

DNA-photocleaving Activities of Water-soluble Carbohydrate-containing Nonionic Homooxacalix[3]arene•[60]fullerene Complex

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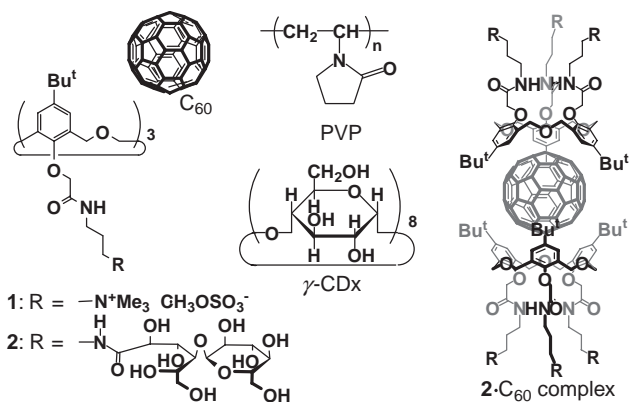
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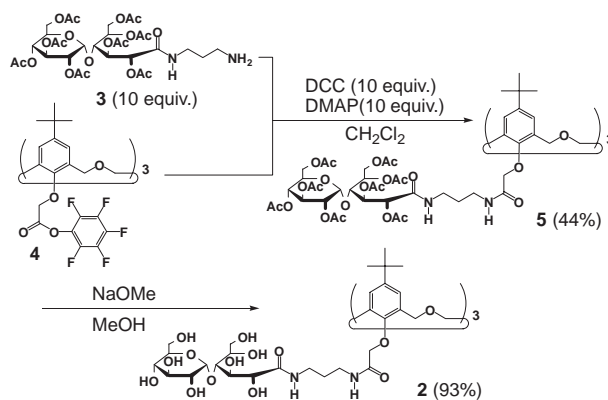
A new water-soluble carbohydrate-containing “nonionic” homooxacalix[3]arene (**2**) was synthesized. **2** could solubilize [60]fullerene (C₆₀) into water and the resultant complex acted as an efficient DNA photocleaving reagent.

The ready availability of fullerenes has increasingly invited exploration of their outstanding new physical and chemical properties. Among them, their easy excitation by visible light irradiation provides great potentials in the application of water-soluble fullerenes for various medicinal uses. For example, they act as a singlet oxygen (¹O₂) photosensitizer^{1,2} to cleave DNA³⁻⁸ and as photoinactivator of viruses.^{9,10} In spite of their high potential, however, the applications have been very limited because of their poor water-solubility. We considered that among several methods for the water-solubilization of C₆₀,¹¹⁻¹⁶ the host-guest method should be superior to others because (i) one can directly use “unmodified C₆₀” which can generate ¹O₂ more efficiently than other chemically modified C₆₀ derivatives,¹⁷ (ii) one can suppress self-quenching of C₆₀ excited states owing to the isolation of C₆₀.¹⁸ In practice, we have found that a cationic homooxacalix[3]arene (**1**) can include C₆₀ in their cavities in water and the resultant **1**•C₆₀ complex is readily bound to DNA and can therein act as an efficient DNA photocleaving reagent.¹⁹



It is known that carbohydrate-containing molecules have a retentivity for a specific organ.²⁰ Therefore, a carbohydrate coated C₆₀ is anticipated to be attracted to a targeted organ. With this objective in mind, we designed a new water-soluble nonionic homooxacalix[3]arene (**2**) bearing tris(maltose) groups.

Water-soluble calixarene **2** was synthesized according to Scheme 1 and identified by ESI-TOF MS spectroscopic evidence and elemental analysis.²¹



Scheme 1.

The critical aggregate concentration (CAC) of **2** was estimated by surface tension (Wilhelmy method). The surface tension of **2** in pure water abruptly decreased at ca. 0.30 mM (1 M = 1 mol dm⁻³). One can consider, therefore, that **2** shows discrete dispersion and forms the aggregate only above this concentration. The CAC value of **2** is smaller than that of **1** (ca. 3 mM). The lower CAC value for neutral **2** is ascribed to the absence of electrostatic repulsion. In subsequent experiments, we set the concentration of **2** to 0.10 mM where **2** exists discretely in aqueous solution. A poly(vinylpyrrolidone) (PVP)•C₆₀ system and the γ -cyclodextrin (γ -CD)•C₆₀ complex were prepared according to the methods described in previous reports.^{8,14}

A solid of C₆₀ (3.6 mg) was extracted into water containing **2** (0.10 mM, 5 mL) by sonication (1 h) followed by stirring (8 days). After centrifugation (10000 rpm, 15 min, 20 °C), a brown-colored solution was obtained. A UV-vis absorption spectrum of the **2**•C₆₀ complex solution gives absorption maxima at 263, 340, and 429 nm assignable to solubilized C₆₀, which are similar to those of the **1**•C₆₀ complex. These results consistently support the view that C₆₀ is solubilized into water by inclusion in **2**. The concentration of C₆₀ in the **2**•C₆₀ complex, determined by measuring the absorbance of the solution at 340 nm (a molar extinction coefficient for the water-soluble **1**•C₆₀ complex of $\epsilon_{420} = 4.87 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$), was 0.045 mM in water. This indicates that 90% of **2** formed a 2:1 complex between **2** and C₆₀. To examine whether the **2**•C₆₀ complex was self-aggregated or not, we measured the particle size of the **2**•C₆₀ complex in solution by a dynamic light-scattering method (DLS: Otsuka Electronic DLS-6000HL). Over the CAC ([**2**] = 0.50 mM and [C₆₀] = 0.23 mM), an average particle size was estimated to be around 50.4 nm, indicating that the self-assembled complex

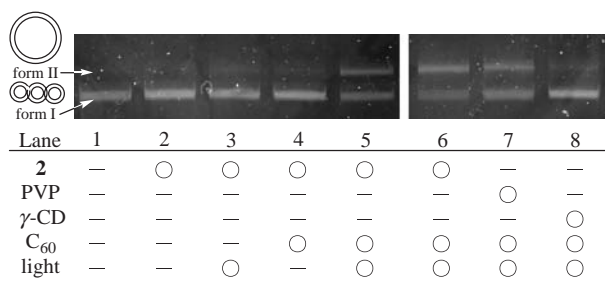


Figure 1. Agarose gel electrophoretic patterns of DNA nicked by the $2 \cdot C_{60}$ complex. The reaction samples contained 1.3 mg/L of ColE1 supercoiled plasmid. Lane 1: no chemicals in distilled water. Lanes 2 and 3: 10 μ M of **2**. Lanes 4, 5, and 6: 10 μ M of **2** and 4.5 μ M of C_{60} . Lane 7: 0.9 wt % PVP and 4.5 μ M of C_{60} . Lane 8: 17.8 μ M of γ -CD and 4.5 μ M of C_{60} . Lanes 3 and 5–8: incubated under visible light irradiation at a distance of 10 cm by a 500-W Xe-arc lamp (UI-502Q; Ushio, Inc.) at 25 $^{\circ}$ C for 2 h. Lanes 1, 2, and 4: incubated in the dark for 2 h. After the addition of 5 μ L of 10% SDS solution and loading buffer (Wako) in this order, electrophoresis was performed by using 0.9% agarose gel. The gel was stained with 0.5 μ g L⁻¹ ethidium bromide and visualized on a UV transilluminator.

is formed in aqueous solution. On the other hand, the formation of such aggregate was not observed for the $2 \cdot C_{60}$ complex under the CAC ($[2] = 0.10$ mM and $[C_{60}] = 0.045$ mM) because of no detection of any scattered light.

The $2 \cdot C_{60}$ complex was applied to the photocleavage of ColE1 supercoil plasmid. Under dark conditions, DNA was not cleaved in the presence of **2** and the $2 \cdot C_{60}$ complex (Figure 1: lanes 2 and 4). Under visible light irradiation, the $2 \cdot C_{60}$ complex clearly showed a DNA-cleaving activity (lane 5). In lane 5, about 30% of supercoiled DNA (form I) was converted to nicked DNA (form II). On the other hand, **2** showed no DNA-cleaving activity by itself (lane 3). For comparison, we carried out a control experiment using the PVP· C_{60} system and the γ -CD· C_{60} complex.⁸ These concentrations of C_{60} were adjusted to be the equivalent values of the $2 \cdot C_{60}$ complex. The DNA-cleaving activities of the PVP· C_{60} system (lane 7; the yield of form II was 17%) and the γ -CD· C_{60} complex (lane 8; the yield of form II was 3%) were obviously lower than that of the $2 \cdot C_{60}$ complex (lane 5) under visible light irradiation. Consequently, one can conclude that C_{60} included in **2** is transported onto DNA with the aid of multipoint hydrogen bonding between the carbohydrate moieties of **2** and the phosphoric acids of DNA.^{20,22}

Foote et al.²³ have proposed that two mechanisms have to be taken into consideration: type I is the photoinduced electron transfer from the guanine unit to C_{60} and type II is the reaction with 1O_2 photochemically generated by C_{60} . These two mechanisms can be distinguished by comparison of the results of the aerobic conditions with those of the anaerobic ones. We found that there is no significant difference between these two conditions: the yields of form II were 30% under the aerobic conditions and 28% under the anaerobic (Ar) conditions. The results suggest that the mechanism mainly operative in the present DNA photocleaving system is type I, which is basically in line with Foote's results.²³

In conclusion, the present paper demonstrates that "nonionic" **2** can solubilize C_{60} into water and transport it to DNA. The

nonionic $2 \cdot C_{60}$ complex showed stronger photocleaving activity than the nonionic PVP· C_{60} system and γ -CD· C_{60} complex. These findings imply, therefore, that the concepts cultivated in host-guest chemistry of C_{60} and its homologues are more fruitfully applied to medicinal chemistry.

References and Notes

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- Compound **2**, Anal. Calcd for $C_{87}H_{138}N_6O_{42} \cdot 6H_2O$: C, 52.41; H, 5.98; N, 3.13%. Found: C, 52.48; H, 5.99; N, 3.33%. ESI: m/z 1961 $[M + Na]^+$, 992 $[M + 2Na]^{2+}$.
- In the presence of excess amount of maltose, maltoheptaose, or γ -CD, the $2 \cdot C_{60}$ complex scarcely showed no DNA-cleaving activity.
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