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Structural Investigation on Ovalbumin Solution

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Mesoscopic structures of ovalbumin solutions with several NaCl concentrations are investigated by a small-angle neutron scattering (SANS) method. The SANS profile of the ovalbumin solution with 0.1 M NaCl shows no peak and is described well with a hard sphere model. On the other hand, the SANS profiles of the ovalbumin solution with NaCl concentrations less than 0.01 M have a peak around q=0.057 Å^{-1}. It is deduced that in the ovalbumin solution with the low NaCl concentration an electric long range interaction exists to maintain the distance between the ovalbumin particles and in the ovalbumin solution with 0.1 M NaCl the interaction is shielded by the ions.

Key words : ovalbumin, hard sphere model, small-angle neutron scattering

1 INTRODUCTION

It is well known that egg white contains several kinds of proteins, in which the main component, ~60%, is ovalbumin (Mw~45,000). Under watery conditions, the ovalbumin has a globular shape with a radius of ~30 Å[1] and the ovalbumin globules exhibit a self-organized mesoscopic structure.[2, 3] This mesoscopic structure seems to be governed by several conditions: temperature, the concentration of the ovalbumin, the species and the concentration of cations and anions around the ovalbumin particles and so on.

In the present study, we have investigated the mesoscopic structure of the ovalbumin solution and the influence of NaCl-addition. In order to observe the mesoscopic structure, we adopted a small-angle neutron scattering (SANS) method. It is one of the most useful techniques to investigate the mesoscopic structure of organic materials because of the possibility of utilizing a contrast matching technique with heavy water[4], and actually the ovalbumin heavy-water solutions were prepared as starting specimens in the present study, since deuterons have a smaller incoherent scattering cross section than protons.

With the SANS technique, we successfully made observations of the structural change caused by adding NaCl into the ovalbumin solution.

2 EXPERIMENTAL

2.1 Sample

A commercially obtained salt-free ovalbumin (Sigma, Grade V, lyophilized and crystallized) was used without further purification. The ovalbumin powder was dissolved into heavy water with a concentration of 5 wt% and then NaCl was added into the solution. We prepared 4 samples with NaCl concentrations of 0.1 M, 0.05 M, 0.01 M and 0.0 M(not added).

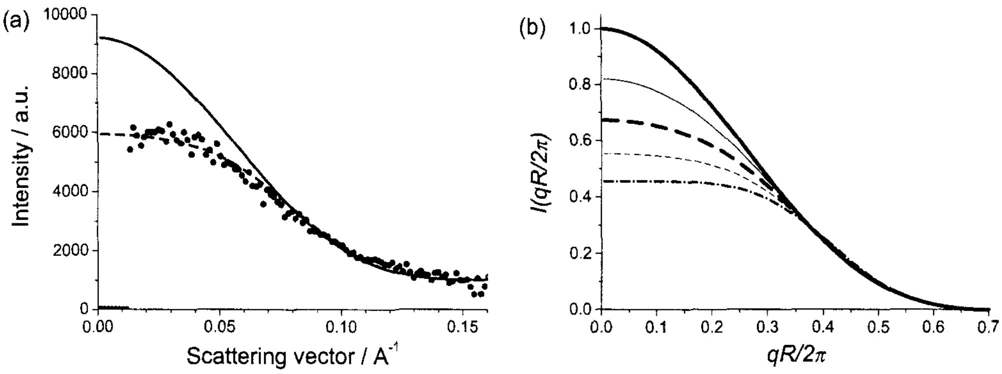

Fig. 1. (a) SANS profile of a 5 wt% ovalbumin D_2O solution with an NaCl concentration of 0.1 M. The circles indicate the observed scattering intensities. The solid line shows the scattering curve of a single particle with a radius of 29.6 Å and the dashed line shows the result of the least square fitting with Percus-Yevics approximation of a hard sphere model. (b) Calculation of the scattering curves from the particle solutions with the various volume fractions. Thick solid, thin solid, thick dashed, thin dashed and dash-dotted lines show the scattering curves with the volume fraction of 0 (single particle), 0.025, 0.05, 0.075 and 0.1, respectively.

2.2 Small-angle neutron scattering

The SANS experiments were performed with a KUR-SANS spectrometer installed at Kyoto University Reactor, Kumatori, Osaka, Japan. Scattering intensities with a scattering vector (q) range of 1.2×10^{-2} to 1.6×10^{-1} Å^{-1} were measured with a 5.6 Å-wavelength neutron beam. The q-range corresponds to the scale range of 5.2×10^{2} to 4.0×10^{1} Å in the real space. The samples were sealed into quartz cells of 2 mm thickness and their scattering intensities were observed at room temperature for 3 hours. After the corrections of the transmission and measuring time, the scattering intensities from the cell and solvent were subtracted from the observed intensities. With these corrections and normalization, the value of the final data straightforwardly corresponds to the scattering from the ovalbumin globules.

3 RESULTS AND DISCUSSION

Figure 1 (a) shows the SANS profile of a 5 wt% ovalbumin D_2O solution with an NaCl concentration of 0.1 M. At first, we adopted a single particle model (dilution limit) to explain this scattering intensity since it monotonically decreases. The scattering intensity $I_s(q)$ from a single particle with a radius of R is given by

$$I_s(q) = I_s(0)\frac{J_{3/2}(qR)^2}{(qR)^3}. \qquad (1)$$

Here, J_ν is Bessel function of order ν. As shown with the solid line in Fig. 1 (a), equation (1) dose not explain this observed scattering intensity, especially for small q values. It is deduced that interference between the ovalbumin particles made the discrepancy between the observed scattering intensity and equation (1), since the particle interference is ignored in the single particle model. Taking the particle interference into consideration, we revise the scattering function $I(q)$ as follows.

$$I(q) = I_s(q)\cdot S(q). \qquad (2)$$

Here, $S(q)$ is a structure factor of the solution. To calculate the structure factor, we assumed potential of the particle, $U(r)$, as follows (hard sphere model).

$$U(r) = \begin{cases} \infty, & 0 \le r \le R, \\ 0, & r > R, \end{cases} \qquad (3)$$

where r is a distance from the particle center. This model describes the particle system having a short range interaction very well. Under Percus-Yevics approximation[5], the structure factor is given by,

$$\begin{aligned} S(q)^{-1} = & 1 - 24\phi\Big\{-\frac{2A_1}{(2qR)^4} + \frac{24A_3}{(2qR)^6} \\ & -\frac{\cos(2qR)}{(2qR)^2}(A_0 + A_1 + A_3) \\ & +\frac{\sin(2qR)}{(2qR)^3}(A_0 + 2A_1 + 4A_3) \end{aligned}$$

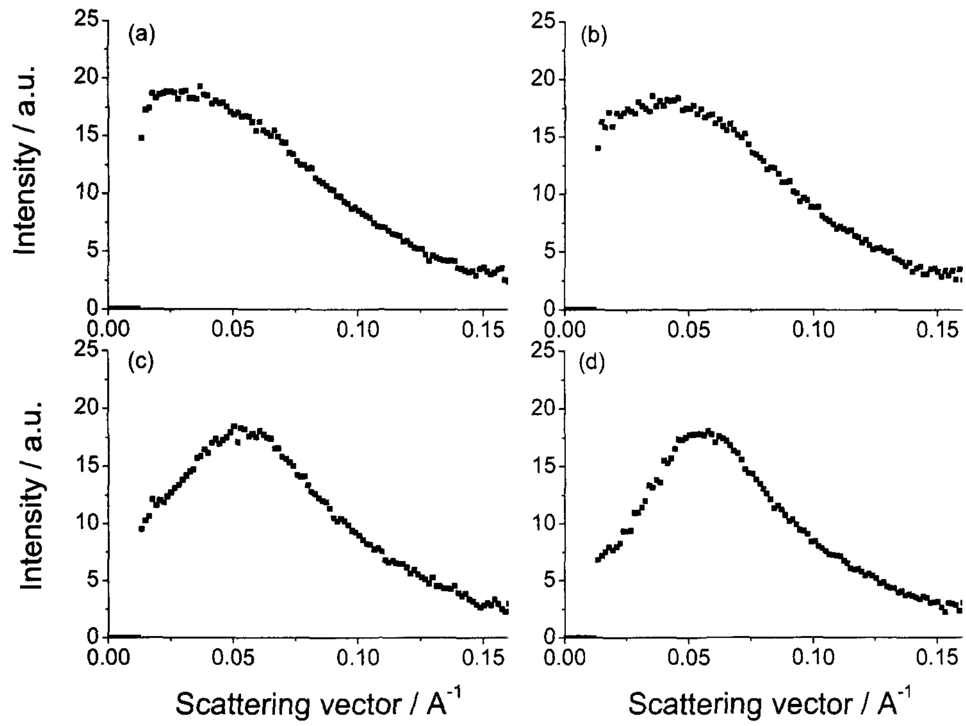

Fig. 2. SANS profiles of the ovalbumin solutions with various NaCl concentrations. The NaCl concentrations are (a) 0.1 M, (b) 0.05 M, (c) 0.01 M, and (d) no NaCl, respectively.

$$
\begin{aligned}
&+\frac{\cos(2qR)}{(2qR)^4}(2A_1+12A_3)\\
&-24A_3\frac{\sin(2qR)}{(2qR)^5}\\
&-24A_3\frac{\cos(2qR)}{(2qR)^6}\Bigg\}, \qquad (4)
\end{aligned}
$$

$$
\begin{aligned}
A_0 &= -\frac{(1+2\phi)^2}{(1-\phi)^4},\\
A_1 &= \frac{6\phi(1+0.5\phi)^2}{(1-\phi)^4},\\
A_3 &= \frac{\phi A_0}{2},
\end{aligned}
$$

where ϕ is the volume fraction of the particle. Figure 1 (b) shows the scattering curves calculated by equations (1), (2) and (4) for various volume fractions. In the small q-region, the scattering intensity decreases with the increase of the volume fraction. Therefore, we adopted the hard sphere model to describe the scattering intensity of the 5 wt% ovalbumin D_2O solution with 0.1 M NaCl, since the observed scattering intensity was weaker than that calculated from the single particle model in the small q-region. The result is shown with a dashed line in Fig. 1 (a) and the radius of the ovalbumin particle was found to be 29.6 Å. This value agrees with that observed by a light scattering.[6]

As shown in Fig. 2, the scattering profiles, *i.e.* the structure of the ovalbumin solution is deeply influenced by adding NaCl. In the solutions with NaCl concentrations less than 0.01 M, the scattering profiles have a peak around q=0.057 Å^{-1} as shown in Fig. 2 (c) and 2 (d). This value corresponds to a scale of 110 Å in the real space, which is different from the size of the ovalbumin particles (~60Å in diameter). Therefore, we deduced that there exists an electric long range interaction keeping a distance of about 110 Å between the ovalbumin particles in these solutions whereas the long range interaction would be shielded by the ions in the solutions with the higher NaCl concentrations. More detailed investigations are in progress.

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