Bovine serum albumin refolding at acid solution by small angle neutron scattering

A Patriati, N Suparno, and E G R Putra

1 Neutron Scattering Laboratory, National Nuclear Energy Agency of Indonesia (BATAN), Kawasan Puspiptek Serpong, Tangerang 15314, Indonesia
2 Polytechnic Institute of Nuclear Technology, National Nuclear Energy Agency of Indonesia (BATAN), P.O.Box 6101/YKKB, Sleman, Yogyakarta 55281, Indonesia

*Email: arum@batan.go.id

Abstract. A series of study of the conformational change of bovine serum albumin (BSA) induced by pH and additive has been conducted by small angle scattering (SANS) technique. The unfolding process occur by increasing the solvent acidity of the buffer. The previous work from SANS scattering profile shows that BSA unfolded into a cylinder-like structure at pH 2. In this work, the role of sorbitol in refolding process of BSA in acid solution was studied. Sorbitol, at concentrations from 0 to 3 M, led to the progressive restoration of BSA globular structure. The SANS scattering data show the conformational change from cylinder-like structure of pure BSA at pH 2 undergo to ellipsoid-like structure in the addition of 3M sorbitol. In the absence, 1M and 2M sorbitol, the SANS scattering profile of BSA fitted to flexible cylinder model. Meanwhile in the present of 3M sorbitol, it fitted to triaxial ellipsoid model. These results are confirmed with ab initio low-resolution shape calculation model analysis using GNOM and DAMMIN obtaining the three-dimensional structure model.

1. Introduction

Protein play important role in biological process of many organisms. The stability and function of protein are sensitive to their environment. Some changes in protein environment including temperature, pH and ionic strength has great effect on protein stability

To the best of our knowledge, no report is available on the three dimensional of protein conformational change after refolding by osmolytes. The intrinsic structure such as helical and sheet content of the refolded protein has given important insight into this issue. However, those intrinsic value did not give comprehensive information on the complete refolding process. Here, we report the conformational
change on bovine serum albumin (BSA) as a model of protein refolding by osmolytes using small angle neutron scattering (SANS).

Serum albumin is the most abundant protein in the circulatory system. The crystal structure of the serum albumin is reported as a heart-shaped, divided into three similar structural domains, each subdivided into two subdomains[12]. Serum albumin has ability to undergo a conformational change by decreasing their environmental pH value in the range of pH 7 to 2[13]. This ability makes serum albumin as ideal model to study unfolding and refolding protein.

The small angle neutron scattering technique has been widely used for revealing three-dimensional structure of protein[4,14,15]. The advantage of this technique is relied on its ability to do the observation in solution. In situ experiment with many different environmental conditions, such as additives or ionic strength, are possible to be done by SANS[16–18].

2. Materials and Methods

2.1. Materials and sample preparation

The BSA powder (A0281), sorbitol (S1876) and D$_2$O 99.8% (151882,) were purchased from the Sigma Chemical Company. All the buffer reagents were purchased from Merck (Pro Analysis) and were used without further purification.

Buffer solution of pH 2 was made in D$_2$O. The BSA powder was then dissolved in buffer solution. The BSA solution were centrifugated with filter to obtain the monomer solution of BSA. The concentration of BSA after centrifugation is measured by UV spectrometer in 280nm. The concentration of the sample was set as 12 mg/ml for each sample.

Certain amount of sorbitol was added into BSA solution at pH 2. The concentration of sorbitol was varied for 1M, 2M, and 3M.

2.2. SANS measurement

The measurements have been performed on the 36 m SANS spectrometer, Neutron Scattering Laboratory BATAN, Indonesia[19]. BSA with sorbitol sample solution was placed in quartz cell with 2 mm inner thickness. For every measurement, the sample was exposed to neutron beam with wavelength of 4.9 Å at room temperature. To cover the momentum transfer 0.02<q<0.35 Å, three configuration sample-detector distances were set at 1.3, 4, and 6 m. The exposure time for each sample-detector distance was 6, 8 and 8 hours, respectively. The buffer as background and cadmium for electronic noise reduction were also exposed by neutron beam in every configuration setting with the same exposure time of the sample. The sample data were subtracted over scattering background from the buffer solution and cadmium using GRASP data reduction program[20].

2.3. Data analysis

The SANS scattering data represent the elastic neutron scattering, occurred as the inreaction of neutron with nuclei of the sample. Therefore, in this scattering data contain the size and the shape of the sample in dimension of 1 to 100 nm. The scattering neutron at certain angle was transformed into momentum transfer, q. Momentum transfer was defined as q=4π sin θ/λ, where 2θ is scattering angle and λ is neutron wavelength.

The SANS scattering data for protein solution as a monodisperse system, is expressed as [21]

\[ I(q) = n(\rho_m - \rho_s)^2V^2P(q)S(q) \]  

where n denotes the number density of scatterers / particles, ρm and ρs are the scattering length densities of the protein molecules and the solvent, respectively. The term (ρm-ρs) is called contrast factor. V is the volume of a protein molecule. P(q) is the intra-particle structure factor and depends on the shape and size of the particles. S(q) is the inter-particle structure factor and is defined by the inter-particle distance and the particle interaction. For dilute solution S(q) ~ 1, so the analysis can be conducted to measure the
size and shape of the sample. The corrected data was then analyzed by Igor NIST data analysis program [22].

For ab initio reconstruction methods the data were further analyzed with ATSAS package program [23]. In this analysis, several programs were used to determine the intra-particle distance distribution function in real space by GNOM [24], to reconstruct low-resolution structure models using DAMMIF [25] which is a development of DAMMIN [26] that run faster.

3. Result and Discussion

It is known that the BSA has undergone unfolded in low pH value. The previous work on revealing confirmation of BSA at pH 2 using SANS technique, has gained a flexible cylinder structure of BSA with contour length, Kuhn length and diameter are 26 nm, 3.6 nm, and 2 nm respectively [SANS data not shown]. The illustration of flexible cylinder model used in this analysis is shown in figure 1. The structure of BSA consists of six subdomain which is constructed by structured α-helix [12]. The flexible cylinder model can be analogue as the unfolding of the BSA subdomain.

![Figure 1. The flexible cylinder model of SANS data analysis [22].](image1)

In figure 2, SANS scattering data of BSA at pH 2 with sorbitol 1M show the effect of the addition of sorbitol into BSA conformation. The SANS data analysis resulted the flexible cylinder structure of BSA with some change on its dimension. The contour length of BSA decrease to 24 nm while its Kuhn length gets longer to 4 nm, and its diameter keep at 2 nm. The decreasing of contour length indicated that sorbitol has restructing effect to BSA conformation that leads to BSA refolding. This result is coincide with the other report on BSA refolding at low pH with sorbitol addition [8].

![Figure 2. SANS scattering data of BSA at pH 2 with 1M sorbitol addition.](image2)

The refolding of BSA at pH 2 continued as the concentration of sorbitol added to the solution increase up to 2M. SANS data analysis, in figure 3, gained the shorter contour length into 15 nm, while the Kuhn length increase up to 5.7 nm with unchanged diameter.
Figure 3. SANS scattering data of BSA at pH 2 with 2M sorbitol addition.

At high concentration of sorbitol, the SANS data analysis, shown in figure 4, was fitted to the triaxial ellipsoid model. This indicated that the addition of 3M sorbitol made BSA refold and gain its globular structure. It is known that BSA in neutral pH has globular structure [27]. The triaxial ellipsoid of BSA with 3M sorbitol has dimension of 3 nm, 2 nm, and 16 nm for three axes of ellipsoid form.

Figure 4. SANS scattering data of BSA at pH 2 with 3M sorbitol addition.

Those SANS data clearly show the effect of sorbitol in the BSA refolding at acid solution. Sorbitol is a polyol that can bind with water molecule via several hydrogen bonding. The interaction between sorbitol and water will decrease the protein solvation by water. This leads to the decreasing of protonation to BSA in acidic solution. As the unfolding BSA was occurred by protonation, the decreasing of water solvation into BSA, make BSA restore their secondary native structure.

However, from the SANS data analysis, the dimension of globular BSA at pH 2 with 3M sorbitol is different to the BSA native structure in neutral pH. The three-dimensional of BSA in pH 2 with sorbitol were shown in figure 5.

In figure 5, the addition of 1M, 2M and 3M sorbitol has successfully refolded the unfolding BSA in acid solution. It is reported that BSA at pH 2 lost 40% of its α-helix content and the addition of 1M sorbitol reported to restore its α-helix up to nearly 80% from the initial at neutral pH [8]. The model of three-dimensional structure of BSA at pH 2 coincides with those result. However, the α-helix reformation as sorbitol addition did not make BSA gained their tertiary structure. The three-dimensional of BSA at pH 2 with 3M sorbitol addition are different with the BSA native structure in neutral pH. The tertiary structure of protein did not only drive by its α-helix content but also the interaction of every amino acid with the solvent. The three-dimensional model of BSA in pH 2 with 3M sorbitol, show an open-heart shape with two arms. This result indicated that in the addition of 3M sorbitol, it did not fully restructure the BSA especially in domain-II and left the domain I and domain III did not close each other.
Figure 5. The three-dimensional structure of BSA obtained by SANS data modelling.

Sorbitol is not only interact with water to minimize the interaction of protein with their solvent but also interact with the hydrophilic group of protein by electrostatic interaction [28]. The polar interaction of sorbitol with several hydrophilic amino acid may affect the tertiary structure of a protein. Therefore, restructuring of α-helix of a protein by sorbitol addition did not automatically reform the tertiary structure of a protein [29]. This explained the SANS data scattering result of refolding BSA at pH 2 by sorbitol addition that cannot gained the heart-shape structure of BSA in neutral pH.

4. Conclusion
Sorbitol addition in solvent can propose the refolding of BSA at pH 2. The addition up to 3M sorbitol did not restore the conformation of BSA in acid solution into its native structure. The interaction of sorbitol not only with water but also with BSA itself make the tertiary structure of the refolding BSA did not gain its initial native form of BSA.

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