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A molecular-thermodynamic model for the interactions between globular proteins in aqueous solutions: Applications to bovine serum albumin (BSA), lysozyme, α-chymotrypsin, and immuno-gamma-globulins (IgG) solutions

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Abstract

To investigate globular protein–protein and protein–salt interactions in electrolyte solutions, a potential of mean force including hard-core repulsion, van der Waals attraction and electric double layer repulsion is proposed in this work. Both van der Waals attraction and double-layer repulsion are represented using hard spheres with two-Yukawa tails. The explicit analytical solution of osmotic pressure is derived from the first-order mean spherical approximation. From the comparison between the calculated and experimental values of osmotic pressures for aqueous bovine serum albumin (BSA), lysozyme, α-chymotrypsin, and immuno-gamma-globulins (IgG) solutions, we found that the proposed model is adequate for the description of the interactions between proteins at low ionic strength and small self-association of protein molecules. At high ionic strength, the charge inversions of protein molecules should be taken into account.

Keywords: Osmotic pressure; Globular proteins; Yukawa potential; Bovine serum albumin; Lysozyme; α-Chymotrypsin; IgG; Equation of state

1. Introduction

The globular proteins, such as bovine serum albumin (BSA), lysozyme, α-chymotrypsin, and immuno-gamma-globulins (IgG) in aqueous media have been widely used in ultrafiltration membrane studies. They are involved in many actual applications such as waste water treatment, drug delivery, etc. [1]. The development of these applications is partly controlled by our understanding of the physicochemical properties of proteins in aqueous electrolyte solutions. Osmotic pressure is one of the thermodynamic properties to show the non-idealities of protein solutions [2].

There are many experimental data of osmotic pressure of globular proteins in aqueous electrolyte solutions. Most of them were measured using membrane osmometry [2–7]. Nevertheless, the osmotic properties of globular proteins in aqueous electrolyte solutions can be investigated using the static light scattering [8].

Theoretical studies of the thermodynamic properties of aqueous protein solutions have yielded a number of models useful for dilute solutions. Some efforts have been made for the concentrated protein solutions. A free solvent model proposed by Yousef et al. [6,7] has been successfully applied to predict the osmotic pressure of several globular proteins at moderate salt concentrations. However, the free solvent model fails to predict the osmotic pressure in low ionic strength solutions because of its principal assumption, i.e., when in low ionic strength solutions, due to insufficient Debye screening, the assumption that the solutions behaves ideally is invalid. A promising theoretical description for the concentrated protein solution is to use the potential of mean force. For example, Vilker et al. [3] employed this concept and developed a more theoretical approach to the virial expansion model. They listed and compared various contributions to the potential energy of interaction and concluded that the dominant pair potentials were the repulsive charge–charge interaction and the attractive dispersion interaction. The virial expansion model is insightful since the virial coefficients can be directly related to solute–solute interaction potentials for dilute solutions. However, even

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though protein concentrations are always very dilute, the virial expansions truncated to a few terms often fail to predict osmotic pressure for the full range of concentrations [9]. Haynes et al. [2] used the relation between virial coefficients and potentials of mean force to determine the interactions of charged protein molecules.

Because the potential of mean force for the proteins in the electrolyte solution is composed of several interactions, an accurate potential of mean force is difficult to obtain. In practice, the simple square-well potential or Yukawa potential is adopted to describe the interactions between two globular protein molecules in an electrolyte solution. When the square-well potential is used, the energy parameter in the square-well potential needs to be represented as a function of ionic strength [10]. The hard-core two-Yukawa potential has been used to correlate and predict the osmotic pressures and diffusion coefficients of globular protein in electrolyte solutions [11–13]. The advantage of the hard-core two-Yukawa model is that all the potential parameters are constants for a specific system. However, in the previous applications the expression of osmotic pressure is empirically calculated as a sum of the infinite order expansion of the Duh and Mier-y-Teran equation of state [13] twice directly. More strictly, Guérin [14,15] derived pressure and internal energy based on a second-order inverse temperature expansion of the free energy within the mean spherical approximation, which performed well on various two-Yukawa fluids modeling either simple or chain fluids. Their expressions lead to easy calculations, the result of which are in general good agreement with the full numerical MSA and the MC simulations. Recently, Tang and co-workers [16] have derived a restrict solution for the hard-core multi-Yukawa fluid based on the first-order mean spherical approximation, which is very accurate when compared with molecular simulation data. This makes it possible to predict osmotic pressure of protein solution in a more reasonable way.

In this paper, the explicit expression for osmotic pressure of globular protein solutions are derived from the Helmholtz free energy of the two-Yukawa fluids based on the first-order mean spherical approximation [16]. The model is applied to analyses the osmotic pressures of charged BSA, lysozyme, α-chymotrypsin, and IgG solutions.

2. Theory

2.1. Intermolecular potential of mean force

In an aqueous protein solution, interactions between two proteins can be quantitatively described by a two-body potential of mean force. In this paper the charged protein–electrolyte solution is considered as a pseudo one-component system, and water molecules are treated as a continuous medium with dielectric constant D. Similar to Lin et al., hard-core two-Yukawa potential of mean force $u(r)$ is employed to describe the interactions between two globular protein molecules in an electrolyte solution, i.e.,

$$u(r) = \begin{cases} \infty, & r < \sigma, \\ -\sum_{i=1}^{2} \varepsilon_i \frac{\exp[-\lambda_i(r/\sigma-1)]}{r/\sigma}, & r \geqslant \sigma, \end{cases} \tag{1}$$

where r is the center to center distance between two globular proteins, σ is the globular protein diameter, λ_i and ε_i are two parameters of interaction range and strength, respectively. The potential of mean force is represented as $u(r) = u^{\mathrm{dis}}(r) + u^{\mathrm{cc}}(r)$ at $r \geqslant \sigma$, where $u^{\mathrm{dis}}(r)$ and $u^{\mathrm{cc}}(r)$ represent the dispersion interaction and the double-layer repulsive charge–charge interaction between charged protein molecules, respectively. The dispersion interaction can be expressed using the first Yukawa tail, i.e.,

$$u^{\mathrm{dis}}(r) = -\frac{\varepsilon_1 \exp[-\lambda_1(r/\sigma - 1)]}{r/\sigma} \quad (r > \sigma), \tag{2}$$

where ε_1 is the dispersion energy parameter and λ_1 is the range parameter. Because the Lennard–Jones potential is the best potential for the dispersion interaction, and when $\lambda_1 = 1.8$, the Yukawa potential yields results comparable with those obtained from Lennard–Jones potential [17]. Therefore $\lambda_1 = 1.8$ is adopted in this work.

The double-layer repulsive charge–charge interaction between protein molecules can also be described using another Yukawa tail,

$$u^{\mathrm{cc}}(r) = -\frac{\varepsilon_2 \exp[-\lambda_2(r/\sigma - 1)]}{r/\sigma} \quad (r > \sigma). \tag{3}$$

From the classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, we can obtain the Yukawa parameters for the electric double-layer repulsive charge–charge interaction between two globular protein molecules.

$$\varepsilon_2 = -\frac{z_{\mathrm{p}}^2 e^2}{\sigma D(1 + \kappa\sigma/2)^2} \quad \text{and} \quad \lambda_2 = \kappa\sigma, \tag{4}$$

where z_{p} is the protein charge number, e is the charge of an electron, D is the dielectric constant of water and κ is the Debye screening parameter, determined by

$$\kappa^2 = \sum_i \frac{\rho_i e^2 z_i^2}{D k_{\mathrm{B}} T}, \tag{5}$$

where k_{B} is the Boltzmann constant, T is the absolute temperature, ρ_i and z_i are the number density and the valence of microion i, respectively.

2.2. Equation of state

Given the interaction between proteins represented by two-Yukawa form, the Ornstein–Zernike (OZ) equation can be solved analytically within mean spherical approximation (MSA) and the analytical expression for the radial distribution function can be obtained. From energy equation and the fundamental relation between internal energy and Helmholtz free energy, one can easily derived the analytical expression for Helmholtz free energy. For the multi-Yukawa potential, the first-order mean spherical approximation (FMSA) solution for the attractive part is straightforwardly a linear combination of individual one-Yukawa solutions. Based on Laplace transform of the resulting radial distribution function, Tang et al. [16] derived the following residual Helmholtz free energy

$$\frac{A}{N k_{\mathrm{B}} T} = \frac{A_0}{N k_{\mathrm{B}} T} + \frac{A_1}{N k_{\mathrm{B}} T} + \frac{A_2}{N k_{\mathrm{B}} T}, \tag{6}$$

where A_0 is the residual free energy of hard spheres, A_1 and A_2 are the perturbation terms of free energy. They can be expressed as

$$\frac{A_0}{Nk_BT} = \frac{4\eta - 3\eta^2}{(1-\eta)^2}, \tag{7}$$

$$\frac{A_1}{Nk_BT} = -12\eta \sum_{i=1}^{2} \frac{\beta\varepsilon_i L(\lambda_i)}{(1-\eta)^2 Q_0(\lambda_i)\lambda_i^2}, \tag{8}$$

$$\frac{A_2}{Nk_BT} = -6\eta \sum_{i=1}^{2}\sum_{j=1}^{2} \frac{\beta^2\varepsilon_i\varepsilon_j}{(\lambda_i+\lambda_j)Q_0^2(\lambda_i)Q_0^2(\lambda_j)}, \tag{9}$$

where N is the number of protein molecules, $\eta = \pi\rho_p\sigma^3/6$, $\rho_p = N/V$, V is the volume of the system, $\beta = 1/k_BT$ and

$$L(\lambda) = (1+0.5\eta)\lambda + 1 + 2\eta, \tag{10}$$

$$Q_0(\lambda) = \frac{S(\lambda) + 12\eta L(\lambda)e^{-\lambda}}{(1-\eta)^2\lambda^3}, \tag{11}$$

$$S(\lambda) = (1-\eta)^2\lambda^3 + 6\eta(1-\eta)\lambda^2 + 18\eta^2\lambda - 12\eta(1+2\eta). \tag{12}$$

The compressibility factor can be derived easily through the standard relations with Helmholtz free energy, i.e.,

$$\frac{P}{\rho_p k_B T} = \frac{1+\eta+\eta^2-\eta^3}{(1-\eta)^3} + \frac{\eta(\partial A_1/\partial\eta)_{T,N}}{Nk_BT} + \frac{\eta(\partial A_2/\partial\eta)_{T,N}}{Nk_BT}. \tag{13}$$

2.3. Osmotic pressure of protein solution

Using McMillan–Mayer solution theory, the osmotic pressure Π of aqueous protein–electrolyte solutions can be expressed as

$$\frac{\Pi}{\rho_p k_B T} = Z^{\text{Donnan}} + \frac{1+\eta+\eta^2-\eta^3}{(1-\eta)^3} + \frac{\eta(\partial A_1/\partial\eta)_{T,N}}{Nk_BT} + \frac{\eta(\partial A_2/\partial\eta)_{T,N}}{Nk_BT}, \tag{14}$$

where superscript "Donnan" represents the contribution of the Donnan effect. In protein–electrolyte solution, to maintain electroneutrality and equilibrium, the concentrations of microions on both sides of a membrane will be unequal, which may bring additional osmotic pressure and is called the Donnan effect. The contribution of Donnan effect to osmotic compressibility factor Z^{Donnan} can be expressed as

$$Z^{\text{Donnan}} = \left(\rho_+^{\text{in}} + \rho_-^{\text{in}} - \rho_+^{\text{out}} - \rho_-^{\text{out}}\right)/\rho_p, \tag{15}$$

where ρ_p, ρ_+, and ρ_- are the number densities of protein, microcation, and microanion, respectively. Considering a single electrolyte, the equations of electroneutrality and the equal ionic concentration products on both sides of the membrane are related by

$$z_p\rho_p + z_+\rho_+^{\text{in}} + z_-\rho_-^{\text{in}} = 0, \tag{16}$$

$$z_+\rho_+^{\text{out}} + z_-\rho_-^{\text{out}} = 0, \tag{17}$$

$$\left(\rho_+^{\text{in}}\right)^{z_-}\left(\rho_-^{\text{in}}\right)^{z_+} = \left(\rho_+^{\text{out}}\right)^{z_-}\left(\rho_-^{\text{out}}\right)^{z_+}, \tag{18}$$

where z_p, z_+, and z_- are the charge numbers of protein, microcation, and microanion, respectively. The superscripts "in" and "out" represent the protein side and the microion side, respectively. The number densities of microcation and anion is defined by

$$\rho_i = 1000C_iN_0 \quad (i = +, -), \tag{19}$$

where C_i is the molar concentration of microcation and anion, in unit mol/L, N_0 is the Avogadro number (6.023×10^{23}).

3. Results and discussion

We have used the theory described above to analyze osmotic pressure of aqueous BSA, lysozyme, α-chymotrypsin, and IgG solutions at different pH and ionic strength. In the calculation, the hard-sphere diameters and molecular weights of globular proteins are taken from the literature. Generally, the charge number per protein molecule at different pH and ionic strength is the titration data also from the literature if it is available, and only the dispersion energy parameter ε_1/k (at $\lambda_1 = 1.8$) is adjusted to fit the experimental data.

3.1. BSA solutions

The osmotic pressure of aqueous BSA is investigated at selected values of temperature, pH, ionic strength, and protein concentration in ammonium sulfate solutions. The values of the parameters used in the calculation of the osmotic pressure of aqueous BSA are collected and listed in Table 1.

In Figs. 1 and 2, we compare the calculated osmotic pressure of aqueous BSA–$(NH_4)_2SO_4$ solution with the experimental data at temperature $T = 298.15$ K and ionic strength $I = 1.0$ and 3.0 mol/L, respectively. Three pH values are considered in the figures. From the figures we can see that the calculated osmotic pressures are in good agreement with the experimental data. In Fig. 3, we present the results for BSA solutions in a mixture with 0.15 mol/L NaCl at 298.15 K and pH 7.4, 5.4, and 4.5. Figs. 4 and 5 show osmotic pressure in aqueous BSA–NaCl solutions with pH 7.4 and 4.5 at ionic strength $I = 1.0$ and 5.0 M.

From Figs. 1–5 we can see that at low ionic strength and high pH values (pH $\geqslant$ 5.4), the predicted osmotic pressures from the hard-core two-Yukawa model are in excellent agreement with the experimental data, while at pH 4.5, the theory overestimates

Table 1
Parameters used to evaluate osmotic pressure for aqueous BSA solution

Parameter	Value	Reference
M_p	69,000 g/mol	[3]
σ_p	6.26 nm	[3]
ε_1/k_B	91.3 K	[11]
z_p	+20 at pH 4.0, −13.5 at pH 6.0, −18.8 at pH 7.0, −22.9 at pH 8.0, $I = 1.0$ mol/L	[5]
	−20.4 at pH 7.4, −9.1 at pH 5.4, +4.5 at pH 4.7, $I = 0.15$ mol/L	[3]

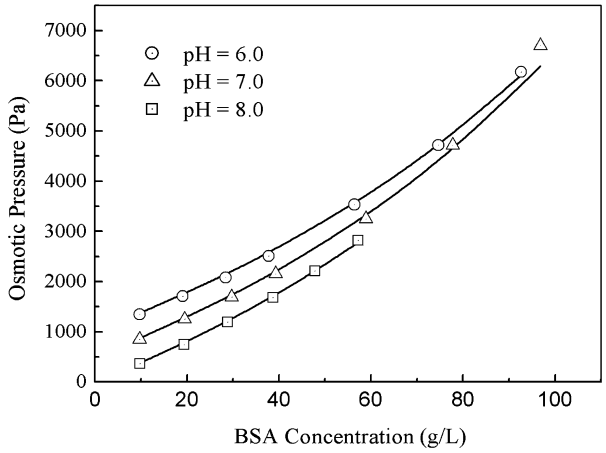

Fig. 1. Osmotic pressure in aqueous BSA–ammonium sulfate solutions at $T = 298.15$ K, $I = 1.0$ mol/L, and different pH values (pH 6, 7, and 8). The symbols and solid curves represent the experimental data [5] and the calculated results from this work. For clarity, the results at pH 7 and 6 are shifted upward by 500 and 1000 Pa, respectively.

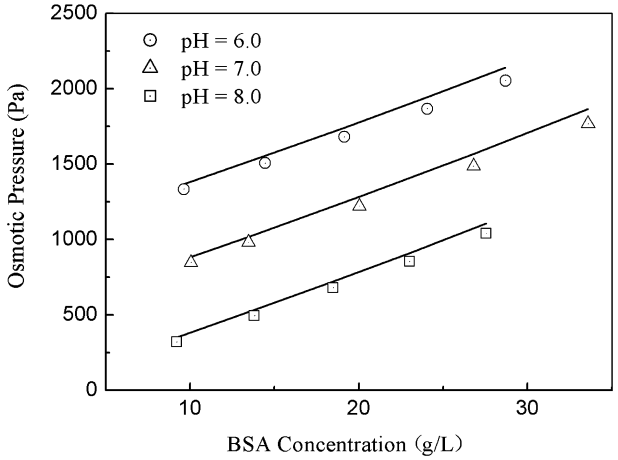

Fig. 2. Osmotic pressure in aqueous BSA–ammonium sulfate solutions at $T = 298.15$ K, $I = 3.0$ mol/L, and different pH values (pH 6, 7, and 8). The symbols and solid curves represent the experimental data [5] and the calculated results from this work. For clarity, the results at pH 7 and 6 are shifted upward by 500 and 1000 Pa, respectively.

the osmotic pressure at all ionic strengths. This is because the theory does not include the self-association between proteins. The analysis of Druchok et al. [18] shows that for BSA, a small association with fraction of dimmers less than 10% at pH $\geqslant$ 5.4 and the degree of association is larger at pH 4.5 (degree of dimerization is about 50%).

On the other hand, from Figs. 4 and 5 we find a relatively larger deviation between the theoretical and experimental osmotic pressures of aqueous BSA solution. This is due to the neglect of the ionic strength effect on the charge number of proteins. From the previous studies on charged colloids, we know that at enough high ionic strength and protein charge number, a charge inversion phenomenon will occur [19]. But this effect is not considered in the present theory. Therefore, the present theory may be improved by considering self-association and charge inversion of proteins in electrolyte solutions.

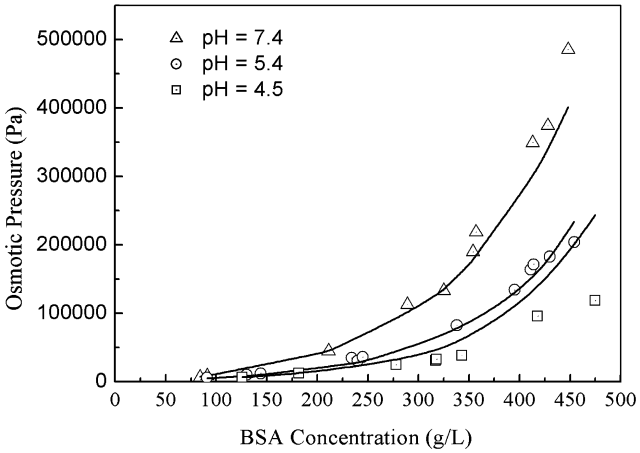

Fig. 3. Correlation of osmotic pressure in aqueous BSA–NaCl solutions at 298.15 K and $I = 0.15$ mol/L. The curves are calculated from this work and the symbols are experimental data of Vilker et al. [3].

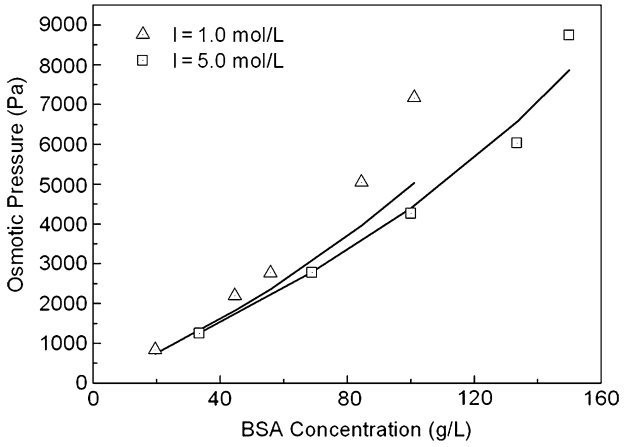

Fig. 4. Osmotic pressure in aqueous BSA–sodium chloride solutions at $T = 298.15$ K, pH 7.4, and $I = 1.0$ and 5.0 mol/L. The curves are calculated from this work and the symbols represent experimental data taken from Wu and Prausnitz [4].

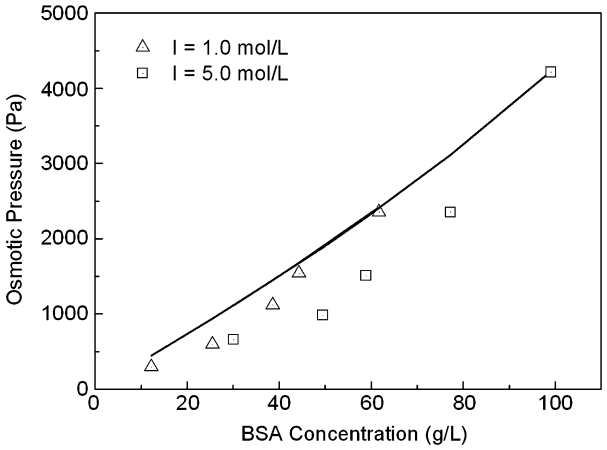

Fig. 5. Osmotic pressure in aqueous BSA–sodium chloride solutions at $T = 298.15$ K, pH 4.5, and $I = 1.0$ and 5.0 mol/L. The curves are calculated from this work and the symbols represent experimental data taken from Wu and Prausnitz [4].

3.2. Lysozyme solutions

The present theory is also applied to calculate the osmotic pressure of aqueous lysozyme at different pH and ionic strength. In the calculation, the molecular weight and hard-sphere diameter of lysozyme are, respectively, 17,000 g/mol and 3.44 nm, which are taken from Moon et al. [5]. The net charge numbers at pH 4.0, 7.0, and 8.0 are +14, +8, and +7.5, respectively [5]. In addition, we assume that the net charge number per lysozyme molecule does not change with the ionic strength in the solution. The dispersion energy parameter ε_1/k_B is determined from the experimental data and has a value of 303.9 K for lysozyme. Figs. 6 and 7 present the effect of pH on the osmotic pressure of lysozyme solutions at ionic strength $I = 1.0$ and 3.0 mol/L. The comparison of the calculated osmotic pressures with the experimental data indicates that the theory is able to describe the variation of osmotic pressure with protein concentration, pH, and ionic strength accurately.

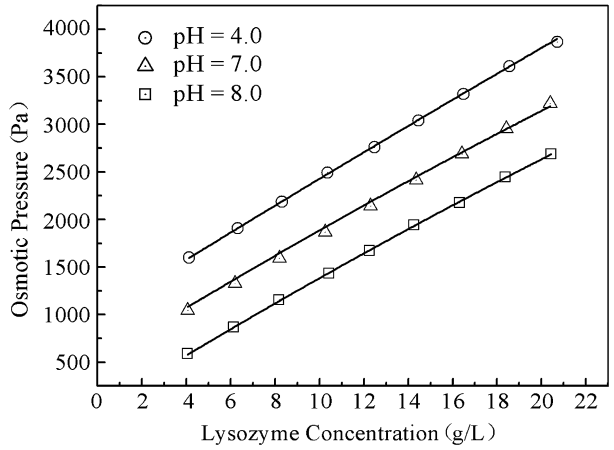

Fig. 6. Osmotic pressure in aqueous lysozyme–$(NH_4)_2SO_4$ solutions at $T = 298.15$ K, $I = 1$ mol/L, and different pH values (pH 4, 7, and 8). The symbols and solid curves represent the experimental data [5] and the results from the present theory, respectively. For clarity, the results at pH 7 and 4 are shifted upwards by 500 and 1000 Pa, respectively.

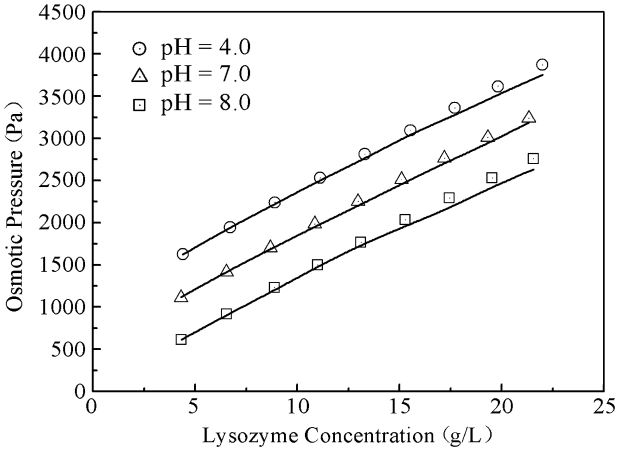

Fig. 7. Osmotic pressure in aqueous lysozyme–$(NH_4)_2SO_4$ solutions at $T = 298.15$ K, $I = 3.0$ mol/L, and different pH values (pH 4, 7, and 8). The symbols and solid curves represent the experimental data [5] and the results from the present theory, respectively. For clarity, the results at pH 7 and 4 are shifted upwards by 500 and 1000 Pa, respectively.

In Fig. 8, the calculated osmotic pressures of lysozyme in aqueous $(NH_4)_2SO_4$ solutions are compared with the corresponding experimental data [5,20]. The results from the square-well models of Grigsby et al. [21] and Chang and Bae [10] are also included in the figure. All three models show satisfactory results over the entire protein concentration range at pH 4.0. However, at pH 7.0, the simple square-well model of Grigsby et al. gives unexpected maximum on the curve of osmotic pressure as a function of protein concentration. The square-well model of Chang and Bae slightly overestimates osmotic pressures of lysozyme solutions in all cases shown in Fig. 8. Because there is almost no self-association for lysozyme in electrolyte solution at pH 4.0, the present hard-core two-Yukawa model gives very accurate osmotic pressures at this pH. Only at pH 7.0 and $I = 1.0$ mol/L, the present theory underestimates the osmotic pressures of lysozyme solution.

3.3. α-Chymotrypsin solutions

For α-chymotrypsin, the molecular weight and hard-sphere diameter are taken from the literature, i.e., 31976.8 g/mol and 4.3356 nm, respectively. The dispersion energy parameter ε_1/k_B for α-chymotrypsin is regressed from the osmotic pressure data of Haynes et al. [2], and has a value of 466.32 K. We regard the molecular weight and dispersion energy parameter ε_1/k_B for a specific protein as constants when pH and ionic strength in solutions are changed. The used net charge of α-chymotrypsin molecule is the titration data of Marini and Wunsch [22], and Shiao et al. [23], which can be found in Table 2.

We compare the calculated osmotic pressures of aqueous α-chymotrypsin–K_2SO_4 solutions with the corresponding experimental data [2] at 298.15 K in Figs. 9 and 10. It can be seen from Figs. 9 and 10 that at pH $\leqslant$ 10, the agreement between the calculated osmotic pressure and the experimental values is excellent. However, at pH 11.0 and 12.0, and high α-chymotrypsin concentrations, the present theory overestimates the osmotic pressure of α-chymotrypsin solutions. At pH 11.0 and 12.0, the net charge number on the surface of α-chymotrypsin molecule are high, and the charge inversion will be possible in the solution. It is the high net charge number which causes the theoretical overestimation of the osmotic pressure of α-chymotrypsin solutions.

3.4. IgG solutions

The osmotic pressure as a function of IgG concentration in phosphate-buffered solution at pH 7.4 and ionic strength $I = 0.13$ mol/L were measured by Yousef et al. [6]. The heterogeneity of IgG is most obvious in its isoelectric chromatograph, where it is found to have prominent values between 5.8 and 8.5. Thus IgG solutions at pH 7.4 have components consisting of both positively and negatively charged species. In the theoretical calculation, we regard the IgG molecule has zero charge at pH 7.4. The molecular weight of IgG is 159,000 g/mol, which

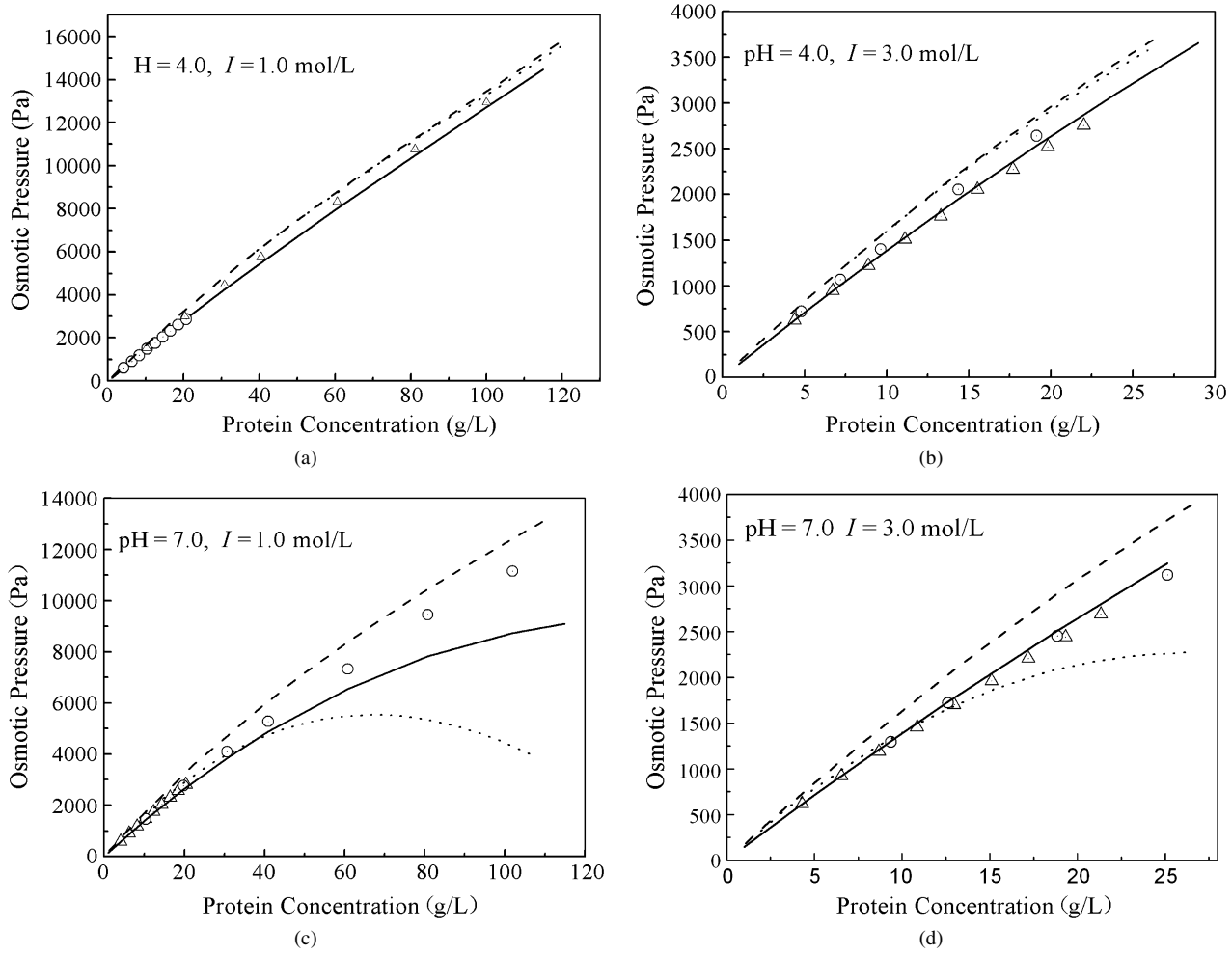

Fig. 8. Osmotic pressure of aqueous lysozyme–$(NH_4)_2SO_4$ solutions at 298.15 K. The symbols represent the experimental data [5,20]: (a) pH 4.0, $I = 1.0$ mol/L; (b) pH 4.0, $I = 3.0$ mol/L; (c) pH 7.0, $I = 1.0$ mol/L; and (d) pH 7.0, $I = 3.0$ mol/L. The dotted, dashed, and solid curves represent the results from the square-well model of Grigsby et al. [21], the model of Chang and Bae [10], and the present theory, respectively.

Table 2
Net charges of α-chymotrypsin in aqueous electrolyte solutions at different pH values [2]

pH	3.0	4.0	4.5	5.0	6.0	7.0	8.0	8.25	9.0	10.0	11.0	12.0
Net charge	14.2	10.2	7.7	5.2	3.1	2.0	0.2	0.0	−1.0	−6.0	−12.0	−15.6

is reported in the literature [24]. From the experimental osmotic pressure data, we obtain the dispersion energy parameter (corresponding to $\lambda_1 = 1.8$) and the hard-sphere diameter of IgG with values of 117.95 K and 7.88 nm, respectively.

In Fig. 11 we compare the predictions from various forms of the virial equation, free solvent model and present theory based on two-Yukawa potential with the experimental data. It can be seen from Fig. 10 that various forms of the virial equation fail to accurately predict the osmotic pressure over the entire range of concentrations, although the expansion up to third virial coefficient only slightly over predicts osmotic pressure at moderate concentrations. This implies that the osmotic pressure of IgG, based on virial expansion with a composition variable in units of grams per liter of solution, has a concentration-dependent virial coefficient. Both predictions from the free solvent model and the two-Yukawa potential reproduce the experimental data

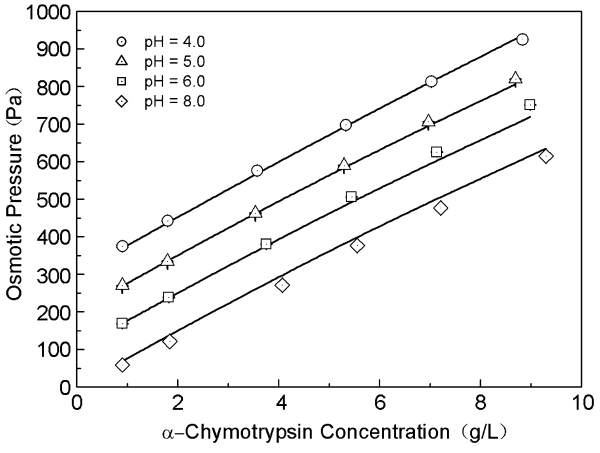

Fig. 9. Osmotic pressure of α-chymotrypsin in aqueous K_2SO_4 solutions at pH 4.0, 5.0, and 6.0, and $I = 0.1$ mol/L. The symbols and solid curves represent the experimental data [2] and the results calculated from present theory, respectively. For clarity, the results at pH 4.0, 5.0, and 6.0 are shifted upwards by 300, 200, and 100 Pa, respectively.

well. However, Yousef et al. [7] found that the parameters of free solvent model are dependent on salt concentration and the pH of solutions, thus free solvent model cannot predict

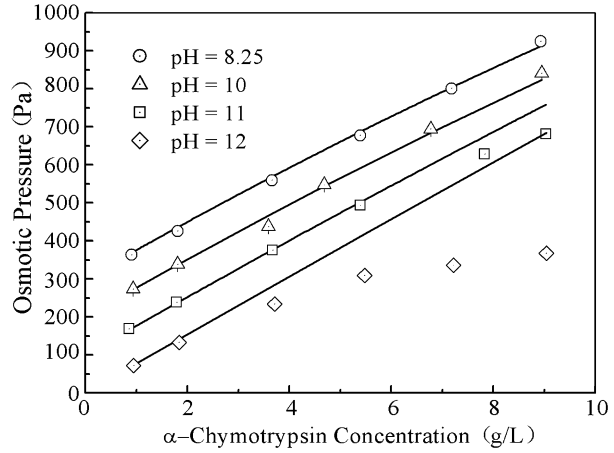

Fig. 10. Osmotic pressure of α-chymotrypsin in aqueous K_2SO_4 solutions at pH 8.25, 10, 11, and 12, and $I = 0.1$ mol/L. The symbols and solid curves represent the experimental data [2] and the results calculated from present theory, respectively. For clarity, the results at pH 8.25, 10, and 11 are shifted upwards by 300, 200, and 100 Pa, respectively.

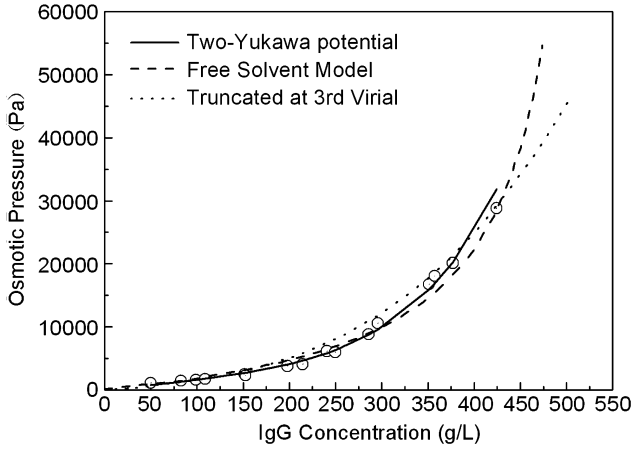

Fig. 11. Osmotic pressure versus IgG concentration in phosphate-buffered solutions at pH 7.4 and $I = 0.13$ mol/L. The experimental data points are taken from Yousef et al. [6].

the osmotic pressure of the systems at high salt concentrations from the parameters obtained from the systems at low salt concentration. However, in the present theory, the only adjusted parameter ε_1/k_B is independent of pH and ionic strength in the protein solution.

4. Conclusion

In this paper, the hard-core two-Yukawa model is used to describe the repulsive charge–charge interaction and the attractive dispersion interaction between charged globular protein molecules in electrolyte solutions. Different from the work of Lin et al., we adopt a more restrict Helmholtz free energy from the first-order mean spherical approximation (FMSA) to construct the expression of osmotic pressure of aqueous protein solutions, which makes the theory more accurate and reasonable.

Extensive comparisons of the theoretical results with the experimental osmotic pressure data of aqueous BSA, lysozyme, α-chymotrypsin, and IgG solutions indicate that the proposed theory is very accurate at not too high charge density on the protein surface and low to moderate ionic strength in the solution. In the case where self-association or charge inversion (i.e., high surface charge density of proteins and high ionic strength in the solutions) occurs, the present theory usually overestimates the osmotic pressure of protein solution. The inclusion of the association and charge inversion effects will definitely improve the present theory. Compared with the virial equations and the free solvent model, one important advantage of present theory is that the molecular weight and dispersion energy parameter are unique for a specific protein, unchanging as the pH and ionic strength are varied.

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