DNA Conformational Switching by Use of an Intercalator and Its Receptor

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DNA conformational switching between B and Z forms was controlled with tilorone (intercalator) and sulfocalix[8]arene (C8AS, receptor for tilorone). When tilorone was added to the *Z*-DNA that had been beforehand induced by cobalt hexamine, the conformational transition from Z to B form was induced. The resultant *B*-DNA was turned back to *Z*-DNA by adding C8AS. This change was a clear-cut transition because most of the intercalated tilorone molecules were pulled out and captured by C8AS. This mechanism was established by isothermal titration calorimetry in which the binding constant of tilorone to C8AS was shown twice larger than that of tilorone/DNA.

Discovery of *Z*-DNA intrigued many researches to investigate its conformational details and other issues such as what makes the reverse Z conformation stable and triggers the conformational transition from B or A form to Z form.[1] The biological importance of *Z*-DNA was recognized since *Z*-DNA-binding proteins was found.[2] The control of B–Z transition seems important to regulate the biological functions of DNA. Sage and Leng[3] showed that *N*-acetoxy-*N*-acetyl-2-aminofluorene intercalated into B-formed poly(dG-dC)•poly(dG-dC) and the intercalation converted the part of the DNA into Z form. Subsequent addition of EDTA to this partial Z form DNA reduced the Z form content. In this case, the molecular mechanism of this Z to B form transition is not clear for us and the transition itself was not distinct. The present paper makes an alternative attempt to clearly switch between the B to Z forms by use of an intercalator and its receptor.

Fluorene is a tricyclic aromatic hydrocarbon and contains a five-membered ring. Fluorene seems to perfectly possess structural requirements for DNA intercalators. There have been several studies for the fluorene derivatives that are interacting with DNA, including for tilorone (2,7-bis[(diethylamino)-ethoxy]fluoren-9-one, see Figure 1),[4] 9-fluoren-β-*O*-glycoside,[5] and 2,7-bis[(dialkylamino)acetylamino]fluoren-9-one derivatives (fluoramides).[4b] It has been reported that tilorone has the same magnitude of the binding constant (K_b) as ethidium bromide and increases the melting temperature of intercalated *B*-DNAs.[4a,6] This result implies that tilorone stabilizes the B form of DNA. Figure 2A presents the circular dichroism changes when *Z*-DNA was titrated with tilorone, where the *Z*-DNA was

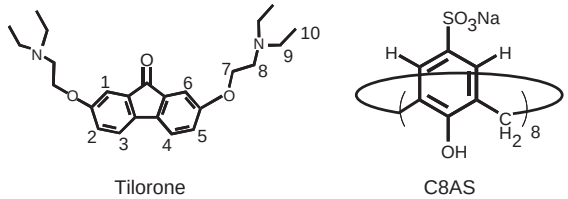

Figure 1. Structures of tilorone and sulfocalix[8]arene (C8AS).

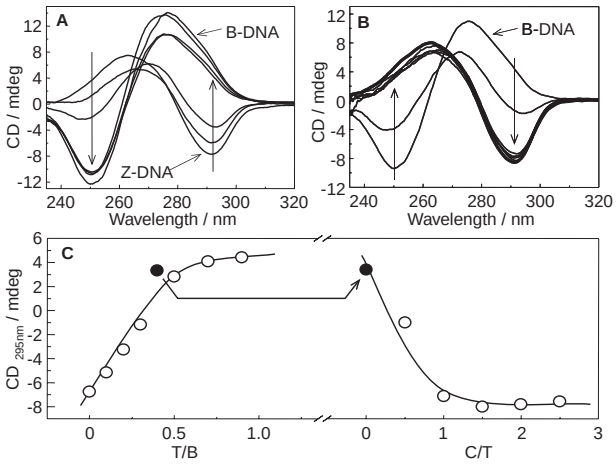

Figure 2. (A) Tilorone concentration dependence of the CD spectra of poly(dG-dC)•poly(dG-dC) in 10 mM Tris-HCl buffer solution (pH 7.5) at 10 °C measured 24 h after mixing. Tilorone concentrations: 0, 3.0, 6.0, 9.0, 12.0, 15.0, 21.0, and 27.0 μM. (B) C8AS concentration dependence of the CD spectra. The DNA had been converted to B form by adding tilorone (filled point in Panel C). The bold line shows the spectrum at C/T = 2.5 after 24 h. C8AS concentrations: 0, 6.0, 12.0, 18.0, 24.0, and 30.0 μM. In both experiments, final concentration of DNA, $[Co(NH_3)_6]Cl_3$, NaCl, and DMSO were 30 μM, 40 μM, 25 mM, and 1%, respectively. (C) The difference of the CD intensity at 295 nm plotted against the tilorone/base pair (T/B) and C8AS/tilorone (C/T) molar ratio.

beforehand induced in a cobalt hexamine aqueous solution.[1a] Similarly to Walker et al.,[7] who titrated *Z*-DNA with ethidium bromide, the addition of tilorone induced the conformational transition from Z to B forms. Figure 2C plots the CD values at 295 nm against the tilorone/base pair molar ratio. Since the transition was a relatively slow process, the CD values after 24 h are presented in the Figure 2. This is presumably because of involvement of taking-out cobalt hexamine, and tilorone intercalation. By the way, the maximum number of intercalators that can bind to DNA helices is one intercalator versus two base pairs. The transition in Figure 2C completed at tilorone/base pair = 0.5. This ratio coincides with the neighbor-exclusion principle. This feature suggests that the tilorone intercalation is the driving force to the changes in Figure 2A.

As long as we know, there has not been any good receptor reported for tilorone, although cyclodextrins and calixarenes are well-known to include fluorene owing to the hydrophobic interactions. On the analogy of fluorene, we found that sulfocalix[8]arene (C8AS) is one of the best receptor of tilorone.

Figure 3 shows the ITC titration thermogram when a tilorone solution was added to a C8AS solution at 10 °C. The net release of heat during the binding (the lower panel in Figure 3)

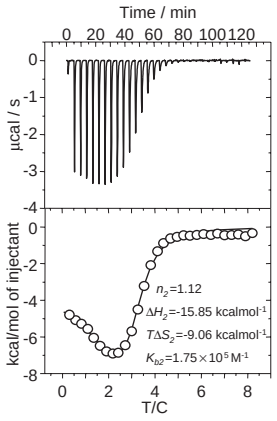

Figure 3. Microcalorimetric titration of tilorone and C8AS in 10 mM Tris-HCl buffer solution (pH 7.5) at 10 °C. Final concentration of Tilorone, C8AS, NaCl, and DMSO were 2 mM, 0.05 mM, 25 mM, and 1%, respectively. Volume of first injection was 2 µL, and that of the rest of injections were 9 µL per injection. (Upper) Raw data for the heat effect during titration. (Lower) The binding isotherms.

indicates that binding is exothermic, and that the binding saturates around the molar ratio = 5.0. Best fits to the data in the panel required a two-site binding model,[8] and the analyses resulted in the binding parameters: $K_b = 1.8 \times 10^5\ M^{-1}$, $\Delta H = -15.9$ kcal/mol, $T\Delta S = -9.1$ kcal/mol, and $n = 1.1$ for the second binding. The first binding should be related to electrostatic interaction between the amino group of tilorone and the sulfonic group of C8AS. Therefore, we conclude that the inclusion interaction between tilorone and C8AS is related to the second mode of the binding. Considering that $K_b = 1.0 \times 10^5\ M^{-1}$ for DNA/tilorone interaction,[6] the binding between tilorone and C8AS is more favorable than that of tilorone and DNA almost by twice.

Figure 4A shows the ^{1}H NMR spectra of C8AS, tilorone, and two mixtures with the tilorone/C8AS ratios (T/C) of 0.5, 1.0, and 1.5. Here, the tilorone protons were observed as a single resonance because of fast exchange between the two species. Additions of tilorone to C8AS resulted in dramatic up-field shift of the aromatic protons of tilorone (the change of the chemical shifts ($\Delta\delta$) summarized in Table S1.[9] The shift was most enhanced for H2/H5 and H3/H4 comes to the second. These results indicate that the fluorene moiety was deeply captured by the hydrophobic cavity of C8AS and H2/H5 should be located in the bottom of the cavity, as presented by the schematics in Figure 4B. These conclusions were supported by ROESY data (see Supporting Information Figure S2[9]). The side chain protons (H-8, H-9, and H-10) were up-field shifted, indicating that the side chain of tilorone was also included in the hydrophobic cavity of C8AS. Interestingly, $\Delta\delta$ values of H-8, H-9, and H-10 reached the maximum at T/C = 0.5, and the order of $\Delta\delta$ value was H10 > H9 ≫ H8. This phenomenon can be related to the presence of the second binding mode between C8AS and tilorone, which is most likely ionic interaction between two C8AS and one tilorone.

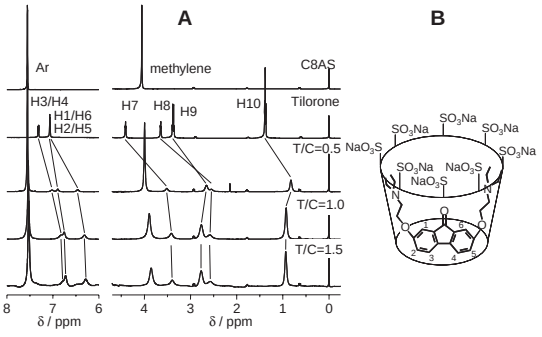

Figure 4. (A) ^{1}H NMR spectra of C8AS, tilorone, and mixture of C8AS and tilorone in 10 mM phosphate buffer solution (pH 7.5) at 25 °C. Final concentration of NaCl was 25 mM. 3-(Trimethylsilyl)propanesulfonic acid was used as an internal standard. (B) The deduced binding model of tilorone and C8AS.

The magnitude difference in K_b between the intercalation ($K_b = 1.0 \times 10^5\ M^{-1}$) and inclusion ($K_b = 1.8 \times 10^5\ M^{-1}$) of tilorone suggests that C8AS can take out the intercalated tilorone from DNA. Figure 2B shows the CD spectral changes of *B*-DNA when C8AS was added (at the filled point in Panel 2C). With increasing the C8AS concentration, the 250 nm-band increased and the 290 nm-band decreased. These changes indicate that the amount of *B*-DNA was reduced. Twenty four hours after added C8AS at C/T > 1.0, the CD spectrum indicated that the *B*-DNA was converted completely to *Z*-DNA. This fact suggests that all of the added C8AS captured tilorone. Quantitatively speaking, this feature may not be compatible with the K_b difference between the intercalation and inclusion. The discrepancy is now under investigation.

To sum up, ITC and NMR studies showed that tilorone can be included in the cavity of C8AS with a larger binding constant. Tilorone induces the conformational transition of poly(dG-dC)·poly(dG-dC) from *Z*-DNA to *B*-DNA in cobalt hexamine solution and the addition of C8AS to this *B*-DNA turned back to *Z*-DNA by taking out the intercalated tilorone from DNA.

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9 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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