Emulsification of Coenzyme Q₁₀ Using Gum Arabic Increases Bioavailability in Rats and Human and Improves Food-Processing Suitability

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Summary We evaluated the characteristics of a coenzyme Q₁₀ (CoQ₁₀) formulation created with gum arabic. We defined the formulation's "modulus of inclusion," a reference index of the emulsified state, as the CoQ₁₀ not extracted by hexane as a percentage of the total CoQ₁₀ content of the formulation. The emulsified CoQ₁₀ formulation had a smaller particle size and larger modulus of inclusion value than the equivalent unemulsified formulation. In a kinetic study in rats, serum CoQ₁₀ levels were significantly greater with the emulsified CoQ₁₀ formulation than with the equivalent unemulsified formulation, which barely increased the levels. In a human study, oral intake of the emulsified formulation significantly increased plasma CoQ₁₀ levels, which peaked 6 h after intake, compared with the equivalent unemulsified formulation or CoQ₁₀ bulk powder. There was a significant positive correlation between baseline plasma CoQ₁₀ and total cholesterol levels, but no correlation was observed between absorption of CoQ₁₀ and baseline CoQ₁₀ levels. The emulsified CoQ₁₀ formulation was highly stable against heat and high humidity and in the presence of some materials (magnesium oxide, vitamin C, and vitamin E). In conclusion, emulsification of CoQ₁₀ using gum arabic increased bioavailability in both rats and humans and improved suitability for food processing.

Key Words CoQ₁₀, emulsification, bioavailability, rat, human

Coenzyme Q₁₀ (CoQ₁₀) is synthesized in the living body and is present in almost all cells, particularly in the organelles such as mitochondria, golgi bodies, and microsomes. It is involved in the electron-transport system for the synthesis of adenosine 5'-triphosphate (ATP), a source of biological energy (1–3). CoQ₁₀ that exists in a reduced form (as ubiquinol-10) outside the mitochondria has an antioxidative effect (4, 5) and is considered to be involved in protection against the aging caused by oxidative stress.

After oral intake, CoQ₁₀ is absorbed from the small intestine and transferred into the blood circulation (6). There have been many reports of CoQ₁₀ deficiency in patients taking statins for the treatment of hyperlipidemia (7, 8). In humans, CoQ₁₀ supplementation has produced improvements in tachycardia heart failure (9), exercise performance, and vitality (10, 11). In addition, skin-care effects (12), improvements in periodontal disease (13), and fatigue alleviation (14) have been reported.

CoQ₁₀ has been widely used as a dietary and health supplement because of its important biological roles.

Because diverse CoQ₁₀ supplements are now needed everywhere, the production of CoQ₁₀ formulations suitable for a variety of types of intake would provide more choice for CoQ₁₀ use as a dietary and health supplement. Improved bioavailability would reduce the intake required. Because of its lipophilia, CoQ₁₀ is poorly absorbed in humans when taken orally, especially under fasting conditions (15, 16). Therefore, various methods of emulsification with modified food starch (17), lecithin (18), gum arabic (19), or polysorbate 80 (18, 20), or inclusion of γ -cyclodextrin (15), have been used to decrease the hydrophobicity of CoQ₁₀. The strategy of emulsification of drugs to decrease hydrophobicity and boost bioavailability has already been used and reported (21, 22).

In addition, CoQ₁₀ is usually used in mixtures with various kinds of materials in food supplements. Therefore, it is important to develop CoQ₁₀ formulations that not only excel in bioavailability, but also excel in compatibility with various types of CoQ₁₀ processing. Here, we selected gum arabic, which is commonly and widely utilized as an emulsifier in food processing, and evaluated the characteristics of a CoQ₁₀ formulation emulsified with this substance.

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MATERIALS AND METHODS

Materials. CoQ-10kyowa20 [20% CoQ₁₀ formulation; CoQ₁₀ content 20% (w/w)] and CoQ-10kyowa (CoQ₁₀ bulk powder) were supplied by Kyowa Hakko Bio Co., Ltd. (Tokyo, Japan). CoQ-10kyowa20 is a product emulsified with gum arabic by high-pressure homogenization at 900 kg/cm². A mixture that was equivalent to CoQ-10kyowa20 but was unemulsified was prepared by mixing 72% gum arabic, 22% CoQ-10kyowa, and 6% methylcellulose under dry conditions. Other chemicals and solvents were of analytical grade.

“Modulus of inclusion” of CoQ₁₀ formulations. One hundred micrograms of CoQ₁₀ formulation was mixed with hexane to a total volume of 20 mL. After the mixture had been left for 30 min in the dark, the CoQ₁₀ content of the supernatant was quantified as extractable CoQ₁₀. The amount of CoQ₁₀ not extracted by the hexane was calculated as the difference between the CoQ₁₀ content before treatment and the extractable CoQ₁₀. The “modulus of inclusion” was defined as the amount of CoQ₁₀ not extracted by hexane as a percentage of the CoQ₁₀ content before treatment.

Kinetic study of oral administration in rats. Male rats (6 wk old, Sprague-Dawley) were maintained on 12 h dark/12 h light cycles (7:00–19:00), with free access to water and CE-2 diet (CLEA Japan, Inc., Tokyo, Japan). The rats were orally given CoQ-10kyowa20 (emulsified CoQ₁₀, group 1), CoQ-10kyowa (CoQ₁₀ bulk powder, group 2), or the unemulsified equivalent of CoQ-10kyowa20 (group 3) by feeding needle. Each CoQ₁₀ formulation was suspended in 0.5% methylcellulose 400 (Wako Pure Chemical Industries, Ltd., Osaka Japan). After 16 h of fasting, rats were orally given the CoQ₁₀ suspension at 10 mL/kg body weight (dose: 30 mg/kg CoQ₁₀). Blood samples were collected in tubes from rats in groups 1 and 2 before administration and 1, 2, 4, 6, 8, and 24 h after. Rats were fed after the 8-h blood collection. Blood samples were collected from group 3 rats 2 h after administration of the suspension. Ethanol (99.5%, 0.3 mL) was added to 0.3 mL serum and vortexed. The serum was then extracted three times with *n*-hexane. HPLC analysis was performed in accordance with a procedure described previously (23).

All the animal tests complied with the Animal Experiment Guidelines of the Healthcare Products Development Center of Kyowa Hakko Bio Co., Ltd. and were approved by the Animal Care Committee of the Center.

Kinetic study of oral administration in human subjects. This study was performed by the contract research organization TTC Co., Ltd. (Tokyo Japan) and had a double-blind, crossover design. The subjects were 12 healthy, nonsmoking male volunteers aged 20–29 y who had not taken any CoQ₁₀, vitamin supplements, or medication within the previous 4 wk. The volunteers received a single oral dose of 120 mg CoQ₁₀ in two different formulations, with a 2-wk interval between doses. CoQ-10kyowa20 (Emulsified CoQ₁₀) and CoQ-10kyowa (CoQ₁₀ bulk powder) were each encapsulated in four hard capsules. At each study, following at least 8 h of fasting, the subjects took the CoQ₁₀ capsules with a glass of water. Blood samples were collected in heparinized tubes before CoQ₁₀ intake and 2, 4, 6, 8, and 12 h after. Standardized meals were provided after the 4-h and 8-h blood collections. Plasma CoQ₁₀ levels (both reduced and oxidized forms) were measured by a method described previously (24). Plasma cholesterol levels in blood samples before CoQ₁₀ intake were also measured. LDL and HDL cholesterol levels were measured by a direct assay method using kits supplied by Sekisui Medical Co., Ltd. (Tokyo, Japan). Total cholesterol was measured by an enzymatic analysis method using kits supplied by Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Written informed consent was obtained from all volunteers. The procedures used for human testing complied with the Helsinki Declaration, as revised in 1983, and were approved by the Ethical Board of Kyowa Hakko Bio Co., Ltd. and Shinjyuku Oiwake Clinic Ethics Committee (Tokyo, Japan).

Analysis of CoQ₁₀ formulation properties. The oxidized CoQ₁₀ in the formulations was quantified. The formulation was dissolved in water, mixed with ethanol (99.5%), and extracted with *n*-hexane. HPLC analysis was performed in accordance with a method previously described (23). The particle size of the CoQ₁₀-containing formulations was measured by Sub-Micron Particle Analyzer (N4, Beckman Coulter, CA, USA). The food-processing suitability of the formulation was assessed in terms of preservation stability, thermal stability, hygroscopicity, and stability with coexisting materials. In the preservation stability study, CoQ-10kyowa20 was preserved at 5, 20, or 40°C for 6 mo and then the CoQ₁₀ content was quantified. In the thermal stability study, CoQ-10kyowa20 was enclosed in an aluminum bag and heated at 120, 140, or 160°C for 30 min. In the

Table 1. Characteristics of CoQ₁₀ formulations.

Formulation	CoQ ₁₀ content (%)	Modulus of inclusion (%)	Particle size (nm)	Cmax value in rat study (μg/mL)	AUC _{0–4h} in rat study (μg·h/mL)
CoQ ₁₀ bulk powder	100	0	—	0.035	0.123
20% CoQ ₁₀ formulation	20	98	536±62	0.310	0.749
Unemulsified 20% CoQ ₁₀ equivalent formulation	20	44	>1,000	0.067	0.197

Cmax: maximum plasma concentration, AUC_{0–4h}: 4-h area under the curve.

Fig. 1. Kinetic study of oral CoQ₁₀ administration in rats. A: Mean serum CoQ₁₀ concentrations after a single oral dose of CoQ₁₀. Each point represents the mean $\pm$ SE of 4 rats. B: Effect of emulsification of CoQ₁₀ formulation on absorbability in rats. Serum CoQ₁₀ concentrations 2 h after a single oral dose are shown. Each value represents the mean $\pm$ SE of four rats. * p < 0.05, ** p < 0.01, t -test versus CoQ₁₀ bulk powder.

Fig. 2. Kinetic study of oral CoQ₁₀ administration in humans. Net increase in CoQ₁₀ concentration in plasma after a single oral dose was plotted. Each point represents the mean $\pm$ SE of 12 subjects. ** p < 0.01, *** p < 0.001, t -test versus CoQ₁₀ bulk powder.

hygroscopicity study, CoQ-10kyowa20 was placed in a sealed container at 11, 33, 52, or 75% relative humidity for 18 d. To test stability with coexisting materials, CoQ-10kyowa20 was enclosed in an aluminum bag at a ratio of CoQ₁₀ (2%) with each material (50% magnesium oxide, 50% vitamin C, or 0.1% vitamin E); the mixture was made up to 100% with powdered sugar. The bag was incubated for 6 d at 60°C.

Fig. 3. Relationship between plasma cholesterol and CoQ₁₀. A: Scatter plot of baseline plasma cholesterol and CoQ₁₀. B: Scatter plot of baseline plasma cholesterol and increase in plasma CoQ₁₀ after intake of the 20% CoQ₁₀ formulation. Each point represents a subject. The correlation between baseline plasma CoQ₁₀ and total cholesterol was significant (test of correlation coefficient, p < 0.05).

RESULTS

Particle size and modulus of inclusion

The relationship between particle size and the modulus of inclusion of CoQ₁₀—a reference index of the emulsified state—were evaluated (Table 1). The 20% CoQ₁₀ formulation had a smaller particle size and larger modulus of inclusion of CoQ₁₀ than its unemulsified equivalent.

Kinetic study of oral administration in rats

The serum concentration of total CoQ₁₀ in rats given the 20% CoQ₁₀ formulation reached a peak 2 h (T_{max}) after administration, with a maximum plasma concentration (C_{max}) value of 0.310 μg/mL, whereas there was no peak in rats given the CoQ₁₀ bulk powder (Fig. 1A and Table 1). The total CoQ₁₀ represented the sum of both oxidized and reduced forms of CoQ₁₀. The 20% CoQ₁₀ formulation gave significantly greater serum CoQ₁₀ levels than did the CoQ₁₀ bulk powder 1, 2, and 4 h after administration (* p < 0.05 at 1 h, ** p < 0.01 at 2 and 4 h). The 20% CoQ₁₀ formulation gave an area under the curve for 4-h (AUC_{0-4h}) six times that given by the CoQ₁₀ bulk powder (Table 1).

To assess the mechanism behind the increased absorbability of the 20% CoQ₁₀ formulation, serum concentrations of total CoQ₁₀ 2 h after administration were compared among the three formulations (Fig. 1B). The

Fig. 4. Processing suitability of the 20% CoQ₁₀ formulation. A: preservation stability, B: thermal stability, C: hygroscopic property, D: stability with coexisting materials.

20% CoQ₁₀ formulation had a significantly higher serum concentration ($p < 0.05$) of total CoQ₁₀ than either the bulk powder or the unemulsified 20% CoQ₁₀ equivalent. Administration of the CoQ₁₀ bulk powder or unemulsified 20% CoQ₁₀ equivalent barely increased the serum CoQ₁₀ concentration level, which did not differ significantly between these two dosage forms.

Kinetic study of oral administration in human subjects

When the volunteers took the 20% CoQ₁₀ formulation, the T_{max} for the plasma concentration of total CoQ₁₀ was 6 h and the C_{max} value of 1.21 $\mu\text{g/mL}$, whereas the CoQ₁₀ bulk powder gave no peak. The values of C_{max} and AUC_{0-12h} in patients given the 20% CoQ₁₀ formulation were significantly higher than those in patients given the CoQ₁₀ bulk powder ($p < 0.01$). The changes in plasma CoQ₁₀ concentration from the initial value were plotted (Fig. 2). The 20% CoQ₁₀ formulation gave significantly greater plasma CoQ₁₀ levels 6, 8, and 12 h after intake than did the CoQ₁₀ bulk powder ($p < 0.01$ for 12 h, $p < 0.001$ for 6 and 8 h). There was a significant correlation between baseline plasma CoQ₁₀ and total cholesterol (test of correlation coefficient, $p < 0.05$, Fig. 3A). There was no correlation between absorption of CoQ₁₀ and either baseline CoQ₁₀ (data not shown) or plasma cholesterol level (Fig. 3B). Almost all the CoQ₁₀ quantified in the plasma was in the reduced form, not the oxidized form (data not shown).

Processing suitability of the 20% CoQ₁₀ formulation

To test the preservation stability of the 20% CoQ₁₀ formulation, the formulation was preserved at 5, 20, or 40 °C for 6 mo and then its content of CoQ₁₀ was quantified. The 6-mo incubation did not change the appearance, quality, or water dispersibility of the 20% CoQ₁₀

formulation. The residual percentage of CoQ₁₀ was over 90% at each temperature (Fig. 4A).

To test for thermal stability, the 20% CoQ₁₀ formulation was enclosed in an aluminum bag and heated at 120, 140, or 160 °C for 30 min. The residual percentages of CoQ₁₀ were over 90%, even at the highest temperature (Fig. 4B).

To test for hygroscopicity, the 20% CoQ₁₀ formulation was placed in a sealed container at 11, 33, 52, or 75% relative humidity for 18 d. Although increases in moisture absorption were observed at 33 to 75%, changes in solid state property were observed only at 75% (Fig. 4C).

CoQ₁₀ is unstable in the presence of some materials. More than 90% of the initial CoQ₁₀ in the presence of magnesium oxide or vitamin E, and 85% in the presence of vitamin C, survived after a 6-d incubation at 60 °C (Fig. 4D).

DISCUSSION

Various factors affect the bioavailability of CoQ₁₀. For example, when taken with meals, CoQ₁₀ is better absorbed than under fasting conditions owing to the action of secreted bile acids. Addition of the oils commonly used in CoQ₁₀ soft capsules, or of surfactants such as polysorbates, also increases the bioavailability of CoQ₁₀ (25, 26). The CoQ₁₀ formulation created with cyclodextrin, for example, has increased bioavailability (15, 27). Emulsification is important for increasing the bioavailability of this lipophilic substance. Moreover, because, in supplements, CoQ₁₀ is usually used in mixtures with a variety of food materials, it is important that we develop CoQ₁₀ formulations that excel in bio-

availability and are compatible with the various types of processing.

In the kinetic study in rats, the 20% formulation of CoQ₁₀ emulsified with gum arabic significantly boosted the serum concentration of total CoQ₁₀ compared with CoQ₁₀ bulk powder (Fig. 1A). To test how the emulsifying process affected CoQ₁₀ absorbability, we then compared the 20% CoQ₁₀ formulation with its unemulsified equivalent. The plasma CoQ₁₀ levels yielded by the unemulsified 20% CoQ₁₀ equivalent were as low as those yielded by the CoQ₁₀ bulk powder (Fig. 1B), suggesting that the presence of all the materials alone was insufficient, and that the emulsification process was important to increase bioavailability. Because we assumed a positive correlation between emulsified state and bioavailability of CoQ₁₀, we then evaluated the particle size and modulus of inclusion—a reference index of the emulsified state—for the 20% CoQ₁₀ formulation and the unemulsified equivalent (Table 1). The 20% CoQ₁₀ formulation had a small particle size and large modulus of inclusion, and these two variables were negatively correlated. The small particles of emulsified CoQ₁₀ may be absorbed more easily from the digestive tract. Surrounding the CoQ₁₀ particles with the gum arabic could prevent CoQ₁₀ from interacting with, and adhering to, each other.

Hatanaka et al. reported that a CoQ₁₀ formulation with gum arabic showed greater absorbability than crystalline CoQ₁₀ (19). They used an emulsified formulation of CoQ₁₀ with a mean particle size of 770 nm; it was prepared by homogenization at 500 kg/cm². On the other hand, because the formulation we used was homogenized at a pressure of 900 kg/cm² and had a mean particle size of 536 nm (Table 1), our formulation may have had greater bioavailability and stability than the formulation of Hatanaka et al. Homogenization pressure may be a critical factor in the stability of emulsified CoQ₁₀.

The absorbability of CoQ₁₀ formulations in human subjects was also tested. The 20% CoQ₁₀ formulation, compared with the CoQ₁₀ bulk powder, significantly increased plasma CoQ₁₀ levels (Fig. 2). Emulsification enabled the CoQ₁₀ to remain in aqueous solution after intake. In this state, a micellar structure including the CoQ₁₀ could easily form with secreted bile acid and could therefore be promptly absorbed from the digestive tract. Because the 20% CoQ₁₀ formulation, with its high absorbability and small particle size, also had a high modulus of inclusion, it may be possible to use this newly defined parameter to predict bioavailability.

Absorbed CoQ₁₀ is taken up into lipoproteins and transferred to the blood (6). There have been many reports of increased blood concentrations of CoQ₁₀ following its administration (28, 29). It has also been reported that CoQ₁₀ is, for the most part, carried to the liver and then excreted through the bile duct, so that only part of the amount ingested is carried to other organs (30). In a study in which ¹⁴C-labeled CoQ₁₀ was given intravenously to rats to investigate the distribution of exogenous CoQ₁₀, radioactivity in the mitochon-

dria—particularly in the inner membranes—increased with time for up to 72 h after administration and then remained stable for at least 22 d (31). Oral administration of CoQ₁₀ to patients who underwent coronary bypass surgery increased CoQ₁₀ levels in the mitochondrial fraction of the heart (29). Further investigation is necessary to reveal the details of CoQ₁₀ distribution.

The plasma concentration of total CoQ₁₀ in our human subjects peaked 6 h after intake of the 20% CoQ₁₀ formulation. The timings of this peak are similar regardless of whether or not subjects are fasting when they take CoQ₁₀ (16, 25). In a previous rat study, serum CoQ₁₀ also peaked 6 h after administration of CoQ₁₀ (16), but we tested our rats earlier than 6 h after administration and found that the T_{max} of serum CoQ₁₀ occurred 2 h after administration (Fig. 1A). Because, unlike in humans, the major form of CoQ in rats is not CoQ₁₀ but CoQ₉, exogenously administered CoQ₁₀ might be absorbed without physiological metabolism. Alternatively, this temporal difference could have resulted simply from the difference in body size.

Baseline CoQ₁₀ levels were almost the same in the same individual in two different trials, consistent with the results of a previous report (32). However, there was a difference among individuals in intrinsic CoQ₁₀ levels, as previously reported (15, 25, 26, 33), and also in absorbed CoQ₁₀ levels (34). In human studies, the experimental conditions largely affect the bioavailability of CoQ₁₀. For instance, two studies of the bioavailability of the same CoQ₁₀ formulation have exhibited different results (26, 35).

There was a significant positive correlation between baseline plasma CoQ₁₀ and total cholesterol (Fig. 3A), consistent with previous reports (26, 34). This may be derived from the fact that the two molecules share a common synthetic pathway. There was no correlation between absorption of CoQ₁₀ and baseline CoQ₁₀ level (Fig. 3B), suggesting that endogenous and exogenous CoQ₁₀ move into the blood in an independent manner. Moreover, a single dose of CoQ₁₀ might not be sufficient to affect the endogenous CoQ₁₀ state.

Almost all CoQ₁₀ quantified was in the reduced form of ubiquinol-10. This is consistent with a report that ubiquinol-10 is the most common form of CoQ₁₀ in vivo (36, 37).

The 20% CoQ₁₀ formulation was highly stable against heat and high humidity (Fig. 4B, C). The emulsification did not affect the stability of CoQ₁₀ in light (data not shown). Although CoQ₁₀ is unstable in the presence of some materials commonly used in various supplements (magnesium oxide, vitamin C, and vitamin E), the 20% CoQ₁₀ formulation was stable in their presence (Fig. 4D), suggesting that coating of CoQ₁₀ particles by gum arabic prevents their interaction with other materials. CoQ₁₀ by itself barely survived under the same conditions (data not shown). The undetected CoQ₁₀ was considered to be in the reduced form, because ubiquinol-10 is produced by the interaction of CoQ₁₀ with vitamin E and/or vitamin C in CoQ₁₀ supplements (38). The lipophilic part of the gum arabic

molecule is responsible for the molecule's emulsifying ability (39). Because this CoQ₁₀ formulation emulsified with gum arabic excels in processing suitability, its use should expand the range of uses of CoQ₁₀ in foods. The strategy of emulsification with gum arabic to reduce hydrophobicity should be useful not only for CoQ₁₀, but also for various drugs and food materials.

Although the physicochemical properties and absorbability of CoQ₁₀ emulsified with gum arabic have been studied previously in rats (18), the new information obtained here on the compound's absorbability in humans and stability under various conditions should be useful for the manufacture of products such as food supplements containing CoQ₁₀.

In conclusion, emulsification of CoQ₁₀ with gum arabic increased its absorbability in rats and humans and also improved its food processing suitability. The emulsified CoQ₁₀ formulation had a small particle size and large modulus of inclusion, a reference index of the emulsified state. Furthermore, the emulsified CoQ₁₀ was highly stable under various conditions, including heat, high humidity, and the presence of other materials.

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