# Investigation on the Mechanism of Crystallization of Soluble Protein in the Presence of Nonionic Surfactant

Yanwei Jia, Janaky Narayanan, Xiang-Yang Liu, and Yu Liu
Department of Physics, National University of Singapore, Singapore 117542

ABSTRACT The mechanism of crystallization of soluble, globular protein (lysozyme) in the presence of nonionic surfactant $C_8E_4$ (tetraoxyethylene glycol monooctyl ether) was examined using both static and dynamic light scattering. The interprotein interaction was found to be attractive in solution conditions that yielded crystals and repulsive in the noncrystallizing solution conditions. The validity of the second virial coefficient as a criterion for predicting protein crystallization could be established even in the presence of nonionic surfactants. Our experiments indicate that the origin of the change in interactions can be attributed to the adsorption of nonionic surfactant monomers on soluble proteins, which is generally assumed to be the case with only membrane proteins. This adsorption screens the hydrophobic attractive force and enhances the hydration and electrostatic repulsive forces between protein molecules. Thus at low surfactant concentration, the effective protein-protein interaction remains repulsive. Large surfactant concentrations promote protein crystallization, possibly due to the attractive depletion force caused by the intervening free surfactant micelles.

## INTRODUCTION

The difficulty of growing protein crystals of suitable size for x-ray crystallography is an impediment to molecular structure determination of proteins. In aqueous solutions, membrane proteins tend to form amorphous aggregates. In the presence of nonionic surfactant, membrane proteins maintain their physiological properties and functions and hence remain soluble. Most importantly, membrane proteins can still be crystallized by more or less conventional means within such an environment (1,2). Therefore, nonionic surfactants are widely used to crystallize membrane proteins since two membrane proteins, bacteriorchodopsin and porin, were successfully crystallized for the first time in 1980 (1,2). As soluble proteins share, to some extent, the aggregation problems of the membrane proteins, the mild nonionic surfactant may also be of use in crystallizing them. A few groups have successfully crystallized soluble proteins with nonionic surfactants (3,4). However, the mechanism of the soluble protein crystallization in the presence of nonionic surfactant is not well understood.

George and Wilson illustrated the importance of intermolecular interactions in the crystal growth of proteins (5). The protein-protein interaction can be characterized by the osmotic second virial coefficient, $B_{22}$. A positive value of $B_{22}$ refers to predominantly repulsive interactions and a negative value to attractive interactions. It has been suggested that an empirical criterion for soluble protein crystallization is $-8 \times 10^{-4} < B_{22} < -2 \times 10^{-4}$ ml mol/g$^2$. The applicability of this criterion to the crystallization of membrane proteins in the presence of nonionic surfactants has been investigated recently (6). However, it is also desirable to examine the mechanism of soluble protein crystallization in the presence of nonionic surfactants or other amphiphilic molecules and elucidate the importance of $B_{22}$.

In this work, we use lysozyme as the model protein to examine the protein interactions in nonionic surfactant solutions using static light scattering (SLS) and dynamic light scattering (DLS). The validity of the criterion for $B_{22}$ is discussed by comparing the experimental results with predicted values. At the end, the role played by nonionic surfactant in the modification of protein interactions is investigated in an effort to unravel the mechanism of crystallization.

## MATERIALS AND METHODS

### Materials

Hen egg white lysozyme (six times purified by crystallization) purchased from Seikagaku (East Falmouth, MA) was used as the model protein. Tetraoxyethylene glycol monooctyl ether ($C_8E_4$) purchased from Sigma (St. Louis, MO) was used as the model nonionic surfactant in protein crystallization. High purity deionized water (~18.2 MΩ) from Millipore (Billerica, MA) Milli-Q system (0.22 $\mu$m) was used for preparing 0.1 M sodium acetate buffer at pH 4.5. The protein was dissolved in the buffer and filtered (0.2 $\mu$m) to make a stock solution and was kept in the refrigerator at 4°C for further use. Protein stock solution, surfactant, and buffer were mixed together in required stoichiometric ratios to obtain the desired concentration of the protein and surfactant for the following experiments. Both protein and surfactant were used as received without further purification. All measurements were carried out at 20°C (±0.1°C).

### Protein crystallization

Protein crystallization was carried out by batch method using a sample volume of 40 $\mu$l. The mixture of $C_8E_4$/lysozyme solutions were kept in the Eppendorf tubes with the cap sealed by parafilm. The samples were kept in water bath at 20°C. Observation was made under microscope periodically.

### Refractive index increment (*dn/dc*)

Abbe refractometer, T4 type (ATAGO, Tokyo, Japan) was used to measure the refractive index, $n$, of the mixed solution of lysozyme and $C_8E_4$ with an

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Address reprint requests to Xiang-Yang Liu, E-mail: phyliuxy@nus.edu.sg.

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accuracy of 0.001. The light source was a sodium lamp (wavelength = 589 nm). The measurements were carried out at 20°C for a series of lysozyme concentrations, $c$, in the range 0–50 mg/ml, in $C_8E_4$ solutions of different concentrations. The refractive index increment, $dn/dc$, was obtained from the regression analysis of the plot of $n$ versus $c$.

## Static light scattering

All light scattering measurements were performed at 20°C using a Brookhaven light scattering instrument (Holtsville, NY) with BI 9000AT correlator. The light source was a 5 mW He-Ne laser with wavelength, $\lambda$, of 633 nm and vertical polarization. Since the molecular size of each of the samples used was $<\lambda/20$, no angular dependence for the excess scattered intensity was expected and all light scattering data were recorded at an angle of 90°.

For dilute protein solutions, the SLS equation is given by Brown (7),

$$\frac{Kc}{R_{90}} = \frac{1}{M_w} + 2B_{22}c, \quad (1)$$

where

$$K = \frac{4\pi^2 n_0^2 (dn/dc)^2}{N_A \lambda^4}, \quad (2)$$

$n_0$ is a refractive index of the solvent, $dn/dc$ the refractive index increment for the protein/solvent pair, $N_A$ the Avogadro number, $\lambda$ the wavelength of the incident light in vacuum, $c$ the concentration of the protein (g/ml), and $R_{90}$ the excess Rayleigh ratio at an angle of 90°. The protein concentration was <10 mg/ml.

The absolute $R_{90}$ values were determined from the excess scattered intensities (intensities above that due to solvent, which in our case is the 0.1 M NaAc with/without added surfactant, and background) by calibration of the photometer using toluene as standard. Equation 1 indicates that from a plot of $Kc/R_{90}$ versus $c$, the molecular weight of the protein $M_w$ can be obtained from the intercept and the second virial coefficient $B_{22}$ can be obtained from the slope of the curve.

## Dynamic light scattering

DLS measurements utilize the temporal correlations of the scattering intensity fluctuations, which are related to the Brownian motion of the solute (8,9). For dilute solutions,

$$D_m(c) = D_0(1 + k_d c), \quad (3)$$

where $D_m(c)$ is the mutual diffusion coefficient which is concentration dependant. The parameter $D_0$ is related to $R_H$, the hydrodynamic radius of scatterers, and $\eta$, the solvent viscosity, through the Stokes-Einstein equation,

$$D_0 = \frac{k_B T}{6\pi\eta R_H}. \quad (4)$$

The parameter $k_d$ can be expressed as

$$k_d = 2M_w B_{22} - k_f - 2\nu, \quad (5)$$

where $k_f$ is the coefficient of the linear term in the development of the friction coefficient factor and $\nu$ is the partial specific volume of the protein molecule. $k_f$ can be determined from self-diffusion measurements using pulsed gradient spin-echo (PGSE) NMR. Combining Eqs. 3 and 5, the self-diffusion coefficient $D_0$ can be obtained from the intercept of linear regression plot of $D_m$ versus $c$. Moreover, because both $k_f$ and $\nu$ are always positive, a positive $k_d$ indicates a positive $B_{22}$, and a negative $B_{22}$ gives negative values for $k_d$.

## Surface tension

Surface tension was determined using the Wilhelmy plate method with a K14 Krüss tensiometer (accuracy 0.01 mN/m) (Hamburg, Germany). The measurements were performed at 20°C by gradual increase of surfactant concentration. An appropriate volume of protein stock solution was added to keep the protein concentration constant at 0.2 mg/ml or 2 mg/ml while changing the surfactant concentration. The solution was stirred and allowed to rest for 10 min before each run of measurement. The equilibrium surface tension was calculated by averaging 10 data points collected at the end of each run until the standard deviation became <0.01 mN/m.

# RESULTS AND DISCUSSION

## Crystallization

A series of lysozyme concentrations from 40 mg/ml to 100 mg/ml and $C_8E_4$ concentrations from 0.5% to 30% were screened for crystallization. The results are shown in Table 1. Irrespective of the protein concentration, no crystallization occurred in the 0.5% $C_8E_4$ solution, and the solution remained clear even after 3 months. In 30% $C_8E_4$, lysozyme crystals appeared in all the trials (with lysozyme concentration >40 mg/ml) in ~1 month. For the intermediate $C_8E_4$ concentration, amorphous aggregates and/or crystals were obtained at some protein concentrations. In some cases, crystals grew up from the amorphous aggregates. However, these cases were less reproducible.

The crystallization of soluble proteins such as lysozyme, horse heart cytochrome c, bovine red blood cell ubiquitin etc., in the presence of nonionic surfactant, $C_8E_4$, has been investigated by Mustafa et al. (3). They used the vapor diffusion method (sitting drop) and batch method (layering 5 $\mu$l of surfactant solution on top of 5 $\mu$l protein solution and allowing slow evaporation of water by keeping one end unsealed). Our results show the same optimal crystallization condition as obtained by them in the batch method. Furthermore, in our study, the concentration of both protein and surfactant were fixed in crystallization trials by sealing the crystallization tube. This would benefit the study of protein molecular interactions by the light scattering experiment as the solvent condition becomes a known parameter.

## Refractive index increment ($dn/dc$)

The refractive index increment, $dn/dc$, is a prerequisite for the data analysis of SLS (vide Eqs. 1 and 2). As shown in Table 2, the refractive index of the solvent ($C_8E_4$ in buffer),

**TABLE 1 Lysozyme crystallization results in the presence of $C_8E_4$**

| Concentration of $C_8E_4$ (v/v %) | Concentration of lysozyme (mg/ml) | Crystallization results |
|---|---|---|
| 0.5% | 40–100 | CS |
| 10% | 50–100 | CS/AA |
| 20% | 40–80 | CS/AA |
| | 90 | crystal |
| 30% | 40–100 | crystal |

CS, clear solution; AA, amorphous aggregation, with crystal growth from aggregates in a few cases.

**TABLE 2 Refractive index and refractive index increment, *dn/dc*, of lysozyme in $C_8E_4$ solution**

| Solvent | $n_s$ | *dn/dc* (ml/g) |
|---|---|---|
| Buffer only | 1.335 | 0.177 |
| 0.5% $C_8E_4$ | 1.335 | 0.167 |
| 5% $C_8E_4$ | 1.341 | 0.185 |
| 10% $C_8E_4$ | 1.347 | 0.142 |
| 20% $C_8E_4$ | 1.360 | 0.118 |
| 30% $C_8E_4$ | 1.371 | 0.114 |

$n_s$, the refractive index of solvent (0.1 M NaAc buffer at pH 4.5 with/without added surfactant $C_8E_4$).

$n_s$, increased with $C_8E_4$ concentration gradually. In the case of buffer alone, $n_s$ was 1.335, whereas for 30% v/v $C_8E_4$, $n_s$ was 1.371. In the absence of $C_8E_4$, the *dn/dc* of lysozyme in buffer was 0.177 ml/g, consistent with that from the literatures (10,11). In the presence of $C_8E_4$, the variation of *dn/dc* of lysozyme with $C_8E_4$ concentration was not very large for low surfactant concentrations, but at high $C_8E_4$ concentration (30% v/v), the *dn/dc* decreased to 0.114 ml/g, ~60% of that without $C_8E_4$.

We have to mention here that generally dialysis needs to be performed, and the dialysate should be used for *dn/dc* and SLS measurements. This is to keep the chemical potential of all other solutes except protein a constant during these measurements. The *dn/dc* of protein in the presence of SDS (sodium dodecyl sulfate) had been studied by other groups (12–15). Dialysis time varied from 1 day to several months (12,14,15). However, results show that the dialysis equilibrium is difficult to achieve even after several weeks and is a function of the cutoff size of the membrane used (12). The *dn/dc* keeps increasing with time because the solution is not in equilibrium. The reason for the long equilibration time is the slow diffusion of surfactant. The micelles cannot pass through the membrane. The higher the micelle concentration, the longer the time needed to reach equilibrium. Very high surfactant concentrations were used in our crystallization trials. Hence we expected the same tendency of *dn/dc* in dialysis while using $C_8E_4$ solutions. Also a huge amount of $C_8E_4$ is needed to perform dialysis. The *dn/dc* values reported here are at constant solute concentration rather than at constant chemical potential. Thus we estimate apparent rather than true molecular weights and second virial coefficients.

## Static light scattering

Fig. 1 shows the plot of $Kc/R_{90}$ against the lysozyme concentration, $c$, in the presence of different concentrations of $C_8E_4$. As per Eq. 1, the intercept corresponds to the reciprocal molecular weight of protein, and the slope gives the value of the second virial coefficient. The data do not coincide at a single point on the ordinate axis. The molecular weight for lysozyme in buffer is estimated to be 13.7 kDa (Table 3), close to the value given by the manufacturer and the literature (14.3 kDa). In the presence of $C_8E_4$, the estimated molecular weight of lysozyme is from 17.9 kDa to 46.8 kDa, much higher than that obtained in the absence of $C_8E_4$. Apart from the approximation in *dn/dc* values as discussed above, this large value of estimated molecular weight can be attributed to the adsorption of surfactant monomers on protein molecules. This adsorption has been confirmed by the surface tension measurements, which will be discussed later. The molecular weight of $C_8E_4$ monomer is 306 Da (16). The increase of the molecular weight of protein indicates that ~70 $C_8E_4$ monomers are adsorbed. This number is less than the aggregation number of $C_8E_4$ micelle, which has been measured by pulsed-gradient nuclear magnetic resonance (PGNMR) and found to be 85 (17). The binding of nonionic surfactant on protein has been verified for bovine serum albumin (BSA) and is attributed to the hydrophobic interaction (18). It has been found that the strength of binding depends inversely on the number of oxyethylene units in the surfactant. The high number of $C_8E_4$ adsorbed on lysozyme may be because of the four oxyethylene units of $C_8E_4$.

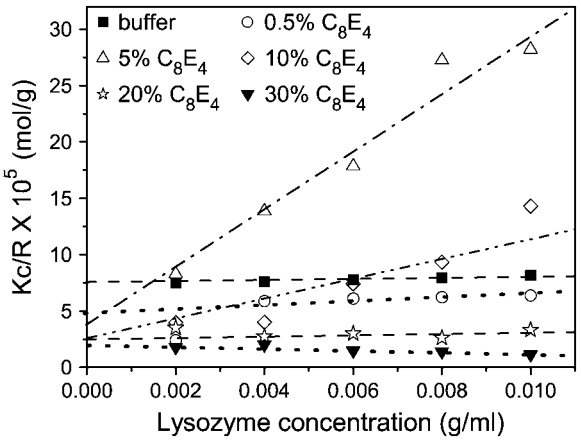


FIGURE 1 Plot of $Kc/R$ versus lysozyme concentration, $c$, for different $C_8E_4$ concentrations.

The second virial coefficients obtained from slopes are summarized in Table 3. For lysozyme in buffer, 0.5% and 5% $C_8E_4$, the $B_{22}$ values were positive and increased with $C_8E_4$ concentration, from $4.08 \times 10^{-4}$ to $233 \times 10^{-4}$ ml mol/g$^2$. This indicates an increase in repulsive interactions between protein molecules in the presence of small amounts of $C_8E_4$, which stabilizes the protein solution. When the $C_8E_4$ concentration increases to 10%, $B_{22}$ starts decreasing. In 20% and 30% $C_8E_4$, negative values were obtained for $B_{22}$, indicating an attractive interaction between protein molecules. Comparing with crystallization results, it can be inferred that for the noncrystallization condition the interaction is repulsive, whereas for crystallization conditions the interaction is attractive and falls in the crystallization window defined for $B_{22}$. The amorphous aggregation observed in some protein samples in 10% and 20% $C_8E_4$ cannot be explained by the $B_{22}$ values. However, a possible

**TABLE 3 Variations with $C_8E_4$ concentration of parameters obtained from SLS and DLS according to Eqs. 1–5**

| Solvent | $B_{22}$ ($10^{-4}$ ml mol/g$^2$) | Crystallization result | $M_w$ (kDa) | $D_0$ ($10^{-7}$ cm$^2$/s) | $k_d$ (ml/g) | $R_H$ (nm) |
|---|---|---|---|---|---|---|
| Buffer | 4.08 | CS | 13.7 | 12.08 | −2.85 | 1.85 |
| 0.5% $C_8E_4$ | 4.11 | CS | 17.9 | 8.40 | 23.15 | 2.66 |
| 5% $C_8E_4$ | 233 | CS | 32.1 | 8.17 | 61.90 | 2.73 |
| 10% $C_8E_4$ | 130 | CS/AA | 31.3 | 7.07 | 8.10 | 3.16 |
| 20% $C_8E_4$ | −2.64 | CS/AA/crystal | 31.4 | 7.62 | −3.94 | 2.93 |
| 30% $C_8E_4$ | −4.72 | crystal | 46.8 | 8.42 | −27.27 | 2.65 |

CS, clear solution; AA, amorphous aggregation.

explanation is that some free micelles may locally link protein molecules studded with surfactant monomers, causing amorphous aggregation.

## Dynamic light scattering

The findings of SLS measurements on interactions of lysozyme molecules in $C_8E_4$ were verified by determining the mutual diffusion coefficient, $D_m$, using DLS as shown in Fig. 2. The parameters obtained from SLS and DLS are summarized in Table 3. According to Eqs. 3 and 5, the positive slopes for 0.5%, 5%, and 10% $C_8E_4$ suggest that $B_{22}$ is positive for these conditions, whereas negative slopes for 20% and 30% $C_8E_4$ indicate negative $B_{22}$ and a resultant attractive force between lysozyme molecules. For pure lysozyme in buffer, the slope is slightly negative. This is because $B_{22}$ has a small positive value as obtained in SLS ($4.08 \times 10^{-4}$ ml mol/g$^2$). As shown in Eq. 5, $k_d$ can be negative for small positive value of $B_{22}$ because $k_f$ and $\nu$ are always positive. The mutual diffusion coefficient values, $D_m$, for pure lysozyme found in this study are consistent with those found by other authors (13,19,20).

The intercept of $D_m$ versus $c$ gives the self-diffusion coefficient, $D_0$, of the scatterers. The hydrodynamic radius, $R_H = 1.85$ nm, evaluated from the intercept $D_0$ using Eq. 4 agrees well with values reported in the literature for pure lysozyme in the absence of $C_8E_4$ (11,13,20). However, in the presence of $C_8E_4$, irrespective of its concentration, the $D_0$ value is found to be $\sim 8 \times 10^{-7}$ cm$^2$/s. The viscosity of $C_8E_4$ was found to almost double for every 10% increase in concentration. Equation 4 was used to extract the $R_H$ value using solvent viscosity $\eta$ as that of buffer. The $R_H$ value evaluated in the presence of $C_8E_4$ was ~2.7 nm. The reason for this higher $R_H$ value may be twofold: 1), the adsorption of $C_8E_4$ monomers on lysozyme molecules increases the size of scatterers, and 2), the light scattering of micelles dominates over that of lysozyme molecules. The critical micelle concentration (CMC) of $C_8E_4$ is 0.2%, as found by surface tension measurements. At all the surfactant concentrations studied here, micelles already exist in a large amount. Therefore, when the protein concentration is low, micelles are predominant scatterers in the solution. Since DLS measures the collective diffusion of macromolecules (protein and micelles), the intercept, $D_0$, may correspond to the more prominent scatterers, namely, the micelles. The size of $C_8E_4$ micelles was measured to be 2.5 nm using PGNMR (17). If the size of protein molecules with adsorbed $C_8E_4$ monomers is the same as that of micelles, they may not be distinguished by light scattering. Similar observation has been made by Valster et al. for BSA-$C_{12}E_8$ complexes (12). They concluded that at high surfactant concentration, the hydrodynamic radius of scatterers obtained is the same as free micelles. The dispute over $R_H$ notwithstanding, the variation of $D_m$ with protein concentration gives supportive evidence for the $B_{22}$ measurements of the SLS study.

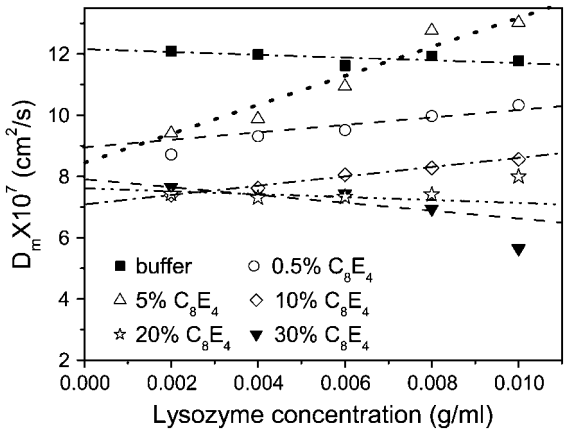


FIGURE 2 Plot of mutual diffusion coefficient versus lysozyme concentration for various $C_8E_4$ concentrations.

## Surface tension measurement

Composed of hydrophobic and hydrophilic amino acids, protein is surface active in aqueous solution (21). Thus surface tension measurement is an effective method to reveal the properties of surfactants and proteins. To explain the origin of changes in lysozyme molecular interactions in the presence of $C_8E_4$, surface tension measurement was performed.

The surface tension of pure $C_8E_4$ in buffer solution was determined before the measurements involving $C_8E_4$/lysozyme mixtures. Although the measurement of the surface tension of $C_8E_4$ in pure water has been reported before (22), a surface tension profile for $C_8E_4$ in sodium acetate buffer

under the specific ionic strength of 0.1 M is not available in the literature. Fig. 3 shows the surface tension plot of $C_8E_4$ in buffer, together with the profile measured for the mixture under fixed lysozyme concentrations of 0.2 mg/ml and 2 mg/ml. The CMC of $C_8E_4$ in acetate buffer remains the same (0.2%) as that in pure water—an expected result for nonionic surfactant in salt solution.

Compared with pure $C_8E_4$ in buffer, the surface tension profiles for the mixed $C_8E_4$/lysozyme system are shifted slightly upward for $C_8E_4$ concentrations $<$ CMC. This is suggestive of the adsorption of $C_8E_4$ monomers on lysozyme molecules at these concentrations. According to Gibbs's equation (23),

$$\Gamma = -\frac{1}{RT}\frac{d\gamma}{d\ln a}, \tag{6}$$

where $\Gamma$ is the adsorption amount of surfactant at the interface, $R$ is the gas constant, $T$ is the absolute temperature, $\gamma$ is the surface tension, and $a$ is the surfactant activity. The surface tension decreases in proportion to the amount of surfactant adsorbed at the interface. When some surfactant monomers are adsorbed on the protein, the activity of surfactant will decrease as the number being adsorbed on the solution surface is less. Therefore, the surface tension will be higher than that without protein. The larger the protein concentration, the greater will be the surface tension, as observed for 0.2 and 2 mg/ml protein and depicted in Fig. 3. Once the hydrophobic sites on the protein are occupied by the monomers, the surface tension profile follows the same path as in the absence of protein. This can be seen at $C_8E_4$ concentrations $\geq$ CMC as shown in Fig. 3.

A similar result has been obtained for the system of mixed human serum albumin and dodecyl dimethyl phosphine oxide, HAS/$C_{10}$DMDO, solutions (24). The quantitative fluorescence studies by Velev et al. also revealed the surfactant adsorption on the protein crystal matrices (25).

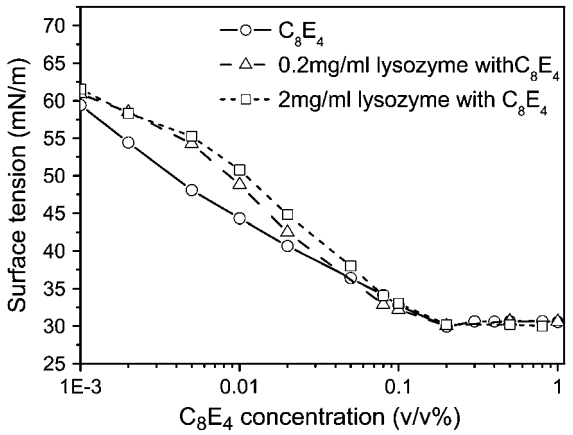


FIGURE 3 Plot of equilibrium surface tension of $C_8E_4$ at different concentrations with and without mixed lysozyme.

## Mechanism of protein crystallization with nonionic surfactant

As discussed in the above sections, $C_8E_4$ monomers tend to adsorb on hydrophobic parts of lysozyme molecules. This adsorption screens the hydrophobic attractive interactions between protein molecules and increases the hydrophilicity of protein surface. Solvation becomes more favorable and results in a higher repulsive protein-protein interaction. As $C_8E_4$ concentration is increased to 20% or 30% v/v, the interaction becomes attractive and falls in the crystallization window. According to Loll et al., the crystallization slot for membrane protein in the presence of nonionic surfactant lies near the cloud point of the surfactant (6). However, the cloud points for all the solution conditions used by us were above 35°C, far higher than the crystallization temperature, 20°C. These excluded the clouding and phase separation of micelles as a reason for proteins to partition into aqueous phase and crystallize.

Asakura and Oosawa proposed a model to describe the ''depletion attraction'' between colloidal particles on addition of nonadsorbing polymers (26). Using this model, the globular protein can be approximated as a sphere of radius $R$, and the free micelle in solution as a sphere of radius $R_g$. There is a shell of thickness $R_g$ around the protein into which the micelle cannot penetrate (Fig. 4 *a*). When far apart, a uniform osmotic pressure is exerted on protein molecules. As protein molecules come closer, micelles no longer penetrate the shaded region in Fig. 4 *b*. As a consequence, the osmotic pressure is unbalanced and drives protein molecules close together. Thus a net attractive potential is set up between protein molecules. When the concentration of free micelles is large, this excluded volume effect becomes predominant, enhancing the depletion attractive forces, which are short ranged.

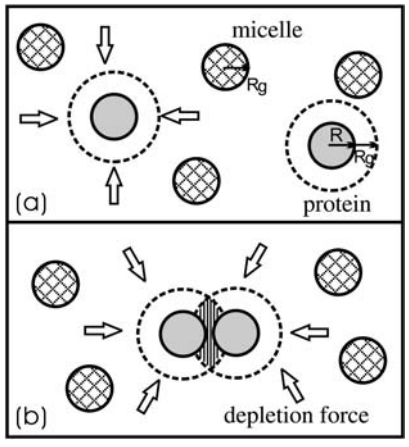


FIGURE 4 Depletion mechanism. (*a*) When far apart, a uniform osmotic pressure is exerted on the protein molecules of radius $R$. (*b*) Micelles of radius $R_g$ cannot enter the region between the closely spaced molecules of radius $R$. The result is net attraction between protein molecules of radius $R$.

This model explains why at higher $C_8E_4$ concentration, the interaction between lysozyme molecules becomes attractive. The depletion force in the region $R_g > R$ in aqueous protein-poly(ethylene glycol) (PEG) solution has been investigated by Kulkarni et al. (27). It was found that the change in $B_{22}$ of protein solution is nonmonotonic as the concentration of PEG is increased. The radii of lysozyme and $C_8E_4$ micelle studied by us are ~1.85 nm and 2.5 nm, respectively, i.e., $R_g > R$. Our results also show a change from initial repulsive interaction to an attractive interaction with increasing $C_8E_4$ concentration. In a certain range, ~20%–30% $C_8E_4$, the $B_{22}$ falls in the crystallization window resulting in the protein crystallization.

From the above analysis, a mechanism of protein crystallization in the presence of nonionic surfactant can be proposed. As illustrated in Fig. 5 *a*, protein molecules dissolved in buffer have certain hydrophobic patches on the otherwise hydrophilic surface. Small amounts of nonionic surfactant added will adsorb on the hydrophobic parts of the protein (Fig. 5 *b*). Thus the protein solution is further stabilized by screening off the attractive hydrophobic force and increasing the repulsive hydration force. When the amount of added surfactant is large and the number of free micelles is high enough, the attractive depletion force becomes appreciable and $B_{22}$ falls in the crystallization window (Fig. 5 *c*). Therefore, the protein molecules with adsorbed nonionic surfactant are brought closer and form crystals (Fig. 5 *d*). The short range of the depletion force enables a slow nucleation process and hence crystal growth.

## CONCLUSION

The nonionic surfactants found useful in membrane protein crystallization can also promote soluble protein crystallization under certain conditions. Several experimental techniques were used to investigate the crystallization of the model soluble protein, lysozyme, in the presence of nonionic surfactant, $C_8E_4$. Our results suggest the following mechanism for protein crystallization. The adsorption of surfactant on hydrophobic parts of protein molecules screens the hydrophobic attraction between protein molecules, increases the solvation, and results in an increase in repulsive interaction. However, at high surfactant concentration, the attractive depletion force due to the presence of free micelles becomes dominant and enables the protein molecules to assemble in crystalline order.

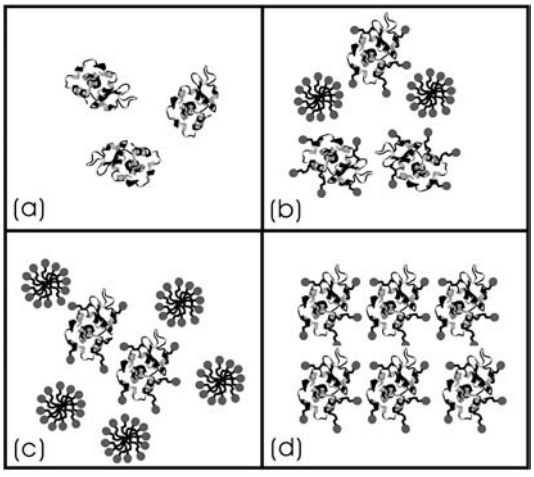


FIGURE 5 Mechanism of protein crystallization in the presence of nonionic surfactant. (*a*) Protein in solution; (*b*) hydrophobic tails of nonionic surfactant monomers adsorb on the hydrophobic parts of protein molecules; (*c*) when the micelle concentration is high enough, the depletion force is predominant and fine tunes the crystallization; and (*d*) protein crystal with adsorbed nonionic surfactant.

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