Experimental report

18/12/2018

Proposal: CRG-2535 **Council:** 4/2018

Title: Pressure effects on the thermal denaturation of Myoglobin: role of protein dynamics and resilience

Research area:

This proposal is a new proposal

Main proposer: Fabio LIBRIZZI

Experimental team: Aline CISSE
Bastien GERVASONI
Judith PETERS
Antonino CALIO
Fabio LIBRIZZI

Local contacts: Judith PETERS

Samples: myoglobin at different hydration levels in D2O

Instrument	Requested days	Allocated days	From	To
IN13	7	7	03/09/2018	11/09/2018

Abstract:

Proposal CRG-2535 on IN13, 03 – 10/09/2018
F. Librizzi, R. Carrotta, A. Cupane, A. Calio, J. Peters

It has long been known that exposure of proteins to high pressure conditions leads to their so-called cold denaturation. In spite of the very large number of studies dedicated to it, this phenomenon (of extreme biophysical and biotechnological relevance) is not yet thoroughly understood. Clearly, pressure effects are related to volume variations upon protein denaturation; however, the volume change can have several contributions arising mainly from elimination of cavities, exposure of polar/nonpolar groups, structure of hydration water, electrostriction, hydrophobic interaction etc. The problem is that these contributions can have opposite signs while being of the same order of magnitude, so that a generally accepted picture of pressure effects on protein folding/unfolding has not been reached yet (for discussions on the various contributions to the pressure effect see refs. [1] and [2] and references therein).

We recently studied the effect of pressure on the dynamical properties of myoglobin and measured the mean square displacements (MSD) of the hydrogen atoms of the protein as a function of both temperature and pressure [3]. This enabled us to evidence pressure effects on the energy landscape of myoglobin. Based on these findings, our working hypothesis about the pressure effects on protein denaturation is that a relevant role might be played by protein dynamics and in particular by the protein resilience, as defined by J. Zaccai in his 2000 seminal paper on protein softness measured by elastic neutron scattering [4]. To get experimental evidence in favor (or against) our hypothesis we proposed now to perform EINS measurements on myoglobin hydrated powders in the temperature interval 273-363 K and in the pressure range $P_{ATM} < P < 5$ kbar. To assure a good pressure transmission, the sample was in contact with the liquid used for it, Fluorinert^{TM}.

The proposal received 8 days beamtime on IN13 and the experiments have been performed in September 2018. Figure 1 reports the normalized scattering intensity as a function of temperature at four pressure values, integrated in the q range from 0.19 to 2 Å^{-1}.

At low temperatures the behaviour of the 0 and 1 kbar curves is similar, while the 2 and 3 kbar curves show a bump in the region around 300 K. In the denaturation region (above 330 K), the behaviour changes: intensity is decreasing with pressure increasing from 0 to 2 kbar, and it increases again at 3 kbar. This indicates that, when denatured, protein molecules become more flexible at higher pressure only up to 2 kbar, and stiffer again at 3 kbar.

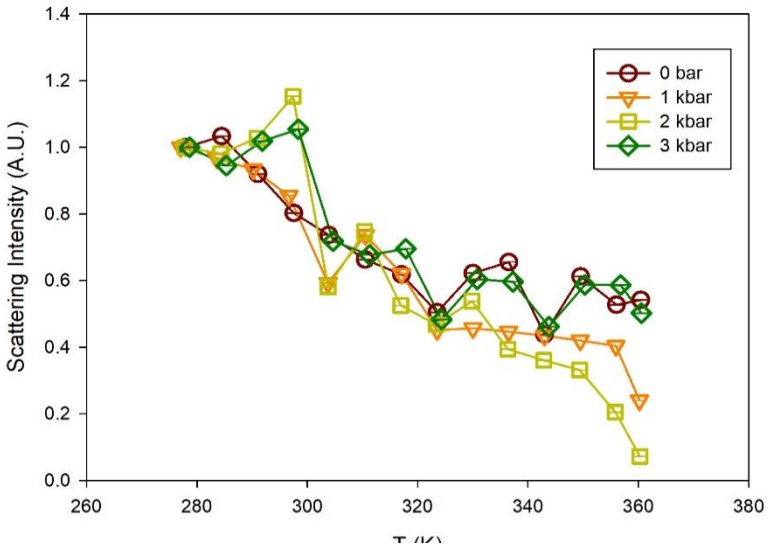

Figure 1: Normalized summed intensities of myoglobin as function of temperature and pressure.

This is somewhat counterintuitive, since the most observed effect of pressure is the reduction of protein dynamics. It could be explained by supposing the existence of two populations of protein, native and denatured, which presents different dynamical properties: pressure could lead to the increase of the population in the denatured state, and thus to the decrease of the integrated intensity.

Moreover, 3 kbar is believed to be the necessary pressure to achieve a complete denaturation. In fact, we observe here an increase in the integrated intensity, meaning that the complete population transfer between the native and denatured state has been achieved somewhere between 2 and 3 kbar, so the further increase of pressure is limiting the dynamics of the totally denatured sample.

We did in parallel an investigation by Differential Scanning Calorimetry (DSC) at the ILL. The denaturation temperature in Fluorinert was found to be shifted with respect to the one in D_2O, and the peak area was much lower (see Figure 2). This could be due to the hydrophobic nature of Fluorinert. Since denaturation causes the exposure to the solvent of hydrophobic residues of the protein, the interaction with a hydrophobic environment could lower the thermal energy needed to activate the unfolding transition. Moreover, an unknown peak appeared in the 300-320 K region, similarly to the bump seen in neutron data. It was certainly not related to denaturation, since it appeared also in the scans done on the samples after the neutron experiments, while the denaturation peak did not. The origin of this peak was not clear, but we suspect un unexpected interaction with Fluorinert.

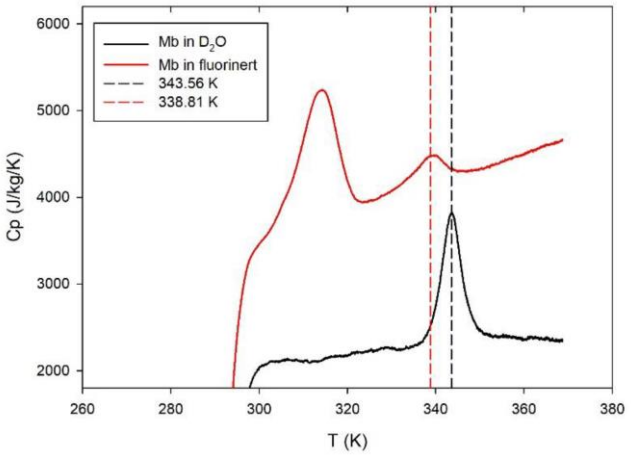

Figure 2: Thermograms for native Myoglobin in Fluorinert™ and in D_2O.

We investigated also the behaviour of Lysozyme by DSC in presence or not of Fluorinert and we did not observe the anomalous peak due to Fluorinert. This indicates that analogous experiments can be performed with Lysozyme rather than Myoglobin to avoid such effects.

References

[1] C.A. Royer, Biochim. Biophys. Acta 1595 (2002), 201-209.
[2] J.R. Grigera & A.N. McCarthy, Biophysical J. 98 (2010), 1626-1631
[3] F. Librizzi, R. Carrotta, J. Peters and A. Cupane, Scientific Reports 8 (2018), 2037.
[4] G. Zaccai, Science 288 (2000), 1604-1607.