Effect of Proteins on the Zeta Potential of Dispersed Vesicular Globules in W/O/W Emulsions

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Synopsis. The zeta potential of dispersed vesicular globules in W/O/W emulsions was found to be reduced systematically at neutral pH by increasing the isoelectric point of proteins immobilized in the inner aqueous phase of the globules. We considered the effect of proteins on the total interactions between vesicular globules.

Dispersed globules of W/O/W emulsions can be characterized by a vesicular structure with an inner aqueous phase separated from an aqueous suspending fluid by a thin layer of the oil-phase components.[1,2] Globules of this type of emulsion also encapsulate a certain amount of water-soluble ingredients including proteins.[3–5] The authors have found that the dispersion state of the globules in W/O/W emulsions is influenced by a kind of proteins immobilized in the inner aqueous phase. Despite the fact that the globules have already been charged negatively in the aqueous phase due to differences in the affinity of the oil and the aqueous phases for ions,[6] the above finding may result from changes in the charged potential of the globules due to the surface activity of proteins, i.e. protein molecules adsorbed tightly onto the inside surface of the oil layer from the inner aqueous phase. It is the purpose of this study to obtain information concerning the correlation between the zeta potential of the vesicular globules and the isoelectric point of proteins immobilized in the globules of W/O/W emulsion systems.

Experimental

The W/O/W emulsions to be tested were divided into liquid-paraffin and olive-oil systems, respectively. The former was stabilized by Span 80 (sorbitan monooleate, produced by ICI America, Inc., U.S.A.) and Tween 80 (poly(oxyethylene) sorbitan monooleate, produced by Difico Laboratories, Inc., U.S.A.), while the latter was prepared by use of TGCR (tetraglyceryl condensed ricinolate) and DGMO (decaglyceryl monooleate). The TGCR and DGMO used were supplied by courtesy of Sakamoto Yakuhin Kogyo, Ltd., Osaka. Each system was also subdivided into four samples according to a kind of proteins immobilized in the inner aqueous phase, i.e. control (without protein), BSA (bovine serum albumin, pI: 4.4—4.9), chymotrypsin (pI: 8.1—8.6), and lysozyme (pI: 10.5—11.0) samples. The liquid paraffin and olive oil were obtained from Wako Pure Chemical Industries, Ltd., Osaka, as reagents of primary standard. BSA and chymotrypsin produced from bovine pancreas were purchased from Sigma Chemical Co., U.S.A., as a grade of biochemical reagents. Purified lysozyme from chicken egg white was provided by Wako Pure Chemical Industries, Ltd., Osaka. These materials were used without further treatment.

All of the W/O/W emulsion samples in a stable form were prepared by a combination[5] of the two separated steps of emulsification[7] and a phase-inversion technique[8,9] in order to immobilize 1 %w/w of each protein in the inner aqueous phase of the samples. According to the results of the preliminary studies, the procedure of emulsification for each sample could be summarized as follows:

(1) A range from 30 to 40 g of the oil phase containing 30 %w/w Span 80 in liquid paraffin or 30 %w/w TGCR in olive oil were placed in a vessel of a pin-mixer (described precisely in Ref. 7).

(2) An aqueous solution of 1 %w/w of each protein was then introduced successively to the oil phase in the vessel, while a stirrer unit of the pin-mixer rotated steadily at 88 rpm at room temperature so as to provide a W/O emulsion. This procedure was carried out over a range from 70 to 80 ml of the amount of the aqueous solution introduced.

(3) A definite amount of the first W/O emulsion, i.e. 90 g for the liquid-paraffin system and 54 g for another, was mixed with 45 ml of an aqueous solution of 1 %w/w Tween 80 in the liquid-paraffin system and with 36 ml of that of 1 %w/w DGMO in the olive-oil system.

(4) The mixture was finally homogenized by means of a mixer at about 5000 rpm of the armature speed for 1 min at room temperature, thus resulting a W/O/W emulsion containing one of the proteins in the inner aqueous phase of the dispersed vesicular globules. Details concerning the mixer used were also described in Ref. 7.

An optical microscopic examination indicated that almost all of the dispersed globules in freshly prepared samples have the vesicular structure with single or multiple compartments of the inner aqueous phase within a range of diameters from 2 to 30 μm.

An assembly with a rectangular cell provided by Mitamura Riken Kogyo, Ltd., Osaka, was employed for evaluating the electrophoretic mobility of the vesicular globules at neutral pH at room temperature. Each W/O/W emulsion was diluted about 100 times by the further addition of an aqueous solution of 1 mmol dm^{-3} KCl immediately before each measurement. The field strengths within a range from 3.3 to 10.0 V cm^{-1} were used to ensure that the electrophoretic mobility is independent of the actual applied voltage. A minimum of 20 readings were taken for each diluted system at any one voltage. The zeta potential (ζ) of the slipping plane of the diffused portion in the electrical double layer on the globules was calculated from the Smoluchowski equation for the electrophoresis of non-conducting spheres,[6] as follows:

$$\mu = \varepsilon_0 D\zeta / \eta, \quad (1)$$

where μ is the electrophoretic mobility, ε_0 is the permittivity of free space, and D and η are the relative permittivity and viscosity of the suspending fluid.

Results and Discussion

The values for the electrophoretic mobility and zeta potential of the vesicular globules in each sample are summarized in Table 1. The effect of proteins on the zeta potential of the globules appears in relation to the different magnitudes for the isoelectric point of the proteins immobilized in the inner aqueous phase, although the values obtained with the liquid-paraffin system are always smaller than those with the olive-oil

Table 1. Micro-Electrophoretic Data of Vesicular Globules in W/O/W Emulsions at Neutral pH at Room Temperature

Inner aqueous phase	Mobility	Zeta potential	Deviation
	$\mu m\,s^{-1}$ per $V\,cm^{-1}$	mV	mV
	Liquid paraffin system		
Without protein	−2.50	−32.1	±0.9
1% BSA	−2.14	−27.4	±0.5
1% Chymotrypsin	−1.67	−21.4	±0.4
1% Lysozyme	−1.06	−13.7	—
	Olive oil system		
Without protein	−5.56	−71.3	2.8[a]
1% BSA	−4.30	−55.1	3.1[a]
1% Chymotrypsin	−3.53	−45.3	2.2[a]
1% Lysozyme	−2.27	−29.0	2.0[a]

a) Standard deviation.

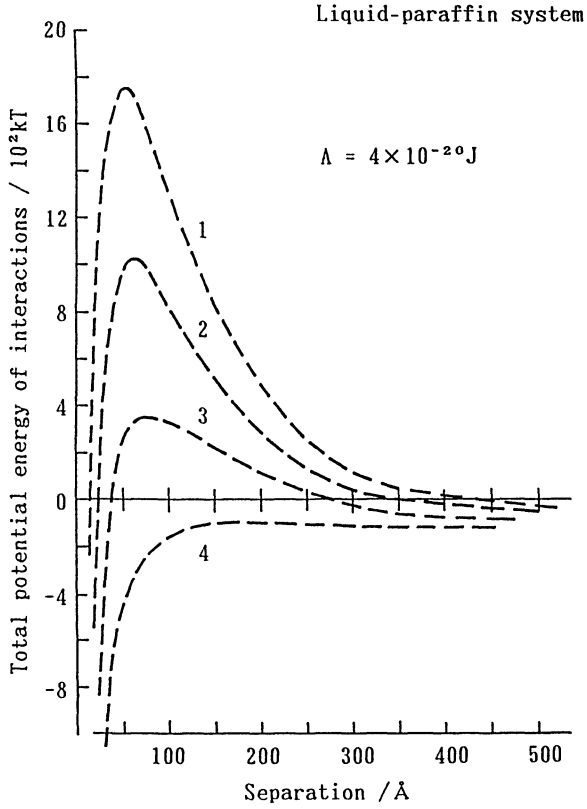

Fig. 1. Effect of proteins immobilized in the inner aqueous phase of a liquid-paraffin system on the total interactions between two vesicular globules at neutral pH at room temperature. Inner aqueous phase: Without protein (1), 1 %w/w BSA (2), 1 %w/w chymotrypsin (3), and 1 %w/w lysozyme (4).

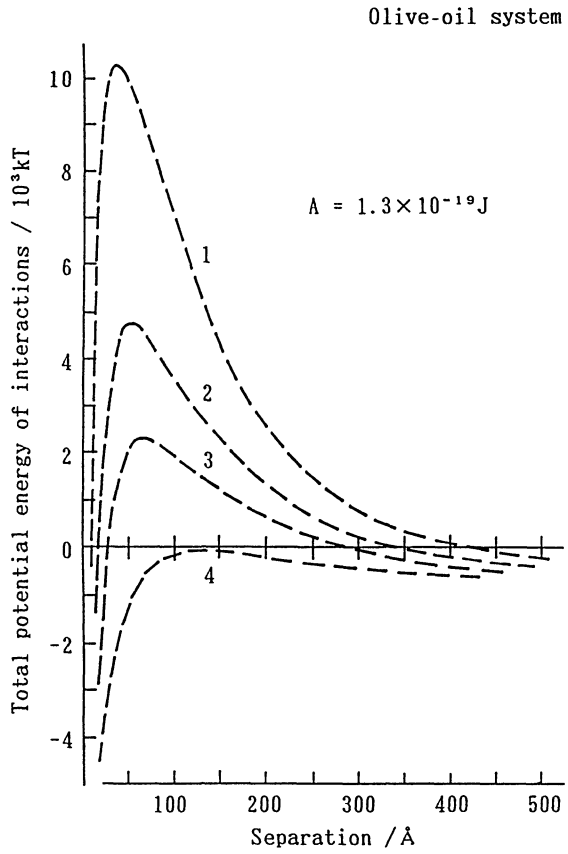

Fig. 2. Effect of proteins immobilized in the inner aqueous phase of an olive-oil system on the total interactions between two vesicular globules at neutral pH at room temperature. Inner aqueous phase: Without protein (1), 1 %w/w BSA (2), 1 %w/w chymotrypsin (3), and 1 %w/w lysozyme (4).

system. Such smaller values of the mobility and zeta potential may be brought about by the relatively longer distance to the slipping plane from the surface of the globules in the liquid-paraffin system, since the globules become to be covered by a deep hydration layer due to the existence of poly(oxyethylene) chains in the adsorbed molecules of Tween 80.[10]

Table 1, however, indicates that the zeta potential of the globules can be ranked with the magnitudes of the isoelectric point of the proteins, irrespective of the kinds of emulsifiers used, while a rapid coagulation occurs irreversively among the vesicular globules in the lysozyme samples showing the lowest zeta potential of the globules in a series of samples to be tested. The surface activity of protein molecules[11] may, more or less, result so as to form an adsorbed layer on the inside surface of the oil layer in the vesicular globules; the adsorbed protein molecules thus seem to play a role in the surface

potential of the vesicular globules according to the value of pH in the two aqueous phases. It is worth noting that the structure of the thin oil layer in a variety of W/O/W emulsions comprises the main lamellar membranes of the hydrophobic emulsifier, according to studies of the water permeation coefficient of the oil layer,[3] although the thickness of such a thin oil layer has not yet been fully investigated.

An attempt was made to estimate the total potential of interactions (V) between the two vesicular globules in each sample as a function of the separation (H) of the slipping planes in light of classical theory,[12] as follows:

$$V = 2\pi\varepsilon_0 D r\zeta^2 \ln[1+\exp(-\kappa H)] - rA/12H, \quad (2)$$

wherer r is the radius of the globules, H the separation, A the Hamaker constant, and κ the Debye–Hückel parameter given by

$$\kappa = (2ne^2z^2/\varepsilon_0 D\, kT)^{1/2}. \quad (3)$$

Here, n, e, and z are the concentration, elementary charge, and valency of counter ions, and kT is the thermal kinetic energy. The authors assumed that there is no energy barrier against the separation between the globules encapsulating lysozyme in each system because of the instability in the dispersion state, as already described. Thus, the value of the Hamaker constant could be estimated as being 4×10^{-20} J for the liquid-paraffin system and 1.3×10^{-19} J for the olive-oil system, respectively. Figures 1 and 2 compare the total potential of interactions against the separation between the two vesicular globules with each sample.

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