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Monitoring of Microenvironmental Changes in the Major and Minor Grooves of DNA by Dan-Modified Oligonucleotides

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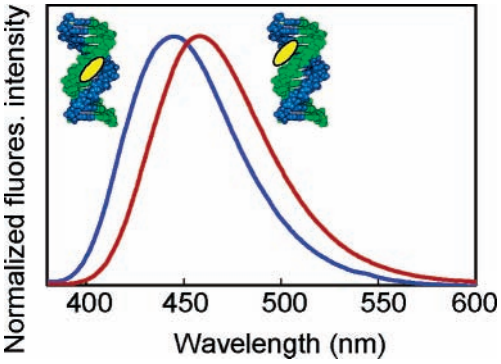
ABSTRACT



We describe the synthesis of new environmentally sensitive fluorescence probes to elucidate DNA structures. DNA oligonucleotides containing fluorophore dan (6-(dimethylamino)-2-acylnaphthalene)-modified dC or dG were able to monitor the microenvironmental changes in both the major and minor grooves of DNA with a B- to A-DNA conformational transition and RNA hybridization.

The structure and dynamics of the grooves of DNA are of significant importance for the recognition of DNA by proteins, drugs, and metal complexes.[1−3] Such DNA complexes are induced in the major or minor grooves of DNA via hydrogen bonding and electrostatic or hydrophobic interactions.[4,5] Therefore, it is important to understand the microenvironment of the major and minor grooves of DNA.

In this study, we selected a dan fluorophore as a probe for the hydration in the grooves of DNA duplexes. It is known that the fluorophore 6-(dimethylamino)-2-acylnaphthalene (dan) undergoes a large charge redistribution upon excitation and has nearly ideal environmental sensor properties.[6−8] Hence, the polarity-sensitive dan fluorophore has been used to understand the environment of the binding site in proteins or DNA.[9,10] We designed novel groove environmentally sensitive fluorescence probes and demonstrated their ability to probe the microenvironmental changes in the major and minor grooves by DNA−drug interactions and conformational transition. It is known that the nonbase-

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Table 1. Stokes Shift ($\Delta\nu$)[a] and Dielectric Constants (ϵ)[b] of Dan Fluoroprobe and Melting Temperatures (T_m)[c] of Duplexes

No.	dioxane (%)[d]	ϵ	sequences[e]	λ_{ex} (nm)	λ_{em} (nm)	$\Delta\nu$ (cm^{-1})	T_m (°C)
	0	78.5	^{dan}C monomer	321	459	9366	
	15	63.3	^{dan}C monomer	322	457	9168	
	30	50.5	^{dan}C monomer	325	454	8743	
	45	37.3	^{dan}C monomer	326	450	8452	
	60	24.0	^{dan}C monomer	328	444	7965	
1		61	5′CGCTTTT^{dan}CAAAACGC3′/3′GCGAAAAGTTTTGCG5′	323	457	9078	50
2		61	5′CGCCAGG^{dan}CCGCACGC3′/3′GCGGTCCGGCGTGCG5′	323	457	9078	72
3		30	5′CGCTTTT^{dan}GAAAACGC3′/3′GCGAAAACTTTTGCG5′	325	443	8196	54
4		27	5′CGCTTTT^{dan}CAAAACGC3′/3′r(GCGAAAAGUUUUGCG)5′[f]	323	437	8076	59
5		56	5′CGCTTTT^{dan}GAAAACGC3′/3′r(GCGAAAACUUUUGCG)5′[f]	322	452	8932	55
6			5′CGCTTTTCAAAACGC3′/3′GCGAAAAGTTTTGCG5′				50
7			5′CGCTTTTGAAAACGC3′/3′GCGAAAACTTTTGCG5′				49

[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] ϵ values were calculated from ref 13. [c] Thermal denaturation profiles were recorded on an Jasco V-530 UV/vis spectrophotometer. Absorbance of the samples was monitored at 260 nm from 10 to 80 °C. [d] Mixed solvents were prepared by stirring distilled water with appropriate volume percent of 1,4-dioxane (spectroscopic grade). [e] DNA samples are duplex concentration of 80 μM (base concentrated), sodium chloride of 100 mM, and pH 7, buffered by 5 mM sodium phosphate solution, at 7 °C in the following conditions. [f] The complementary strand is 2′-O-Me substituted RNA oligomer.

paired substituent on the N4- or N2-exocyclic amino groups of dC and dG in B-DNA extends 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 compared with rigid spacer arms in previous studies (Figure 1).[13,14]

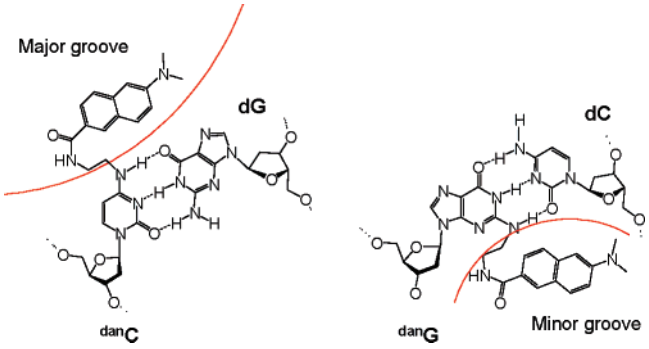

Figure 1. Dan-modified dC (^{dan}C) and dG (^{dan}G).

As the flexible linker between dan fluorophore and nucleobase, a ethylaminolinker was attached to the N4-position of deoxycytidine and the N2-position of deoxyguanosine according to reported procedures.[15,16] Ethylamino linkers of nucleobases were conjugated with 6-dimethyamino-2-naphthoic acid by using activating reagents for peptide synthesis, *N*-hydroxybenzotriazole and *N*,*N*′-dicyclohexylcarbodiimide.

We synthesized a series of dan-labeled dC (^{dan}C) or dan-labeled dG (^{dan}G) modified 15 mer oligodeoxynucleotides (ODNs). The ^{dan}C containing **ODN1** exhibited a melting temperature (T_m) up to about 1 °C higher than the unmodified **ODN6** (Table 1). On the other hand, the T_m of the ^{dan}G-modified **ODN3** notably increased (up to 5 °C) more than the unmodified **ODN7**. The stabilization of the dan-modified duplexes seems to reflect the location of the dan moiety in the major and minor grooves.

ODN1,2 showed a 2-nm blue shift of the dan emission compared with the dan-modified single strand ODNs (λ_{max} = 459 nm). It is known that a subtle difference exists between the hydration of the G/C and A/T base pairs in DNA.[17] However, no sequence-dependent change in the fluorescence property was observed for **ODN1** and **ODN2**. The difference between the hydration of the G/C- and A/T-rich sequence may be too small for detection in the dan-modified nucleobase. A dramatic blue shift of the dan emission was observed for the **ODN3** in which the dan moiety is located in the minor groove. It is known that the blue shift in the fluorescence emission is dependent upon a large decrease in the dipole moment in the fluorescent state compared with that in the ground state.[18] Therefore, the microenvironment of dan fluorophore in the minor groove of B-DNA shows a higher hydrophobicity compared with that of the major groove. These results unambiguously indicated that the dan fluorophore is excellent as a groove microenvironment-sensitive probe of DNA, and the major and minor grooves of DNA have significant differences in polarity.

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To estimate the dielectric constants (ϵ) of the major and minor grooves in DNA, the fluorescence parameters (λ_{ex}, λ_{em}) of the ^{dan}C monomer were measured in media of different dielectric constants generated from varying the ratios of dioxane/water (Table 1). We 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.[14] The Stoke's shift ($\Delta\nu$) value of the major groove-modified **ODN1,2** is 9078 cm^{-1}. These values correspond to a dielectric constant of 61 D. On the other hand, the $\Delta\nu$ of the minor groove-modified **ODN3** was 8196 cm^{-1}, which corresponds to the ϵ value of 30D. The results showed that the major groove (61 D) of DNA is more polar than the minor groove (30 D). Therefore, the dan probe is effective in monitoring the microenvironment of DNA. Jin and Breslauer measured the minor groove environment with bisbenzimide as a probe molecule, which was added to the poly[d(AT)]−poly[d(AT)] duplex without covalent bonding, and reported an ϵ value (ϵ = 20 D) similar to that (ϵ = 30 D) measured in the present study.[19] Moreover, many studies have focused on the hydration process of biomaterials such as DNA, RNA, or protein.[20−22] Therefore, DNA has different hydration conditions in its two grooves. These results correspond to the fact that the major groove is about 2 times wider than the minor groove.

DNA/RNA hybrids are important intermediates in transcription, the normal replication of double-stranded DNA,[23] and the reverse transcription by retroviruses. To compare the microenvironment of the DNA/RNA hybrid duplexes (A-like) with that of the DNA duplexes (B-like), the 2′-OMe-RNA complementary strand such as the RNA-like strand was hybridized to the dan-modified ODN (**ODN4**,**5**). In the major groove-modified A-like **ODN4**, a remarkable blue shift of the dan emission was observed when compared with the B-like (**ODN1,2**). However, in the minor groove (**ODN5**) of the A-like, an interesting red shift was observed. The estimated ϵ values of the major and minor grooves in the A-form are 27 D and 56 D, respectively. It is known that the DNA/RNA hybrid is globally closer to the A-form than the B-form. The X-ray data revealed that the minor and major grooves of the A-form DNA become progressively wider and shallower and deeper and narrower than those of the B-form, respectively. Therefore, it is suggested that the increase in polarity at the minor groove of the DNA/RNA hybrid reflects the enlargement of the groove width in the minor groove. These results suggest that the DNA/RNA hybrid is more similar to the crystal structure of A-form DNA.[24] The 2′-OMe substitution of the RNA strand may lose flexibility of the duplex compared with the native RNA. The microenvironment in the minor groove of the A-like was more polar than that of the major groove. Moreover, the estimated polarity of the minor groove in the A-like (ϵ = 56 D) was lower than the major groove in the B-like (ϵ = 61 D). It should be noted that the former is more polar than that in the minor groove of the B-like (ϵ = 30 D).

Recently, it has been suggested that $Co(NH_3)_6^{3+}$ induces the A-DNA structure in DNA with GC-rich sequences, as evidenced, among other data, by the characteristic changes in their circular dichroism (CD) spectra. It is known that $Co(NH_3)_6^{3+}$ can adhere to guanine bases in the deep major groove of the A-DNA helix, as is evident from the significant direct NOE cross-peaks from the protons of $Co(NH_3)_6^{3+}$ to GH8.[25] Therefore, we performed the titration of $Co(NH_3)_6^{3+}$ with the major groove-modified **ODN1** and **2**. As shown in Figure 2B, the fluorescence spectra of the GC-rich sequence

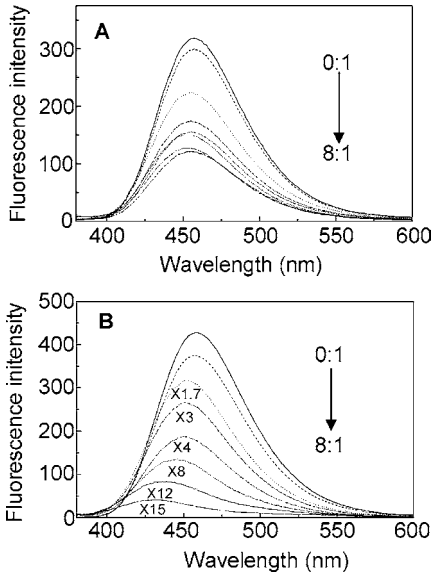

Figure 2. Fluorescence titration of $Co(NH_3)_6^{3+}$ with the solution of dan-modified duplexes. (A) Free **ODN1** duplex and the 0.1:1, 0.2:1, 0.5:1, 1:1, 2:1, 4:1, and 8:1 Co^{3+}/duplex complexes. (B) Free **ODN2** duplex and the 0.1:1, 0.2:1, 0.5:1, 1:1, 2:1, 4:1, and 8:1 Co^{3+}/duplex complexes.

ODN2 showed a dramatic change in the fluorescence property of the dan fluorophore. The fluorescence maxima of **ODN2** shifted from 457 to 435 nm by adding $Co(NH_3)_6^{3+}$. It was noted that this tendency is similar to the A-like DNA/RNA hybrid. It is known that A-DNA is conformationally similar to the DNA/RNA hybrid.[26,27] On the other hand, the AT-rich sequence **ODN1**, in which $Co(NH_3)_6^{3+}$ does not induce A-DNA, had only a 6-nm blue shift under the same conditions. We also observed the intensity decrease of the dan fluorescence in the presence of $Co(NH_3)_6^{3+}$. We observed fluorescence quenching of ^{dan}C monomer with various ratios

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of $Co(NH_3)_6^{3+}$ (data not shown), suggesting that electron transfer occurs between dan and $Co(NH_3)_6^{3+}$.

The CD spectra are good indicators of the DNA conformation. Figure 3B shows the CD spectra of the GC-rich

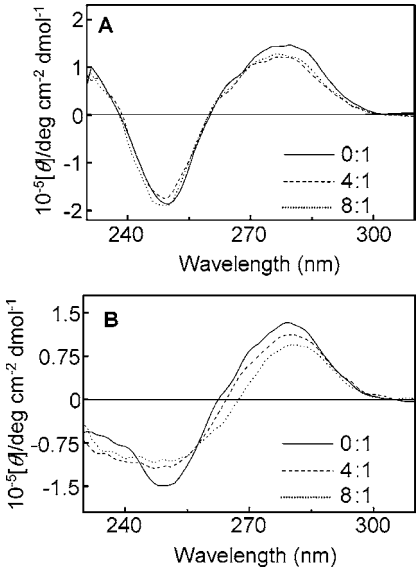

Figure 3. Titration of $Co(NH_3)_6^{3+}$ with the solution of dan-modified duplexes as monitored by their CD spectra. (A) CD spectra of free **ODN1** duplex and the 4:1 and 8:1 Co^{3+}/duplex complexes. (B) CD spectra of free **ODN2** duplex and the 4:1 and 8:1 Co^{3+}/duplex complexes.

ODN2. The characteristic spectral shift toward the longer wavelength associated with a B- to A-DNA transition is evident.[25] However, no change was observed in the CD spectrum of **ODN1** upon the addition of $Co(NH_3)_6^{3+}$ as compared with the GC-rich sequence. These results suggest that a critical blue shift of the dan emission depends on the B- to A-DNA conformational transition of the ODN.

We synthesized the novel dan-modified nucleosides (^{dan}C and ^{dan}G). The ODNs containing a dan-modified nucleoside exhibit interesting fluorescence properties upon binding to DNA and RNA duplexes. We measured the local dielectric constant of the environment in several different DNA grooves. Dramatic fluorescence changes of the dan-modified ODNs were observed upon binding to the minor groove of DNA and the major groove of RNA. These probes can discriminate between the microenvironments in both grooves of the duplexes and also detect the B- to A-DNA conformational transition. Therefore, these probes are applicable to measuring the microenvironment in a variety of DNA structures. The potential of such a probe can be exploited by incorporating ^{dan}C or ^{dan}G into specific sequences for studying the microenvironments in different DNA polymorphs such as Z-DNA,[28,29] triplexes,[30] or G-quadruplex[31] and by observing hydration dynamics at specific sites in DNA.

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Supporting Information Available: Experimental details, synthesis, and characterization of all oligonucleotides, melting curves, and absorption spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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