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The physicochemical interactive mechanism between nanoparticles and raffinose during freeze-drying

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ABSTRACT

New methods of preparing nanoparticles and in vivo studies of their behavior have been the subject of much study. However, there exist few studies on maintaining the nanoparticle size. In this work, we report on the interaction mechanism between raffinose and nanoparticles during freeze-drying.
The mean particle size of the rehydrated freeze-dried raffinose-containing nanoparticles (170.5 nm) was similar to the initial particle size before freeze-drying (156.1 nm), indicating that the particle size was maintained. The powder X-ray diffraction of the freeze-dried raffinose-containing nanoparticles shows a halo pattern, while that of the normal-dried raffinose shows a crystalline pattern. No endothermic peak of the freeze-dried raffinose appeared, while the normal-dried raffinose had an endothermic peak at 84.0 °C. These results suggest that there exists a relationship between the nanoparticles and the raffinose, and that the relationship depends on whether the mixture is freeze-dried or normal-dried.
In the case of normal drying, the raffinose molecules have space and time to arrange themselves into regular arrangement because the nanoparticles and raffinose molecules can move around freely in water. In contrast, in the case of freeze-drying, the moisture was sublimed while the raffinose molecules and nanoparticles were immobilized in the ice, thereby preventing aggregation.

1. Introduction

Recently, numerous studies on the application of nanoparticles have been reported in the pharmaceutical field (Kamiya et al., 2008, 2010a,b). Furthermore, new methods for preparing nanoparticles and in vivo studies of their behavior were reported (Wang et al., 2013; An et al., 2013). However, there are few studies on maintaining the particle size of nanoparticles. The most utilized procedure for protecting nanoparticles from aggregation is the freeze-drying method. Suspended nanoparticles have a tendency to agglomerate, as this is a very thermodynamically favorable process. The particle size of the nanoparticles can be maintained by solidification for a long term using the freeze-drying method. Additives generally play an important role in the protection of the nanoparticles when the freeze-drying method is applied. Therefore, it is essential to choose optimal additives for long-term storage. Although monosaccharides cannot protect against aggregation, disaccharides have been shown to be very helpful in maintaining the particle size of nanoparticles (Hirsjärvi et al., 2006; Layre et al., 2006). There are previous reports on suitable disaccharides or monosaccharides (Kamiya et al., 2006, 2009). However, larger saccharides such as trisaccharides, tetrasaccharides, and pentasaccharides have rarely been investigated. On the contrary, the mechanism to protect protein denaturation during freeze-drying has been heavily studied. However, few or no mechanisms to prevent the aggregation of nanoparticles have been reported. Therefore, in the present study, we look at the nanoparticle aggregation during freeze-drying in a suspension of nanoparticles containing a trisaccharide it is known that disaccharides and trisaccharides can maintain the particle diameter of nanoparticles (Kamiya et al., 2010a,b). In contrast, polysaccharides cannot suppress the aggregation of nanoparticles. Thus, there is an important question as to which kinds of saccharides can preserve the particle size of nanoparticles. Hence, we studied the physicochemical mechanism of interaction between nanoparticles and raffinose molecule. There are many reports on the role of

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raffinose in enteric bacteria and of its stability with respect to changes in pH and heat (Poddar et al., 2008; Gill et al., 2006; Labrie et al., 2005). Raffinose, composed of fructose, galactose, and glucose, is a sort of native oligosaccharide and is classified as a trisaccharide. Raffinose is prepared by extraction and purification from sugar beet or sugarcane. It possesses a low hygroscopicity, high acid and heat resistances, a therapeutic effect for intestinal bacterial flora, and it improves intestinal function (Clavel et al., 2010). However, in general, raffinose has not been used as an additive in the freeze-drying of nanoparticles. Also, the primary interaction mechanism of raffinose with nanoparticles during freeze-drying has not been elucidated.

2. Materials and method

2.1. Materials

Hydrogenated soybean phosphatidylcholine (HSPC; COATSOME® NC-21) and dipalmitoyl phosphatidylglycerol (DPPG; COATSOME® MG-6060LS) were purchased from Nippon Oil and Fats Co., Ltd. Ethanol and raffinose pentahydrate (reagent grade) were purchased from Wako Pure Chemical Industries Ltd. The reagents were used as supplied. Purified water treated by ion exchange was used.

2.2. Methods

2.2.1. Preparation of the nanoparticle suspension

The phospholipids (100 mg; HSPC: DPPG = 4:1 mol ratio) were dissolved in 5 mL of ethanol at 80 °C in a water bath, and the ethanol was evaporated away. The phospholipid (HSPC: DPPG) mixture was dispersed in 100 mL of purified water and sonicated using a sonicator (BIORUPTOR; COSMO BIO Co., LTD. Tokyo, Japan) for 3 h. The parameters for the sonication are as follows: output power of 250 W, run time of 20 s and interval time of 10 s. This suspension was used as the measurement sample in the following experiments.

2.2.2. Size measurement of the nanoparticle suspension

The mean particle size was measured at 24 °C using an electrophoretic light-scattering photometer (ELS; ELS-8000®, Otsuka Electronics Co., Ltd. Tokyo, Japan) at a fixed angle of 90°. The particle sizes were analyzed on the basis of the weight distribution of the nanoparticle suspension. A five-fold diluted nanoparticle suspension was analyzed.

2.2.3. Freeze-drying and rehydration of the nanoparticles

The nanoparticle suspension (2 mL) was collected in separate vials, to which 0 mg, 2 mg, 4 mg, 10 mg, and 20 mg of raffinose were added, respectively to the vials. After each vial was vortexed, the suspensions were drastically frozen at −40 °C and left standing for 4 h. The frozen samples were freeze-dried in a glass chamber for 24 h at room temperature using a vacuum pump (type: GLD-051, ULVAC, Inc. Yokohama, Japan) and a vapor condenser (−45 °C, 8.0 Pa; EYELA® FREEZE DRYER FD-1000, Tokyo Rikakikai Co., LTD. Tokyo, Japan).

Purified water (2 mL) was added to each vial, and the vials were shaken by hand to rehydrate the freeze-dried samples. The mean particle size of the rehydrated GF-lipid nanoparticle suspension was analyzed by ELS.

2.2.4. Normal-dried raffinose-containing nanoparticles

Twenty milligrams of raffinose was added to 2 mL of the nanoparticle suspension. After mixing, the suspension was evaporated in a drying machine (EYELA® NDO-500, Tokyo Rikakikai Co., LTD. Tokyo, Japan) for 24 h at 40 °C.

2.2.5. Measurements of the freeze-dried and normal-dried nanoparticles

Twenty milligrams of raffinose was added into 2 mL of the nanoparticle suspension as a lyoprotectant. The freeze-dried raffinose containing nanoparticles was subjected to powder X-ray diffraction, differential scanning calorimetry (DSC), and Fourier transform infrared (FT-IR) spectroscopy. The morphology of the freeze-dried raffinose was observed by scanning electron microscopy (SEM)

2.2.6. Scanning electron microscopy

The SEM measurements were performed with a JSM-6300F (SCANNING MICROSCOPE: JEOL). The samples were coated with platinum using a magnetron sputtering apparatus (JUC-5000: JEOL).

2.2.7. Powder X-ray diffraction

The freeze-dried raffinose samples containing nanoparticles were subjected to powder X-ray diffraction with Cu Kα radiation at 40 kV, 30 mA, and room temperature using a powder X-ray diffractometer (PW1825 generator, PHILIPS). The analyses of the samples were performed using an X-ray diffractometer (X'PERT DATA COLLECTOR).

The scanning rate was 10 min^{-1}, and the diffraction angle (2θ) was 2–30°.

2.2.8. Thermal analysis of the freeze-dried nanoparticles

The freeze-dried raffinose samples containing nanoparticles were analyzed by DSC (DSC-50, Shimadzu). Each sample (8.0 mg) was weighed in a pan (Open Sample Pan, Shimadzu Co.), which was covered with a plate cover (Open Plate Cover, Shimadzu Co.) and subsequently sealed. The programmed heating rate was 10 °C/min, and the temperature range was 40–160 °C.

2.2.9. Fourier transform infrared spectroscopy (FT-IR)

The freeze-dried raffinose samples containing nanoparticles were analyzed by FT-IR, using the attenuated total reflection (ATR) method. The samples were measured by the transmission diffuse reflection method using an FT-IR spectrometer (FT/IR-4200®, JASCO Co. Tokyo, Japan). Three milligrams of the samples and 57 mg of KBr were ground with a mortar and pestle. The mixture was pressed using a press tool (MP-1®; Mini press, JASCO Co., Tokyo, Japan) to prepare the KBr pellet. Each sample was subjected to 128 scans at a resolution of 4 cm^{-1}.

3. Results and discussion

We have previously reported that disaccharides can prevent aggregation after rehydrating freeze-dried nanoparticles. In contrast, dextrin cannot prevent aggregation after rehydrating freeze-dried nanoparticles. This fact begs the question of what differences exist between disaccharides and polysaccharides that influence the preventative capabilities for particulate aggregation. Also, we have doubts as to whether trisaccharides, tetrasaccharides and/or pentasaccharides prevent aggregation in the same manner. Therefore, we focused on the structural state to shed light on the underlying mechanism.

We began by investigating the relationship between the raffinose/phospholipid ratios and the particle size of the rehydrated nanoparticles. Fig. 1 shows the mean particle size of the rehydrated nanoparticles that were freeze-dried with various raffinose/phospholipid ratios. The mean particle size of the nanoparticles in the suspension before freeze-drying (control group) was 156.1 nm. The mean particle size of the nanoparticles after rehydration was found to be 3000–6000 nm when raffinose/phospholipid ratios of 0, 1, and 2 were used. Meanwhile, the mean