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Research paper

Effect of ethanol on structures and interactions among globular proteins

Sarathi Kundu [a,*], V.K. Aswal [b], J. Kohlbrecher [c]

[a] Soft Nano Laboratory, Physical Sciences Division, Institute of Advanced Study in Science and Technology, Vigyan Path, Paschim Boragaon, Garchuk, Guwahati, Assam 781035, India
[b] Solid State Physics Division, Bhabha Atomic Research Centre, Mumbai 400085, India
[c] Laboratory for Neutron Scattering, Paul Scherrer Institute, CH-5232 PSI Villigen, Switzerland

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ABSTRACT

Structures and interactions among globular proteins BSA and lysozyme are explored by small angle neutron scattering (SANS) technique at pD ≈ 7.0 by varying ethanol concentration. Interaction behaviours are also obtained in presence of monovalent salt (NaCl). SANS analysis shows that for both lower and higher BSA concentrations and in presence of NaCl, combination of intermediate-range repulsion and weak long-range attraction is responsible for the effective interaction behaviours with the variation of ethanol concentration. For lysozyme, interaction nature is same as BSA in absence of NaCl but in presence of NaCl, fractal structure factor explains the interaction behaviours.

1. Introduction

Alcohol is a consumable biomolecule which crosses the biological membrane and can readily distribute throughout the body. Excessive consumption of alcohol disturbs numerous biological pathways causing damage to many organs such as the liver, pancreas, heart and immune system [1–4]. However, as a protein precipitant ethanol is used in plasma fractionation to produce life-saving biopharmaceuticals from pooled blood [5–7]. It is assumed that during the fractionation processes aqueous ethanol does not cause irreversible denaturation of plasma proteins. But at higher concentrations ethanol acts as a protein destabilizer and can cause protein denaturation [8–10]. Ethanol is also used as a disinfectant [11,12]. It disrupt membrane structures of enveloped viruses leading to virus inactivation [13,14]. Such effects are explained in terms of hydrophobic properties of ethanol [15]. However, ethanol can also inactivate non-enveloped viruses at higher concentrations than normally required for enveloped viruses [14]. Ethanol can denature proteins [10,16], often accompanied by transition in secondary structures [17,18] and reduces their solubilities [19,8] and also alters protein–protein interactions. Moreover, it is also evidenced that presence of excess amount of ethanol can form amyloid fibrils of proteins through the lateral association of protofilaments [20]. Like ethanol, different salts also have effects on protein structure and protein-protein interaction. In biology, presence of salts commonly introduces Hoffmeister effects which effectively show the salt dependent different phase formation or precipitation of proteins [21]. It has also been shown that addition of salt in protein solutions enhances attractive interaction between proteins and can show liquid-liquid phase separation and crystallization after a certain salt concentration [22–24].

Protein-protein interactions strongly depend upon the protein concentration, solution pH, dissolved ions in the solution, etc. [25–32]. All complex phenomena shown by the proteins in solutions highly depend upon the interaction behaviours between the protein molecules [25,33]. Phase separation, re-entrant condensation, crystallization, etc. usually occurs when the attractive interaction dominates over the repulsive interaction [28,33]. Actually, the intricate balance between the attractive and repulsive interactions is responsible for obtaining any specific phenomenon [28,33,34]. Although Derjaguin-Landau-Verwey-Overbeek (DLVO) model [25] are used to explain [30] the interaction behaviour of protein molecules in solutions at low ionic strength, however, it has been found that if effective interaction parameters are used then DLVO model works also at higher salt concentration [35,36]. Due to the complex anisotropic structure, irregular shape and surface charge inhomogeneity, DLVO model cannot fully explain the rich behaviours of proteins [37]. Small angle neutron scattering (SANS) study shows that in combination with the short-range attraction and intermediate-range electrostatic repulsion a possible weak long-range attractive interaction may exist between protein molecules [38]. One, two or three attractive or repulsive Yukawa form potentials were used to explain the SANS data [38–40]. In preferred con-

* Corresponding author.
E-mail address: sarathi.kundu@gmail.com (S. Kundu).

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ditions fractal aggregates may also form in protein solutions and the corresponding fractal dimensions represent the interconnectivity between the structure forming materials [41–43]. Although different potential models were used for explaining the protein-protein interactions but much more study is required for complete understanding of the interaction behaviours of protein molecules in solutions especially with the variation of solvent nature, dissolved ions and protein concentration.

In this article, SANS technique is used to study the interaction behaviours among Bovine Serum Albumin (BSA) and lysozyme protein molecules in aqueous solutions with the variation of ethanol concentration for a fixed protein and salt concentrations at pD ≈ 7.0. Using two-Yukawa form potential model the intermediate-range repulsive and weak long-range attractive interactions among the protein molecules are obtained and their relative strength with the variation of the ethanol concentration are extracted. For favored protein, ion and ethanol concentrations, fractal structures form and the corresponding fractal dimensions are extracted.

2. Experimental details

Bovine Serum Albumin (BSA) protein (catalog No. 05480) was purchased from Fluka. Lysozyme (Cat. No. 62970) was purchased from Sigma-Aldrich. Samples for SANS experiments were prepared by dissolving specific amount of BSA and lysozyme in phosphate buffer solutions ($pD \approx 7.0$) of D_2O so that finally both 1 wt% and 5 wt% protein solutions were prepared. The effect of ethanol was studied by varying the ethanol concentration up to 25 vol% in solutions. The interactions among proteins were also studied by dissolving monovalent (NaCl) salt in the protein solutions and for that the salt concentration was constant to 0.1M for BSA and lysozyme solutions. Small-angle neutron scattering experiments were performed on the SANS-I instrument at Swiss Spallation Neutron Source, SINQ, Paul Scherrer Institut, Switzerland [44]. The mean wavelength of the incident neutron beam (λ) was 8 Å with a wavelength resolution ($\Delta\lambda/\lambda$) of approximately 8%. The scattered neutrons were detected using a two-dimensional 96×96 cm^2 detector. The experiments were performed at two sample-to-detector distances of 2 and 8 m, respectively, to cover the data in the wave-vector-transfer Q range of 0.005–0.25 Å^{-1}. The measured SANS data were corrected and normalized to a cross-sectional unit using standard procedures.

3. SANS analysis

Small angle neutron scattering intensity, $I(Q)$, for a system of monodisperse particles is expressed by [45]

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

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. The scattering vector $Q = (4\pi/\lambda)\sin\theta$, where 2θ is the scattering angle. $P(Q)$ is the form factor of a protein molecule, i.e., the scattering from a single protein after orientation averaging. For BSA and lysozyme molecules, specific ellipsoid form factors are used [22,24,31]. $S(Q)$ is the effective inter-particle structure factor and B is the constant term representing incoherent background. $S(Q)$ is calculated using two-Yukawa potential (U_{TY}) with the mean spherical approximation (MSA). The protein molecules are assumed to be a rigid equivalent sphere of diameter $\sigma = 2(ab^2)^{1/3}$, where a and b are, respectively, the semimajor and semiminor axes of the ellipsoidal protein molecules. The two-Yukawa potential (U_{TY}) model [38,40] is used to describe the intermediate-range repulsion and the weak long-range attraction between the protein molecules. The two-Yukawa potential (U_{TY}) is expressed as [38,40]

$$U_{TY}(r) = \begin{cases} \infty & \text{for } r < 1 \\ -K_1 \frac{\exp[-Z_1(r-1)]}{r} - K_2 \frac{\exp[-Z_2(r-1)]}{r} & \text{for } r \geqslant 1 \end{cases} \quad (2)$$

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 repulsive interactions. The specific interaction range is proportional to $1/Z$.

The second virial coefficient (B_2) can be calculated from the interaction potential as [23]

$$B_2 = \frac{2\pi\sigma^3 N_A}{M^2} \int [1 - \exp(U_{TY}(r))] r^2 dr, \quad (3)$$

where N_A is Avogadro's number and M is the molecular weight of the protein.

$S(Q)$ takes fractal structure factor if preferred experimental conditions are achieved depending upon the salt and ethanol concentrations. For fractal structure, $S(Q)$ is expressed by mass fractal model as [46,47]

$$S(Q) = 1 + \frac{1}{(Q\sigma/2)^D} \frac{D\Gamma(D-1)}{[1 + (Q\xi)^{-2}]^{[(D-1)/2]}} \times \sin[(D-1) \times \tan^{-1}(Q\xi)], \quad (4)$$

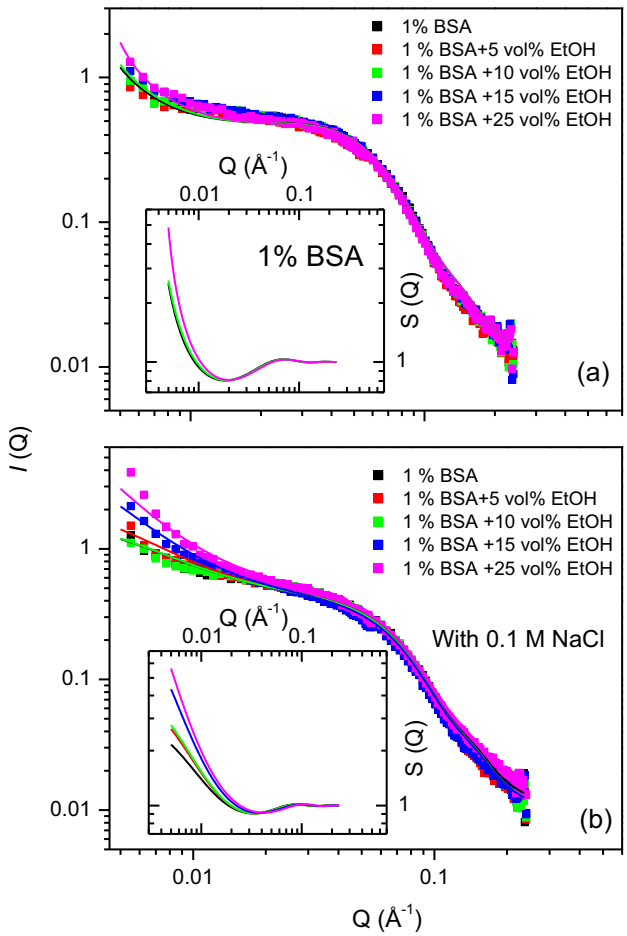

Fig. 1. SANS data (open circle) and fitted curves (solid line) for BSA protein (1.0 wt %) in aqueous solutions at $pD \approx 7.0$ with ethanol concentration variation (0–25 vol %) in (a) absence and (b) presence of NaCl (0.1 M). Insets: inter particle structure factor, $S(Q)$, extracted from the fitting using two-Yukawa potential model.