Photo-induced DNA Cleavage by Water-soluble Cationic Fullerene Derivatives

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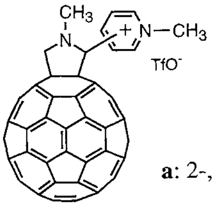
(Received December 17, 1998; CL-980930)

Fullerene derivatives **1a–c** cleaved double stranded DNA under photo-irradiation through the action of singlet oxygen generated by the reaction of the photo-excited fullerenes with oxygen. The photolysis in conventional aqueous solution was more effective than that in the presence of PVP or γ-cyclodextrin.

Biological interest in fullerene is highlighted by its potential in photo-cleaving DNA.[1-3] However, fullerene derivatives reported before[1,4-6] carried only a poor solubility in water, and therefore, dimethylsulfoxide[1] or water-soluble amphiphilic polymers such as poly(vinylpyrrolidone) (PVP)[4,5] and γ-cyclodextrin (γ-CD)[6] had to be used to solubilize the fullerenes in aqueous solution. To examine the exact role of fullerenes in the photo-cleavage of DNA, it is of essential importance to study the process in conventional aqueous solution.

Recently, we found that the cationic fullerene drivatives **1a–c**, carrying pyridinium moieties, possessed fairly good solubility in water and that they could bind to double stranded DNA with diverse affinities.[7] In this paper, we studied their DNA photo-cleavage behavior.



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

A detailed study of DNA-cleaving activity was performed with ligand **1b** under visible light irradiation for 2.0×10^{-4} M aqueous solution of pBR322 supercoiled plasmid.[8] The concentration of **1b** was 7.5×10^{-6} M. Some results are shown in Figure 1. Within 2 h of photo-irradiation, 80% of the supercoiled DNA (form I) was converted to a nicked DNA (form II) (Figure. 1, lane 10). This is the first demonstration of the DNA-cleaving activity of the fullerene derivative in conventional aqueous solution without any additivies. Under dark, none of these fullerene derivatives showed DNA-cleaving activity (data not shown). Under photolysis in the presence of D_2O (55% D_2O and 45% H_2O), the supercoiled DNA was cleaved completely, whereas only 50% was cleaved in ordinary water under the same conditions. Since D_2O increases $^1O_2$ stability,[9] this shows that $^1O_2$ generated from the reaction of photo-excited fullerene with oxygen can cleave a DNA strand by the same mechanism as reported in the aqueous media with additives[1,4] (the guanine base of DNA is suspected to be the reaction site).

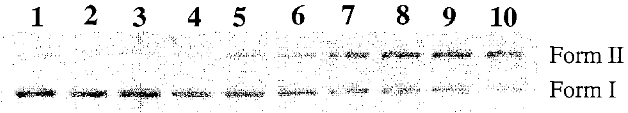

Figure 1. Irradiation-time effect on photocleavage of pBR322 supercoiled plasmid DNA in the presence of **1b**. Lane 1: untreated DNA; Lanes 2 to 10: incubated under visible light irradiation for 0, 1, 5, 10, 20, 40, 60, 90, and 120 min, respectively. The gel was stained with ethidium bromide and photographed to observe fluorescence (red filter, 320 nm excitation).

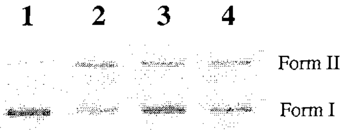

Figure 2. Effect of the presence of PVP and γ-CD. The solution containing 12 μM of fullerene derivative **1b** and 0.1 mM of plasmid was photo-irradiated for 30 min in the presence and absence of 83 mg of γ-CD or 2.6 mg of PVP. Lane 1: untreated DNA; Lane 2: **1b** alone; Lane 3: **1b**+ PVP; Lane 3: **1b**+γ-cyclodextrin.

We also studied the difference in photolysis efficiency among **1a–c**. The solutions containing 5.8 μM of fullerene derivatives and 0.1 mM of plasmid were photo-irradiated for 30 min. Effectiveness of photolysis was assessed measuring the ratio of the amount of form II to form I plasmid DNA.[10] The ratios for fullerene derivatives **1a–c** were 0.23, 0.45, and 0.82, respectively (Fig. 2). Electrochemical study showed some different DNA binding manner among **1a–c**: **1a** having the shortest distance between the fullerene sphere and the pyridinium catioic center seemed to bind to DNA with a substantial contribution from electrostatic interaction, while the contribution from hydrophobic interaction is also important in the binding of **1b** and **1c**.[7] This suggests that **1b** and **1c** are placed in the DNA groove where the fullerene parts are in closer contact with nucleic base than in the case of **1a**. Therefore, the compound which can achieve more effective contact with nucleic base of DNA should possess an increased photolysis ability.

To evaluate the role of the nature of reaction media on cleavage efficiency, we further studied our DNA/**1a-c** system for the effect of added PVP[4,5] and γ-cyclodextrin,[6] which are commonly used to improve the solubility of fullerene derivatives. Figure 3 shows the photolysis ability of **1b** in the presence of varying amount of PVP or γ-cyclodextrin at 30 min irradiation. This indicates that the presence of solubilizing agents hampers photo-cleavage of DNA, but PVP and γ-CD indicate a different nature and degree in their inhibition. In the case of γ-CD, the photo-cleavage was suppressed until the effect leveled off at γ-CD/ **1b** ratio of about 1 (Fig. 3, A). This suggested that a γ-CD molecule associated with one

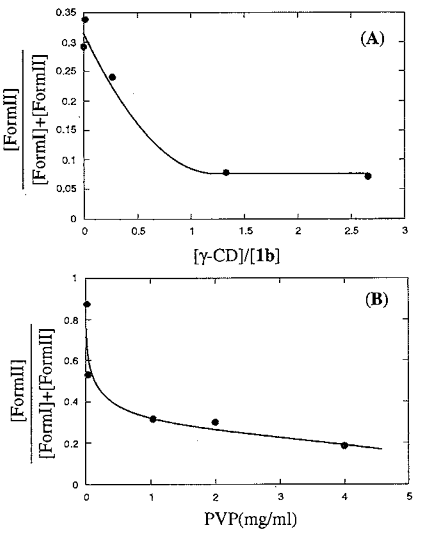

Figure 3. The effect of γ-CD (A) and PVP (B) in the DNA photoclevage by fullerene derivatives **1b**. The solution containing 12 μM of fullerene derivative **1b** and 0.1 mM of plasmid was photo-irradiated for 30 min in the presence and absence of γ-CD or PVP.

molecule of **1b**, and the resulting complex could also bind to DNA: there was no choice-between-the-two-alternative competition between γ-CD and DNA for the binding of **1b**. This is quite reasonable if one considers the structures of **1a**-**c**, where the half sphere remains intact to make it possible to interact with one molecule of γ-CD. The another hemisphere is derivatized with pyridinium ion rendering it a high hydrophilicity, and therefore, no interaction is expected with γ-CD. As is well known, fullerene itself, with its two intact hemispheres, associates with two molecules of γ-CD. On the other hand, PVP shows a rather uniform decrease in photolysis ability as the concentration of PVP increased (Fig. 3, B), which is in accord with a competitive binding of **1b** with PVP and DNA.

In conclusion, fullerene derivatives **1a**–**c** could photo-cleave a DNA double strand, and their relative efficiencies reflected their nature of binding to DNA. PVP and γ-cyclodextrin were found to bind to **1a**-**c** and to reduce the efficiency of DNA photolysis. **1a**-**c** seemed to form a 1:1 complex with γ-CD, where the underivatized hydrophobic hemisphere of the parent fullerene is encapsulated in γ-CD. This encapsulation probably prohibited the fullerene moiety of **1a**-**c** to interact directly with DNA through hydrophobic groove binding, thus leading to their reduced photo-cleaving activity in the presence of γ-CD.

This work was supported in part by the Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. Authors are also grateful to the financial support from the Japan Society for the Promotion of Science.

Reference and Notes

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