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Investigation of the topological shape of bovine serum albumin in solution by small-angle x-ray scattering at Beijing synchrotron radiation facility*

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This paper reports that at a newly constructed small-angle x-ray scattering station of Beijing Synchrotron Radiation Facility, the topological shape of ligand-free bovine serum albumin in solution has been investigated. An appropriate scattering curve is obtained and the calculated value of the gyration radius is 31.2Å±0.25 Å (1Å=0.1 nm) which is coincident with other ones' results. It finds that the low-resolution structure models obtained by making use of *ab initio* reconstruction methods are fitting the crystal structure of human serum albumin very well. All of these results perform the potential of the beamline to apply to structural biology studies. The characteristics, the defects, and the improving measures of the station in future are also discussed.

Keywords: small-angle x-ray scattering, *ab initio* methods, bovine serum albumin, Beijing synchrotron radiation facility

PACC: 6110, 6110F, 3620, 2920

1. Introduction

Over the past decade, tremendous amounts of high resolution biological macromolecules structural information have been obtained by using x-ray crystallography and nuclear magnetic resonance (NMR). However, some disadvantages are more and more obvious about these two methods. Firstly, because of the requirement of perfect crystals for the former method and the low molecular mass requirement of the later one, a significant fraction of proteins cannot be analysed by using these two methods. Secondly, a question often arises with crystallography that whether the macromolecular structures under physiological conditions in solution are preserved in the crystal environment because the interactions between the protein's subunits are considered to be of the same order of magnitude as those between neighbouring molecules in the crystal determined by the crystal packing forces.[1] Thirdly, in the studying of non-equilibrium dynamics of proteins (e.g. oligomerization, (dis)assembly or (un)folding processes), near physiological environment or huge molecular mass is required to determine both of these two methods which have crucial defects.

Small-angle x-ray scattering (SAXS) and small-angle neutron scattering (SANS)[2] are considered as a solution in recent years for their advantages which can provide the size and low resolution three-dimensional structural information of macromolecules in solution with molecular masses ranging from a few kilo Daltons to several million Daltons.[3] Despite the low resolution methods, these techniques allow one to study the structure of native particles in near physiological conditions and to analyse structural changes in response to variations in external conditions. Hence, more and more groups with traditional rotating-anode x-ray sources, synchrotron radiation sources or neutron sources are concerning with SAXS and SANS, and the number of published structural biology papers with these methods is keeping increase in recently years.[4] As to Beijing Synchrotron Radiation Facility (BSRF), though a SAXS & x-ray diffraction (XRD) beamline named 4B9A which can support both SAXS method and XRD method was constructed in 1990,[5−8] merely a few applications of biological macromolecules have been done,[9] restricted by the intensity and instabil-

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ity of the incident x-ray. Companying with a new wiggler insertion device installed at BSRF recently, we constructed a new SAXS beamline named 1W2A with special structure and instruments to improve the situation. All of them were accomplished in Jan. of 2007 and started to receive the users' experimental applications.

In this paper, we describe our beamline's main structure and characteristics, and then present the investigation of the topological shape of the ligand-free bovine serum albumin (BSA) to inspect the beamline's capacity of application to structural biology studies. Low-resolution three-dimensional structure models were obtained by making use of *ab initio* reconstruction methods. As the crystal structure of BSA has not been obtained yet, the crystal structure of human serum albumin (HSA),[10] which has a high homology up to 76% with BSA, was listed to compare the results.

2. Experimental setup

The instrument layout of the 1W2A SAXS beamline can be viewed in Fig.1. The more detailed description of the beamline design can be seen elsewhere.[11] For briefness, using two horizontal focusing triangle-bending crystals of Si(111) and Si(220) respectively, we can capture and monochromatize 2-mrad synchrotron radiation x-ray on horizontal direction of a 7-periods wiggler at a wavelength of 1.54 Å or 0.96 Å (1 Å=0.1 nm), chosen by the users' demands. Then a vertically focusing plane mirror with Rh-coated, which can provide a reflectivity of 90% for 1.54Å wavelength, is used to focus the beam to the detector position, with a focused beam size of 1.5 mm by 0.3 mm in the horizontal and vertical directions. A set of sample holders has been designed to deal with different samples such as films, solutions and solid samples. The scattered x-ray intensity is recorded by a Marccd detector and a wide-angle gas detector, it is a function of the modulus of the scattering vector $s=4\pi\sin\theta/\lambda$, where 2θ is the scattering angle. The distance between the CCD detector and the sample holder can be changed at 1 m, 1.6 m or 5m to satisfy different signals resolution requirements. For restricting the beam divergence, three sets of collimator slits are arranged along the beamline, and the exposure time is controlled by an electronic shutter which can support 0.1 s for shortest time. An ion chamber was settled behind the sample holder to record the intensity of the scattering signals for correcting the absorption of the samples.

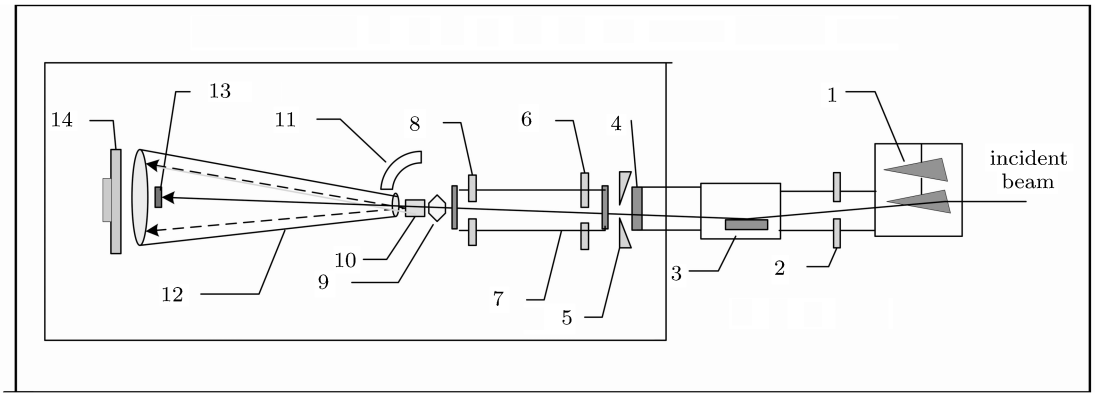


Fig.1. Schematic view of the 1W2A beamline at BSRF: (1) horizontal focusing triangle-bending crystal monochromator; (2), (6) and (8) collimator slits; (3) vertically focusing mirror; (4) Be windows; (5) electronic shutter; (7) and (12) vacuum pipe; (9) sample holder; (10) ion chamber; (11) wide-angle gas detector; (13) beam stop; (14) CCD detector.

We summarize the main characteristics of the 1W2A station in Table 1, and those of X33 at the EMBL-Hamburg[12] which has done excellent works in SAXS, are also listed for comparison. As Table 1 listed, one can note that the 1W2A has a nearly similar performance with X33, except a larger divergence at 1W2A. The wavelength of 0.943 Å can be used to support smaller size range of the samples. However, it should be pointed out that as a newly constructed station, these performances are often affected by some

factors such as the instability of the wiggler, the optimization of the slits, the quality of the sample holder and so on. A lot of works still have to do for the stabile operation of the station in future.

Table 1. The main characteristics of 1W2A and X33.

Option	1W2A	X33
Source	7-Periods Wiggler	Bending Magnet
Monochromator	Two Horizontal focusing triangle-bending crystals	Horizontal focusing triangle-bending crystal
Wavelength	1.54Å, 0.943Å	1.5Å
Beam size at detector	1.5(H)×0.3(V)mm^2	2(H)×0.6(V)mm^2
Beam divergence	3.0(H)×0.5(V)mrad^2	0.5(H) ×0.02(V)mrad^2
Flux at sample	10^{12}ph/s/200mA/1.54Å	5×10^{11}ph/s/100mA
Detector	Two-dimensional Marccd with 2048×2048 pixels, 79μm pixel size One-dimensional wide-angle gas detector with 1024 channels	Two-dimensional Mar345 image plate with 2300×2300 pixels, 150μm pixel size One-dimensional linear gas detector with 1024 channels

3. Material and analysis methods

3.1. Sample and data collection

The ligand-free BSA (Sigma A7030), was dissolved in distilled water at certain concentrations of 2 mg/ml, 6 mg/ml, 8 mg/ml, with sequent purification using centrifuge about 10 minutes. Then the samples were encapsulated inside a columned cell with two 20 μm thick mica windows, sample thickness is 1 mm. The 1.5wt% dithiothreitol (5 μL) was injected into the samples before the measurement to prevent the denaturation of the protein. In the present experiment, the wavelength of the monochromatic x-ray beam was λ=1.536Å and the sample to detector distance was 1600.0mm, allowing the maximum range of scattering vector 4.14 nm^{-1}. The data accumulation time was typically 1.5–5min, depending on the scattering intensity. Each sample was measured three times including two measurements of the buffer before and after the sample with the same exposure time. All scattering profiles were converted to the I (intensity)-S (scattering vector) curves by using FIT-2D program,[13] and then the buffer scattering intensity were subtracted from the solution to obtain the net protein's scattering signals. All the samples of different concentrations produced almost the same scattering profiles, except that the lower concentration samples had a larger noise. Therefore, at the selected concentration range, the contribution from the interparticle scattering could be excluded.

3.2. *Ab initio* reconstruction methods

Following Svergun's *ab initio* reconstruction methods,[14] there are several overall particle parameters should be computed directly from the experimental data ($R_{\rm g}$, $D_{\rm max}$, and the overall asymmetry) at first. The average radius of gyration, $R_{\rm g}$, which is related to the size of the BSA molecule in the solution, was evaluated from the scattering data at low s range using Guinier's law[15] as

$$I(s) = I_{\rm e}Nn^2\exp[-s^2R_{\rm g}^2/3], \tag{1}$$

where $I(s)$ is the experimental scattering data, $I_{\rm e}$ is the scattering intensity per electron, N is the number of the particles, and n is the number of electrons. In order to get the information about the overall asymmetry of the scattering particle as well as its maximum dimension, $D_{\rm max}$, the intraparticle distance distribution function, $P(R)$,[12] which is defined as

$$P(R) = R^2\left\langle\Delta\rho(u)\Delta\rho(u+R)\mathrm{d}u\right\rangle_{\omega}, \tag{2}$$

where $\Delta\rho(u)$ is the excess scattering length density, R is the intraparticle distance, u is the particle position, the subscript ω mean the oriented average. $P(R)$was calculated by making use of the GNOM program,[16] in which the inverse Fourier transform of $I(s)$ is implemented as follows:

$$P(R) = \frac{1}{2\pi^2}\int_0^{\infty} I(s)sR\sin(sR)\mathrm{d}s. \tag{3}$$

After then, two *ab initio* approaches were employed to reconstruct low-resolution structure models. The program DAMMIN[17] represents the particle

shape as an ensemble of densely packed beads inside a spherical search volume with diameter $D_{\max}$. Each packed bead should be ascribed either to particle or to solvent. Starting from a random distribution of beads, we employ simulated annealing to find a compact configuration minimizing the discrepancy χ between the experimental and calculated scattering curves, and χ is defined as follows

$$\chi^2 = \frac{1}{N-1}\sum_i \left[\frac{I_{\exp}(s_i) - cI_{\text{calc}}(s_i)}{\sigma(s_i)}\right]^2, \quad (4)$$

where N is the number of experimental points, c is a scaling factor, and $\sigma(s_i)$ is the experimental error at the point s_i. In a more versatile approach implemented in the program GASBOR,[18] the protein is represented as an assembly of dummy residues (DR). Starting from randomly positioned residues, we find a chain-compatible spatial distribution of DRs inside the same search volume by simulated annealing too. As the *ab initio* methods do not provide unique three-dimensional models, ten independent reconstructions were performed with different starting conditions, and then aligned these models using the program SUPCOMB,[19] which also provides a quantitative measure of dissimilarity of two (low or high) resolution models. After alignment, we use the program DAMAVER[20] to average models representing at a lower resolution and select the most probable one.

4. Results and discussion

The net scattering intensity curve of BSA is presented in Fig.2. Discarding the scattering data at the higher angle dominated by a large noise, we plot the scattering intensities in the range up to s=1.78 nm^{-1}. The value R_{g}=31.2Å±0.25 Å was obtained from the slope of the linear part of $\ln I(s)$ vs. s^2 plot in the range up to $s * R_{\text{g}} \leq 1$. This result is coincident with other ones' results with same samples,[21–24] indicating that our primitive data processing methods are valid, and the macromolecules of the protein have no obvious aggregation.

The interparticle distance distribution function, $P(R)$, is very sensitive to the overall asymmetry and domain structure of the particle. We calculate it based on the net scattering data and show the results in Fig.3. The scattering points both at the initial angle influenced by the beam stop and the higher angle dominated by a large noise were discarded. The value of $D_{\max}$=93.0Å was estimated by trial and error in GNOM with a highest total estimate of 0.695, which mean a reasonable solution. Comparing the shape of $P(R)$ with that calculated based on the sphere model, long rod model and dumbbell model theoretically,[12] we conclude that the shape of BSA molecule, with oriented average, belongs to a dumbbell-like particle with nonequivalent diameter.

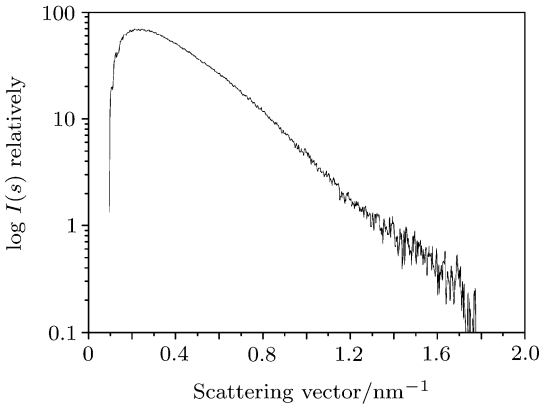


Fig.2. The net scattering intensity of the ligand-free form of BSA in water at a concentration of 6mg/mL. It is obtained by subtracting the scattering intensity of buffer from the intensity of the BSA in solution.

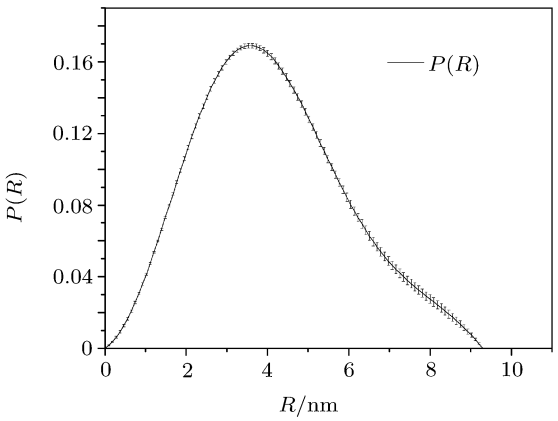


Fig.3. $P(R)$ function obtained from the net scattering data of BSA using GNOM program with the $D_{\max}$=93 Å. The existing shoulder at the tail of the shape indicates that the particle belongs to a dumbbell-like shape.

Figure 4 shows the low-resolution structure models obtained by using the program DAMMIN and GASBOR as being mentioned in Material and Methods. Reinforced an oblate anisomery among ten successive runs, the most probable structural model was obtained with discrepancy χ=0.2(DAMMIN) and χ=0.36(DASBOR). For comparison, a crystal structure of HSA (PDB entry 1BKE), which was oriented to a nearest direction with the low resolution models, is also illustrated in three orthogonal directions. Excepting that an 'ear' stands out which is coincident

with the dumbbell-like shape speculated before, we find that the DAMMIN and GASBOR yielded highly similar results and both of them fit the size and the overall shape of crystal structure of HSA very well. To convince the obtained structures, the scattering intensity curves yielded by the low resolution structures were also calculated and compared with the experimental data in Fig.5. Both of them fit the experimental data very well, this fact indicates that the restored models are reliable. All these results show the performance of the beamline which is suitable to study the protein's non-equilibrium process, as the shape of the protein often changes during the conformational transformation or substrate-bounding process.

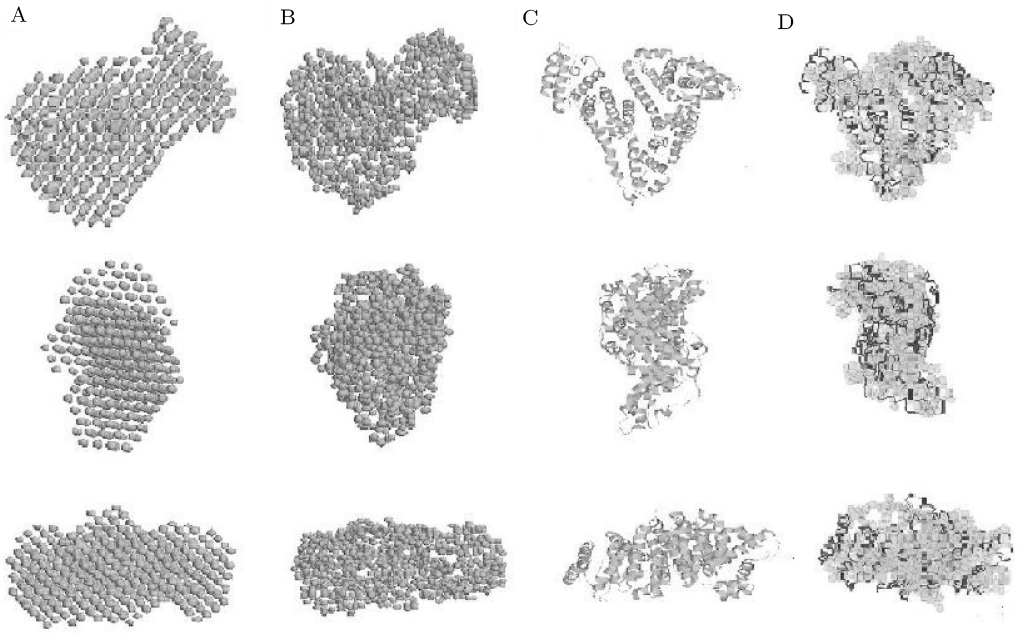


Fig.4. Low resolution structure models obtained with DAMMIN (in A column) and GASBOR (in B column), the crystallographic structure of HSA (in C column), and the model obtained by Svergun at X33 (in D column). The crystal structure of HSA (PDB, entry 1BKE) was oriented to a nearest direction with the low resolution models by using SUPCOMB program. All of them were illustrated in front view (top row), right-side view (middle row, D is left-side view), and top view (bottom row).

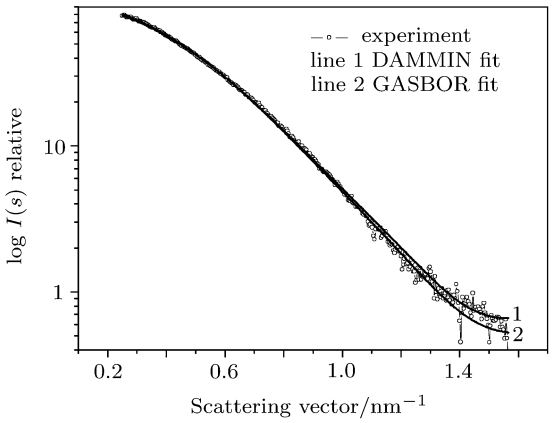


Fig.5. Scattering intensity for BSA from experiment (open circle) and the low resolution structure models yielded by DAMMIN (line 1) and GASBOR (line 2).

However, comparing with the similar results obtained by Svergun at X33[16] (shown in column D of Fig.4), we find that our models are truly rough because of the data range which we collected was up to 1.78 nm^{-1} while in Svergun's collection the data range was extended to 10 nm^{-1}. The main restrained factor of the data range may contribute to two points: firstly, because of the instability of the incident beam, we had to decrease the incident beam size to prevent the direct beam shifting to the outside of the beamstop, leading to the intensity of the incident beam weaken to even ten percent of the origin; secondly, during the experiment, the worse vacuum condition of 10^{-1} Torr (1 Torr=133 Pa) in the vacuum pipe may lead to the scattering signals are absorbed by the residual atmosphere partly. Fortunately, this situation will be improved by some measures in the near future: one is operating a feedback system of the electron beam orbit of BSRF to improve the incident beam's stability;

second is making use of a two-dimension gas detector instead of Marccd for lower 'dark current'; third is changing the vacuum condition by using a new vacuum pump. The fact of the standing out 'ear' will be also checked carefully by collecting higher range scattering data in future.

5. Conclusion

We have constructed a new SAXS beamline name 1W2A at BSRF. The main structure and characteristics of the beamline are described here. Comparing the characteristics with X33 at EMBL-Hamburg, we note that both of them have nearly equal performance except a larger divergence at 1W2A.

By making use of *ab initio* reconstruction methods, the topological shape of the ligand-free BSA in solution has been investigated at the new SAXS beamline. We find that the radius of gyration, R_g =31.2 Å±0.25 Å, is coincident with other ones' results, and the low-resolution structure models are also fitting the crystal structure of HSA in size very well. All of them indicate that the performance of the beamline is suitable to apply to structural biology studies.

However, the defects also exist: one is that our models are truly rough to describe the shape in detail; the other is an 'ear' stands out which seems like a little disparity. To improve the situation, some measures will be carried out in the near future: one is operating a feedback system of the electron beam orbit of BSRF to improve the incident beam's stability; second is using a two-dimensional gas detector instead of Marccd for lower 'dark current', third for improving the vacuum condition.

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