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Small angle neutron scattering studies on protein denaturation induced by different methods

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Abstract. Small angle neutron scattering (SANS) has been used to study conformational changes in protein bovine serum albumin (BSA) as induced by varying temperature and in the presence of protein denaturating agents urea and surfactant. BSA has prolate ellipsoidal shape and is found to be stable up to 60°C above which it denaturates and subsequently leads to aggregation. The protein solution exhibits a fractal structure at temperatures above 64°C, with fractal dimension increasing with temperature. BSA protein is found to unfold in the presence of urea at concentrations greater than 4 M and acquires a random coil Gaussian chain conformation. The conformation of the unfolded protein in the presence of surfactant has been determined directly using contrast variation SANS measurements by contrast matching surfactant molecules. The protein acquires a random coil Gaussian conformation on unfolding with its radius of gyration increasing with increase in surfactant concentration

Keywords. Protein denaturation; gelation and unfolding of protein; small angle neutron scattering.

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1. Introduction

Protein denaturation depends upon the interplay between the different interactions among the residues responsible for its biological functionality. It is one of the most widely studied topics in molecular biology due to its widespread application in the industrial and scientific world. The denaturation process can be brought about by various means and conditions [1,2]. Each different route of denaturation has its own application and advantage in material processing and basic sciences. Along with different applications related with different denaturation processes, these methods have different mechanisms of denaturating the proteins. The conformation of such a denaturated protein molecule will depend upon the process of denaturation and the kind of denaturating medium. Scattering techniques correlate the denaturation with the conformational changes in the three-dimensional structure of the protein

[3]. In the present paper, we have used small angle neutron scattering (SANS) to probe conformational changes during the protein denaturation induced by temperature, urea and surfactant.

2. Experiment

BSA protein, urea and salts were purchased from Fluka. d-SDS surfactant was purchased from Cambridge isotope laboratory. The samples for SANS experiments were prepared by dissolving known amount of BSA, urea, d-SDS and additives in D_2O. The use of D_2O as solvent instead of H_2O provides better contrast for hydrogenous protein in neutron experiments. All the samples were prepared in acetate buffer solution at pH 5.5, which is close to the isoelectric pH of BSA (4.9) to minimize the interparticle interaction among protein molecules. Small angle neutron scattering experiments were performed on the SANS-I instrument at Swiss Spallation Neutron Source, SINQ, Paul Scherrer Institut, Switzerland [4]. The mean wavelength of the incident neutron beam was 6 Å with a wavelength resolution of approximately 10%. The experiments were performed in the wave vector transfer Q range of 0.006 to 0.25 $Å^{-1}$.

3. Results and discussion

In small angle neutron scattering, one measures the coherent differential scattering cross-section per unit volume $[d\Sigma/d\Omega(Q)]$ as a function of Q. For a system of monodispersed interacting protein macromolecules, $d\Sigma/d\Omega(Q)$ can be expressed as [5]

$$\frac{d\Sigma}{d\Omega}(Q) = N_p V_p^2 (\rho_p - \rho_s)^2 \left[\langle F(Q)^2\rangle + \langle F(Q)\rangle^2 (S_p(Q) - 1)\right] + B, \qquad (1)$$

where N_p is the protein's number density and V_p is the volume of the protein macromolecule. ρ_p and ρ_s are the scattering length density of the protein and the solvent, respectively. $F(Q)$ is the single particle form factor and $S_p(Q)$ is the interparticle structure factor, which can be approximated to unity in case of low concentration of protein and at high salt concentration. B is a constant term that represents the incoherent scattering background, which is mainly due to hydrogen in the sample

SANS data for 1 wt% BSA at increasing temperatures are shown in figure 1. It is found that a temperature as high as 60°C has no prominent effect on the structure of protein macromolecule. The protein structure remains stable up to this temperature having prolate ellipsoidal shape with semi-major and semi-minor axes $a = 70.2 \pm 5.1$ and $b = c = 22.2 \pm 0.8$ Å, respectively, which are similar to values reported earlier [6]. SANS data beyond 60°C show build-up of scattering cross-section in the low-Q region with increase in temperature, which is an indication of protein denaturation and subsequent aggregation at higher temperatures due to exposure of hydrophobic groups of protein to water [7]. For temperatures above 65°C, SANS data show a linear region of scattering cross-section on log–log scale for

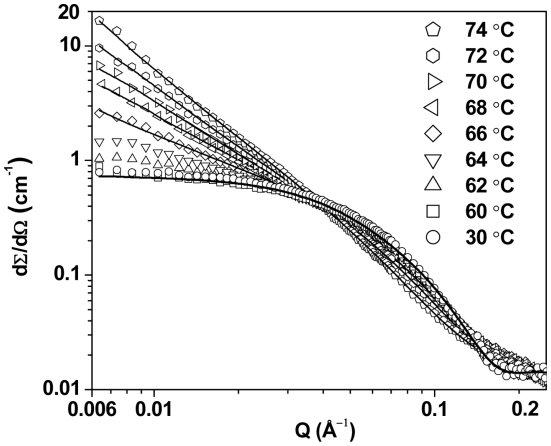

Figure 1. SANS data for 1 wt% BSA at various temperatures.

Table 1. Fitted parameters of SANS analysis for 1 wt% BSA at various temperatures.

Temperature (°C)	Radius R_{p} (Å)	Fractal dimension D
66	24.1 ± 0.9	1.12 ± 0.03
68	25.5 ± 1.0	1.46 ± 0.04
70	25.5 ± 1.0	1.66 ± 0.06
72	25.5 ± 1.0	1.88 ± 0.10
74	25.5 ± 1.0	2.01 ± 0.12

values of $Q < 0.07$ Å^{-1}. This represents the fractal structure of a gel which consists of a network kind of arrangement of protein aggregates in the system. The slope of the scattering data gives the value of the fractal dimension D of the network. The cut-offs of the linear range of the data at low- and high-Q values are, respectively, related to the extent of the aggregated network and the size of the building block of the network. The low Q cut-off is not observed in figure 1, where the lowest Q value $Q_{\mathrm{min}} = 0.006$ Å^{-1}. This means that the aggregated network has a size ξ larger than $2\pi/Q_{\mathrm{min}}$ (i.e. 900 Å). The fitted parameters using a fractal model are given in table 1.

SANS for 1 wt% BSA in the presence of varying concentrations of urea is shown in figure 2. The data show decrease in scattering cross-section with increasing urea concentration. It is observed that up to 4 M concentration of urea, there is a continuous decrease in the scattering cross-section. However, the functionality of the scattering pattern does not change and the structure of protein is found to remain same. The decrease in scattering cross-section can be explained in terms of decrease in contrast $(\rho_{\mathrm{p}} - \rho_{\mathrm{s}})^2$ as the scattering length density of deuterated solvent (ρ_{s}) decreases on addition of hydrogenous urea to protein solution [8]. There is a change in the functionality of the scattering profile beyond 4 M urea and it is interpreted in terms of unfolding of the protein. It is believed that the solvation

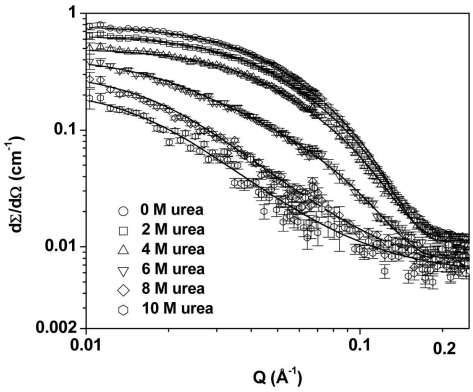

Figure 2. SANS data for 1 wt% BSA as a function of increasing concentrations of urea.

Table 2. Fitted parameters of SANS analysis for 1 wt% BSA in the presence of urea.

Urea (M)	R_g (Å)
6	55.0 ± 2.9
8	84.0 ± 4.1
10	93.5 ± 6.4

of hydrophobic portions of the protein at high urea concentrations leads to the unfolding of a protein. The unfolded protein is fitted as random Gaussian coil. The fitted parameters are given in table 2.

It is known that the binding of ionic surfactant molecules to the protein disrupts the native structure of the protein [7]. At high surfactant concentration, the formation of micelle-like clusters in the complex leads to protein unfolding which has been modelled as fractal structure representing a necklace model of micelle-like clusters randomly distributed along the polypeptide chain. The conformational changes of protein in protein–surfactant complex have been examined by contrast variation SANS by contrast matching the surfactant. The surfactant is contrast-matched using deuterated SDS (d-SDS) and the sample is prepared in D_2O. Figure 3 show the SANS data for 1 wt% BSA in the presence of varying d-SDS concentrations. SANS data show a decrease in the scattering cross-section with increase in d-SDS concentrations. It is observed that up to 20 mM concentration of urea, there is a continuous decrease in the scattering cross-section. However, the functionality of the scattering pattern does not change significantly and the structure of protein is found to remain prolate ellipsoidal with increase in its size dimension along the semi-major axis. The decrease in scattering cross-section can be explained as the contrast of protein decreases as the size of complex increases on addition of surfactant. There is a change in the functionality of the scattering profile beyond 20 mM surfactant concentration and it is interpreted in terms of unfolding of the

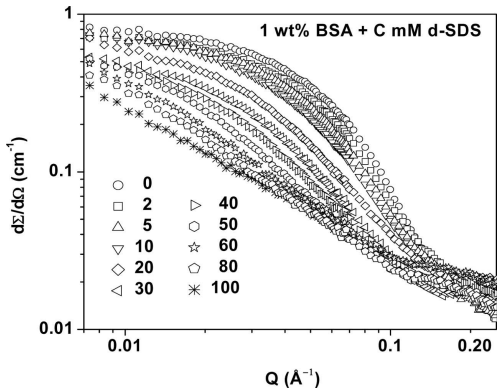

Figure 3. SANS data for 1 wt% BSA as a function of increasing concentrations of d-SDS.

Table 3. Fitted parameters of SANS analysis for 1 wt% BSA in the presence of d-SDS.

	Folded structure		Unfolded structure
d-SDS (mM)	Semi-major axis a (Å)	Semi-minor axis $b = c$ (Å)	Radius of gyration R_g (Å)
0	71.0 ± 5.1	22.2 ± 0.8	–
10	88.0 ± 6.4	23.0 ± 0.9	–
20	94.0 ± 6.7	25.8 ± 1.1	–
40	–	–	60.1 ± 1.6
50	–	–	70.3 ± 1.8
60	–	–	85.5 ± 2.4
80	–	–	102.3 ± 4.6

protein. It is believed that the formation of micelle-like clusters along the hydrophobic portions of the protein molecule leads to protein unfolding. The unfolded protein is fitted as random Gaussian coil. The fitted parameters are given in table 3.

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