Enhancement of Water Solubility of Fullerene by Cogrinding with Mixture of Cycloamyloses, Novel Cyclic α,1,4-Glucans, via Solid–Solid Mechanochemical Reaction

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Received March 5, 2004; accepted May 19, 2004; published online May 21, 2004

Improvement of solubility for fullerene (C60) was studied by cogrinding with cycloamyloses using a ball mill in the solid state. Cycloamylose is a novel cyclic α,1,4-glucan produced from synthetic amylose by enzymatic reaction. Although sample solutions showed a pale yellow for the initial period of cogrinding with cycloamyloses and C60, the color varied to brown after 48 h. Subsequently, the solubility of C60 was improved markedly to 560 μg/ml at 96 h. From powder X-ray diffraction analysis, the peak intensity of crystalline C60 decreased as the cogrinding time was extended. The UV–VIS absorption spectrum of C60 shows absorption bands at 262 and 340 nm in water with cycloamyloses, and 258 and 328 nm in n-hexane. These results suggested that C60 molecules were dispersed into cycloamyloses micellar system and the red-shift of the UV–VIS spectra was due to an inter-molecular interaction between C60 and cycloamyloses.

Key words cycloamylose; fullerene; solubilization; cogrinding; mechanochemical

Cycloamylose is a α,1,4 cyclic glucan produced from amylose by D-enzyme which catalyzes an intramolecular transglycosylation reaction as well as cyclodextrin glucanotransferase and amylo maltase. So far, a number of investigations have been conducted on the improvement of physicochemical property of various drugs by complexation with cyclodextrins, a typical cyclic glucan consisting of 6, 7 and 8 glucopyranose units. Although cycloamylose should have similar basic structure to that of cyclodextrin, the degree of polymerization of cycloamyloses is higher and ranges from 17 to several hundred. In addition, it should be noted that cycloamylose shows much higher solubility into water and less aging, that is, coprecipitation due to aggregation, than cyclodextrin, in spite of their high molecular weight. However, little is known about the physicochemical interaction between cycloamyloses and chemical compounds except for the results of our primitive study.

Fullerenes have attracted much attention for their unique cage-like shape and biological activities such as HIV-1 protease inhibition, photodynamic tumor necrosis and action as an artificial vector for gene transfection. On the other hand, solubilization of fullerenes into water has also been investigated extensively, since their applicability was strictly limited due to poor solubility to polar solvent. In addition to developing chemically modified hydrophilic fullerene derivatives, fullerene (C60) has been solubilized into water combined with β-cyclodextrin, γ-cyclodextrin, polyvinylpyrrolidone and fluoroalkyl oligomer. Complexation between C60 and each solubilization agent was usually performed in the aqueous solution or organic solvent i.e. via solid–liquid reaction. Braun et al. have reported, however, that C60 is concentrated considerably higher when cogrinding with crystalline γ-cyclodextrin than with reflux boiling in γ-cyclodextrin solutions.

In this paper, we describe the improvement of the solubility of C60 by cogrinding with cycloamyloses in the solid state. The molecular state of C60 in the complex obtained was evaluated using powder X-ray diffraction (XRD), UV–VIS spectroscopy and dynamic light scattering measurement.

Experimental

Materials Cycloamyloses were provided by Ezaki Glico Co., Ltd. (Osaka, Japan). Fullerenes (C60) was obtained from Frontier Carbon Co. (Tokyo, Japan). All other chemicals and solvents were reagent grade purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and used without further purification.

High-Performance Anion-Exchange Chromatography (HPAEC) HPAEC was carried out with a Dionex DX-500 system with a pulsed amperometric detector (ED-50, Dionex Co., Sunnyvale, CA). An anion exchange column (CarboPac PA-1, 4.0 mm i.d.×250 mm, Dionex) was used with sodium nitrate and sodium hydroxide solution as the mobile phase using a gradient system according to Koizumi et al. with minor modification.

Particle Size Analysis A powder sample was dispersed or dissolved in water and sonicated for 1 min and passed through a 0.1 μm membrane filter (Millipore) before measurement. Particle size was determined by the dynamic light scattering method using a Nicomp 380ZLS (Particle Sizing Systems, Santa Barbara, CA, U.S.A.) at 25°C with a DPSS laser (325 nm).

Preparation of Grinding Mixture A ground mixture (GM) of C60 and cycloamyloses was prepared using a Desktop Ball Mill (V-1M, Irie Shokai Co., Ltd., Japan). In a typical experiment, 2.5 mg of C60 and 12.5 mg of cycloamyloses was put into a 5.0 ml shade glass vial with glass balls (diameter: 25 mm) and cogrind at 150 rpm at room temperature.

Determination of Solubilized Fullerene C60 After cogrinding, 2.5 ml of distilled water was put into the glass vial and incubated with gentle shaking for 1 h at room temperature. The suspension was then centrifuged at 25°C and 3000 rpm for 1 h and the clear supernatant solution was passed through a membrane (0.45 μm, HLC-DISK 3, Kanto Chemical Co., Inc., Japan). The solution of cycloamyloses with solubilized C60 was subjected to UV–VIS spectroscopy (V-550, Jasco Co., Japan) and HPLC analysis (LC-2000, JASCO Co., Japan). The HPLC conditions were as follows: mobile phase, toluene–acetonitrile mixture (45:55); column; Inertsil ODS-2 (250 mm, GL Science Co., Inc., Japan); flow rate, 1.0 ml/min; column temperature, ambient; detector, UV at 325 nm.

Powder X-Ray Diffractometry (XRD) Powder XRD was carried out with a Rigaku Geigerflex Rad-II (Tokyo, Japan). Measurements were performed at 35 kV voltage, 25 mA current and a scanning speed of 6°/min with a Cu Kα radiation source.

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Results and Discussion

Properties of Cycloamylloses

Cycloamylose is produced from synthetic amylose by enzymatic reaction (Fig. 1). The minimum degree of polymerization (DP) of cycloamylose produced by amylomaltase was 22, whereas the minimum DP of cycloamylose produced by potato D-enzyme was 17.21

Figure 2 shows the HPAEC elution profile of cycloamylloses produced by amylomaltase. Cyclic glucan with DP22 are pointed out with arrows and the number above each peak refers to its DP. It was confirmed that the DP of cycloamylloses used in this study ranged from 22 to around 60 (the corresponding molecular weights were 3567 to 9729). Each cycloamylose might be eluted sequentially in agreement with its DP. This suggested that each cycloamylose existed as a homologous structure in aqueous solution.

There have been some papers on the physicochemical property of each cycloamylose, sometimes called large-ring cyclodextrin, with DP ranging from 9 to 31. Koizumi et al. recently demonstrated that the chemical shifts of all glucose carbons were practically unvarying for DP9 and DP31 in \(^{13}\)C-NMR measurements.19 In addition, we have reported the solubilities, specific rotations and surface tensions of large-ring cyclodextrins with DP ranging from 10 to 17. Although complex formation of cycloamylose with various compounds was expected to improve the physicochemical property of those guest compounds, no surface activity was observed in each large-ring cyclodextrin, and it was still unknown whether larger ring cyclodextrin had surface activity.20 In this paper, we evaluated the physicochemical property of cycloamylloses and its solubilizing ability as a mixture.

The particle size distribution of cycloamylloses solution was measured by the dynamic light scattering method. The mean particle size was 3.5 nm as shown in Fig. 3, when measuring the 2.0% cycloamylloses solution. According to Saenger’s research, the diameter of cycloamylose with DP26 was estimated as approximately 20 Å by calculation from crystallographic data.22,23 Hence, it was suggested that this measurement was performed reasonably and cycloamylloses were presented as a molecular colloid in aqueous solution. The mean particle size and distribution of cycloamylloses did not change for one week, so the colloidal solution of cycloamylloses would be stable at least for a week at ambient temperature. This indicated that a reduction in mean particle size due to chemical degradation did not occur, and neither did an increase in particle size by aggregation of cycloamylloses.

Solubilization of C\textsubscript{60} by Cogrinding

The solubilization of C\textsubscript{60} was studied by cogrinding with cycloamylloses using a ball mill via solid–solid reaction. Figure 4 shows the correlation between cogrinding time and the concentration of solubilized C\textsubscript{60}. Although sample solutions appeared pale yellow for the initial period of cogrinding with cycloamylloses and C\textsubscript{60}, the color varied to brown after 48 h. Subsequently, the solubility of C\textsubscript{60} was improved markedly to 560 (\(\mu\text{g/ml}\)) at 96 h, while the concentration of cycloamylloses was 5.0 (mg/ml). In the preliminary study using an agate mortar and pestle, C\textsubscript{60} was solubilized to 30 (\(\mu\text{g/ml}\)) maximally in optimum conditions. Thus, ball milling may be a suitable grinding method for the solubilization of C\textsubscript{60} by cogrinding with cycloamylloses. After that, the C\textsubscript{60} solubilized with cycloamylloses was stable for one month at room temperature.

The weight ratio of ground mixture strongly affected the solubilization of C\textsubscript{60} (Fig. 5). The solubilized amount of C\textsubscript{60} was significantly reduced as the content of cycloamylloses decreased in the weight ratio, varying from 5 : 1 to 1 : 1 (cycloamylloses : C\textsubscript{60}). Mechanochemical reaction might be unreached to the end-point in the 2 : 1 and 1 : 1 system at this period. In the 5 : 1 cycloamylloses–C\textsubscript{60} system, 5.0 mg cycloamylloses were allowed to solubilize 0.56 mg C\textsubscript{60} in 1 ml water approximately, thereby a 10-fold amount of cycloamy-
loses should interact with C$_{60}$ in practice. In addition, given that the mean molecular weight of cycloamyloses is 6500, the stoichiometry was calculated as 1 : 1 (molecular weight of C$_{60}$ is 720). Table 1 summarizes the solubility of C$_{60}$ as improved by various methods. The solubility of C$_{60}$ with cycloamyloses is greater than those prepared by other methods.

**Evaluation of Molecular State of C$_{60}$ in Solid and Liquid States**

In order to study the molecular state of C$_{60}$ in the solid state, powder XRD was employed for a ground mixture of C$_{60}$ and cycloamyloses. Although cycloamyloses had no crystalline structure, diffraction peaks of crystalline C$_{60}$ were observed at 2$\theta$, equal to 10.8, 17.7 and 20.8°. New diffraction peaks implying the formation of crystalline complex did not appear. The peak intensity assigned to crystalline C$_{60}$ decreased as the cogrinding time was extended (Fig. 6). This suggested that the crystalline structure of C$_{60}$ was disrupted, and then C$_{60}$ molecules were substantially dispersed into amorphous cycloamyloses in the solid state. Accordingly, mechanochemical effect which induced interaction between cycloamylose and C$_{60}$ molecule was observed during the cogrinding process.

To investigate the molecular state of C$_{60}$ in the liquid state, particle size distribution was analyzed using the dynamic light scattering method. The mean particle size was about 50 nm when measuring the aqueous solution of a ground mixture consisting of C$_{60}$ and cycloamyloses (Fig. 7). Accordingly, C$_{60}$ molecules could be dispersed into water with cycloamyloses as the colloidal solution.

Figure 8 shows the UV–VIS absorption spectrum of C$_{60}$ in the cycloamyloses solution, which was almost same as that in the polyvinylpyrrolidone (PVP) solution.$^{10}$ The absorption bands were observed at 264 and 340 nm in water and 258 and 328 nm in $n$-hexane. Thus, cycloamyloses provided a hydrophobic circumstance which accommodated the C$_{60}$ molecule, possibly like a synthetic polymer such as PVP. In addition, these results suggested that C$_{60}$ molecules existed in a cycloamyloses micellar system and the red-shift of the

**Table 1. Comparison of the Solubilized Amount of Fullerene (C$_{60}$) Prepared by Various Methods with Solubilization Agents**

<table>
<thead>
<tr>
<th>Solubilization agent</th>
<th>Solubility of C$_{60}$ (µg/ml)</th>
<th>Concentration of solubilization agents (mg/ml)</th>
<th>Preparation method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-Cyclodextrin</td>
<td>110</td>
<td>8.43</td>
<td>Cogrinding</td>
<td>18</td>
</tr>
<tr>
<td>δ-Cyclodextrin</td>
<td>110</td>
<td>1.4</td>
<td>Cogrinding</td>
<td>24</td>
</tr>
<tr>
<td>Fluoroalkyl oligomer</td>
<td>100</td>
<td>1000</td>
<td>Solvent</td>
<td>17</td>
</tr>
<tr>
<td>PVP</td>
<td>400</td>
<td>50</td>
<td>Evaporation</td>
<td>16</td>
</tr>
<tr>
<td>Cycloamyloses</td>
<td>560</td>
<td>5.0</td>
<td>Cogrinding</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 4. Relationship between the Amount of Solubilized Fullerene C$_{60}$ into Water and the Cogrinding Time**

Cogrinding of cycloamyloses and fullerene (C$_{60}$) was performed at the weight ratio 5 : 1.

**Fig. 5. Effect of the Sample Ratio in the Glass Pot of the Ball Mill on the Amount of Solubilized Fullerene into Water**

Each point indicates the mean ± S.D. of 3 experiments.

**Fig. 6. Change in the Powder X-Ray Diffraction Pattern of Ground Mixture of Cycloamyloses and Fullerene (C$_{60}$) at the Weight Ratio 5 : 1 on Grinding**

Grinding time: (a) 0; (b) 1; (c) 24; (d) 48 h.

**Fig. 7. Particle Size Distribution of Colloidal Solution Consisting of Cycloamyloses and Fullerene (C$_{60}$)**
Conclusion

We found in this study that the cogrinding method with cycloamyloses had some advantages compared to the solubilizing methods previously reported, for following reasons: (i) showed superior solubilization for C\textsubscript{60}, (ii) was done without organic solvent, and (iii) was demonstrated using a natural product. Further study is expected to elucidate the mechanism in the solubilization process and the micellar system, and apply it to various compounds including medicinal substances. In any case, a new member has been added to the family of solubilization agents for poorly water-soluble compounds.

Acknowledgements

This work was supported in part by a Grant from the Ministry of Education, Culture, Sports, Science and Technology to promote multi-disciplinary research projects, Japan.

References and Notes


Fig. 8. UV–VIS Spectra of Fullerene (C\textsubscript{60}) in Water with Cycloamyloses (a) and PVP (b), and in n-Hexane (c)