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Critical Behavior of a Binary Mixture of Protein and Salt Water

Coe Ishimoto
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

and

Toyoichi Tanaka
Department of Physics, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
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Evidence is presented for the existence of a critical mixing point of a protein and salt-water binary mixture. The asymptotic behavior of the osmotic isothermal compressibility and the long-range correlation length near the critical mixing point is consistent with the scaling laws predicted by the mean-field theory. The dynamic behavior of concentration fluctuations is not described by the mode-coupling theory.

The universality of the asymptotic behavior of certain equilibrium and transport thermodynamic properties of binary mixtures near the critical mixing point has been well established over the previous decade.[1,2] In this Letter we present evidence for the existence of a critical binary mixture of a globular protein, lysozyme (molecular weight: 14 388), and a 0.5M aqueous solution of NaCl (pH 5.4). An understanding of critical behavior, and more generally of phase transitions, in protein/solvent mixtures is of considerable interest as a new physico-chemical approach to the study of protein solubility. It is also of great physiological importance in relation to the phenomenon of cold cataract in certain animal lenses.[3,4] The lysozyme molecule is approximately ellipsoidal, with dimensions of $45\times30\times30$ Å^3.[5] The dimensions of the individual molecules of the two components in a binary mixture of lysozyme and salt water are thus different by as much as an order of magnitude. The lysozyme molecule carries 6.5 net positive electrical charges at pH 5.4.[6] Such a mixture of compact globular macromolecules and solvent, or a mixture in which one of the components carries net electrical charges, has not previously been investigated as a critical binary mixture. The salt water is treated as a single component although it contains Na^+ and Cl^- ions as well as water molecules. The salt is required for the critical temperature to be in the range between the freezing point of the mixture (approximately -10°C) and the denaturation temperature of lysozyme (approximately 55°C).[7]

We investigated the asymptotic behavior near the critical mixing point of the osmotic isothermal compressibility and the long-range correlation length by measuring the turbidity of the mixture along the critical isochore. The behavior of these equilibrium thermodynamic properties was found to be consistent with the behavior predicted by the mean-field theory[8] (critical exponents $\beta=\frac{1}{2}$, $\gamma=1$, $\nu=\frac{1}{2}$). The macroscopic shear viscosity showed a large divergence near the critical mixing point; its relationship to the decay rate of concentration fluctuations was not in agreement with the prediction of the mode-coupling theory.[9]

Crystalline flakes of chicken-egg-white lysozyme (3.2.1.17, Worthington; specific activity: 11 700 units) were dissolved in a 0.5M aqueous solution of NaCl (Mallinckrodt, reagent grade, dissolved in distilled water) with precautions taken to avoid denaturation of the protein. The dissolved mixture was centrifuged at 1500g for 5 min in order to remove air bubbles and the small amount of undissolved protein. The volume fraction of protein was determined from a measurement of OD_{280}, after $\times1000$ dilution in water, the extinction coefficient of lysozyme, $E_{1\,\mathrm{cm}}^{1\%}=26.4$, and the partial specific volume of lysozyme, $\bar{v}=0.703$. This volume fraction was divided by a factor of 0.74, so that 100% corresponds to the close-packed arrangement in which the shape of

the lysozyme molecule is approximated by a spherical ball. Thus the adjusted volume fraction (%) was given by $37 \cdot OD_{280}$. Since there was a slow formation of crystals of lysozyme in the mixture, all measurement runs were repeated in order to make certain that crystal formation did not significantly affect the quantity being measured. There were no such effects over a period of 2–3 h following the preparation of the mixture.

The prepared lysozyme/salt-water mixtures were simply placed in glass cuvettes (1×1×4 cm) and sealed with Parafilm for laser-light-scattering and turbidity measurements. The temperature of the sample was regulated to within ±0.05°C and to an absolute accuracy of ±0.05°C. For each type of measurement, the quantity being measured was monitored on a chart recorder to confirm that after each change of temperature the system attains equilibrium before the measurement is recorded; 5–10 min were required for this equilibration. No significant density gradient was formed in the mixture even at temperatures very close to critical mixing temperature.

The coexistence curve and the spinodal curve were determined from the temperature dependence of the intensity of argon-ion laser light (50 mW) scattered at a scattering angle of 90° from mixtures of several different volume fractions. As the temperature is lowered the intensity increases continuously until some temperature T_c is reached at which the mixture suddenly becomes opaque and thus the intensity suddenly drops to a very low value. The inverse of the intensity extrapolates to zero at a lower temperature, T_s. The mixture remains opaque as the temperature is lowered below T_c. Figure 1 shows T_c and T_s for various mxitures. Mixtures of volume fractions greater than about 50% were progressively more difficult to prepare. The curves of T_c and T_s are the coexistence curve and the spinodal curve, respectively, of binary mixtures. These two curves have maxima which coincide at the critical mixing point at a volume fraction φ_c^* of $(33 \pm 5)\%$ and a temperature T_c^* of (10.90 ± 0.05)°C. The coexistence curve is described by $|\varphi_c - \varphi_c^*| \propto \epsilon^\beta$ with the critical exponent $\beta = 0.41 \pm 0.1$, where φ_c is the volume fraction of the mixture on the coexistence curve, and $\epsilon = (T - T_c^*)/T_c^*$ is the reduced temperature.

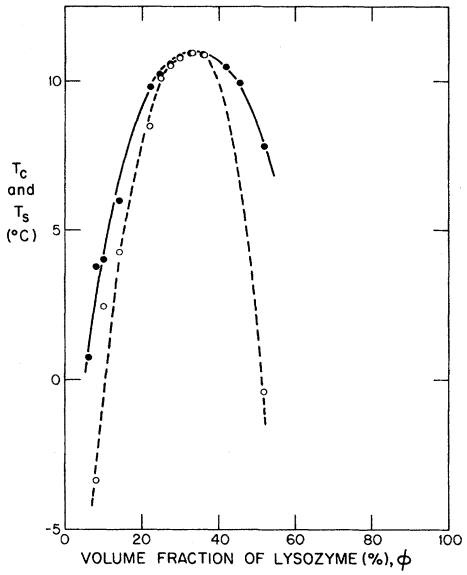

FIG. 1. The coexistence curve (solid circles; the solid line indicates $|\phi_c - \phi_c^*| \propto \epsilon^{0.41}$) and the spinodal curve (open circles; the broken line is drawn to aid the eye) of lysozyme and salt-water mixtures.

In order to investigate the asymptotic behavior of the osmotic isothermal compressibility κ_T and the long-range correlation length ξ, the turbidity was measured along the critical isochore. The advantage of turbidity measurements over the scattered-intensity measurements[10] in reducing the effects of multiple scattering is particularly desirable in the protein/salt-water mixture because of the intense scattering even at large reduced temperatures. The turbidity is obtained by integrating over all angles the light-scattering intensity calculated using the concentration fluctuations given by the Ornstein-Zernike theory. The result is[10]

$$\tau = \frac{\pi^3}{\lambda^4}\left(\frac{\varphi \partial n^2}{\partial \varphi}\right)_T^2 k_B T \kappa_T \left[\frac{2\alpha^2 + 2\alpha + 1}{\alpha^3}\ln(1+2\alpha) - \frac{2(1+\alpha)}{\alpha^2}\right], \quad (1)$$

where λ is the wavelength of light in the salt solution, φ is the volume fraction of the mixture, n is the refractive index of the mixture, k_B is the Boltzmann constant, and T is the absolute temperature. Here $\alpha = 2(k_0\xi)^2$, where k_0 is the wave vector of the incident light in the mixture and ξ is the long-range correlation length. For $\alpha \ll 1$, Eq. (1) becomes

$$\tau_0 = \tfrac{8}{3}(\pi^3/\lambda^4)(\varphi \partial n^2/\partial \varphi)_T^2 k_B T \kappa_T. \quad (2)$$

On the assumption that $\kappa_T \propto \tau_0/T$ has a simple

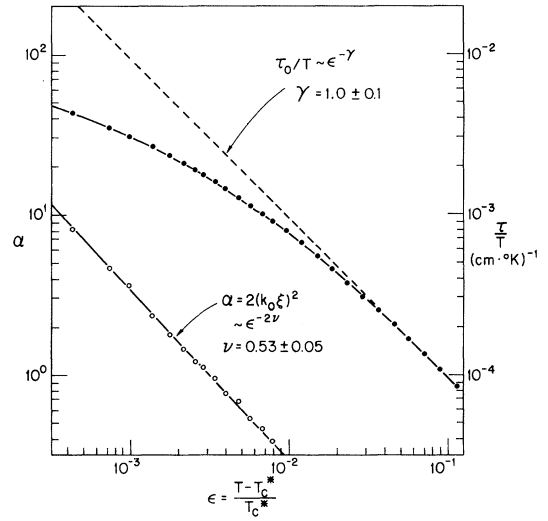

FIG. 2. The log-log plot of τ/T (solid circles) and of α (open circles) versus the reduced temperature along the critical isochore. The broken line represents τ_0/T.

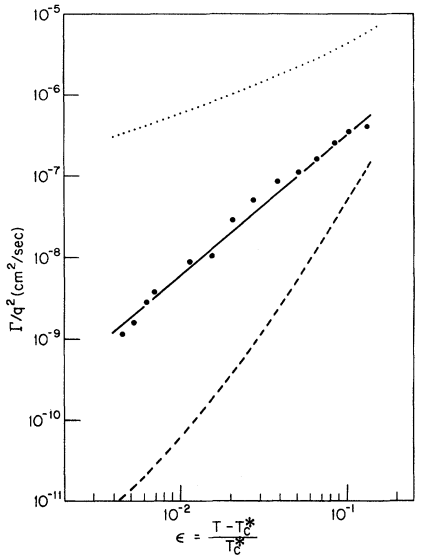

FIG. 3. Log-log plots of Γ/q^2 versus the reduced temperature along the critical isochore. Solid circles indicate experimental values obtained from linewidth measurements. The broken line indicates the curve calculated from Eq. (4) using independently observed values for ξ and η_m. For reference, the dotted line indicates the curve calculated from Eq. (4) using independently observed values for ξ and the viscosity of the salt water.

power-law dependence on ϵ, such a power law should be manifest in the range of large ϵ where $\alpha \ll 1$. The departure of τ/T from simple power-law behavior near the critical point can be used to determine ξ.[10]

The intensity, I, of the beam from a He-Ne laser (5 mW) transmitted through the mixture (1-cm path length) was determined by direct measurement of the photocurrent of a photomultiplier tube. The reference intensity I_0 was determined for the same mixture with the NaCl omitted, since such a mixture is far from its critical mixing point. The turbidity was calculated as $\tau = -\ln(I/I_0)$ cm^{-1}. The measured τ/T is shown as solid circles in Fig. 2 and deviates from τ_0/T shown as the broken line. A linear extrapolation of high-temperature data gives the line for τ_0/T. The large value of ξ_0, however, leads to only a short linear region since there is an upper limit to the experimental range of ϵ at 10^{-1} because of the susceptibility of lysozyme to thermal denaturation. Thus the slope of the line for τ_0/T can only be determined to be in the range 0.9–1.1. The open circles show the calculated results for ξ. These results are described by

$$\kappa_T = \kappa_T^0 \epsilon^{-\gamma} \propto \tau_0/T, \tag{3a}$$

$$\xi = \xi_0 \epsilon^{-\nu}, \tag{3b}$$

with $\kappa_T^0 = (3.1 \pm 0.2) \times 10^{-7}$ cm sec^2/g, $\gamma = 1.0 \pm 0.1$, $\xi_0 = 26 \pm 5$ Å, and $\nu = 0.53 \pm 0.05$. These values of the critical exponents γ and ν are consistent with the predictions of the mean-field theory rather than of the renormalization-group theory[11] ($\gamma = 1.23$, $\nu = 0.63$). This may be because the values of ϵ involved in the measurements are too large for the renormalization-group theory to apply. The value of κ_T^0 is two orders of magnitude greater and the value of ξ_0 an order of magnitude greater than the values which have been determined for critical binary mixtures of nonmacromolecular components.[1] This reflects the smaller number concentration and the larger size of lysozyme molecules.

The decay rate of concentration fluctuations was measured as Rayleigh linewidths of scattered laser light along the critical isochore by optical-mixing spectroscopy using an argon-ion laser and a 19-channel digital autocorrelator. All measurements were in the hydrodynamic regime ($q\xi < 0.1$, where q is the magnitude of the scattering wave vector). In this regime, the prediction of the mode-coupling theory is approximated to within 10% by[12]

$$\Gamma/q^2 = k_B T/6\pi\eta\xi, \tag{4}$$

where η is the macroscopic shear viscosity. The

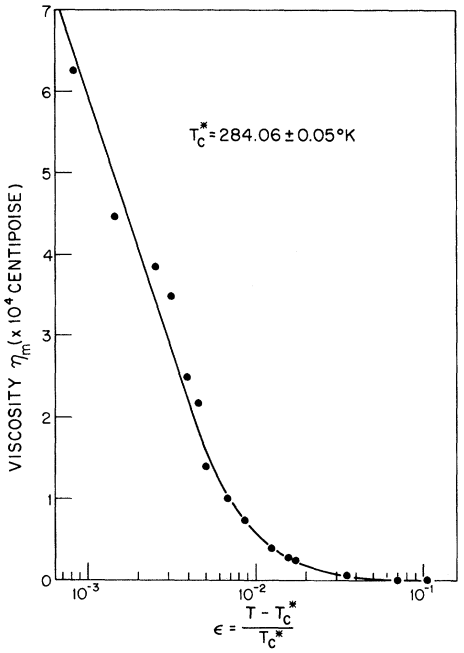

FIG. 4. The macroscopic shear viscosity η_m versus the reduced temperature along the critical isochore.

measured values of Γ/q^2 are shown in Fig. 3. In order to check the validity of Eq. (4) for this mixture, Γ/q^2 was calculated from ξ given by Eq. (3b) and η_m determined by rolling-ball viscometry using a stainless-steel microball (0.025 in. diam and a 100-μl glass micropipette and calibrating against glycerol-water standard solutions. Figure 4 shows the divergence of the macroscopic shear viscosity η_m at smaller values of ϵ as a semilog plot.[13] The viscosity anomaly is extremely large compared to that found in binary mixtures of small molecules. The nonanomalous background is negligibly small. The broken line in Fig. 3 shows the calculated curve for Γ/q^2 using this η_m. There is a discrepancy of several orders of magnitude between the measured and calculated values. This discrepancy is probably due to a difference between the nature of η which appears in Eq. (4) and the nature of the measured η_m.[14]

In summary, we have demonstrated the existence of a critical mixing point in a protein/solvent mixture. The static behavior of the concentration fluctuations is in close agreement with the prediction of the mean-field theory. The dynamic behavior is not in agreement with the mode-coupling theory unless the viscosity of the mixture is properly evaluated.

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[14]The same situation occurs in polystyrene/water mixtures (S. H. Chen, private communication).