Pseudorotaxane-Like Supramolecular Complex of Coenzyme Q10 with γ-Cyclodextrin Formed by Solubility Method

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The purpose of this study is to reveal whether Coenzyme Q10 (CoQ10) forms pseudorotaxane-like supramolecular complex with γ-cyclodextrin (γ-CyD). The poorly soluble complex of CoQ10 with γ-CyD in water was prepared by the solubility method. The X-ray diffraction pattern of the CoQ10/γ-CyD complex was different from that of the physical mixture, but almost the same as that of polypropylene glycol (PPG)/γ-CyD polypseudorotaxane. Also, the differential scanning calorimetrical study and FT-IR study demonstrated the interaction between CoQ10 and γ-CyD in the solid state. The ^{1}H-NMR study and the yield study of the supramolecular complex of CoQ10 with γ-CyD demonstrated that the stoichiometry was 5 : 1 (γ-CyD : CoQ10). The dispersion rate of CoQ10 was markedly increased by the formation of the supramolecular complex with γ-CyD, possibly due to submicron-ordered particle formulation. In fact, CoQ10 was found to form submicron-sized supramolecular particles with γ-CyD, when prepared by the solubility method. Consequently, the present study showed that CoQ10 forms the pseudorotaxane-like supramolecular complex with γ-CyD in water.

Key words Coenzyme Q10; γ-cyclodextrin; supramolecule; complex; pseudorotaxane

Coenzymes Q10 (ubiquinones) are a group of benzoquinone derivatives with one to twelve mono-unsaturated trans-isoprenoid units in the side chain, among which the 10 unit homologue (Coenzyme Q10, CoQ10, Fig. 1) in the most common in animals. CoQ10 is a fat-soluble, vitamin-like, benzoquinone compound that functions primarily as an antioxidant, a membrane stabilizer and a cofactor in the oxidative phosphorylation production of adenosine triphosphate (ATP).[1] It has also been shown to help preserve myocardial sodium-potassium ATPase activity and stabilize myocardial calcium-dependent ion channels.[2,3] Due to the lipophilic property of CoQ10, however, it is not well absorbed when taken orally by humans.[4] Therefore, formulations that could improve aqueous solubility of CoQ10 and enhance its oral bioavailability are necessary.

Cyclodextrins (CyDs) are known to form inclusion complexes with lipophilic drugs and improve their low water solubility, dissolution rate and bioavailability.[5,6] CyDs can generally form inclusion complexes with a number of lipophilic substances as guests and thus have been utilized for improving their water solubility, stability and bioavailability.[7,8] Lutka *et al.* reported that γ-CyD and methyl-β-CyD increased CoQ10 solubility in aqueous solution and stabilized CoQ10 in solid state through inclusion complexation.[9—12] Likewise, we previously reported that heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) significantly enhanced the low solubility and oral bioavailability of CoQ10 in rats.[13] In addition, we recently reported that among α-, β- and γ-CyDs, γ-CyD significantly increases the solubility of CoQ10 at lower CyD concentrations, and the dissolution rate of CoQ10 is significantly enhanced by the complexation with γ-CyD when prepared by the kneading method, probably due to soluble complex formation and/or submicron-sized particle formation, which was reflected in the enhanced oral absorption in dogs.[14] Furthermore, Terao *et al.* reported the enhancement of oral bioavailability of CoQ10 by complexation with γ-CyD in healthy adults.[15]

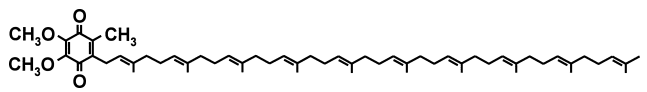

Fig. 1. Chemical Structure of CoQ10

Harada *et al.* first reported the supramolecular assemblies of polyethylene glycol (PEG) and α-CyD, in which a number of cyclic molecules are spontaneously threaded onto the polymer chain, when they were prepared by the solubility method.[16] γ-CyD was also found to form the supramolecular complex with polyethylene oxide (PEO)-based surfactants.[17] In addition, the complex formations between α-CyD as well as γ-CyD and polypropylene glycol (PPG)[18] and the other polymers have been demonstrated.[19] Based on these backgrounds, we hypothesized that γ-CyD forms the pseudorotaxane-like supramolecular complex with CoQ10 including the isoprenoid unit, which is similar structure to PEG and PPG. Then, the purpose of this study is to reveal whether CoQ10 forms pseudorotaxane-like supramolecular complex with γ-CyD. In the present study, we examined the appearance and confirmation of a formation of supramolecular complex prepared by the solubility method using X-ray diffractometer, differential scanning calorimeter (DSC), FT-IR and ^{1}H-NMR. Furthermore, to reveal the pharmaceutical properties of the supramolecular complex of CoQ10 with γ-CyD, we determined the dispersion rate and size distribution of the complex.

Experimental

Materials α-CyD, β-CyD, γ-CyD and 2-hydroxypropyl-γ-CyD (HP-γ-CyD; the average degree of substitution of the hydroxypropyl group=5.4) were obtained from Nihon Shokuhin Kako (Tokyo, Japan). CoQ10 were donated by Nisshin Pharma (Tokyo, Japan). PPG (molecular weight=1000) was obtained from Wako Pure Chemicals Ind. (Osaka, Japan). All other

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chemicals and solvents were of analytical reagent grade and double distilled water was used throughout the study.

Preparation of Solid Complexes of CoQ10 with CyDs The solid complexes were prepared by the solubility method.[16,20] The CoQ10 (44.2 mg, 51.2 μmol) was added to 2.4 ml, 22.0 ml, 2.0 ml and 1.6 ml of aqueous α-CyD (145 mg/ml), β-CyD (18.5 mg/ml), γ-CyD (232 mg/ml) and HP-γ-CyD (360 mg/ml) solution (molar ratio of CoQ10 : CyDs = 1 : 7), respectively, and then the suspensions were sonicated by a US-4 sonicator (AS ONE, Osaka, Japan) for 30 min followed by the incubation at 25 °C for 5 d under nitrogen gas atmosphere and in the dark. After centrifugation (4492×$\boldsymbol{g}$, 10 min), the supernatants were removed and then the resulting precipitates were washed by 2 ml of diethyl ether twice in order to remove the free CoQ10, followed by drying under the reduced pressure overnight. Physical mixture was prepared by simply mixing powder of each component of CoQ10 and CyD using an agate mortar and a pestle in molar ratios of 1 : 5 (CoQ10 : CyD).

Yields of Solid Supramolecules CoQ10 (44.2 mg, 51.2 μmol) was added to 2 ml of aqueous solution containing γ-CyD at the concentrations of 58, 116, 174 and 232 mg/ml, and then the solid supramolecules were prepared as described above. After drying, the yields of the supramolecules were determined by weighing the resulting precipitates, and the values were plotted against the concentration of γ-CyD. The amounts of CoQ10 in the precipitates were determined from the integration values of the proton signals of γ-CyD and CoQ10 by ^{1}H-NMR described below. The experiment regarding the effect of molar ratio of γ-CyD to CoQ10 on the yield was performed using the method reported by Yui and coworkers[21] The CoQ10 (44.2 mg, 51.2 μmol) was added to 0.2 ml, 0.6 ml, 1.0 ml, 1.4 ml, 1.6 ml, 1.8 ml, 2.0 ml or 2.5 ml of aqueous γ-CyD solution (232 mg/ml), and then the same procedure was performed as that described in the section of the preparation of solid complexes of CoQ10 with CyDs. After drying, the yields of the supramolecules were determined by weighing the resulting precipitates, and the values were plotted against the molar ratio of γ-CyD to CoQ10.

Powder X-Ray Diffraction Studies Powder X-ray diffraction patterns were measured by a Rigaku RINT 2500 VL X-ray diffractometer (Tokyo, Japan) with a Ni filtered Cu$K\alpha$ radiation, a voltage of 40 kV, a current of 40 mA, a scanning speed of 1°/min, a time constant of 2 s, and a scan range of 2θ=5—35°, a divergent slit of 1.74 mm (1°), a scattering slit of 0.94 mm (1°) and a receiving slit of 0.15 mm.

DSC Studies CoQ10, γ-CyD, the physical mixture or the complex of CoQ10/γ-CyD was used as samples. The DSC analysis was carried out using a SII EXSTAR DSC6200 (Seiko Instruments, Tokyo, Japan), with a sample weight of 2 mg (equivalent to CoQ10) and a heating rate of 10 °C/min under nitrogen atmosphere.

FT-IR Studies The sample preparation was performed using the KBr method. FT-IR spectra of CoQ10, γ-CyD, and the physical mixture and the complex of CoQ10 and γ-CyD were recorded on a JIR-6500W FT-IR spectrometer (JEOL, Tokyo, Japan) in the range between 4000 and 400 cm^{-1}, with a resolution of 4 cm^{-1} and 32 scans.

^{1}H-NMR Spectroscopic Studies ^{1}H-NMR spectra were taken at 25 °C on a JEOL JNM-ECP500 (Tokyo, Japan) operating at 500 MHz, using a 5-mm sample tube. Deuterated DMSO (DMSO-d_6) was used as a solvent. Supramolecular complex of CoQ10 with γ-CyD was dissolved in DMSO-d_6 at a concentration of 1 mg/ml as CoQ10. The DMSO signal was used as an internal reference for ^{1}H-NMR. Chemical shifts were expressed in parts per million (ppm) relative to that of the DMSO signal (2.49 ppm from sodium 2,2-dimethyl-2-silapentane-5-sulfonate), with an accuracy of ±0.001 ppm.

Dispersion Studies The *in vitro* dispersion rate was measured by the dispersed-amount method.[22] The powder sample (equivalent to 30 mg CoQ10, <100 mesh) was added to 100 ml of degassed water at 37 °C, and stirred at 100 rpm/min. At appropriate intervals, an aliquot (1.0 ml) of the dispersion medium was withdrawn with a syringe and the suspensions were filtered using membrane filters (MWCO 0.8 μm, DISMIC-25 CS, Toyo Roshi, Tokyo, Japan). Five hundred microliters of ethanol solution containing 100 μg/ml of tocopherol acetate as an internal standard were added to 0.5 ml of the filtrate. CoQ10 concentration was measured by high performance liquid chromatography (HPLC) as follows: a JASCO PU-1580 pump and a JASCO UV-970 UV detector (Tokyo, Japan) at 275 nm; a Hitachi D-2500 ChromatoIntegrator (Tokyo, Japan); a Tosoh TSK gel ODS-80TS column (5 μm, 4.6×75 mm, Tokyo, Japan); a mobile phase of ethanol/methanol (3 : 2 v/v); and a flow rate of 1.0 ml/min.

Size Distribution Studies The aqueous suspensions containing CoQ10, the physical mixture or supramolecular complexes of CoQ10 and γ-CyD (equivalent to 30 mg CoQ10, <100 mesh) were vigorously agitated for 30 s, and then were sonicated for 30 min. The resulting suspensions were filtrated through filter membrane (MWCO 0.8 μm). The particle seizes of the filtrates were determined by quasi-elastic (dynamic) light scattering using a Zetasizer nano (Malvern Instruments, Worcestershire, U.K.) at 25 °C.

Statistical Analysis Data are given as means±S.E. Statistical significance of mean coefficient for the studies was performed by analysis of variance followed by Scheffe's test to perform multiple comparisons. p-values for significance were set at 0.05.

Results and Discussion

The solid CyD complex formation can be studied by various physical methods such as thermal analyses, single-crystal or powder X-ray analysis, and solid NMR and FT-IR spectroscopies. Threading of CyD rings onto polymer chains can be demonstrated by Harada's method[16]: formation of channel inclusion complexes generally results in precipitation of the product from aqueous solution containing CyD due to a lack of hydration of the complexes with H_2O molecules. To test whether CoQ10 forms the water-insoluble complex with CyDs, we macroscopically observed appearance of the precipitates after preparation of the complex as described in Experimental. Figure 2 shows the appearance of the suspensions after incubation of CoQ10 with CyDs in aqueous solutions for 5 d. In the absence of CyDs, no precipitate was observed, although CoQ10 undissolved was observed onto the solvent (water). Meanwhile, the solution in the presence of γ-CyD provided yellow precipitates, whereas the solution in the presence of α-CyD, β-CyD or HP-γ-CyD gave no precipitates. These results indicate the formation of poorly soluble complex of CoQ10 with γ-CyD, but not α-CyD, β-CyD or HP-γ-CyD, in water. Recently, we reported that the intrinsic solubility of CoQ10 was 7.5×10^{-7} M in water at 25 °C and the phase solubility diagrams showed a mixed pattern of A_p and B_s-type diagrams defined by Higuchi and Connors[20] in the CoQ10 and γ-CyD system. These findings clearly support the formation of the water-insoluble complex of CoQ10 with γ-CyD by the solubility method.

There are three types of crystal packing of CyD complexes, *i.e.*, channel type, cage type and layer structure.[23,24] The powder X-ray diffractograms are generally useful for the confirmation of the polypseudorotaxanes with CyDs because they provide enough information to distinguish between the herringbone packing free CyDs and the channel packing of inclusion complexes.[19] Figure 3d shows powder X-ray diffraction patterns of γ-CyD complex with CoQ10, in comparison with those of PPG which was used for a positive control because it is known to form polypseudorotaxane with γ-CyD.[18] The diffractogram of the γ-CyD system showed the herringbone packing pattern (Fig. 3b). The X-ray diffraction pattern of the physical mixture of CoQ10 and γ-CyD was simply a superposition of the diffraction patterns of each component (Fig. 3c). Meanwhile, the diffraction peak of CoQ10, *e.g.* at 2θ=18.8°, was significantly reduced by com-

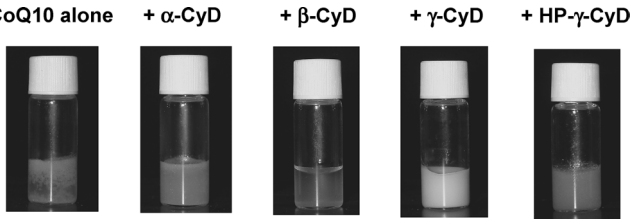

Fig. 2. Appearances of Suspensions of CoQ10/CyD Systems in Water after Agitation, Sonication and Incubation for 5 d at 25 °C

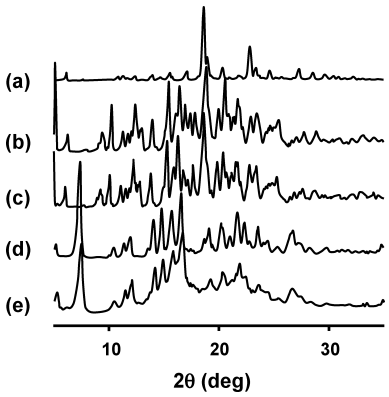

Fig. 3. Powder X-Ray Diffraction Patterns of CoQ10/γ-CyD Systems

(a) CoQ10 alone; (b) γ-CyD alone; (c) physical mixture of CoQ10 and γ-CyD; (d) complex of CoQ10 with γ-CyD; (e) polypropylene glycol (PPG) polypseudorotaxane with γ-CyD.

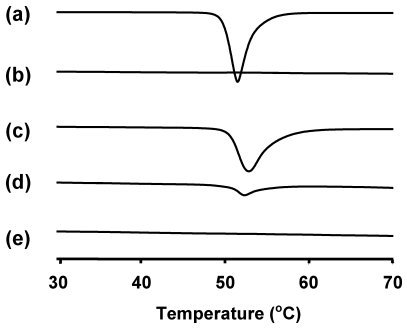

Fig. 4. DSC Thermograms of CoQ10/γ-CyD Systems

(a) CoQ10 alone; (b) γ-CyD alone; (c) physical mixture of CoQ10 and γ-CyD; (d) complex of CoQ10 with γ-CyD; (e) polypropylene glycol (PPG) polypseudorotaxane with γ-CyD.

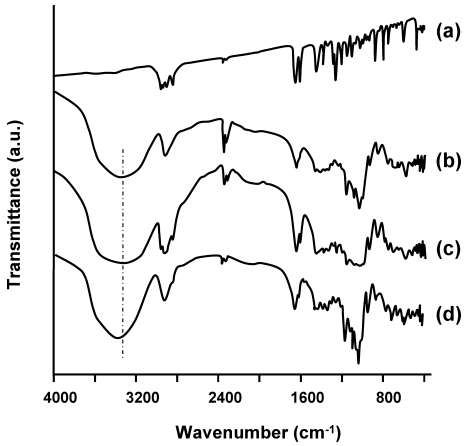

Fig. 5. FT-IR Spectra of CoQ10/γ-CyD Systems

(a) CoQ10 alone; (b) γ-CyD alone; (c) physical mixture of CoQ10 and γ-CyD; (d) complex of CoQ10 with γ-CyD.

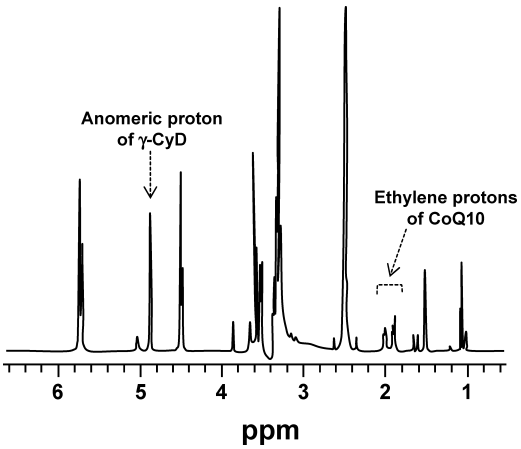

Fig. 6. ^{1}H-NMR Spectrum of Supramolecular Complex of CoQ10 with γ-CyD in DMSO-d_6 at 25 °C

plexation with γ-CyD (Fig. 3d). Hence, the diffraction pattern of the CoQ10/γ-CyD complex was different from those of physical mixture. Interestingly, it was almost the same as that of PPG/γ-CyD polypseudorotaxane. These results suggest that CoQ10 forms the pseudorotaxane-like supramolecular complex with γ-CyD, where the long isoprenoid chain is embedded in the stacked host channel. Figure 4 shows the DSC thermographs of CoQ10 complexes with γ-CyD. CoQ10 showed an endothermic peak at 53 °C in the DSC curve, due to the melting of the drug (Fig. 4a). The intensity of this endothermic peak of CoQ10 was reduced by the complexation with γ-CyD (Fig. 4d), compared with those of the corresponding physical mixture (Fig. 4c). Hence, these results suggest the interaction between CoQ10 and γ-CyD in the solid state. However, this endothermic peak did not completely disappear, suggesting the presence of small amounts of free CoQ10 in the precipitates, which may be due to crystallization of CoQ10. Anyhow, it is highly possible that CoQ10 forms the supramolecular complex with CoQ10.

To confirm the formation of the supramolecular complex, we measured FT-IR spectra of CoQ10, γ-CyD, and the physical mixture and the complex of CoQ10 and γ-CyD (Fig. 5). There was no band between 4000 and 3200 cm^{-1} in the CoQ10 alone system (Fig. 5a). The extremely broad bands between 4000 and 3000 cm^{-1} are normally assigned to the O–H stretching modes for γ-CyD.[25] A broad peak observed at 3372 cm^{-1} in the γ-CyD system and the physical mixture system seems to be shifted from 3385 cm^{-1} in the complex system (Figs. 5b, c, d). This shift is likely due to hydrogen bonds of the O–H groups of γ-CyD with the included the isoprenoid side chain, as previously reported in the supramolecular complex of triblock copolymer with γ-CyD.[25] Therefore, these results strongly suggest that CoQ10 forms the supramolecular complex with γ-CyD.

To calculate the stoichiometry of the supramolecular complex, we measured ^{1}H-NMR spectrum of the CoQ10 complex with γ-CyD after dissolving the solid complexes in DMSO-d_6 (Fig. 6). Under the present experimental conditions, only the information regarding the stoichiometry of the supramolecular complex, not the formation of the complex, can be provided because the γ-CyD molecules should dissociate from the complex. As shown in Fig. 6, the proton peaks of the secondary hydroxyl groups of γ-CyD and the peaks of the isoprenoid group were observed at *ca.* 5.8 ppm and 1.9—2.1 ppm, respectively. These peak areas of the anomeric proton of γ-CyD and the ethylene protons of CoQ10 of the complex were calculated, and the number of γ-CyD molecule encapsulated a CoQ10 molecule was estimated to be 5.03. Additionally, these facts suggest that the coverage of the long isoprenoid side chain of CoQ10 by γ-CyD was approximately 100%, when assumed that the two isoprenoid repeat

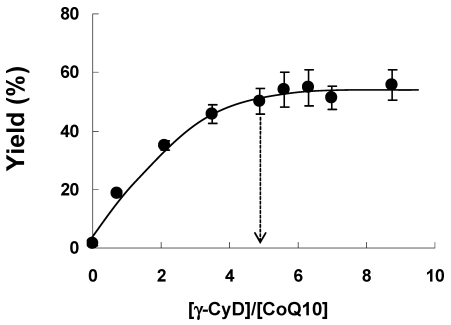

Fig. 7. Changes in Yield of CoQ10/γ-CyD Supramolecular Complex as a Function of the Ratio of γ-CyD to CoQ10

Each point represents the mean±S.E. of 4—5 experiments.

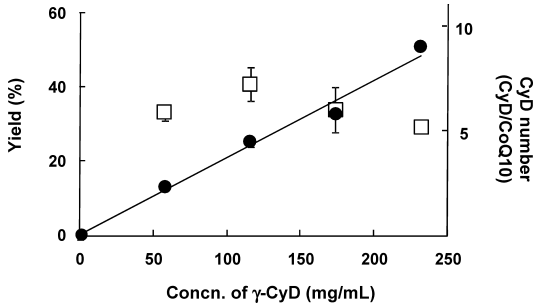

Fig. 8. Relationship between Yield of CoQ10 Supramolecular Complex with γ-CyD and the Number of γ-CyD Encapsulated CoQ10 as a Function of the Concentration of CyDs

Closed circle; yield; open square, stoichiometry. Each point represents the mean±S.E. of 4—5 experiments.

units are included in one γ-CyD cavity, consistent with the polypseudorotaxane of PPG with γ-CyD. Moreover, our preliminary study demonstrated that in the case of some compounds having the isoprenoid chains of the different length, the same coverage value was shown (data not shown). These results support the presumption that CoQ10 forms the pseudorotaxane-like supramolecular complex with γ-CyD by inserting the isoprenoid moiety of CoQ10 into γ-CyD cavities.

To confirm the stoichiometry of the solid CoQ10/γ-CyD supramolecule, we calculated the yield of the pseudorotaxanes using the method reported by Yui and coworkers.[21] Figure 7 shows the change in yield of the supramolecular complex of CoQ10 with γ-CyD as a function of the ratio of γ-CyD to CoQ10. The yield of the CoQ10 complex lineally increased as the ratio increased, and reached to a plateau at the molar ratio of 5 of γ-CyD to CoQ10. These results suggested that five γ-CyD molecules were involved in the inclusion of one CoQ10 molecule, consistent with the value calculated from the ^{1}H-NMR experiments. To clarify the change in stoichiometry of the CoQ10 complex when γ-CyD at the various concentrations was added, the relationship between yield and the stoichiometry of the supramolecular complexes as a function of the γ-CyD concentration was evaluated. As shown in Fig. 8, the yield lineally increased as the concentration increased. Meanwhile, the stoichiometry of 5 (γ-CyD to CoQ10) was almost the same up to the γ-CyD concentration of 232 mg/ml. These results suggested that the supramolecular complex having the stoichiometry of 5 (γ-CyD/CoQ10) is formed even at the low concentrations of γ-CyD, although it

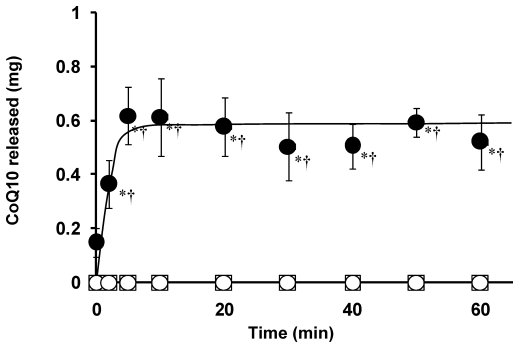

Fig. 9. Dissolution Profiles of CoQ10, Its Physical Mixture with γ-CyD and Its Supramolecular Complex with γ-CyD in Water at 25 °C

Open square, CoQ10 alone; open circle, physical mixture with γ-CyD; closed circle, supramolecular complex. Each point represents the mean±S.E. of 3—4 experiments. $*p<0.05$, compared to CoQ10 alone. $\dagger p<0.05$, compared to physical mixture.

is probably that the remaining CoQ10 and its complex having the stoichiometry (γ-CyD to CoQ10) less than 5 may dissolve in the suspension.

We determined the dispersion rate using the dispersed amount method[22] with a slight modification to evaluate the pharmaceutical properties of the supramolecular complex of CoQ10 with γ-CyD. Figure 9 shows the dispersion profiles of the CoQ10 complexes with CyDs in water at 37 °C. Here we could not detect the CoQ10 dissolved completely in water after filtration of samples through a filter membrane (MWCO 0.2 μm) because of extremely low aqueous solubility of CoQ10. Thereby, we measured the CoQ10 concentration in the filtrate filtrated through a membrane filter (MWCO 0.8 μm). Actually, the dispersion rate of CoQ10 in the CoQ10 alone system was markedly low. Likewise, the physical mixture of CoQ10 with CyDs did not exhibit any increase in dispersion. Interestingly, it is evident that the dispersion rate of CoQ10 complex with γ-CyD markedly increased. These results suggested the rapid dispersion of the complex of CoQ10 with γ-CyD, compared to CoQ10 alone and its physical mixture with γ-CyD. However, the pseudorotaxane-like supramolecular complex would be water-insoluble as described above. Thereby, the contradictory results may be explained by the presumption that the complex is particles with submicron size less than 0.8 μm (MWCO).

To address the hypothesis, we grossly observed the turbidity of the solution after agitation for 30 s, sonication for 30 min, and filtration through a filter membrane (MWCO 0.8 μm). As shown in Fig. 10, the CoQ10 alone system and the physical mixture system caused phase-separation. After filtration through the membrane, the CoQ10 alone system and the physical mixture system provided almost transparent solution. Meanwhile, the γ-CyD system provided white suspension after agitation and then white and fine-dispersed suspension after sonication, followed by slightly cloudy suspension even after filtration through the membrane-filter (MWCO 0.8 μm). These results suggest that the supramolecule of CoQ10 with γ-CyD has submicron-sized particles having the sizes in the range of 0.2—0.8 μm (MWCO).

Next, we measured the particle size and distribution of the supramolecular complex of CoQ10 with γ-CyD by a quasi-elastic (dynamic) light scattering method. As shown in Fig. 11, the mean particle sizes were 626 nm, 594 nm and 318 nm after agitation, sonication and a membrane filtration (MWCO

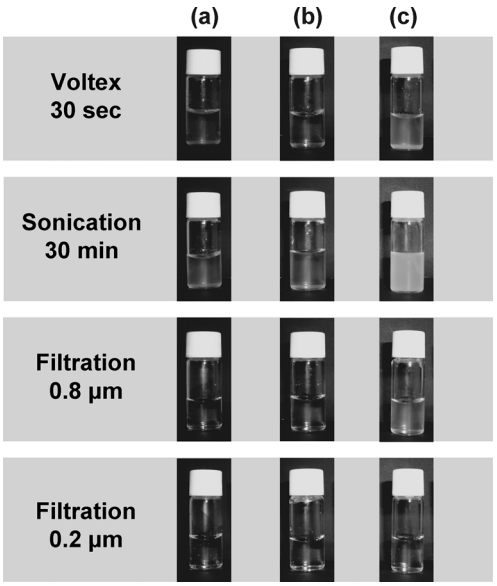

Fig. 10. Appearances of Suspensions of CoQ10/γ-CyD Systems in Water after Agitation, Sonication and Incubation for 5 d at 25 °C Followed by Filtration through Membranes (MWCO 0.8 μm and 0.2 μm)

(a) CoQ10 alone; (b) physical mixture with γ-CyD; (c) supramolecular complex.

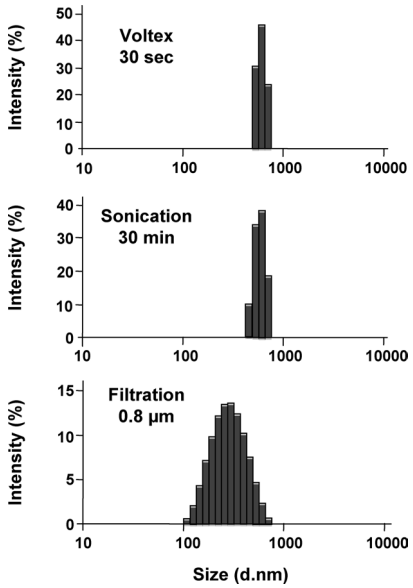

Fig. 11. Size Distributions of the Supramolecular Complex of CoQ10 with γ-CyD

0.8 μm membrane-filter), respectively. In addition, the size distributions of the γ-CyD suspension were 500—800, 300—800 and 100—800 nm after agitation, sonication and membrane filtration, respectively. Thus, these results suggest that the CoQ10 formed submicron-sized supramolecular particles with γ-CyD, when it was prepared by the solubility method, consistent with the complexes prepared by the kneading method as reported previously.[14)]

In the present study, of three natural CyDs and HP-γ-CyD, only γ-CyD formed the supramolecular complex with CoQ10. We previously reported that the phase solubility experiment showed that apparent solubility of CoQ10 in the presence of 1.5 mM CyDs increased in the order of α-CyD$<\beta$-CyD$<\gamma$-CyD.[14)] In addition, it is acknowledged that CyD derivatives such as methylated CyDs hardly form the polypseudorotaxane supramolecular complexes with PEG and PPG because of only very weak hydrogen bond formation between the CyD molecules.[26)] Hence, the strongest interaction of γ-CyD with CoQ10 among the natural CyDs and the formation of the hydrogen bond between CyD molecules may result in the formation of the supramolecular complex.

The question remains whether the complex of CoQ10 with γ-CyD prepared by the solubility method possesses the same structure as that prepared by the kneading method. Our previous study suggested that the X-ray diffractogram of the complex prepared by the solubility method is somewhat different from that prepared by the kneading method. Under the present experimental conditions, CoQ10 was incubated with γ-CyD for 5 d in the solubility method, whereas CoQ10 was kneaded with γ-CyD for 2—3 h in the kneading method.[14)] In addition, our preliminary data indicate that temperature is actually important factor to prepare the pseudorotaxane-like complexes of CoQ10 with γ-CyD, when the complex was prepared by the kneading method. Hence, it is possible that the chemical structure and/or component of the supramolecular complex of CoQ10 with γ-CyD are distinguished by the preparation methods, and the difference in those of the complex may be associated with the preparation time and temperature. Further studies are currently underway to investigate this difference in the detailed structure and physicochemical properties of the supramolecular complexes of CoQ10 with γ-CyD.

In conclusion, the present study showed that CoQ10 forms the pseudorotaxane-like complex with γ-CyD when it is prepared by the solubility method. There are a number of chemical compounds including isoprenoid chains, and thereby it is likely that the diverse and complicated supramolecular complexes with isoprenoids with CyDs may be formed, as partly described above. The extensive and elaborate studies may be further required to elucidate the formation of supramolecular complexes with various isoprenoids and their structures and pharmaceutical properties.

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