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Small angle neutron scattering studies on the interaction of cationic surfactants with bovine serum albumin

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Abstract. The structure of the protein–surfactant complex of bovine serum albumin (BSA) and cationic surfactants has been studied by small angle neutron scattering. At low concentrations, the CTAB monomers are observed to bind to the protein leading to an increase in its size. On the other hand at high concentrations, surfactant molecules aggregate along the unfolded polypeptide chain of the protein resulting in the formation of a fractal structure representing a necklace model of micelle-like clusters randomly distributed along the polypeptide chain. The fractal dimension as well as the size and number of micelles attached to the complex have been determined.

Keywords. Small angle neutron scattering; protein solution; cationic surfactants.

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1. Introduction

The protein–surfactant interactions have been a subject of extensive research ever since the surfactants were found to be the denaturants of water soluble proteins [1,2]. The formations of complexes between anionic surfactants and proteins in aqueous solutions have been well established [3]. Cationic surfactants have been found to interact with the proteins to a lesser extent compared to anionics mainly as a consequence of smaller relevance of electrostatic interactions at the pH's of interest [4]. However, the binding isotherms of both these type of surfactants have been found to be similar [5]. The interaction of surfactants with proteins is of importance in a wide variety of industrial, biological, pharmaceutical and cosmetic systems. The mechanism of unfolding of proteins on addition of the surfactant has been studied by several techniques such as circular dichroism (CD), nuclear magnetic resonance (NMR), microcalorimetry, light scattering and small angle scattering. Among the several proposed models of the protein–surfactant complexes such as rod-like, flexible helix model and the necklace model, the necklace model is the most accepted for understanding the interaction of these two components in their complex formation [6].

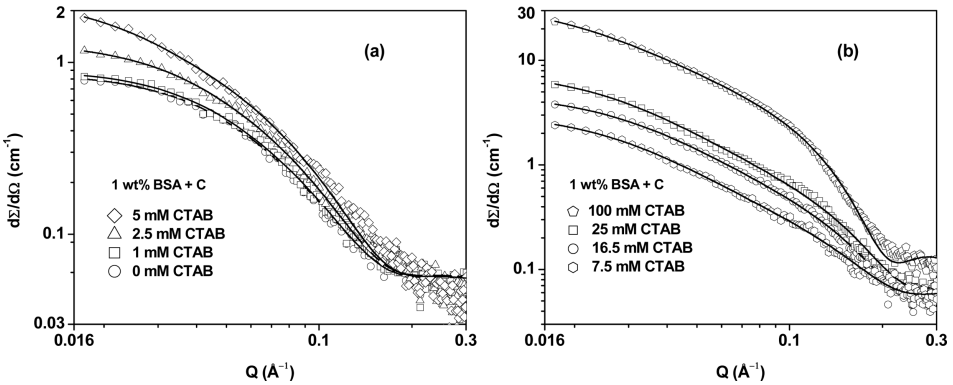

Figure 1. Fitted parameters of SANS analysis for 1 wt% BSA on addition of (**a**) 0 to 5 mM CTAB surfactant and (**b**) 7.5 to 100 mM CTAB surfactant.

This paper deals with the effect of varying the hydrocarbon chain length of the cationic surfactant on the protein–surfactant complex as studied by SANS. The investigations were carried out on BSA.

2. Experimental

BSA protein and the surfactants CTAB, TTAB and DTAB were purchased from Sigma Chemicals, USA. Samples of SANS experiments were prepared by dissolving a known amount of BSA and the surfactants in a phosphate buffer solution (pH = 7) of D_2O. Small angle neutron scattering experiments were performed on the SANS instrument at Dhruva reactor, BARC, Mumbai [7]. The mean wavelength of the incident neutron beam was 5.2 Å with a wavelength resolution of ∼15%. The data were collected in the wave vector transfer magnitude Q range of 0.018 to 0.35 $Å^{-1}$. The measurements were made for 1 wt% BSA in the presence of varying concentrations of the surfactants. The measured SANS data were collected and normalized to a cross-sectional unit using standard procedures [8].

3. Results and discussion

SANS data for 1 wt% BSA of varying CTAB concentrations in a buffer solution of pH 7.0 are shown in figures 1a and 1b. Based on the features of the scattering profiles, the data can be grouped in two different sets. The first set corresponds to proteins at low surfactant concentrations (0 to 5 mM) where the scattering data show behaviour similar to that of the pure protein solution (figure 1a). In this data set, the overall cross-section increases with increase in surfactant concentration. SANS data in this system of monodisperse interacting protein macromolecules can be expressed in terms of prolate ellipsoidal shape, the equation for which is [9]

$$\frac{\mathrm{d}\Sigma}{\mathrm{d}\Omega}Q = N_\mathrm{p}V_\mathrm{p}^2(\rho_\mathrm{p} - \rho_\mathrm{s})^2\left\{\langle F^2(Q)\rangle + \langle F(Q)\rangle^2[S(Q) - 1]\right\} + B, \qquad (1)$$

Table 1. Fitted parameters of SANS analysis for 1 wt% BSA in the presence of CTAB surfactant.

System	Semi-major axis (Å)	Semi-minor axis (Å)
0	70.7 ± 5.1	21.1 ± 0.7
1	71.9 ± 5.2	21.2 ± 0.7
2.5	85.7 ± 6.4	21.1 ± 0.7
5	138.4 ± 10.1	21.0 ± 0.7

where N_{p} is the number densities of the protein, ρ_{p} and ρ_{s} are the scattering length densities of the protein and the solvent and V_{p} is the volume of the protein molecule. $F(Q)$ is the single particle form factor and $S_{\mathrm{p}}(Q)$ is the interparticle structure factor. B is a constant term that represents the incoherent scattering background, which is mainly due to hydrogen in the sample. The corresponding table 1 gives the calculated structural parameters for SANS data in figure 1a. It is found that in pure protein solution, the protein macromolecules have a prolate ellipsoidal shape with semi-major and semi-minor axes as 70.7 and 21.0 Å, respectively. This result is in good agreement with those reported earlier [10]. In the first data set, the protein macromolecules maintain their folded structure on addition of surfactant. It is believed that individual surfactant molecules bind to the protein at low surfactant concentration. Table 1 shows changes in the dimensions of the protein on increasing binding of surfactant molecules as a function of the surfactant concentration. The semi-minor axis remains almost the same while the semi-major axis increases with increasing surfactant concentration. It is believed that six protein sub-domains forming BSA remain intact but separate from each other, leading to an elongation of the protein on addition of the surfactant [11].

The features of the scattering profile in the second set at higher surfactant concentrations ($\geq$7.5 mM) are very similar to those of the first data set. One of the interesting features is the linearity of the scattering profiles on log–log scale in the intermediate Q range with the linearity increasing with surfactant concentration. This is an indication of fractal structure by the protein–surfactant complex. The build-up of scattering cross-section in the higher cut-off of the linearity of the scattering data suggests the formation of surfactant aggregates and the lower cut-off corresponds to the overall size of the protein–surfactant complex. Based on the linearity of the scattering data on the log–log scale, the second data set in figure 1b has been explained using fractal structure of protein–surfactant complex by the following equation [1,12]

$$\frac{\mathrm{d}\Sigma}{\mathrm{d}\Omega}(Q) = \frac{N_1}{N_{\mathrm{p}}N}(b_{\mathrm{m}} - V_{\mathrm{m}}\rho_{\mathrm{s}})^2 P(Q) S_{\mathrm{f}}(Q) + B, \tag{2}$$

where N_1 is the number density of the total surfactant in solution, V_{m} the volume of the micelle and N the number of such micelles attached to a polypeptide chain, b_{m} represents the scattering length of the surfactant molecule. $P(Q)$ denotes the normalized interparticle structure factor ($\langle F^2(Q)\rangle$) of a single micelle-like cluster, $S_{\mathrm{f}}(Q)$ has been calculated using fractal structure for the necklace model of protein–surfactant complex [13].

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

CTAB (M)	Fractal dimension	Micellar radius (Å)	Correlation length ξ (Å)	Number of micelles in the complex
7.5	2.23 ± 0.15	17.0 ± 0.6	34.3 ± 1.5	2
16.5	2.16 ± 0.14	17.0 ± 0.6	32.0 ± 1.4	2
25	2.23 ± 0.15	17.0 ± 0.6	37.4 ± 1.8	2
100	1.74 ± 0.04	20.5 ± 0.7	62.0 ± 4.2	6

Table 3. Fitted parameters of SANS analysis for 1 wt% BSA in the presence of DTAB/TTAB.

System	Fractal dimension	Micellar radius (Å)	ξ (Å)	Number of micelles in the complex
25 mM DTAB	2.21 ± 0.15	13.7 ± 0.4	42.5 ± 2.0	3
25 mM TTAB	2.26 ± 0.15	14.6 ± 0.5	36.0 ± 1.8	2

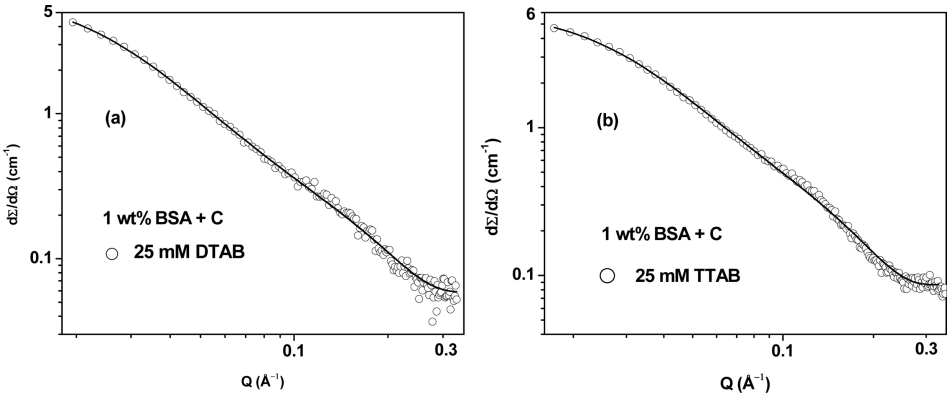

Figure 2. Fitted parameters of SANS analysis for 1 wt% BSA on addition of (**a**) DTAB surfactant and (**b**) TTAB surfactant.

The fractal structure of the complex on the basis of the necklace model considers micelle-like clusters of the surfactant formed along the unfolded polypeptide chain of the protein. The slope of the scattering data on log–log scale gives the value of the fractal dimension D of the complex. The cut-off of the linear range of the data at low- and high-Q values are, respectively, related to the formation of the extent of the complex and the size of the individual micelles in the complex. The fitted parameters of the analysis are given in table 2. It is found that the fractal dimension decreases and shows a relatively similar behaviour up to 25 mM of surfactant concentration. There is significant decrease in fractal dimension corresponding to increase in correlation length. Also the size of the micelle-like clusters and the number of such clusters do not change till the surfactant concentration

reaches 25 mM while an increase in the size as well as number is observed at a surfactant concentration of 100 mM.

SANS studies on interaction of BSA with cationic micelles having different chain length to that of CTAB were also performed. Figures 2a and 2b show SANS data on 1 wt% BSA in the presence 25 mM of DTAB and TTAB concentrations. It is found that both TTAB and DTAB show behaviour similar to that of CTAB surfactant at higher concentration. The SANS data profile shows a linear region in the intermediate Q range indicating fractal formation. The fitted parameters of the analysis are given in table 3. It is observed that DTAB has a slightly higher unfolding efficiency as the fractal dimension is observed to be lesser and overall correlation length (ξ), that is the measure of extent of unfolding of the polypeptide chain, bigger than that in the case of TTAB.

To conclude, SANS measurements show that the binding of ionic surfactant to protein disrupts the native structure of the protein. At low surfactant concentration an increase in the dimension of the ellipsoidal protein is observed. A fractal structure at higher concentration suggests the formation of micelle-like clusters in the protein–surfactant complex.

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