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Cloud and solubility temperatures versus ionic strength in model lysozyme solutions

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Abstract

We report on a DLVO (Derjaguin–Landau–Verwey–Overbeek) theory determination of cloud and solubility temperatures as a function of the salt molarity in lysozyme solutions. The model is able to reproduce—with a fair accuracy—the experimentally observed linear dependence on the logarithm of the ionic strength, within a protein concentration range spanning from 90 to 140 g l^{-1}. A short discussion of the results is also given in connection with previous applications of the same DLVO model to protein solutions.

In a previous paper [1] (hereafter referred to as I) we showed that a globular protein solution, composed of lysozyme in water and added NaCl salt, can be modelled successfully in terms of a DLVO (Derjaguin–Landau–Verwey–Overbeek) theory [2]. Specifically, the experimental protein-rich/protein-poor coexistence lines can be reproduced qualitatively if the DLVO potential parameters are derived from the fit of physical quantities directly related to static or dynamic light scattering data of the solution. Prompted by the continuing interest in simple models of protein systems (see, for example, [3, 4]), here we continue the investigation initiated in paper I and show that the DLVO approach is able to reproduce other experimental features of the protein solution phase behaviour. In particular, we study the dependence of cloud and solubility temperatures on the salt molarity of the solution, for which a linear dependence on the logarithm of the ionic strength has been reported in experimental studies [5, 6].

We recall that the DLVO potential is written as the sum of a short-range attractive van der Waals term,

$$v_{\mathrm{HA}}(r) = -\frac{A_{\mathrm{H}}}{12}\left[\frac{\sigma^2}{r^2} + \frac{\sigma^2}{r^2-\sigma^2} + 2\ln\frac{r^2-\sigma^2}{r^2}\right], \tag{1}$$

and a Debye–Hückel-like contribution,

$$v_{\mathrm{DH}}(r) = \frac{1}{4\pi\epsilon_0\epsilon_{\mathrm{r}}}\left[\frac{z_{\mathrm{p}}e}{1+\chi_{\mathrm{DH}}\sigma/2}\right]^2 \frac{\exp[-\chi_{\mathrm{DH}}(r-\sigma)]}{r}, \tag{2}$$

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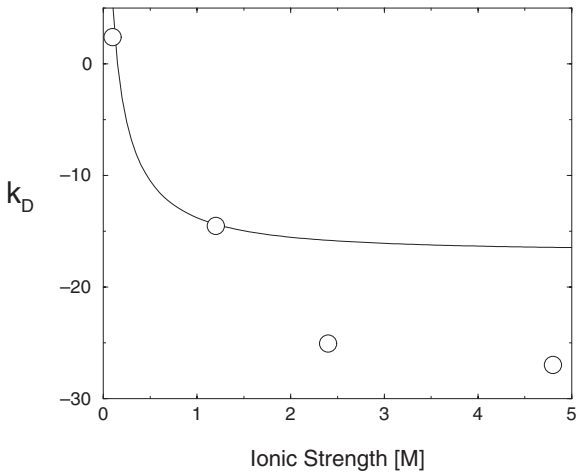

Figure 1. The slope of the collective diffusion coefficient versus the solution ionic strength in lysozyme solutions (pH = 4.5): circles—experimental points; curve—DLVO fit of k_D at $I_s = 1.2$ M. All data are taken from [9].

combined to form the interaction potential

$$v_{\mathrm{DLVO}}(r) = \begin{cases} \infty & r < \sigma + \delta \\ v_{\mathrm{HA}}(r) + v_{\mathrm{DH}}(r) & r \geqslant \sigma + \delta. \end{cases} \tag{3}$$

Here, σ represents the effective protein diameter, A_H is the Hamaker constant, $Q = z_p e$ is the net charge on the protein in electron units, ϵ_r and ϵ_0 are the (solution) relative and vacuum dielectric constants respectively, and χ_{DH} is the inverse Debye screening length. The Stern layer thickness δ, which is related to the intrinsic size of counter-ions that condense on the macro-molecule surface, is introduced in equation (3) to circumvent the singularity of the van der Waals term entering the DLVO theory [7, 8].

As discussed in detail in paper I, the parameters of the DLVO potential were fixed in experimental works [7, 9], in order to rationalize static and dynamic light scattering data on lysozyme in water and NaCl or $(NH_4)_2SO_4$ salt solutions. In particular, Beretta *et al* [9] reproduced the slope of the collective diffusion coefficient k_D as a function of the solution ionic strength I_s, in terms of the DLVO representation. In what follows, we shall use their theoretical curve crossing the single datum at ionic strength $I_s = 1.2$ M (reported in figure 1), obtained with parameters $A_H = 8.0\ k_B T$, $Q = 10$ e, $\delta = 0.164$ nm, and $\sigma = 3.6$ nm. In paper I, we used this specific set of parameters to determine the fluid–fluid coexistence lines, through Gibbs ensemble Monte Carlo (GEMC) simulations [10] at 0.51, 0.85 and 1.2 M ionic strengths; these salt molarities correspond to the experimental conditions for which Muschol and Rosenberger [5] determined the phase diagram of lysozyme solutions. For the benefit of the reader, in figure 2 we reproduce the GEMC fluid–fluid coexistence lines obtained in paper I.

Now, Broide and co-workers [6] have shown that the cloud temperature, T_{cloud}, of lysozyme solutions varies linearly as a function of the logarithm of ionic strength, at fixed protein concentration. Hinging on the simulation data reported in figure 2, we perform the same investigation within our DLVO model for three different protein concentrations. It appears from figure 3 that the theoretical T_{cloud} behaviour is fairly linear in $\log I_s$. As can be seen in figure 3(b), the critical temperature T_{cr} also exhibits the linear dependence observed experimentally by

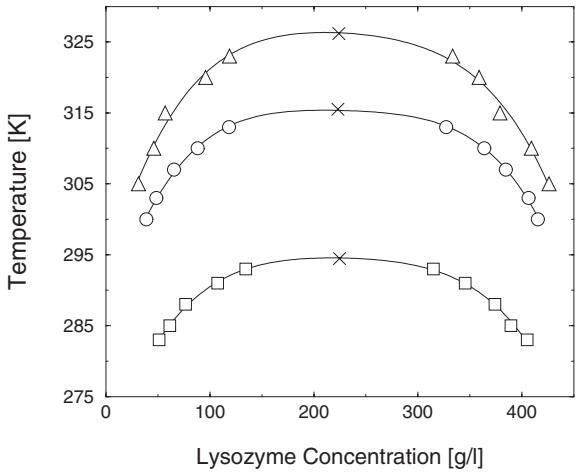

Figure 2. GEMC cloud points of lysozyme model solutions at ionic strengths $I_s = 0.51$ M (squares), 0.85 M (circles), and 1.2 M (triangles). The curves are interpolations of the simulation data with corresponding estimates for the critical points (crosses). All data are taken from paper I.

Muschol and Rosenberger [5]. The shift between the simulation and experimental curves reflects the systematic over-estimate of the true critical points, as already documented in paper I.

A similar linear dependence of the crystallization temperature, $T_{\rm crys}$, on the logarithm of the ionic strength has been also reported in [6]. In paper I, the solid–fluid equilibrium was determined through free-energy calculations of both the fluid and the solid phases. In particular, the free energy of the fluid phase was determined in the framework of the thermodynamically self-consistent hybrid mean-spherical approximation [11, 12], while the free energy of the solid phase was obtained through a standard first-order perturbation theory [13]. However, the latter was based on a reference fcc hard-sphere solid (not suited for representing the structure of the real protein crystal), resulting in a large over-estimate of the true solubility envelope. We then resort to a different expression for the chemical potential of the crystal, namely

$$\mu_{\rm crys} = \mu_0 - \tfrac{1}{2} n_{\rm s} \epsilon_{\rm DLVO} - k_{\rm B} T \ln[(\lambda - 1)^3]. \tag{4}$$

Equation (4) constitutes a simplified version of cell theory [14], applied extensively to systems characterized by short-range interactions [15]. This approach provides a direct link to the essential properties of protein crystals, namely the average number of contacts, $n_{\rm s}$, and the translational freedom along one axis, λ, of the protein inside the unit cell. In equation (4) μ_0 is the standard part of the chemical potential and $\epsilon_{\rm DLVO}$ is the minimum of $v_{\rm DLVO}(r)$, thus establishing a direct connection with our basic model. We assume in our calculations that $\lambda = 0.0225$ nm and $n_{\rm s} = 9$—values which lie approximately at the centre of the commonly accepted experimental ranges of $\lambda = 0.017$–0.03 nm [16] and $n_{\rm s} = 8$–10 [17]. The results for the crystallization temperatures $T_{\rm crys}$ are shown in figure 4, along with the experimental points of [5, 6]. The DLVO $T_{\rm crys}$ exhibits the correct linear trend as a function of the logarithm of ionic strength; the discrepancy with the results of Broide *et al* [6] can be explained by noting that the latter refer to a solution with a pH value that is different to that investigated in [9]. We also observe that the discrepancy with the only experimental datum available at approximately the same pH [5] barely exceeds 1%. Actually, the entire reproduction of the experimental solubility curves reported in paper I can be greatly improved by adopting the simple expression (4) to characterize the solid phase [18].

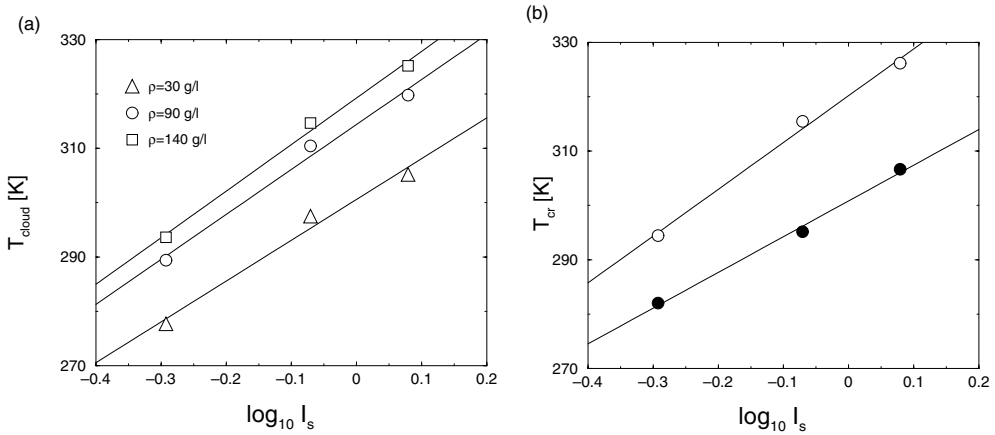

Figure 3. (a) The symbols represent GEMC cloud points as a function of the logarithm of ionic strength at different protein concentrations. (b) Experimental (full circles [5]) and simulation (open circles) critical points as a function of log I_s. The curves are linear fits of the simulation and experimental points.

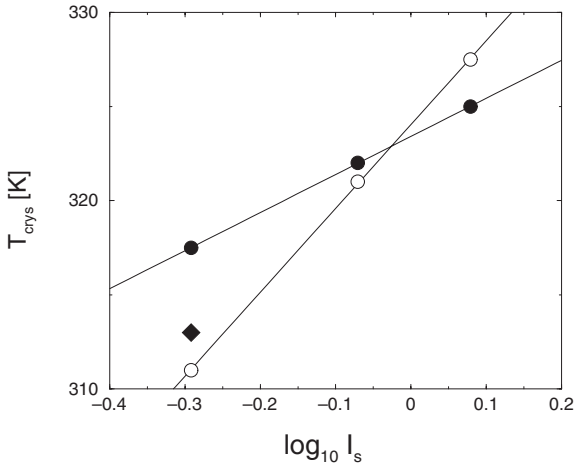

Figure 4. Solubility points versus the ionic strength for lysozyme solutions at the fixed protein concentration of $\rho = 90$ g l^{-1}: experimental data—full circles from [6] (pH = 7.8) and diamond from [5] (pH = 4.5); open circles—DLVO results.

In conclusion, the present results and those reported in paper I and [18] indicate that the DLVO model reproduces with overall accuracy the relevant features of the lysozyme solution phase diagram. We remark that the interactions between proteins in solution are generally much more complex than those emerging from a straightforward central potential description, as for the DLVO model adopted here. As we discussed extensively in paper I and [18], further tests and applications are thus needed in order to ascertain the overall capabilities and limitations of the proposed model.

References

[1] Pellicane G, Costa D and Caccamo C 2003 *J. Phys.: Condens. Matter* **15** 375
[2] Derjaguin B V and Landau L V 1941 *Acta Physicochim. USSR* **14** 633
Verwey E J W and Overbeek J T G 1948 *Theory of Stability of Lyophobic Colloids* (Amsterdam: Elsevier)

[3] Rosenbaum D F, Zamora P C and Zukoski C F 1996 *Phys. Rev. Lett.* **76** 150
[4] Piazza R 1999 *J. Cryst. Growth* **196** 415
[5] Muschol M and Rosenberger F 1997 *J. Chem. Phys.* **107** 1953
[6] Broide M, Tominc T M and Saxowsky M D 1996 *Phys. Rev.* E **53** 6325
[7] Muschol M and Rosenberger F 1995 *J. Chem. Phys.* **103** 10424
[8] Nägele G 1996 *Phys. Rep.* **272** 215
[9] Beretta S, Chirico G and Baldini G 2000 *Macromolecules* **33** 8663
[10] Panagiotopoulos A Z 1987 *Mol. Phys.* **61** 813
[11] Zerah G and Hansen J-P 1986 *J. Chem. Phys.* **84** 2336
[12] Caccamo C and Pellicane G 2002 *J. Chem. Phys.* **117** 5072
[13] Hansen J-P and McDonald I R 1986 *Theory of Simple Liquids* 2nd edn (London: Academic)
[14] Lennard-Jones J E and Devonshire A F 1937 *Proc. R. Soc.* A **163** 53
[15] Asherie N, Lomakin A and Benedek G B 1996 *Phys. Rev. Lett.* **77** 4832
Lomakin A, Asherie N and Benedek G B 1996 *J. Chem. Phys.* **104** 1646
Sear R P 1999 *J. Chem. Phys.* **111** 4800
Warren P B 2002 *J. Phys.: Condens. Matter* **14** 7617
[16] Doucet J and Benoit J 1987 *Nature* **325** 643
[17] Janin J and Rodier F 1995 *Proteins: Struct. Funct. Genet.* **23** 580
[18] Pellicane G, Costa D and Caccamo C 2003 submitted