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Small angle X-ray scattering study on the intermolecular interactions between proteins

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1 Introduction

While naturally occurring globular proteins are water-soluble, artificially designed proteins are prone to precipitate and aggregate [1]. This hinders its production and the subsequent characterization, thereby slows down a progress of studies on designing proteins. To address the issue, we focus on an interaction potential between protein molecules, $V(r)$, which in part governs the solubility and the aggregation. We measured a small angle X-ray scattering (SAXS) of protein solution to determine a structure factor, $S(q)$, called inter-particle interference. $S(q)$ is originating from spatial arrangement of protein molecules in solvent, i.e. protein-protein radical distribution. According to integral equation theory for pure liquid, $S(q)$ is connected to $V(r)$. In fact, however, the experimental $S(q)$ cannot directly be converted to $V(r)$ because $S(q)$ is unavailable in an infinite range of scattering parameter. As well as an indirect method that determines a particle distance distribution function from a form factor, $P(q)$, there is an indirect method to analyze $S(q)$: assuming functions representing the potential such as Derjaguin–Laudau–Verwey–Overbeek (DLVO), the variable parameters are optimized to reproduce the experimental $S(q)$ [2]. However, it remains unknown whether the model potential appropriately represents the interaction between proteins. In addition, assuming the model limits our interpretation of $V(r)$ to the framework of the theoretical model. To improve this situation, we developed a quasi-direct method to determine $V(r)$ without assuming model potentials by modifying an integral equation theory [3]. Then, we elucidate $V(r)$ of a soluble protein, lysozyme, by applying this method to the SAXS data. This will be a step toward understanding the insolubility of designed proteins.

2 Experiment

The SAXS experiments were performed at the beam line BL-10C. The X-ray wavelength was 0.1488 nm; the camera length was 957 mm. X-ray intensities were recorded using PILATUS 300K-W (DECTRIS Ltd., Switzerland). Lysozyme from hen egg white (Sigma Aldrich, St. Louis, MO) was dissolved in 25 mM bis-Tris buffer at pH 7.

3 Results and Discussion

A closure relation to solve Ornstein-Zernike equation in the integral equation theory is given by

$$h(r) = \exp[-V(r)/k_BT+h(r)-c(r)+B(r)]-1, \quad (1)$$

where $h(r)$, $c(r)$ and $B(r)$ are total correlation function, the direct correlation function and the bridge function, respectively. We divide both $c(r)$ and $V(r)$ into two terms, i.e. hard sphere term and an excess term,

$$c(r) = c_{HS}(r) + c_{ex}(r),\ V(r) = V_{HS}(r) + V_{ex}(r), \quad (2)$$

and then take $c_{ex}(r)$ as $-V_{ex}(r)/k_BT$. As a result, we obtain

$$h(r) = \exp[-V_{HS}(r)/k_BT+h(r)-c_{HS}(r)+B(r)]-1, \quad (3)$$

which does not require use of model potentials in the iterative calculation because $V(r)$ does not explicitly appear in the equation. Alternatively, we use the experimental $S(q)$ for calculating $h(r)$ in the right side, with the relation, $S(q) = nh(q) + 1$ (n is number of density). We call this model-potential free (MPF) method. More details of the method are described in ref [3].

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

$$I(q) = ckP(q)S(q), \quad (4)$$

where c is the protein concentration, k is a constant. The SAXS profile of the dilute protein solution (0.26 wt%), at the concentration of which the interparticle interferences are negligibly small, yields $kP(q)$. From the experimental $S(q)$ of lysozyme at the concentration of 10 wt% shown in Fig. 1(a), MPF determines $V(r)$ shown in Fig. 1(b). The corresponding $S(q)$ calculated in MPF agrees well with the experimental $S(q)$ (Fig. 1(a)).

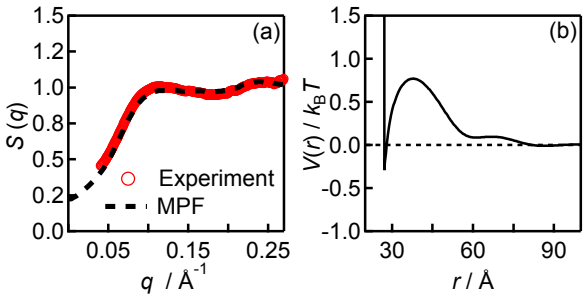

Fig. 1: (a) The structure factor, $S(q)$, of lysozyme at the concentration of 10 wt%. (b) the intermolecular interaction potential, $V(r)$, of lysozyme calculated by MPF using the experimental $S(q)$.

The minimum of $V(r)$ is found at the distance (r) that the protein molecules contact each other. A large activation barrier is found between the first and second minimums (at ~ 40 Å). This yields a picture that lysozyme molecules that contact are the most stable, but the aggregation is suppressed by the large activation barrier. We further studies for unraveling the origin of the activation barrier.

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References

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Research Achievements

H. Imamura, T, Morita, T. Sumi, Y. Isogai, M. Kato, K. Nishikawa, 33rd International Conference on Solution Chemistry, Selected speaker, Kyoto, Japan, July 2013.

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