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Surface Activity of Crude Ginseng Saponin

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Abstract ☐ The critical micelle concentration (CMC) of crude ginseng saponin in water was determined by fluorometry and surface-tension measurement. These two methods gave the CMC value, 0.015g/100ml and 0.013g/100ml, respectively. The surface excess of the saponin and the area occupied by a saponin molecule at the monolayer adsorbed at air and waterinterface were calculated employing Gibbs adsorption equation. The presence of salt increased the surface activity of the saponin: it decreased the CMC, the surface tension at the CMC and the area occupied by a saponin molecule at the monolayer, which should be due to the salting-out effect of the salt.

Keywords ☐ Surface activity, Critical micelle concentration, Ginseng saponin.

The chemical compositions and pharmacol ogical activities of panax ginseng have been extensively studied by many workers. Comparing with the large volume of these studies, the physical properties of the components of this herb medicine have been relatively neglected. Ginseng saponins which are amphiphilic compounds are expected to have moderate surace activity: they might have a tendency toward hydrophobic self-aggregation in aqueous solution, and have ability to solubilize and emulsify fats and lipids. Many of pharmacological activities of ginseng have been suggested to originate from the ginsenosides, and to be closely related with their surface activity.[1-8] Although systematic studies on the surface activity of ginseng compononts are essential for understanding their pharmacology, studies along this line have been lacking.

Joo and his coworkeers[1] already investigated some of the surface activities of crude ginseng saponin. They determined the critical micelle concentration (CMC) by osmometry. However, the CMC value they reported seemed unreasonably high comparing with CMC values of other nonioninc surfactants. In this research, we investigated the CMC and some other surface properties of crude ginseng saponin by fluorometry and surface-tension measurement.

EXPERIMENTAL METHODS

Materials

Crude ginseng saponin was prepared according to the procedure described by Namba.[9] From 1.2 kg of powdered Korean white ginseng roots, 16g of ginseng saponin was obtained. It was purified with recrystallization in chloroform. Water was double-distilled. All other reagents were reagent grades, and used without further purification.

Apparatus

Fluorescence measurements was performed on spectrofluorometer, Shimadzu Model RF-510. The temperature was maintained constant with thermobath, Shimadzu Model TB-85 during the measurement. Shaking water bath, Fisher Model 125 was employed to solubilize pyrene in aqueous solution of the saponin or sdoium dodecyl sulfate.

Surface tension was measured with DuNouy tensiometer Fisher Model 20.

Measurement of the CMC by Fluorometry

The desired concentrations of the saponin were prepared by volumetric dilution of the stock solution of the crude ginseng saponin with 0.1M phosphate buffer of pH 7.4. To a 50-ml erlenmyer flask containing 20ml of the saponin solution, 5mg of pyrene was added. After capped and covered with aluminum foil, it was placed in the shaking bath for 3 days at 25°C. This time period was proven earlier to be long enough to reach the solubilization equilibrium of pyrene. After this time, an appropriate volume of the clear solution was decanted and the fluorescence spectrum was recorded from 300nm to 600nm with 340nm excitation with water circulating at 25°C through the sample cell holder. The spectral band widths for excitation and emission were kept below 5nm.

Surface-Tension Measurement

The vessels were employed after rinsed in sulfuric acid-potassium dichromate mixture. The measurement was performed after the solution was adequately aged. The surface tension of the solution was measured in a temperature-controlled double-jacketed glass vessel at 25°C.

RESULTS AND DISCUSSION

Fluorescence Spectrum of Pyrene in Aqueous Solution of the Crude Ginseng Saponin

Fluorescence spectra of pyrene in an aqueous solution of the crude ginseng saponin and sodium dodecyl sulfate were shown in Fig. 1. Pyrene was solubilized in micelllar solution of the saponin, which strongly emitted fluorescence at 390nm. However, it did not emit fluorescence at 470nm significantly. Pyrene solution in micellar system of sodium dodecyl sulfate was

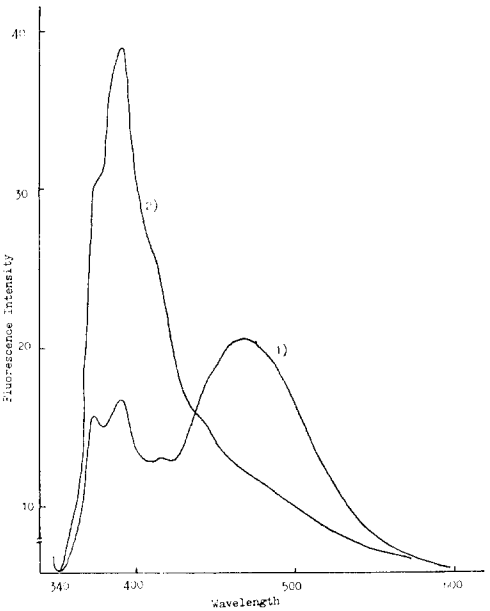

Fig. 1: Fluorescence spectra of pyrene in aqueous solution of sodium dodium dodecyl sulfate (1mM) and the crude ginseng saponin(0.1g/100ml) from 340nm to 600nm at the excitation wavelength 340nm.

included for comparison, which showed strong fluorescences at 390nm and 470nm. This emission at 470nm is known to be due to the formation of an excimer of pyrene.[10] This result suggests that the micellar size of the crude ginseng saponin is not large enough to accomodate more than one molecule of pyrene per micelle, whereas the micellar size of sodium dodecyl sufate is relatively large to accomodate more than one pyrene molecule per micelle, where pyrene molecules are approached closely enough to form dimers.

The Critical Micelle Concentration of the Crude Ginseng Saponin

From the plot of the fluorescence intensity at 390nm vs. the concentration of the saponin or the surface tension vs. the logarithm of the concentratation of the saponin, the CMCs were

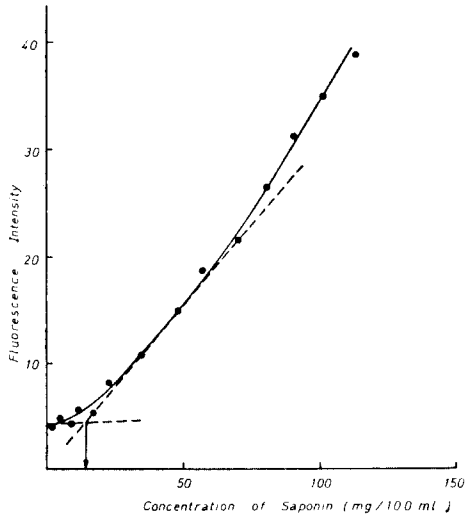

Fig. 2: Fluorescence intensity of pyrene at 390nm as a function of the concentration of the saponin in water at the excitation wavelength 340nm.

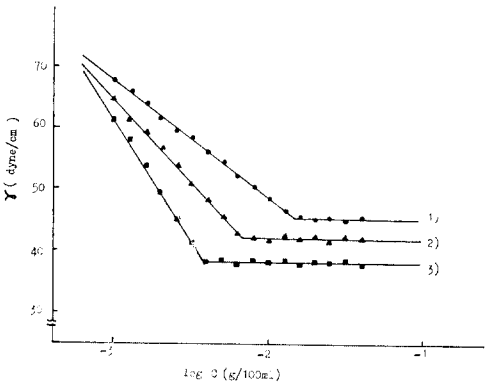

Fig. 3: Surface tension versus logarithm of the concentration of the crude ginseng saponin 1) in water 2) 0.2M NaCl 3) 0.5M NaCl

estimated by the usual procedure of linear extrapolation of data below and above the CMC[11,12] The intersection of these two extrapolated lines was taken as the CMC. The data are plotted in Fig. 2 and 3. The CMC values of the crude ginseng saponin in water and aqueous solutions of sodium chloride were determined by the two methods and are listed in

Table I: The critical micelle concentrations of the crude ginseng saponin determined by fluorometry and surface-tension measurement.

Solvent	CMC, g/100ml	
	Fluorometry	Tensiometry
Water	0.015	0.013
0.2M NaCl	0.010	0.007
0.5M NaCl	0.006	0.004

Table I. The data were that the CMC of the crude ginseng saponin in water was 0.015g/100ml by fluorometry and 0.013g/100ml by surface-tension measurement. These two values agreed fairly well. The CMCs obtained in this experiment were far lower than 2g/100ml reported by Joo et al.[1] The CMC values obtained in this experiment are in reasonable range of the CMCs of nonionic surfactants. For example the CMC of triton X-100, typical nonionic surfactant is 0.012g/100 ml.[13] The algycones of ginseng saponins are fairly hydrophobic, sugars are highly hydrophilic and the HLB of these molecules are expecsed to be well balanced to give moderate turface activity. The result of this experiment coincided with this expectation. The presence of sodium chloride in the solution lowered the CMC, and the effect was proportional to the concentration of the salt.

Surface Area of Saponin Molecule Adsorbed at Air-Water Interface

The surface excess of amphiphilic compound at the monolayer of the liquid/gas interface can be calculated employing the Gibbs adsorption equation:

$$\Gamma = -\frac{1}{RT}\frac{d\gamma}{d\ln C}$$

where γ is surface tenshon and C is concentration of the amphiphilic compound. The area

Table II: Surface tension at the CMC, surface excess and area occupied by a saponin molecule at monolayer.

Solvent	γcmc (dyne/cm)	$\Gamma \times 10^{10}$ (mole/cm^2)	A(Å^2)
Water	45.5	3.5	47
0.2M NaCl	42.3	4.8	35
0.5M NaCl	38.2	7.8	22

occupied by a molecule at the monolayer adsorbed at air and water interface, A can be calculated by:

$$A=\frac{1}{\Gamma N}$$

where N is Avogadro's number. From the plot of the surface tension vs. the logarithm of the concentration of the saponin, these values were calculated, and listed in Table II. The surface tension at the CMC, γcmc was included in this table. The γ cmc was 45.5 dyne/cm in water, not so low as those of typical nonionic surfactants, which are usually in the range between 40 to 40 dyne-cm. This means that the crude ginseng saponin is not so surface-active as typical nonionic surfactants. The area occupied by a saponin molecule was 47.4Å^2, which is bulky comparing with the area occupied by straightchain hydrocarbon compounds. The γ cmc and A decreased as the concentration of salt increased: the salt increased the surface activity. This might be due to the increased hydrophobicity of the saponin by the salting-out effect of the electrolyte.

The CMC value measured in this experiment is not the value of pure ginseng sapon, but the value of the mixture of crude ginseng saponins, and accordingly has only limited meaning. However, it could provide a parameter which evaluate the nonspecific surface activity of the crude drug.

LITERATURE CITED

1) Joo, C.N., Choi, R.S., Lee, S.J., Cho, S.H. and Son, M.H.: Biochemical studies on ginseng saponins (II). *Korean Biochem. J.* **6**, 185 (1973).

2) Glauert, A.M., Dingle, J.J. and Lucy, J.A.: Action of saponin on biological cell membrane. *Nature* **196**, 953 (1962).

3) Joo, C.N. and Lee, S.J.: Biochemical studies of ginseng saponins (IX). *Korean Biochem. J.* **10**, 59 (1977).

4) Ko, J.S. and Chun, S.Y.: Effect of Kroean ginseng on cell surface membranes in relation to its various pharmacological effects. *Korean Biochem. J.* **11**, 17 (1978).

5) Segal, R. and Milo-Goldzweig, J.: The susceptability of cholesterol depleted erythrocytes to saponin and sapogenin hemolysis. *Biochim. Biophys. Acta* **512**, 223 (1978).

6) Joo, C.N., Kim, D.S. and Koo, J.H.: Biochemical studies on hypercholesterolemia induced by prolonged cholesterol feeding in rabbits. *Korean Biochem. J.* **13**, 51 (1980).

7) Akiyama, I, Takagi, S., Sankawa, U., Inari, S. and Saito, H.: Saponin-cholesterol interaction in the multibilayers of egg yolk lecithin as studies by D^{2}NMR: Digitonin and its analogues. *Biochemistry* **19**, 1904 (1980).

9) Namba, T., Yoshizaki, M., Tomimori, T., Kobashi, K., Mitaui, K. and Hase, J.: Fundamental studies on the evaluation of the crude drugs. III. Chemical and biochemical evaluation of ginseng and related crude drugs. *Yakugaku Zasshi* **94**, 252 (1974).

10) Vanderkooi, J.M. and Callis, J. B.: Pyrene, a Probe of lateral diffusion in the hydrophobic region of membrane. *Biochemistry* 13, 4000 (1974).

11) Schick, M.J.: *Nonionic Surfactant*, Chap. 15, Marcel Dekker, New York, (1967).

12) Lucassen-Reynders, E.H.: *Anionic surfactants*, Vo. 11, Chap. 2, Marcel Dekkeer, New York (1980).
13) Roe, J.M. and Barry, B.W.: Measurement of critical micelle concentrations by photon correlation spectroscopy. *J. Colloid and Interface Sci.* **94**, 580 (1983).