

Aqueous micellar systems in membrane protein crystallization

Partial miscibility of a nonionic surfactant in the presence of salt or polyethylene glycol

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The effects of NaCl and polyethylene glycol (PEG) 4000 on the lower consolution boundary (LCB) of a nonionic surfactant (C_8E_5) were studied and compared. Micellar systems where NaCl and PEG 4000 are present are often used in membrane protein crystallization. While sodium chloride shifts the surfactant LCB to lower temperatures without a significant change in the shape of the boundary, PEG produces a large solubility change strongly depending on the surfactant concentration. The salt effect is explained by a reduced interaction of the micellar oligooxyethylene chains with the water and the PEG effect by an unfavourable configurational interaction between the C_8E_5 micelles and PEG molecules.

Detergent Crystallization Membrane protein Polyethylene glycol

1. INTRODUCTION

Well-ordered 3-dimensional crystals of several membrane proteins have recently been obtained after solubilizing the proteins by various nonionic surfactants. At least the following proteins have been crystallized by now: *E. coli* matrix and malto porin [1,2], bacteriorhodopsin [3], the photosynthetic reaction center from *Rhodospseudomonas viridis* [4] and *R. sphaeroides* [5] and the light-harvesting complex B800-850 from *R. capsulata* [6]. Crystallographic analysis of the *R. viridis* reaction center complex has proceeded to a resolution of 0.3 nm [7]. Several crystal space groups and morphologies were observed depending on the pH, concentration of salt and PEG and surfactant chemical structure. Two basic approaches were used to obtain the crystals, based on established methods for hydrophilic proteins [8]: Garavito et al. [1,2] used short-chain nonionic surfactants (α -octylglucoside and octyl oligooxyethylenes) in combination with NaCl and PEG, while Michel

used primarily a surfactant/ammonium sulfate/co-surfactant (e.g. 1,2,3-heptanetriol) system [3,4]. It seems that crystals preferentially form either in the isotropic micellar phase, close to the phase border [9], or in the surfactant-rich phase after phase separation (crystals grow in small isolated droplets) [2]. Critical properties of the crystallization mixtures and the position of phase boundaries are thus of primary importance. Binary mixtures of nonionic surfactants in water often show a well-defined phase separation, at a surfactant-specific temperature. The critical temperature with, e.g. the alkyl oligooxyethylene glycol (C_nE_m) series, depends on the number of methylene and oxyethylene units (values range from 4 to 120°C [10]). Since micelles are composed of amphiphile monomers they can freely change their size and shape. Both 1H -NMR linewidth and self-diffusion measurements [11,12] and neutron scattering measurements [13,14] indicate no substantial micellar growth as a function of temperature or concentration for several species of the C_nE_m series

(C_8E_5 , $C_{12}E_6$, $C_{12}E_8$, $C_{16}E_8$). The micelles thus retain an essentially spherical form (2.5–6.0 nm radius) and the fast exponential increase of, e.g. the apparent hydrodynamic radius, with temperature is due to strong interparticle interactions. The phase separation process can thus be analyzed, as with ordinary binary liquid mixtures, in terms of critical exponents for static and dynamic scattering properties [15]. The extent of aggregate clustering is then roughly proportional to the temperature or concentration distance from the demixing point [15]. After phase separation one of the phases contains practically all the surfactant (surfactant-rich phase), while the other contains most of the solvent water and the hydrophilic components. The micellar geometry is retained after phase separation in the condensed phase with most C_nE_m surfactants [16]. In the surfactant-rich phase one has a continuous structure of close-packed (mixed) micelles, but some membrane proteins may denature there, e.g. bacteriorhodopsin [9]. We present results on how NaCl and PEG 4000 change the phase separation boundary (1–50 vol%) of the surfactant C_8E_5 in water.

2. MATERIALS AND METHODS

The nonionic surfactant *n*-octyl pentaoxyethylene glycol, C_8E_5 , was obtained from Bachem (Bubendorf, Switzerland). The surfactant is of high purity and monodisperse to a high degree with respect to the alkyl and oligooxyethylene chain lengths [17]. No aging effects were detected even after a storage for 4 months in a desiccator at room temperature; the demixing temperature, of 2 vol% C_8E_5 solutions, remained the same to within 0.1°C . PEG 4000 of analytical grade was obtained from Fluka (Switzerland) and used without further purification. Two PEG concentrations of 40 and 80 mg/ml were prepared in double-distilled water. The solutions, in quantities of 0.5 ml, were made by weighing the surfactant and aqueous PEG 4000 solutions. These were allowed to equilibrate overnight before measurement. Some of the samples containing PEG and a high (40 or 50 vol%) surfactant concentration had already separated into 2 phases at room temperature.

Phase separation (or clouding) temperatures of the solutions in quantities of 0.4 ml were deter-

mined in 2-ml cylindrical glass tubes which were carefully closed and mounted in an aluminium holder with 12 tube positions. The holder was fully immersed in a large-volume (20 l) Schott viscosity bath with windows for observation on the front and back sides. The samples were visually observed while the temperature of the water bath was slowly ($0.08^\circ\text{C}/\text{min}$) increased with a Lauda temperature controller. Clouding occurred for all the studied solutions homogeneously, and at a well-defined temperature. If the temperature was stopped at the clouding temperature, separation into 2 phases could be observed after 2–3 min. We have shown that the cloud point temperature with small sample volumes is the same ($\pm 0.1^\circ\text{C}$) in the volume range 0.05–0.4 ml [18].

3. RESULTS AND DISCUSSION

3.1. Effect of sodium chloride

The consolution boundaries for C_8E_5 in the presence of NaCl at 4 concentrations, 0.2, 0.5, 0.9 and $1.3\text{ mol}/\text{dm}^3$, are shown in fig.1 (data from [18]). Added NaCl thus shifts the binary C_8E_5 – H_2O boundary in temperature without greatly changing

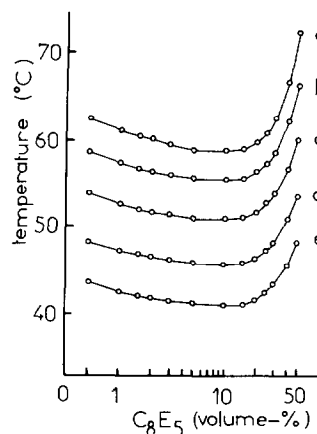


Fig.1. Lower consolution boundaries (0–50 vol%) of the nonionic surfactant C_8E_5 in (a) water, (b) $0.2\text{ mol}/\text{dm}^3$ NaCl, (c) $0.5\text{ mol}/\text{dm}^3$ NaCl, (d) $0.9\text{ mol}/\text{dm}^3$ NaCl and (e) $1.3\text{ mol}/\text{dm}^3$ NaCl. Circles correspond to experimental clouding temperatures. Below the curve: isotropic micellar (surfactant) phase. Above curve: 2-phase area; the upper phase is surfactant-rich, the lower (aqueous) phase contains surfactant at low concentration ($<2\text{ vol}\%$).

its shape. In fig.3a we show how the phase separation temperature depends on NaCl concentration. Demixing observed with C_nE_m surfactants is most probably due to a decrease in oligoxyethylene-water interaction [12–14]. This interaction is sensitive to various external perturbations due to directional hydrogen bonding between the ether oxygens and water molecules and a simultaneous accommodation of the ethylene groups in the overall solvent structure [19]. Increasing the temperature disfavours the arrangement and ultimately leads to a phase separation. Salting out of C_8E_5 by various monovalent salts follows the prediction of the classical Hofmeister or lyotropic series [20]; the solubility shifts are mainly determined by the anions. This is surprising since it is known that anions are less hydrated than cations [21]. The salt effect is thus not due to a water competition effect but must be related to how the ions interact with the solute water interface [18,22,23]. This reasoning is based on surface tension measurements [24,25] which show that anions are repelled from a dielectric discontinuity like the water/air or the water/oil interface in the order $F^- > Cl^- > Br^- > I^-$. Of these iodide even shows a net attraction towards the interface. The cations all seem to be strongly repelled [25]. A repulsive force between the ion and the interface leads to the formation of salt-deficient regions around the solute, and the size of the regions will depend on the strength of the repulsion. It has not yet been established if the resulting salting out is predominantly an entropic effect due to the existence of the salt-deficient regions, or an enthalpic effect due to dehydration of the solute with increasing salt concentration.

3.2. The effect of polyethylene glycol 4000

Phase separation temperatures for ternary mixtures of C_8E_5 -PEG 4000-water were determined in the surfactant concentration range 1–50 vol%. Fig.2 shows the effect of 2 PEG concentrations of 40 and 80 mg/ml and fig.3b shows the lowering of the clouding temperature from the binary (C_8E_5 -water) value as a function of surfactant concentration. At low C_8E_5 concentrations PEG 4000 has only a small effect and the critical temperature is essentially determined by the component with the lowest solubility, i.e. the nonionic micelles. PEGs exhibit similar lower consolution boundaries to the C_nE_m surfactants but at much higher temperatures

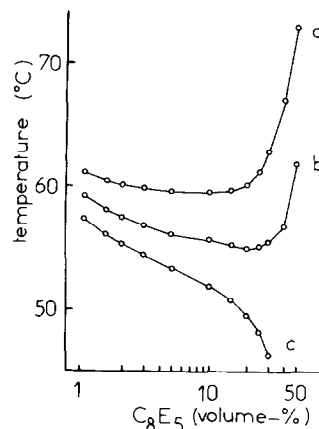


Fig.2. Lower consolution boundaries (0–50 vol%) of the nonionic surfactant C_8E_5 in (a) water, (b) 40 mg/ml PEG 4000 and (c) 80 mg/ml PEG 4000. Below curve: isotropic micellar (surfactant) phase. Above curve: 2-phase area; the upper phase is surfactant-rich, the lower phase contains essentially all the PEG (>95% as deduced from the intensity of scattered light). Solutions with 40 or 50 vol% C_8E_5 (80 mg/ml PEG 4000) were phase separated at room temperature.

(>150°C) [26]. Since the phase separation values of the ternary solutions approach the binary C_8E_5 values at low surfactant concentration, the oligoxyethylene-water interaction must be essentially unaffected by the presence of PEG molecules. When the surfactant concentration is increased there is a strong deviation of the demixing values from the binary values. With 40 mg/ml PEG 4000 the boundary is still of a similar shape to that in water, but with 80 mg/ml PEG 4000 the high-concentration branch turns towards low temperatures (fig.3), and solutions with 40 or 50 vol% C_8E_5 are phase separated already at room temperature. The strong lowering of the critical temperatures with increasing surfactant concentration could be due to transient cross-linking of adjacent micelles by the PEG molecules, but this is unlikely since oxyethylene-water interaction still strongly dominates over oxyethylene self-interaction at 60°C. A comparison of the surfactant consolution boundary in water and in aqueous PEG solutions (fig.2) indicates that a simple excluded volume effect does not explain the data [27]. We propose that the PEG effect is due to an unfavourable configurational (entropic) effect between the micelles and the PEG molecules, i.e. the distribution of the 2

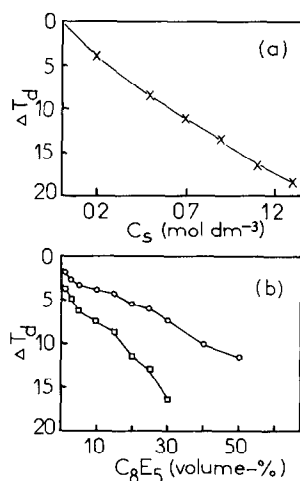


Fig.3. (a) The lowering (ΔT_d) of C_8E_5 (2 vol%) phase separation (clouding) temperatures as a function of NaCl concentration (C_s). (b) Difference (ΔT_d) between phase separation temperatures of C_8E_5 in water and in (○) 40 mg/ml PEG 4000, (□) 80 mg/ml PEG 4000.

components is nonuniform on a microscopic scale through the solution. With aqueous protein solutions the PEG effect has been attributed to a local clustering of the protein molecules beyond their solubility limit [28] or to an 'unfavourable thermodynamic interaction between the PEG molecules and the proteins' [29].

3.3. Application to membrane protein crystallization

We have thus shown that NaCl and PEG change the micellar partial miscibility by 2 different mechanisms. Sodium chloride changes the interaction of the micellar oligooxyethylene chains with water and thus shifts the boundary to lower temperatures without strong surfactant concentration dependence. With PEG the lowering of phase separation temperatures depends on surfactant concentration (fig.3); solutions with 40 or 50 vol% surfactant are already separated at room temperature. When the micelles consist of the surfactant plus an integral membrane protein the addition of salt can be used to change electrostatic protein-protein interactions. If the surfactant concentration is increased at constant temperature, e.g. in a vapour diffusion experiment, the tendency of the entities to form clusters will increase, due to the presence of PEG, until phase separation occurs.

Additives which induce a similar inversion of the surfactant demixing boundary to PEG are potentially interesting for crystallization experiments.

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