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Water-soluble Inclusion Complexes of [60]Fullerene Derivatives Using γ-Cyclodextrin

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Pyrrolidine and *N*,*N*-dimethylpyrrolidinium derivatives of C_{60} could be solubilized in water with the aid of γ-cyclodextrin. The complexes were found to be soluble in water at high concentrations over 1.5 mM. These solubility values are significantly higher than those of other C_{60} derivatives reported previously. Furthermore, these solutions are stable for at least one month at room temperature.

Cyclodextrins (CDxs) can form host–guest inclusion complexes with a lipophilic guest molecule, such as drug molecules, within the lipophilic central cavity.[1] Consequently, CDxs have been used in pharmaceutical formulations generally to improve drug properties such as solubility in water, stability, and absorption.[2] Fullerenes are also solubilized in water using γ-CDx,[3] and these complexes can deliver fullerenes into cells directly[4] or indirectly using lipid-membrane-incorporated fullerenes[5] prepared by the exchange reaction from the complexes. In particular, water-solubilization of fullerenes (C_{60} and C_{70}) has recently received attention for potential photosensitizers because C_{60} and C_{70} are efficient visible-light triplet-sensitizers and have high photoproduction abilities of $^1O_2$ (energy transfer) and anion radicals (electron transfer).[6] However, difficulties in achieving water-soluble and functional fullerenes have often been observed, because water-soluble monoadduct fullerenes tend to form micelles in water. A solution to the problem and the generation of a compound with low cytotoxicity compared with unmodified C_{60}[7] involved the preparation of γ-CDx·C_{60} derivative complexes. Such complexes represent important compounds for pharmaceutical applications. However, previous examples of the γ-CDx·C_{60} derivative complexes are very limited.[3b,8] Moreover, the solubilities of the C_{60} derivatives in water are well below 0.1 mM.[3b] This paper presents pyrrolidine and *N*,*N*-dimethylpyrrolidinium derivatives of C_{60} that are solubilized in water by γ-CDx with similar concentrations to unmodified C_{60}.

Compounds **1**,[9] **2**,[10] **3**,[11] and **4**[12] (Chart 1) were synthesized according to previously reported methods. All γ-CDx·C_{60} derivative complexes were prepared using a mechanochemical high-speed vibration milling apparatus (HSVM) according to a Komatsu method.[3b] Vigorously mixed γ-CDx (27.8 mmol) and C_{60} derivatives (6.94 mmol) were dissolved in 0.9% w/v NaCl solution (1.5 mL) to produce a brown emulsion and centrifuged to remove the nondispersed C_{60} derivatives.

To confirm the formation of the complexes and determine the concentrations of the C_{60} derivatives, UV–vis absorption and ^{1}H NMR spectra were acquired (Figures 1 and S1[15]). The γ-CDx·**1** and ·**2** mixtures (red and blue lines in Figure 1) exhibited broadened absorptions in the 200–600 nm range. This broadening resulted from the presence of **1** and **2**. In water, **1** and **2** existed in

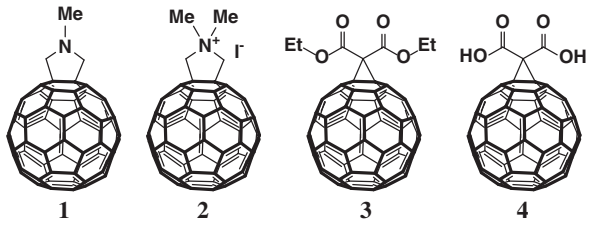

Chart 1.

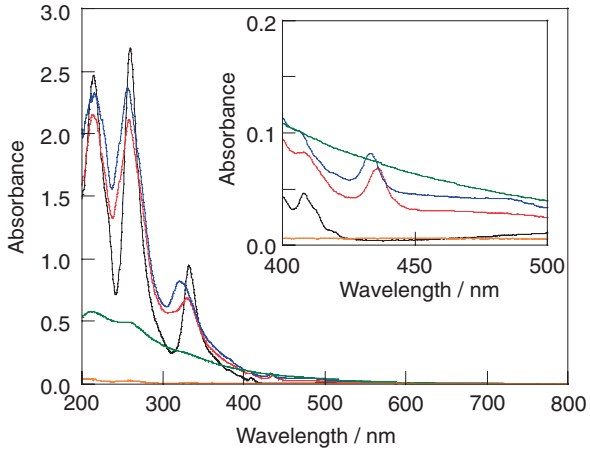

Figure 1. UV–vis absorption spectra of the γ-CDx·C_{60} complex (black line), γ-CDx·**1** (red line), γ-CDx·**2** (blue line), γ-CDx·**3** (orange line), and γ-CDx·**4** (green line) mixtures in water. All spectra were recorded at 25 °C with a 1 mm cell. All solutions were diluted to 1:10. The inset shows the 400–500 nm region.

an isolated (i.e., disaggregated) state, because (i) these broadened peaks compared with those of the γ-CDx·C_{60} complex (black line in Figure 1) are virtually identical to the spectra of **1** in cyclohexane and (ii) the clearly observed sharp absorptions at 436 and 433 nm for **1** and **2**, respectively, are characteristic of most C_{60} derivatives (see inset of Figure 1). Figure 1 clearly shows that the γ-CDx·**4** mixture (green line) gave rise to a noticeably broader spectrum than the spectra recorded for the γ-CDx·**1** and ·**2** mixtures. This indicates that **4** forms self-aggregates in water, as detailed below. In contrast, Figure 1 shows that γ-CDx barely solubilized **3**.[13] This observation is primarily due to the steric bulkiness of the diethyl malonate moiety in **3**. As shown in Figure S1A,[15] it is known that several new peaks assigned to the γ-CDx·C_{60} complex appeared separately from those assigned to free γ-CDx.[14] In the same manner, new peaks assigned to the γ-CDx·**1** and ·**2** mixtures

appeared separately (Figures S1B and S1C),[15] thereby indicating that **1** and **2** are solubilized in water by inclusion in γ-CDx. However, the complexities of these peaks compared to the γ-CDx·C_{60} complex are due to the unsymmetrical nature of the C_{60} derivatives. The stoichiometries of the γ-CDx and C_{60} derivatives **1** and **2** are 2.2:1 and 1.3:1, respectively, as determined from the peak intensities between γ-CDx and **1** or **2** in the γ-CDx·**1** and ·**2** complexes. These results are predicted when taking into consideration the γ-CDx·**1** complex is 2:1 and the γ-CDx·**2** complex is a 2:1 and 1:1 mixture. On the other hand, Figure S1D shows that no assignable peak appeared for the γ-CDx·**4** complex,[15] indicating that **4** self-aggregates to form as globular water-soluble micelles.[13] The self-aggregation of **4** was supported by DLS measurements (average particle size: 183.8 nm).

The concentrations of the C_{60} derivatives **1** and **2** were evaluated using the peak intensity ratios of the C_{60} derivatives vs. the sodium 3-(trimethylsilyl)propanesulfonate of their ^{1}H NMR signals (Figure S1).[15] The molar absorption coefficients at 330 nm for the γ-CDx·**1** complex and 321 nm for the γ-CDx·**2** complex were determined by the absorption spectra of the same solutions (10-fold dilution measured) in which the concentrations of **1** and **2** were determined using ^{1}H NMR spectra (**1**: $\varepsilon_{330} = 4.11 \times 10^4\,dm^3\,mol^{-1}\,cm^{-1}$, **2**: $\varepsilon_{321} = 4.31 \times 10^4\,dm^3\,mol^{-1}\,cm^{-1}$). The solubility order of the C_{60} derivatives was determined to be: C_{60} > **2** > **1** (solubilities of C_{60} and its derivatives 2.21, 1.90, and 1.67 mM).

The formation of 2:1 complexes was also supported by electrospray ionization mass spectrometry (ESI-MS). When an aqueous solution of the γ-CDx·**1** or ·**2** mixtures was subjected to ESI-MS, weak peaks appeared at 3372.9 and 3386.8, which were assigned to [2:1 γ-CDx·**1** complex + H]$^+$ and [2:1 γ-CDx·**2** complex − I]$^+$, respectively (Figure 2).

To characterize the γ-CDx·C_{60} derivative complexes, size distributions studies were carried out using dynamic light scattering. Although the polydispersity indices were not small, the average diameter of the γ-CDx·C_{60}, γ-CDx·**1**, and γ-CDx·**2** complexes was primarily 1.7 nm (Table S1 and Figure S2),[15] indicating that these complexes did not form large self-aggregates. Herein, the solutions of the γ-CDx·C_{60}, γ-CDx·**1**, and γ-CDx·**2** complexes were pH 6.1, 6.6, and 5.2, respectively.

The aqueous solutions of the γ-CDx·**1** and ·**2** (0.2 mM) complexes could be stably stored for at least one month at room temperature.

In summary, we succeeded in the preparation of γ-CDx·C_{60} derivatives **1** and **2** complexes in water at concentrations of 1.67 and 1.90 mM, respectively. In contrast, γ-CDx hardly solubilized **3** because of the steric bulkiness of the diethyl malonate moiety of **3**. We are currently extending our studies to other C_{60} derivatives using HSVM.

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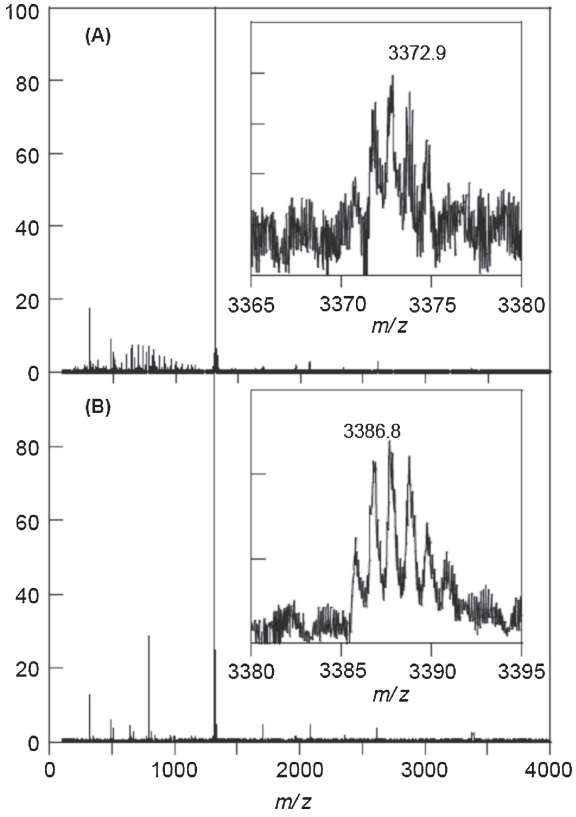

Figure 2. ESI-MS spectra of (A) the γ-CDx·**1** and (B) ·**2** mixtures ([**1**] = [**2**] = 1.0 mM in H_2O).

References and Notes

1 K. A. Connors, *Chem. Rev.* **1997**, *97*, 1325.
2 T. Loftsson, D. Duchêne, *Int. J. Pharm.* **2007**, *329*, 1.
3 a) T. Andersson, K. Nilsson, M. Sundahl, G. Westman, O. Wennerström, *J. Chem. Soc., Chem. Commun.* **1992**, 604. b) K. Komatsu, K. Fujiwara, Y. Murata, T. Braun, *J. Chem. Soc., Perkin Trans. 1* **1999**, 2963.
4 A. Ikeda, M. Matsumoto, M. Akiyama, J. Kikuchi, T. Ogawa, T. Takeya, *Chem. Commun.* **2009**, 1547.
5 a) A. Ikeda, T. Sato, K. Kitamura, K. Nishiguchi, Y. Sasaki, J. Kikuchi, T. Ogawa, K. Yogo, T. Takeya, *Org. Biomol. Chem.* **2005**, *3*, 2907. b) A. Ikeda, Y. Doi, K. Nishiguchi, K. Kitamura, M. Hashizume, J. Kikuchi, K. Yogo, T. Ogawa, T. Takeya, *Org. Biomol. Chem.* **2007**, *5*, 1158. c) A. Ikeda, Y. Doi, M. Hashizume, J. Kikuchi, T. Konishi, *J. Am. Chem. Soc.* **2007**, *129*, 4140. d) Y. Doi, A. Ikeda, M. Akiyama, M. Nagano, T. Shigematsu, T. Ogawa, T. Takeya, T. Nagasaki, *Chem.—Eur. J.* **2008**, *14*, 8892. e) A. Ikeda, T. Sue, M. Akiyama, K. Fujioka, T. Shigematsu, Y. Doi, J. Kikuchi, T. Konishi, R. Nakajima, *Org. Lett.* **2008**, *10*, 4077. f) A. Ikeda, M. Nagano, M. Akiyama, M. Matsumoto, S. Ito, M. Mukai, M. Hashizume, J. Kikuchi, K. Katagiri, T. Ogawa, T. Takeya, *Chem. Asian J.* **2009**, *4*, 199. g) A. Ikeda, Y. Kawai, J. Kikuchi, M. Akiyama, *Chem. Commun.* **2010**, *46*, 2847. h) A. Ikeda, M. Akiyama, T. Ogawa, T. Takeya, *ACS Med. Chem. Lett.* **2010**, *1*, 115.
6 T. Hamano, K. Okuda, T. Mashino, M. Hirobe, K. Arakane, A. Ryu, S. Mashiko, T. Nagano, *Chem. Commun.* **1997**, 21.
7 C. M. Sayes, J. D. Fortner, W. Guo, D. Lyon, A. M. Boyd, K. D. Ausman, Y. J. Tao, B. Sitharaman, L. J. Wilson, J. B. Hughes, J. L. West, V. L. Colvin, *Nano Lett.* **2004**, *4*, 1881.
8 S. Samal, K. E. Geckeler, *Chem. Commun.* **2000**, 1101.
9 D. M. Guldi, H. Hungerbühler, K.-D. Asmus, *J. Phys. Chem. A* **1997**, *101*, 1783.
10 A. M. Cassell, W. A. Scrivens, J. M. Tour, *Angew. Chem., Int. Ed.* **1998**, *37*, 1528.
11 C. Bingel, *Chem. Ber.* **1993**, *126*, 1957.
12 I. Lamparth, A. Hirsch, *J. Chem. Soc., Chem. Commun.* **1994**, 1727.
13 At present, it is not clear whether **3** and **4** were released from CDx cavity before or after the extraction by 0.9% NaCl solution.
14 T. Andersson, G. Westman, O. Wennerström, M. Sundahl, *J. Chem. Soc., Perkin Trans. 2* **1994**, 1097.
15 Supporting Information is also available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

