Study of the DNA Interaction with Water-soluble Cationic Fullerene Derivatives

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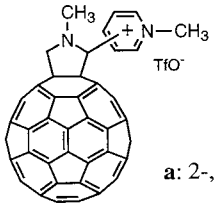
The cationic fullerene derivatives **1a–c** are fairly well soluble in water and bind to calf thymus DNA due to the electrostatic interactions at the phosphate anion sites and the hydrophobic interactions in the grooves.

Interaction of fullerene derivatives with DNA is interesting from the viewpoint of the biological activities of fullerene.[1-5] One of its possibilities is to cleave DNA by singlet oxygen ($^1O_2$) generated from the reaction of photoexcited fullerene with oxygen.[1-4] Unfortunately, fullerene and its known derivatives are sparingly soluble in water. To overcome this problem, the poly(vinylpyrrolidone)(PVP) has been used to form a polymer micelle to impose fullerene derivatives a solubility in water.[4,5] However, under such conditions, there is no direct evidence for the interaction of fullerene derivatives with DNA. Since a diameter of fullerene molecule is comparable with the dimension of the grooves of a double stranded DNA (ca. 10 Å), there is a possibility of hydrophobic interaction of fullerene with DNA grooves.

Recently, we synthesized the fulleropyrrolidines, **1a–c**, carrying pyridinium units and have found that they are soluble in water. In this paper, we studied their interactions with DNA by spectrophotometric and electrochemical methods.



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a: 2-, **b**: 3-, **c**: 4-

Isomeric fulleropyrrolidines **1a–c**[6] were synthesized by the reaction of pyridinecarboxyaldehyde, sarcosin, and fullerene[7] followed by methylation with methyl triflate. Synthesis of N-methyl-2-(4'-pyridyl)-3,4-fulleropyrrolidine, the precursor of **1c**, was reported in the previous paper.[8] Use of o-chlorobenzene as a solvent in the synthesis of the precursors of **1a** and **1b** gave the yields of 62 and 44%, respectively. The reaction of methyl triflate with the precursor was performed in toluene (6 h, refluxing) for **1a**, and cholorobenzene (4 h, refluxing) for **1b** and **1c**. The yields of **1a-c** were 75, 92, and 92%, respectively.

The saturated aqueous solutions of **1a–c** were obtained by shaking a solid suspension in water for one day under photoshielded conditions. The uv/vis absorption spectra of the solution thus obtained show the peak maxima at 209, 267, and 335 nm, which are in good agreement with the reported spectra of fullerene in hexane.[5] The saturation concentrations of **1a–c** in water were determined as follows. The saturated solutions of **1a–c** (each 3 ml) were dried up in vacuum and dissolved in a proper amount of chlororbenze. The concentrations were determined by using the known molar absorptivities of **1a-c** in chlorobenzene at 335 nm. The concentrations of **1a**, **1b**, and **1c** in saturated aqueous solutions were 26.0, 29.0, and 6.3 μM, respectively.

When the aqueous solutions of **1a–c** (ca. 10^{-5} M) were added to sonicated calf thymus DNA, the absorbance at 335 nm decreased by about 5% for all the compounds. The reason of this hypochromic effect is not clear, but it may come from the difference in micro environment between water and a hydrophobic DNA groove. The absorbances of ligands **1b** and **1c** decreased with the charge ratio of the added DNA phosphate anion to ligand cation until the absorbance change leveled off at the charge ratio (DNA/ligand) of 1, whereas the absorption changes continued up to the ratio of 2 for ligand **1a**. This suggests that one molecule of **1a** binds to two phosphate groups of DNA while for **1b** and **1c** one ligand nolecule binds to one phosphate group of DNA. There was no precipitation in the solution of the DNA complex with **1a–c**. The difference in the binding behavior of **1a**, **1b**, and **1c** is reasonable if one considers their molecular structures. The distance between the cationic site and fullerene moiety in **1a** is smaller than those in **1b** and **1c**. Therefore, the strong interactions between the DNA phosphate group and the cationic site in **1a** may result in the diminished interaction of its fullerene part onto DNA groove.

Cyclic voltammetry (CV) was applied to study the electrochemical behavior of the DNA complexes. First, CV was run in the electrolytes containing about 10^{-5} M of **1a–c** and 0.1 M KCl in aqueous solution. However, only ill-separated, broadened current peaks resulted. Therefore, the CV traces were obtained using the DNA-fullerene coated electrode in the electrolyte containing 0.15 M of butylammonium tetrafluoroborate (TBAF) in chlorobenzene, with Pt counter electrode and Ag/Ag^+ reference electrode at room temperature. The DNA-fullerene coated electrode was prepared according to the following procedure: ten microliters of the solution which contained 10 pmol of calf thymus DNA and was saturated with fullerene derivative, **1a**, **1b**, or **1c** was placed on the top of the glassy carbon electrode (Area, 2.5 mm^2) and dried up in air.

Figure 1 shows the CV recorded on the electrode coated with DNA-**1a** complex (a) as well as that on a bare glassy carbon electrode in 0.4 mM solution of **1a** (b).[9] No peaks were observed on DNA (without fullerene ligands)-coated electrodes (data not shown). The electrochemical data for **1a–c** are summarized in Table 1. The CV of **1a** in solution shows four redox couples: $E_{1/2}$ = -808, -1239, -1615, and -1941 mV vs. Ag/Ag^+ corresponding to the $E^{0/1-}$, $E^{1-/2-}$, $E^{2-/3-}$, and $E^{3-/4-}$ redox steps, respectively. The potentials of these redox couples are shifted to positive side comparing with the corresponding fullerene derivative before methylation, indicating that the reduction is facilitated by the introduction of cationic charge.

The CV on the electrode coated with DNA-**1a** complex shows three redox couples with a disappearance of some of the E_{pc} and E_{pa} peaks and a negative shift of some of the $E_{1/2}$ values.

Table 1. Comparison of electrochemical responses for **1a–c**[a]

Compound	Condition	$E^{0/1-}$	$E^{1-/2-}$	$E^{2-/3-}$	$E^{3-/4-}$	$\Delta E^{0/1-}$	$\Delta E^{1-/2-}$	$\Delta E^{2-/3-}$	$\Delta E^{3-/4-}$
1a	solution[b]	-808	-1239	-1615	-1941	-112	-39	-[d]	11
	DNA film[c]	-920	-1278	-[d]	-1930				
1b	solution[b]	-927	-1411	-1835	-2122	97	9	-28	-8
	DNA film[c]	-830	-1409	-1863	-2130				
1c	solution[b]	-846	-1258	-1576	-1927	17	-55	-[d]	-83
	DNA film[c]	-829	-1313	-[d]	-2010				

[a]The Es denote the average of anodic and cathodic peak potentials (the midpoint potential, mV). The ΔE values (mV) stand the difference in E values, i.e., E on DNA-fullerene electrode subtracted from E on bare electrode in fullerene solution. All experiments were performed in the electrolyte containing 0.15 M TBAF in chlorobenzene. All data was collected at 100 mV/s. [b]The same condition as Fig. 1 (b). [c]The same conditions as in Fig. 1 (a). [d]Not detected.

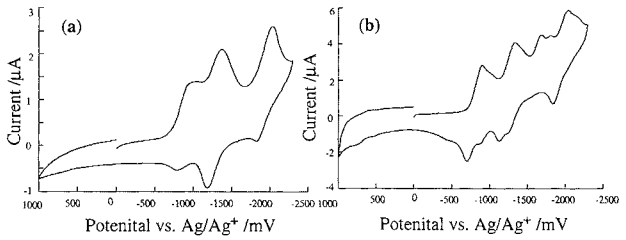

Figure 1. Cyclic voltammogram on the electrode coated with calf thymus DNA–**1a** complex (amount of DNA is 12 pmol on phosphate unit) (a) and that on glassy carbon electrode (b). The solution contained 0.4 mM of **1a** and 0.15 TBAF in chlorobenzene; the Pt wire counter electrode and Ag/Ag^+ reference electrode were used, the scan rate in all studies being 100 mV/s.

Bard and co-workers[12] studied the electrochemical behavior of metal complexes in the presence of DNA and discussed the shift of $E_{1/2}$ when bound to DNA through hydrophobic and electrostatic interactions. In our present study, the first redox step ($E^{0/1-}$) was used for inspection to the binding behavior to avoid complexity associated with other peaks which include additional electron transfer steps. If one follows the Bard's consideration,[10] the negative shift of $E_{1/2}$ ($\Delta E^{0/1-}$ = -112 mV) suggests an electrostatic contribution in DNA-ligand interactions. The electrostatic interaction is reasonable in the reaction of cationic **1a** with polyanionic DNA. However, the $E_{1/2}$ values of **1b** and **1c** were shifted to positive side after complexation with DNA. These results suggest that the origin of the DNA interaction with **1b** and **1c** is not only electrostatic in nature but also involves hydrophobic interaction. This is in agreement with the structural features of the ligands and the proposed binding modes discussed above.

In conclusion, the fullerene derivatives **1a–c** dissolve in water by themselves and bind to DNA. The affinities of **1a–c** to DNA are different as a result of the variation in the distance between cationic site and fullerene part: **1a** having the shortest distance binds to DNA mainly by electrostatic interaction, while the contribution from hydrophobic interaction is also important in the binding of **1b** and **1c**. The CV features of the DNA complexes could not be fully accounted for yet, but these findings deserves note for the further study of fullerene-DNA interactions.

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6 **1a**: ^{1}H-NMR (270 MHz, DMSO-d_6) δ 3.28 (3H, s), 4.60, 5.15 (each 1H, d, J=9.7 Hz), 4.69 (3H, s), 6.28 (1H, s), 8.08-8.20 (1H, m), 8. 74 (1H, dd, J=8.0, 7.7 Hz), 8.84 (1H, d, J=7.6 Hz), 9.10 ppm (1H, d, J=5.9 Hz). MS (FAB) 868 $(M\text{-}TfO)^+$, 720 $(C_{60})^+$. **1b**: ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.76 (3H, s), 4.43 (3H, s), 4.42, 5.15 (each 1H, d, J=10.1 Hz), 5.43 (1H, s), 8.25 (1H, dd, J=8.0, 6.3 Hz), 9.00-9.10 (2H, m), 9.53 ppm (1H, d, J=7.6 Hz). MS (FAB) 868 $(M\text{-}TfO)^+$, 720 $(C_{60})^+$. **1c**: ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.76 (3H, s), 4.35 (3H, s), 4.44, 5.16 (each 1H, d, J=10.1 Hz), 5.55 (1H, s), 8.59 (2H, d, J=6.3 Hz), 9.04 ppm (2H, d, J=6.3 Hz). MS (FAB) 868 $(M\text{-}TfO)^+$, 720 $(C_{60})^+$.
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