MEASUREMENT OF THE ISOTHERMAL COMPRESSIBILITY OF HYDRATED MYOGLOBIN BY SMALL-ANGLE NEUTRON SCATTERING

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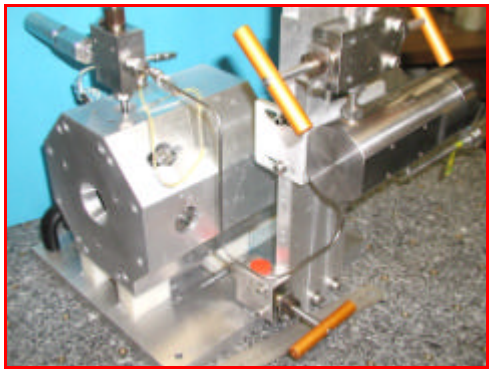
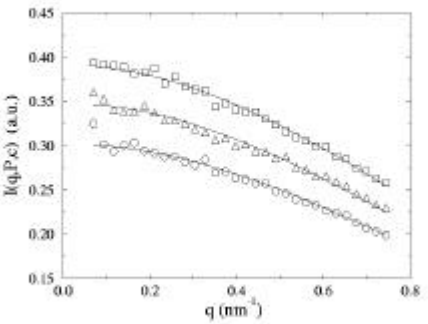
The isothermal, k_T, and adiabatic, k_S, compressibilities are important quantities because their values give an estimate of the magnitude of the different type of movements in a system. k_S is proportional to the amplitude of longitudinal phonons whereas $(k_T - k_S)$ is proportional to that of the diffusive motions associated with heat diffusion. Therefore the measurement of the isothermal compressibility of a protein provide information on the amplitude of its internal density fluctuations. These fluctuations may modulate its function.

Proteins are also sensitive to pressure. For instance binding of a ligand to a protein is affected by pressures lower than 400 MPa. Furthermore protein denaturation and unfolding may occur at higher pressures. The effects of pressure on hemeproteins have been the subject of numerous investigations. Optical absorption, fluorescence, Fourier-transform infrared, Raman, and nuclear magnetic resonance spectroscopies, and laser flash photolysis have all shown that pressures near 300 MPa leads to subtle local rearrangements of the protein structure and that some intermediate states preceding unfolding probably appear. Therefore is it important to determine whether the modifications observed at the level of the active site of myoglobin (Mb) and the reorganization of the secondary structure are related to a change in the tertiary structure of the protein.



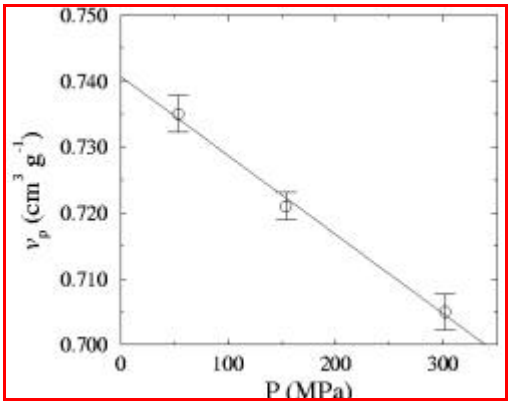
To this end small-angle neutron scattering (SANS) experiments were carried out at room temperature on pD 6.6 solutions of horse heart azidometmyoglobin (MbN_3) at pressures up to 300 Mpa [1]. The measurements were performed using various concentrations of MbN_3 in order to determine the second virial coefficient of the protein solution and the actual radius of gyration of the protein. The results shows that the interactions between the macromolecules are always strongly repulsive, even if their magnitude decreases with increasing pressure, whereas the radius of gyration of the protein remains constant. This indicates that the compactness of MbN_3 is not significantly altered by pressures up to 300 MPa. However it is possible that a molten globule forms at the highest pressures. This structural change cannot be observed by means of SANS experiments [2].



Scattering spectra I(q,P,c) of MbN_3 at p^2H 7, as a function of the wave-number transfer q. The measurements were performed at 20°C. The protein concentration at atmospheric pressure (0.1 MPa) was c(0.1) = 11.7 mg cm^{-3} and the pressures, P, were: 54 (?), 154 (?), and 302 Mpa (?). Fits of the Guinier approximation to the data are shown as full lines.

Taking advantage of the pressure-induced contrast variation of the protein these experiments allow the partial specific volume, v_p, of MbN_3 to be accurately determined as a function of pressure. It is found that v_p = (0.741 ± 0.003) cm^3 g^{-1} at atmospheric pressure and that its value decreases by about

5.4% at 300 MPa. In this pressure range the isothermal compressibility of hydrated MbN_3 is found to be $(1.6 \pm 0.1)\ 10^{-4}$ MPa^{-1} at about 20°C. Therefore, hydrated MbN_3 is about two to three times as incompressible as light or heavy water at the same temperature.



Densimetry would have seem to be the simplest way to measure the isothermal compressibility of a protein. However the only suitable commercial densimeter available today does not allow such measurements to be carried out at pressure higher than 100 Mpa. SANS allows much higher pressures to be reached with a comparable accuracy on the partial specific volume measurements. A new high-pressure cell has already been tested at pressures up to 1 Gpa, pressure at which water freezes at room temperature. It is going to be used in future experiments on proteins.

References

[1] C. Loupiac, M. Bonetti, S. Pin and P. Calmettes, *Eur. Jour. Biochem.* **269** (2002) 4731.
[2] D. Russso, D. Durand, P. Calmettes and M. Desmadril, *Biochemistry* **40**, (2001) 3958.