Chemical Physics Letters 610–611 (2014) 405–410

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

Modification of interactions among proteins with the lowering of solution *p*D toward the isoelectric point in presence of different valent ions

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ARTICLE INFO

Article history:
Received 21 May 2014
In final form 16 July 2014
Available online 23 July 2014

ABSTRACT

Bovine serum albumins show a short-range attraction and a long-range electrostatic repulsion among them and these interactions are modified depending upon the solution p*D* and different dissolved counterions in the solution. Small angle neutron scattering study shows that for equal mono-valent (Na^+) and di-valent (Ni^{2+}) ion concentrations, both the attractive and repulsive interaction decreases with lowering the solution p*D* toward the protein isoelectric point, however for the tri-valent (Fe^{3+}) ion attractive interaction increases and repulsive interaction decreases. Interaction variation for the equal ionic strength of the three different valent ions becomes prominent as the p*D* decreases toward the isoelectric point.

1. Introduction

Protein–protein interactions and protein aggregation phenomena are very important field of research to explore the idea about some diseases like Alzheimer, Parkinson, etc. [1–3]. Understanding their interaction behaviors is also very important for obtaining protein crystals as the crystalline structure of protein strongly determines their biochemical role [1,4,5]. In solution, protein can be considered as charged, colloidal particle and hence Derjaguin-Landau-Verway-Overbeek (DLVO) potential [6] can be applied to obtain the protein behavior in solution [7,8]. Although the interaction nature of some globular proteins is explained only by considering short-range attraction [7,9,10] but both short-range attraction and long-range repulsion among the protein molecules in solution exists [8,11,12] that can explain the interaction behavior of proteins effectively. However, DLVO model cannot fully explain the rich behavior of proteins [1,8,13,14] due to the anisotropic property, irregular shape, inhomogeneous surface charge, etc., of proteins [1].

The DLVO model describes the interactions and phase behaviors of proteins under low ionic strength [7,8,15] considering the hard-sphere, electrostatic and van der Waals interactions in combination, although some nonspecific interactions such as hydration forces and hydrophobic interactions may also be included [8,15,16]. It has been observed that the actual physicochemical parameters strongly affect the protein–protein interactions and biochemical functions of proteins [8,12,15,17–19]. Ionic strength, temperature, *p*H, concentration of proteins, etc., modifies the nature of interactions among proteins in solution [20–23]. At higher ionic strength and for multivalent ions a reentrant condensation phenomenon of protein molecules is observed in solution [24]. A square-well type attractive interaction potential was considered to explain that. The intricate balance of attractive and repulsive forces among the protein molecules is responsible for the specific structures and properties of proteins. Small angle neutron scattering (SANS) study shows that a possible weak long-range attractive interaction between protein molecules may exist [25] in combination with the short-range attraction and intermediate-range electrostatic repulsion. One, two or three attractive or repulsive Yukawa form potentials were used to explain the nature of the SANS data [25–28]. Although different potential models have been used but a complete understanding about the effective interactions among protein molecules in solution with the variation of ions and solution *p*H is under study.

In this work, by using SANS technique we have shown the interaction variation of bovine serum albumin (BSA) protein with the variation of the solution *p*D in presence of mono-, di-, and tri-valent ions. Considering two Yukawa potential and by varying the relative strength of the attractive/repulsive interactions, the SANS profiles and hence the interaction nature is studied. It has been observed

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http://dx.doi.org/10.1016/j.cplett.2014.07.032
0009-2614/

that for the mono-valent (Na^+) and di-valent (Ni^{2+}) ions, both attractive and repulsive interactions decreases with lowering the solution p*D* toward the BSA isoelectric point (≈4.8), however for the tri-valent (Fe^{3+}) ion the attractive interaction increases and repulsive interaction decreases. Variation in the interaction nature for the equal ionic strength of the three different valent ions becomes prominent as the *p*D decreases toward the isoelectric point.

2. Experimental details

Bovine serum albumin (BSA) protein (catalog no. 05480) was purchased from Fluka. Samples for SANS experiments were prepared by dissolving a weighted amount of BSA in a buffer solution of D_2O. 10 wt% BSA was taken for all the measurements as final solution. Sample *p*D was adjusted at ≈7.0, 6.0 and 5.0 using phosphate buffer and hydrochloric acid solutions. The interaction among proteins is studied by dissolving mono-valent (NaCl), di-valent ($NiCl_2$) and tri-valent ($FeCl_3$) salts in the protein solutions. Salt concentrations were varied from 10 mM to 500 mM depending upon the valency of the counterions. Any precipitation of phosphate salt was not observed and among all these salts a faint turbidity was observed only for iron salt at higher ion concentration. *p*D of the sample solutions were measured before and after the BSA addition and the adjusted final *p*D is reported here. For getting equal ionic strength of the above mentioned chloride salts, the mono-valent and di-valent salt concentrations should be six and two times higher than the tri-valent salt concentration. Hence, one set of equal ionic strength solutions, which we have taken in our experiments is 120, 40 and 20 mM for mono-, di- and tri-valent salts respectively. For a particular salt concentration three different *p*D (≈7.0, 6.0 and 5.0) were used where the lowest *p*D was very close to the BSA isoelectric point. Small-angle neutron scattering experiments were carried out at Dhruva Reactor, Bhabha Atomic Research Centre, Trombay [29]. The mean incident wavelength was 5.2 Å with $\Delta\lambda/\lambda = 15\%$. The data were collected in the Q range of 0.017–0.35 $Å^{-1}$. The temperature for all the above measurements was kept fixed at 25 °C. The measured SANS data were corrected and normalized to a cross-sectional unit using standard procedures.

3. SANS analysis

SANS measures the scattered neutron intensity, $I(Q)$, where the scattering vector $Q = 4\pi/\lambda \sin\theta$, and 2θ is the scattering angle. For a system of monodisperse particles the scattering intensity is expressed by [30]

$$I(Q) = n_p V_p^2 (\rho_p - \rho_s)^2 P(Q) S(Q) + B \tag{1}$$

where n_p is the number density of protein molecules in solution, V_p is the volume of the single protein, ρ_p and ρ_s are, respectively, the scattering length densities of the protein molecules and the solvent. $P(Q)$ is the form factor of a protein molecule, i.e., the scattering from a single protein after orientation averaging. An ellipsoid form factor was used to model BSA molecule [31]. $S(Q)$ is the effective interparticle structure factor and B is the constant term representing incoherent background.

$$P(Q) \equiv \left\langle \left|F(Q)\right|^2 \right\rangle = \int_0^1 d\mu \left| \frac{3(\sin x - x\cos x)}{x^3} \right|^2 \tag{2}$$

$$x = Qb\left[\left(\frac{a}{b}\right)^2 \mu^2 + (1-\mu^2)\right]^{1/2}$$

where a and b are, respectively, the semimajor and semiminor axes of the ellipsoidal protein macromolecules and μ is the cosine of the angle between the directions of a and the wave vector transfer Q.

$S(Q)$ specifies the correlation between the centers of different particles and it is the Fourier transform of the radial distribution function $g(r)$ for the mass centers of the particles. $S(q)$ are calculated by solving the Ornstein-Zernike (OZ) equation with the mean spherical approximation (MSA) closure involving an effective pair potential $U(r)$, which can take different specific forms like hard-sphere, screened coulomb, Yukawa, square-well potentials, etc. [16,25,27,32–35]. The protein molecules are assumed to be a rigid equivalent sphere of diameter $\sigma = 2(ab^2)^{1/3}$ interacting through a screened Coulomb potential, $U_{SC}(r)$. In presence of different ions, the interaction between proteins modifies and the two-Yukawa potential (U_{TY}) model [25,27] is used to describe the short-range attraction and the relatively long-range repulsion. The two-Yukawa potential (U_{TY}) is expressed as [25,27]

$$U_{TY}(r) = \begin{cases} \infty & \text{for } r < 1 \\ -K_1 \dfrac{\exp[-Z_1(r-1)]}{r} - K_2 \dfrac{\exp[-Z_2(r-1)]}{r} & \text{for } r \geq 1 \end{cases} \tag{3}$$

where K_1 and K_2 are normalized by k_BT, k_B is the Boltzmann constant and T is the absolute temperature, and r is the interparticle distance normalized by the particle diameter σ. Positive values of K_1 and K_2 are for attractive interactions, whereas negative values are for the repulsive interactions. In our data analysis, K_1 takes positive values but K_2 takes negative values. The specific interaction range is proportional to $1/Z$. Regarding the fitting procedure we have calculated the structure factor considering the effective sphere obtained from the form factor. Form factor was constant initially for obtaining the others fitting parameters of reasonable values and after that it was free during fitting, although it was nearly unchanged after fitting. Smearing effect was not considered.

4. Results and discussion

SANS data obtained from the BSA solutions for three different solutions *p*D from ≈7.0 to ≈5.0 in presence of monovalent ions, Na^+, and the corresponding fitted curves are shown in Fig. 1. An oblate form factor $P(Q)$ of dimensions ≈9 Å × 39 Å × 39 Å together with specific structure factor $S(Q)$ were used to fit the SANS data. $S(Q)$ includes two-Yukawa potential model as stated before and the corresponding fitting parameters are shown in Table 1. The dimensions of the BSA molecule obtained from the form factor are relatively less in comparison with the values obtained by the other groups [36,37]. Although initially it was modeled as a prolate ellipsoid [37] of dimensions ≈70 Å × 20 Å × 20 Å, later it was considered as an oblate ellipsoid [38] of dimensions ≈12.5 Å × 42 Å × 42 Å as this oblate structure fits the data properly. The larger volumes are mainly due to the effects of scattering length density and the visibility of the hydration shell around the protein molecule [38,39]. The dimensions obtained from our study are little smaller that the reported values of the oblate spheroid [38]. Probably it depends upon the specificity of the BSA molecules used and also upon the experimental conditions. Another point that is related with the BSA dimensions is the value of the volume fraction which we kept constant during the fitting as 0.09 as we used 10 wt% BSA and the specific density of the BSA was taken as 1.2 gm/cm^3. Regarding $S(Q)$, only one Yukawa potential is not enough to fit the data as in the lower Q value there is a upturn in the scattering intensity. This is more pronounced for di- and tri-valent ions. Moreover, square well potential cannot fit all the data obtained in presence of di- and tri-valent ions. Only two-Yukawa potential model can fit all the data and is thus very much effective to explain the attractive and repulsive interaction nature depending upon the variations of physicochemical parameters. It has been obtained from the data fitting that with the lowering of the solution *p*D from ≈7.0 to ≈5.0, both the attractive and repulsive strength (K_1