Electronic Supplementary Information

Fullerene-Porphyrin Hybrid Nanoparticles that Generate Activated Oxygen by Photoirradiation

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MATERIALS AND METHODS

Materials
γ-Cyclodextrin (γ-CD), trimethyl-β-cyclodextrin (TMe-β-CD), and tetraphenylporphyrin (1) were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Poly(ethylene glycol) monomethyl ether (PEG, Mₙ = 2,000) and 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (5) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Zinc tetraphenylporphyrin (1), 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (4), and 5,10,15,20-tetrakis(4-aminophenyl)porphyrin (6) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Fullerene C₆₀ (>99.5%) was acquired from MER Co. (Tucson, AZ). 5,10,15,20-Tetrakis(4-methoxyphenyl)porphyrin (3) was synthesized, as previously reported.¹

Preparation of the C₆₀-γ-CD complex¹
C₆₀ (5.0 mg, 6.9×10⁻⁶ mol) and γ-CD (36.0 mg, 2.8×10⁻⁵ mol) were placed in an agate capsule containing two agate mixing balls. The mixture was mixed vigorously at 30 Hz for 20 min using a high-speed vibration mill (MM 200; Retsch Co., Ltd., Haan, Germany). The solid mixture was suspended in deionized water or deuterium oxide (1.5 mL) to produce a black emulsion. After centrifugation (14,000 rpm, 20 min), the non-dispersed C₆₀ was removed from the solution. The concentration of C₆₀ in the C₆₀-γ-CD complex was determined by measuring the absorbance of the solution at 332 nm (the molar extinction coefficient for the water-soluble C₆₀-γ-CD complex is ε₃₃₂ = 4.27×10⁴ dm³ mol⁻¹ cm⁻¹).

Preparation of the porphyrin (por)-TMe-β-CD complex²
Following the same procedure as that used to prepare the C₆₀-γ-CD complex, the por-TMe-β-CD complexes were obtained by complexation of 1, 2, 3, 4, 5, or 6 (5.1 mg) with TMe-β-CD (23.3 mg, 1.6×10⁻⁶ mol) in water (1.5 mL). The concentration of each porphyrin in the corresponding por-TMe-β-CD complex was determined by measuring the absorbance of the solution at each absorption maximum (the molar extinction coefficients for 1-, 2-, 3-, 4-, 5-, and 6-TMe-β-CD complexes are: ε₄₁₅ = 3.30×10⁴ dm³ mol⁻¹ cm⁻¹, ε₄₁₅ = 1.81×10⁵ dm³ mol⁻¹ cm⁻¹, ε₄₁₈ = 3.49×10⁵ dm³ mol⁻¹ cm⁻¹, ε₄₁₉ = 3.25×10⁵ dm³ mol⁻¹ cm⁻¹, ε₄₁₈ = 3.77×10⁵ dm³ mol⁻¹ cm⁻¹, and ε₄₂₅ = 2.70×10⁵ dm³ mol⁻¹ cm⁻¹, respectively).

Preparation of water-dispersible C₆₀-nanoparticles (NPs) by the guest exchange method
An aqueous solution of the C₆₀-γ-CD complex (0.2 mL, [C₆₀] = 1.0 mM) and an aqueous solution of PEG (0.2 mL, 50 g/L), and water (1.6 mL) were mixed and heated at 80 °C for 1 h in a glass vial.
Preparation of water-dispersible C₆₀-por NP by the guest exchange method

Typically, an aqueous solution of the C₆₀-γ-CD complex (0.2 mL, [C₆₀] = 1.0 mM), an aqueous solution of the 1-TMe-β-CD complex (0.2 mL, [1] = 1.0 mM), an aqueous solution of PEG (0.2 mL, 50 g/L), and water (1.4 mL) were mixed and stirred at 80 °C for 1 h in a 5-mL glass vial.

Characterization of nC₆₀ and C₆₀-por NP

Light absorption by aqueous dispersions of nC₆₀ or nC₆₀-por was measured using a ultraviolet-visible (UV/Vis) spectrophotometer (UV-3600, Shimadzu, Kyoto, Japan). Particle morphology and size were characterized using transmission electron microscopy (TEM; JEM-1400, JEOL Ltd., Tokyo, Japan). 100 NPs were counted to calculate the average diameter of the NPs from the TEM micrographs. High-resolution TEM (HRTEM) images were obtained using a JEM-2010 microscope (JEOL Ltd., Tokyo, Japan). Lattice fringe spacing was determined from HRTEM micrographs analysis using ImageJ software by averaging at least 100 measurements. ζ-potential and additional size measurements were performed by dynamic light scattering using a Zetasizer Nano ZS analyzer (Malvern Instruments Ltd., Worcestershire, UK) equipped with a He-Ne laser operating at 633 nm and 4 mW.

¹H NMR spectroscopy

¹H NMR and ¹³C NMR data were recorded using a 400-MHz Varian 400-MR spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) relative to the peak assigned to water (δ = 4.8 ppm) and dimethyl sulfoxide-d₆ (δ = 39.5 ppm).

References

(1) A. Ikeda, S. Satake, T. Mae, M. Ueda, K. Sugikawa, H. Shigeto, H. Funabashi, A. Kuroda, ACS Med. Chem. Lett., 2017, 8, 555.
(2) A. Ikeda, T. Sato, K. Kitamura, K. Nishiguchi, T. Sasaki, J. Kikuchi, T. Ogawa, K. Yogo and T. Takeya, Org. Biomol. Chem., 2005, 3, 2907.
**Figures**

*Fig. S1* $^1$H NMR spectra of fullerene-$\gamma$-cyclodextrin ($C_{60}$-$\gamma$-CD) complex aqueous solutions (i) before and (ii) after incubation for 1 h at 80 °C, with PEG. ([C$_{60}$] = 0.1 mM, [PEG] = 5 g/L). Open circles: free $\gamma$-CD and filled circles: C$_{60}$-$\gamma$-CD complex.

*Fig. S2* $^1$H NMR spectra of aqueous solutions of the 1-trimethyl-$\beta$-cyclodextrin (TMe-$\beta$-CD) complex after incubation for 1 h, at (i) room temperature with poly(ethylene glycol) monomethyl ether (PEG), (ii) 80 °C with PEG, (iii) room temperature without PEG, and (iv) 80 °C without PEG ([I] = 0.1 mM, [PEG] = 0 or 5 g/L). Open diamonds: free TMe-$\beta$-CD and filled diamonds: 1-TMe-$\beta$-CD complex.
Fig. S3 $^1$H NMR spectra of mixed solutions comprising C$_{60}$-γ-CD and (a) 2-TMe-β-CD, (b) 3-TMe-β-CD, (c) 4-TMe-β-CD, (d) 5-TMe-β-CD, and (e) 6-TMe-β-CD complexes ([C$_{60}$] = [2, 3, 4, 5, or 6] = 0.1 mM) with PEG (5 g/L) (i) before and (ii) after heating at 80 °C for 1 h. Open circles: free γ-CD, filled circles: C$_{60}$-γ-CD, open diamonds: free TMe-β-CD, and filled diamonds: porphyrin-TMe-β-CD complex. The spectra between 7.6–8.5 ppm are amplified five-fold.
Fig. S4 (a) Ultraviolet-visible (UV/Vis) absorption spectra of 1-TMe-β-CD before (dashed line) and after (solid line) incubation for 1 h at 80 °C, in the absence of C₆₀-γ-CD. [1] = 0.1 mM, [PEG] = 5.0 g/L.

Fig. S5 UV/Vis absorption spectra of the mixed solution comprising C₆₀-γ-CD and (a) 2- or (b) 3-TMe-β-CD complexes ([C₆₀] = [2 or 3] = 0.1 mM), respectively, with PEG (5 g/L) before (dashed line) and after (solid line) heating at 80 °C for 1 h.
Fig. S6 Low-magnification transmission electron microscopy images of (a) nC$_{60}$ and nC$_{60}$-1 prepared with (b) 0.05 mM and (c) 0.1 mM of the 1-TMe-β-CD complex. Scale bars represent 200 nm.