Enhanced dissolution and oral bioavailability of coenzyme Q_{10} in dogs obtained by inclusion complexation with γ-cyclodextrin

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Abstract

Purpose: The solubility of coenzyme Q_{10} (CoQ_{10}) in water is extremely low, resulting in a low oral bioavailability. In this study, we attempted to improve the low dissolution and bioavailability of CoQ_{10}, by means of the complexation with natural α-, β- and γ-cyclodextrins (CyDs) and 2-hydroxypropyl-β-CyD (HP-β-CyD). **Methods:** The complexation of CoQ_{10} with CyDs was studied by the solubility method. Solid CoQ_{10}/CyD complexes with different molar ratios were prepared by the kneading method using ethanol/water (1:1) mixed solvent. **Results:** Among these CyDs, γ-CyD significantly increased the solubility of CoQ_{10} at lower CyD concentrations. The powder X-ray diffraction peaks of CoQ_{10} disappeared following solid complexation with γ-CyD, although the endothermic peak due to the melting of the drug did not disappear completely. The dissolution rate of CoQ_{10} was significantly increased by complexation with γ-CyD, probably due to soluble complex formation and/or nanometer-sized particle formation, which was reflected in the enhanced oral absorption in dogs. **Conclusion:** The results indicated that γ-CyD is useful for improving the oral bioavailability of CoQ_{10}.

Keywords: Coenzyme Q_{10}, Cyclodextrin, Inclusion Complex, Solubility, Oral bioavailability

1. Introduction

Coenzymes Q (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 Q_{10}, CoQ_{10}, Fig. 1) is the most common in animals [1,2]. CoQ_{10} is used as an antioxidant and in the treatment of a variety of cardiovascular disorders including angina pectoris, hypertension, and congestive heart failure. Furthermore, CoQ_{10} is widely used commercially as a nutritional supplement in oil-based and powder-filled capsule formulations, because it is involved in electron transport in the mitochondrial membrane and is essential for the production of cellular energy in the form of ATP [3]. However, the solubility of CoQ_{10} in water is extremely low due to its highly hydrophobic isoprenoid side chain, resulting in a low oral bioavailability.

Cyclodextrins (CyDs) are cyclic oligosaccharides consisting of usually six, seven, or eight glucose units (α-, β- and γ-CyDs, respectively) bound by 1,4-glycosidic linkages [4]. CyDs are known to form inclusion complexes with lipophilic drugs and improve their low water solubility, dissolution rate and bioavailability [5-7]. When a drug is to be formulated as a CyD complex, it is important to select the most suitable CyD for the drug, because the solubilization effect of CyDs is dependent on their cavity size. In a previous paper [8], we reported that 2,6-di-O-methyl-β-CyD (DM-β-CyD) significantly enhances the low solubility and oral bioavailability of CoQ_{10}. However,
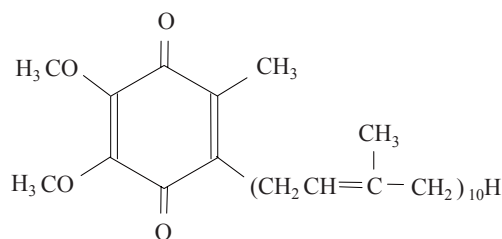

Fig. 1. Chemical structure of CoQ_{10}.

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natural CyDs or other hydrophilic CyDs may be more suitable for improvement of low bioavailability of nutritional supplements such as CoQ$_{10}$ from a viewpoint of safety and bioadaptability, because nutritional supplements are daily taken for long periods and sometimes in large amounts, and DM-β-CyD has a membrane-perturbing effect, resulting in hemolysis and local irritation at higher doses [5]. In this study, therefore, we studied the effect of natural α-, β- and γ-CyDs and hydrophilic 2-hydroxy-propyl-β-CyD (HP-β-CyD) on the aqueous solubility of CoQ$_{10}$ to select a more suitable CyD to improve the low oral bioavailability of CoQ$_{10}$.

2. Materials and methods

2.1. Materials

α-CyD, β-CyD, γ-CyD and HP-β-CyD (degree of substitution 5.4) were obtained from Nihon Shokuhin Kako Co. (Tokyo, Japan). CoQ$_{10}$ and CoQ$_9$ were donated by Nisshin Pharma Inc. (Tokyo, Japan) and used as supplied. All other chemicals and solvents were of analytical reagent grade and double-distilled water was used thoughout the study.

2.2. Solubility studies

The solubility was investigated using the method of Higuchi and Connors [9]. An excess of CoQ$_{10}$ was added to brawn screw-capped vials containing CyD solutions at various concentrations in water. After shaking the vials for 7 days at 25°C, the solutions were centrifuged (3,000 r/min, 5 min) and filtered through a cotton plug. To the filtrates (0.25 ml) were added 0.25 ml ethanol and 20 μl methanol containing an internal standard, vitamin E. CoQ$_{10}$ was extracted from the solutions by shaking with diethyl ether (5 ml) for 30 min. The organic phase (4 ml) was evaporated under reduced pressure and the residue was dissolved in 200 μl methanol, and 50 μl of this was subjected to HPLC analysis under the following conditions: a YMC A-302 ODS column (5 μm, 4.6 × 150 mm, Kyoto, Japan), a mobile phase of ethanol/methanol (3:2 v/v), a flow rate of 1.0 ml/min, and detection at 275 nm.

2.3. Preparation of solid complexes of CoQ$_{10}$ and CyDs

The solid complexes of CoQ$_{10}$ with CyDs with different molar ratios (guest:host 1:1, 1:3, 1:5 and 1:10) were prepared by the kneading method [10], *i.e.* the calculated amounts of CoQ$_{10}$ and CyDs were weighed and triturated with ethanol/water (1:1 v/v) solution in a mortar and the slurry was thoroughly kneaded for 2–3 h while adding the mixed solvent dropwise. After evaporation of the solvent, the solid material was dried at room temperature for 3 d under reduced pressure. Powder X-ray diffraction patterns of the complexes were taken on a Rigaku Rint 2,500 diffractometer, operating under the following conditions of Ni-filtered Cu-Kα radiation, a voltage of 40 kV, a current of 40 mA, and a scanning rate of 1°/min. Differential scanning calorimetric (DSC) analysis was carried out using a Perkin-Elmer DSC-7 analyzer, with a sample weight of 5.0 mg and a heating rate of 10°C/min. The particle sizes of the complexes or CoQ$_{10}$ were measured using a COULTER LS230 particle analyzer at 25°C.

2.4. Dissolution Studies

The dissolution rates of CoQ$_{10}$/CyD complexes were measured by the dispersed amount method [11]. The powder sample (equivalent to 30 mg drug, <100 mesh) was added to 100 ml degassed water at 37°C, and the suspension was stirred at 100 r/min. At appropriate intervals, an aliquot (2.0 ml) was withdrawn with a cotton-plugged pipet, and the CoQ$_{10}$ content measured by HPLC as described above.

2.5. In Vivo Absorption Studies

Male beagle dogs (9–11 kg) were fasted for about 24 h before drug administration. The sample powder (equivalent to 30 mg CoQ$_{10}$, <100 mesh) was placed in a gelatin capsule (content 0.95 cm^3) and administered orally with 50 ml water. At appropriate intervals, blood samples (2 ml) were withdrawn from the cephalic vein using a heparinized injection syringe and centrifuged at 1,100 × g for 10 min. To the plasma (0.5 ml) were added 20% SDS/sodium chloride solution (0.5 ml), 20% ethanol (0.1

ml), 50 μl ethanol containing an internal standard, CoQ$_9$, and 2.0 ml methanol/propanol (95: 5 v/v), and the mixture was shaken for 30 min. The drug and the internal standard were extracted with *n*-hexane (5.0 ml) and the organic phase (4.5 ml) was evaporated under reduced pressure. The resulting residue was dissolved in 100 μl methanol, and 20 μl of this was analyzed for the drugs by HPLC as described above.

3. Results and discussion

3.1. Interaction and preparation of CyD complexes

Fig. 2 shows the phase solubility diagrams of CoQ$_{10}$ with α-CyD, β-CyD, γ-CyD and HP-β-CyD in water at 25°C. The α-CyD and HP-β-CyD systems showed a typical A$_L$-type solubility diagram [9], where the solubility of CoQ$_{10}$ increased linearly as the CyD concentration

increased. The apparent 1: 1 stability constant (K$_c$) of the α- and HP-β-CyD complexes were calculated from the straight line of the solubility diagrams according to the equation, K$_c$= slope/[S$_o$(1–slope)], where S$_o$ (7.5 × 10^{-7} mol/l at 25°C) and the slope represent the intrinsic solubility of CoQ$_{10}$ and the slope of the diagram, respectively [9]. The K$_c$ values of the α-CyD and HP-β-CyD complexes were 350 ± 40 l/mol and 30 ± 5 l/mol, respectively. The β- and γ-CyD systems showed a mixed-pattern of A$_P$ and B$_S$-type diagrams, where the solubility of CoQ$_{10}$ gradually increased with an upward curvature up to about 3 mmol/l β-CyD and 18 mmol/l γ-CyD, followed by a plateau region, and then decreased with further increase in CyD concentrations [9, 12]. The initial upward increases in the solubility of CoQ$_{10}$ may be attributable to high-order complexation with CoQ10, because the drug has a long isoprenoid side chain in its structure. The reductions in the solubility of CoQ$_{10}$ at higher CyD concentrations
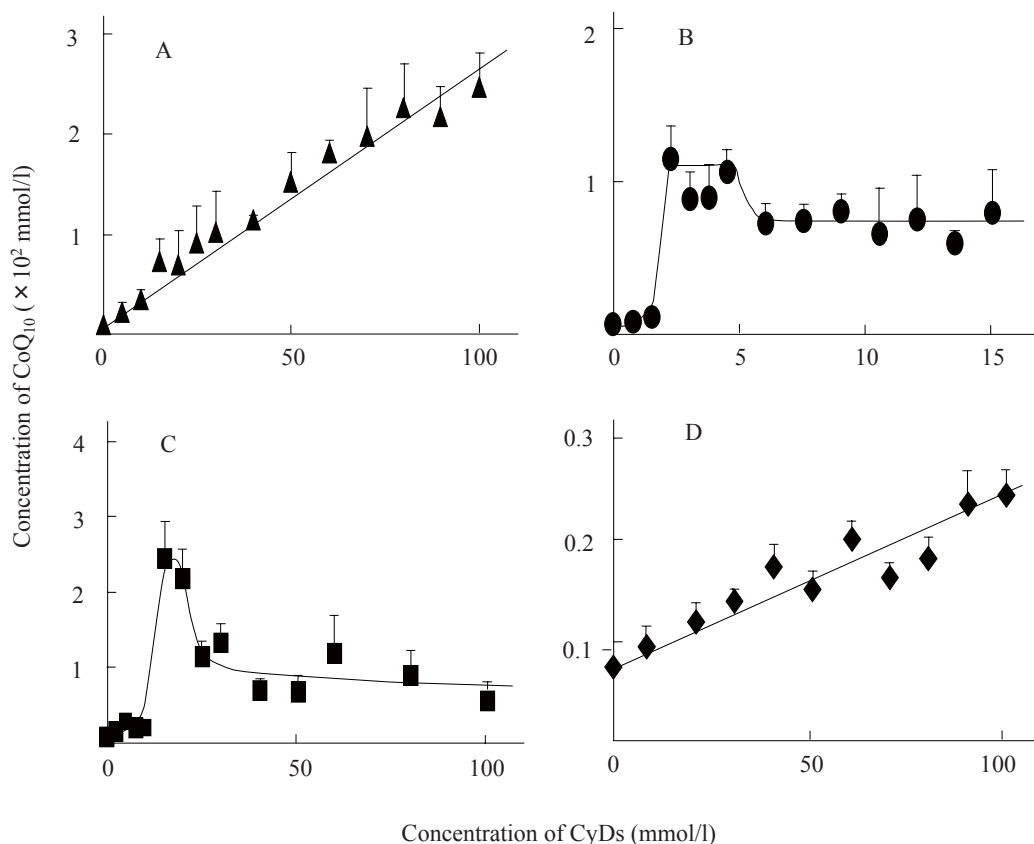

Fig. 2. Phase solubility diagrams of CoQ$_{10}$/CyD systems in water at 25°C. (A) α-CyD system, (B) β-CyD system, (C) γ-CyD system, (D) HP-β-CyD system. Each point represents the mean ± standard error (SE) of 3−5 experiments.

can be ascribed to the precipitation of solid complexes of the drug with β- and γ-CyDs. Unfortunately, it was difficult to determine the K_c values of the β- and γ-CyD complexes, because complete separation of solid particles by filtration was difficult due to the formation of very small particles of CoQ_{10} or its solid complexes, as described later. We attempted to determine the stoichiometry of the β- and γ-CyD complexes by chemical analysis of the solids precipitated at higher CyD concentrations, but it was difficult because free CoQ_{10} solids always contaminated in the precipitated complexes even after vigorous shaking for more than 7 days. Among these CyDs, γ-CyD appea-

red to be the most appropriate host molecule for the solubilization of CoQ_{10}, because of its high solubilizing ability at lower concentrations, *i.e.* the apparent solubility of CoQ_{10} in the presence of 1.5 mmol/l CyDs increased in the order of HP-β-CyD < α-CyD< β-CyD < γ-CyD, as is apparent from Fig. 2.

Solid complexes of CoQ_{10} with α-CyD, β-CyD, γ-CyD and HP-β-CyD with different molar ratios were prepared by the kneading method [10]. Fig. 3 shows the powder X-ray diffractograms and DSC curves of CoQ_{10}/γ-CyD solid complexes with 1:1, 1:3, 1:5 and 1:10 molar ratios (guest: host), in comparison with those of their physical mix-
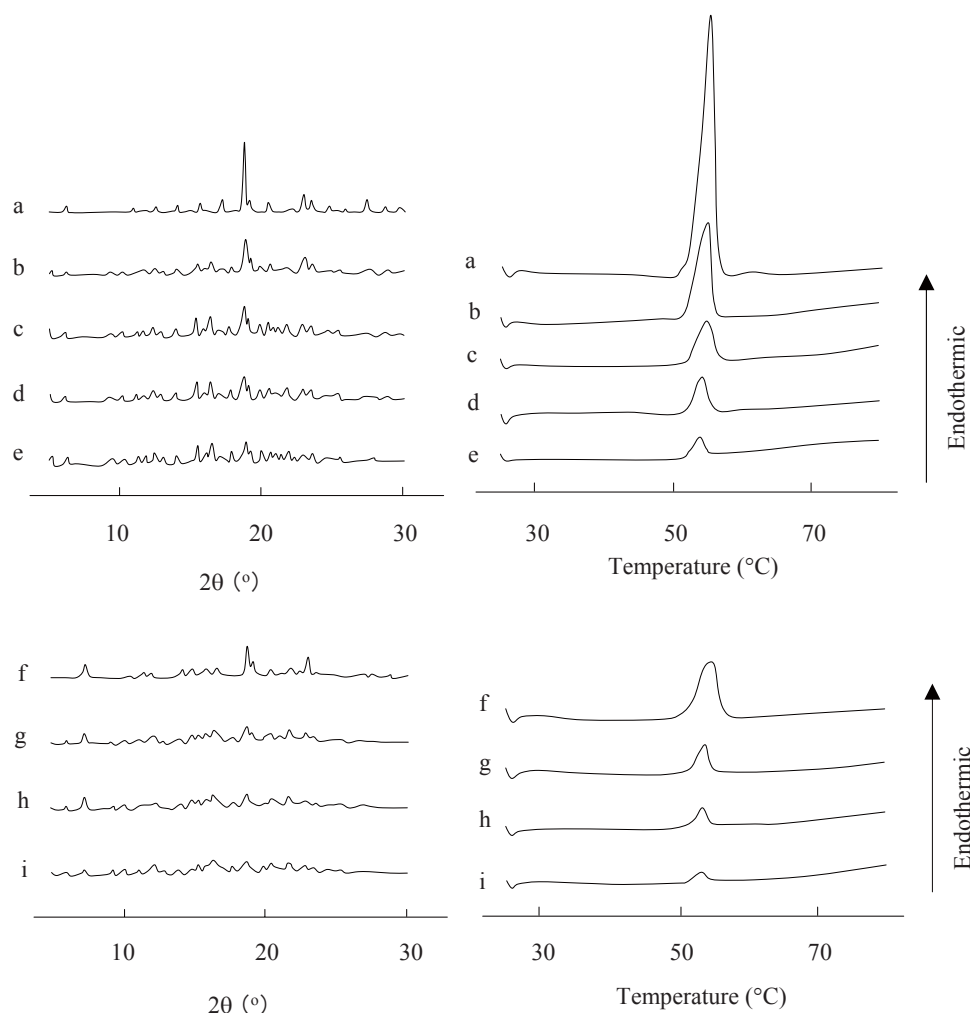

Fig. 3. Powder X-ray diffraction patterns (left) and DSC curves (right) of CoQ_{10}/γ -CyD systems with different molar ratios. a, CoQ_{10} alone; b-e, physical mixtures of 1:1, 1:3, 1:5 and 1:10 (CoQ_{10}/γ-CyD) molar ratios, respectively; f-i, complexes prepared in 1:1, 1:3, 1:5 and 1:10 (CoQ_{10}/γ-CyD) molar ratios, respectively.

tures. The X-ray diffraction pattern of the physical mixt-ures of CoQ$_{10}$ and γ-CyD was simply a superposition of the diffraction patterns of each component. On the other hand, the diffraction peak of CoQ$_{10}$, *e.g.* at 2θ = 18.8°, was significantly reduced by complexation with γ-CyD, particularly above a molar ratio of 1:10. CoQ$_{10}$ showed showed an endothermic peak at 53°C in the DSC curve, due to the melting of the drug. The intensity of this end-othermic peak of CoQ$_{10}$ was reduced by complexation with γ-CyD, compared with those of the corresponding physical mixtures. However, this endothermic peak did not completely disappear even after longer kneading tim-es such as 2–3 h, suggesting the presence of small amou-nts of free CoQ$_{10}$ in the kneading products, which may be due to crystallization of CoQ$_{10}$ during/after the kneading or incomplete dissolution of the drug in the solvent, eth-anol/water (1:1 v/v). This incomplete complexation of CoQ$_{10}$ was much more significant in the α-CyD, β-CyD and HP-β-CyD complexes, *i.e.* incomplete disappearan-ces of the powder X-ray diffraction peaks and the endo-thermic peak of CoQ$_{10}$ following complexation with these CyDs.

3.2. In vitro dissolution and in vivo absorption studies

Fig. 4 shows the dissolution profiles of CoQ$_{10}$/CyD (1:10) solid complexes in water at 37°C, measured by the disp-ersed amount method [11]. It is apparent that the dissolu-tion rate of CoQ$_{10}$ significantly increased following com-plexation with γ-CyD and moderately increased follow-ing complexation with β-CyD, whereas α-CyD and HP-β-CyD exhibited only slightly increased dissolution. The physical mixtures of CoQ10 with α-, β- and γ-CyDs and HP-β-CyD did not exhibit any increase in dissolution. When the γ-CyD complex was vigorously agitated in water, the solution was changed into a white, milky susp-ension, as shown in Fig. 5, and the β-CyD system chang-ed into a white suspension, whereas in the case of the α-CyD and HP-β-CyD systems, large yellow particles were suspended in solution. The γ-CyD suspension was slightly cloudy even after filtration through 0.8 μm mem-brane, while the filtrates of the α-CyD, β-CyD and HP-β-CyD suspensions were almost transparent, as shown in Fig. 5. Fig. 6 shows the particle size distribution profiles of particle for the suspensions of β-CyD and γ-CyD com-plexes in water. The average particle size and distribution of the β-CyD suspension were 3.8 μm and 0.60–63 μm,
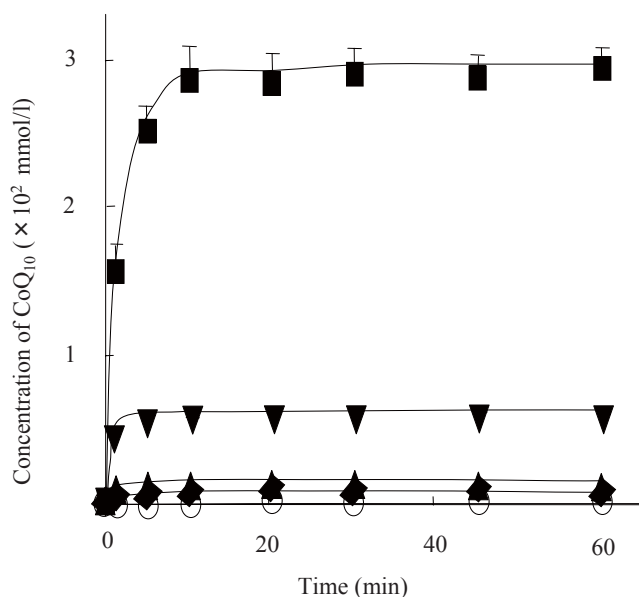

Fig. 4. Dissolution profiles of CoQ$_{10}$/CyD (1:10) complexes prepared by kneading method, measured by dispersed amount method in water at 37°C. (○) CoQ$_{10}$ alone; (▲) α-CyD complex; (▼) β-CyD complex; (■) γ-CyD complex; (♦) HP-β-CyD complex.
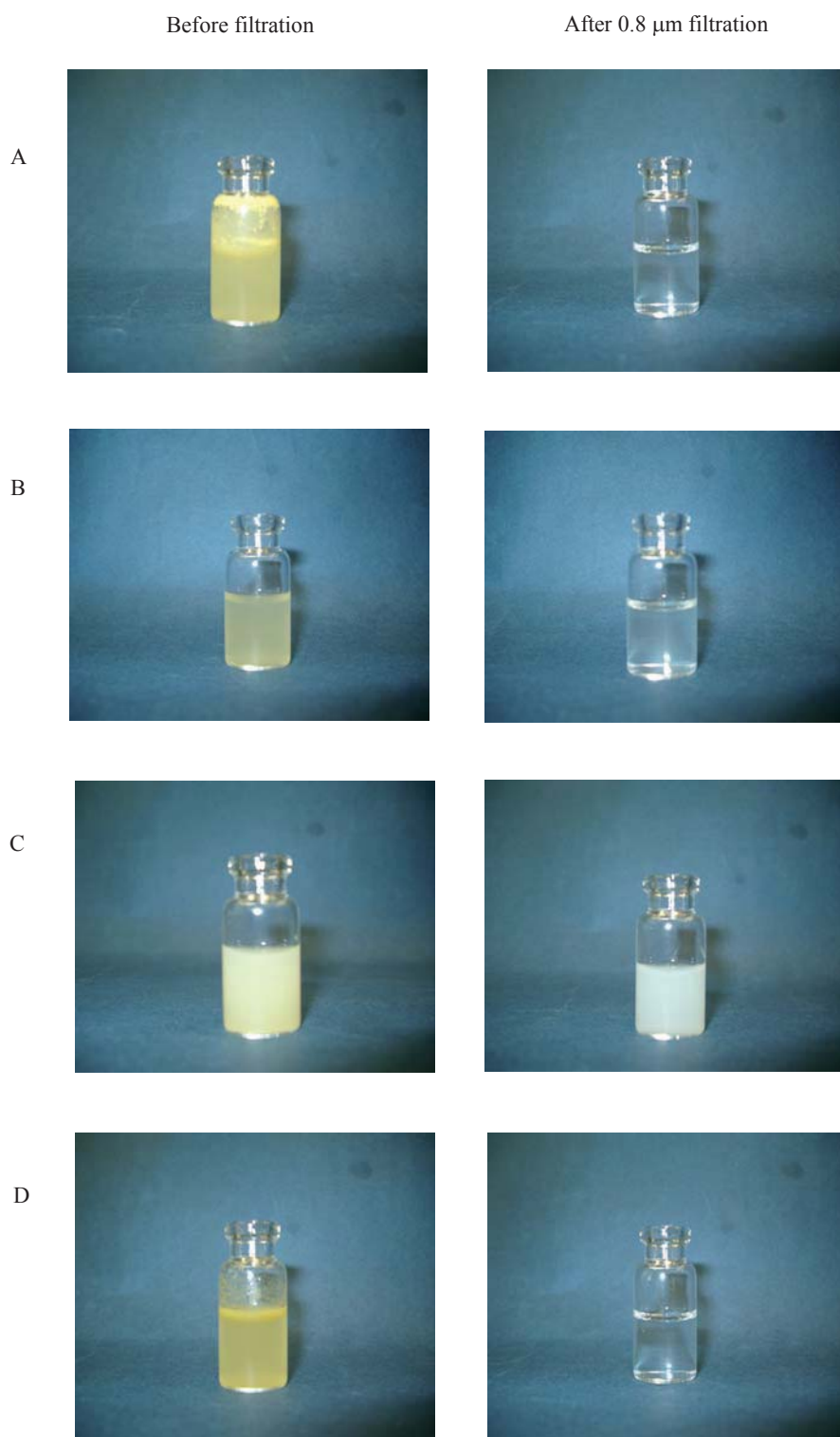
Before filtration   After 0.8 μm filtration

A

B

C

D

Fig. 5. Appearance of suspensions and filtrates (0.8 μm) of CoQ$_{10}$/CyD (1: 10) systems in water. (A) α-CyD system, (B) β-CyD system, (C) γ-CyD system, (D) HP-β-CyD system.

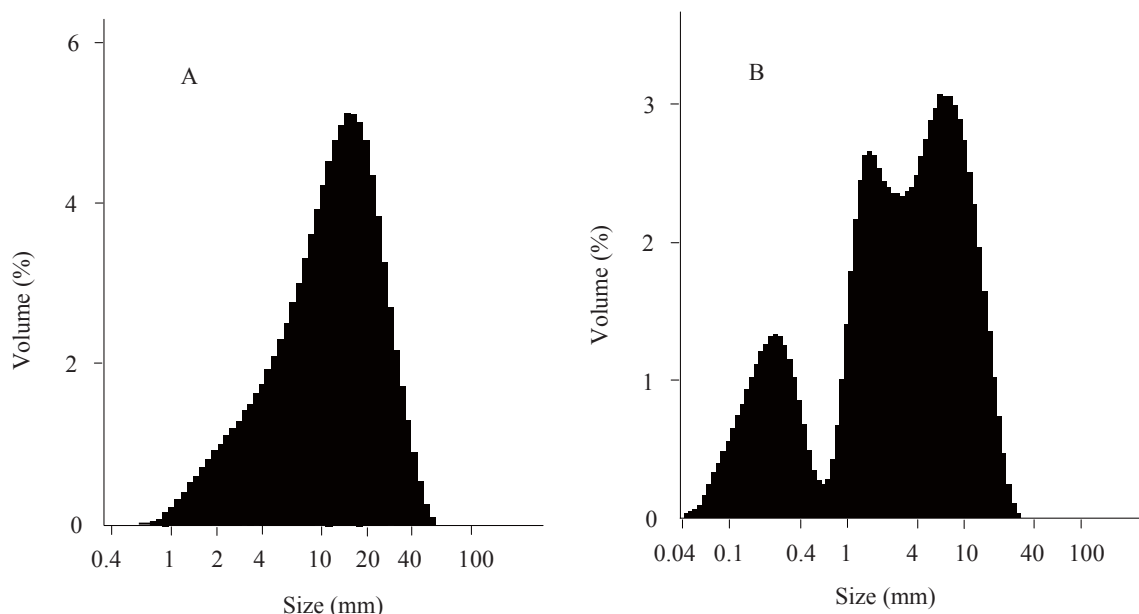Fig. 6. Particle size distribution profiles for suspensions of CoQ$_{10}$/β-CyD (A) and CoQ$_{10}$/γ-CyD (B) systems.

respectively. However, the γ-CyD suspension showed three peaks around 0.04–0.6 μm, 0.6–3 μm and 3–30 μm in the distribution profile, giving an apparent average size of 4.7 μm. It is apparent that part of CoQ$_{10}$ or the complex particles suspended in γ-CyD solutions are of nanometer size, as reported by Yamamoto *et al.* [13]. Fig. 7 shows the plasma levels of CoQ$_{10}$ after oral administration of its solid γ-CyD complex (in a molar ratio of 1:10) in dogs, in comparison with those of CoQ$_{10}$ alone and the CoQ$_{10}$/γ-CyD physical mixture. In the case of the drug alone and the physical mixture, the plasma levels were only negligibly increased and almost identical to the endogenous CoQ$_{10}$ level (about 0.4 μg/ml). On the other hand, the CoQ$_{10}$ levels were significantly increased when the drug was administered in the γ-CyD complex, and were comparable with those when the drug was administered as a complex with 2,6-di-O-methyl-β-CyD, as reported previously [8]. A similar increased oral bioavailability of CoQ$_{10}$ following γ-CyD complexation was also observed in healthy adult volunteers, and this will be reported elsewhere [14].

4. Conclusion

In this paper, we have shown that γ-CyD significantly improves the low solubility, dissolution and oral bioavai-

lability of CoQ$_{10}$, probably due to soluble complex formation and/or nanometer-sized particle formation. Further studies are needed to identify the detailed mechanisms of the nanometer-sized particle formation and investigate the inhibitory effect of γ-CyD on the aggregation of CoQ$_{10}$, together with the stability profiles of the particles during storage. The present results indicate that γ-CyD is useful for improving the oral bioavailability of CoQ$_{10}$, and this may assist the design of formulations of nutritional supplements such as CoQ$_{10}$, from a safety viewpoint [15].
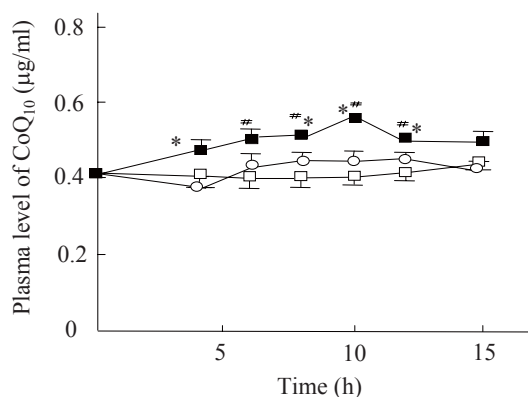

Fig. 7. Plasma levels of CoQ$_{10}$ following oral administration of capsules containing CoQ$_{10}$/γ-CyD systems (equivalent to 30 mg CoQ$_{10}$) in dogs. (○) CoQ$_{10}$ alone; (□) 1:10 physical mixture; (■) 1:10 complex. Each point represents the mean ±SE of 3 experiments. *$P<0.05$ versus CoQ$_{10}$ alone, #$P<0.05$ versus CoQ$_{10}$/γ-CyD physical mixture.

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