and ^{dan}G). These results showed that the microenvironments in both grooves of the Z-DNA are more hydrated than those of B-DNA.

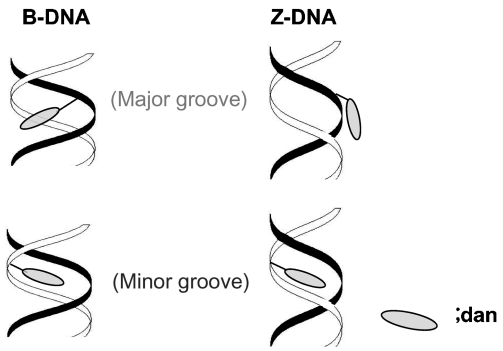


Fig. 3 Schematic diagram of dan in the major and minor grooves of B- and Z-DNA.

While the minor groove of Z-DNA has a hydrophobic site, the microenvironment in the major groove had a greater hydrophilicity. Therefore, the major groove of Z-DNA has no binding site. On the other hand, the minor groove of Z-DNA may be suitable for protein or drug binding. However, no protein that interacts with the minor groove of Z-DNA has yet been found. In fact, the human editing enzyme, double stranded RNA adenosine deaminase (ADAR1), contacts the sugar-phosphate backbone of Z-DNA.[4] The minor groove of Z-DNA is too deep for proteins to recognize bases. Such dan-modified dC and dG have the potential for use as new types of optical DNA structural sensors.

This work has been partly supported by a Grant-in-Aid for Scientific Research on Priority Area (417), 21st Century COE Research and others from the Ministry of Education, Culture, Sport, Science and Technology (MEXT) of the Japanese Government.

Notes and references

1 F. M. Poul and T. M. Jovin, *J. Mol. Biol.*, 1972, **67**, 375.
2 T. Kimura, K. Kawai and T. Majima, *Chem. Commun.*, 2004, 268.
3 L. F. Liu and J. C. Wang, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 7024.
4 T. Schwaltz, M. A. Rould, K. H. Lowenhaupt and A. Rich, *Science*, 1999, **284**, 1841.
5 S. C. Ha, K. Lowenhaupt, A. Rich, Y. G. Kim and K. K. Kim, *Nature*, 2005, **437**, 1183.
6 D. W. Pierce and S. G. Boxer, *J. Phys. Chem.*, 1992, **96**, 5560.
7 K. Yamana, T. Mitsui and H. Nakano, *Tetrahedron*, 1999, **55**, 9143.
8 R. Svensson, C. Grenö, A. S. Johansson, B. Mannervik and R. Morgenstern, *Anal. Biochem.*, 2002, **311**, 171.
9 B. E. Cohen, T. B. McAnaney, E. S. Park, Y. N. Jan, S. G. Boxer and L. Y. Jan, *Science*, 2002, **296**, 1700.
10 J. K. Kamal, L. Zhao and A. H. Zewail, *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 13411.
11 K. Nakatani, C. Dohno and I. Saito, *J. Am. Chem. Soc.*, 2002, **124**, 6802.
12 X. J. Tang and I. J. Dmochowski, *Org. Lett.*, 2005, **7**, 279.
13 M. Behe and G. Felsenfeld, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 1619.
14 T Kimura, K. Kawai, S. Tojo and T. Majima, *J. Org. Chem.*, 2004, **69**, 1169.
15 D. A. Barawkar and K. N. Ganesh, *Nucleic Acids Res.*, 1995, **23**, 159.
16 T. Kimura, K. Kawai and T. Majima, *Org. Lett.*, 2005, **7**, 5829.
17 C. Reichardt, *Chem. Rev.*, 1994, **94**, 2319.

