Solution conformation of the response regulator proteins from Deinococcus radiodurans studied by SAXS*

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Abstract: In this paper the solution conformation of the response regulator proteins from Deinococcus radiodurans was studied by small-angle X-ray scattering (SAXS). The SAXS curves of Dr-rrA in solutions were obtained at Beamline 1W2A of Beijing Synchrotron Radiation Facility (BSRF). Two possible conformations of the response regulator proteins, compact and incompact conformations, have been represented by the known crystallographic structures. And theoretical solution scattering curves of the two possible conformations were calculated and fitted to the experimental scattering curve of Dr-rrA, respectively. The result indicates that the solution conformation of the response regulator proteins is inclined to the compact one, which is in agreement with the result of biochemical experiments.

Key words: small-angle X-ray scattering, response regulator, Dr-rrA, solution conformation

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1 Introduction

The gram-positive bacterial Deinococcus radiodurans is one of the most resistant species in the world [1, 2]. It can survive even after suffering intensive gamma radiation at dosages higher as 5 kGy, or ultraviolet, hydrogen peroxide and desiccation. The DNA damage which occurs during such severe conditions can be easily repaired without any errors in Deinococcus radiodurans and its corresponding responses to its environment are amazingly appropriate to compensate for the impairs [3]. The protein encoded by gene DR_2418 in Deinococcus radiodurans is predicted as a response regulator of a two-component signal transduction system. This protein is also called Dr-rrA and has been found to be very important to the extreme radioresistance of Deinococcus radiodurans [4].

The crystal structures of the response regulator proteins from other bacterium demonstrate two domains, e.g., the receiver domain and the effecter domain [5, 6]. The relative orientations and positions of the two domains are essential to the DNA binding activities of this kind of protein. However, during the process of crystallization, the interaction between protein molecules, which is essential for the crystal packing, may alter the physiological conformation of the protein. Therefore, the conformations of proteins in solutions are necessary for the understanding of their functions.

Small-angle X-ray scattering (SAXS) is an effective method to study the low-resolution structure of biological macromolecules under nearly physiological solutions and this method is applicable in many fields [7]. For the sensitivity of SAXS curve to the overall shape and quaternary structure of the sample particles, comparisons between the experimental scattering curves and those evaluated from the crystal structures can be used to verify the structural similarity between macromolecules in crystals and in solution, and also to validate the theoretically predicted mod-

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els. Methods to compute the solution scattering patterns from atomic models have been developed.

There are two beamlines, 4B9A and 1W2A, used for SAXS experiments at the Beijing Synchrotron Radiation Facility. The 4B9A was built in 1990 from a bending magnet. The beamline 1W2A was established in 2007 from a new wiggler insertion device. The flux of 1W2A can reach 10^{12}phs/s at the sample. The capability of this beamline was verified by the scattering experiments of the ligand-free bovine serum album (BSA) in solution. The low-resolution three-dimensional structure model of BSA was obtained by ab initio reconstruction methods in 2008, which indicated that the performance of Beamline 1W2A was suitable for the studies of biological macromolecules [8]. In the present study, the experimental SAXS curve of Dr-rrA solution was obtained. Two possible conformations of the response regulator proteins, compact and incompact conformations, have been represented by crystallographic structures of proteins RegX3 (PDB code: 20QR) and Mtra (PDB code: 2GWR), respectively, which have high homologies of about 40% with Dr-rrA. Through the comparison between the experimental curve and those calculated from crystal structures, the conformation of response regulators Dr-rrA can be confirmed.

2 Materials and analysis methods

2.1 Sample preparation and data collection

The Dr-rrA protein was expressed in E. coli and purified by using a Ni^{2+}-chelating affinity column. The purity of the target protein was examined by SDS-PAGE. The proteins used for SAXS measurements were diluted at a series of concentrations of 3 mg/ml, 6 mg/ml, 9 mg/ml and 15 mg/ml. The sample was checked by SDS and native PAGE before measurements. On the gels, there was a single band, indicating that the sample used was of high purity.

SAXS experiments were carried out on Beamline 1W2A of the BSRF. The SAXS data were collected by a high-sensitivity charge coupled device (CCD) detector with a pixel size of 79 μm. Each scattering profile was collected at 18 ℃ for 100 s and 200 s. The wavelength of incident X-ray was 1.55 Åand the distance from sample to detector was 1.585 m. The sample container was sealed by two 20 μm thick mica windows and the sample thickness was 1 mm. A reducing agent (2 mM DTT) was added to the sample before the experiments in order to diminish the damage or aggregation of the proteins during data collection. The scatterings of the corresponding buffers were measured before and after each measurement of the protein solutions, and treated as backgrounds in data reductions. A series of concentration of proteins (3, 6, 9, 15 mg/ml) was used in the measurements.

2.2 Data reduction

Raw data acquired from SAXS experiments were processed by FIT2D [9] first, converted to I (intensity)- s (scattering vector) curves, where $s = 4\pi\sin\theta/\lambda$ and 2θ was the scattering angle. Data reduction was done by the PRIMUS program [10]. The buffer scattering intensity was subtracted from the solutions, then the net protein scattering intensities could be obtained. The curves of different exposure times up to 200 s show no difference, which means that aggregation did not occur during the experiments. Therefore, the exposure time of 200 s is applicable. The curves of different concentrations were also collected. The scattering patterns were coincident at higher angles. However, a deviation between the concentration of 15 mg/ml and the others was found at low scattering vectors, where it was influenced by attractive or repulsive interactions of the protein molecules in the case of high concentration. Therefore, the data were extrapolated to zero concentration in order to eliminate the effect of protein concentration at very small scattering angles and the resulting curve was merged with the scattering from the sample of 15 mg/ml at higher angles to acquire the final experimental curve [10].

2.3 Evaluating the solution scattering

The similarity between the structures in crystals and solutions for proteins and also nucleic acids can be verified by comparing the scattering curves from experiments and the one calculated by theoretical models [7]. CRYSOL, a program for calculating the solution scattering from atomic models of macromolecular structures, is presented in the program package ATSAS [11, 12]. The program computes the scattering from an atomic model of the particle in solution as

$$\begin{aligned} I(s) &= \langle |\, A(\vec{s}\,)\,|^2 \rangle_\Omega \\ &= \langle |\, A_{\rm a}(\vec{s}\,) - \rho_0 A_{\rm c}(\vec{s}\,) + \delta\rho_{\rm b} A_{\rm b}(\vec{s}\,)\,|^2 \rangle_\Omega, \end{aligned} \quad (1)$$

where $A_{\rm a}(\vec{s}\,)$ is the scattering amplitude from the particle in vacuum, $A_{\rm c}(\vec{s}\,)$ and $A_{\rm b}(\vec{s}\,)$ are the scattering amplitudes from the excluded volume and the hydration shell respectively. In the equation ρ_0 is the average scattering density of the solvent and $\delta\rho_{\rm b}$ is

the effective scattering density of the hydration shell. Eq. (1) indicates that the program takes into account the hydration shell surrounding the macromolecules in solution.

For a given experimental scattering curve, CRYSOL also can adjust two parameters, the average displaced solvent volume per atomic group $r_{\rm a}$ and the contrast of the hydration shell $\delta\rho_{\rm b}$, to minimize the discrepancy

$$\chi^2(r_0,\delta\rho)=\frac{1}{N_{\rm p}}\sum_{j=1}^{N_{\rm p}}\left[\frac{I_{\rm exp}(s_{\rm j})-cI(s_{\rm j},r_0,\delta\rho)}{\sigma(s_{\rm j})}\right]^2, \quad (2)$$

where $N_{\rm p}$ is the number of experimental points, r_0 is the effective atomic radius, $I_{\rm exp}$ is the experimental curve, I is the calculated scattering curve by CRYSOL, $\sigma(s_{\rm j})$ are the experimental errors and c is the scale factor.

3 Results and discussion

The net experimental scattering intensity curve of Dr-rrA extrapolated to zero concentration is presented in Fig. 1. The largest recorded scattering vector s is up to 2.44 nm^{-1}. The gyration radius $R_{\rm g}$ can be calculated by PRIMUS according to the Guinier approximation [13], $R_{\rm g}$=20.1±0.4 Å. The distance distribution function $P(r)$ was computed by the GNOM program [14], as shown in Fig. 2. The function $P(r)$ contains the same information as the scattering intensity $I(s)$ and represents more straightforward information about the particle shape [15]. 25 points were discarded at a higher angle for a large noise. The maximum particle diameter $D_{\rm max}$=63 Å was obtained by GNOM with the highest total estimate of 0.918, which indicates an excellent solution.

Figure 3 shows two possible conformations of response regulator proteins in solution, incompact and compact conformations, represented by crystallographic models of RegX3 and Mtra, respectively.

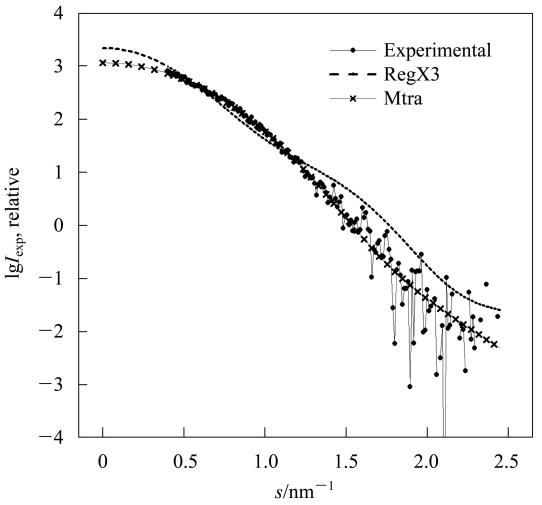

Fig. 1. The net experimental scattering intensity curve of Dr-rrA extrapolated to zero concentration in solution, fitted by the theoretical solution scattering curves calculated from RegX3 and Mtra by CRYSOL.

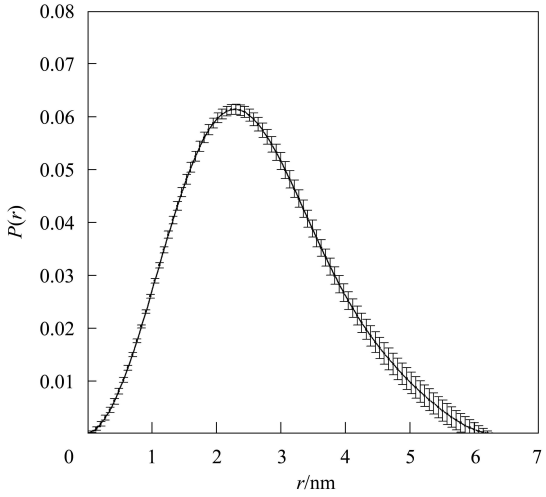

Fig. 2. The distance distribution function $P(r)$ obtained from the above net scattering data computed by GNOM. The range of $I(s)$ used for $P(r)$ calculation is 0.40 to 2.15 nm^{-1}.

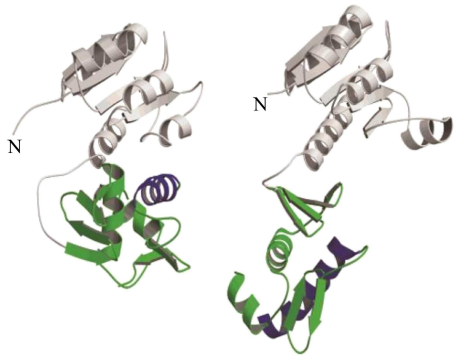

Fig. 3. The crystallographic models of RegX3 (right) and Mtra (left), represent the incompact-conformation and compact-conformation, respectively.

Firstly, the $R_{\rm g}$ and $D_{\rm max}$ of RegX3 and Mtra were calculated respectively by CRYSOL and were compared with those evaluated from the solution scattering curve (Table 1). From Table 1 we can find that the values of $R_{\rm g}$ and $D_{\rm max}$ obtained from the experimental solution scattering profile are closer to those evaluated from Mtra, which indicates that the

solution conformation of the response regular protein Dr-rrA is inclined to the compact conformation. To prove this point further, theoretical solution scattering curves of RegX3 and Mtra are calculated and fitted to the experimental solution scattering curve of Dr-rrA by CRYSOL, respectively, and the results are shown in Fig. 1. CRYSOL also presents the fitting discrepancies χ=1.455 ($r_{\rm a}$=0.14nm and $\delta\rho_{\rm b}$=75 e/nm^3) for RegX3 and χ=0.749 for Mtra ($r_{\rm a}$=0.14 nm and $\delta\rho_{\rm b}$=65 e/nm^3). Comparison of the discrepancies indicates that the compact conformation provides a better overall fit to the experimental data than the incompact conformation. In other words, the solution conformation of the response regulator protein Dr-rrA is inclined to the compact conformation, which is in agreement with the result from Table 1. This result is supported by the following biochemical experiments. The compact conformation of the response regulator depicts a low activity one. In the study by Lianyan Wang et al. it is observed that Dr-rrA will bind to DNA only at high protein concentrations, which proves the solution conformation of Dr-rrA is inclined to the compact conformation with weak binding abilities or activities [4].

Table 1. Comparison between the $R_{\rm g}$ and $D_{\rm max}$ of RegX3 and Mtra calculated, respectively, by CRYSOL and those evaluated from the solution scattering curves.

	$R_{\rm g}$/Å	$D_{\rm max}$/Å
RegX3	23	77
Mtra	18	58
experimental	20	63

4 Conclusion

The solution structure of Dr-rrA was studied by SAXS. Two possible conformations of the proteins, compact and incompact conformations, have been constructed according to the known crystallographic structures. The analysis of the SAXS data indicates that the most possible conformation of Dr-rrA in solution is compact. This result is in agreement with the result of biochemical experiments.

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