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Effect of pH on phase separation of globular protein

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Abstract−A molecular-thermodynamic framework is proposed to describe protein precipitation by inorganic salt. The equation of state consists of a hard-sphere reference contribution and a perturbation term. The reference term is derived based on the modified Chiew's model to describe the pre-aggregation effect of protein at various solution pH. In this study, we discuss protein-protein effective two-body potentials. The distribution and magnitude of charges on the surface of a protein vary significantly with pH. It changes the magnitude of charge-charge repulsion, charge-dipole attraction, dipole-dipole attraction, and induced dipole-induced dipole attraction forces between protein pairs in solution. The distribution of the charge fluctuation is slightly effective in solution pH. To investigate the effect of pH, modified charge fluctuation distribution model is proposed. Using the proposed model, we successfully describe the pH dependence of the protein precipitation.

Key words: pH Dependence, Protein Precipitation, Interaction Potentials, Pre-aggregation

INTRODUCTION

In the early days of protein chemistry, the only practical way of separating different types of proteins was by causing a part of a mixture to precipitate through the alternation of some properties of the solvent. Non-covalent forces including Coulombic, van der Waals and hydrophobic forces govern protein interactions. While these forces are understood on the level of small molecules, they remain obscure for complex macromolecules such as proteins.

Protein precipitation is the simplest and the oldest practical way to separate different proteins from a solution mixture. Protein precipitation and crystallization are fundamental procedures to recover and characterize all proteins in application fields such as biotechnology and the pharmaceutical industry. However, because protein interactions are governed by many factors, such as pH, surface hydrophobicity, surface-charge distribution, salt-type and salt concentration, the protein phase behavior is not well understood. Separation is achieved through the addition of precipitation agents such as inorganic salts, nonionic polymers, polyelectrolytes, and organic solvents [7,11,18,20,21]. A variety of researches have studied the protein precipitation behavior by using various experimental techniques. Shih et al. [21] observed the solubility of lysozyme, α-chymotrypsin and bovine serum albumin in an aqueous electrolyte solution as a function of ionic strength, pH, the chemical nature of salt, and the initial protein concentration. Coen et al. [5] studied the salting-out phase equilibria for lysozyme and α-chymotrypsin from the concentrated ammonium-sulfate solution. Their experimental results suggest that the protein salting-out may be considered a fluid-fluid phase separation resulting in a supernatant fluid phase with a dense precipitate fluid phase. And the degree of separation is characterized by the partition coefficient, K, which is defined as the ratio of the protein concentration in the dense phase to that in the supernatant phase. These experimental results also show the dependence on the factors, such as ionic strength, temperature, salt concentration and solution pH. Theoretically, many researchers [2,6,8,10,23,24,27] reported models to describe the phase behaviors of these complex systems by using the one-component mean-force potential approximation. Mahadevan and Hall [16,29], Vlachy and Prausnitz [25], and Vlachy et al. [26] have proposed a model to describe aqueous globular proteins in solutions of low salt concentration, and Chiew et al. [4] and Kuehner et al. [14] employed a similar approach for solutions of high salt concentartion. Thermodynamic models with a properly chosen potential of mean force should lead to a satisfactory description of the phase behavior of a protein solution. Thermodynamic properties and phase-separation conditions of protein solutions described by such models have been computed by using a number of different statistical-mechanical approximation methods. These methods can be charaterized as based on the osmotic virial expansion, statistical-mechanical perturbation theory, integral-equation theory, and the random-phase approximation.

By using the second-order Baker and Henderson perturbation theory with the Asakura Oosawa osmotic attraction [2,3] as the dominant contribution of the mean force potential, Gast et al. [8] and Mahadevan and Hall [16,29] have studied the polymer-induced phase separations of aqueous and nonaqueous colloidal and nonadsorbed protein systems. Predictions based on this method led to solid-fluid phase transition rather than fluid-fluid phase separation observed experimentally by de Hek and Vrij [6] for colloidal systems. However, they have shown that the perturbation theory combined with the Asakura-Oosawa potential is able to predict both fluid-fluid and solid-fluid transitions when very large polymer molecules are present.

Based on the experimental studies of de Hek and Vrij [6] for colloidal systems and of Shih et al. [21] for protein solutions, they suggest that a salt- (or polymer-) induced protein (or colloid) precipitation may be more appropriately viewed as a phase separation resulting in two fluid phases. Grimson [10], Vlachy et al. [26], Chiew et al. [4] and Kuehner et al. [14] have used the random-phase approximation to describe a fluid-fluid phase separation for a similar mean-force potential to that used in the previously mentioned perturba-

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tion theory calculations. The major advantage of the random-phase approximation is its simplicity, as little computational effort is required to calculate the whole phase diagram.

Several theoretical studies [14,16,22,17,29] have been developed to predict the phase separation behavior of protein solution. In these studies, most dependencies on the factors are discussed; however, the pH dependence yet has not been sufficiently studied.

In this study, we present a molecular-thermodynamic framework to describe the protein precipitation by inorganic salt. It consists of a hard-sphere reference contribution and a perturbation term. The reference term is derived based on the modified *Chiew*'s model to describe the pre-aggregation effect of protein, which can express the conformation change with various solution pH. We also discuss protein-protein effective two-body potentials. The distribution and magnitude of charges on the surface of a protein vary significantly with pH. It changes the magnitudes of charge-charge repulsion, charge-dipole attraction, dipole-dipole attraction, and induced dipole-induced dipole attraction forces between protein pairs in solution. The charge fluctuation distribution is modified to describe the pH dependence of the protein partitioning.

MODEL DEVELOPMENT

1. Potential of Mean Force

Protein interaction can be described quantitatively by a two-body potential of mean force. The overall potential between two different protein molecules is equal to:

$$W^{overall}(r)=W^{elec}(r)+W^{disp}(r)+W^{osmotic}(r)+W^{specific}(r)+W^{fluctuaion}(r) \quad (1)$$

where r is the center to center distance and $W^{elec}(r)$, $W^{disp}(r)$, $W^{osmoic}(r)$, $W^{specific}(r)$ and $W^{fluctuaion}$ are the electrostatic potential, the dispersion potential, the osmotic attraction potential, the specific potential and the distribution of the charge fluctuation, respectively. The electrostatic potential is the sum of five potentials, such as charge-charge, charge-dipole, charge-induced dipole, dipole-dipole and dipole-induced dipole potential.

$$W^{elec}(r)=W^{q\_q}(r)+W^{q\_\mu}(r)+W^{q\_i\mu}(r)+W^{\mu\_\mu}(r)+W^{\mu\_i\mu}(r),\ r\geq d+2\Delta r \quad (2)$$

The charge-charge potential is defined

$$\frac{W^{q\_q}(r)}{kT}=\frac{z^2e^2\exp[-\kappa(r-d)]}{4\pi\varepsilon_0\varepsilon_r r(1+\kappa d/2)^2} \quad (3)$$

where z is the valance of the protein, which gives the average protein surface charge varying with the solution pH, e is the unit of electron charge, is the hard-sphere diameter of protein, $4\pi\varepsilon_0$ is the dielectric permittivity of free space, ε_r is the relative dielectric permittivity of water, Δr is the effective-space hydration/stern layer and κ is the Debye parameter given by $\kappa^2=(2e^2N_A I)/(kT\varepsilon_0\varepsilon_r)$.

The charge-dipole potential is

$$\frac{W^{q\_\mu}(r)}{kT}=-\frac{2}{3kT}\frac{z^2e^2\mu^2\exp[-\kappa(r-d)]}{(4\pi\varepsilon_0\varepsilon_r)^2kTr^4}\times\left[\frac{3(1+\kappa\tau)\exp[-\kappa(r-d)]}{(1+\kappa d/2)\left(2+\kappa d+(\kappa d/2)^2+(1+\kappa d/2)\frac{\varepsilon_s}{4\pi\varepsilon_0\varepsilon_r}\right)}\right] \quad (4)$$

where μ is the dipole moment, which is pH dependent parameter, ε_s is the effective dielectric constant at the surface of the protein.

The charge-induced dipole potential is

$$\frac{W^{q\_i\mu}(r)}{kT}=-\frac{z^2e^2\alpha}{(4\pi\varepsilon_0\varepsilon_r)^2kTr^4} \quad (5)$$

where α represents the polarizability.

The dipole-dipole potential is

$$\frac{W^{\mu\_\mu}(r)}{kT}=-\frac{2}{3kT}\frac{\mu^4}{(4\pi\varepsilon_0\varepsilon_r)^2kTr^6}\frac{3^4[2+2\kappa\tau+(\kappa\tau)^2]^2\exp[-2\kappa(r-d)]}{\left[2+\kappa d+(\kappa d/2)^2+(1+\kappa d/2)\frac{\varepsilon_s}{4\pi\varepsilon_0\varepsilon_r}\right]^4} \quad (6)$$

The dipole-induced dipole potential is

$$\frac{W^{\mu\_i\mu}(r)}{kT}=-\frac{2\mu^2\alpha}{(4\pi\varepsilon_0\varepsilon_r)^2kTr^6} \quad (7)$$

The attractive Hamaker dispersion interaction [12,23] is

$$W_{disp}(r)=-\frac{H}{6}\left[\frac{d^2}{r^2}+\frac{d^2}{r^2-d^2}+2\ln\left(1-\frac{d^2}{r^2}\right)\right],\ r\geq(d+2\Delta r) \quad (8)$$

where H is the Hamaker constant.

In the concentrated electrolyte solution, ions occupy a significant fraction of the total solution volume. Protein molecules are so close together that ions are excluded from the region between the protein particles. It causes an imbalance in the local osmotic pressure exerted by the ions on the proteins. The osmotic pressure difference is approximated by the ideal osmotic pressure of the bulk solution [$\Pi_{id}=\rho_s kT$]. The resulting potential between proteins is expressed simply by

$$\frac{W_{osmotic}(r)}{kT}=-\frac{4}{3}\pi d_{ps}^3(\rho_s k_B T)\left[1-\frac{3r}{4d_{ps}}+\frac{r^3}{16d_{ps}^3}\right],\quad d+2\Delta r<r<2d_{ps}+2\Delta r \quad (9)$$

where $d_{ps}=(d+d_s)/2$.

The specific interaction can be represented by a site-specific square-well potential [14,15]. The interaction includes an identity, a hydrogen bonding effect, a hydrophobicity of surface amino acid residues and surface roughness, etc.

$$\frac{W^{specific}}{kT}=-\varepsilon_{sp},\ (d+2\Delta r)\leq r\leq(d+\delta+2\Delta r) \quad (10)$$

where ε_{sp} and δ are model parameters.

For a protein in aqueous buffer solution at a given pH, the binding of ions onto the protein is described by a dynamic equilibrium with a very small time constant. Thus, when we speak of the net charge of the protein, we are referring to mechanics that indicate that the total charge carried by each protein monomer in solution varies rapidly with time.

The existence of charge fluctuations and their influence on the perturbation potential was first noted by Kirkwood and Shumaker [13], who used statistical-mechanical arguments to relate the time correlation between fluctuations in charge to the distribution of charge

associated with fluctuations in the number and configuration of ions bound to the protein. Further analysis by Phillies [19] led to the following expression for the charge fluctuation contribution to the potential:

$$\frac{W^{fluctuation}(r)}{kT} = -\frac{1}{2(kT)^2}\frac{\langle Q_i^2 Q_i^2\rangle - \langle Q_i Q_i\rangle^2}{\varepsilon^2}\frac{\exp[-2\kappa r]}{r^2}\left[\frac{\exp(\kappa d/2)}{1+\kappa d/2}\right]^4 \quad (11)$$

where Q_i is the formal charge on a protein monomer i. In Eq. (11), the quantity $\langle Q_i^2 Q_i^2\rangle - \langle Q_i Q_i\rangle^2$ can be identified as $[\langle z^2\rangle^2 - \langle z\rangle^4]e^4 = \Delta z^4 e^4$, where z is the net charge on each protein monomer and Δz is the variance in the average charge of the monomer. This parameter is dependent on the solution pH. The negative sign in front of the right-hand side of Eq. (11) indicates that $W^{fluctuation}(r)$ provides an attractive force contribution to the overall perturbation potential.

2. Equation of State

In perturbation theory, an assembly of hard spheres is used as the reference system, while the remaining interactions are treated as perturbations:

$$\frac{P}{\rho k_B T} = \left(\frac{P}{\rho k_B T}\right)_{ref} + \left(\frac{P}{\rho k_B T}\right)_{pert} = \left(\frac{P}{\rho k_B T}\right)_{ref} + \frac{\omega_{PA}^2 \rho U}{2 k_B T} \quad (12)$$

where ρ is the density of protein molecules, P is the pressure, ω_{PA} is the average degree of the pre-aggregation, and U is the perturbation energy per unit density, given by

$$U = 4\pi\int W^{overall}(r) r^2 dr \quad (13)$$

The reference hard-sphere equation of state is given by

$$\left(\frac{P}{\rho kT}\right)_{ref} = 1 + 4\omega_{PA}^2\eta\frac{1-\frac{\eta}{2}}{(1-\eta)^3} - (\omega_{PA}-1)\left[\frac{1-\frac{\eta}{2}}{(1-\eta)^3} - 1\right] \quad (14)$$

The total equation of state is the sum of the reference and the perturbation, given by

$$\left(\frac{P}{\rho kT}\right) = 1 + 4\omega_{PA}^2\eta\frac{1-\frac{\eta}{2}}{(1-\eta)^3} - (\omega_{PA}-1)\left[\frac{1-\frac{\eta}{2}}{(1-\eta)^3} - 1\right] + \frac{\omega_{PA}^2\rho U}{2k_B T} \quad (15)$$

where the packing fraction $h = 4\pi\rho d^3/6$.

The Helmholtz energy is derived by using the following relation:

$$\frac{A}{N\omega_{PA}kT} = \frac{A^0}{N\omega_{PA}kT} + \int_0^{\rho\omega_{PA}}\left(\frac{P}{\rho\omega_{PA}kT} - \frac{1}{\omega_{PA}}\right)\frac{d(\rho\omega_{PA})}{\rho\omega_{PA}} + \ln(\rho\omega_{PA}kT) \quad (16)$$

Then, the chemical potential is

$$\mu = \left(\frac{\partial A}{\partial N}\right)_{T,V} \quad (17)$$

At equilibrium, pressure and composition are calculated from

$$\Delta\mu^s = \Delta\mu^d,\ P^s = P^d \quad (18)$$

Table 1. Contributions to the potential of mean force between protein particles

Type	Mean-force potential	Screening parameter, $\zeta(r)$	Reference number
$W^{q\_q}(r)$	$\frac{z^2e^2\zeta(r)}{4\pi\varepsilon_0\varepsilon_r r}$	$\frac{z^2e^2\exp[-\kappa(r-d)]}{4\pi\varepsilon_0\varepsilon_r r(1+\kappa d/2)^2}$	[7]
$W^{q\_\mu}(r)$	$-\frac{2}{3}\frac{z^2e^2\mu^2\zeta(r)}{(4\pi\varepsilon_0\varepsilon_r)^2kTr^4}$	$\left[\frac{3(1+\kappa r)\exp[-\kappa(r-d)]}{(1+\kappa d/2)\left(2+\kappa d+(\kappa d/2)^2+(1+\kappa d/2)\frac{\varepsilon_s}{4\pi\varepsilon_0\varepsilon_r}\right)}\right]^2$	[23], [25]
$W^{q\_i\mu}(r)$	$-\frac{z^2e^2\alpha\zeta(r)}{(4\pi\varepsilon_0\varepsilon_r)^2r^4}$	unknown (near unity)	[23], [25]
$W^{\mu\_\mu}(r)$	$-\frac{2}{3}\frac{\mu^4\zeta(r)}{(4\pi\varepsilon_0\varepsilon_r)^2kTr^6}$	$\frac{3^4[2+2\kappa r+(\kappa r)^2]^2\exp[-2\kappa(r-d)]}{\left[2+\kappa d+(\kappa d/2)^2+(1+\kappa d/2)\frac{\varepsilon_s}{4\pi\varepsilon_0\varepsilon_r}\right]^4}$	[23], [25]
$W^{\mu\_i\mu}(r)$	$-\frac{2\mu^2\alpha\zeta(r)}{(4\pi\varepsilon_0\varepsilon_r)^2r^6}$	unknown (near unity)	[23], [25]
$W_{disp}(r)$	$-\frac{H}{6}\left[\frac{d^2}{r^2}+\frac{d^2}{r^2-d^2}+2\ln\left(1-\frac{d^2}{r^2}\right)\right],\ r\geq(d+2\Delta r)$		[7], [27]
$W_{osmotic}(r)$	$-\frac{4}{3}\pi d_{ps}^3(\rho_s k_B T)\left[1-\frac{3r}{4d_{ps}}+\frac{r^3}{16d_{ps}^3}\right],\ d+2\Delta r<r<2d_{ps}+2\Delta r$		[15]
$W_{specific}(r)$	$-\varepsilon_{ps},\ (d+2\Delta r)\leq r\leq(d+\delta+2\Delta r)$		[19], [26]

Symbol definitions are as follows: z is the valance of the protein, which gives the average protein surface charge varying with the solution pH, e is the unit of electron charge, d is the hard-sphere diameter of protein, $4\pi\varepsilon_0$ is the dielectric permittivity of free space, ε_r is the relative dielectric permittivity of water, Δr is the effective-space hydration/stern layer and κ is the Debye parameter given by, $\kappa^2 = (2e^2N_AI)/(kT\varepsilon_0\varepsilon_r)$, μ is the dipole moment, which is pH dependent parameter, ε_s is the effective dielectric constant at the surface of the protein, α represents the polarizability, H is the Hamaker constant, $d_{ps} = (d+d_s)/2$, ε_{sp} and δ are model parameters

where superscripts s and d denote the supernatant and dense phases, respectively.

RESULTS AND DISCUSSION

1. The pH Dependence of the Electrostatic Potentials

The electrostatic potential in aqueous solution is significantly affected by the solution pH, especially the charge-charge potential and the distribution of the charge-fluctuation.

In electrostatic potential, the protein surface charge and the dipole moment are a function of pH. It changes the magnitude of charge-charge repulsion, charge-dipole attraction, and dipole-induced dipole attraction forces between protein pairs in solution.

Fig. 1 shows the pH dependence of the electrostatic perturbation energies, when the charge-pH profile is set to the hen-egg-white lysozyme (Isoelectric point (pI)=11.3). As shown in Fig. 1, the charge-charge perturbation energy is more affected by the solution pH than that of any other electrostatic perturbation energies. The positive

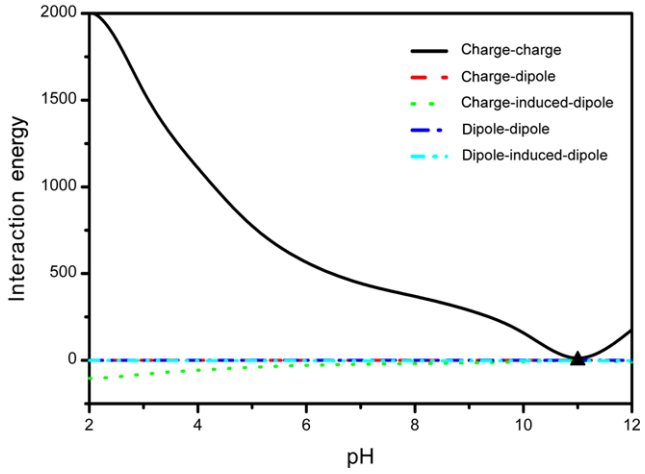

Fig. 1. Contribution to the electrostatic interaction energy as a function of solution pH: I=5.5 M, H/kT=8.9, Δr=0.8 Å, ε/kT=4.5 Å, δ=4 Å, d_s=0.694 Å, d_{11}=3.5 nm, d_{22}=3.4 nm. Dark triangle is the pI (=11.3) of the system.

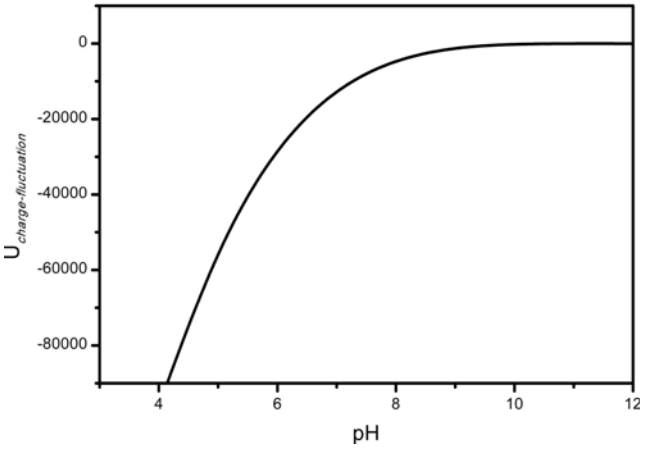

Fig. 2. Charge fluctuation contribution to the interaction energy as a function of solution pH: I=0.03 M, H/kT=8.9, Δr=0.8 Å, ε/kT=4.5 Å, δ=4 Å, d_s=0.694 Å, d_{11}=3.5 nm, d_{22}=3.4 nm.

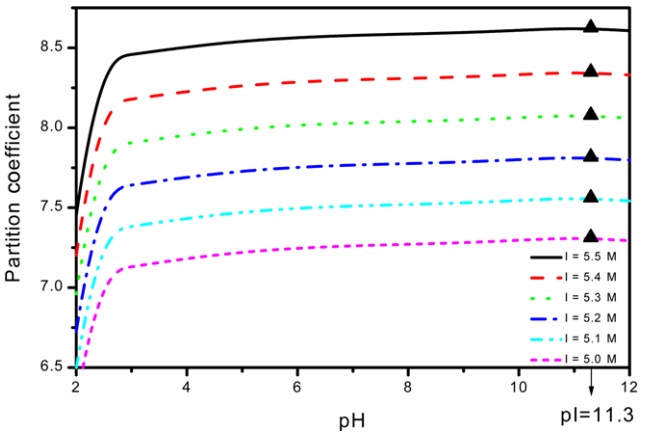

Fig. 3. The calculated partition coefficient K plotted as a function of solution pH for the system which takes into account the overall perturbation energy containing the distribution of the charge fluctuation effect with I=5.5 M, H/kT=8.9, ε_{sp}/kT=3.7, δ=4 Å, d_s=6.94 Å, d=34.3 Å, and Δr=0.8 Å in the case of fixed ω_{PA}=1.3. Dark triangles are maximum points at pI=11.3.

sign of the charge-charge interaction energy implies the repulsion between protein molecules. Overall electrostatic perturbation energy is the smallest at pI (pH 11.3). It explains that at pI, protein molecules aggregate and precipitate more favorably.

2. The pH Dependence of the Charge Fluctuation Distribution

Fig. 2 shows the pH dependence of the perturbation energy of the charge fluctuation contribution. In this plot, we assume that the dependence of Δz is zero at pI of protein and increases with pH above the pI.

Fig. 3 represents the calculated partition coefficient K plotted as a function of solution pH for the system, which takes into account the overall perturbation energy including the distribution of the charge fluctuation effect with I=5.5 M, H/kT=8.9, ε_{sp}/kT=3.7, δ=4 Å, d_s=6.94 Å, d=34.3 Å, and Δr=0.8 Å at the fixed ω_{PA}=1.3. The partition coefficient shows the maximum value at pI. This result is coincident with previous work [1,21].

3. The Effect of the Conformation Change of Protein Molecules

Protein molecules have both hydrophilic and hydrophobic groups in their structures. In the aqueous solution, protein molecules fold to globular form and the most hydrophobic group is found to exist inside [1]. Because of its structural restriction, however, hydrophobic groups cannot be absolutely folded inside and the residues remain on the surface. Changes in solution pH can also alter the conformation of a protein [28]. These conformational changes affect the distribution of the surface hydrophobic residues. Due to these hydrophobic residues, protein particles are partially pre-aggregated. We expect that the pre-aggregation of protein molecules can be explained by the effect of solution pH. As shown in Fig. 3, the maximum points (dark triangles) appear at pI (=11.3).

Fig. 4 shows the effect of the pH dependence when the degree of the pre-aggregation is given as a function of pH at various ionic strengths. In this figure, the dependence of the ionic strength shows the same behavior as that of the commonly observed feature in salting-out [5,14]. The tendency of the dependence on pH is more effective than that of Fig. 3.

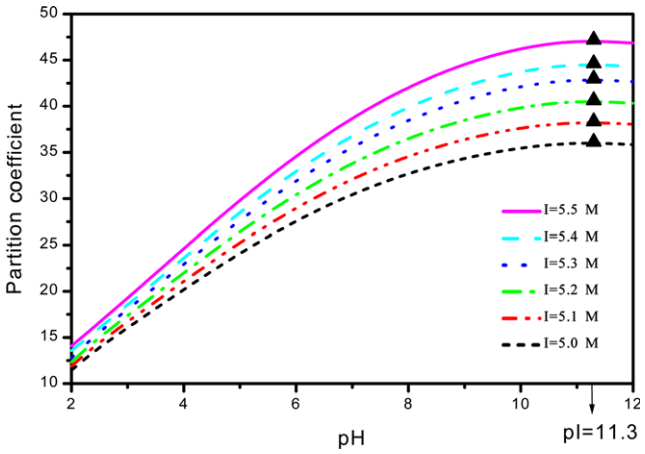

Fig. 4. The calculated partition coefficient K plotted as a function of solution pH for the system considering the pre-aggregation with I=5.5 M, H/kT=8.9, ε_{sp}/kT=3.7, δ=4 Å, d_s=6.94 Å, d=34.3 Å, and Δr=0.8 Å. Dark triangles are maximum points at pI=11.3.

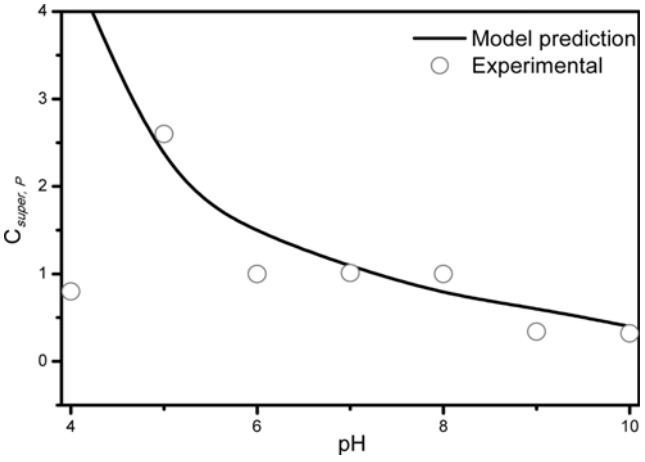

Fig. 5. Experimental and correlated values of $C_{p, super}$ as a function of solution pH in the case of hen-egg-white lysozyme at I= 3 M, H/kT=8.9, ε_{PA}/k_BT=4.5, δ=4.2 Å, Δr=0.8 Å and ω_{PA}= 2.25. Open squares are experimental data from Shih et al. and the solid line is calculated values using the proposed model.

Shih et al. [21] have conducted precipitation experiments for the hen-egg-white lysozyme in NaCl solution at various ionic strengths and pH. Fig. 5 shows experimental data and calculated values of $C_{p, super}$ as a function of solution pH for the hen-egg-white lysozyme at ionic strength=3 M. Hamaker constant and the thickness of the hydration/stern layer (Δr) were 8.9 and 0.8 Å, respectively. These values are coincident with the data reported by Kuhner [15], who indicated that the Hamaker constant depends on the thickness of the hydration/stern layer. As shown in Fig. 5, calculated supernatant concentrations are in good agreement with experimental data [21], for ε_{PA}/k_BT=4.5, δ=4.2 Å, I=3 M and ω_{PA}=2.25 at pI. But, in case of pH 4, calculated results show large deviation with experimental data. The possible reason is that there could be an experimental error since biopolymer is very sensitive to the experimental condition.

CONCLUSION

We have proposed a molecular-thermodynamic framework to describe protein precipitation by inorganic salt. The model is based on statistical mechanical perturbation theory and the reference term is derived from the modified *Chiew*'s equation to express the pre-aggregation effect that takes into account the conformation change with various pH. We discussed protein-protein effective two-body potentials. The distribution and magnitude of charge on the surface of a protein change significantly with pH. It varies the magnitudes of charge-charge repulsion, charge-dipole attraction, dipole-dipole attraction, and induced dipole-induced dipole attraction forces between protein pairs in solution and the distribution of the charge fluctuation.

Equilibrium supernatant concentration calculated by the proposed model is in good agreement with experimental results for hen-egg-white lysozyme in NaCl solution when the pre-aggregation is set to the linear function of pH. Our results also show that the pre-aggregation effect of protein plays an important role in the precipitation of proteins.

NOMENCLATURE

$C_{p, super}$: protein concentration in the supernatant phase
d : hard-sphere diameter of protein
d_{ps} : mean diameter of protein and salt
e : unit of electron charge
H : hamaker constant
I : ionic strength
p : osmotic pressure
Q_i : formal charge on a protein monomer I
r : center to center distance
U : perturbation energy per unit density
z : net charge on each protein monomer
Δr : effective-space hydration/stern layer
Δz : variance in the average charge of the monomer.
$4\pi\varepsilon_0$: dielectric permittivity of free space
N : Avogadro's number
A : helmholtz free energy
k : Boltzmann's constant
W : potential of mean force
T : temperature

Greek Letters

α : polarizability
δ, ε_{sp} : model parameters
ε_r : relative dielectric permittivity of water
ε_s : effective dielectric constant at the surface of the protein
ρ : density of protein molecules
κ : Debye parameter
μ : dipole moment
ω_{PA} : average degree of pre-aggregation

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