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SANS STUDIES ON THE BOVINE SERUM ALBUMIN DENATURATION IN THE PRESENCE OF SDS

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ABSTRACT

SANS STUDIES ON THE BOVINE SERUM ALBUMIN DENATURATION IN THE PRESENCE OF SDS. The effect of the presence of sodium dodecyl sulfate (SDS) on the denaturation of bovine serum albumin (BSA) has been studied using 36m small-angle neutron scattering (SANS) BATAN spectrometer (SMARTer). The neutron scattering data reduction used the Graphical Reduction and Analysis SANS Program (GRASP) software, and the fitting process used the IGOR SANS Analysis software. The denaturation process was identified by observing the changes BSA globular structure. The experimental results showed the addition of SDS at low concentrations (2mM, 5mM, 10mM) into BSA solution at pH 7 do not cause a significant change in the size of the BSA globular structure. The SANS scattering profile of BSA fitted with the triaxial ellipsoid model, a simple shape approach for protein globular structure. The fitting result showed the semi-axis B for BSA in the addition of 2mM, 5mM, 10mM SDS were 33.8Å, 33.8Å, and 37.8Å, respectively. While the semi-axis A and semi-axis C were constant for those three variations at 14.6Å and 32.2Å, respectively. In higher addition of SDS, the globular structure of BSA unfolded into flexible cylinder structure with the radius of 14.4Å and length of 83.5Å. The denaturation of BSA was clearly showed by the addition of 40mM SDS. The structure of BSA in this condition fitted to fractal structure with fractal dimension of 1.1, the block radius of 16.7Å and the correlation length of 42.5ÅThese results indicated that the addition of SDS at low concentrations has not caused the denaturation of BSA. Meanwhile, the addition of SDS at high concentrations made BSA to unfold that lead to the denaturation of BSA. In this study,

Keywords: BSA, SDS, Denaturation, SANS

ABSTRAK

STUDI PENGARUH PENAMBAHAN SDS PADA PROSES DENATURASI BOVINE SERUM ALBUMIN (BSA) MENGGUNAKAN TEKNIK SANS. Telah dilakukan studi pengaruh penambahan sodium dodecyl sulfate (SDS) pada proses denaturasi bovine serum albumin (BSA) menggunakan 36m small-angle neutron scattering (SANS) BATAN spectrometer (SMARTer). Material penelitian terdiri dari 10 wt% BSA pada PH 7, konsentrasi SDS 2 mM, 5 mM, 10 mM, 20 mM, and 40 mM. Proses reduksi data hamburan neutron menggunakan software Graphical Reduction and Analysis SANS Program (GRASP), dan proses fitting menggunakan software IGOR SANS Analysis. Proses denaturasi diidentifikasi dengan mengamati perubahan struktur BSA. Hasil eksperimen menunjukkan bahwa penambahan SDS pada konsentrasi rendah (2 mM, 5 mM, 10 mM) tidak menyebabkan perubahan yang signifikan pada ukuran molekul BSA. Hal tersebut dibuktikan dengan profil hamburan yang hampir serupa dengan struktur globular BSA sesuai dengan model triaxial ellipsoid dimana nilai radius terukur untuk masing – masing sampel tidak memiliki perbedaan yang

besar, yaitu Semi-axis A (nilai terkecil) dan Semi-axis C (nilai terbesar) konstan untuk tiga variasi data yaitu 14,6Å dan 32,2Å, Semi-axis B berturut - turut 33,8Å, 33,8Å, dan 37,8Å. Pada konsentrasi yang lebih tinggi (20 mM) SDS, struktur globular BSA mengalami unfolding menjadi bentuk flexible cylinder dengan radius sebesar 14.4Å dan panjang 83.5Å. Proses denaturasi BSA ditunjukkan pada penambahan SDS 40 mM, hal ini dibuktikan dengan perubahan struktur BSA menjadi fractal, dengan radius blok 16.7Å, panjang korelasi 42.5Å dan dimensi fractal sebesar 1,1. Berdasarkan penelitian, dapat disimpulkan bahwa SDS pada konsentrasi rendah belum menyebabkan proses denaturasi BSA. Penambahan SDS dengan konsentrasi tinggi menyebabkan BSA mengalami unfolding yang menyebabkan denaturasi protein BSA.

Kata kunci: BSA, SDS, Denaturasi, SANS

INTRODUCTION

The interaction between protein and surfactant has a significant role in material research in the field of food, washing, and pharmaceutical industries. Protein-surfactant interaction has applied to food allergen labeling and food emulsifier [1][2]. The ability of the surfactant to bind protein is important to develop the detergent material for enhancing the ability of the detergent product to get away organic dirt[3]. In pharmacy, a surfactant-based drug delivery device needs the information of the interaction between protein and surfactant during their loading administration and distribution in body fluids[4]. The stability and conformational change of protein as an impact of interacting with the surfactant influences the efficacy of the drug delivery device. The protein unfolding to denaturation as a common effect of protein-surfactant interaction has beneficial applications on the pharmaceutical products based on surface absorption and protein solubilization. It is well-known that one of the important factor in protein denaturation is the denaturant concentration[5].

Bovine serum albumin (BSA) is a globular protein naturally found in cow's blood or milk with a molecular weight of 66.5 kDa and its estimated dimensions are 80 × 80 × 30 Å[6]. The primary structure of BSA is composed of a single polypeptide chain containing 582 amino acid residues, while the secondary structure contains 50 - 68% alpha-helices and 16-18% beta-folds. The tertiary structure of BSA is composed of 3 homologous domains that have angles to each other and the stability of the BSA tertiary structure is supported by a system of covalent interactions in globule proteins and disulfide covalent bonds[7]. BSA is quite easy to obtain, ease of purification, low cost, and has unusual ligand-binding properties made BSA a good model to study the protein surfactant interaction. Sodium dodecyl sulfate (SDS) is an anionic surfactant that is known to have good binding ability to protein compared to several other surfactant molecules such as guanidinium chloride or urea[8].

Previous studies have investigated the interaction between BSA and SDS using fluorescence spectroscopy, conductivity, circular dichroism (CD) spectroscopy, atomic force microscopy, molecular docking computational simulation [5], and surface tension, to understand the intrinsic properties behavior of BSA regarding SDS interaction. The conformational change of BSA as SDS addition has been revealed by small-angle X-ray scattering (SAXS) [3], small-angle neutron scattering (SANS), and dynamic light scattering (DLS) techniques[9]. In general, those methods can show the denaturation process of BSA proteins from breaking the bond, partially unfolding to fully denaturation. However, only small angle scattering technique, both SANS and SAXS, that can give the information of the BSA shape and size in solution. Small angle scattering is a powerful tool to study protein structure in solution [10]–[12]. Furthermore, as the best of our knowledge, the study of the conformational change due to the denaturation of a high concentration BSA as the addition of SDS have not been reported yet.

Therefore, in this work, the conformational change of BSA in high concentration during its denaturation process due to SDS addition was revealed by SANS technique. The SDS concentration was set from below to above SDS critical micellar concentration (CMC). All the effect of SDS addition will be revealed using SANS that will give information of BSA conformational change during denaturation.

EXPERIMENTAL METHOD

Material and Sample Preparation

BSA powder, SDS, and D_2O powder purchased from Sigma Aldrich, and the buffer reagent purchased from Merck used without any further purification. BSA was dissolved in buffer at pH 7 up to a concentration of

10 wt%. The buffer solution was made in D_2O, to reach a good contrast between BSA-SDS and their surroundings. The other benefit of using D_2O is the low incoherent background regarding the SANS experiment. The CMC of SDS was reported at 8.3mM. Therefore, the SDS concentration was set from below (2 mM and 5 mM) to above SDS critical micellar concentration (CMC) i.e 10 mM, 20 mM, and 40 mM, to better understand the interaction between BSA and SDS.

SANS Measurement

SANS measurements were carried out at the BATAN neutron scattering laboratory, Serpong, Indonesia using 36m small-angle neutron scattering BATAN Spectrometer (SMARTer)[13]. The wavelength of the neutron used is 3.74 Å. Neutron scattering is detected using a 2D-PSD (2- Dimensional Positive Sensitive Detector) detector with a diameter of 640 x 640 mm divided into 128 x 128 channels with a resolution of 5 mm. The sample and detector distances are 2 m and 6 m to cover the momentum transfer Q range 0.01 - 0.25 $Å^{-1}$. During the experiment, the samples were inserted into a 2 mm quartz cell.

SANS Data Analysis

The small angle scattering data was obtained from the scattered radiation particle after interact-ing with the sample. The scattering position depend to their momentum transfer q= 4 *sin / in which related to the position of each scattering body of the particle in the sample. Thus, the overall data contain information about shape, size and distribu-tion of particle in their environment. Therefore, small angle scattering technique was widely ap-plied to study protein in solution [14]-[16].

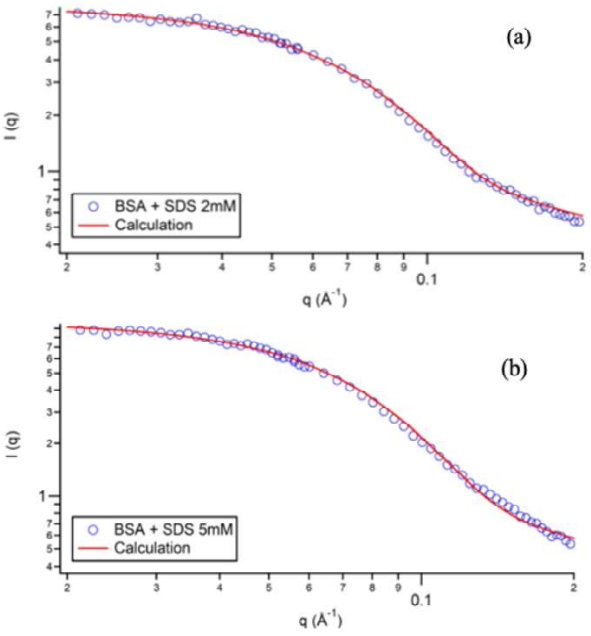

Figure 1. SANS data for 10 wt% BSA with low SDS concentration (a) 2 mM, (b) 5 mM fitted with triaxial model fitting calculation.

The neutron scattering data reduction used the Graphical Reduction and Analysis SANS Program (GRASP) software[17], and the fitting process used the IGOR SANS Analysis software[18]. The soft-ware has advantages, such as any number of SANS data sets can be analyzed at the same time, and documents for each processing step and analysis model, as well as detailed examples and test data, are included.

The triaxial ellipsoid model, the flexible cylinder model, and the fractal structure model were applied from model available in SANS NIST Igor Analysis without modification.

RESULT AND DISCUSSION

The neutron scattering profile can be grouped into two different sets according to increasing SDS concentrations. The first data set corresponds to proteins at low surfactant concentrations below CMC (Critical Micelle Concentration). SDS as a surfactant will form micelle after reach a certain concentration, called CMC. The CMC of SDS was reported at 8.6mM[19].

In this study, the SANS scattering data of BSA in addition of SDS below CMC fitted with the triaxial ellipsoid model, a simple shape approach for protein globular structure. It is previously reported that the initial interaction of SDS and protein is the binding of SDS molecule to the loop and the b-sheet part [20]. The addition of 2mM SDS led to invasion of SDS molecule to the loop of BSA and it seemed that the further addition of 5mM SDS still happened in the loop part as BSA do not have b-sheet content. In this state, the interaction between SDS molecules and BSA was dominated by hydrophobic interaction as the SDS was anionic surfactant and BSA were in above of their pI which made BSA has more negative group. Additionally, the considered low concentration of SDS into high concentration of BSA might be the reason of the unsignificant effect to BSA conformation.

The second data set corresponds to proteins at surfactant concentrations above CMC. Starting from the SDS concentration of 10 mM, the SANS data showed that the binding of SDS to BSA made the globular

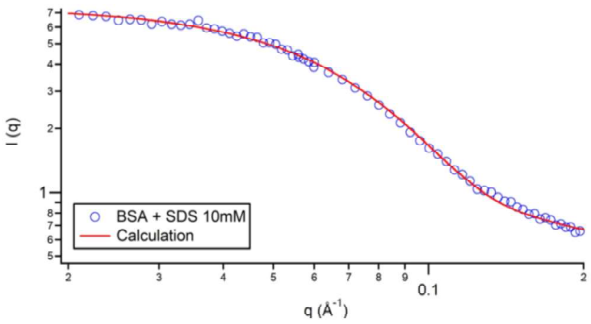

Figure 2. SANS data for 10 wt% BSA with SDS concentration 10 mM.

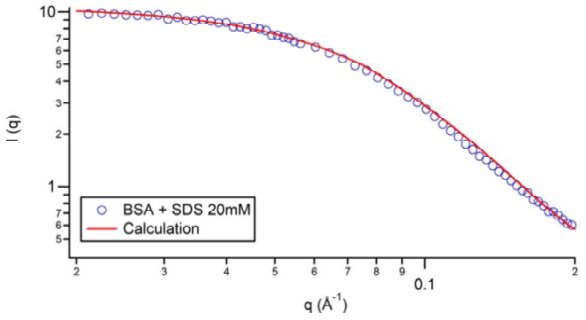

Figure 3. Neutron scattering profile fitted with a flexible cylinder model for BSA 10 wt% in the presence of SDS 20 mM.

structure of BSA loose and elongated. The experimental results showed a slight increase in the size of the semi-axis B radius in the addition of 5mM to 10mM SDS from 33.8Å to 37.8Å respectively. Figure 2 shows the SANS scattering profile of 10 wt% BSA in the presence of SDS 10 mM. Table 1 shows the change in protein radius with increasing surfactant concentration.

The anionic surfactant like SDS interact with protein mainly in the loop and β-sheet part of the protein. The addition of SDS allow the hydrocarbon tails of the SDS surfactant to bind to the hydrophobic patch of BSA protein via hydrophobic attraction, whereas the main group of anionic surfactants can bind to the protein cationic groups via electrostatic attraction[9]. The addition of 10mM SDS, which is above CMC, made more invasion of SDS molecules in the loop via hydrophobic or electrostatic attraction and then started alter the BSA conformation [3]. Those interactions disrupt the secondary structure of BSA that led to a conformational change of BSA's tertiary structure.

In the higher addition of SDS at 20 mM into the BSA solution, there was an increase in the hydrsophobic interaction of the hydrophobic SDS chain with the hydrophobic BSA backbone and the electrostatic attraction of the SDS head and BSA polar group. These interactions might occur in the α-helix part which caused the internal structure of the protein to break up. Since BSA mostly consist of α-helix, the alteration of the helical part have greater effect to the tertiary structure of BSA. Therefore, a complete unfolding of the BSA occured and the BSA elongation process continued that led the globular protein turn into a kind of flexible cylinder. Figure 3 shows the neutron scattering profile fitted with a flexible cylinder model, suggesting that the globular structure of BSA unfolded into a flexible cylinder structure with a radius of 14.4Å and a length of 83.5Å.

Table 1. Fitted parameters of SANS data analysis of BSA 10wt% and SDS low concentration with a triaxial ellipsoid model.

SDS concentration (mM)	2	5	10
Semi-axis A [smallest](Å)	14.6	14.6	14.6
Semi-axis B (Å)	33.8	33.8	37.8
Semi-axis C [largest](Å)	32.2	32.2	32.2

The denaturation of BSA was clearly showed by the addition of 40mM SDS. The effect of further addition of high concentration of SDS after they completely unfolded, was the micellization around the protein backbone. The interaction between anionic head of SDS to the cationic group of BSA still remain while the SDS molecules were forming micelle [20]. Those SDS micelles formed bead-like along the BSA chain. Therefore, the shape of the BSA changed from a globular to a fractal form. The SANS data of BSA in this condition fitted to a fractal structure with a fractal dimension of 1.1, the block radius of 16.7Å, and the correlation length of 42.5Å. The block radius of this fractal referred to the SDS micellar shape and size. Fractal dimension of 1.1 depicted the fractal growth in one dimension. Those informations, confirmed the formation of SDS-BSA complex with bead necklace-like form.

The results of this research agreed with other previous studies with different techniques, for example, Y. Ding et.al [8] reported from the FF-TEM data that BSA has loosened their globular structure at low concentration of SDS. The further expand of the BSA occured in the high concentration of SDS. Furthermore,

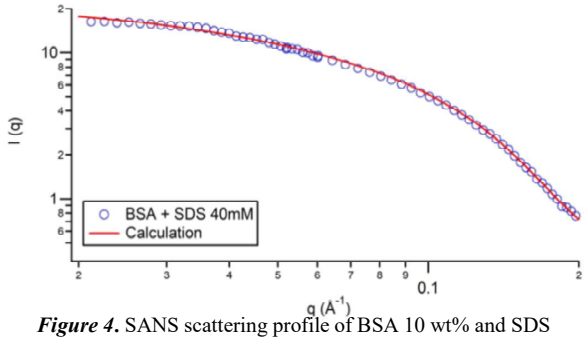

Figure 4. SANS scattering profile of BSA 10 wt% and SDS 40 mM fitted with fractal structure model.

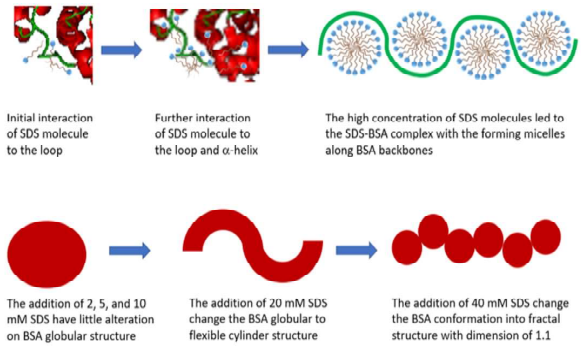

Figure 5. The ilustration of BSA denaturation in addition of SDS.

it was reported that BSA has changed from a spherical shape to a rod-like shape.

On the other hand, B. Shweitzer et.al [9] reported that the BSA formed complexed with the SDS in aqueous buffered (sodium acetate) solution. In that studythe SAXS data showed that the SDS formed micelle-like aggregates along the denatured BSA chain.

The mechanism of BSA denaturation based on other reports [3], [20] in addition of SDS and the suitability with the SANS data analysis was summarized in Figure 5.

CONCLUSION

The experimental results indicated that the addition of SDS at low concentrations has not caused the denaturation of BSA, but the binding of SDS to BSA makes globular structures loose and elongated. Meanwhile, the addition of SDS at high concentrations made BSA to unfold that lead to the denaturation of BSA. According to the SANS data analysis, the addition of SDS to SBA caused changes in protein shape and size, from the globular structure of BSA, then unfolded into a flexible cylinder structure and fitted to the fractal structure.

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