Observation of Polymerlike Phase Separation of Protein-Surfactant Complexes in Solution

Xuan-Hui Guo and Sow-Hsin Chen

Department of Nuclear Engineering and Center for Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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Protein solution at appropriate *p*H and ionic strength has been shown to phase separate upon addition of a suitable amount of anionic surfactant, sodium dodecylsulfate (SDS). We determine a series of cloud-point curves for bovine serum albumin-SDS complexes in aqueous solutions at different *p*H. Small-angle neutron-scattering and light-scattering studies give strong evidence that these denatured proteins in solution are multicomponent polymerlike objects. We show that the generalized Flory-Huggins theory for polydisperse polymers can describe the cloud-point curves satisfactorily.

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Understanding the structure and thermodynamics of polymer-surfactant and polyelectrolyte-surfactant complexes in solution is important from both a theoretical and a practical point of view, and as such it has attracted much attention in recent years.[1] On the other hand, phase-separation phenomena in polymer and block copolymer solutions are well known and the research in various aspects associated with the phase separation, such as the critical phenomena and spinodal decomposition, continue to be very active.[2] It has been observed that some micellar systems, consisting of nonionic, cationic, and zwitteronic surfactants in aqueous solution, also show some analogous phase-separation behavior with polymer solutions.[3] Proteins are block copolymers, but they generally fold into three-dimensional structures with rather high packing density in solution, mainly due to the hydrophobic interaction.[4] Therefore, their thermodynamic and conformational behavior cannot be treated simply by applying the polymer theory. However, it is probable that the polymer phase-separation theory can be applied to the protein-surfactant complexes in solution in which the proteins are unfolded due to the binding of surfactants.

We have recently studied the structure of bovine serum albumin–sodium dodecylsulfate (BSA-SDS) complexes in solution by small-angle neutron scattering (SANS).[5,6] From the quantitative interpretation of SANS intensity distributions, we were able to describe the structure of BSA-SDS complexes in terms of a necklace model,[7] which assumed that denatured polypeptide chains are flexible in solution and micellelike clusters of SDS are formed around hydrophobic patches of the protein backbone along the unfolded polypeptide chain. The parameters extracted from SANS data are the following: the average micelle size and its aggregation number; the fractal dimension characterizing the conformation of the micellar chain; the correlation length giving the extent of the unfolded polypeptide chains; and the average number of micellelike clusters in a complex. The results suggest that BSA-SDS complexes in high ionic strength solution are polymerlike objects despite some uncertainty about the detail of the local structure. In particular, the fractal dimension D of the complex varies from 2.0 to 1.6 depending on the amount of surfactant binding and is consistent, respectively, with the fractal dimensions of monodisperse (i.e., fractionated) and polydisperse randomly coiled polymers.[8] We have thus conjectured that the BSA-SDS system may undergo phase separation similar to copolymers in solution[9] when some specific conditions are met, namely, low *p*H, high ionic strength, and moderate concentrations. In this paper, we report the first cloud-point curve (CPC) determination of protein-surfactant complexes in solution. We measured the light scattering, both static and dynamic, along an isotherm above CPC in the one-phase region to determine the critical volume fraction and also in the two-phase region below CPC to confirm that the phase separation is a protein-protein type. We applied the generalized Flory-Huggins (FH) theory for polydisperse polymers to describe the CPC. Our analysis establishes that the BSA-SDS complexes in solutions are indeed multicomponent polymerlike objects.

BSA is an important lipid transport protein having 581 amino-acid residues with a known amino-acid sequence.[10] Its molecular weight is 66114 and $pI=5.0$. Its native conformation is a prolate ellipsoid with major and minor axes of 70×20 Å^2, respectively, which has been confirmed by a recent x-ray crystallography investigation.[11] In this study, BSA was obtained from Sigma (A-7638, lot 77F-9369) and SDS from BDH (product No. 44244); both were used without further purification. First, three buffer solutions containing $0.1M$ sodium acetate and $0.5M$ sodium chloride were prepared, and individually adjusted to $p\mathrm{H}=5.00$, 4.90, and 4.80 by dropping acetic acid. Next, 10-wt.% BSA solution and 30-wt.% SDS solution were prepared separately using the same buffer. Then equal volume of these two solutions were mixed slowly in a 1.2-cm-diam glass vial which has an airtight cap preventing evaporation. At the same time a tiny magnetic bar was put into the vial and the

solution was stirred with a magnetic stirrer at a temperature of 55 °C. After the mixing, the solution was maintained at the same condition for another 2 h. This assured that the complexation reached equilibrium before the CPC was measured. Specific volumes of BSA (0.73 cm^3/g) and SDS (0.847 cm^3/g) were used to compute the solute volume fraction in the initial solution and then to calculate the actual BSA to SDS weight ratio (=1/2.6 in our procedure) in the solution after mixing. The total volume fraction of BSA and SDS was also calculated. In the same way, solutions with different BSA-SDS weight ratios were prepared. For the light-scattering measurement, a He/Ne laser of wavelength 6328 Å and 25-mW power together with a Brookhaven Instrument Model Bi2030AT 78-channel digital correlator were used to perform intensity and linewidth measurements. The scattering angle was fixed at 90°. The temperature of the sample was controlled to within ±0.1 °C by a circulating water bath.

For the cloud-point (CP) determination, the solution was immersed in a temperature-controlled water bath having a temperature stability of ±0.05 °C. First, the solution was kept in the one-phase region, a visually clear region, at temperature well above the CPC. Then, the CP was obtained by slowly lowering the temperature of the solution of known solute volume fraction and noting the temperature at which the solution became cloudy visually. By repeating this procedure, a whole CPC for a given BSA-SDS weight ratio was obtained by successively diluting the same solution from high to low volume fraction. The CPC at three different *p*H (4.80, 4.90, and 5.00) are presented in Fig. 1. One obvious feature of the CPC is its highly asymmetric shape with the maximum occurring at a rather low volume fraction, similar to polymers solution. This is in sharp contrast to simple liquid mixtures and pure protein solutions[12] for which

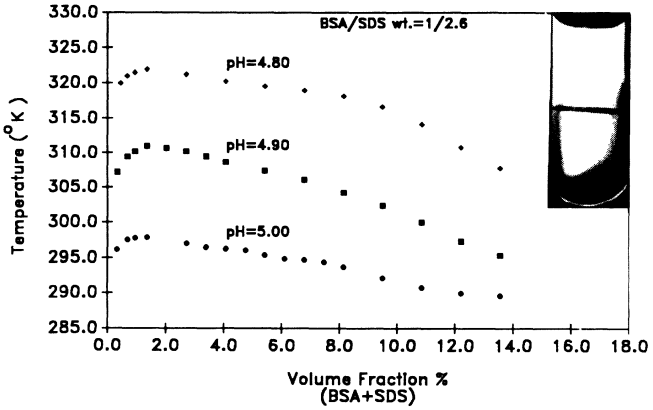

FIG. 1. Cloud-point curves (CPC) of BSA-SDS complexes in solution at three different *p*H values as indicated. BSA to SDS weight ratio is kept constant at 1/2.6. Inset: Photograph of a phase-separated solution at *p*H =4.90 and volume fraction ϕ_s =5.4%.

the coexisting curves are more symmetric and the critical volume fraction occurring at higher volume fractions (17% for γ-crystalline lens protein). It is interesting to observe that the CPC is easily shifted upward by just slightly lowering the *p*H. Lowering of *p*H below 5.0 in BSA solution is known to have two effects: swelling of the structure of the native BSA (Ref. 13) and changing the net charge of a BSA molecule to a positive value. How these effects are related to the shifting of CPC remains to be answered. It should be mentioned that no phase separation was observed down to temperature 10 °C for *p*H above 5.0. According to Fig. 1, the extrapolated CPC for *p*H =5.10 would be around 10°. This temperature is far below the Kraft point of SDS in water and one can expect the binding properties of SDS to the protein may be dramatically changed. The CPC with various BSA to SDS weight ratios at *p*H =4.90 were measured but not all presented here. We found that there is no substantial change of CPC for BSA to SDS weight ratio varying between 1/2.2 and 1/3.2, except the shape of CPC becomes slightly flatter with increasing SDS to protein weight ratio. Therefore, in the following, the theoretical treatment of CPC will focus on a typical BSA to SDS weight ratio of 1/2.6 at *p*H =4.90.

Figure 2(a) presents the light-scattering measurements along an isotherm (T=4.2 °C). The maximum of the light-scattering intensity and the minimum of the mutual diffusion coefficient establish that the critical volume fraction ϕ_{sc} is located on the right branch of the CPC. A similar case was found for polydisperse synthetic polymer solutions, although whether the intensity maximum could be used to precisely locate the critical volume fraction was in dispute.[14] In order to convince ourselves, a solution with an initial solute volume fraction of 5.4% was set at 1 °C below CP for one day, then a photograph (Fig. 1, inset) was taken to show that the phase volumes of the upper and the lower phases in equilibrium were approximately equal. This supports that the critical volume fraction is indeed around the location of the two light-scattering extremes. Furthermore, we observed that the upper phase was a transparent liquid with low viscosity and the lower phase was a slightly turbid liquid with very high viscosity; this phenomenon is very similar to the rheological property of a phase-separated synthetic polymer solution.

A direct observation proved that there were BSA molecules in both phases. We first allowed two phases to reach equilibrium at a temperature of 1 °C below the CP. The two liquid layers were transparent. We then lowered the temperature slightly. Both liquid layers became cloudy. This was evidence that there was protein present in both layers, because the SDS-H_2O system does not have phase separation in this concentration range. Next, a solution with volume fraction of 6.8% in the one-phase region was allowed to phase separate at a temperature of 32 °C. The two phases in equilibrium

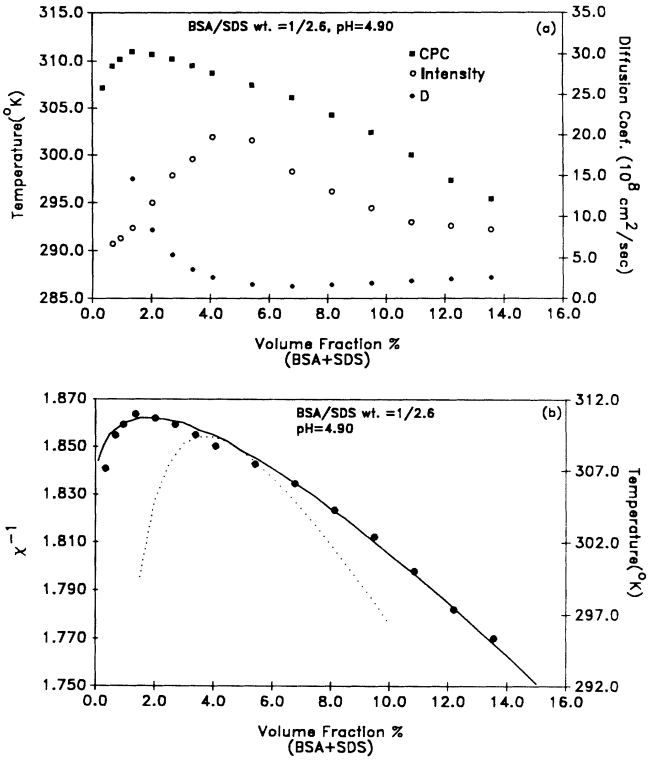

FIG. 2. (a) Light-scattering, both static and dynamics, measurements along an isotherm at $T=42\,^{\circ}$C. The open circles are the intensity and the solid circles are the mutual diffusion coefficient. The mutual diffusion coefficients were deduced from the first cumulant. The cloud-point curve (solid squares) are also shown for comparison. (b) Comparison of the experimental cloud-point curve (CPC) with the theoretical CPC calculated using generalized Flory-Huggins theory for polydisperse polymers in solution. Solid circles are the experimental cloud-point temperature, and vs ϕ_s curve; solid line the theoretical χ^{-1} calculated using a polydispersity index $Z=1$ and an average effective chain length $\lambda_1=676$. The dashed line is the spinodal line corresponding to CPC. It should be noted that $(d\chi_{sp}^{-1}/d\phi_s)\phi_{sc}=(d\chi^{-1}/d\phi_s)\phi_{sc}$.

were then removed and diluted, respectively, to low volume fractions where light scattering gave the similar intensities. The dilution factors for the upper and the lower phases were 4.4 and 30, respectively. Then, the size characterization of the complexes in both phases was made by dynamic light scattering at a temperature of 40°C. The normalized correlation functions $g^{(1)}(t)$ from the measurements were analyzed by assuming a distribution of sizes, namely, $g^{(1)}(t)=\langle e^{-\Gamma t}\rangle$, where $\Gamma=k_BTq^2/6\pi\eta R$ and the size distribution function is taken to be a Schultz distribution,

$$f_s(R)=[(Z+1)/\bar{R}]^{Z+1}R^Z \times\exp[-(Z+1)R/\bar{R}]/\Gamma(Z+1).$$

From this analysis we found that $\bar{R}=50$ Å and $Z=3$ were able to fit the correlation functions from both phases satisfactorily. The small-Z value indicates that the complexes in both phases are very polydisperse, about 50% polydispersity. But the average size is about the same, 50 Å, as compared to the hydrodynamic radius of a native BSA which is 36 Å.

We now turn to characterize the CPC from a thermodynamic approach. A generalized FH theory for a polydisperse polymer solution had been developed by Solc and Koningsveld, respectively.[9] If the interaction parameter $\chi\sim$const/T is assumed to be independent of the volume fraction of polymers, the CPC, which is defined as the functional relationship between χ^{-1} and ϕ_s at a given polydispersity of chain length P, is obtained by solving the following equations [(1) and (2)]:

$$2\chi\phi_s(\bar{\lambda}_0-1)=K\sigma+\ln[(1-\phi_s)/(1-\bar{\lambda}_0\phi_s)], \quad (1)$$

$$\tfrac{1}{2}K\sigma(1+\bar{\lambda}_0)+(\bar{\lambda}_0-1-\bar{\lambda}_{-1}+\lambda_{-1}) +[\phi_s^{-1}-(1+\bar{\lambda}_0)/2]\ln[(1-\bar{\lambda}_0\phi_s)/(1-\phi_s)]=0, \quad (2)$$

$$\phi_{sc}=(1+\lambda_1^{3/2}\lambda_2^{-1/2})^{-1}. \quad (3)$$

In these two equations ϕ_s is the volume fraction of the solute (polymers); σ is a parameter to be eliminated from Eqs. (1) and (2) in the process of the solution; λ_l is the lth moment of the effective chain-length distribution $f_s(P)$ which is assumed to be a Schultz distribution; and $\bar{\lambda}_l=\int_0^\infty P^l\bar{f}(P)dp$, where $\bar{f}(P)=f_s(P)\exp(KP\sigma)$; $K=1$ (if $\phi_s<\phi_{sc}$) or -1 (if $\phi_s>\phi_{sc}$), where ϕ_{sc} is the critical volume fraction given by Eq. (3). Using the Schultz distribution, we obtain $\bar{\lambda}_l$ and λ_l explicitly in the following forms:

$$\bar{\lambda}_l=\frac{[(Z+1)/\lambda_1]^{Z+1}(l+Z)!}{Z![(Z+1)/\lambda_1-K\sigma]^{-(l+Z+1)}}$$

and

$$\lambda_l=[(Z+1)/\lambda_1]^{-l}(l+Z)!/Z!.$$

We now assume that the maximum of light-scattering intensity and the minimum of the mutual diffusion coefficient occur at the critical volume fraction ϕ_{sc}. From Fig. 2(a) we have $\phi_{sc}=4.5\%$. Then for a given polydispersity parameter Z, λ_1 can be obtained by solving Eq. (3). Knowing λ_1 we can compute all λ_l and $\bar{\lambda}_l$. Subsequently, χ is solved from Eqs. (1) and (2) numerically for every ϕ_s assigned. It was found that only $Z=1$ gave the result in satisfactory agreement with experiment. The calculated (solid lines) and measured (solid circles) CPC are shown in Fig. 2(b). The spinodal curve can be obtained from the equation $\chi_{sp}=0.5[(1-\phi_s)^{-1}+(\lambda_1\phi_s)^{-1}]$. It turns out that $\chi=\chi_{sp}$ at $\phi_s=\phi_{sc}$. The spinodal curve is plotted as the dashed line in Fig. 2(b).

In summary, the thermodynamic approach successfully captures the essential features of the CPC we have discovered, namely, the general shape and the skewness. The picture of the complexes emerging from the CPC analysis is consistent with that from our previous SANS

studies. The globular protein-SDS complexes in solution behave like flexible polymers, at least when the *p*H is at or below the *p*I of the protein and the ionic strength of the solution is sufficiently high. It is of interest to further explore the complete ternary phase diagram of the protein-SDS-H_2O system and to locate the critical point more precisely.

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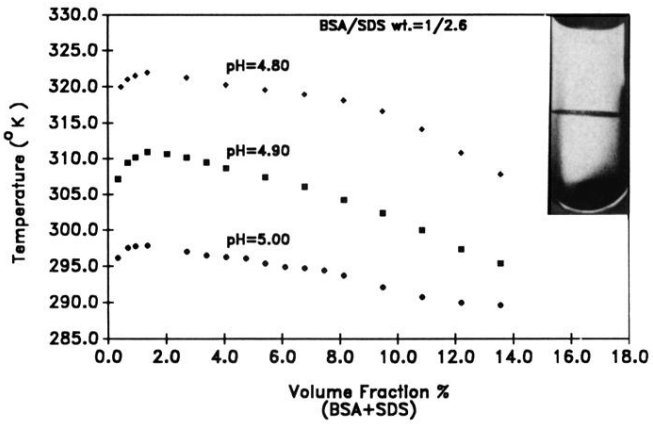

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