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Published on Web 03/20/2007

An Extremely Effective DNA Photocleavage Utilizing Functionalized Liposomes with a Fullerene-Enriched Lipid Bilayer

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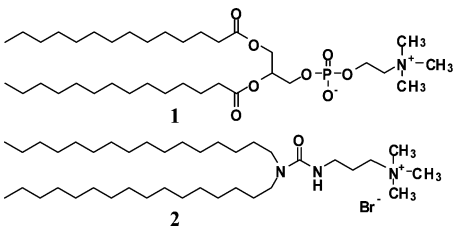
Visible-light cleavage of DNA is an important subject for next-generation medical treatments such as photodynamic therapy. Many suggestions have been put forth to build "artificial photonucleases".[1,2] Photosensitized DNA cleavage takes place via generation of "active oxygen" {singlet-oxygen ($^1O_2$) and superoxide anion radical (O^{2-})} or direct electron transfer between DNA and a photoexcited sensitizer. Among candidate materials, fullerenes are anticipated as efficient visible light triplet sensitizers. However, their poor water solubility (poor absorption of light) has limited their biochemical applications extremely. Several attempts have been made to improve the solubility: introduction of water-soluble substituents;[3,4] mixing with water-soluble polymers[5] or lipid membranes;[6] and solubilization in γ-cyclodextrin (γ-CDx)[7] or water-soluble calixarenes.[8,9] Of those options, we adopted a strategy of dispersing unmodified fullerenes into lipid membranes for three reasons: (i) for their photoproduction abilities of $^1O_2$ (energy transfer) and anion radicals (electron transfer), unmodified fullerenes are far superior to other sensitizers, including chemically modified fullerenes;[10] (ii) various surface-functionalized vesicles can be prepared by selecting lipids and they can exhibit target affinity as drug carriers;[11] and (iii) size control of vesicles is promising for enhanced permeability and retention (EPR) effects.[12,13] Very recently, we proposed that lipid-membrane-incorporated fullerenes (LMICx) can be prepared easily by transferring fullerenes from water-soluble host−guest complexes to lipid membranes. Actually, $LMIC_{60}$ was obtained through exchange with C_{60}•γ-CDx. It showed higher DNA cleavage ability than C_{60}•γ-CDx.[14] However, the optical density of $LMIC_{60}$ was still insufficient. Photoreactivity of $LMIC_{60}$ was not superlative despite the excellent photoreactivity of C_{60}.

We now report on an advanced preparation and developed reactivities of a surface-functionalized, fullerene-enriched liposome. A C_{70}-enriched liposome, $LMIC_{70}$, was obtained using the exchange from the unstable water-soluble complex of fullerene, C_{70}•γ-CDx. Biological activities of $LMIC_{70}$ toward DNA were assayed under visible-light irradiation. Results showed that $LMIC_{70}$ serves as a superlative system of DNA photocleavage.[15]



We used a cationic liposome because cationic $LMIC_{60}$ has higher DNA photocleaving ability than neutral and anionic $LMIC_{60}$.[14] Liposomes were prepared by sonication of an aqueous dispersion of dimyristoylphosphatidylcholine (**1**) and a cationic lipid **2** in a 9:1 molar ratio with a cup-type sonicator at 50 W for 1 h. The $LMIC_{60}$ was prepared by an exchange reaction between the liposomes and the C_{60}•γ-CDx complex[7b] by heating at 80 °C for 1 h, as described in a previous paper.[14] On the other hand, the $LMIC_{70}$ was prepared using an exchange reaction between the liposomes and the C_{70}•γ-CDx complex[7b] at as low as 30 °C for only 1 min (Scheme S1 and Figure S1).[16] The $LMIC_{60}$ cannot be prepared using an exchange reaction at 30 °C. Therefore, the exchange rate in the C_{70} system is much faster than that in the C_{60} system. We suggest two explanations for these different rates: (i) $LMIC_{70}$ is much more stable than $LMIC_{60}$; and (ii) the C_{70}•γ-CDx complex is less stable than the C_{60}•γ-CDx complex. Explanation (i) probably contributes little to this rate difference because the selectivity between C_{60} and C_{70} is very small compared to that of host molecules with a size-controlled cavity.[17] However, supportive evidence for explanation (ii) is that, in the absence of liposomes, C_{70} can be released from the γ-CDx cavity more easily and self-aggregate at 80 °C for 3 min.[18] Size distributions of the liposomes were studied using dynamic light scattering (DLS). Table S1 summarizes the average diameters of all liposomes before and after the exchange reactions of C_{60} and C_{70}. An initial concentration of C_{60} and C_{70} in the fullerenes•γ-CDx complex, as determined by measuring the absorbance of the solution at 332 and 381 nm (a specific extinction coefficient for the C_{60}•γ-CDx complex of $\epsilon_{332} = 4.27 \times 10^4$ dm^3 mol^{-1} cm^{-1} and the C_{70}•γ-CDx complex of $\epsilon_{381} = 3.80 \times 10^4$ dm^3 mol^{-1} cm^{-1}),[7] was 0.20 mM in an aqueous solution (1.0 mL). After an aqueous solution of lipids (10 equiv of C_{60}) was added to the solution (1.0 mL, 2.00 mM), final concentrations of the respective components were evaluated using integral intensities of the ^{1}H NMR spectrum,[19] where [γ-CDx] = 1.02 mM, [C_{60}] = 0.10 mM, and [lipids] = 1.00 mM and [γ-CDx] = 1.90 mM, [C_{70}] = 0.10 mM, and [lipids] = 1.00 mM (γ-CDx/C_{60}/lipids = 10.2:1:10 and γ-CDx/C_{70}/lipids = 19:1:10).

These $LMIC_{60}$ and $LMIC_{70}$ values were applied to photocleavage of the ColE1 supercoil plasmid. The DNA was cleaved neither under dark conditions in the presence of these reagents (Figure 1, lanes 3 and 5) nor under visible-light irradiation in the absence of C_{60} and C_{70} (Figure 1, lane 2). Under visible-light irradiation ($\lambda_{ex} >$ 350 nm) for 2 h, the $LMIC_{60}$ and $LMIC_{70}$ values showed a distinct DNA cleaving activity (lanes 4 and 6). In lane 4, about 26% of supercoiled DNA (Form I) was converted to nicked DNA (Form II) and linear DNA (Form III). Furthermore, in lane 6, the $LMIC_{70}$ showed markedly higher photocleaving performance (>92%) than the $LMIC_{60}$. Figure 2 shows the photocleavage of DNA by the $LMIC_{60}$ and $LMIC_{70}$ as a function of irradiation time (Figures S2−S4). This reaction curve also shows that a quantitative DNA conversion can be established by sufficient light illumination to $LMIC_{70}$. The difference of buildup curves between $LMIC_{60}$ and

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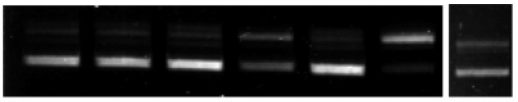
10.1021/ja070243s CCC: $37.00



Lane	1	2	3	4	5	6	7
C_{60}	—	—	○	○	—	—	—
C_{70}	—	—	—	—	○	○	○
Solubilizer	—	—	Lipid	Lipid	Lipid	Lipid	CDx
Light	—	○	—	○	—	○	○
Form II + Form III / %	0	1	4	26	4	92	30

Figure 1. Agarose gel electrophoretic patterns of DNA nicked by $LMIC_{60}$ and $LMIC_{70}$. Reaction samples contained 2.2 mg L^{-1} of ColE1 supercoiled plasmid. Lanes 1 and 2: the distilled water contained no chemicals. Lanes 3−6: 300 μM of lipids and 30 μM of C_{60} or C_{70}. Lane 7: 570 μM of γ-CD and 30 μM of C_{70}. Lanes 2, 4, 6, and 7: incubated under visible-light irradiation at a distance of 10 cm using a 500 W Xe arc lamp (UI-502Q; Ushio Inc.) at 25 °C for 2 h under aerobic conditions. Lanes 1, 3, and 5: incubated in the dark for 2 h under aerobic conditions. After addition of each 2.5 μL of 10% SDS solution and loading buffer (Wako Pure Chemical Industries Ltd.) in that order, electrophoresis was performed using a 0.9% agarose gel. The gel was stained with SYBR Gold (1:10000 dilution of stock supplied by Molecular Probes Inc., Eugene, OR) and viewed on a UV transilluminator. This experiment was performed at least five times; the reported values of photocleavage efficiency are averages of these separate runs.

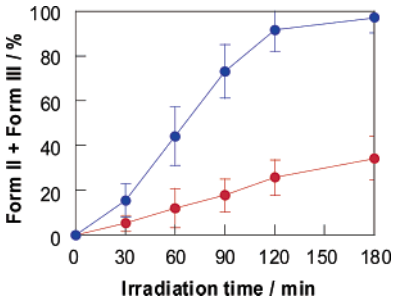


Figure 2. Photocleavage by $LMIC_{60}$ (red line) and $LMIC_{70}$ (blue line). Experimental conditions are identical to those described in Figure 1. Form % was determined by agarose gel electrophoretic patterns of the nicked DNA. Each experiment was repeated five times.

$LMIC_{70}$ is simply derived from amounts of light absorption in the 400−600 nm region (Figure S5). The reason is that reactivity of triplet C_{60} is much the same as that of C_{70}, which can be estimated from Rehm−Weller relations.[20a] In fact, it has been reported that C_{60} and C_{70} show similar quantum yields for photoinduced reduction using various electron donors.[20b] For comparison, we conducted a control experiment using the C_{70}•γ-CDx complex. The DNA cleaving activity of **1**-incorporated C_{70} (lane 6, 92%) was appreciably higher than that of the C_{70}•γ-CDx complex (lane 7, 30%).[21] From these findings, it was revealed that $LMIC_{70}$ is one of the most potent DNA cleavers compared with the conventional photoreactions.

In conclusion, this study revealed that $LMIC_{70}$ with high C_{70} concentrations is easily prepared at room temperature in a few minutes using the exchange from an unstable complex in water. The DNA photocleavage ability of $LMIC_{70}$ is 3.5-fold higher than that of $LMIC_{60}$ in the same photon flux (>350 nm). In this aqueous photoreaction system, two photochemical strategies exploited the full potential of fullerenes, namely, (i) an affinity of vesicles for DNA derived from their cationic surface, and (ii) sufficient optical densities in the visible region. This is a case in which the product fulfilled all the necessary conditions of aqueous fullerene photochemistry. These findings have important implications for various applications in biological, medicinal, and material chemistry because material-incorporated C_{70} can be prepared easily using the exchange method. Applications of these systems are being studied in our laboratories.

Acknowledgment. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supporting Information Available: Supporting Table S1, Scheme S1, and Figures S1−S6. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Photoinduced DNA cleaving activities of C_{60}•γ-CDx and C_{70}•γ-CDx complexes in the presence of NADH as an electron donor have been described, but a comparison of their activities has not been reported; See: Yamakoshi, Y.; Sueyoshi, S.; Fukuhara, K.; Miyata, N. *J. Am. Chem. Soc*. **1998**, *120*, 12363−12364.
(16) The resulting $LMIC_{70}$ solution exhibited no precipitation for at least 3 days at room temperature.
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(18) In the absence of lipids, the C_{70}•γ-CDx complex quickly aggregates by itself at 80 °C. Finally, a precipitate was observed.
(19) Figure S6 shows that the peaks assignable to the C_{70}•γ-CDx complex (3.72, 4.01, and 5.06 ppm) disappeared at 30 °C for 10 min after addition of the **1** + **2** vesicle. This result indicates that all C_{70} were transferred from the γ-CDx cavity to lipid membranes to yield vesicle-incorporated C_{70}. These results also show that concentrations of C_{70} in all vesicles are equal to the initial concentration of the C_{70}•γ-CDx complex (0.10 mM).
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(21) A brown precipitation of the C_{70}•γ-CDx complex was formed after photoirradiation for 2 h.

JA070243S