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The influence of a medium pressure on the structure and dynamics of a bovine pancreatic trypsin inhibitor protein

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Abstract

A new hydrostatic pressure cell designed for small-angle neutron scattering (SANS) and quasi-elastic neutron scattering (QENS) experiments on biomolecular solutions has been developed at the Laboratoire Léon Brillouin. SANS and QENS experiments on bovine pancreatic trypsin inhibitor (BPTI), a small protein, have been performed and show us that this pressure cell is well adapted to this kind of experiments.

Interesting results about the influence of pressure (1–6200 bar) on structure and dynamics of BPTI have been obtained.

1. Introduction

The mechanisms allowing proteins to have a well-defined structure are at the origin of the folding process and are not yet well understood; the relation between the sequence in amino acid and the secondary and tertiary structures (local structure and global conformation) is not fully established. The study of the structure and the dynamics of proteins as a function of various thermodynamic parameters (temperature, pressure, pH, viscosity) is a necessary way to understand them.

While the temperature produces simultaneous changes in both volume and thermal energy, high pressure applied on protein solutions perturbs the environment in a continuous controlled way by changing only intermolecular distances [1]. Within a range of a few kilobar, the unfolding of proteins induced by the pressure is widely reversible. Therefore, pressure is a suitable parameter for the study of folding and unfolding pathways.

In this purpose, we have performed small-angle neutron scattering (SANS) and quasi-elastic neutron scattering (QENS) experiments on bovine pancreatic trypsin inhibitor (BPTI), a small protein of 58 amino acid residues (6.5 kDa). The crystallographic structure of BPTI has

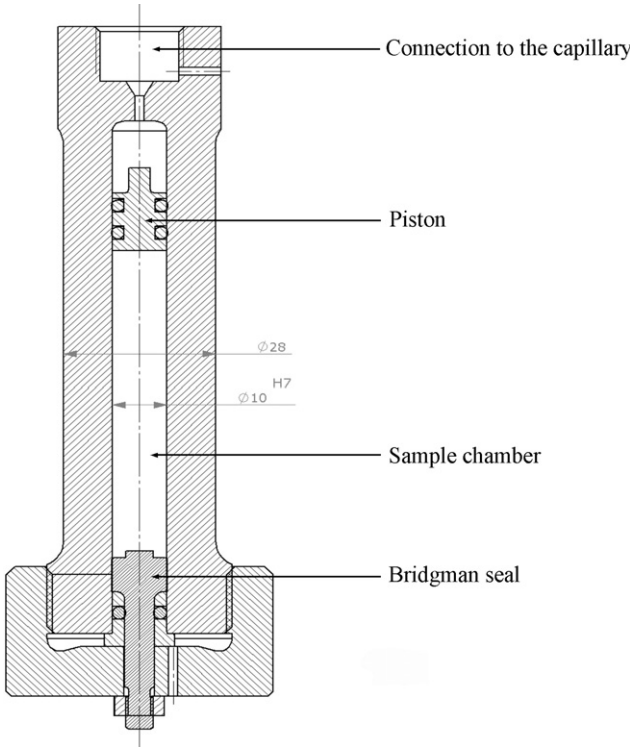

Figure 1. Scheme of the CuBe pressure cell.

been resolved at 1.5 Å resolution [2]. This protein contains three disulfide bridges, which gives its great stability. Because of this stability, pressures as high as 12 kbar are requested to unfold this protein. During these experiments the applied pressure has been limited to 6200 bar.

2. Materials and methods

2.1. A new pressure cell

To perform high-pressure experiments, a new hydrostatic pressure cell designed for SANS and QENS experiments has been developed at Laboratoire Léon Brillouin (LLB) (see figure 1). This cell is made with a CuBe alloy (98%Cu–2%Be), the neutron scattering cross-sections of which are given in table 1. It has an internal diameter of 10 mm and an external diameter of 28 mm. This design allows a maximum pressure of 7000 bar. The cell can contain up to 7.4 ml of solution, but a cylindrical insert of 5 mm has been put inside the sample chamber to reduce the sample volume. The cell was connected to the hydraulic pressurizing system via a capillary; the pressurizing medium was liquid D_2O. The tightness is ensured by a double Bridgman seal. At the end of each pressure experiment, the concentration of the solution is measured by UV–visible absorbance spectroscopy.

This pressure cell has been especially developed for studying protein folding and for comparing the conformation and dynamics of native and pressure-denatured states of proteins in solution. It has been optimized to allow both SANS and QENS experiments. With this cell, pressure experiments on NEAT multichopper spectrometer (HMI, Berlin) have been

Table 1. Neutron scattering cross-sections of the constituents of the cell. The calculated transmission at 2 Å for the SANS experiment is $T = 0.15$.

Element	σ_{coh} (barn)	σ_{inc} (barn)	σ_{abs} at 1.8 Å (barn)
Cu	7.49	0.55	3.78
Be	7.63	1.8×10^{-3}	7.6×10^{-3}
Alloy	7.49	0.54	3.70

successively performed at a given pressure using the two configurations of the spectrometer: SANS with the multidetector and time of flight (TOF) configurations. These two configurations have been described by Ruffle *et al* [3].

2.2. *Experimental conditions and sample description*

The sample was a BPTI solution at 85 mg ml^{-1} in 50 mM acetic acid buffer (D_2O) with 100 mM of $(NH_4)_2SO_4$. This buffer has been chosen to avoid aggregation and intermolecular interactions. SANS measurements at ambient pressure in classical Hellma cell have established that the solution is monodisperse [4].

Concerning QENS experiments, we have measured, respectively, at atmospheric pressure, the spectrum of the empty cell and that of a vanadium sample to get the resolution function of the spectrometer and to normalize the intensities of the spectra. Spectra of the solution and of the buffer have been measured at each pressure and at room temperature.

Concerning SANS experiments, we have measured, respectively, at atmospheric pressure, the spectrum of the empty cell and that of an H_2O sample to normalize the intensities of the spectra. Spectra of the solution and of the buffer have been measured at each pressure and at room temperature.

So, we have been able, by performing the adequate transmission corrections and subtractions of the buffer, to obtain the quasi-elastic contribution and the SANS contribution of BPTI in solution. We have worked at room temperature and at pressures of 1 bar, 3 kbar, 5 kbar and 6 kbar on IN5 (ILL, Grenoble) and NEAT spectrometers. We have also made measurements by going back to atmospheric pressure in order to test the reversibility of the phenomena. The QENS measurements were carried out with a resolution of 100 μeV on both IN5 and NEAT (HMI, Berlin) spectrometers. The calculated transmissions at 5.2 Å for the sample and for the buffer are respectively 0.658 and 0.724.

2.3. *Corrections, subtractions and data treatment*

The transmission corrections for the SANS and QENS spectra of BPTI in solution were undertaken according to adapted standard procedures available at LLB, ILL and HMI.

Examples of QENS spectra, after vanadium normalization, are displayed at the top of figure 2. There is a significant difference between the intensities of these spectra. Thus the subtraction of the empty pressure cell from the BPTI solution can be properly done (see bottom of figure 2). SANS spectra of the empty pressure cell and of the BPTI solution are displayed in figure 3. The empty cell contribution is here a large part of the signal, but still the subtraction can be properly done. It appears that the pressure cell is well adapted to perform both QENS and SANS experiments on NEAT spectrometer.

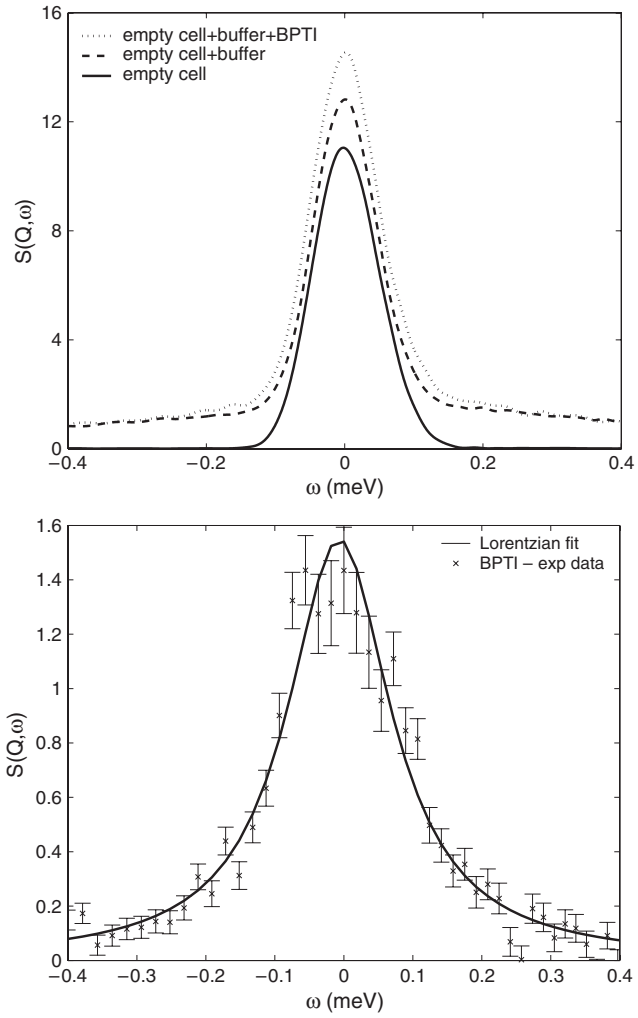

Figure 2. Top: spectra of the empty pressure cell, of the buffer in the cell and of the BPTI solution in the cell. Bottom: spectrum of the BPTI solution after subtraction of CuBe cell. The resolution is 100 μeV and the wavevector is 0.9 Å^{-1}. These data have been recorded using NEAT TOF configuration at ambient pressure.

3. Results

3.1. Quasi-elastic neutron scattering experiments

After subtraction of the buffer contribution to the solution spectrum, the incoherent dynamic structure factor of BPTI, $S(Q, \omega)$, is obtained at each pressure (IN5 and NEAT spectrometers). A fast Fourier transform procedure leads to the incoherent intermediate scattering function $S(Q, t)$. We have fitted these $S(Q, t)$ using the following model:

$$S(Q,t) = DW(Q)(A_0(Q)\mathrm{e}^{-t/\tau_1} + (1 - A_0(Q))\mathrm{e}^{-t/\tau_1}\mathrm{e}^{-t/\tau_2}) \tag{1}$$

where $DW(Q)$ is the Debye–Waller factor, $A_0(Q)$ is the elastic incoherent structure factor (EISF), $\tau_1(Q)$ is the characteristic time of translational diffusion and $\tau_2(Q)$ is the characteristic

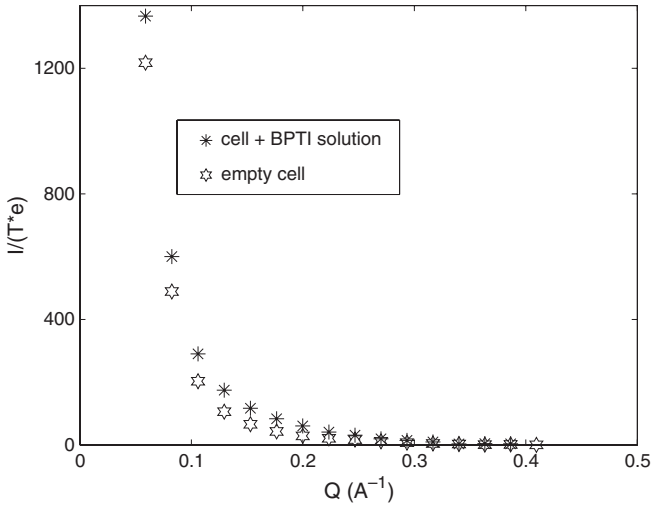

Figure 3. Spectra of the empty pressure cell and of the cell filled with BPTI solution (NEAT SANS configuration using the multidetector). Each spectrum has been normalized with respect to the transmission coefficient T and the thickness of the cell e.

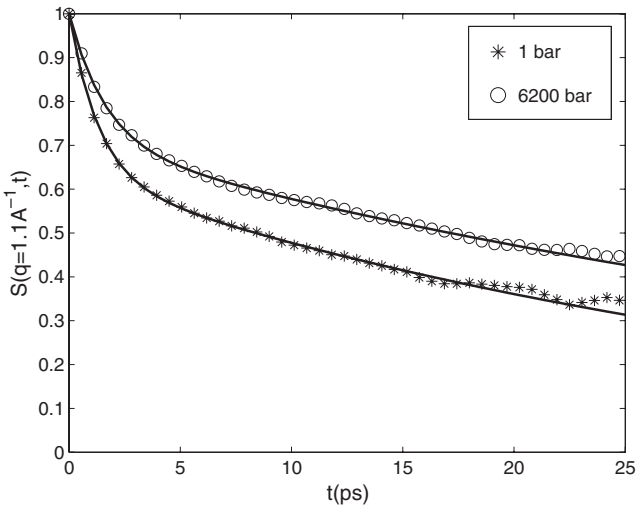

Figure 4. Incoherent intermediate scattering function $S(Q = 1\,\text{Å}^{-1}, t)$ of BPTI at ambient pressure ($*$) and at 6200 bar (O) fitted with the model given in equation (1) (full line).

time of internal motions [5]. Examples of fit are shown in figure 4. One can see a significant pressure effect on the intermediate scattering function $S(Q, t)$. For each pressure, the evolution of each characteristic time has been obtained as a function of Q. As shown in figure 5, $1/\tau_1$ is proportional to Q^2, giving access to the translational diffusion coefficient D. The values of the diffusion coefficient obtained at ambient pressure respectively on IN5 and NEAT spectrometers are close: $18.9 \pm 0.4 \times 10^{-7}$ cm^2 s^{-1} for the IN5 experiment and $17.8 \pm 0.6 \times 10^{-7}$ cm^2 s^{-1} for the NEAT experiment. In the case of a 65 mg ml^{-1} solution in H_2O, the diffusion coefficient has been found to be equal to $12.0 \pm 0.2 \times 10^{-7}$ cm^2 s^{-1} [6]. As expected, the diffusion coefficient decreases when the pressure is increased.

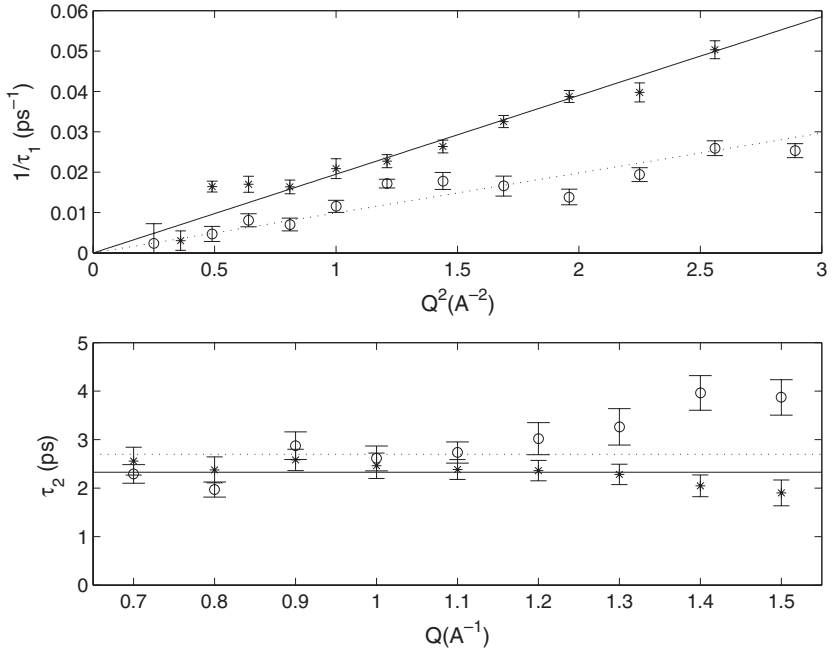

Figure 5. Evolution of τ_1 and τ_2 of BPTI in solution as a function of the wavevector Q at ambient pressure ($*$) and at 6 kbar (O). These values are extracted from the QENS experiment on IN5.

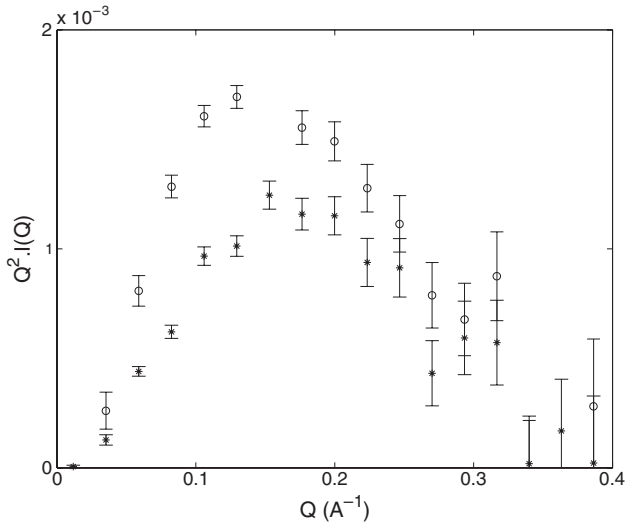

Figure 6. Kratky plot of the spectra of BPTI at ambient pressure (stars) and at 6 kbar (O).

The characteristic time τ_2, which is almost independent of Q (figure 5), is the characteristic time of internal motions. The average value of τ_2 varies between 2.2 and 2.7 ps when the pressure is raised up to 6200 bar. This demonstrates that the effect of pressure is to slow down internal motions in BPTI.

3.2. Small-angle neutron scattering

In the Guinier regime [7], the radius of gyration of BPTI in solution has been obtained and its evolution as a function of pressure determined. The value of the radius of gyration at room temperature and at ambient pressure ($R_g = 15.3$ Å) is similar to that obtained in previous SANS experiments using a classical Hellma cell. By using a Kratky representation [8] (figure 6), modifications of the conformation of BPTI between 1 bar and 6 kbar have been detected.

4. Conclusions

Despite the high resolution of the QENS experiments, characteristic times τ_2 of 3 ps (ten times lower than the resolution) have been extracted. We have been able to determine the diffusion coefficient of BPTI in solution with a reasonable accuracy. The value of the diffusion coefficient decreases when the pressure increases up to 6200 bar while the relaxation time τ_2 increases, which demonstrates that the effect of pressure is to slow down internal motions in BPTI. From the analysis of SANS spectra and in the Guinier regime, the radius of gyration of BPTI in solution has been obtained and its evolution as a function of pressure determined. Moreover, using a Kratky representation, modifications in the conformation of BPTI have been detected.

A new pressure device allowing to reach pressures up to 14 000 bar is under development.

Acknowledgments

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