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Enhanced Water Dispersibility of Coenzyme Q_{10} by Complexation with Albumin Hydrolysate

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Supporting Information

ABSTRACT: The biologically important coenzyme Q_{10} (CoQ_{10}) is widely used as a drug for chronic heart failure, as a nutritional supplement, and in cosmetics. However, the oral bioavailability of CoQ_{10} is poor due to its extremely low solubility in aqueous media. In this study, complexation of CoQ_{10} with albumin hydrolysate as a peptide mixture (Pep) was shown to enhance the water dispersibility of CoQ_{10}. An aqueous solution of Pep and an acetone solution of CoQ_{10} were mixed and lyophilized to obtain a white-yellow powder containing peptides and CoQ_{10} complex (Q10-Pep). The water dispersibility of Q10-Pep was much higher than that of CoQ_{10} alone and increased with the quantity of Pep. The particle size of Q10-Pep in aqueous media was 170−280 nm, suggesting that Q10-Pep was present as a hydrocolloidal material. Characterization of Q10-Pep using differential scanning calorimetry showed that CoQ_{10} was incorporated in the hydrocolloid in an amorphous state.

KEYWORDS: *coenzyme Q_{10}, dispersibility, albumin, peptide, hydrocolloid, nanoparticle*

INTRODUCTION

Coenzyme Q_{10} (CoQ_{10}, Figure 1), also known as ubiquinone or ubidecarenone, is a naturally occurring lipophilic chemical that

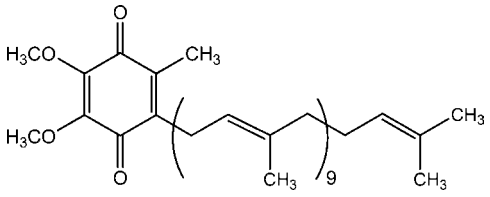

Figure 1. Molecular structure of CoQ_{10}.

is synthesized by mammals and plants and serves as a potent antioxidant. CoQ_{10} is involved in various essential cellular processes: cellular energy production in the synthesis of adenosine triphosphate (ATP), an antioxidant, and a membrane stabilizer.[1−3] In clinical application, the therapeutic value of CoQ_{10} as an adjunct to standard medical therapy in congestive heart failure has been recognized.[4−6] CoQ_{10} biosynthesis decreases with age, and its deficit in tissues is associated with degenerative changes.[5] CoQ_{10} deficiency can be ameliorated with oral supplements.[7] However, oral bioavailability of CoQ_{10} is poor due to its extreme low solubility in aqueous media. The water solubility and/or water dispersibility of such insoluble supplements and drugs can often be improved by formulation.[8,9] Various techniques have been developed for the enhancement of the water dispersibility and oral bioavailability of CoQ_{10}.

Complex formation between CoQ_{10} and cyclodextrins increases the water solubility or water dispersibility of CoQ_{10}.[10−12] Additionally, thermal- and photostability of CoQ_{10} was also improved by the complexation with cyclodextrins. The oral absorption and bioavailability of CoQ_{10} in healthy adult volunteers were enhanced by complexation with γ-cyclodextrins.[13] CoQ_{10} forms a pseudorotaxane-like complex with γ-cyclodextrin.[14,15]

Emulsification is also a major technique for improving the oral bioavailability of poor water-soluble drugs.[16] Self-emulsifying drug delivery systems (SEDDS) of CoQ_{10} using polyglycolyzed glycerides as emulsifiers have been developed.[17] A 2-fold increase in bioavailability was observed for the self-emulsifying system compared with a powder formulation. A pharmacokinetic study in rats for the formulation based on SEDDS composed of oil, surfactant, and cosurfactant also showed an increase in the bioavailability of CoQ_{10} compared with a powder suspension formulation.[18] A self-nanoemulsified drug delivery system (SNEDDS) based on the eutectic property of CoQ_{10} with essential oils has been developed.[19,20] Nanostructured lipid carriers composed of cetyl palmitate and caprylic/capric triacylglycerols were also developed to incorporate CoQ_{10}.[21] The lipid carrier showed a mean particle size of 180−240 nm and was negatively charged, providing a good physical stability.

Nanoparticles based on a polymeric matrix are also used as carriers for CoQ_{10}.[22] The particles in a poly(ethyleneimine)−dodecanoate complex exhibit hydrodynamic diameters in the range 80−150 nm and were developed as a carrier for CoQ_{10}.[23] Recently, ABC miktoarm polymers (A = polyethylene glycol, B = polycaprolactone, C = triphenylphosphonium bromide), which self-assemble into nanosized micelles, have been developed as a nanocarrier for CoQ_{10} loading.[24] The micelles of the miktoarm polymers showed high loading capacity for CoQ_{10} in aqueous media. Improved transdermal delivery of

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CoQ_{10} using a block copolymer-based nanoparticle has also been reported.[25]

In this study a peptide mixture, prepared as a protein hydrolysate by enzymatic hydrolysis, was used as a complexing agent for CoQ_{10}. As the protein hydrolysate consists of peptides (Pep), which have variable molecular weight and amino acid sequence, some may exhibit affinity to CoQ_{10}, resulting in the formation of a hydrophilic complex. Bovine serum albumin (BSA) was used as a model protein to prepare the modifier. Heme iron preparation (HIP) is a precedent as a water-dispersible complex between poorly water-soluble substances and peptides.[26−29] HIP was prepared by the hydrolysis of hemoglobin using protease followed by enrichment using ultrafiltration. HIP, obtained as the complex between heme iron and peptide fragments, is more readily absorbed by the human body than heme iron alone.[30−32] Hence, complexation with a peptide mixture may be an effective method for enhancing the water solubility of other poorly water-soluble materials.

This study aims to investigate the complexation between CoQ_{10} and albumin hydrolysate as a means to enhance the water dispersibility of CoQ_{10} to improve its bioavailability. Figure 2 shows the flowchart for the preparation of the complex between CoQ_{10} and Pep (Q10-Pep). Pep was prepared by enzymatic hydrolysis of albumin using α-chymotrypsin. The aqueous solution of Pep and an acetone solution of CoQ_{10} were mixed. After lyophilization of the mixture, Q10-Pep was obtained as a white-yellow powder. The results of water dispersibility tests showed improved Q10-Pep dispersibility compared with CoQ_{10} alone. Q10-Pep was prepared under different conditions by changing the volume ratio of the solutions, the quantity of Pep, and the pH, to determine optimal conditions for the preparation of water-dispersible materials. To estimate the particle size and material distribution of Q10-Pep, the aqueous suspension of Q10-Pep was fractionated by centrifugation followed by membrane filtration. The fractions were analyzed by dynamic laser scattering (DLS) and UV−vis spectroscopy. Additionally, Q10-Pep was analyzed using differential scanning calorimetry (DSC) to study the crystalline form of CoQ_{10} incorporated in Q10-Pep.

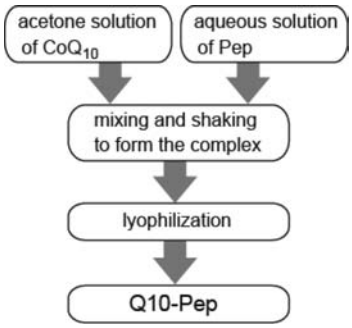

Figure 2. Flowchart for the preparation of Q10-Pep.

MATERIALS AND METHODS

Materials. Coenzyme Q_{10} was obtained from Tokyo Chemical Ind. (Tokyo, Japan). Bovine serum albumin was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). α-Chymotrypsin was purchased from Sigma-Aldrich (Tokyo, Japan). All other reagents were analytical grade. Disposable cellulose acetate membrane filters (Φ 0.80, 0.45, or 0.20 μm) were purchased from Advantec Toyo (Tokyo, Japan).

Preparation of Albumin Hydrolysate. BSA (25 g) was dissolved in 100 mM aqueous sodium hydroxide (500 mL) with stirring (300 rpm at 30−35 °C) for 6 h to induce denaturation for enzymatic hydrolysis. This solution was neutralized with 1.0 M hydrochloric acid (50 mL), and the pH adjusted to pH 8.0 with aqueous phosphate buffer (0.2 M). The denatured BSA was enzymatically hydrolyzed with α-chymotrypsin (125 mg) with stirring (500 rpm at 35−40 °C) for 18 h. After lyophilization of the mixture, the resulting albumin hydrolysate (Pep) containing a peptide mixture from BSA and salts was obtained as a white powder. On the basis of mass balance, the content of peptides in the resulting material was 77%. The molecular weight distribution of Pep was determined using gel filtration chromatography/high-performance liquid chromatography (GFC-HPLC; Shimadzu Prominence Isocratic System, Kyoto, Japan). Isocratic HPLC was performed using a Shimadzu LC-10ADvp pump unit (flow rate: 0.5 mL/min) together with Shimadzu Inertsil WP300 Diol (5 μm, 250 mm ×7.6 mm i.d.) and Inertsil Diol (5 μm, 250 mm ×7.6 mm i.d.) columns and detection of ultraviolet (UV) absorption with a Shimadzu SPD-10Avp UV−vis detector at 230 nm.

Preparation of the Complex between CoQ_{10} and Albumin Hydrolysate (Q10-Pep) and Water Dispersibility Test. The CoQ_{10} and albumin hydrolysate complex (Q10-Pep) was prepared according to the flowchart in Figure 2. An acetone solution containing CoQ_{10} and an aqueous solution containing Pep were mixed and shaken. The mixture was lyophilized, and Q10-Pep was obtained as white-yellow powder. Q10-Pep was prepared under various conditions as follows, to understand the optimal conditions to prepare a more water-dispersible Q10-Pep.

Effect of Volume Ratio of Solutions Used in Preparation. Organic solutions of CoQ_{10} were prepared by dissolving CoQ_{10} (8.63 mg) in different volumes of acetone (7.0, 6.0, 4.0, 2.0, and 1.0 mL). Aqueous solutions of Pep were prepared by dissolving 40 mg of Pep into different volumes of distilled water (1.0, 2.0, 4.0, 6.0, and 7.0 mL). Corresponding solutions of CoQ_{10} and Pep were mixed to give the same final volume of the mixture (approximately 8.0 mL). The volume ratios of these solutions (CoQ_{10} solution:Pep solution) were 7:1, 3:1 1:1, 1:3, and 1:7. Each mixture was shaken in a thermostatic water bath (30 °C, 120 rpm) for 24 h and then lyophilized to obtain Q10-Pep as a white-yellow powder.

To evaluate the water dispersibility of Q10-Pep, 5 mg of each sample was added to 5 mL of 10 mM aqueous phosphate buffer solution (pH 8.0). The aqueous mixture was swirled with a vortex mixer at room temperature for 15 s. After filtration of the aqueous mixtures through a Φ 0.80 μm membrane filter, the turbidity of the filtrate was monitored by the absorbance at 555 nm using a UV−vis spectrophotometer (JASCO V-660, Tokyo, Japan).[33]

Effect of the Mass Ratio between Pep and CoQ_{10}. Six solutions of CoQ_{10} were prepared by dissolving CoQ_{10} (21.6 mg, 10 mM) in acetone (2.5 mL). Aqueous solutions of Pep were prepared by dissolving 5.0, 10, 30, 40, 60, and 80 mg of Pep in distilled water (20 mL). The acetone and aqueous solutions were mixed in sample tubes and shaken (120 rpm) at 30 °C for 24 h. After shaking, each mixture was lyophilized to obtain Q10-Pep. Reference mixtures (abbreviated as Q10-Pep-R) were prepared by mixing the same quantities of CoQ_{10} (21.6 mg) and Pep (5.0, 10, 30, 40, 60, 80 mg) in the dry state, to study the effect of the mixing method.

The complex between CoQ_{10} and BSA (Q10-Alb) was also prepared in a similar manner to Q10-Pep. Aqueous solutions of BSA were prepared by dissolving 5.0, 10, 30, 40, 60, and 80 mg of BSA in 20 mL of distilled water. The solution of CoQ_{10} (21.6 mg 10 mM, 2.5 mL) and an aqueous solution of BSA (20 mL) were mixed and shaken at 30 °C for 24 h. After shaking, each mixture was lyophilized to obtain Q10-Alb as a white-yellow powder.

To evaluate the water dispersibility of Q10-Pep, Q10-Pep-R, and Q10-Alb, each sample (5 mg) was added to 5 mL of aqueous phosphate buffer solution (10 mM, pH 8.0). The aqueous mixture was swirled with a vortex mixer at room temperature for 15 s and filtered through a 0.80 μm membrane filter. The turbidity of the filtrate was determined using a UV−vis spectrophotometer.

Extraction of CoQ_{10} from the aqueous suspension of Q10-Pep (10 mg) was examined. Thus, an aqueous suspension of Q10-Pep (10 mg) was passed through a 0.80 μm membrane filter, and 10 mL of the filtrate treated with 5.0 mL of hexane. After shaking the mixture at 30 °C (120 rpm) for 12 h, the phases were separated and the UV−vis spectrum of the hexane phase was observed to confirm the presence of CoQ_{10} in the filtrate.

Effect of pH. CoQ_{10} (86.3 mg, 10 mM) was dissolved in acetone (10 mL). Pep (400 mg) was dissolved in distilled water (90 mL). The acetone solution of CoQ_{10} and aqueous solution of Pep were mixed and shaken at 30 °C (120 rpm) for 2 h and then lyophilized to obtain Q10-Pep. A blank sample (Q10-Blank) was prepared by mixing acetone (10 mL) containing 10 mM CoQ_{10} and distilled water (90 mL), without Pep.

To evaluate the water dispersibility of Q10-Pep, Q10-Pep (5.0 mg) or Q10-Blank (5.0 mg) was added to 5.0 mL of 10 mM sodium phosphate buffer solutions with pH ranging from 3 to 11. The aqueous mixtures were shaken and filtered using a 0.80 μm membrane filter. The pH of the filtrate was measured using a pH meter (DKK-TOA Co., HM-30G, Tokyo, Japan). The turbidity of the filtrate was determined using a UV−vis spectrophotometer.

Material Distribution and Particle Size Analysis for Q10-Pep. Q10-Pep was prepared in a similar manner to that shown above by mixing an acetone solution (10 mL) containing 86.3 mg of CoQ_{10} (10 mM) and an aqueous solution (90 mL) containing 400 mg of Pep. Q10-Pep (30 mg) was added to 30 mL of distilled water. The aqueous suspension was fractionated as shown in Figure 3. The Q10-Pep

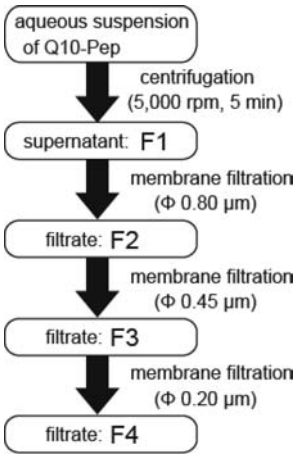

Figure 3. Flowchart for fractionation of an aqueous suspension of Q10-Pep.

suspension was centrifuged at 5000 rpm for 5 min, and the supernatant was analyzed as centrifuged sample F1. The supernatant was filtered using a 0.80 μm membrane filter to obtain the filtrate (F2). Filtration using a 0.45 μm membrane filter followed by using a 0.20 μm membrane filter was performed to obtain fractions F3 and F4, respectively.

The turbidities of the fractions F1−F4 were monitored by the absorbance at 555 nm using a UV−vis spectrophotometer. The particle size of Q10-Pep in fractions F1−F4 was measured using a DLS particle size/zeta potential analyzer (Horiba SZ-100 Nanopartica, Kyoto, Japan).

Zeta Potential of Q10-Pep. Q10-Pep was prepared in a similar manner to that shown above. Q10-Pep or Pep (5.0 mg) was added to 5.0 mL of 10 mM sodium phosphate buffer solution with pH ranging from 3 to 11. The aqueous mixture was shaken and filtered using a 0.80 μm membrane filter. The pH of the filtrate was measured using a pH meter. The zeta potential of the filtrate was measured using a DLS particle size/zeta potential analyzer (Horiba SZ-100).

Differential Thermal Analysis for Q10-Pep. DSC analysis for CoQ_{10}, Q10-Pep-R, and Q10-Pep (prepared as described above) was performed using a Shimadzu DTG-60/60H thermal analyzer (Kyoto, Japan) from 30 °C to 80 °C at a heating rate of 10 °C/min under nitrogen.

RESULTS AND DISCUSSION

Water Dispersibility of CoQ_{10} and Peptide Complex. GFC-HPLC analysis showed that Pep had a wide molecular weight distribution. The molecular weights of the major components calculated from a calibration curve using a peptide standard were 1100, 1500, 2600, and 5900 Da.

In preliminary experiments, the effect of the shaking time in the preparation of Q10-Pep on the water dispersibility of Q10-Pep was studied. The water dispersibility of Q10-Pep increased with shaking time in the preparation up to 2 h. The turbidity of Q10-Pep slightly decreased by shaking over 2 h, probably because of the precipitation of CoQ_{10}. In the present study, Q10-Pep was prepared by shaking the mixture of the CoQ_{10} solution and Pep solution for 2−24 h.

The photograph in Figure 4 shows the aqueous suspension of CoQ_{10} alone, Q10-Pep-R, and Q10-Pep. As CoQ_{10} is a

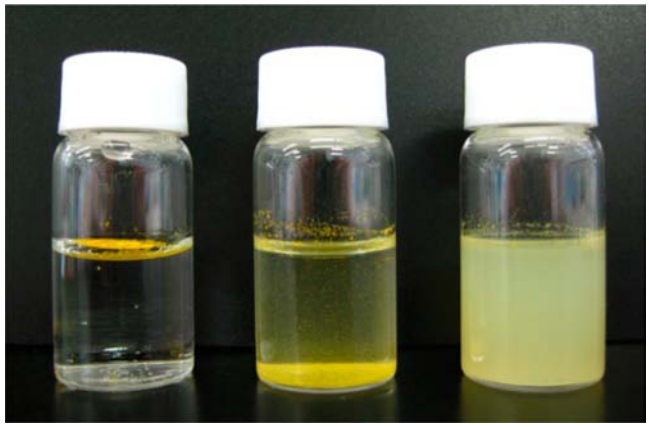

Figure 4. Image of aqueous suspensions of CoQ_{10} (left), Q10-Pep-R (center), and Q10-Pep (right).

lipophilic material and is almost insoluble in water, CoQ_{10} does not disperse in the aqueous media at all. Similarly, yellow crystalline CoQ_{10} in Q10-Pep-R does not disperse in aqueous media. In contrast, Q10-Pep reasonably disperses in aqueous media, and the solution was yellow-white. Additionally, Q10-Pep was more dispersible than the commercially available CoQ_{10}−cyclodextrin complex in aqueous media. To evaluate the water dispersibility of Q10-Pep and the references, the turbidity of the filtrate that passed through a ⌀ 0.80 μm membrane filter was analyzed by the absorbance at 555 nm.

CoQ_{10} dissolved in acetone should precipitate when the solution is mixed with an aqueous Pep solution. The complexation between CoQ_{10} and Pep should be competitive with the precipitation of CoQ_{10}. Table 1 summarizes the effect of the volume ratio of the CoQ_{10} solution and the Pep solution on water dispersibility of Q10-Pep. The turbidity showed a maximum value when the volume ratio was 1:7 (CoQ_{10} solution:Pep solution). From the result, a small volume of the CoQ_{10} solution should be mixed with a larger volume of the Pep solution. The kinetic balance between complexation and precipitation of CoQ_{10} is still not clear.

Figure 5 shows the effect of the mass ratio between Pep or BSA and CoQ_{10} on the turbidity of the aqueous suspensions of Q10-Pep, Q10-Pep-R, Q10-Alb, and Pep at pH 8. Pep is soluble in water, and the filtrate of Pep that passed through a

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Table 1. Effect of the Volume Ratio of CoQ_{10} and Pep Solutions on the Water Dispersibility of Q10-Pep[a]

volume ratio (CoQ_{10} solution:Pep solution)	$Abs_{555\ nm}$
7:1	0.0143
6:2	0.0053
4:4	0.0093
2:6	0.0114
1:7	0.1427

[a]Sample, 5.0 mg; solution volume, 5.0 mL; pH 8.0.

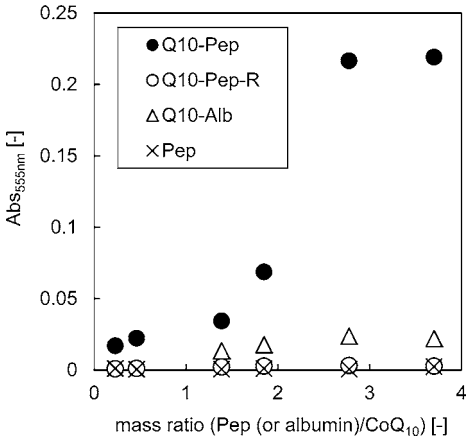

Figure 5. Effect of the mass ratio between Pep or BSA and CoQ_{10} on the turbidity of the aqueous suspensions of Q10-Pep, Q10-Pep-R, Q10-Alb, and Pep (sample, 5.0 mg; solution volume, 5.0 mL; pH 8.0).

0.8 μm membrane filter was clear. By contrast, the turbidity of the filtrate for Q10-Pep, which passed through a 0.80 μm membrane filter, increases with increasing the quantity of Pep. As the turbidity of the filtrate for Q10-Alb is less than that of Q10-Pep, Pep was more effective in forming a water-dispersible complex than BSA. Therefore, Pep was confirmed to be effective for the enhancement of water dispersibility of CoQ_{10}.[34] The dispersibility of Q10-Pep-R did not increase, suggesting that CoQ_{10} and Pep should be mixed as solutions to form the water-dispersible complex. Extraction of CoQ_{10} with hexane from the aqueous suspension of Q10-Pep was examined. The UV−vis spectrum of the hexane phase showed an absorption peak at around 400 nm, which originates from CoQ_{10} (see Supporting Information). Thus, the aqueous suspension that passed through a 0.80 μm membrane filter contains CoQ_{10}.

Figure 6 shows the dependence of the pH on the water dispersibilities of Q10-Pep and Q10-Blank. The water dispersibility of Q10-Blank, which was prepared without Pep, was quite small. The water dispersibility of Q10-Pep is much higher than that of Q10-Blank. However, the turbidity of the filtrate of Q10-Pep decreased at pH around 5−6. The decrease of water dispersibility of Q10-Pep is due to the aggregation of Q10-Pep at a pH around the isoelectric point of Pep. The zeta potential of Q10-Pep supports this result. Thus complexation with Pep was found to enhance the water dispersibility of CoQ_{10}. The water dispersibility of CoQ_{10} increases with increasing the quantity of Pep for complexation, except under weakly acidic conditions. In the case of heme iron preparation, which is the complex between heme iron and peptide fragments, hydrophobic interaction is believed to be one of the major interactions between heme iron and peptides.[30] Similarly, a hydrophobic interaction should contribute to the

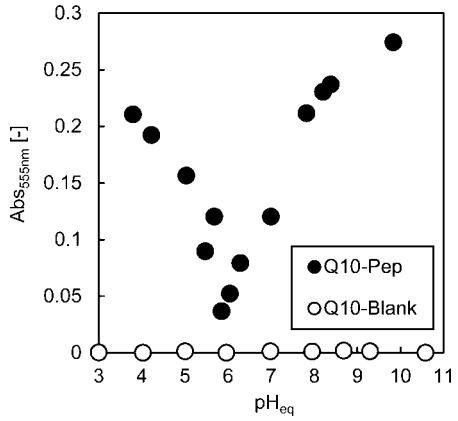

Figure 6. Effect of pH on the turbidity of the aqueous suspensions of Q10-Pep and Q10-Blank (sample, 5.0 mg; solution volume, 5.0 mL; pH 3−11).

complexation between CoQ_{10} and Pep. Albumin acts as the main fatty acid binding protein in extracellular fluids.[35,36] Plasma albumin possesses about seven binding sites for fatty acids with moderate to high affinity. Pep, which was prepared from BSA, should also have binding domains to interact with CoQ_{10} mainly by hydrophobic interaction. The reason that Pep was more effective in forming the water-dispersible complex than BSA is not clear. To understand the structure of Q10-Pep, the following experiments were conducted.

Material Distribution and Particle Size Analysis. The aqueous suspension of Q10-Pep was fractionated by centrifugation and filtration using membrane filters (⌀ 0.80, 0.45, and 0.20 μm).[37] Figure 7 shows a typical particle size

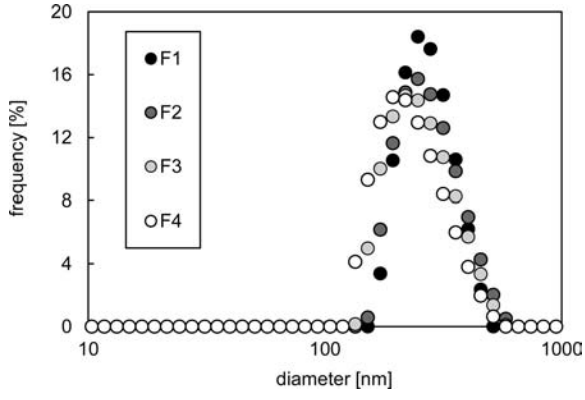

Figure 7. Particle size distribution of Q10-Pep in aqueous media determined using dynamic light scattering (DLS).

distribution of fractions F1−F4 of the aqueous suspensions of Q10-Pep (Figure 3) determined using DLS. The particle sizes for F1−F4 of Q10-Pep were 200−280 nm. Figure 8 shows the UV−vis spectra of the fractions F1−F4 of aqueous suspensions of Q10-Pep. The absorbance of Q10-Pep decreases from F1 to F4, indicating that the aqueous suspensions of Q10-Pep are gradually captured by membrane filters. Therefore, Q10-Pep is present as a colloidal material with wide particle size distributions. Here, the absorbance at 555 nm, a measure of the turbidity, is an index of the presence of Q10-Pep containing CoQ_{10}.[33,38,39] On the other hand, the absorption at 280 nm, which mainly originates from tryptophan residues, is an indication of the quantity of peptides present. Table 2

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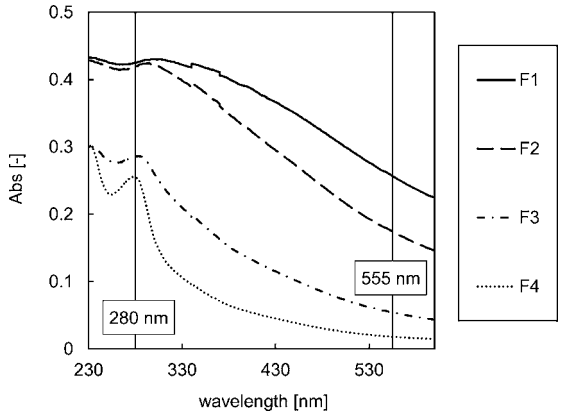

Figure 8. UV−vis spectra of fractions F1−F4 of the aqueous suspensions of Q10-Pep.

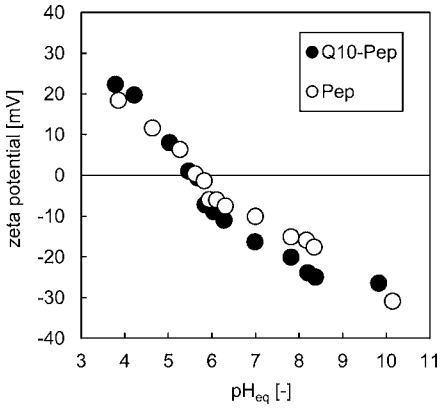

Figure 9. Zeta potential of aqueous suspensions of Q10-Pep and Pep as a function of pH.

summarizes the average particle size from DLS and absorbances at 555 and 280 nm by UV−vis for the fractions of Q10-Pep. The DLS results showed Q10-Pep formed colloidal structures with diameters of 200−280 nm. The $Abs_{555\ nm}/Abs_{F1,\ 555\ nm}$ value is the absorption ratio at 555 nm for each fraction against F1. As the particles of Q10-Pep were captured by filtration using 0.80, 0.45, and 0.20 μm membrane filters, the $Abs_{555\ nm}/Abs_{F1,\ 555\ nm}$ values for F2−F4 decreased from 0.680 to 0.074. On the other hand, the absorption ratio between 555 and 280 nm ($Abs_{555\ nm}/Abs_{280\ nm}$) for each fraction would be an index for the mass ratio between CoQ_{10} and peptide in each fraction. The $Abs_{555\ nm}/Abs_{280\ nm}$ values decreased by membrane filtrations giving fractions F1−F4. The result suggests that the mass ratio between CoQ_{10} and Pep is variable depending on the Q10-Pep particle size. Thus greater amounts of CoQ_{10} are incorporated in larger Q10-Pep particles compared with smaller Q10-Pep particles. From the results of mass distribution of CoQ_{10} and particle analysis by DLS for the fractions of Q10-Pep, the complex was found to exist as a hydrocolloidal material.

Zeta Potential of Q10-Pep. Figure 9 shows the zeta potential of aqueous suspensions of Q10-Pep and Pep as a function of pH.[40−42] The zeta potential of Q10-Pep decreased with increasing pH. As Pep consists of a mixture of peptides, Pep behaves as a mixture of polyelectrolytes. The zeta potential of Q10-Pep closely agrees with that of Pep. The result indicates that Pep dominates the surface charge of Q10-Pep. The zeta potential of Q10-Pep supports the dependence of the water dispersibility of Q10-Pep on pH shown in Figure 6: As the zeta potential of Q10-Pep at pH 5−6 approaches 0, the water dispersibility of Q10-Pep decreases due to the aggregation of Q10-Pep under weakly acidic conditions. As the absolute values of the zeta potential of Q10-Pep in acidic and basic conditions are close to 30 mV, the hydrocolloid of Q10-Pep is stable in aqueous media because of electric repulsion between particles.[43−45] Therefore, Q10-Pep particles are stably dispersed in acidic and basic conditions, but precipitate under weakly acidic conditions.

Differential Thermal Analysis for Q10-Pep. Differential thermal analysis was performed to evaluate the complexation between Pep and CoQ_{10}.[10,19−21,24] Figure 10 shows the DSC

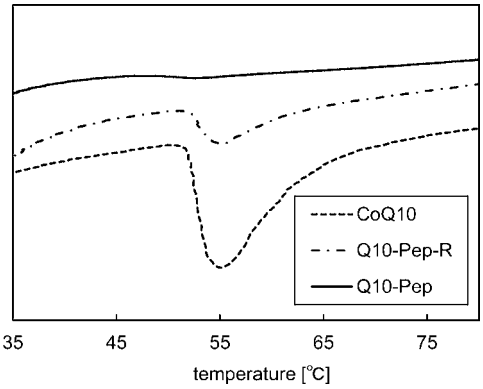

Figure 10. Differential scanning calorimetry (DSC) curves of CoQ_{10}, Q10-Pep-R, and Q10-Pep.

thermograms for CoQ_{10}, Q10-Pep-R, and Q10-Pep. The DSC thermogram of CoQ_{10} showed an endothermal peak at around 50 °C, attributed to the melting point of CoQ_{10} (48 °C). The DSC thermogram of Q10-Pep-R also showed an endothermal peak at around 50 °C, suggesting that CoQ_{10} in Q10-Pep-R is present as crystalline. In contrast, no endothermic peak at around 50 °C was observed for Q10-Pep. This suggests that CoQ_{10} in Q10-Pep is incorporated into the peptide matrix and is not present as a crystalline structure.

The water-dispersible complex is expected to enhance the oral bioavailability for poorly water-soluble CoQ_{10}. Permeability studies of CoQ_{10} in Q10-Pep through the Caco-2 cell

Table 2. Average Particle Size from DLS and Absorbance at 555 and 280 nm by UV−Vis for the Fractions of Q10-Pep

	DLS		UV−vis			
fraction	median diameter (nm)	cumulant diameter (nm)	$Abs_{555\ nm}$	$Abs_{280\ nm}$	$Abs_{555\ nm}/Abs_{F1\ 555\ nm}$	$Abs_{555\ nm}/Abs_{280\ nm}$
F1	252	285	0.2567	0.4251	1.000	0.604
F2	249	260	0.1744	0.4189	0.680	0.416
F3	231	227	0.0542	0.2854	0.211	0.190
F4	209	206	0.0189	0.2540	0.074	0.075

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monolayer are under way.[46,47] Moreover, the incorporation of CoQ_{10} in a peptide matrix is expected to enhance the photochemical stability of CoQ_{10}.

■ ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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