Improved Solubility of Quercetin by Preparing Amorphous Solid with Transglycosylated Rutin and Isoquercitrin

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The effect of isoquercitrin (IQC) addition on quercetin (QUE) solubility was investigated. Evaporated particles (EVPs) were prepared by rotary evaporator with QUE, α-glucosyl rutin (Rutin-G), and IQC. Differential scanning calorimetry and powder X-ray diffraction analysis revealed the amorphization of QUE and IQC by evaporation with Rutin-G. No diffraction peaks were detected in EVPs even after storage in sealing condition for 8 weeks at 40°C. The amount of dissolved QUE from the physical mixture was enhanced according to the increase of Rutin-G ratio because QUE was solubilized in the aggregated structure of Rutin-G formed in proportion to Rutin-G concentration. In the case of EVPs, the concentration of dissolved QUE increased when IQC was added in QUE/Rutin-G binary formulation. The concentration of dissolved QUE from EVPs of QUE/Rutin-G/IQC (1/7/3, w/w/w) and QUE/Rutin-G/IQC (1/5/5, w/w/w) was much higher than that with QUE/Rutin-G/IQC (1/10/0, w/w/w). These results suggested that IQC inhibit the re-crystallization from an amorphous QUE in dissolution medium, resulted in the enhancement in stability of an amorphous QUE in the supersaturated state. In conclusion, the addition of IQC into QUE/Rutin-G binary system could obviously improve the solubility of QUE.

Keywords: amorphous, α-glucosyl rutin, isoquercitrin, quercetin, solubility enhancement

INTRODUCTION

Self-medication is an important self-care practice performed in daily life when people experience common health problems that they feel to not require a hospital visit. Self-medication is defined as the use of any over-the-counter drug, functional foods, and/or supplements for treatment of an illness or condition without consulting a doctor. Oxidative stress caused by reactive oxygen species is involved in lifestyle-related diseases, such as arteriosclerosis (Harrison et al., 2003), diabetes (Johansen et al., 2005), and cancer (Valko et al., 2006). Antioxidant plays an important role in the body to prevent damage by oxidative stress. Therefore, an intake of antioxidant from supplements and functional foods will be useful to prevent the occurrence of lifestyle-related disease.

Quercetin (QUE) is well-known flavonoid widely existing in vegetables and fruits, and normally exists as glycosides in them. Many researchers have reported for its pharmacological effects, such as anti-inflammatory (Guardia et al., 2001), anti-atherosclerotic (Juzwiak et al., 2005), and antioxidant (Boots et al., 2008). In various effects, antioxidant effect of QUE has been expected for prevention of lifestyle-related disease because of its very strong antioxidant effect in flavonoids. One of the most difficult points in the practical use of QUE is its poor solubility. QUE solubility at room temperature and pH 3 was reported as ca. 400 ng/mL (Zheng et al., 2005). The low solubility of QUE resulted in its poor absorption after oral administration in human (Li et al., 2009). Therefore, improvements in the dissolution properties of QUE will be important challenges to enhance its bioavailability. Various attempts have been reported to enhance the dissolution and absorption of QUE with amorphous formulation (Kakran et al., 2011), nanocrystal formation (Sahoo et al., 2011), co-crystal formulation (Smith et al., 2011), and inclusion complexation (Borghetti et al., 2009). Among them, the amorphous formulation is often used in the pharmaceutical area for enhancing the dissolution properties of poorly soluble drugs. It is prepared by evaporation, spray drying, and melt extrusion with excipients such as a water-soluble polymer, and a crystalline compound is changed to amorphous form through the dispersion into excipients or interaction between excipient and compound (Li et al., 2013). The dissolution of crystal compound occurs after the disruption of crystal packing. A crystal compound has higher crystal packing energy than an amorphous compound. Amorphous compound shows the higher dissolution rate and apparent solubility compared to crystal one because it has low packing energy (Brough and Williams, 2013). Therefore, the preparation of an amorphous formulation is able to enhance the solubility and absorption of poorly soluble compounds.

The purpose of this study was to investigate the effect of isoquercitrin (IQC) addition to QUE/α-glucosyl rutin (Rutin-G) formulation on QUE solubility. We previously reported that Rutin-G enhanced the apparent solubility of QUE with the loading ratios of Rutin-G due to the formation of the molecular aggregation in water (Tozuka et al.,...
2012; Fujimori et al., 2015). IQC, which is hydrophobic flavonoid with a similar chemical structure to QUE, was investigated the possibility as a stabilizer of supersaturated state of QUE in QUE/Rutin-G formulation. An amorphous formulation of QUE was prepared by rotary evaporator with Rutin-G and IQC. The physiochemical properties of evaporated particles (EVPs) were evaluated by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and powder X-ray diffractometry (PXRD). The solubility of QUE from EVPs with QUE/Rutin-G/IQC was compared to that from physical mixtures (PMs) with QUE/Rutin-G/IQC in distilled water. The effect of IQC addition into QUE/Rutin-G formulation on the apparent solubility of QUE was investigated.

MATERIALS AND METHODS

Materials
Quercetin dihydrate (QUE, Fig. 1(a)), isouercitrin (IQC, Fig. 1(b)) and α-glucosyl rutin (Rutin-G, Fig. 1(c)) were supplied by Toyo Sugar Refining Co., Ltd. (Tokyo, Japan). All other chemicals and solvents were reagent grade and used without further purification.

Preparation of evaporated samples
QUE and IQC were completely dissolved in ethanol using sonication, and Rutin-G was dissolved in distilled water. Ethanol solution was added to distilled water and the solution was mixed for 1 min at room temperature. Mixed solution was processed by rotary evaporator (R-3; Buchi, Tokyo, Japan) under 102 mbar of pressure in a water bath at 50°C and then evaporated particles (EVPs) were prepared by removing the solvents completely with the rotary evaporator under 20 mbar of pressure in a water bath at 50°C. Each formulation is shown in Table 1. Each formulation was prepared three times.

Morphology of EVPs
The morphology of samples was observed by scanning electron microscopy (SEM) (TM3030, HITACHI, Japan). The acceleration voltage of 15 kV during each observation was performed. Samples were attached onto carbon sticky tape mounted on SEM stubs. Prior to observation, samples were sputtered with a thin layer of platinum under a vacuum.

Crystallinity of EVPs
Differential scanning calorimetry (DSC) (Perkin Elmer, USA) was used to detect the crystallinity of QUE, Rutin-G and IQC samples. 2–3 mg of the sample was placed in the aluminum open-pan, and DSC analysis was performed at a nitrogen flow rate of 40 mL/min. The range of temperature was from 35 to 200°C at a heating rate of 10°C/min. Powder X-ray diffraction (PXRD) (Rigaku Corporation, Tokyo, Japan) was performed at room temperature. The scanning rate was 4°/min over a 2θ range of 4–35° at a step size of 0.02°.

QUE solubility
Solubility test was performed in distilled water at 37°C under shaking at 100 s/min. Samples at a QUE concentration of 20 mg were added in 10 mL distilled water, and one-milliliter samples were withdrawn at 1 and 4 h. Collected samples were filtered through a 0.2 μm filter, and the concentrations of QUE were determined by HPLC (SPD-10A, Shimadzu Co., Ltd., Kyoto, Japan). An ENDURO C18 (5 μm, 150 mm × 4.6 mm, SGE Analytical Science, Melbourne, Australia) was used. The mobile phase was 0.1 (w/w) % acetic acid and acetonitrile (65:35, v/v). The flow rate was controlled at 1.0 mL/min with a 10 μL injection volume. QUE was eluted at 40°C and quantified at 254 nm. Each concentration of QUE (μg/mL) was calculated as average of dissolved QUE from individual three samples, and the error of result from individual three samples was presented as the mean ± standard error.

RESULTS AND DISCUSSION

Physicochemical properties of EVPs of QUE/Rutin-G/IQC
Morphology of untreated QUE, IQC, Rutin-G, and EVPs of QUE/Rutin-G/IQC were evaluated by a SEM (Fig. 2). Untreated QUE and Rutin-G showed the needle-like particles and spherical particles, respectively. An aggregation of the fine particle was observed in the untreated IQC. All EVPs formed the agglomerate with the smooth surface in association with the disappearance of original QUE, IQC, and Rutin-G.

DSC and PXRD analysis were performed to evaluate the crystallinity of EVPs. In DSC analysis, the endothermic peaks derived from QUE and IQC crystal were observed in untreated QUE and IQC although EVPs did not show any characteristic peaks (Fig. 3). The PXRD

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Table 1: Preparation conditions of evaporated particles (EVPs).

<table>
<thead>
<tr>
<th>QUE/Rutin-G/IQC (w/w/w)</th>
<th>1/10/0</th>
<th>1/9/1</th>
<th>1/7/3</th>
<th>1/5/5</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUE (mg)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Rutin-G (mg)</td>
<td>2000</td>
<td>1800</td>
<td>1400</td>
<td>1000</td>
</tr>
<tr>
<td>IQC (mg)</td>
<td>—</td>
<td>200</td>
<td>600</td>
<td>1000</td>
</tr>
<tr>
<td>Water (mL)</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>150</td>
</tr>
<tr>
<td>EtOH (mL)</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>150</td>
</tr>
</tbody>
</table>

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Fig. 1: Chemical structure of (a) quercetin, (b) isouercitrin, and (c) α-glucosyl rutin.
result of untreated QUE and IQC showed peaks originating in QUE and IQC crystal (Fig. 4). When QUE and IQC were processed by rotary evaporator with Rutin-G, several diffraction peaks of untreated QUE and IQC completely disappeared in all EVPs. These results suggested that QUE and IQC existed as an amorphous particle or molecularly dispersed state in EVPs. Figure 5 shows the PXRD profile of EVPs after storage in sealing condition for 8 weeks at 40°C. Crystal transition from an amorphous solid to crystal during the storage is a serious problem because amorphous products are physically unstable compared to the crystal products. Storage temperature is one of the most important factors for the stability of an amorphous compound. When an amorphous products are exposed to high temperature, re-crystallization from an amorphous products is caused by the increase of molecular mobility (Hancock et al., 1995). Dissolution profile of the crystal compound is lower than that of an amorphous compound, resulting in the decrease in bioavailability due to change of crystal form (Wong et al., 2006). PXRD profile of EVPs of QUE/Rutin-G/IQC
showed the halo pattern even after storage for 8 weeks at 40°C. This result indicated that re-crystallization from an amorphous QUE and IQC was inhibited in EVPs during storage at 40°C.

Solubility of QUE from EVPs of QUE/Rutin-G/IQC

The solubility of QUE from physical mixtures (PMs) and EVPs were evaluated in distilled water at 37°C. PMs of QUE/Rutin-G/IQC were prepared by simple mixing with the vortex mixer for 1 min. The solubility of untreated QUE was ca. 1.4 µg/mL in distilled water after incubation at 37°C for 4 h (Fig. 6). QUE is a highly lipophilic compound, and the absorption in the gastrointestinal tract is limited by the poorly water-soluble property. Therefore, it is important to enhance the dissolution property of QUE in order to improve its bioavailability. The amount of dissolved QUE from PMs of QUE/Rutin-G/IQC (1/10/0), QUE/Rutin-G/IQC (1/9/1), and QUE/Rutin-G/IQC (1/5/5) after incubation at 37°C for 4 h were 101.5±2.6, 69.1±2.6, 73.4±3.4 and 56.3±4.6 µg/mL, respectively. Apparent solubility of QUE showed the highest value in PMs of QUE/Rutin-G/IQC (1/10/0). We previously reported that Rutin-G formed the molecular aggregation above the critical aggregation number of ca. 5 mg/mL (Tozuka et al., 2012). Rutin-G could solubilize poorly water-soluble compounds in that aggregated structure in proportion to the amount of Rutin-G. Therefore, the apparent solubility of QUE from PMs of QUE/Rutin-G/IQC showed the highest value. Figure 7 shows the amount of dissolved QUE from EVPs of QUE/Rutin-G/IQC. The dissolved QUE from EVPs increased significantly compared to PMs. From results of DSC and PXRD, it was estimated that QUE existed as amorphous solid in EVPs. An amorphous solid, with the thermodynamically metastable state, does not have the long-range order like a crystal and has lower crystal packing energy compared to crystal. Therefore, an amorphous solid shows the higher solubility and wettability compared to the crystal (Hancock and Parks, 2000). Changes of crystalline form from crystal to amorphous solid resulted in improved solubility of QUE. In the case of EVPs, the improvement of QUE solubility after 1 h did not depend on the additive ratio of Rutin-G. EVPs of QUE/Rutin-G/IQC (1/7/3) and QUE/Rutin-G/IQC (1/5/5) showed higher QUE solubility than EVPs of QUE/Rutin-G/IQC (1/10/0) although those solubility decreased after 4 h. The improved solubility after 1 h was interpreted as the stabilization of supersaturated state of amorphous QUE in the dissolution medium. An amorphous solid shows the higher solubility than crystal. However, its solubility significantly decreases according to re-crystallization from the amorphous solid caused by contact of dissolution medium. Solid dispersion system with water-
soluble polymers is often used to keep the supersaturated state of the active substance (Kojima et al., 2012). One of the mechanism is the inhibition of re-crystallization from an amorphous solid through the interaction between the polymer and the active substance. IQC and QUE, are lipophilic compounds, were dissolved in ethanol. On the other hand, Rutin-G has a hydrophilic property and was dissolved in distilled water before evaporation process. IQC and QUE precipitated prior to Rutin-G during evaporation process because ethanol was firstly removed from the mixed solution of water/ethanol (50/50, v/v). Therefore, IQC has a possibility to interact more strongly with QUE compared to Rutin-G although specific interaction cannot be detected due to their similar structures.

In conclusion, IQC may inhibit re-crystallization from amorphous QUE and stabilize the supersaturated state of an amorphous QUE in dissolution medium owing to the interaction between IQC and QUE produced by evaporation. Two effects of the stabilization of the supersaturated state by IQC and solubilization in aggregated structure formed by Rutin-G may contribute to the increase of QUE solubility.

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REFERENCES


