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Dynamic protein clusterization in supercritical region of the phase diagram of water–protein–salt solutions

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ABSTRACT

A protein solution phase diagram, constructed on the basis of the thermodynamic description of protein–solvent interaction, is discussed. A thermodynamic mechanism of formation of protein clusters-oligomers and mesoscopic clusters in the supercritical region of a protein solution phase diagram, is considered. Variation in the chemical potential of water, which depends on solution composition, is the parameter which describes the interaction. Equations for the spinodal and critical points of the phase diagram, in which the critical composition (protein to salt concentration ratio) of the system is related to the physico-chemical characteristics of protein (charge, number of adsorbed ions) and salt (activity), are proposed. The equations are used to construct the phase diagrams of protein solution in the water chemical potential, protein and salt concentration planes, protein solubility and salt concentration plane and to relate effective temperature and critical composition at which a critical point and a spinodal are achieved. The approach proposed provides a deeper insight into the formation of globular-reticular and cellular structures in water–protein solution, induced by phase transformation in the pre-critical and in the supercritical region of the phase diagram.

1. Introduction

The phase diagrams (PD) of globular protein solutions [1,2] are essential for the understanding of protein crystallization, analysis of association and aggregation phenomena in physiological and biotechnological processes and structural-dynamic changes in proteins responsible for the pathology of condensation diseases.

The interaction of globular proteins in concentrated solutions is often described using a model of solid spheres with a short-range attraction potential [3]. In this case, two types of phase transitions (PT): (1) liquid–solid (L–S), characterized by the co-existence of crystalline and liquid phases, and (2) liquid–liquid (L–L), in which both a diluted and a concentrated phase exist, are expected. Such an approach results in one of the versions of phase equilibrium diagrams known for systems of gas–liquid type, where first-order PT is transformed into critical PT at certain temperature and concentration. Here, protein molecules in diluted solution are regarded as gas and protein molecules in a concentrated dense phase as liquid. As protein molecules in solutions exhibit attractive interactions with a range about 1/4 of the particle diameter, L–L type PT is found to be metastable relative to L and S. Such PT is revealed systematically in protein lysozyme solutions [4], some γ-crystallines [5] and antibodies [6]. As temperature decreases, droplets of more concentrated protein solution, which is in metastable equilibrium with a less concentrated phase, are formed. Two liquid layers, differing in density, may be formed some time later or upon the centrifugation of such solutions [6,7].

Fig. 1b shows schematically a phase diagram of protein solution used most commonly to analyze its phase state [1,2] and, particularly, L–L type PT. Here, the presence of stable, metastable and unstable regions and their boundaries – binodal and spinodal lines – makes it possible to establish the one-to-one correspondence of special points on these curves with the points on the curves for the water chemical potential $(\mu_1 - \mu_1{}^0)$ of protein solution versus protein concentration m_2 [8]. The isotherm $(\mu_1 - \mu_1{}^0)$, shown in Fig. 1a, is similar to the isotherm which describes first-order PT gas–liquid and a loop, analogous to van der Waals loop, is formed by the isotherm between points A and D.

Within the spinodal (curve 3 in Fig. 1b, which separates the PD regions of instability and metastability) any fluctuation of concentration results in phase transition with instant gel formation. Metastable and stable PD regions are separated by binodal (curve 4). The metastable phase equilibrium L↔L may arise on the curve, if concentration fluctuations result in sufficiently high heterogeneity. Equilibrium protein concentration in solution in the presence of a crystalline phase L↔S is described by a solubility

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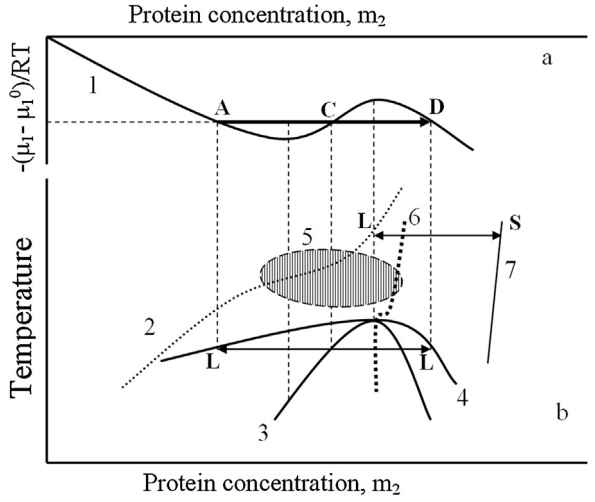

Fig. 1. Schematic representation of isotherms (a) and a typical phase diagram (b) of the model system water–protein in the water chemical potential, protein concentration ($\mu_1 - \mu_1{}^0, m_2$) plane (a), and in the temperature, protein concentration plane (b). Figures denote: isotherm with a van der Waals loop (1), solubility line (dotted, 2), spinodal line (3), binodal line (4), region of experimentally observed oligomers and dynamic mesoscopic protein clusters (hatched, 5), gelation line (heavy dotted, 6), solidus line (7). L↔S denotes equilibrium of liquid and solid phases. L↔L denotes metastable equilibrium of diluted and dense phases. Point C is the isotherm inflection point. Combination of inflection points for a set of isotherms in the supercritical region gives a quasi-spinodal [19].

(saturation) line (thin dotted line 2). Line 7 is the solid phase boundary, and heavy dotted line 6 is the arbitrary line of gel formation. Here, the common point of the binodal and spinodal determines the upper critical solution temperature (UCST) at a certain protein concentration. Above this point, the solution is expected to be macroscopically homogeneous.

One of the most intriguing results from the point of view of supercritical phenomena in protein solutions, obtained in the past few years, is the data on small protein oligomers (clusters), which comprise several protein molecules [9–11], and dynamic mesoscopic protein clusters, hundreds of nanometers in diameter [12–14], in the supercritical region of the phase diagram (for systems with UCST, these are supercritical temperatures, and in Fig. 1b this region is hatched). Their emergence is related to a spinodal for the solution to crystal phase transition [1]. This supercritical spinodal (quasi-spinodal) is located near the gel formation line (dotted line 6 in Fig. 1b) and corresponds to slightly smaller protein concentrations. The structures formed here can also be closely connected to gel formation processes in protein solutions (incomplete gelation or amorphous precipitation [15]). On the other hand, the presence of mesoscopic clusters can be coupled to either the formation of crystalline phase nuclei or protein polymerization [13].

It appears that the presence of both clusters in the seemingly homogeneous region of the phase diagram above the critical point is typical of protein, amino acid and many inorganic systems [16]. In addition, some distinctive methods (small-angle neutron and X-ray scattering) and relevant experimental procedures are mainly used to observe small clusters [11], while other methods (dynamic light scattering, atomic force microscopy, brownian microscopy) are employed to examine mesoscopic clusters [1,14]. However, the physico-chemical and thermodynamic arguments, proposed in favour of the existence of such structures, are clearly insufficient [14–18]. On the other hand, it is the thermodynamic theory of continuous phase transitions which predicts [19] that the determinant and coefficients of stability for a supercritical phase take minimum values at the quasi-spinodal line, where the highest development of the nonequilibrium fluctuation embryos of both low-temperature boundary phases is reached under these conditions, creating a distinctive mesophase. This is because it still partly retains the properties of low temperature phases.

Based upon the theory of thermodynamic stability to diffusion [20], adapted to the heterogeneous water–protein–salt system [21,22], we propose a thermodynamic analysis of phase formation phenomena near the critical point and cluster formation in the supercritical region of the phase diagram. Instead of protein–protein interaction we consider water–protein interaction and examine the free energy of mixing of the model system biopolymer (protein)-low molecular liquid (water + salt). Phase diagrams with UCST, common for model protein systems characterized by a critical point, a spinodal and a binodal, can thus be obtained. In this approach, however, the role of solvent and the properties of protein itself are considered to a much greater extent than in the analysis of mere protein-protein interaction. The quality of the solvent, responsible for its thermodynamic affinity to a biopolymer, is controlled by the addition of salt. However, not only ionic strength but also salt ion–protein interaction and the concentration of the salt used should be considered here. This is also essential from the biological point of view because physiological protein solutions contain certain evolutionally approved salts and because proteins are crystallized dominantly in the presence of salts. Such an approach is assumed to provide additional evidence for the thermodynamics of protein interaction in the critical and supercritical regions of the phase diagram that underlie cluster formation, gel formation, crystallization and polymerization.

2. Results

The equations, describing the isotherm of the water chemical potential $(\mu_1 - \mu_1{}^0) = \Delta\mu_1$ of protein solution versus molar concentrations of protein m_2 and salt m_3, the critical curve and the spinodal (quasi-spinodal) [21,22], are shown below in analytical form. They are presented graphically in corresponding coordinates in Figs. 2 and 3.

The equations for $\Delta\mu_1(m_2, m_3)$ is of the form:

$$\mu_1 - \mu_1^0 = -\mathrm{RT}\frac{1+\Delta}{m_1 \Delta z^2}\left\{\frac{2m_3}{\nu(2+\Delta)}\ln\ [4m_3^2(2+\Delta) - \Delta z^2 m_2^2] + \frac{4m_3^2}{\Delta z^2 a}\ln\ \frac{a+m_2}{a-m_2} - m_2\right\} + \text{const}, \quad (1)$$

where $\alpha^2 = (2+\Delta)4\,m_3{}^2/\Delta z^2$.

In this equation, protein is described by the parameters z and ν, where z is protein charge and ν is the number of salt ions adsorbed on a protein molecule in the specific sites of sorption. The variable Δ describes the rate of change in the salt activity coefficient with the salt concentration (in this case, ionic strength ζ):

$$\Delta \approx -m_3 \frac{\partial}{\partial m_3}\left(\frac{A\zeta^{1/2}}{1+r\kappa\zeta^{1/2}} - \sum \alpha_i \zeta^i\right) \quad (2)$$

In the parentheses, under the differential sign, is Debye–Huckel equation for the activity coefficient of electrolyte extended for high salt concentrations by introducing the empirical corrections α_i. As the concentration dependence of the salt activity coefficient commonly has a near-parabolic form, then Δ as a derivative from a parabolic function is a negative value at small and moderate salt concentrations (one parabola branch) and a positive value at high salt concentrations (another parabola branch).

The equation for the critical point is of the form:

$$\frac{m_2}{m_3} = 2\frac{1+\sqrt{1+(2+\Delta)^2\nu^2 z^{-2}}}{\nu(2+\Delta)}. \quad (3)$$