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Structure and interaction among protein and nanoparticle mixture in solution: Effect of temperature

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ABSTRACT

Structure and interaction among globular protein bovine serum albumin (BSA) and silica nanoparticle mixtures in solutions have been studied using small angle neutron scattering technique by varying the solution temperature. Our study shows that in absence of nanoparticles and up to 70 °C, an intermediate range repulsive and one long range attractive interaction potential between the proteins exist. Above that temperature, fractal structure forms. In presence of nanoparticles, fractal structures form even at room temperature by both the protein and nanoparticles. Fractal dimension increases with the increase of BSA concentration and solution temperature, and this temperature induced structural transition is irreversible.

1. Introduction

Studies on protein and nanoparticle mixture in solution are very important as proteins can adsorb on the nanoparticle surface or can form specific structures which are important for both basic and applied point of view. Protein-coated nanoparticles can show enhanced biocompatibility and can have potential applications in the field of drug delivery and biosensors [1,2]. There are numerous studies for identifying and improving the interaction of nanoparticles with proteins to enhance the properties of the protein –nanoparticle complexes [3,4]. The properties of the complex systems not only depend upon the nanoparticle and protein but also upon the various solution conditions like temperature, pH, ionic strength, etc . [5–8]. For oppositely charged protein and nanoparticle, like lysozyme and silica nanoparticle, strong adsorption of protein on nanoparticle occurs [5,7,8], however, for similarly charged protein and nanoparticle, like BSA and silica nanoparticle, no adsorption takes place which can lead to the depletion forces between the silica nanoparticle [9–11] and as a result, fractal aggregation takes place. Therefore, it is interesting to study the structures and interactions in the protein –nanoparticle complexes and to tune them for various potential applications.

In the absence of nanoparticle, globular proteins like BSA can form gel when heated as with heating the attractive interaction among protein increases [12–15]. Gel formation can occur even at room temperature if the heat treated protein molecules are added with salts [16,17]. Gel formation generally depends upon the different physicochemical parameters, which modifies the attractive and repulsive interactions among proteins and hence the structure and properties of gels [18–20]. Gelation of globular proteins has attracted much more attention because of its physical and industrial significance as they have low elasticity and high water-holding capacity and are therefore used immensely in food and drug related products. Like proteins, different colloidal dispersions also show gelation or glass transition which takes place from the fluid phase [21–23]. Microstructure analysis of protein gels shows the formation of closely packed fractal aggregates throughout the system [24–27]. The formation of fractal aggregates and the corresponding fractal dimensions represent the strength of the gels [20,28,29]. With increasing the fractal dimension the interconnectivity between the structure forming materials increases that enhances the strength of the gel. Small angle neutron scattering (SANS) technique is using to investigate the structure of such fractal aggregates in detail over a wide length scale and also to study the kinetics of the gelation [13,29–31]. In our previous study [27,30], gelation of BSA protein in presence of ions has already been reported. In those studies the effects of preheating temperature, ion concentration, types of ions, etc. have been explored. Although there are several studies on structures and properties of protein gels, and also on protein –nanoparticle complexes, but the effect of nanoparticle on protein gelation and the variation of nanoparticle aggregation with gel formation is not yet clear.

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In the present work, by using the SANS technique the structure and interactions of BSA protein in presence and absence of silica nanoparticles have been studied. BSA proteins are heated gradually to get the gel like structure and the corresponding interaction behaviours. The modifications of interaction and gelation have been studied in the presence of nanoparticles with the variation of solution temperature, and protein and nanoparticle concentrations. The structure, interactions and fractal dimensions obtained from the SANS study for different experimental conditions have been explored.

2. Experimental details

Bovine serum albumin (BSA) protein (catalogue no. 05480) was purchased from Fluka. Electrostatically stabilized colloidal suspension of Ludox HS40 silica nanoparticles (catalogue no. 420816) was purchased from Sigma –Aldrich. The nanoparticles as obtained have 40 wt% concentration and kept at pH ≈9.0–9.5 for stabilization. Samples for SANS experiments were prepared by dissolving a weighted amount of BSA in a buffer solution of D_2O. The use of D_2O as solvent instead of H_2O provides better contrast for hydrogenous protein in neutron experiments. Sample *p*D was adjusted at ≈7.0 using 20 mM phosphate buffer solution. 1, 2 and 5 wt% BSA were taken for the SANS measurements as final solutions. Silica nanoparticle concentration was fixed at 1 wt% for all the experiments. At *p*H ≈7.0, 1 wt% HS40 silica nanoparticles have been found to be quite stable [32,33] and no gelation was observed for a month time. Actually, silica nanoparticles are stabilized against gelation by causing the silica particles to become charged. This is done by addition of small amounts of alkali e.g. NaOH which reacts with the silica surface to produce the negative charge. Thus, at high *p*H, silica nanoparticles are stable because of high particle charge. As *p*H drops, particle charge decreases but sufficient hydroxyl ions remain to catalyze cross linking, and stability reaches a minimum at around *p*H ≈5. The silica nanoparticle are found to be quite stable at *p*H ≈7.0 and measured zeta potential of this system has a value of −15 mV. All the samples for SANS experiments were prepared freshly prior to the experiments. Mixtures of protein and nanoparticle were done by varying the BSA concentration keeping the nanoparticle concentration fixed. Mixtures of BSA and nanoparticles were done in both D_2O and H_2O buffer solutions at two different contrast conditions. The solution temperature was varied from 30 to 80 °C for both the pure BSA and nanoparticle mixed BSA solutions and SANS data were taken at five different solution temperatures, i.e., at 30, 60, 70, 75 and 80 °C. During heat treatment, samples were heated gradually (∼1 °C/min) to reach the final target temperature. For each sample, after heat treatment up to 80 °C, the solution was slowly cooled down at room temperature (≈30 °C) and then again SANS data was taken to see the temperature effect. Small-angle neutron scattering experiments were performed on the SANS-I instrument at Swiss Spallation Neutron Source, SINQ, Paul Scherrer Institut, Switzerland [31]. The mean wavelength of the incident neutron beam (λ) was 0.8 nm with a wavelength resolution (Δ λ/λ) 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 6 m, respectively, to cover the data in the scattering vector (Q) range of 0.06–2.4 nm^{-1}. 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 [34]

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

where n_p is the number density of particles in solution, V_p is the volume of the particle, ρ_p and ρ_s are, respectively, the scattering length densities of the particle and the solvent. $P(Q)$ is the form factor of a particle, i.e., the scattering from a single particle after orientation averaging. A spherical form factor for silica nanoparticles, $P_s(Q)$, and an ellipsoid form factor for BSA protein, $P_e(Q)$, were used [35]. $S(Q)$ is the effective inter-particle structure factor and B is the constant term representing incoherent background.

$$P_s(Q) = \left[\frac{3\left\{\sin(QR) - (QR)\cos(QR)\right\}}{(QR)^3}\right]^2$$
$$P_e(Q) = \int_0^1 d\mu \left[\frac{3(\sin\ x - x\cos\ x)}{x^3}\right]^2 \tag{2}$$
$$x = Qb[(a/b)^2\mu^2 + (1-\mu^2)]^{1/2}$$

where R is the radius of spherical nanoparticle, 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.

For a polydisperse particle system, $I(Q)$ is modified by the size distribution of particles as [36]

$$I(Q) = \int I(Q, R) f_s(R) dR + B \tag{3}$$

where $f_s(R)$ is the size distribution function. It is usually accounted for by log-normal distribution as given by

$$f(R) = \frac{1}{R\sigma\sqrt{2\pi}} \exp\left[-\frac{\ln(R/R_{med})^2}{2\sigma^2}\right] \tag{4}$$

where R_{med} and σ are the median value and standard deviation, respectively. The mean (R_m) and median values are related as $R_m = R_{med}\exp(\sigma^2/2)$.

$S(Q)$ specifies the correlation between the centres of different particles and it is the Fourier transform of the radial distribution function $g(r)$ for the mass centres 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 [35,37,38]. In the case of silica nanoparticles $S(Q)$ is considered unity whereas it has been modelled for protein because of their large number density (small effective size) than nanoparticles. BSA molecules are considered as a rigid equivalent sphere of diameter $2R = 2(ab^2)^{1/3}$. In the absence of nanoparticles in the heat treated BSA, i.e., when gel was not formed, two-Yukawa potential (U_{TY}) model [37,38] is used to describe the attractive and repulsive interactions between proteins. The two-Yukawa potential (U_{TY}) is expressed as [37,38]

$$U_{TY}(r) = \begin{cases} \infty & \text{for} \quad r < 1 \\ -K_1\dfrac{\exp[-Z_1(r-1)]}{r} - K_2\dfrac{\exp[-Z_2(r-1)]}{r} & \text{for} \quad r \geq 1 \end{cases} \tag{5}$$

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 $2R$. Positive values of K_1 and K_2 are for attractive interactions, whereas negative values are for the repulsive interactions. The specific interaction range is proportional to $1/Z$. $S(Q)$ takes the fractal structure when the protein becomes gel due to the heat treatment and also due to the