Preparation of Hydrophilic Nanoparticles of C_{60} with High Resistance to Aggregation during Storage, using 2-Hydroxypropyl-β-cyclodextrin

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Hydrophilic nanoparticles of C_{60} were prepared by cogrinding with 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) or γ-CyD in a molar ratio 1:2 using an automatic magnetic agitating mortar for 3 h at 4 °C and reduced pressure. The nanoparticles (about 55 nm) in the γ-CyD colloidal solution were significantly aggregated during storage at 25 °C, increasing in size to about 800 nm. On the other hand, the size of the C_{60}/HP-β-CyD nanoparticles (about 90 nm) remained small for 28 days. C_{60}/HP-β-CyD nanoparticles had a high propensity for generating superoxide anions. HP-β-CyD is useful for preparation of hydrophilic nanoparticles of C_{60} and preferred as an efficient stabilizing agent for pharmaceutical application of C_{60}.

Fullerenes are currently of great interest for practical applications that take advantage of their unique electronic properties and biological activities. The fullerene family, especially C_{60}, has appealing photo-, electro-chemical, and physical properties, which can be exploited in various medical fields. For example, it can be used as radical scavenger, antioxidant, and photosensitizer.[1] In addition, fullerenes have been used as a carrier for gene and drug delivery systems. However, the extremely low solubility of fullerenes in water has impeded further pharmaceutical applications. Furthermore, it is well known that fullerenes are easily aggregated, giving large particles or insoluble aggregates, depending on environmental conditions such as concentrations and solvent polarity. Therefore, it is important to control the aggregation behavior of fullerenes, because the aggregation significantly affects their photophysical properties and biological activities.[2] Several methods have been described for the preparation of water-soluble C_{60}, such as chemical modification of C_{60}, formation of water-soluble host–guest complexes, or solubilization by surfactants. Host–guest complexes are most often used as a solubilization method for C_{60} in water. Notably, cyclodextrins (CyDs) have been widely investigated because of their high solubilizing ability through the inclusion. Among various CyDs, the large cavity size of γ-CyD has been widely investigated for solubilization of C_{60}.[3] However, the solubilization methods are often complicated and require the use of nonpolar solvents such as hexane and toluene, moreover, there are no medicinal products on the market using γ-CyD as solubilizing agent. Therefore suitable preparation procedures yielding C_{60} with superior pharmaceutical properties are needed.

This paper deals with a simple procedure of stable aqueous colloidal solutions of C_{60} by using HP-β-CyD with superior biocompatibility, in comparison with γ-CyD. Further, the aggregation behavior and superoxide anion generation efficiency of C_{60} nanoparticles in HP-β-CyD and γ-CyD solutions were compared.

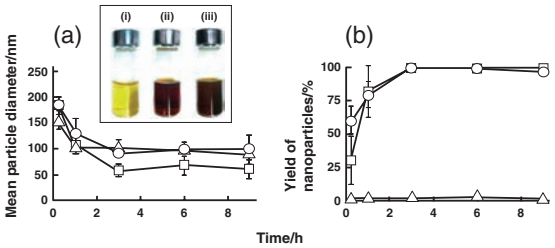

Figure 1. Effects of CyDs and grinding time on particle size (a) and yield (b) of C_{60} nanoparticles prepared by using a magnetic agate mortar at 4 °C and reduced pressure. Inserts are the appearance of C_{60}/β-CyD (i), γ-CyD (ii), or HP-β-CyD (iii) colloidal solutions syringe-filtered with a pore size of 0.8 μm. Each point represents the mean ±S.E. of 3–6 experiments. ○: C_{60}/HP-β-CyD nanoparticles, △: C_{60}/β-CyD nanoparticles, □: C_{60}/γ-CyD nanoparticles.

Powders of C_{60} alone or mixtures of C_{60} and CyDs (molar ratio 1:2) were ground for 3 h at 4 °C and reduced pressure, then the pulverized samples were dispersed in water by ultrasonication for 5 min, and the resulting suspensions were syringe-filtered through a filter of 0.8 μm pore size. Transparent solutions without any colors were obtained, when C_{60} alone was ground, probably because C_{60} existed in a large aggregated state and was completely filtered out by the 0.8 μm filter. On the other hand, dark brown colored solutions were obtained when C_{60} was dispersed in water after the cogrinding with γ-CyD or HP-β-CyD (inserts Figures 1(ii) and 1(iii)). Light yellow colored solutions were obtained in the case of parent β-CyD (insert Figure 1(i)), because large amounts of C_{60} were trapped on the filter with 0.8 μm pore size. The mean particle diameter of the C_{60}/CyDs colloid particles obtained from the cogrinding was determined by dynamic light scattering machine. The mean particle diameter of C_{60}/CyDs nanoparticles decreased with increasing the grinding time, as shown in Figure 1a, and reached the constant sizes of 100, 90, and 55 nm for the β-CyD, HP-β-CyD, and γ-CyD systems, respectively, after 3 h cogrinding. As shown in Figure 1b, the yield of C_{60} nanoparticles in the HP-β-CyD and γ-CyD systems reached 100% after 3 h grinding, suggesting C_{60} was quantitatively changed to the small sized particles and passed completely through the 0.8 μm filter. On the other hand, the parent β-CyD system gave yield of 3% after 3 h cogrinding, suggesting almost all C_{60} existed as large particles that were hard to pass through the filter. These results suggest that the cogrinding method using HP-β-CyD or γ-CyD is useful to obtain hydrophilic nanoparticles of C_{60} with high yields.

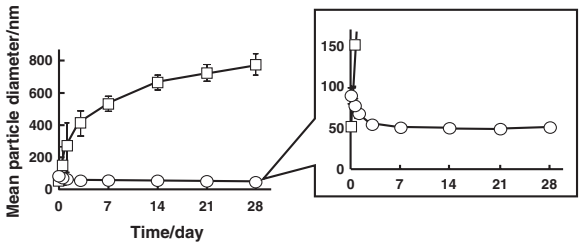

Figure 2. Changes in particle size of C_{60}/HP-β-CyD or C_{60}/γ-CyD nanoparticles as a function of storage time at 25 °C. Each point represents the mean ±S.E. of 3–6 experiments. ○: C_{60}/HP-β-CyD nanoparticles, □: C_{60}/γ-CyD nanoparticles.

Figure 2 shows changes in size of the nanoparticles in C_{60}/CyD colloidal solutions during storage at 25 °C in the dark. The initial C_{60}/γ-CyD nanoparticles (55 nm) increased markedly with time and reached about 800 nm after 28 days, accompanying precipitation of dark colored powders of C_{60} and its γ-CyD complex particles. In sharp contrast, the aggregation of C_{60}/HP-β-CyD nanoparticles was significantly inhibited, even decreasing in size to about 50 nm and maintaining the small size for 28 days. The ζ-potentials of C_{60}/γ-CyD and HP-β-CyD colloidal solutions obtained from the cogrinding were −20.8 and −19.7 mV, respectively. The negative ζ-potentials of the C_{60}/CyD colloidal solutions hardly changed during the storage, in spite of the different aggregation behavior (data not shown). It was difficult to explain the different aggregation behavior of the C_{60}/CyDs colloidal solutions, merely from a viewpoint of the change in ζ-potential. One possible explanation is that HP-β-CyD interacts with the surface of the small C_{60} nanoparticles, inhibiting the aggregation of the clusters.[4] The hydroxypropyl groups of HP-β-CyD may contribute to the hydrophobic interaction of the host molecule with surface of the nanosized C_{60} clusters, probably due to the moderate surface activity of HP-β-CyD (surface tensions of 2 mM γ-CyD and HP-β-CyD were 71 and 62 mN m^{-1}, respectively). However, detailed studies should be done to elucidate not only the host/guest interaction but also the solubilizing and inhibiting mechanisms of HP-β-CyD at the molecular level.

To evaluate the photosensitizing ability of C_{60}/HP-β-CyD and C_{60}/γ-CyD nanoparticles, the superoxide anion generation efficiency with visible light irradiation was measured by employing a cytochrome *c* method.[5] Aqueous solution (5 mL) of 80 μM cytochrome *c* was mixed with 5 mL of colloidal solution containing C_{60}/CyDs (C_{60} = 80 μM). The resulting colloidal solution was placed into a conical flask and exposed to visible light supplied from a fluorescence lamp (3500 lux, 400–700 nm, 2 cm from the flask bottom). Increase in the absorbance at 548 nm of the reduced cytochrome *c* was measured with a spectrophotometer. In the presence of C_{60}/HP-β-CyD or C_{60}/γ-CyD nanoparticles, the absorbance of the reduced form increased with the irradiation time and the effects of C_{60}/HP-β-CyD nanoparticles was significantly higher than that of the C_{60}/γ-CyD nanoparticles as shown in Figure 3. Such increase of the absorbance was not observed for the CyD solutions. The increase of the absorbance was completely suppressed by the addition of superoxide dismutase (SOD). These results indicate that C_{60}/HP-β-CyD nanoparticles effectively generate superoxide anions, reacting with cytochrome *c* to form the reduced form, and the C_{60}/HP-β-CyD nanoparticles have more potential as a photosensitizer, compared to the C_{60}/γ-CyD nanoparticles.

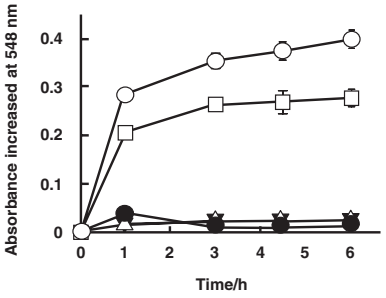

Figure 3. Absorbance increment of reduced cytochrome *c* with visible light irradiation in the absence and presence of C_{60}/HP-β-CyD or C_{60}/γ-CyD nanoparticles. Each point represents the mean ±S.E. of 3–4 experiments. ○: C_{60}/HP-β-CyD nanoparticles, □: C_{60}/γ-CyD nanoparticles, △: HP-β-CyD, ▼: γ-CyD, ●: C_{60}/HP-β-CyD nanoparticles with SOD (600 unit/mL).

A number of papers[3] have reported that the large cavity of γ-CyD is favorable for inclusion of C_{60} and forms a 1:2 (C_{60}:γ-CyD) complex, whereas β-CyD is thought to be difficult to form a complex at the molecular level because of the smaller cavity size. However, our results indicated that HP-β-CyD had a high solubilizing ability for C_{60}, keeping the high efficiency of the superoxide anion generation. Especially, the inhibiting effect of HP-β-CyD on the aggregation of C_{60} during storage was markedly larger than that of γ-CyD. HP-β-CyD is clinically used as a safe pharmaceutical solubilizing agent for several preparations.[6] Therefore, the present cogrinding method using HP-β-CyD may be much more useful for preparation of hydrophilic C_{60} nanoparticles with superior pharmaceutical properties.

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