BL-10C/2014G165

The structure factor of myoglobin determined by using the experimental and the calculated form factor

Hiroshi Imamura[1], Tomonari Sumi[2], Hiroyo Ohgi[1], Yasuhiro Isogai[3,*]
1. Graduate School of Advanced integration Science, Chiba University, 1-33 Yayoi, Inage-ku, Chiba 263-8522, Japan
2. Department of Chemistry, Faculty of Science, Okayama University, 3-1-1 Tsushima-Naka, Kita-ku, Okayama 700-8530, Japan
3. Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama, 939-0398, Japan

1 Introduction

Self-association of proteins including the formation of quaternary structure, crystallization and aggregation is governed by a self-interaction potential of proteins. The self-interaction of the extant natural proteins is likely optimized [1], while the underlying physicochemical mechanism and the biological relevance are yet to be elucidated. We have used small angle X-ray scattering (SAXS) to "monitor" the self-interaction potential with the aid of liquid state theory [2]. An inhomogeneous protein distribution in a solvent gives the corresponding scattering called a structure factor. Recently, we have developed a method for extracting the self-interaction potential from the structure factor without assuming model potentials by modifying an integral equation (Model-Potential-Free method) [3-6]. Application of this method to lysozyme scattering revealed that the contact between the protein molecules should be stabilized [4]. To further the study, we targeted myoglobin molecule, a well known soluble protein despite its small net charge. The present study determined the structure factor by using the experimental form factor and compared it with the structure factor by using the form factor calculated based on the crystal structure.

2 Experiments

The SAXS experiments were performed at the beamline BL-10C. The X-ray wavelength was 0.1488 nm; the camera length was 1048 mm, which was calibrated by use of a scattering pattern of silver behenate. X-ray intensities were recorded by PILATUS3 2M (DECTRIS Ltd., Switzerland). Myoglobin from equine skeletal muscle (Sigma-Aldrich, St. Louis, MO) was dissolved in ice-cooled ultrapure water (Milli-Q, Millipore). The solution was centrifuged. The supernatant solutions diluted with the ultrapure water were used for the measurements. The sample solutions were kept on ice until the measurement. To obtain the scattering that is used as the form factor, a myoglobin solution of 6 mg/mL was measured. During the measurement, the sample was flowed to avoid the damage by X-ray radiation. For decreasing noises at large q values, 450 images were collected; an image was recorded for 2 sec of the exposure time. The temperature was controlled to be 25.0 ± 0.1°C. The circular 1D average of the image was performed by the program *Nika* [7]. The 1D data were averaged, eliminating the data for the sample damaged by radiation.

3 Results and Discussion

The SAXS intensity of a protein, $I(q)$, is described by

$$I(q) = ckP(q)S(q), \qquad (1)$$

where c is the protein concentration, k is a constant including the factor depending on the setup of the measurement, $P(q)$ is a form factor, and $S(q)$ is a structure factor. We used the SAXS intensity of the dilute protein solution, at the concentration of which the interparticle interferences are negligible, as $P(q)$. $P(q)$ was also obtained by calculation based on the atomic coordinates of the molecule with a use of a CRYSOL program [8]. Here, the atomic coordinates of myoglobin (PDB: 1YMB) [9] and the default parameters in the program were used for the calculation. The arrows in Fig. 1(a) indicate the difference between the experimental $P(q)$ and the calculated $P(q)$ at $q > 0.2$ Å^{-1}. According to eq. (1), $S(q)$ was determined by using the SAXS intensity of the concentrated protein solution (108 mg/mL) with the experimental $P(q)$ or the calculated $P(q)$ as shown in Fig. 1(b). $S(q)$ determined by use of the calculated $P(q)$ gave a strong oscillation, while $S(q)$ determined by use of the experimental $P(q)$ did not. Obviously, the oscillation in the former $S(q)$ appeared an artifact. Previous study suggested that the calculation of $P(q)$ at large q in the

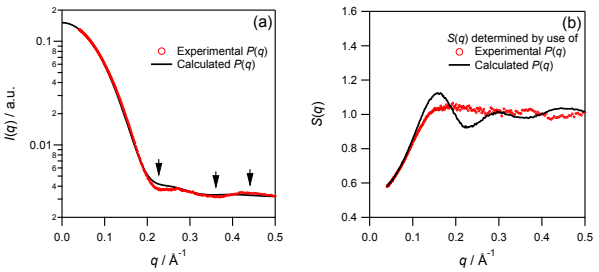

Figure 1: (a) The SAXS intensities derived from the experimental $P(q)$ and the calculated $P(q)$ based on the atomic coordinates of myoglobin. (b) The structure factor, $S(q)$, determined by use of the experimental $P(q)$ and the calculated $P(q)$.

small angle region should require more elaborate analysis involving computation of a hydration effect, i.e., an inhomogeneous distribution of waters around a protein molecule [10]. $S(q)$ at large q in the small angle region represents the interaction potential at short range distance between the molecules [4, 5], which is of interest. Thus, the experimental $P(q)$ should be used for the following analysis of $S(q)$. The present examination suggests that use of the calculated $P(q)$ would be the potential pitfall for studies on the interaction potential by SAXS.

Acknowledgement

This work was supported in part by Izumi Science and Technology Foundation (to H.I.).

References

[1] H. Imamura, Y. Isogai, M. Kato, *Biochemistry* **51**, 3539 (2012).
[2] H. Imamura, T. Morita, T. Sumi, Y. Isogai, M. Kato, K. Nishikawa, *J. Synchrotron Rad*. **20**, 919 (2013).
[3] H. Imamura, T. Sumi, H. Ohgi, Y. Isogai, Photon Factory Activity Report 2013 PART B, No. 62, (2014).
[4] T. Sumi, H. Imamura, T. Morita, Y. Isogai, K. Nishikawa, *Phys. Chem. Chem. Phys.* **16**, 25492 (2014)
[5] T. Sumi, H. Imamura, T. Morita, K. Nishikawa, *J. Mol. Liq*. **200**, 42 (2014)
[6] H. Imamura, T. Sumi, H. Ohgi, Y. Isogai, Photon Factory Activity Report 2014 PART B, No. 32, (2015).
[7] J. J. Ilavsky, *J. Appl. Crystallogr*. **45**, 324 (2012).
[8] D. Svergun, C. Barberato, M. H. J. Koch, *J. Appl. Crystallogr*. **28**, 768 (1995).
[9] S. V. Evans, G. D. Brayer, *J. Mol. Biol.* **213**, 885 (1990).
[10] Y. Seki, T. Tomizawa, N. N. Khechinashvili, K. Soda, *Biophys. Chem*. **95**, 235 (2002).

* yisogai@pu-toyama.ac.jp