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Model-potential-free analysis of an inter-particle interference of protein solution scattering: insights into solvent effects on the intermolecular interaction

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1 Introduction

The intermolecular interaction between proteins, self-interaction, governing its aggregation or precipitation increasingly attracts attention as a fundamental issue in biophysics and biopharmaceutical field. A mechanism avoiding aggregation is likely to exist in naturally occurring soluble proteins, while that is yet to be elucidated. In fact, the present artificially designed proteins are prone to aggregate [1]. An inter-particle interference, called $S(q)$, in a small angle X-ray scattering captures a radial distribution of protein molecules in solution, which is related to the self-interaction potential, $V(r)$, through liquid theory [2]. To determine $V(r)$ from $S(q)$, a classical method optimizes variable parameter in a model potential such as Derjaguin–Laudau–Verwey–Overbeek (DLVO) to reproduce the experimental $S(q)$. However, it is often ambiguous whether the model potential selected is appropriate for the system. To overcome this drawback, we developed a method that determines $V(r)$ without assuming model potentials by modifying an integral equation theory [3-5]. Then, we elucidate $V(r)$ of a soluble protein, lysozyme, by applying this model-potential-free (MPF) method to the experimental SAXS data. This demonstrates solvent effect on the intermolecular interaction between proteins.

2 Experiments

The SAXS experiments were performed at the beam line BL-10C. The X-ray wavelength was 0.1488 nm; the camera length was 957 or 1048 mm, which was calibrated by use of a scattering pattern of silver behenate. X-ray intensities were recorded using PILATUS (DECTRIS Ltd., Switzerland). Lysozyme from hen egg white (Sigma Aldrich, St. Louis, MO) was dissolved in 25 mM bis-Tris buffer at pH 7. To obtain the scattering of the form factor, a lysozyme solution of 2.6 mg/mL was measured. During the measurement, the sample was flowed to avoid the damage by X-ray radiation. The exposure time was 80 minutes for decreasing noises at large q values.

3 Results and Discussion

The model-potential-free (MPF) method has been described in our papers [3-5] in detail. Briefly, a closure relation to solve Ornstein-Zernike (OZ) 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 $h(r)$, $V(r)$, k_B, T, $c(r)$ and $B(r)$ are the total correlation function, the pair interaction poteintial, the Boltzmann constant, the thermodynamic temperature, the direct correlation function and the bridge function, respectively. We divide both $c(r)$ and $V(r)$ into two terms,

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

$$c(r) = c_{HS}(r) + c_{ex}(r). \quad (3)$$

The subscripts ''HS'' and ''ex'' denote hard sphere and excess, respectively. Next, we introduce the following assumption for $V_{ex}(r)$:

$$-V_{ex}(r)/k_BT = c_{ex}(r). \quad (4)$$

Accordingly, we obtain

$$h(r) = \exp[-V_{HS}(r)/k_BT+h(r)-c_{HS}(r)+B(r)]-1, \quad (5)$$

where the excess potentail, $V_{ex}(r)$, does not explicitly appear. The experimental $S(q)$ is converted to $h(r)$ in the right side of the eq. (5), with the relation, $S(q) = nh(q) + 1$ (n is number density). After iterative numerical calculation, $V_{ex}(r)$ is determined through OZ eq. and eq. (3-5).

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

$$I(q) = ckP(q)S(q), \quad (6)$$

where c is the protein concentration, k is a constant. The SAXS profile of the dilute protein solution (2.6 mg/mL),

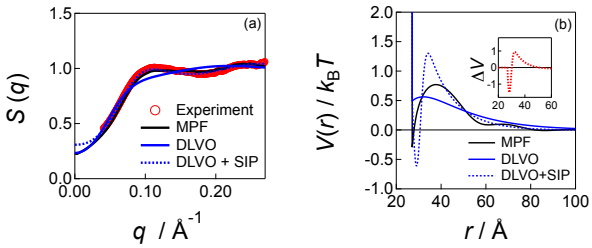

Fig. 1: (a) The experimental (red circle), MPF-derived (black line), DLVO-derived (blue line), DLVO+SIP-derived (blue dashed line) structure factors, $S(q)$, of lysozyme at the concentration of 100 mg/mL. (b) the intermolecular interaction potentials, $V(r)$, of lysozyme calculated by MPF, DLVO and DLVO+SIP using the experimental $S(q)$. In the inset of (b), the difference between $V(r)/k_BT$ of DLVO+SIP and that of DLVO is shown.

at the concentration of which the inter-particle interferences are negligibly small, yields $kP(q)$. We determined the experimental $S(q)$ of lysozyme at the concentration of 100 mg/mL shown in Fig. 1(a). The corresponding $S(q)$ calculated in MPF agrees well with the experimental $S(q)$ (Fig. 1(a)). We also analyzed the experimental $S(q)$ using the classical procedure assuming DLVO-type model potential, where the effective charge of lysozyme is 8. This does not give the sufficient $S(q)$ comparable to the experimental $S(q)$ (Fig. 1(a)). Thus, we introduced an additional potential called "solvent-induced potential (SIP)" [4]. The DLVO + SIP potential shown in Fig. 1(b) reproduces the experimental $S(q)$ well (Fig. 1(a)). The difference between $V(r)/k_BT$ of DLVO+SIP and that of DLVO shown in the inset of Fig 1(b), ΔV, indicates what DLVO lacks to reproduce the experimental $S(q)$: ΔV shows the repulsion, which decays rapidly with increasing the distance between the proteins, and the attraction exists in ΔV at short range. According to theoretical studies [6,7], we attribute both the characteritic ΔV potential to solvent effects: when the protein molecules approach each other to form the contact configurations, it needs energy to remove water molecules in the hydration shell surrounding the protein, and the contact configurations are stablized not only by direct interactions, but by translational motion of water molecules.

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Research Achievements

H. Imamura, The 9th Mini-Symposium on Liquids (MSL), Invited talk, Fukuoka, Japan, July 2015.

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