**Review Article**

**Enhancement of Oral Bioavailability of Functional Ingredients by Complexation with Cyclodextrin**

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**Abstract**

Natural lipophilic bioactives that possess human health benefits, such as coenzyme Q10 (CoQ10) and R-α-lipoic acid (RALA), often have undesirable characteristics that limit their use as nutraceuticals and cosmeceuticals. These bioactives are usually unstable against oxygen, ultraviolet light, low pH and heat. Furthermore, their watersolubility is low because of their hydrophobic nature or instability and this leads to low bioavailability. Therefore, systematic studies have been performed to investigate improvements in the stability, water-solubility and bioavailability of lipophilic bioactives through complexation with cyclodextrins (CDs). The solubility of CoQ10 in water is extremely low, resulting in low bioavailability when administered orally. Bioavailability of CoQ10 was enhanced significantly by complexation with γ-CD. CoQ10 generally agglutinates, but the dissociated CoQ10 from γ-CD was captured by bile acid to form micelles without aggregation and therefore both solubility and bioavailability were enhanced. RALA is available as a functional food ingredient but is unstable to heat or acid. To stabilize RALA, complexation with γ-CD was investigated. RALA was unstable molecule, whereas RALA-γ-CD complex was stable under the acidic conditions of the stomach and was easily absorbed in the intestine. CD complexation is a promising technology as a formulation aid for oral delivery of insoluble or unstable ingredients such as CoQ10 and RALA.

**ABBREVIATIONS**

CD: Cyclodextrin; CoQ10: Coenzyme Q10; RALA: R-α-Lipoic Acid; SALA: S-α-Lipoic Acid; TCNa: Sodium Taurocholate; GZK2: Dipotassium Glycyrrhizate; Cmax: Maximum Plasma Concentration; AUC: Area Under the Plasma Concentration-time Curve; Tmax: Time to Reach Maximum Plasma Concentration; T1/2: Half-life

**INTRODUCTION**

Cyclodextrins (CDs) are non-reducing, chiral cyclic oligosaccharides, in which D-(+)-glucopyranose units are linked by α-(1,4)-glycosidic bonds to form a ring structure. Depending on the number of D-(+)-glucopyranose units, and thus also on the size of the ring, a distinction is made between α-CD, β-CD and γ-CD. α-CD consists of six units, β-CD consists of seven units and γ-CD consists of eight glucose units. α-CD is widely used as dietary fiber because it is not enzymatically digested and thus has no nutritional value. In contrast, γ-CD is degraded into monosaccharides by α-amylase and therefore functions as an energy source. CDs are capable of forming complexes with a variety of ionic and lipophilic substances by taking the entire molecule or part of the molecule into their cavity. The formation of such molecular complexes affects many of the physicochemical properties of the guest molecules, such as their aqueous solubility, stability or bioavailability [1-3].

Because the inside of CDs is hydrophobic and the outer surface is hydrophilic, CDs can enclose various hydrophobic substances in their cavities and form inclusion complexes. α-CD forms inclusion complexes with relatively smaller-sized molecules such as carbon dioxide gas and short-chain fatty acids (SCFAs) such as...
acetate, propionate and butyrate. SCFAs stimulate colonic blood flow, and fluid and electrolyte uptake [4]. β-CD forms inclusion complexes with middle-sized molecules including monoterpenes and flavonoids such as hesperidin, which is found abundantly in citrus fruits and acts as an antioxidant to contribute to the integrity of the blood vessels, and reduce LDL cholesterol and blood pressure [5,6]. γ-CD forms inclusion complexes with larger-sized molecules such as macrocyclic compounds and lipophilic vitamins including vitamins A, D, E, and K, coenzyme Q10 (CoQ10) and R-α-lipoic acid (RALA), which are used as nutraceutical ingredients in supplements and health foods having various human health benefits.

Irrespective of whether the guest molecule is in a gaseous, liquid, or solid state, the resultant inclusion complexes are always solid powders. Stable powders are much easier to handle than unstable volatile substances such as aromatic oils. Converting volatile and unstable active substances into inclusion complexes facilitates the process of adding precise amounts of the substances and storing them stably until use in medicinal food and cosmetic products.

CDs are generally used in nutraceuticals, pharmaceuticals and cosmetics for the following purposes:

- Solubilizing hydrophobic bioactive compounds in water-phase systems.
- Enhancing bioavailability for nutraceuticals used as preventive medicines, such as vitamins, CoQ10, curcumin and α-lipoic acid (ALA).
- Stabilization of unstable bioactive compounds like unsaturated fatty acids and carotenoids against oxidation, hydrolysis, photoreaction and thermal decomposition during storage.
- Reduction of the bitter taste and unpleasant smell of various plants extracts containing bioactive compounds such as catechin from green tea and capsaicin from chili peppers.
- Long lasting controlled release of medically active substances.

CoQ10 (Figure 1a) is a fat-soluble, vitamin-like, benzoquinone compound that functions as an antioxidant, as a membrane stabilizer and as a cofactor in the production of adenosine triphosphate (ATP) by oxidative phosphorylation. The solubility of CoQ10 in water is extremely low, resulting in very poor bioavailability when administered orally. Recently, it was found that the bioavailability of CoQ10 was enhanced by complexation with γ-CD, yielding the CoQ10-γCD complex, and the plasma CoQ10 level after a single oral administration of the CoQ10-γCD complex was extended [half-life, \(T_{1/2}\), increased] [7]. This long-lasting high CoQ10 concentration in plasma can provide various health benefits.

RALA (Figure 1b) is a cofactor for mitochondrial enzymes such as pyruvate dehydrogenase and alpha-keto-glutarate dehydrogenase, and thus plays a central role in energy metabolism [8,9]. ALA has a chiral center at its C6 carbon leading to two enantiomers: R- and S-ALA (SALA, Figure 1c), of which RALA is the naturally occurring compound.

Further, RALA and its dihydro-form, which is produced via metabolic reduction, are powerful antioxidants because of their radical scavenger properties and their synergistic interaction with other antioxidants. Dihydro-RALA, the reduced form of RALA, reduces glutathione, which is an important antioxidant for many physiological processes. As an amphiphile molecule, RALA is soluble in both aqueous and non-aqueous media. ALA is widely used as a pharmaceutical or nutraceutical ingredient.

Although it is possible to separate the ALA enantiomers (the bioactive RALA and SALA), commercially available ALA is the chemically synthesized racemate with the mixture containing the same amount of RALA and SALA. This occurs because the pure bioactive enantiomer RALA is unstable when exposed to low pH, light or heat [10]. RALA decomposes gradually at room temperature and easily polymerizes at temperatures higher than its melting point, which is 46–49 °C. Low pH also causes RALA to polymerize so quickly that it decreases RALA bioavailability. Therefore, finding solutions to stabilize RALA is of industrial interest and several studies aiming to stabilize RALA have been carried out. Among these attempts to stabilize RALA, for example, is complex formation or encapsulation with chitosan [11,12], which appears to be a promising approach. Unfortunately, encapsulation efficiency of chitosan with RALA has proven to be limited. Recently, it was found that complex formation with CDs was more efficient to stabilize RALA and enhance the bioavailability. The aim of this work was review of systematic studies in the stability, water-solubility, and bioavailability of lipophilic bioactives as like CoQ10 and RALA through complexation with CDs.

**RESULTS AND DISCUSSION**

**Coenzyme Q10**

The biological absorption of CoQ10 after oral administration of a CoQ10-γCD complex was greatly enhanced despite the poor soluble characteristics of the complex in water. The most reasonable explanation of this phenomenon is an increase in the water-solubility of CoQ10 by sodium taurocholate (TCNa), which is a major component of bile acid in the small intestine. By adding TCNa to a water suspension of the CoQ10-γCD complex, the guest molecule, CoQ10 is replaced with TCNa and forms a water-soluble TCNa-γCD complex. This occurs because TCNa
has a higher association constant with γ-CD than that of CoQ10. Generally, CoQ10 molecules agglutinate in water to form visible particles. However, a CoQ10 molecule dissociated from the γ-CD cavity can be soluble in water by micelle formation with TCNa. Here, the dissociated CoQ10 in water is captured by a TCNa micelle and locates to the central region of the micelle and is thus soluble [13,14].

Bioavailability enhancement of CoQ10 and its mechanism

The bioavailability of CoQ10 was enhanced by complexation with γ-CD showing a unique profile. The CoQ10-γCD complex shows excellent pharmacokinetic properties with a significantly higher area under the CoQ10 concentration curve in blood plasma from 0–48 hours (AUC) and higher maximum plasma concentration (Cmax) [7]. This complex also shows mean plasma levels even after 24 and 48 hours that are significantly higher after administration of CoQ10-γCD to healthy adult volunteers when compared with the same administration of commercially available CoQ10 formulation with dietary fatty oil-based emulsifiers (so-called “Water Soluble CoQ10”) and CoQ10 formulation with microcrystalline cellulose (MCC) (Figure 2). Moreover, administration of CoQ10-γCD also gives much longer T1/2 values when compared with the other two administered γ-CD formulations. This study was performed using 72 healthy human subjects and statistically calculated. Why was a significant enhancement of the bioavailability of CoQ10 observed despite the poor aqueous solubility of the CoQ10-γCD complex? The mechanism, as mentioned in the last section, is due to the significant increase in the aqueous solubility of CoQ10 with the aid of TCNa [13,14], as described in more detail below.

According to the study, by adding TCNa to a water suspension of the CoQ10-γCD complex, a water-soluble TCNa-γCD complex was formed by substitution of the guest molecule from CoQ10 to TCNa, which has a higher association constant with γ-CD than CoQ10. The association constants of γ-CD with TCNa and CoQ10 are 4800 M⁻¹ and 2200 M⁻¹, respectively [15]. The CoQ10 molecule dissociates from the γ-CD complex and this molecule is captured by a TCNa micelle to form a “nanometer molecular captured micelle”. In aqueous solution, the hydrophobic CoQ10 molecule generally agglutinates to form visible particles.

The aqueous solubility of CoQ10 through the combination of the CoQ10-γCD complex with TCNa was ~100 times higher than that of a commercially available oil emulsifier formulation, “Water Soluble CoQ10”, whose particle size was controlled to be around 100–200 nm in diameter [13,14]. The formation of the “nanometer molecular captured micelle” was found to facilitate a significantly higher AUC via effective absorption into intestinal epithelial cells. The same bioavailability-enhancing effect of complexing γ-CD with various hydrophobic nutraceuticals with human health benefits, such as curcumin and tocotrienols, was also achieved.

We applied this technique which combine CoQ10-γCD complex with TCNa in nutraceuticals to cosmeceuticals. Instead of TCNa, dipotassium glycyrrhizate (GZK) was used, because it had also high association constant with γ-CD, and similar chemical structure with TCNa (Figure 3) [16,17]. Here, a much higher CoQ10 absorption into a human epidermis structure model by formulation of a CoQ10-γCD complex with GZK, was observed when compared with other cosmetic formulations using liposome or fatty oil-based emulsifiers.

Study on water-solubility of CoQ10 captured in TCNa micelles after complete degradation of γ-CD by α-amylase

The water solubility and bioavailability of CoQ10 is enhanced significantly by combining the CoQ10-γCD complex with TCNa. The hypothetical mechanism is described above and presented in Figure (4). If the hypothesis is correct γ-CD would not affect the water-solubility of CoQ10 in the system. Therefore, to confirm this hypothesis, the water-solubility change in CoQ10 was evaluated after confirming a water-solubility increase in CoQ10 by adding TCNa to the CoQ10-γCD complex followed by the complete degradation of γ-CD by α-amylase [14].

To a buffer solution (pH 6.0), α-CD, γ-CD and pancreatic α-amylase were added. The solution was kept for 1 h at 37°C. More than 80% of the non-complexed γ-CD was degraded by pancreatic α-amylase, whereas α-CD (control) was not degraded. On the other hand, γ-CD encapsulating CoQ10 was not degraded after 24 h incubation in a solution containing the CoQ10-γCD complex and pancreatic α-amylase. Presumably the pancreatic α-amylase could not approach the γ-CD encapsulating CoQ10 to perform the enzymatic reaction because γ-CD did not exist in the liquid phase but in the solid phase owing to the insoluble characteristics of the CoQ10-γCD complex in water.

However, by adding TCNa to the solution, γ-CD encapsulating CoQ10 was completely degraded under the same conditions after 24 h. Then, pancreatic α-amylase can readily hydrolyze γ-CD because of the high water-solubility of the complex. Since γ-CD is converted into linear dextrins by this ring cleavage reaction, formation of a complex between CoQ10 and γ-CD can no longer occur. Therefore, CoQ10 that has dissociated from the CoQ10-γCD complex with the aid of TCNa could be sustainably absorbed in the small intestine because of the very slow degradation
reaction of γ-CD with pancreatic α-amylase while releasing CoQ10 at a slow rate. This most likely explains why the $T_{1/2}$ of CoQ10 plasma concentration after oral administration of the CoQ10-γCD complex was prolonged.

Studies on the solubility-enhancing effects of hydrophobic bioactives by adding TCNa or GZK$_2$ to the γ-CD complex

Effect of adding TCNa to the CoQ10-γCD complex: CoQ10 is poorly soluble in water because of its lipophilic side chain composed of 10 mono-unsaturated trans-isoprenoid units. Even after sonication, the water-solubility of CoQ10 is only 0.3μg/mL. Complex formation with γ-CD only enhances the water-solubility of CoQ10 by modest amount, with the CoQ10 concentration in the complex reaching 2.9 μg/mL. In contrast, the addition of TCNa to the CoQ10-γCD complex enhanced the solubility of CoQ10 significantly. The addition of TCNa to a cloudy water suspension of CoQ10-γCD complex led to the solution appearing as transparent clear solution. The CoQ10 concentration of the solution obtained from the formulation of CoQ10-γCD complex with TCNa was surprisingly high at 1147.5 μg/mL, which is more than 100 times higher than that of the "Water Soluble CoQ10" formulation using the amino acid cationic surfactant, CAE (11.4 μg/mL), as shown in Figure (5) [13,14].

Effect of adding GZK$_2$ to the CoQ10-γCD complex: The structure of TCNa consists of a hydrophilic region and a hydrophobic region. The hydrophobic region is entrapped by an excellent fit in the γ-CD cavity. The hydrophilic region is placed outside of the cavity and is accordingly thought to be the reason for the high water-solubility of the TCNa-γCD complex. GZK$_2$ is also known to form a water-soluble complex with γ-CD owing to its similar structure to TCNa, as described in Figure (3). Therefore, to examine this water-solubility feature of GZK$_2$, water-solubility changes in CoQ10 following the addition of GZK$_2$ to the CoQ10-γCD complex was compared with a mixture of pure CoQ10 and GZK$_2$. The water-solubility of CoQ10 was not enhanced by addition of GZK$_2$ to the suspension of pure CoQ10 in water. However, addition of GZK$_2$ to the CoQ10-γCD complex suspension increased the water-solubility of CoQ10, and this increase correlated with increasing amounts of added GZK$_2$. Significantly high CoQ10 water-solubility (more than 2000 μg/mL) was achieved with a higher GZK$_2$/CoQ10 molecular ratio than 10 (Figure 6) [14]. The results suggest that only one molecule of GZK$_2$ is required for the formation of the GZK$_2$-γCD complex, but ~10 molecules of GZK$_2$ are essential for solubilizing one molecule of dissociated CoQ10 by the formation of a molecular micelle of GZK$_2$ that entraps CoQ10.
CoQ10-γCD complex is the most effective natural CD complex for performing solubility and bioavailability enhancement of CoQ10 and other lipophilic bioactives in the small intestine because of the existence of bile acid, which has a high association constant with γ-CD as well as GZK₂. In fact, Takahashi et al. investigated CoQ10 absorption in humans (n = 5) by oral administration of its β-CD and γ-CD complexes containing 0.3 g of CoQ10 compared with uncomplexed CoQ10. This study showed that the highest bioavailability of CoQ10 was achieved when administered as a γ-CD complex [19].

Effect of adding GZK₂ and 1-adamantane carboxylic acid to three CoQ10-natural CD complexes: The water-solubility changes of CoQ10 in complex with three CDs following addition of GZK₂ are showing Figure (7A). An increasing molecular ratio of GZK₂ to CoQ10 gave a substantially higher CoQ10 concentration for the CoQ10-γCD complex (CoQ10γ) mixed with GZK₂ whereas no significant increase was observed when adding GZK₂ to the CoQ10-αCD (CoQ10α) or the CoQ10-βCD complexes (CoQ10β). This excellent solubility-enhancing effect of GZK₂ is probably because of the high association constant of GZK₂ toward γ-CD to form the GZK₂-γCD complex by substituting the guest molecule from CoQ10 followed by the formation of a CoQ10 molecular captured micelle with GZK₂. The association constants of GZK₂ with α-CD and β-CD are too weak to substitute CoQ10 for the formation of GZK₂-CD complexes.

1-Adamantane carboxylic acid (AdCA) is known to have an extremely high association constant with β-CD as well as a high association constant between GZK₂ and γ-CD [18]. Therefore, we evaluated the effect of AdCA on the water-solubility changes of CoQ10 by the combination of three natural CD complexes with GZK₂ [13,14]. As shown in Figure (7B), a significant increase in the CoQ10 solubility was observed by the addition of AdCA to the CoQ10β with GZK₂ with almost the same CoQ10 solubility increase observed when the γ-CD complex is combined with GZK₂. We believe that this observation supports our mechanism, in which, initially a GZK₂-γCD complex or AdCA-βCD complex is formed followed by CoQ10 molecular captured micelle formation with GZK₂. These results suggest that the CoQ10-γCD complex is the most effective natural CD complex for performing solubility and bioavailability enhancement of CoQ10 and other lipophilic bioactives in the small intestine because of the existence of bile acid, which has a high association constant with γ-CD as well as GZK₂.
**In vitro study on the epidermis absorption-enhancing effect of CoQ10 by adding GZK to its γ-CD complex**

A CoQ10 absorption study using an in vitro digestion Caco-2 cell model was reported by Bhagavan et al [20]. In their study, various commercially available CoQ10 formulation products were subjected to simulated digestion to mimic their passage through the gastrointestinal tract in order to generate micelles containing CoQ10 and bile acids such as taurocholate. The micelles prepared from the CoQ10 formulation products were added to monolayers of Caco-2 cells to determine the amounts of CoQ10 uptake. Their data demonstrated a significant enhancement of uptake of CoQ10 from the CoQ10-γCD complex when compared with that of pure CoQ10 powder and other formulations. Here, similar results were obtained using an in vitro human epidermis structure model. Importantly, high CoQ10 uptake by human epidermis cells (keratinocytes) was observed when a solution prepared from CoQ10-γCD complex with GZK, was applied. In comparison, other standard cosmetic formulations using liposome and fatty oil-based emulsifiers showed negligible uptake (Figure 8).

The processes of aging and photo-aging are associated with an increase in cellular oxidation. This is partly due to a decline in the levels of the endogenous cellular antioxidant CoQ10. Hoppe et al., demonstrated that topical application of CoQ10 is effective against UVA-mediated oxidative stress in human keratinocytes [21]. Furthermore, the topical application of CoQ10 was able to suppress significantly the expression of collagenase in human dermal fibroblasts and was effective in reducing wrinkle depth. However, in cosmetic formulations, a higher concentration of CoQ10 than 0.3 wt% was required to prevent many of the detrimental effects of photo-aging; despite the upper limit of CoQ10 concentration in cosmetics being set to 0.03 wt% in Japan. Accordingly, this finding may contribute to the development of effective photo-aging care products containing CoQ10.

**R-α-lipoic acid**

We have used α-CD, β-CD or γ-CD to stabilize RALA by complex formation. There are reports on how the physicochemical properties or characteristics of the lipophilic guest molecules such as the CoQ10, curcumin, astaxanthin and docosahexaenoic acid change when using γ-CD as a host molecule [22,23]. There is a study on the physicochemical properties of racemic ALA-CD complexes that shows that complex formation with CD improves ALA solubility and stability towards heat exposure [24]. However, in other stabilization technique for ALA such as complex formation with chitosan [21] the racemic ALA was analyzed, while in our studies we focus on the bioactive enantiomer RALA.

In our studies, we prepared RALA-CD complexes to stabilize RALA and have examined their formation, shape and stability under heated conditions using high-pressure liquid chromatography (HPLC) and scanning electron microscopy (SEM). Furthermore, focusing on the use of the RALA-CD complexes for oral consumption, we mimicked the stomach environment and evaluated the stability of the complexes under acidic conditions. Furthermore, the bioavailability of RALA-CD complexes was evaluated in animals and healthy human subjects.

**Physicochemical properties of the RALA-CD complexes**

RALA-CD complexes were prepared as described previously [25]. Pure RALA was dissolved in water and mixed with a corresponding molar amount of α-CD, β-CD and γ-CD to yield a 1:1 ratio. The solution was mixed well and the pH adjusted. The freshly prepared suspension was frozen overnight and lyophilized the next day. For the physical mixtures, RALA and the corresponding molar amounts of the different CDs were mixed to yield a 1:1 ratio.

We analyzed the prepared complexes using SEM with 300, 500, 1000 and 5000 times magnification. SEM experiments showed that the shape and aspect of the complex particles differed considerably and depended on the CD used for complex formation. Figure (9) shows SEM images with the highest magnification of RALA+CD physical mixtures and RALA-CD complexes. RALA+CD physical mixtures contain particles that appear cracked and wrinkled, and shapes that are uneven and particle sizes that vary considerably in the physical mixtures with the maximum RALA+CD physical mixture particle size exceeding 50μm. There was no considerable change observed between the physical mixtures with RALA in SEM analysis. In contrast, the particle size distribution of RALA-αCD, RALA-βCD and RALA-γCD complexes appeared to be more homogenous than in the RALA+CD physical mixtures (Figure 10). Particles of RALA-CD complexes form larger aggregates that appear to stack.

Additionally, the RALA-βCD and RALA-γCD complexes are shaped like prisms with parallel sides and in the case of the rod-shaped RALA-γCD particles, there are tetragonal and orthorhombic particles observed in the SEM images. While the
RALA-βCD and RALA-γCD complex particles have rather smooth surfaces, the particles that constitute the RALA-αCD complex are rougher and the shapes formed by these particles seldom show square and parallel surfaces. These results show that the morphological characteristics of the RALA-CD complexes are different from RALA+CD physical mixtures.

In the thermal stability test, RALA and RALA-CD complexes were exposed to 100% relative humidity for 30 min, 1 h, 2 h, 5 h, 24 h or 48 h at 70 °C, which is above the melting point of RALA. After incubation, the remaining RALA was measured by HPLC.

Apart from studying the stability of the RALA-CD complexes towards heat exposure, we also analyzed their stability under acidic conditions. Similar to heat and humidity, low pH also induces RALA polymerization, leading to poor absorption in the stomach. In order to imitate the acidic environment of the stomach, we exposed free RALA and the RALA-CD complexes to pH 1.2 and incubated the samples at 37 °C for 1 h. While free RALA was very unstable [43% of the incubated RALA remained], complex formation with any of the tested CDs improved the residual RALA significantly. However, RALA-αCD and RALA-γCD showed the highest stabilization of the incorporated RALA, with stability values of almost 100%.

From our physicochemical data including stability, we conclude that γ-CD is the best suited CD for RALA complex formation (Table 1). The RALA-γCD complex was the most stable towards heat, humidity and low pH, as determined by quantifying the residual RALA by HPLC. The morphology of the RALA-CD complexes changes upon complex formation and differs depending on the CD used for complex formation. The RALA-γCD complex formed the most distinctive type of particle, namely rod-shaped particles.

Further studies including in vivo experiments are required to explore the bioavailability and biological activity of the RALA-CD complexes in order to evaluate their potential use as nutraceuticals, pharmaceuticals and cosmeceuticals. We are currently studying the absorption mechanism of orally administrated RALA-CD in rats. McCormick and co-workers investigated the metabolism of dl-[1, 6-14C] lipoic acid in rats and found that most of the racemate is metabolized via β-oxidation of the valeric acid side chain [26]. Having found a way to feed enantiopure RALA, it would also be interesting to investigate whether this or its administration as a complex affects its metabolism.

Absorption after single oral administration of RALA-CD complexes in animals

Cremer et al. calculated that the 50% lethal dose and the no-observed-adverse-effect level for racemic ALA in rats to be more than 2000 mg/kg and 61.9 mg/kg/day, respectively, based on acute and subchronic toxicity studies [27]. Therefore, racemic ALA has been administered at doses between 600 and 1800 mg/day in many clinical trials [28-31]. On the other hand, Gal reported that SALA was more toxic than RALA in thiamine deficient rats [32]. Thus, RALA would be preferred to racemic ALA as a nutritional supplement owing to safety concerns. In this study, we stabilized RALA through complex formation with α-, β- and γ-CD, and the bioavailability of the prepared RALA-CD complexes were evaluated in rats. The plasma concentrations of RALA were measured after a single oral administration of RALA or RALA-CDs (20 mg R-LA/kg, 2 mL/kg, (Figure 11)) to rats, and the

<table>
<thead>
<tr>
<th>(Sample)</th>
<th>Thermal stability (70 °C, 48h)</th>
<th>Acidic stability (pH1.2, 37 °C, 1h)</th>
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<tr>
<td>RALA-αCD</td>
<td>98 ± 1.25%*</td>
<td>~100%**</td>
</tr>
<tr>
<td>RALA-βCD</td>
<td>69 ± 1.83%*</td>
<td>95 ± 0.14%*</td>
</tr>
<tr>
<td>RALA-γCD</td>
<td>~100%**</td>
<td>~100%**</td>
</tr>
</tbody>
</table>

* mean ± S.D.; ** Almost complete recovery. RALA-αCD: R-α-lipoic acid/α-cyclodextrin inclusion complex; RALA-βCD: R-α-lipoic acid/β-cyclodextrin inclusion complex; RALA-γCD: R-α-lipoic acid/γ-cyclodextrin inclusion complex.
Table 2: Pharmacokinetic parameters of R-α-lipoic acid after oral administration of R-α-lipoic acid or R-α-lipoic acid/cyclodextrin complexes to rats.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>RALA</th>
<th>RALA-αCD</th>
<th>RALA-βCD</th>
<th>RALA-γCD</th>
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<tbody>
<tr>
<td>Route</td>
<td>po</td>
<td>po</td>
<td>po</td>
<td>po</td>
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<tr>
<td>Dose (mg/kg)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>C_{max} or C_{0}</td>
<td>1.7 ± 0.9</td>
<td>1.4 ± 0.6</td>
<td>1.6 ± 1.9</td>
<td>3.4 ± 2.5</td>
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<tr>
<td>T_{max} (min)</td>
<td>11.8 ± 14.1</td>
<td>10.7 ± 10.7</td>
<td>33.3 ± 44.0</td>
<td>9.0 ± 10.7</td>
</tr>
<tr>
<td>AUC_{0-t} (µg·min/mL)</td>
<td>56 ± 35°</td>
<td>56 ± 12°</td>
<td>50 ± 19°</td>
<td>121 ± 24°</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters are shown as mean ± S.D. (n = 6). RALA: R-α-lipoic acid; RALA-αCD: R-α-lipoic acid/α-cyclodextrin inclusion complex; RALA-βCD: R-α-lipoic acid/β-cyclodextrin inclusion complex; RALA-γCD: R-α-lipoic acid/γ-cyclodextrin inclusion complex; C_{max}: maximum plasma concentration; C_{0}: initial concentration; T_{max}: time of maximum drug concentration; AUC_{0-t}: area under the plasma concentration versus time curve (from initial to last points); po: per os; and °: P < 0.05 compared with RALA-γCD. Statistical analysis was performed using analysis of variance followed by Tukey’s multiple comparison tests.

Figure 11 Plasma concentration-time profiles of RALA after oral administration of RALA (A), RALA-αCD complex (B), RALA-βCD complex (C) and RALA-γCD complex (D) and after intravenous administration of RALA sodium salt (RALA-Na) (E) to rats. Data are shown as mean ± S.D. (n = 6).

pharmacokinetic parameters were calculated (Table 2). Although there were no significant differences in the C_{max} and time to reach maximum plasma concentration (T_{max}) among the groups after oral administration, the AUC_{0-t} of RALA after administration of the RALA-γCD complex sample was higher than that of the other complexes (p < 0.05, Table 2).

We reported that RALA-γCD was suitable for pharmaceutical formulation when considering pharmaceutical processing [25]. On the other hand, there have been many reports that CD complexes can achieve good oral bioavailability of poorly absorbable drugs [33]. Specifically, several studies have revealed that γ-CD complexes enhanced the bioavailability of drugs such as digoxin in dogs, diazepam in rabbits, and artemisinin and CoQ10 in humans [7,34-36], but there have been no studies on RALA. In this study, comparing the AUC 0-120min of RALA after oral administration of α-, β-, and γ-CD complexes to rats, it was found that RALA-γCD showed the highest plasma exposure among the groups (Figure 11), (Table 2). This result demonstrated that RALA by γ-CD inclusion was the preferable complex to the other CDs with respect to oral absorption. Then, the detailed mechanisms by which complex formation with γ-CD could enhance absorption of RALA were evaluated.

To examine the absorption from the stomach, non-complexed RALA or the RALA-γCD complex was administered to rats after pylorus ligation; plasma concentrations in excess of the endogenous level of RALA were detected only 2 min after dosing with either formulation [37,38]. This result indicates that RALA is rapidly absorbed from the stomach regardless of the formulation in rats. Furthermore, the concentration profiles of the two formulations were almost equal in extent and velocity. A similar observation was reported in an in situ absorption study by Peter and Borbe [39]. Moreover, the gastric pH of humans in the fasted state is ~1.2, whereas that of rats is ~4.0 [40,41]. Therefore, RALA in the rat stomach was sufficiently stable, so that there was no difference in the absorption from the two formulations. When administered to humans, RALA-γCD might be more stable in the stomach than RALA and may achieve higher exposure.

On the other hand, the absorption of RALA was greater...
after intraduodenal administration of RALA-γCD than after administration of non-inclusion RALA ($p < 0.05$). Furthermore, results of X-ray imaging using a contrast medium (data not shown) suggested that both formulations reach the small intestine within a few minutes after oral administration. These results showed that the difference in absorption between RALA-γCD and non-complexed RALA occurred mainly in the small intestine, even at <5 min after oral administration (data not shown). As anticipated, a large portion of the administered dose was retained in the stomach at 30 min, leading to AUC values after oral administration (data not shown).

Results showed that the difference in absorption between RALA-γCD and non-complexed RALA occurred mainly in the small intestine within a few minutes after oral administration. These results indicate that some factors in the small intestine enhance RALA absorption by γ-CD complex formation.

In 2012, Uekaji et al., reported that CoQ10, as a guest molecule in γ-CD complexes, was liberated by bile acids to form micelles with bile acids and represents a mechanism for enhancing absorption of CoQ10 [13]. If the same process also occurs in the rat intestine in the case of RALA, liberation of RALA molecules could increase and be absorbed effectively. However, the AUC of RALA after intraduodenal administration of RALA-γCD was no different between the bile duct ligation (BDL) group and the respective sham operation group, indicating that bile acid did not associate with RALA after liberation from γ-CD.

The complex form with a guest molecule and its liberation from CD was reversible and in equilibrium [42]. Therefore, γ-CD, which releases the guest molecule, would be present at defined proportions in the small intestine. If γ-CD in the small intestine were digested to maltose or glucose by pancreatic amylase, liberation of RALA molecules from γ-CD would likely be promoted and the plasma RALA concentration could increase. Therefore, we elucidated whether α-amylase activity had an effect on the liberation of RALA from γ-CD and on its absorption. However, the AUC$_{0-\infty}$ of RALA after intraduodenal administration of RALA-γCD was no different between groups treated with and without acarbose, an α-amylase inhibitor (data not shown). This result indicated that α-amylase activity was also not associated with the absorption of RALA after its liberation from γ-CD.

The pharmacokinetic parameters after intraduodenal administration of RALA-γCD and the water-soluble RALA-sodium salt were almost comparable, i.e., RALA-γCD dissolved immediately in small intestinal fluid and RALA might be liberated continuously from the complex. From the study by Trentin on the stability constant of the lipoate anion with CDs [43], γ-CD had a lower stability constant than α- or β-CD. This lower stability is because γ-CD has the biggest cavity among the CDs. According to these results we consider the dissolution process to be a key factor in the mechanism for enhancement of RALA-γCD, and not the liberation of RALA from γ-CD.

Furthermore, we also considered whether CD increases the paracellular permeability of intestinal membranes by opening the tight junctions. Several reports have revealed that the permeability of nasal or cutaneous membranes to drugs was enhanced by β-CD [44,45]. Those studies, however, required pre-treatment for a few hours to open the tight junctions. However, RALA was rapidly absorbed after administration and thereby this mechanism was thought to be not associated with the enhancement of RALA absorption.

**Plasma pharmacokinetics of RALA-CD in healthy human subjects**

It is well documented that 600 mg of racemic ALA has been dosed in pharmacokinetic or pharmacology studies [46-48], and in many clinical trials, racemic ALA has been administered at doses between 600 and 1800 mg/day to diabetic patients [28-31]. Breithaupt-Grögler et al. monitored the plasma ALA concentration 10 min after dosing and demonstrated that the area under the AUC showed dose linearity after single doses of 50 to 600 mg ALA in the fasted volunteers. They also showed that there were no drug-induced effects in healthy male subjects [47]. Gleiter et al., showed that food intake influenced the bioavailability of ALA and the mean plasma ALA concentration of the fed healthy volunteers who received a single dose of 600 mg racemic ALA was lower than that of the fasted healthy volunteers [46]. Gleiter et al., and Breithaupt-Grögler et al., also reported in 1996 and 1999, respectively, that the bioavailability of the RALA enantiomer was higher than that of SALA when a racemic mixture of ALA (RALA:SALA=50:50) was administrated [46,47]. Our recent animal study showed similar enantioselective pharmacokinetics of ALA [49]. Carlson et al., (2007) conducted a clinical study to determine the pharmacokinetics of RALA in healthy human subjects administered with 600 mg RALA as its sodium salt[50]. This was the first reported study to demonstrate the pharmacokinetics of enantiopure RALA. Since then, several pharmacokinetic clinical studies of RALA have been conducted, primarily in Germany and the United States, but there has been no scientific research conducted in Japanese subjects. In this study, we were aiming to evaluate the bioavailability of RALA-CD in healthy human subjects and analyze the pharmacokinetics of the plasma RALA levels after a single oral administration of 600 mg RALA or 6 g RALA-CD (equivalent amount of 600 mg of RALA) as described in our report [57].

The mean plasma RALA concentration versus time profiles after oral administration of RALA or RALA-γCD is shown in Figure (12). The pharmacokinetic (PK) parameters are listed in Tables (3,4). For all subjects, the plasma concentrations of RALA after a single oral dose of RALA-γCD were significantly higher than those

![Figure 12 Plasma concentrations of RALA following a single oral dose of 600 mg RALA or 6 g RALA-γCD in healthy volunteers. Values are the mean ± S.D. from six subjects. Note: 6 g RALA-γCD is the equivalent amount of 600 mg RALA.](image-url)
of RALA at 5, 15, 30, 45 and 60 min after oral administration. At every measured point, the mean plasma concentrations of RALA after an oral dose of RALA-γCD were higher than those of RALA. The mean Cmax values were 1.68 ± 1.01 and 4.10 ± 0.96 µg/mL (mean ± S.D.) for RALA and RALA-γCD administration, respectively. The Cmax value of RALA-γCD was significantly higher than that of RALA. The mean AUC0–180min (µg min/mL) values were 78.0 ± 43.5 and 195.9 ± 17.7 µg·min/mL (mean ± S.D.) for RALA and RALA-γCD administration, respectively. The mean AUC0–100min, of RALA-γCD was 2.5 times higher than that of RALA and was also statistically significant. The Tmax and the T1/2 were not different between RALA-γCD. At 180 min, the mean plasma RALA concentrations had returned to nearly base line levels for both RALA and RALA-γCD.

RALA-CD was stable under acidic conditions found in the stomach and could be easily absorbed in the intestine. The mean AUC0–100min of RALA in the subjects orally administered 6 g of RALA-CD (equivalent amount of 600 mg RALA) was 2.5 times higher than that of the subjects administered 600 mg of RALA. Thus, our results suggest that complexation of RALA with γ-CD significantly enhanced its bioavailability in healthy human volunteers, making it a promising technology for delivering functional but unstable ingredients like RALA. Additionally, there were no drug-induced side effects observed. These results indicate that 6 g of RALA-CD is suitable for nutraceutical purposes.

**MATERIALS AND METHODS**

CoQ10 was obtained from Mitsubishi Gas Chemical Company, Inc. (Tokyo, Japan). CoQ10-γCD complex containing 20 % (wt/wt) CoQ10 was supplied from Wacker Chemie AG (München, Germany) as product named CAVAMAX® W8 CoQ10. CoQ10-γCD complex and CoQ10-βCD complex were prepared by a conventional spray-dry method. RALA was purchased from Toyo Hakko Co., Ltd (Obu, Japan). CAVAMAX® W6 FOOD (α-CD), CAVAMAX® W7 FOOD (β-CD) and CAVAMAX® W8 FOOD (γ-CD) were purchased from Wacker Chemie AG (München, Germany). GZKα was donated by Tokiwa Phytochemical Co., Ltd. (Chiba, Japan). TCNa was purchased from Wako Pure Chemical Industries, Ltd., (Osaka, Japan). AdCA was purchased from Tokyo Chemical Industry Co., Ltd., (Tokyo, Japan). The human three-dimensional cultured epidermal model “LabCyte EPI-MODEL” was purchased from Japan Tissue Engineering Co., Ltd. (Aichi, Japan). This is multi-layered epidermal model cultured by using normal human skin cells. It consisted of multiple and viable cell layers and contained basal layer, stratum spinosum epidermidis, granular layer and stratum corneum.

**Bioavailability enhancement of CoQ10 and its mechanism**

This open-label, single-dose crossover study was conducted as previously described [7]. Three different formulations, CoQ10-γCD complex, CoQ10 with fatty oil based emulsifier so called “Water Soluble CoQ10” and CoQ10-MCC mixture were comparative. The concentrations of plasma CoQ10 were calculated by changing all of the contents of CoQ10 into reduced form (ubiquinol), which was determined using an HPLC with an electrochemical detector and online reduction system [7].

**Study on water-solubility of CoQ10 captured in TCNa micelles after complete degradation of γ-CD by α-amylase**

500 mg of CoQ10-γCD complex and 90 mg of α-CD were added to 15 mL of MES buffer with 165 mg of TCNa in vials. α-CD was degraded to TCNa micelles after complete degradation of γ-CD by α-amylase.

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**Table 3: Pharmacokinetic parameters of individual subjects administrated with a single oral dose of 600 mg R-α-lipoic acid or 6 g R-α-lipoic acid/γ-cyclodextrin.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>RALA</th>
<th>RALA-γCD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cmax (µg/mL)</td>
<td>AUC0–100min (µg min/mL)</td>
</tr>
<tr>
<td>1</td>
<td>1.02</td>
<td>43.6</td>
</tr>
<tr>
<td>2</td>
<td>1.03</td>
<td>46.7</td>
</tr>
<tr>
<td>3</td>
<td>1.83</td>
<td>74.9</td>
</tr>
<tr>
<td>4</td>
<td>1.02</td>
<td>53.8</td>
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<tr>
<td>5</td>
<td>3.62</td>
<td>158.8</td>
</tr>
<tr>
<td>6</td>
<td>1.55</td>
<td>90.5</td>
</tr>
</tbody>
</table>

RALA-γCD: R-α-lipoic acid/γ-cyclodextrin; RALA: R-α-lipoic acid; Cmax: maximum plasma concentration; and AUC: area under the plasma concentration-time curve.

**Table 4: Pharmacokinetic parameters for subjects orally administered with 600 mg R-α-lipoic acid or 6 g R-α-lipoic acid/γ-cyclodextrin.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>CoQ10</th>
<th>RALA</th>
<th>RALA-γCD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cmax (µg/mL)</td>
<td>AUC0–180min (µg min/mL)</td>
<td>Cmax (µg/mL)</td>
</tr>
<tr>
<td>1</td>
<td>1.68 ± 1.01</td>
<td>20.8 ± 10.7</td>
<td>17.5 ± 6.1</td>
</tr>
<tr>
<td>2</td>
<td>20.8 ± 12.2</td>
<td>23.3 ± 10.3</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean ± S.D. from six subjects, **p<0.01; RALA-γCD: R-α-lipoic acid/γ-cyclodextrin; RALA: R-α-lipoic acid; Cmax: maximum plasma concentration; and AUC: area under the plasma concentration-time curve; T0.5: time to the maximum plasma concentration; T1/2: half-life.
is known as a non-degradable dextrin by α-amylase and is hence used as a standard substance to evaluate the degradation of γ-CD. After shaking at room temperature for a 1 hour, the resultant suspension was filtered through a 0.2 μm filter. 12 mL of the filtrated solution was pre-incubated at 37°C for a few minutes. Then 200 μL of pancreatic α-amylase reagent was diluted five times beforehand with MES buffer, and was added to the solution at 37°C in a water bath. At 0 and 60 minutes after the addition of diluted pancreatic α-amylase, each 0.5 mL of the suspension was taken into 1 mL of dimethylformamide in micro tubes. They were heated at 90°C for 5 minutes, and filtrated through a 0.2 μm filter. A Shimadzu HPLC system (LC-2010C, Shimadzu Corporation, Kyoto, Japan) was used for the content measurement of α-CD and γ-CD. An X-chipine HPLC column (amide: 4.6 mm i.d. × 150 mm) was used. Column temperature was set at 30°C. The mobile phase of acetonitrile : distilled water (65:35) was used at a flow rate of 0.9 mL/min. CDs were detected using a RI detector [14].

**Effect of adding TCNα to the CoQ10-γCD complex**

CoQ10 samples were prepared as previously described [13,14]. A Shimadzu HPLC system (LC-2010C, Shimadzu Corporation, Kyoto, Japan) was used for the measurement of CoQ10 concentration in the aqueous solution. A Phenomenex HPLC column (Luna 5μ C18(2) 100Å: 4.6 mm i.d. × 150 mm) was used. Column temperature was set at 35°C. A mixture of methanol, ethanol, and distilled water (80:10:10) was used as the mobile phase with a flow rate of 0.8 mL/min. CoQ10 was detected using an UV detector at 275 nm.

**Effect of adding GZKα to the CoQ10-γCD complex**

1000 μg/mL of CoQ10 were mixed with 1 mL of Milli-Q water (3 mL). Pure CoQ10 (12 mg) and GZKα (molar ratios against CoQ10 are 0, 0.5, 1, 2.5, 5, 10 and 20) was added to the vials, followed by the addition of Milli-Q water (3 mL). Pure CoQ10 (12 mg) and GZKα (molar ratios against CoQ10 are 0, 0.5, 1, 2.5, 5, 10 and 20) was added to the vials, followed by the addition of Milli-Q water (3 mL). These resultant suspensions were sonicated for 30 minutes, and filtered through a 0.2 μm PTFE filter to obtain a transparent solution containing CoQ10. The concentrations of CoQ10 for all samples were measured by HPLC. The same Shimadzu HPLC system (LC-2010C, Shimadzu Corporation, Kyoto, Japan) was used as in the above experiment [14].

**Effect of adding GZKβ and AdCA to three CoQ10-natural CD complexes**

The CoQ10-natural CD complex (10 mg) was weighed in a vial. GZKβ (molar ratios against CoQ10 are 0, 0.5, 1, 2.5, 5, 10 and 20) was added to the vials, followed by the addition of Milli-Q water (5 mL). The resultant suspensions were sonicated for 30 minutes, and filtered through a 0.2 μm PTFE filter to obtain a transparent solution containing CoQ10. The concentrations of CoQ10 for all samples were measured by HPLC. The same Shimadzu HPLC system (LC-2010C, Shimadzu Corporation, Kyoto, Japan) was used as in the above experiment [13,14].

**In vitro study on the epidermis absorption-enhancing effect of CoQ10 by adding GZKβ to its γ-CD complex**

CoQ10 samples containing 1000 μg/mL of CoQ10 were prepared as previously described [13,14]. The tissue cultures were preincubated for 18 hours at 37°C in a 5% CO₂ environment. Then the tissues were placed into a well plate with 1 mL assay medium dispensed. After each 0.2 mL of test materials had been added to the tissues, they were incubated for more than 6 hours at 37°C in a 5% CO₂ environment. The tissues were rinsed five times with 1 mL of 0.1 M phosphate-buffered saline (PBS). The content of absorbed CoQ10 in the tissues was extracted with 5 mL of chloroform : methanol (1 : 1) by shaking for 30 minutes. Then the extracted solvent was evaporated using a centrifugal concentrator. After drying, to the evaporated residue was added 0.7 mL ethanol including 0.1 mL of iron chloride in ethanol solution (1 mg/mL) as an oxidizing reagent. This was filtered through a 0.2 μm PTFE filter, and its CoQ10 content in the tissue culture was measured with a Shimadzu LCMS system (LCMS-2020, Shimadzu Corporation, Kyoto, Japan). A Phenomenex HPLC column (Luna 5 μm C18(2) 100 Å: 4.6 mm i.d. × 150 mm) was used. Column temperature was set at 35°C. The mobile phase was used with a mixture of acetonitrile and isopropanol (8:7) containing 0.5% formic acid and 0.1% triosodium citrate aqueous solution (1 mg/mL), with a flow rate of 0.2 mL/min. The mass spectrometer fitted with electro-spray ionization (ESI) source was used for analysis. It was operated in the positive ion mode with the following parameters: probe voltage +4.50 kV (+ESI), nebulizer gas flow 1.5 L/min, drying gas flow 15.0 L/min, block heater 200°C, DL temperature 250°C.

**Measurement of RALA content by HPLC**

RALA contents in the RALA-CD complexes were analyzed by HPLC using a chiral column (CHIRALPAK AD-RH Daicel; 4.6 mm i.d. x 150 mm) with a mobile phase consisting of 5 mM H₃PO₄/acetonitrile (70:30, v/v) at a flow rate of 0.6 mL/min and at a temperature of 25°C. Lipoic acid was detected at 215 nm based on current literature [25] and the injection volume was 10 μL. racemic DL-alpha lipoic acid (Fluka, Sigma-Aldrich, MO, USA) was used as a standard; the stock solution was prepared at 0.5 mg/mL in 25 mM H₃PO₄ buffer (pH 3.5) / acetonitrile (50:50, v/v) and filtered (ADVANTEC DISMIC-25, 0.2 μm).

**Measurement of plasma RALA content by LC-MS/MS**

Plasma concentrations of RALA were determined using an API 3200™ (AB SCIEIX, Framingham, MA, USA) liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) system interfaced with a Shimadzu Prominence HPLC system (Shimadzu, Kyoto, Japan) as previously described [37].

**Morphological characterization via scanning electron microscopy (SEM) analysis**

For scanning electron microscopy analysis, the RALA-CD complexes were sprinkled onto conductive glue on a palladium SEM stub and sputter coated with gold for 3 min. Then, the RALA-CD complexes were measured at 15 kV with the SEM S-4500 (HITACHI, Tokyo, Japan), for morphology analysis [25]. Three different fields within each sample were randomly chosen and 4 images of each field were taken at the magnifications 300, 500, 1000 and 5000 giving a total number of 12 images per sample.

**Particle size distribution of RALA-CD complexes**

The particle size analysis of RALA-CD complexes using SEM data was conducted by software Scandium (OLYMPUS, Japan).
Tokyo, Japan). The unit length was set on the SEM picture and calibration was done. Particles were automatically detected using the contrast by the software. Then the detected particles were painted and the area of the painted particles was calculated by the software.

CONCLUSIONS

Natural lipophilic bioactives possessing human health benefits, such as CoQ10 and RALA, often have unfavorable characteristics with respect to their use as nutraceuticals and cosmeceuticals. They are usually unstable against oxygen, ultraviolet light, low pH and heat. Their low water-solubility or instability leads to low bioavailability to the human body. Therefore, studies have investigated improvements in the stability, water-solubility and bioavailability of lipophilic bioactives through complexation with CDs. As a breakthrough in a series of such studies, a new nanotechnology for nanomedicinal food and personal care applications, “nanometer molecular captured micelle formation”, has been developed using the combination of TCNa (bile acid) or GZK2 with γ-CD complexes of bioactives.

Previously known micro- or nano-encapsulation technologies in the food and cosmetic fields are generally based on approaches that discover how to minimize particle size physically, followed by micelle formation or encapsulation using liposome or dietary emulsifiers such as fatty acid base surfactants. The bioavailability of lipophilic bioactives, e.g., CoQ10, are enhanced by improving their dissolution rates using such nano-encapsulation technologies [51].

On the other hand, this new nanotechnology (“nanometer molecular captured micelle formation” by the combination of TCNa (bile acid) or GZK2 with γ-CD complexes of bioactives) is a completely different approach from the preparation of nanoparticles starting from a single molecular capsule, a nanometer host-guest complex. The addition of TCNa or GZK2 to the water suspensions of lipophilic bioactives does not help solubilize them because of their sizable and visual particle formation characteristics in water. However, bioactives can be complexed with γ-CD for molecular separation, which leads to the formation of nanometer molecular captured micelles (Figure 13). As a result, the bioavailability-enhancing effect of CoQ10 via intake of a CoQ10-γCD complex was much higher.

This innovative molecular captured micelle formation nanotechnology, introduced here, which is useful for the enhancement of bioavailability and epidermis permeability, is applicable not only for CoQ10 but also for other lipophilic...
bioactives such as curcumin and tocotrienols.

The absorption of RALA appears to differ to that of CoQ10 absorption. The plasma RALA profile after a single administration of RALA-CD in healthy human subjects changed from that observed after a single administration of non-complexed RALA, which is different to the change observed with CoQ10. RALA in the absence of a host carrier is unstable, whereas RALA-CD is stable under the acidic conditions found in the stomach and was easily absorbed in the intestine. The mean AUC0-180 min of RALA in the subjects orally administered 6 g of RALA-CD (equivalent amount of 600 mg RALA) was 2.5 times higher than that of the subjects administered 600 mg of RALA. On the other hand, we observed no changes in Tmax and T1/2 in response to a single oral dose of RALA or RALA-CD. Our results suggest that complexation of RALA with γ-CD significantly enhances RALA bioavailability in healthy human volunteers, making it a promising technology for delivering functional but unstable ingredients like RALA. Additionally, there were no drug-induced side effects observed. These results indicate that 6 g of RALA-CD is suitable for nutraceutical purposes.

The complexation effects of natural α-CD, β-CD and γ-CD on water-solubility and bioavailability were evaluated for particular hydrophobic nutraceuticals such as CoQ10 [7,22], curcumin [52-54], tocotrienol [55,56], RALA [37,57] and astaxanthin [58] (Table 5). Formation of complexes for these hydrophobic nutraceuticals with natural CDs did not generally enhance aqueous solubility. However, the bioavailability of most of the examined nutraceuticals is significantly enhanced when γ-CD was used.

- There are three types of approaches for bioavailability enhancement of bioactives, which focus on achieving the Cmax of the bioactives and T1/2 of the Tmax of bioactives in blood plasma (Figure 14). The first (Type 1) is the Cmax enhancer. RALA-CD falls into this category. A commercially available “Water Soluble CoQ10” formulation using a dietary fatty oil-based emulsifier is a typical example for enhancing Cmax, but usually does not prolong T1/2 [59].
- The second approach (Type 2) functions to prolong T1/2. For example, glucosidated bioactives such as ascorbic acid 2-glucoside give T1/2 prolongation of the plasma

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Type</th>
<th>CD</th>
<th>PK change (fold)</th>
<th>Reference</th>
<th>Species</th>
<th>Physicochemical change via CD complexation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coenzyme Q10</td>
<td>Type 3</td>
<td>γ</td>
<td>Cmax: 3.4 *</td>
<td>[7]</td>
<td>human</td>
<td>Solubility</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>AUC: 18.4 *</td>
<td></td>
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<td>Tmax: no change</td>
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<tr>
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<td></td>
<td></td>
<td>T1/2: prolonged</td>
<td></td>
<td></td>
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<tr>
<td>Curcumin</td>
<td>Type 3</td>
<td>γ</td>
<td>Cmax: 96.7 **</td>
<td>[52]</td>
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<td>AUC: 39.1 **</td>
<td></td>
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<td>Tmax: shortened</td>
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<td>T1/2: prolonged</td>
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<tr>
<td>γ-tocotrienol</td>
<td>Type 3</td>
<td>γ</td>
<td>Cmax: 2.3 #</td>
<td>[55]</td>
<td>rat</td>
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<td>C3h: 2.1 ##</td>
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<tr>
<td>R-α-lipoic acid</td>
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<td>γ</td>
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<td>[57]</td>
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<td>Astaxanthin</td>
<td>Type 3</td>
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Table 5: Comparison of the plasma concentration and PK response after oral administration of nutraceuticals.
ascorbic acid concentration because of the slow cleavage of the glucose bond by glycosidase in the intestine [60]. However, owing to the slow release of the bioactive into the bloodstream, $C_{\text{max}}$ becomes lower and no increase in the AUC is observed.\[\text{1/2}\]

- In comparison with the above two approaches, the inclusion complex formation of hydrophobic substances with γ-CD functions to both enhance $C_{\text{max}}$ and prolong $T_{1/2}$ and therefore gives the highest AUC (Type 3). The CoQ10-γCD complex belongs to this approach (Type 3).

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REFERENCES
