SANS Study of Interaction of Silica Nanoparticles with BSA Protein and their Resultant Structure

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Abstract. Small angle neutron scattering (SANS) has been carried out to study the interaction of anionic silica nanoparticles (88 Å) with globular protein Bovine Serum Albumin (BSA) (M.W. 66.4 kD) in aqueous solution. The measurements have been carried out on fixed concentration (1 wt %) of Ludox silica nanoparticles with varying concentration of BSA (0-5 wt %) at pH7. Results show that silica nanoparticles and BSA coexist as individual entities at low concentration of BSA where electrostatic repulsive interactions between them prevent their aggregation. However, as the concentration of BSA increases (≥ 0.5 wt %), it induces the attractive depletion interaction among nanoparticles leading to finally their aggregation at higher BSA concentration (2 wt %). The aggregates are found to be governed by the diffusion limited aggregation (DLA) morphology of fractal nature having fractal dimension about 2.4.

Keywords: Nanoparticle-protein interactions, fractal structure, particle aggregation, SANS.

PACS: 82.70.Dd, 61.43.-j, 64.75.Xc

INTRODUCTION

There are numerous areas where system of nanoparticles are of significant scientific and technological interest. Particularly, nanoparticles-proteins system over a great extent used in drug delivery, catalysis, biological imaging, biosensors, and bio fuel cell, to name but a few [1]. The intrinsic characteristic of nanoparticles (e.g. size, shape, charge etc.) and the forces (e.g. electrostatic, hydrogen bonding, steric forces etc.) at the bio-nano interface control the biological response of nanoparticles and can be tuned by varying different solution parameters such as ionic strength, pH, temperature and concentration as required for different applications [2-3]. The functional property of biomolecules may change when it come in contact to nanoparticles [4]. This in turn is also important for the stability of nanoparticles and their resultant structure. That's why understanding of the nanoparticles-biomolecules complex is crucial to the safe and beneficial use of nanoparticles. Colloidal dispersions of inorganic nanoparticles in matrix of proteins provide a model system to study the interaction of nanoparticles with biomolecules. Silica nanoparticles are one of the most

commonly used model inorganic nanoparticles for studying their interaction with different proteins as it is non-toxic and biocompatible as well less expensive and commercially available [5]. The globular protein BSA is known to be highly stable, available with high purity and soluble in water. In this study we have characterized the resultant structure of silica nanoparticles in presence of BSA using Small Angle Neutron Scattering (SANS). SANS with the possibility to vary contrast is a useful technique to study the structure and interaction in colloids.

EXPERIMENT

Spherical silica NPs (Ludox HS-40) and protein Bovine Serum Albumin (BSA) were purchased from Sigma-Aldrich and used as obtained. Samples for SANS experiments were prepared by dissolving weighted amount of nanoparticles and BSA in 20 mM phosphate buffer at pH 7 in D₂O. Samples for SANS experiments were prepared in D₂O instead of H₂O because of high contrast for hydrogenous sample in D₂O. Small angle neutron scattering experiments were performed at the SANS-I instrument at the Swiss spallation neutron source, SINQ, Paul Scherrer

Institute, Switzerland [6]. The mean wavelength of the incident neutron beam was 6 Å with the wavelength resolution of approximately 10%. The scattered neutrons were detected by using 96×96 cm² detector. The experiments were performed at two sample-to-detector distances of 2 and 8 m, respectively to cover the data in scattering vector ($Q = 4\pi\sin(\theta/2)/\lambda$, where θ is scattering angle) range of 0.006-0.25 Å⁻¹. All the measurements were carried out for fixed concentration (1 wt %) of silica nanoparticles and varying the concentration of BSA in the range 0.1 to 5 wt %. The freshly prepared samples were held in HELMA quartz cells having thickness 2 mm and temperature kept constant at 30 °C during the measurements. The data were corrected and normalized to absolute scale using standard procedure.

SANS ANALYSIS

In SANS experiment, the coherent differential scattering cross section per unit volume ($d\Sigma/d\Omega$) is measured as a function of scattering vector. In the case of monodispersed particles dispersed in a medium, it can be written as

$$\frac{d\Sigma}{d\Omega}(Q) = nV^2(\rho_p - \rho_s)^2 P(Q)S(Q) + B$$

where n is number density and V particle volume. ρ_p and ρ_s are scattering length densities of particle and solvent, respectively. $P(Q)$ is intraparticle structure factor giving information about shape and size of the particle whereas $S(Q)$ is interparticle structure factor provides information about interaction in the system. B is a constant term representing incoherent background. Data have been analyzed by considering appropriate models for $P(Q)$ and $S(Q)$. Fitted parameters were obtained by non-linear least square fitting of the experimental data using above equation.

RESULTS AND DISCUSSION

Fig.1 shows the scattering profiles of 1 wt% silica nanoparticles with varying concentration (0-5 wt %) of BSA protein. There are no significant changes in SANS data of 1 wt % silica nanoparticles on addition of BSA up to 0.2 wt % concentration and data of silica nanoparticles-BSA system overlap to that of pure silica nanoparticles. On further addition of BSA there is consistent build up in scattering at low as well at higher Q values. At higher BSA concentration (≥ 2 wt %), scattering at low Q shows saturation but

build up at higher Q remains with increasing BSA concentration.

FIGURE 1. Scattering profiles of 1 wt % silica nanoparticles with varying concentration (0-5 wt %) of BSA.

To understand the behaviour of scattering profiles in fig. 1, we compared (fig. 2) the scattering data of silica nanoparticles-BSA system for one of the BSA concentrations (1 wt%) to that of individual added scattering data of pure silica nanoparticles and BSA. It is clear from fig. 2 that measured data is matching to that of added data at higher Q and deviate in lower Q region. The matching of data at higher Q region arises because of dominance of scattering from BSA. Deviation in scattering profile in the low Q region is expected either due to change in interaction between nanoparticles and/or by the evolution of structure such as formation of aggregates of silica in presence of BSA [7].

FIGURE 2. Scattering data of 1wt% silica nanoparticles with 1wt % BSA along with the pure components. Inset shows the comparison of measured scattering data to that of added scattering profiles of individual components.

The fitting of SANS data in fig. 3 provides the interaction and structural information in silica nanoparticle-BSA system under going as a function of BSA concentration. Data in fig. 3 were fitted for three different BSA concentration regimes: (i) a low concentration regime where calculated scattering is sum of individual contributions from nanoparticles and free protein i.e. nanoparticles and protein remain as individual entities in solvent. In this regime, electrostatic repulsion between them prevents their aggregation since both components are similarly charged under the experimental conditions used. (ii) Data for BSA concentration (0.5-1wt %) are modeled by two Yukawa potential taking account of both repulsive and attractive interactions. The attractive depletion interaction similar to electrostatic repulsion is found to be a long-range. The magnitude of depletion over the repulsion increases with the BSA concentration. (iii) The high concentration regime of BSA (2-5 wt %) induces the aggregation of silica nanoparticles. It is believed to be as a result of non-adsorption of BSA induces the depletion force that causes the aggregation of nanoparticles at higher concentrations. The fractal aggregates are fitted coexisting with free protein. The fractal dimension of the aggregates is found to be about 2.4 in consistent with that of the diffusion limited aggregation (DLA) morphology of fractal nature [8].

FIGURE 3. Fitted SANS data of 1wt% silica nanoparticles with varying concentration (0-5 wt %) of BSA.

CONCLUSIONS

SANS has been used to study the interaction of silica nanoparticles with BSA. The electrostatic repulsive interaction prevents the adsorption of BSA on silica nanoparticles surface and hence silica nanoparticles

and BSA remain as individual entities. At higher concentrations, BSA induces depletion force that dominates over electrostatic repulsion leading to aggregation of silica nanoparticles. The resultant interaction is modeled by two Yukawa potential combining long range repulsion and long range attraction. The aggregates are characterized by DLA fractals having fractal dimension of 2.4.

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