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BBA Report

Phase separation of Triton X-100 micelle solution induced by osmotic stress

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We have found out that the phase separation of Triton X-100 micelle solution was caused by the addition of poly(ethylene glycol) (PEG) above a critical concentration. The critical concentration of PEG depended on its molecular weight and temperature, larger molecular weight or higher temperature giving lower critical concentration. These results were analyzed on the basis of the osmoelastic coupling theory recently proposed by us (Biochemistry (1989) 28, 3710–3715; Biochemistry (1989) 28, 5626–5630).

Nonionic detergents containing polyoxyethylene chains as hydrophilic moieties have been used for solubilization of membrane proteins and their characterization [1–3]. For example, Triton X-100 micelle has been extensively applied to the solubilization of membrane proteins because of its high efficiency to solubilize the proteins and its low cost. It is known that Triton micelle suspensions separate into two phases with a raise in temperature [4]. This phenomenon is utilized to characterize the properties of hydrophobic membrane proteins [5]. However, the mechanism of the phase separation is not well understood [4,6–9].

In this report, we have studied the phase separation of Triton X-100 micelle solution caused by the addition of high molecular weight of PEG. The mechanism of this phase separation was reasonably explained according to the osmoelastic coupling theory recently proposed by us [10–12].

Triton X-100 (polyoxyethylene glycol(10)-*p*-*t*-octylphenyl ether) and PEG 20K, PEG 6K, PEG 4K, PEG 1K, PEG 400 and ethylene glycol were purchased from Wako Chemical Co. The nominal average molecular weights of these PEGs are 20000, 7500, 3000, 1000 and 400, respectively. Octadecyl Rhodamine B (R_{18}) was purchased from Molecular Probes Inc. Triton X-100 in Pipes buffer (10 mM Pipes, 140 mM NaCl (pH 7.5)) were mixed with various concentrations of PEG and equilibrated at an appropriate temperature from 4°C to 70°C. For light scattering measurement, a Hitachi F 3000 spectrofluorimeter was used. The wavelength and the angle of scattering were 450 nm and 90°, respectively.

The addition of PEG abruptly increased the intensity of light scattering of Triton X-100 micelle solution at a critical concentration, 16% (w/v) of PEG 6K and 11% (w/v) of PEG 20K, respectively (Fig. 1). A short centrifugation (15000 × *g* for 15 min) separated the micelle solution into two layers above the critical concentration of PEG. The concentrations of Triton X-100 in the upper layer, determined by the absorbance at 277 nm, was greatly decreased; in the presence of 20% (w/v) of PEG, the concentration was 12% of that of the PEG-free solution. In addition, almost all of the hydrophobic fluorescence probe, R_{18}, was incorporated into the lower layer. These results indicate that the addition of PEG caused the Triton suspension to separate into two phases; one phase is composed of concentrated micelles and the other composed of the diluted ones. The abrupt increase in the light scattering observed should be attributed to this phase separation.

The critical concentration of PEG for the abrupt increase in the light scattering depended on the molecular weight of PEG, and the smaller weight of PEG required the higher concentration (Table I). For example, PEG 20K caused the phase separation at 11% (w/v), whereas PEG 1K, PEG 400 and ethylene glycol

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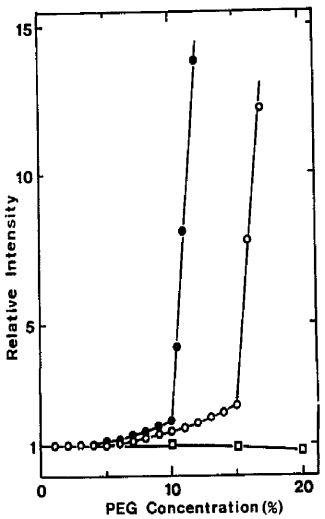

Fig. 1. Increase in the light scattering of 2% (v/v) Triton X-100 solution caused by PEGs with different molecular weights and ethylene glycol at 24°C; PEG 20K (●), PEG 6K (○), PEG 400 and ethylene glycol (same data; □). The ordinate gives the relative intensity of the light scattering represented as the ratio of the intensity in the presence of PEG to that in the absence of it.

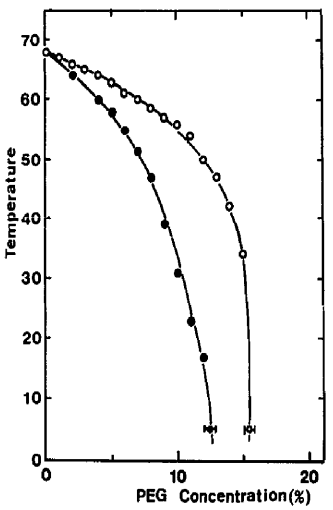

Fig. 2. Temperature dependence of the critical concentrations of PEG 20K (●) and PEG 6K (○) in 2% (v/v) Triton X-100 solution.

could not cause it even at 50% (w/v) (Fig. 1 and Table I). The critical concentration of PEG also depended on the temperature, decreasing with an increase in temperature (Fig. 2).

PEG-induced phase separation of Triton X-100 micelle solution might be due to direct interactions of PEG with Triton X-100 molecules. However, the following results suggest that the phase separation might not be due to the direct interaction; (1) the efficiency causing phase separation was strongly dependent on the molecular weight of PEG, (2) the head group of Triton X-100, which faces the aqueous phase, has the same chemical composition as PEG, and so it might not be able to interact specifically with PEG.

Alternatively, indirect interaction of PEG may bring about the phase separation. Regarding the mechanism of such indirect interaction, two different theories have been proposed so far.

One of them is the so-called 'volume exclusion theory', such as proposed by Tilcock and Fisher [13] in PEG-induced aggregation of phospholipid vesicles and by Arnold and Zschornig [14] in PEG-induced aggregation of low density lipoprotein. According to this theory, the Triton X-100 micelle should be forced to be aggregated at high concentrations of PEG, because the volume occupied by PEG is so large that the micelles should be excluded from the occupied region. However, as recognized in the PEG-induced aggregation of phospholipid vesicles or lipoproteins, this theory fails to give any satisfying explanation of the effects of physicochemical properties of the suspension and suspended particles such as pH and surface charge, and also any quantitative explanation of the molecular weight dependence of PEG [10,13,14].

The other, more favorable theory is the 'osmophobic association theory' recently proposed by Yamazaki et al. [10] and Ito et al. [11], which is thermodynamically equivalent to the 'preferential exclusion theory' proposed by Timasheff and his collaborators [15,16]. PEG molecules of high molecular weight should be preferentially excluded from the region adjacent to the surface of the micelle (exclusion layer) by steric hindrance, as is well recognized by our recent studies in protein solutions or phospholipid membranes [10–12,16,17]. Such preferential exclusion should cause an imbalance in osmolarity between the exclusion layer and bulk phase. The osmotic stress due to the imbalance in osmolarity

TABLE I

Molecular weight dependence of the critical concentrations for the PEG-induced abrupt increase in light scattering of Triton X-100 micelle solution

The concentration of Triton X-100 was 2% (v/v), and the light scattering was measured at 24°C.

PEG No	Molecular weight (av)	Critical concentration (%)
20000	20000	11
6000	7500	16
4000	3000	31
1000	1000	> 50

should be counterbalanced by the elastic pressure resulting from elastic compression of the micelle (osmoelastic coupling). The osmoelastic coupling may be followed by an increase in free energy of the dispersed micelle with the increased concentration of PEG. Consequently, above a critical concentration of PEG, the homogeneous suspension should be separated into two phases; one is the concentrated micelle phase and the other the diluted one (osmophobic association). Larger molecular weight of PEG may be able to cause the phase separation at lower concentration, since it may be excluded from the exclusion layer more effectively (Table I) [10,16].

An abrupt increase in the light scattering of Triton micelle solution is also observed above a critical temperature, which is known as 'cloud point phenomenon' [1,5,6]. The cause of this phenomenon may be due to an increase in the free energy of micelles in dispersed state with raised temperature. This temperature effect may explain the decrease in the critical intensity of osmotic stress with the raised temperature (Fig. 2).

The temperature-induced phase separation of Triton micelle solution is useful to extract membrane proteins at temperatures above the critical one [5]. The phase separation presented here, caused by the addition of poly(ethylene glycol), should be more useful, since the extraction could be carried out at a temperature low enough to prevent the protein denaturation.

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