Physico-Chemical Properties of Monodesmoside Saponins of Sapindaceae (*Sapindus mukurossi* Gaertn) at Air/Water and Oil/Water Interfaces

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Received July 27, 1995

The surface activity, emulsion stability and foamability for two kinds of monodesmoside saponins of *Sapindus mukurossi* Gaertn (I and II) and the zeta potentials of emulsions prepared with these saponins in a water/kerosene system were investigated. There were no differences in the surface activity, foamability, emulsion stability or zeta potential of the emulsion droplet between the two kinds of saponins (I and II) of sapindaceae. The surface activity, emulsion stability and foamability of both I and II were higher than those of soyasaponin I (SI). The zeta potential of emulsion droplets prepared with I or II was lower than that prepared with SI. The water separation of both the I and II emulsions by creaming were higher than that of the SI emulsion.

Keywords: saponin, sapindaceae, surface activity, foamability, emulsion stability, zeta potential, creaming

Sapindus mukurossi Gaertn. (Sapindaceae), a tall deciduous tree (Japanese name: Mukuroji), grows in Japan and Southeast Asia, and contains various kinds of saponins in its pericarp (Kimata *et al.*, 1983; Takagi *et al.*, 1980; Yoshikawa *et al.*, 1982). We previously reported that the surface activity and the zeta potential of three kinds of soybean saponins were influenced by the number of polysaccharide chains and by the dissociation degree of the carboxyl group of the glucuronic acid residue in the polysaccharide chain (Gohtani *et al.*, 1985, 1986, 1989, 1990; Gohtani & Yamano, 1985, 1987, 1990). For some bisdesmoside Sapindaceae saponins, the surface tension in aqueous solution was investigated by Nakayama *et al.* (1986). The surface activities (including surface tension) of α -L-arabinopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranoside of hedragenin (I) and α -L-xylopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranoside of hedragenin (II) have not yet been investigated because of their low solubility in water. These monodesmoside saponins contain a carboxyl group bound to an aglicon (hedragenin). The solubility of these saponins may vary with pH, in view of the fact that the solubility of soyasaponin I (SI), a monodesmoside saponin with a carboxyl group, varies with pH (Gohtani *et al.*, 1986; Shimoyamada *et al.*, 1993). The present paper deals with the physicochemical surface properties of I and II dissolved in water.

Materials and Methods

Materials Chloroform, methanol and ethyl acetate of extra pure reagent grade and NaOH of guaranteed reagent grade from Nacalai Tesque Co. (Kyoto) were used without any further purification. The water was deionized (5.46×10^{-8} S/cm) and filtered through a 0.22 μ m membrane filter using a water purifier (Mili-Q Labo:Milipore Co., Ltd., Tokyo).

Sample preparations Sapindaceae saponins (I and II) were purified from a crude sapindaceae saponin powder (Mukuroji Ekisu Pauda (powder): Maruzen) containing

about 20% saponin. This powder was subjected to column chromatography on silica gel using CHCl_3 -MeOH- H_2O (6 : 2 : 1 v/v, lower layer) to obtain a fraction containing I and II (detected by TLC). The fraction was separated by repeated column chromatography on silica gel using $\text{CH}_3\text{COOCH}_2$ - CH_3 -EtOH- H_2O (14 : 2 : 1) affording I and II. Compounds I and II were identified based on their ^{13}C nuclear magnetic resonance spectra (Kimata *et al.*, 1983) as α -L-arabinopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranoside of hedragenin and α -L-xylopyranosyl(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl(1 $\rightarrow$ 2)- α -L-arabinopyranoside of hedragenin, respectively. Soyasaponin I (SI) was purified from a crude soybean saponin powder (Daizu saponin, Tokiwa Kanpou Seiyaku) by the previously described method (Gohtani *et al.*, 1986). Aqueous sample solutions I, II and SI were prepared by the neutralization of the acidic suspensions of the saponins with an aqueous NaOH (0.01 M) solution as per the previously described method (Gohtani *et al.*, 1986; Gohtani & Yamano, 1987). The pH of the sample solutions used in this study was 7.5 ± 0.2 .

Measurement of surface activity The surface tension of saponin solutions and the interfacial tension between kerosene and the saponin solution were measured using a Wilhelmy type interfacial tensiometer (CVBP A-3: Kyowa Kaimen Kagaku Co., Ltd., Tokyo) with a glass plate at $25 \pm 0.1^\circ\text{C}$.

Foamability was estimated in terms of the specific volume of foam prepared by vigorous stirring (15,000 rpm, 2 min) for each saponin solution (1.0 mM, 5 g) using a high speed disperser (Ultra-Turrax T-25, Janke & Kunkel, German) while foam stability was estimated by the change in specific volume of the foam with time.

A series of O/W emulsions were obtained by stirring a mixture (1 : 1, W/W, 10 g) of saponin solution and kerosene using the high speed disperser. The mean droplet diameter of the emulsion was determined by optical microphotography

using an image analyzer (Luzex III-U, Nireco Co., Ltd., Tokyo) according to the method of Yamano *et al.* (1984). The creaming rate was determined by measuring the water separation of the emulsion at $25 \pm 0.2^\circ\text{C}$ with the image analyzer (Chen *et al.*, 1993b).

pH titration An aqueous saponin solution of 0.1 mM and pH 9 was used for the pH titration with 0.001 M HCl aqueous solution.

Zeta potential of emulsion droplets The zeta potential of each dispersed droplet in the emulsions was determined using an electrophoretic light scattering spectrophotometer (ELS-800, Otsuka Electronics Co., Ltd., Hirakata) at $25 \pm 0.1^\circ\text{C}$.

Results

Surface and interfacial tension Figure 1 shows the surface tensions of aqueous saponin solutions plotted as a function of the saponin concentration. The surface tensions of both solutions I and II dropped with a small increase in the concentration of I and II, and showed a constant value of 40 mN/m above a 0.02 mM saponin concentration, i.e. lower than that of SI solution. The interfacial tensions between kerosene and the aqueous saponin solutions I and II significantly decreased with an increase in saponin concentration

Fig. 1. Surface tension of aqueous solution of compound I (●), II (○) and soyasaponin I (○) at 25°C .

Fig. 2. Interfacial tension between kerosene and aqueous solution of compound I (●), II (○) and soyasaponin I (○) at 25°C .

(Fig. 2), and reached a constant value of 4 mN/m at 0.05 mM, which was markedly lower than that of SI.

These results of both the surface and interfacial tensions indicate that the surface activities of the two Sapindaceae saponins (compounds I and II) were higher than that of SI and that the differences in molecular structures between these Sapindaceae saponins and SI may have brought about the differences in surface activity.

Foamability and foam stability The specific volumes of foam immediately after stirring of the aqueous I, II and SI solutions were 3.5, 3.5 and 2.0 cm^3/g and the specific volumes at 2 h after stirring were 0.5, 0.5 and 0 cm^3/g , respectively (Fig. 3). These results show that I and II have similar foamability and foam stability and are both stronger than in SI. This foamability of I and II is a direct reflection of the low surface tension shown in Fig. 1.

Emulsifying ability and emulsion stability The average droplet diameters of 0.1 mM saponin (I, II, and SI) emulsions

Table 1. Average droplet diameters (μm) of 0.1 mM saponin emulsions immediately after and 6 h after preparation and ratio of increase over 6 h.

Aging time (h)	I	II	SI
0	3.31	3.49	4.49
6	4.13	4.36	7.21
Increase ratio	1.25	1.25	1.61

Fig. 3. Change in specific foam volume prepared from 5 g of saponin solutions (1.0 mM) at 25°C . Compound I: ●, II: ○, SI: ○.

Fig. 4. Change in water ratio separated from saponin emulsions (1.0 mM of saponins) by creaming at 25°C . Compound I: ●, II: ○, SI: ○.

Fig. 5. Titration curve of 1.0 mM compound I solution (10 ml) with 0.001 N HCl.

of 0 and 6 h age after preparation and their rates of increase over 6 h are shown in Table 1. The average droplet diameters of the I and II emulsions were barely different from each other at 0 h aging and were smaller than that of the SI emulsion. This result shows that the emulsifying abilities of both I and II are higher than that of SI. This order of emulsifying ability for these saponins is reasonable in that it agrees with the result of the interfacial tension shown in Fig. 2. The ratio of increase of the average droplet diameters of both I and II emulsions of over 6 h was 1.25, lower than that of the SI emulsion at 1.61. This result indicates that the emulsions of both I and II have a higher emulsion stability than that of SI.

Creaming of emulsion The change in the water-phase separation of the emulsions due to creaming at 25°C is shown in Fig. 4. The water separation in both the I and II emulsions was larger than that in the SI emulsion and there was little difference in the water separation between the I and II emulsions. This result shows that the creaming stability of I and II was lower than that of SI.

pH titration curve The pH titration curve of aqueous I solution with 0.001 M HCl is shown in Fig. 5. From the pH titration curve, it is clear that most of compound I dissociates above pH 7.5.

Discussion

In the present study, it has been confirmed that the physicochemical properties, surface activity, emulsifying ability and emulsion stability of the Sapindaceae saponins I and II are stronger than those of SI. The creaming stability of I and II was, however, weaker than that of SI. We think that these differences in physicochemical properties are due to the differences in molecular structures between the Sapindaceae saponins (I and II) and SI. Each saponin examined in this study has one carboxyl group (Kitagawa *et al.*, 1982; Yoshikawa *et al.*, 1982). While the carboxyl group of the SI bonds to the trisaccharide, which is hydrophilic, the carboxyl group of the Sapindaceae saponins (compounds I and II) bonds to the triterpenoid, which is hydrophobic. From the pH titration results (Fig. 5), most of the carboxyl group might be expected to dissociate if the group existed in aqueous solution, since the pH of the sample solutions was fixed at

7.5 ± 0.2 in this experiment. We believe, however, that the ratio of dissociation of the carboxyl group is very low in this system, because most of the carboxyl groups bonded to the triterpenoid exist in the kerosene phase. Some proportion of the carboxyl groups of SI are believed to dissociate in the aqueous phase because they are bonded to the trisaccharide. The free carboxyl anion increases the solubility of a molecule with the carboxyl anion in aqueous solution. In fact, the solubility of SI is lower when below pH 7 than above pH 7.5 (Gohtani *et al.*, 1986; Shimoyamada *et al.*, 1993). We think that the hydrophile and lyphophile balance is influenced by the dissociation of the carboxyl group and that the balance of I and II has a greater propensity than SI to adsorb at the water/kerosene interface. We believe that this is the reason why both the surface and interfacial tensions of the I and II solutions were lower than those of the SI solution (Figs. 1 and 2).

The ratios of increase of the average droplet diameters of the I, II and SI emulsions indicate that the emulsions of both I and II have higher stability than that of SI (Table 1). It is well known that the factors determining emulsion stability are electrical double layer repulsion, mechanical strength of the interfacial film made by the surfactant, and viscosity of the continuous phase. It is considered that the viscosities of the continuous phases of the I, II and SI emulsions were almost the same for our present experimental systems since the viscosities of the aqueous saponin solutions were the same as water in the preliminary experiments. Usually the electrical double layer repulsion is estimated by the zeta potential (Yamano *et al.*, 1995). In this study, the zeta potentials of the emulsion droplets of compounds I, II and SI were -45 , -43 and -96 mV at pH 7.5, respectively. The zeta potential values of both the I and II emulsion droplets were lower than that of the SI emulsion droplet. This result suggests that the electric double layer repulsion estimated by the zeta potential does not contribute to the high stability of both the I and II emulsions. We think that the mechanical strength of the interfacial films made by the saponins used in this experiment influences the stability of the emulsions. It is presumed that the higher stability of both the I and II emulsions is attributable to the difference in the strength of the interfacial membrane of the emulsion droplets. The results of foam stability (Fig. 3) may also suggest that the interfacial membranes of I and II is stronger than that of SI. The cause of this difference in the strength of the interfacial membrane will be discussed later.

The zeta potential values of both the I and II emulsion droplets were lower than that of the SI emulsion droplet, as already described. These results imply that the adsorption amount of I and II on the surface of the emulsion droplets is smaller than that of SI because of the well-known theory that the zeta potential of a droplet depends on the amount of surface adsorption of the active ions (Shaw, 1992). However, as already described, both the results of the interfacial tension (Fig. 1) and pH titration of the saponins (Fig. 5) indicate that the amount of adsorption of compounds I and II at the interface are greater than that of SI. We consider that the reason why both the I and II emulsions have a lower zeta potential than the SI emulsion, in spite of their higher

adsorption of I and II than SI is that the positions of the carboxyl group are different. Based on the result of interfacial tension, as already discussed, we think that most of the carboxyl groups of SI exist in the water phase and dissociate, while most of those for both I and II exist undissociated in the kerosene phase. It is clear that carboxyl ions contribute to the surface charge of the emulsion droplets. Since the zeta potential depends on the surface charge of the droplet, the zeta potential of the emulsion prepared by SI with a dissociated carboxyl group showed a higher value than those prepared by either compounds I or II with an undissociated carboxyl group. We believe that the zeta potential results prove the hypothesis, i.e., most of the carboxyl groups of SI exist in the water phase and dissociate while most of those for both I and II exist undissociated in the kerosene phase.

We propose one hypothesis. The difference in dissociation of the carboxyl group between Sapindaceae saponins and SI may cause the difference in the mechanical strength of the interfacial membrane of emulsion droplets and bubbles. We presume that the Sapindaceae saponins I and II are packed more tightly at both the air/water and oil/water interface than SI, since a lateral repulsion by electrostatic force, based on the dissociated carboxyl group, between Sapindaceae saponins at the interface is lower than that between Soyasaponins. Thus the mechanical strength of the interfacial membrane of I and II is stronger than that of SI. However, further detailed studies including measurement of the surface and interfacial viscosity are necessary to confirm this hypothesis.

The creaming stability of I and II was lower than that of SI as shown in Fig. 4. It is well known that the settling rate, according to Stokes' law, is proportional to the square of the particle diameter under dilute conditions (Bergensstahl & Claesson, 1990). For emulsions, the creaming rate has been reported to increase with an increase in droplet diameter and decrease with an increase in volume fraction of the oil phase (Chen *et al.*, 1993a). The results of creaming in this experiment are in conflict with the previously described results, since the emulsions of I and II, which have a smaller droplet size than that of SI (Table I), showed a higher creaming rate than did SI. We previously reported that the creaming rate of the SI emulsion increased with decreasing pH below pH 4 and that the zeta potential values were lower than -65 mV below pH 4 (Gohtani & Yamano, 1990). The zeta potentials of the emulsion droplets of I and II were -45 and -43 mV, respectively, as already described. It is clear that the low values of the zeta potential lead to the high creaming rate. However, the coalescence rate of droplets in both the I and II emulsions was slower than that of the SI emulsion as already described (Table I). The creaming rate of the aggregates of droplets is faster than that of unaggregated droplets. We think that the aggregating frequency between the I or II emulsion droplets is higher than that between the droplets of the SI emulsion since the zeta potential of both the I and II emulsion droplets is smaller than that of SI.

It is concluded that Sapindaceae saponins I and II have surface activity, emulsifying ability and emulsion stability higher than those of SI. The creaming stability of I and II was, however, lower than that of SI. These differences in the physicochemical properties may be due to the difference in

molecular structures between the Sapindaceae saponins (I and II) and SI. We think that the degree of dissociation of the carboxyl group, which depends on the binding position in the saponin molecule, influences these properties at the air/water and oil/water interfaces.

Acknowledgments The authors are grateful to Graham Page of Language House Inc. for the proofreading of this manuscript.

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