Development of High-Pressure Cell for X-ray Solution Scattering Optimized for BL45XU

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1. Introduction

Hydrostatic pressure is considered to be as a good parameter to control the balance of hydrophobic and hydrophilic interactions of proteins. For example, the pressure induced dissociation of oligomeric proteins is observed below 200 MPa [1]. According to Le Chatlier's principle, increase in pressure reduces the volume of a system. Since small-angle X-ray scattering (SAXS) is a powerful technique for probing volume changes, measurement of high-pressure SAXS (HP-SAXS) becomes very useful for studying the effects of pressure in biology. Kato and Fujisawa (KF) [2] developed the first high-pressure cell for SAXS with diamond windows for protein solutions. Because of the low parasitic scattering of diamond window material compared to beryllium, it was capable of recording on quantitative scattering profile. The subunit-subunit interaction of oligomeric protein was studied using an HP-SAXS [3]. The cell, however, had the following disadvantages; first, the sample volume was large (1.5~2.0 ml). Second, the cell often leaked after changing the sample. We therefore developed a new cell to conquer these disadvantages.

2. Experiments

The improved cell was designed to achieve 500 MPa. It has a column like structure and is compact: 50 mm in diameter, 100 mm in length, and approximately 1500 g in weight, as shown in Fig. 1. The pressure pump is situated outside the shielding hutch, by which one can control pressure very easily. The pressure media (water) is supplied to the cell in medium pressure (less than 25 MPa). The final pressure is obtained by a pressure-intensifier (×20) incorporated in the cell. By employing the pressure-intensifier we can reduce leakage trouble in relation to high-pressure tubes. The port for exchanging the sample was attached, which also reduced leakage trouble. The sample-volume required for the measurement was designed to be several times smaller than that of KF-type [2], *i.e.*, less than 400 μl, which is an important requirement for the application of protein solutions. The length of the optical path in the sample chamber is 4 mm. The windows for X-rays are made of synthetic diamonds (Sumicrystal, Sumitomo Denco Co., type Ib) 1 mm thick and 5 mm in diameter. The opening for X-rays is 2 mm in diameter.

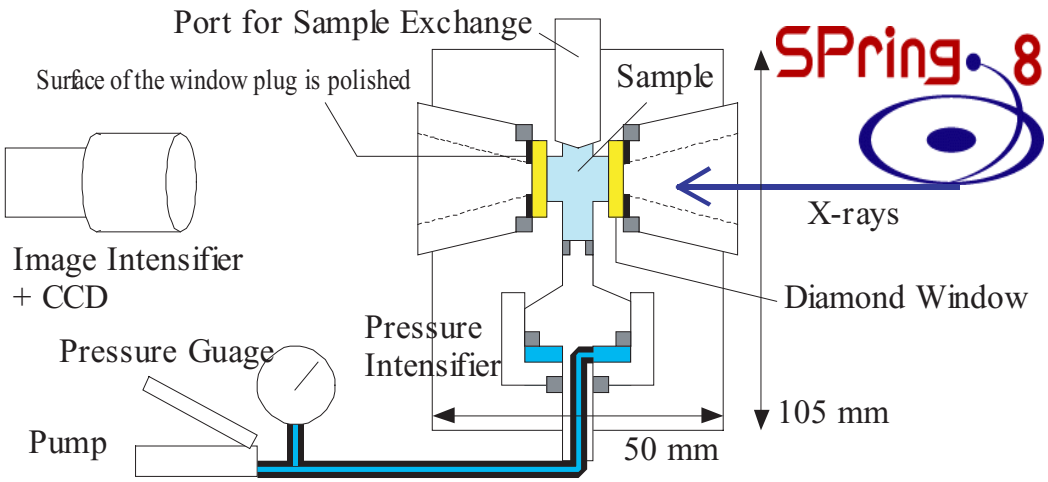

Fig. 1. Schematic illustration of the high-pressure cell and the optical geometry.

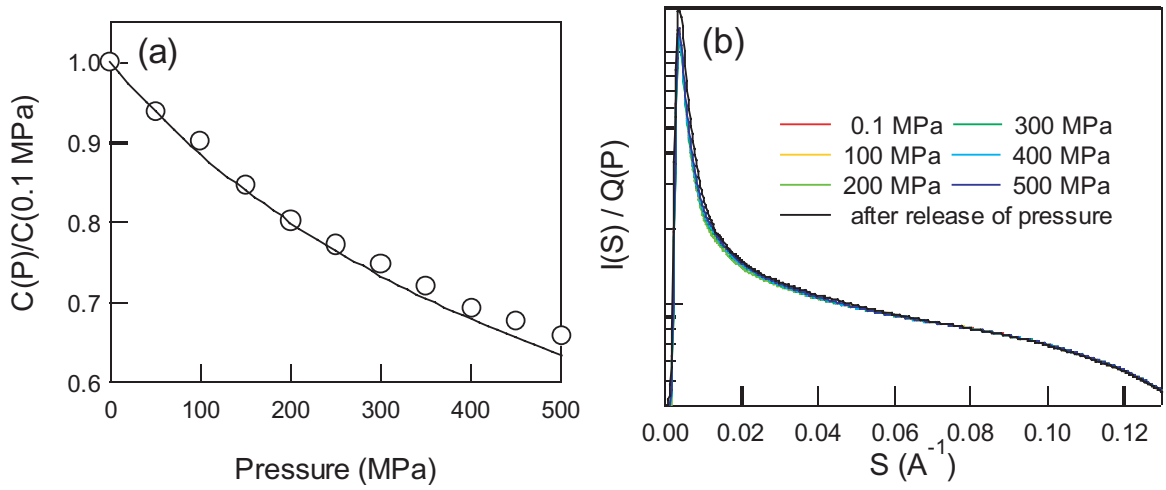

Fig. 2. (a) Pressure dependence of the transmission of water reduced by that of 0.1 MPa. The solid line is calculated from the density of the water reported in ref. 6. (b) The SAXS profiles of water scaled by Q(P). All profiles coincided with a single curve.

All SAXS measurements were performed at the BL45XU SAXS station [4]. The wavelength of X-rays was 1 Å. The short wavelength is preferable in order to reduce the X-ray absorption of diamond windows and water. The camera length was 594 mm, and the size of the beam stop was 3 mm. The scattering intensities were acquired by the CCD coupled with X-ray image intensifier (XR-II+CCD) [5]. Here we use S as the scattering vector, $S = 2\sin\theta/\lambda$; 2θ and λ are the scattering angle and the wavelength of the incident, respectively.

Cytochrome-C solutions were used as the sample. 5.0, 2.5 and 1.0 mg/ml of Cytochrome-C solutions were prepared by dissolving in the buffer solution containing 10 mM Tris-HCl (pH 7.0). The protein and buffer solutions were applied in turn to the SAXS measurements, that is, a pressure series of scattering images of a protein solution, and subsequently the corresponding series of buffer solution.

3. Results and Discussion

Tests of the high-pressure cell were performed by using water as a sample. 500 MPa was achieved constantly without any leakage. Transmission, C, of the water is calculated from the absorption of X-rays, as follows,

$$C \propto \exp\left[-\frac{\mu_0}{\rho_0}\rho\ell\right] , \qquad (1)$$

where μ_0/ρ_0, ℓ and ρ are a liner absorption coefficient per a unit mass, the optical path length inside the cell, and the density of the sample, respectively. Both μ_0/ρ_0 and ℓ are constant against the change of pressure, P, while ρ is a variable. As increasing the pressure ρ increases and C decreases. C is proportional to the integral intensity, Q. Figure 2(a) shows the pressure dependence of C calculated form Q, *i.e.*, $C(P)/C$ (0.1 MPa). Theoretical transmission calculated from the density of water [6] was also plotted as a solid line. The agreement was very good. The deviation between the measured and theoretical transmissions at 500 MPa was 3.5%, which may be due to the precision of our pressure gauge connected to the medium-pressure tube: the error of the pressure gauge is magnified 20 times. It can be concluded that the pressure intensifier works well and 500 MPa was achieved in the sample chamber. Figure 2(b) shows the scattering profiles of water reduced by $Q(P)$. All profiles with different P coincided with a single curve. The profile obtained after release of pressure was also identical with that before applying pressure, except for the small S region, which assures the parallelism of the two windows over P ranging from 0.1 to 500 MPa.

Figure 3(a) shows the background-subtracted profiles obtained from different concentrations at 0.1 MPa. The large intensity of 1.0 mg in the small S region is caused by a subtraction error, because the parasitic scattering is intense in $S < 7\times10^{3}$. The lower limit of S depends on sample quantity. The parasitic scattering presents serious problems for low S/N data, *i.e.*, the small amount of proteins. For $S < 7\times10^{3}$, the profile obtained by using the standard static cell [6]

agreed well with that obtained by using a high-pressure cell. The radius of gyration (*Rg*) obtained by $\ln I(S) = \ln I(0) - (2\pi S)^2 Rg^2/3$ gave 13.68±0.02 Å at 0.1 MPa, which is consistent with the value reported previously [7]. The data plotted in a Guinier plot (Fig. 3(b)) showed good linearity and no indication for systematic deviation over observed pressure range. We therefore concluded that the quality of the data obtained from 1.0 mg/ml was sufficient for quantitative analysis up to 500 MPa. This concentration range is about 1/10 of other high pressure probes, such as NMR [8] and FT-IR [9]. A lower concentration is always preferable in order to remove aggregation effects, which often lead to misinterpretation of the data. As a result, it will offer a high-pressure probe for a wider range of application, which may include unstable proteins.

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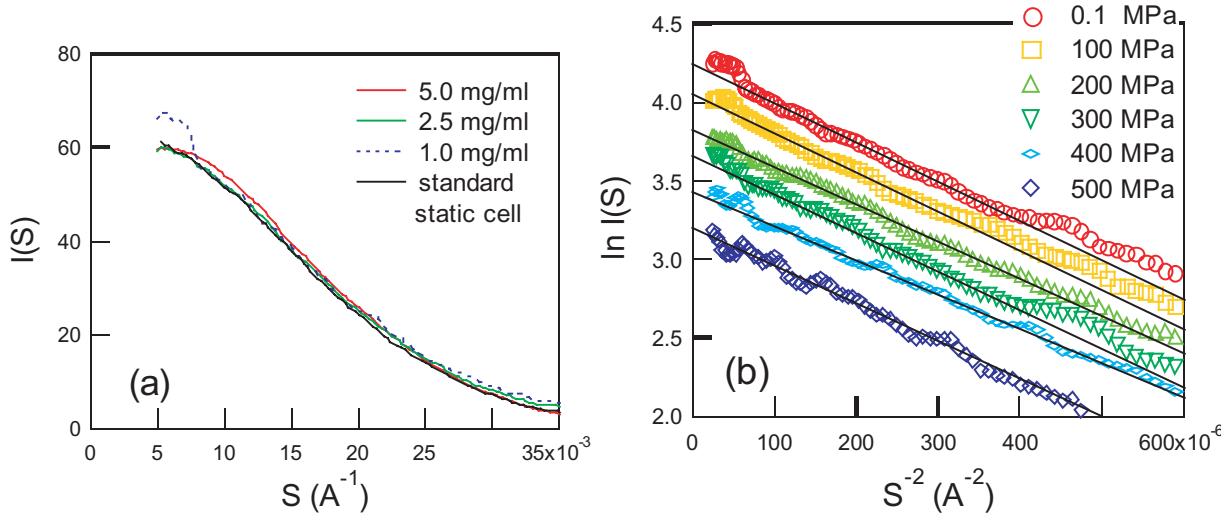

Fig. 3. (a) Concentration effects on the scattering profiles. (b) Guinier plots for the data obtained from 1.0 mg/ml.