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Aggregate formation of glycyrrhizic acid

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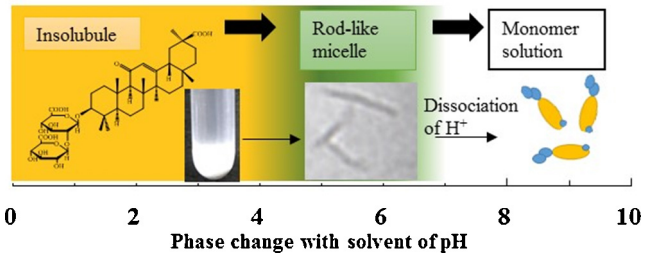
HIGHLIGHTS

- The aqueous solubility of glycyrrhizic acid was relatively low (0.15 mM).
- The aggregate formation strongly depended on the solvent of pH.
- The cmcs were 2.9 mM and 5.3 mM at pH 5 and 6, respectively.
- The glycyrrhizic acid formed rod-like micelle in the range of pH 5–6.

GRAPHICAL ABSTRACT



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ABSTRACT

Glycyrrhizic acid is derived from the licorice root. The compound is amphiphilic with a large hydrophobic aglycone group, and two glucuronic acids and a carboxyl group constituting the hydrophilic part. Therefore, glycyrrhizic acid can form aggregates or lower the surface tension of aqueous solutions owing to its specific amphiphilic structure. The aqueous solubility of glycyrrhizic acid is relatively low (0.15 mM) and dependent on the solvent pH due to the weak acidity resulting from the carboxylic groups. Glycyrrhizic acid is quite soluble in a buffer solution at pH 4.5, and its ability to affect the surface tension of the buffer solution increases from pH 5. The critical micelle concentration (cmc) was estimated to be 2.9 mM (γ_{cmc} = 55.2 mN/m) and 5.3 mM (γ_{cmc} = 56.8 mN/m) at pH 5 and 6, respectively. The surface tension also decreased gradually at pH 7, but the critical point was not observed in the curve. At pH 7, the pyrene fluorescent probe method and light scattering measurements did not show a clear cmc. Small angle X-ray scattering experiments revealed that the aggregates were rod-like micelles with an estimated radius and length of 1.5 nm and 21 nm, respectively, at 5 mM in a pH 5 solvent. Transmission electron microscopy confirmed that glycyrrhizic acid forms rod-like micelles. These results suggest that glycyrrhizic acid has potential applications as a biosurfactant in various fields.

1. Introduction

Glycyrrhizic acid is a sweetener derived from the licorice root, and its sweetness is more than 30 times that of glucose [1]. Therefore, its function as a sweetener with a unique flavor is famous. Glycyrrhizin acid is also widely used in cosmetics, shampoos, hair tonics, and expectorants. As a naturally derived compound, glycyrrhizin acid is relatively safe, unless consumed in large quantities [2,3]. Glycyrrhizic acid is classified as a terpene saponin, as shown in Fig. 1. The molecule has an aglycone structure with a large hydrophobic group and two glucuronic acids as a hydrophilic group [4]. The amphiphile must have the properties of both anionic and

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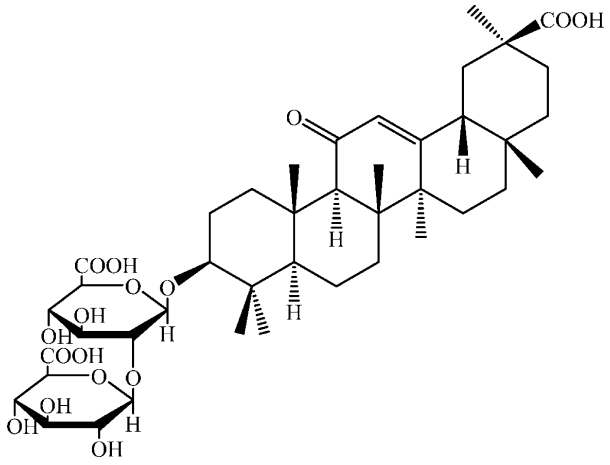

Fig. 1. Chemical structures of glycyrrhizic acid.

nonionic surfactants because of the specific hydrophilic groups (several carboxylic and hydroxyl groups). Because this molecular structure is amphipathic, it shows surface-active action in aqueous solutions. Moreover, this plant-derived material may be able to form aggregates in aqueous solutions.

Gilbert and James carried out conductivity and surface tension measurements and reported that glycyrrhizic acid does not form micelles in aqueous solution [5]. On the other hand, Sunada et al. determined the critical micelle concentration (cmc) for glycyrrhizic acid using several measurements [6]. For example, the cmc at 298.2 K was found to be approximately 0.25 mM (M mol dm^{-3}) and 0.38 mM at pH 5.1 and pH 5.5 in buffer solutions, respectively. However, they did not clarify the micelle structure for glycyrrhizic acid in detail but indicated that its aggregation behavior was similar to that of bile salt. Moreover, Polyakov et al. reported that glycyrrhizic acid easily make complex of aggregate with organic compounds depending on their concentration [7,8].

The present paper reports the aqueous-solution properties of glycyrrhizin acid in terms of surface tension and aggregate formation in relation to the solvent pH. The aim of this study was to carefully determine the cmc for glycyrrhizin acid solutions using surface tension, fluorescence probe, and light scattering measurements. Moreover, small angle X-ray scattering and transmission electron microscopy analyses were performed to determine the shape of the aggregate of glycyrrhizin acid. These findings will provide important basic information for the practical applications of glycyrrhizic acid.

2. Materials and methods

2.1. Materials

Glycyrrhizic acid (>98%, HPLC) was obtained from Kurita and used as received. The water used in the experiments was distilled once after an ion-exchange treatment. The glycyrrhizic acid crystals were dissolved in a buffer solution (pH 5–7) with sonication. Each objective pH buffer solution was adjusted by mixing appropriate doses of acetic acid (0.05 M), neutral phosphate salt (0.05 M), and sodium hydroxide (0.05 M).

2.2. Solubility of glycyrrhizic acid in the buffer solution

Two milliliters of each pH buffer solution and excess glycyrrhizic acid crystals were used to produce a saturated solution in a 10-mL injector tube. The system reached equilibrium within 24 h. The separation of solids was performed by filtration through a membrane filter with a pore size of 0.2 μm (Millipore Co., FGLP01300) by applying pressure to the injector in a thermostat-controlled bath at 298.2 ± 0.3 K. The glycyrrhizic acid concentration in the filtrates was determined using an ultraviolet-visible (UV-vis) spectrophotometer (JASCO V-630 iRM), which measured the absorbance (wavelength of 260 nm at the maximum absorbance). These operations were performed three times to determine the mean value.

2.3. Surface tension measurement

The surface tension of the glycyrrhizic acid solution was measured by the Wilhelmy plate technique using a surface tensiometer (DyneMaster DY-300YM, KYOWA), at 298.2 ± 0.3 K. The measurement conditions are described elsewhere [9]. The cmc is defined as the point of intersection of the tangents to the two slopes of the surface tension curve.

2.4. Fluorescence measurement (cmc determination)

A stock concentrated aqueous buffer solution of the glycyrrhizic acid was introduced stepwise into a saturated solution of pyrene, whose concentration was less than 6.7×10^{-7} M. In the present experiment, pyrene was dissolved in each buffer solution depending on the pH. The fluorescence emission spectra were monitored using a fluorescence spectrophotometer FP-8200ST (JASCO, Japan), where the temperature was controlled to within 298.2 ± 0.3 K by circulating thermostated water. The excitation and emission slit widths were set to 5.0 and 1.5 nm, respectively, where the excitation wavelength was 336 nm and the emission wavelength was measured over the range, 350–450 nm. The ratio of I_1 (at 372 nm)/I_3 (at 384 nm) was plotted as a function of the glycyrrhizic acid concentration.

2.5. Light scattering measurement (cmc determination)

The light scattering measurements were performed using a laser light scattering photometer (ALV-5000, Germany). The light source was a 200 mW Nd:YAG laser with a wavelength of 532 nm. The temperature was controlled to within 298.2 ± 0.3 K by circulating thermostated water. The glycyrrhizic acid solutions were filtered through a membrane filter with pore size of 0.22 μm (MILLEX-GV). The light scattering intensity in the 90° direction was measured by changing the glycyrrhizic acid solution.

2.6. Small angle X-ray scattering (SAXS) measurement

The shape of the molecular aggregates was estimated by small angle X-ray scattering (SAXS) at the BL40B2 beamline at SPring-8. The wavelength of X-rays for the SAXS measurement was 0.7 Å. The distance between the sample and detector was 2 m and the exposure time was 1 min. The solution was introduced to a flat cell with a 3.0 mm path length made from a quartz cell. The X-ray scattering was detected using a Rigaku imaging plate R-AXIS IV++ (30 × 30 cm, position resolution 0.1 mm). The final scattering data were determined by subtracting the solvent X-ray scattering from the sample scattering. The scattering profiles $I(q)$ were observed over the q-range, 0.005–1 $Å^{-1}$.

2.7. Transmission electron microscopy (TEM)

A droplet of a glycyrrhizic acid solution was placed on a carbon-coated grid for two minutes. The excess liquid was removed by touching one end of the grid with a filter paper. After the grid was partially dried, a drop of a staining solution (2% uranyl acetate) was placed on the grid for two minutes. The excess liquid was removed by filter paper and the grid was dried at room temperature. All