DNA with γ-Aminopropyltriethoxysilane Switches between B- and C-Form Structures under Thermal Control

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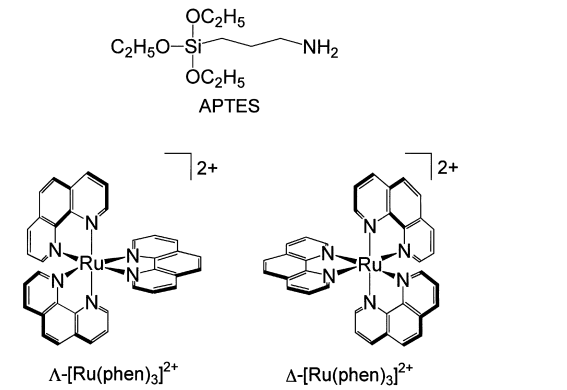
DNA, the most important genetic material of living organisms, has a double-stranded structure.[1] This double-stranded structure allows the DNA to have various specific functions, which include intercalation,[2] groove binding,[2] and electron transfer.[3] Double-stranded DNA in living organisms possesses various structures, such as the A, B, and C forms.[1] B-form DNA generally represents the native DNA in living organisms.[1] In addition, A-form DNA has been reported to occur in a DNA – protein complex.[1] However, the conformation of DNA in the C form is not well understood. C-form DNA has been considered to be a variant of the B form under special salt conditions or an intermediate between the A and B forms.[1,4] These DNA structures are sequence-dependent and/or are induced by various conditions, which include ionic strength and the solvent present. However, the DNA structures are not significantly affected by thermal changes.

Organofunctional silanes, such as 3-glycidoxypropyltrimethoxysilane and 3-methacryloxypropyltrimethoxysilane, are used as adhesion promoters or cross-linking reagents for surface modification.[5] A γ-aminopropyltriethoxysilane (APTES)-modified polymer has been used for the immobilization of DNA.[6] In contrast, organosilanes form stable siloxane oligomers through cross-linking reactions in aqueous solution.[5] The interaction of siloxane oligomers and DNA has not yet been evaluated.

In this paper, we describe the structural analysis of the DNA – APTES complex at various temperatures with circular dichroism (CD) spectroscopy. The DNA – APTES complex has the B- and C-form structures at different temperatures. Additionally, the binding affinity of the DNA – APTES complex to tris-(1,10-phenanthroline)ruthenium(II) $[Ru(phen)_3]^{2+}$ was also examined at various temperatures.

A salmon-sperm double-stranded DNA aqueous solution ($Mw \approx 5 \times 10^6$, 100 μL, 10 mg mL^{-1}) was incubated with APTES (10 μL) at 4 °C for 24 h. The chemical structure of APTES is shown in Scheme 1. The structure of the DNA – APTES complex was analyzed at various temperatures by using CD spectroscopy (Figure 1). The CD spectrum of the DNA – APTES complex at 4 °C



Scheme 1. *Chemical structures of γ-aminopropyltriethoxysilane (APTES) and the two forms of tris-(1,10-phenanthroline)ruthenium(II) $[Ru(phen)_3]^{2+}$.*

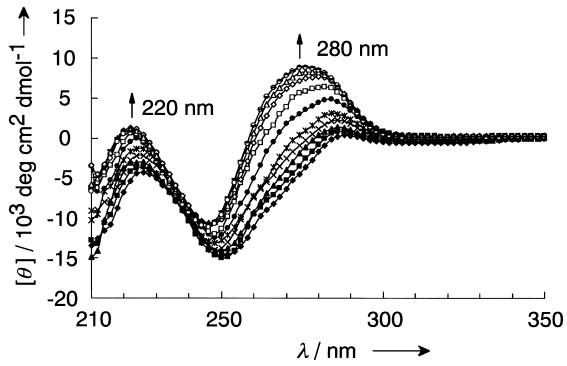


Figure 1. *CD spectra of the DNA – APTES complex at different temperatures: ◆, 4 °C; ■, 5 °C; ▲, 6 °C; ×, 8 °C; ✳, 10 °C; ●, 15 °C; □, 20 °C; ◇, 25 °C; ▲, 30 °C; ○, 35 °C; —, 40 °C. Arrows indicate the direction of change of the spectrum during heating.*

showed two maximum peaks at 225 nm and 280 nm and a minimum peak at 250 nm. When the temperature was increased, the two maximum peaks were shifted to 222 nm and 275 nm and the θ_{230} and θ_{280} values of the DNA – APTES complex increased until they reached a constant value at 35 °C. During the cooling process (from 40 °C to 4 °C), the CD spectral changes were reversed. These results indicate that this structural change by thermal control is reversible. The CD spectrum of DNA alone was similar to that of the DNA – APTES complex at 35 °C (data not shown). Additionally, APTES alone did not show any CD spectral features over the range 4 – 40 °C (data not shown).

The CD spectrum of DNA in the presence of 5 M NH_4Cl indicated the presence of the C-form structure, with a maximum peak at 230 nm and a minimum peak at 250 nm (Figure 2a, closed circles) as previously described.[4] The CD spectrum of

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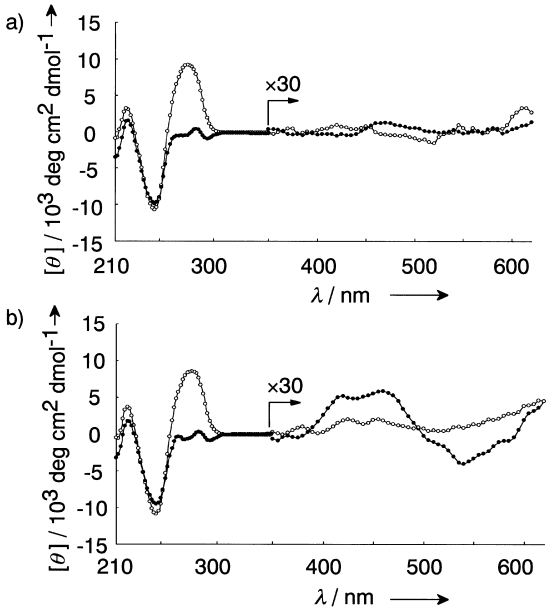

Figure 2. *CD spectra of B- and C-form DNAs in the absence (a) and presence (b) of 1 mM $[Ru(phen)_3]^{2+}$ complex. The spectra of the B- and C-form DNAs were measured in 100 mM tris(hydroxymethyl)aminomethane (Tris) – HCl (pH 7.4) in the absence (○, B-form DNA) or presence (●, C-form DNA) of a 5 M NH_4Cl solution.*

DNA in the absence of 5 M NH_4Cl indicated that the DNA had the B-form structure,[7] with two maximum peaks at 230 nm and 280 nm and a minimum peak at 250 nm (Figure 2a, open circles). The CD spectrum of the DNA – APTES complex at 4 °C is similar to that of DNA in a 5 M NH_4Cl solution. These results also suggest that the DNA – APTES complex possesses the C-form structure at low temperature (4 °C) and the B-form DNA structure at high temperature (35 °C).

Next, we examined the interaction of $[Ru(phen)_3]^{2+}$ with DNA in the absence or presence of 5 M NH_4Cl (Figure 2b, open and closed circles). $[Ru(phen)_3]^{2+}$ exists in the Δ and Λ forms (Scheme 1), and these two forms have different affinities for double-stranded DNA; the Δ form mainly interacts with DNA during intercalation and base stacking, and the Λ form binds to the surface of DNA by electrostatic interaction.[8] When racemic $[Ru(phen)_3]^{2+}$ was added to the DNA solution in the absence of 5 M NH_4Cl, the CD spectrum did not change (Figure 2b, open circles). When racemic $[Ru(phen)_3]^{2+}$ was added to the DNA solution in the presence of 5 M NH_4Cl, a maximum peak at 420 nm and a minimum peak at 530 nm were induced (Figure 2b, closed circles). However, the CD spectrum did not change at with temperature (4 – 40 °C; data not shown). Additionally, racemic $[Ru(phen)_3]^{2+}$ alone did not produce any CD spectral peaks (data not shown). These results suggest that $[Ru(phen)_3]^{2+}$ specifically interacts with C-form DNA in the presence of 5 M NH_4Cl.

When the conformation of the DNA – APTES complex in the presence of $[Ru(phen)_3]^{2+}$ was examined at different temperatures by CD spectroscopy, five isodichroic points were observed at 253, 291, 348, 389, and 475 nm (Figure 3). The CD spectrum of the DNA – APTES complex in the presence of $[Ru(phen)_3]^{2+}$ at 4 °C showed a maximum peak at 420 nm and a minimum peak at

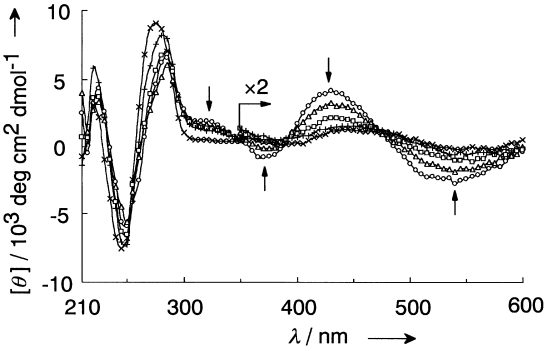

Figure 3. *CD spectra of DNA – APTES with the $[Ru(phen)_3]^{2+}$ complex at different temperatures: ○, 4 °C; △, 10 °C; □, 20 °C; +, 30 °C; ×, 40 °C. Arrows indicate the direction of change of the spectrum during heating.*

530 nm, similar to the spectrum of DNA – $[Ru(phen)_3]^{2+}$ in the presence of 5 M NH_4Cl. These results suggest that the DNA – APTES complex has the C-form DNA structure at 4 °C and specifically interacts with $[Ru(phen)_3]^{2+}$ (Figure 2b, closed circles). When the DNA – APTES complex was examined at higher temperature, the maximum peak at 420 nm and the minimum peak at 530 nm disappeared (Figure 3). The structural switch induced by thermal changes was reversible (data not shown). These results suggest that the DNA in the DNA – APTES complex reversibly switches between the C and B forms under thermal control.

APTES has been reported to construct water-soluble oligomers with siloxane cross-links in H_2O.[9] These APTES oligomers are positively charged and aggregate with DNA by electrostatic interaction. As a result, at low temperature (4 °C), water molecules around the double-stranded DNA are excluded and the structure of DNA is switched from the native B-form to the C-from structure. Water molecules are critical for constructing the B-form structure of DNA, in which water molecules interact with oxygen atoms of the deoxyribose unit and the phosphate group.[10] In fact, the B-form structure is not induced when the binding water is excluded under high ionic strength conditions (Figure 2a, closed circles).[4, 10] At high temperature, the aggregation of DNA and the APTES oligomer is reduced and the structure of DNA in the complex returns to the B-form structure through the interaction of water molecules. These results suggest that the DNA – APTES complex can be reversibly switched between the C- and B-form structures by thermal control. The C-form structure of DNA is not well understood.[1, 4] The DNA – APTES complex is useful for studying this C-form structure of DNA.

Recently, DNA has been used as a component of functional materials, such as biomaterials,[11–14] electronic materials,[15, 16] and optical materials.[17] We previously described the preparation of a water-insoluble DNA matrix with an intermolecularly cross-linked structure induced by UV irradiation.[11] The UV-irradiated DNA selectively accumulated harmful compounds with a planar structure, such as dioxin and polychlorinated biphenyl (PCB) derivatives, benzo[*a*]pyrene, ethidium bromide, and acridine orange.[12, 13] Moreover, this UV-irradiated DNA condensed heavy metal ions from water containing harmful metal ions, such as

mercury, cadmium, and lead ions.[14] The DNA – APTES complex has potential utility not only for fundamental research of DNA structure but also as a novel DNA material with applications such as optical resolution with thermal control, the thermal separation of mutagenic molecules, and thermal sensing.

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[1] W. Saenger, *Principles of Nucleic Acid Structure*, Springer-Verlag, Berlin, **1987**.
[2] a) M. J. Waring, *Annu. Rev. Biochem.* **1981**, *50*, 159 – 192; b) H. M. Berman, P. R. Young, *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 87 – 114.
[3] a) E. M. Boon, J. E. Salas, J. K. Barton, *Nat. Biotechnol.* **2002**, *20*, 282 – 286; b) S. O. Kelley, J. K. Barton, *Science* **1999**, *283*, 375 – 381; c) S. O. Kelley, N. M. Jackson, M. G. Hill, J. K. Barton, *Angew. Chem.* **1999**, *111*, 991 – 996; *Angew. Chem. Int. Ed.* **1999**, *38*, 941 – 945.
[4] a) J. Portugal, J. A. Subirana, *EMBO J.* **1985**, *4*, 2403 – 2408; b) N. J. Rhodes, A. Mahendrasingam, W. J. Pigram, W. Fuller, J. Brahms, J. Vergne, R. A. J. Warren, *Nature* **1982**, *296*, 267 – 269.
[5] F. Beari, M. Brand, P. Jenkner, R. Lehnert, H. J. Metternich, J. Monkiewicz, H. W. Siesler, *J. Organomet. Chem.* **2001**, *625*, 208 – 216.
[6] a) A. Ulman, *Chem. Rev.* **1996**, *96*, 1533 – 1554; b) M. Tanigawa, T. Okada, *Anal. Chim. Acta* **1998**, *365*, 19 – 25.
[7] a) W. Fuller, M. J. Waring, *Ber. Bunsen-Ges.* **1964**, *68*, 805 – 808; b) S. Hanlon, S. Brudno, T. T. Wu, B. Wolf, *Biochemistry* **1975**, *14*, 1648 – 1660.
[8] a) J. K. Barton, *Science* **1986**, *233*, 727 – 734; b) J. K. Barton, J. M. Goldberg, C. V. Kumar, N. J. Turro, *J. Am. Chem. Soc.* **1986**, *108*, 2081 – 2088; c) K. E. Erkkila, D. T. Odom, J. K. Barton, *Chem. Rev.* **1999**, *99*, 2777 – 2795; d) A. Yamagishi, *J. Phys. Chem.* **1984**, *88*, 5709 – 5713.
[9] E. P. Plueddemann, *Silane Coupling Agents*, 2nd ed., Plenum Press, New York, **1991**.
[10] G. G. Prive, U. Heinemann, S. Chandrasegaran, L. S. Kan, M. L. Kopra, R. E. Dickerson, *Science* **1987**, *238*, 498 – 504.
[11] M. Yamada, K. Kato, M. Nomizu, N. Sakairi, K. Ohkawa, H. Yamamoto, N. Nishi, *Chem. Eur. J.* **2002**, *8*, 1407 – 1412.
[12] M. Yamada, K. Kato, K. Shindo, M. Nomizu, M. Haruki, N. Sakairi, K. Ohkawa, H. Yamamoto, N. Nishi, *Biomaterials* **2001**, *22*, 3121 – 3126.
[13] M. Yamada, K. Kato, M. Nomizu, K. Ohkawa, H. Yamamoto, N. Nishi, *Environ. Sci. Technol.* **2002**, *36*, 949 – 954.
[14] M. Yamada, K. Kato, M. Nomizu, M. Haruki, K. Ohkawa, H. Yamamoto, N. Nishi, *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1627 – 1632.
[15] K. Tanaka, Y. Okahata, *J. Am. Chem. Soc.* **1996**, *118*, 10679 – 10683.
[16] Y. Okahata, T. Kobayashi, K. Tanaka, M. Shimomura, *J. Am. Chem. Soc.* **1998**, *120*, 6165 – 6166.
[17] Y. Kawabe, L. Wang, S. Horinouchi, N. Ogata, *Adv. Mater.* **2000**, *12*, 1281-1283.

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