H2. INFLUENCE OF TEMPERATURE AND PRESSURE ON STRUCTURE AND DYNAMICS OF A MODEL PROTEIN BELONGING TO THE REGULATION OF THE ENZYMATIC CATALYSIS : THE BOVINE PANCREATIC TRYPSIN INHIBITOR : A NEUTRON SCATTERING STUDY

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Motions in proteins occur at different time scale from millisecond for enzymatic reaction to femtosecond for electronic transitions. Quasielastic Neutron scattering allows to probe picosecond to nanosecond time scale internal motions [1].Bovine Pancreatic Trypsin Inhibitor is a small protein belonging to the enzymatic catalysis. This protein is a model system because of its small amount of residues (58 amino acid residues) and low molecular weight value (6500 Da), these characteristics allowed molecular dynamic simulation studies [2]. It was also studied by several other techniques : BPTI has a very high stability since it cannot be denatured at temperature below 95°C as it have been shown by Raman spectroscopy [3] or at pressure below 14 kbar as shown by Fourier Transform Infrared spectroscopy [4,5]. This stability is due to the presence of three disulphide bridges and three salt bridges. We have studied the structure and the dynamics of native state and thermal [6] and pressure [7] denatured states of BPTI by neutron scattering technique. For our high pressure study, we used a hydrostatic pressure cell developed at the Laboratoire Léon Brillouin [8].

The structural investigation by small angle neutron scattering allowed us to observe an increase of the radius of gyration of the protein in solution at 95°C and a reduction of this radius under 6000 bar. (Figure 1)
The ellipsoidal shape of the molecule in the native state do not change between 22°C et 95°C but we have observed an increase of the volume of BPTI. Indeed, the shape of BPTI is modified from an ellipsoidal one to a spherical one at 3000 bar, while it is well represented by a micelle when applied pressure values reach 5000 and 6000 bar. (Figure 2)
Further experiments by infrared spectroscopy and by UV-visible spectroscopy as a function of temperature and pressure allowed us to confirm our results [6].
Quasielastic neutron scattering allowed us to observe an opposite effect of temperature and pressure on translational diffusion coefficient and internal relaxation time of BPTI in solution (Figure 3). Increasing temperature induces a faster dynamics of these global and internal motions whereas increasing pressure induces a slowing down of these motions.

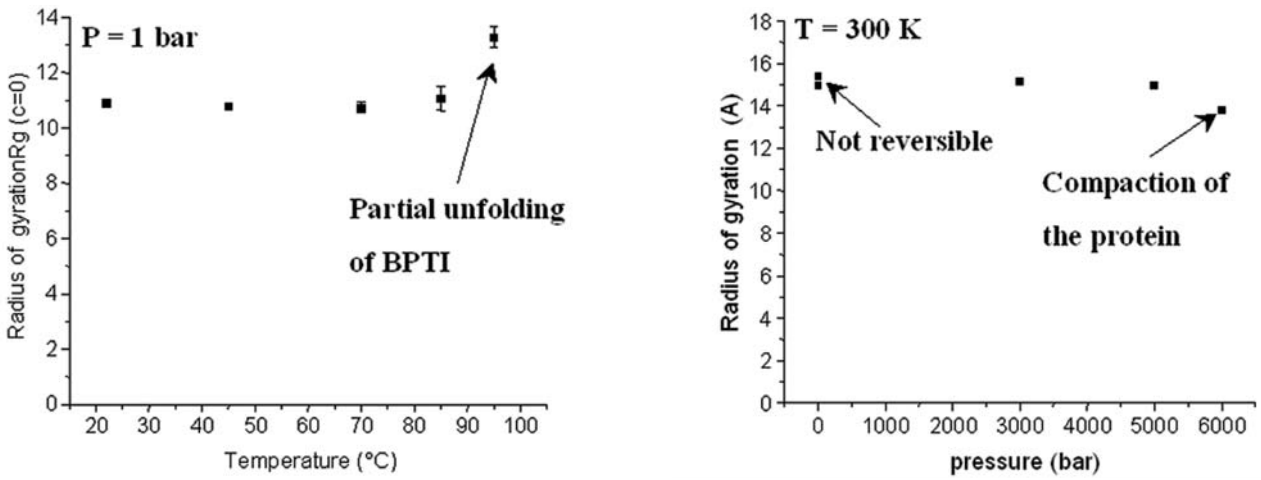

Figure 1 : Evolution of the radius of gyration of BPTI as a function of pressure (left) and as a function of temperature (right).

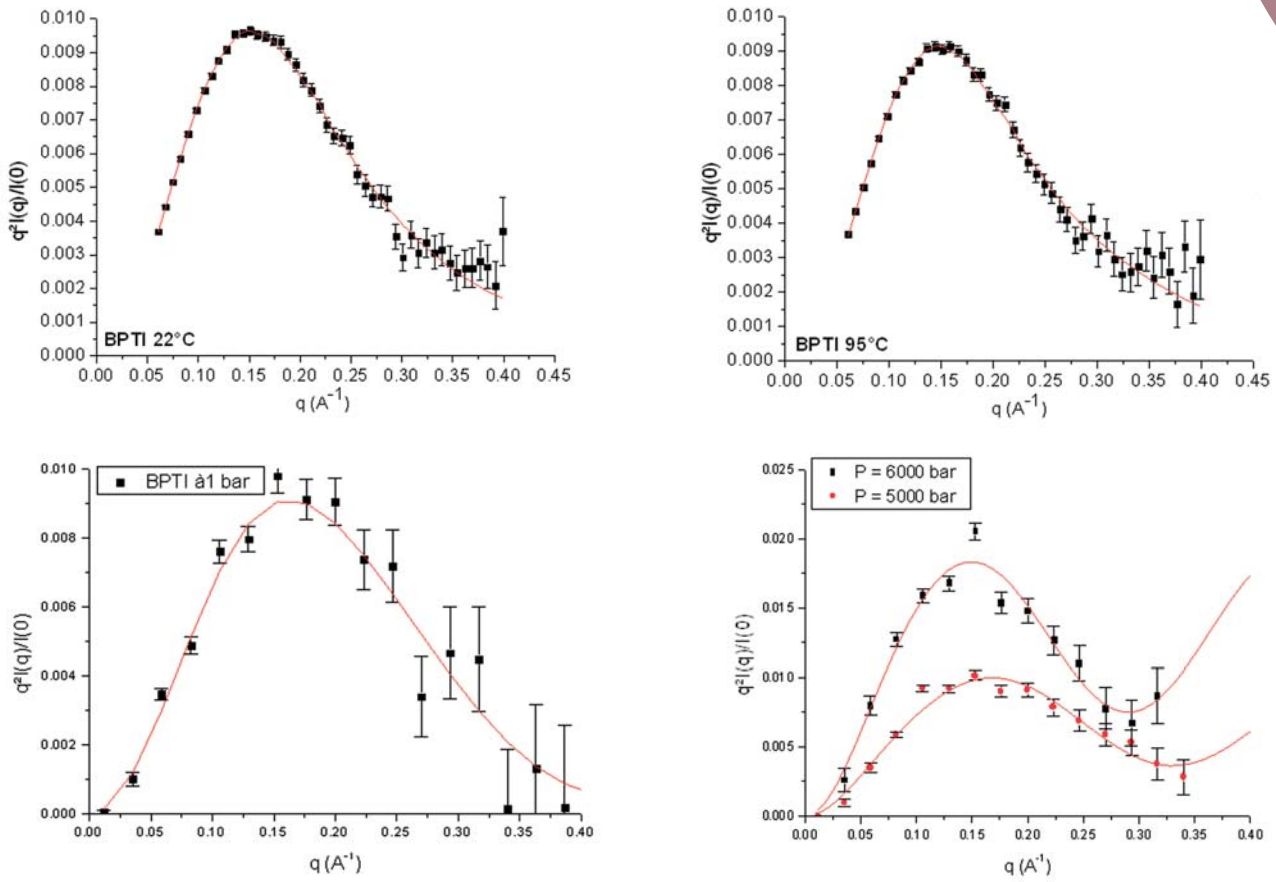

Figure 2 : Kratky plot of SANS spectrum for BPTI at ambient and high temperature (top) and at atmospheric pressure and high pressure (bottom).

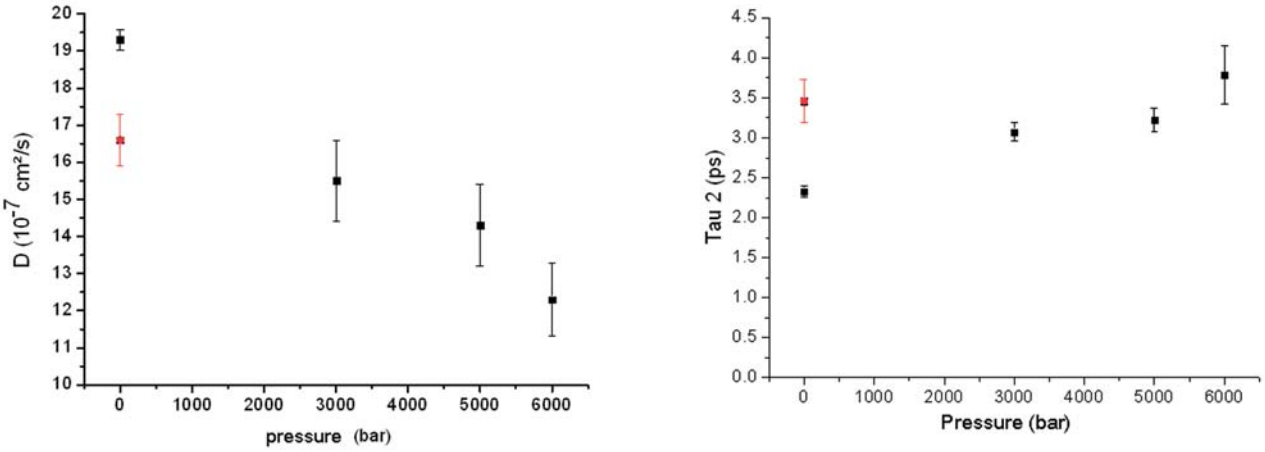

Figure 3 : Effect of pressure on global (left) and internal motions (right)

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