Experimental report

14/06/2016

Proposal: 8-04-737 **Council:** 10/2014

Title: Beta-Lactoglobulin partial and kinetical unfolding process probed through dynamics under high pressure.

Research area: Physics

This proposal is a continuation of LTP-8-4

Main proposer: Jeremie MARION

Experimental team: Judith PETERS
Jeremie MARION

Local contacts: Jacques OLLIVIER

Samples: Protein Beta-Lactoglobulin in heavy water

Instrument	Requested days	Allocated days	From	To
IN5	3	2	29/04/2015	01/05/2015

Abstract:

The protein folding/unfolding process represents today in science a deep open question. We thus decided to investigate the unfolding process of beta-lactoglobulin (LG) which involves different steps and did already preliminary studies under high pressure. Within the framework of the long term proposal LTP 8-4 (see ILL report LTP 8-4) we probed the protein on D16 and IN16 under high pressure and got interesting results with respect to the large scale structure and dynamics. Some of the involved processes take a rather long time to be fully achieved (several hours or days). This emerged through changes over time during measurements at a specific pressure and temperature. Indeed several competitive effects may participate to the unfolding mechanism as aggregation, partial unfolding and water insertion. We thus wish to further investigate the system to better understand the consecutive steps and their meaning.

Beta-Lactoglobulin partial and kinetical unfolding process probed through dynamics under high pressure

Experiment 8-04-737 on the instrument IN5: 29 – 30/04/2015 (wavelength 10 Å)

Experimental team: Jérémie Marion, Judith Peters, *Local contact*: Jacques Ollivier

The protein folding/unfolding process represents a debated question in science. We thus decided to investigate the unfolding process of beta-lactoglobulin (βLG) which involves different steps as we did already preliminary studies under high pressure. Within the framework of the long term proposal LTP 8-4 (see ILL report LTP 8-4) we probed the protein on D16 and IN16 under high pressure and got interesting results with respect to the large scale structure and dynamics. Some of the involved processes take a rather long time to be fully achieved (several hours or days). This emerged through changes over time during measurements at a specific pressure and temperature. Indeed, several competitive effects may participate to the unfolding mechanism as aggregation, partial unfolding and water insertion. We thus wish to further study the system to better understand the consecutive steps and their meaning.

The protein βLG presents a molten globule state (1,2) known as a change in the global structure with partial unfolding of the molecule, but without complete denaturation. To better understand such intermediate meta-stable state, we did a first experiment in 2012 on D16 under high pressure (3). It occurred that the process was lasting over a broad pressure range to reach its final state of (partial) unfolding. The main question is thus to better understand the evolution of the process and how much time of pressure exposure is needed to reach this state. We did furthermore an experiment on IN16 (3) to clarify (through quasi-elastic and elastic scans) how the dynamics of the protein in solution behaves at the specific pressure points where the curve of the radius of gyration showed inflexion points (around 2700 and 4000 bar). For that we scanned the pressure range from 1 bar to 5000 bar using elastic incoherent neutron scattering and quasi-elastic neutron scattering (QENS) at the four specific points of 1, 2700, 4000 and again 1 bar. The QENS intensity was rather low due to the highly absorbing pressure cell and to the fact that half of the analyser crystals were already transferred to IN16B. As consequence it was almost impossible to reasonably analyse the QENS data, however the long duration measurement at these specific pressure points revealed an important increase in intensity over time in the elastic incoherent scattering, corresponding to a high dynamical modification of the system due to relaxation. Therefore, we repeated now this measurement on IN5 at the same energy resolution as available on IN13 to gain in flux and to follow the evolution of the intensity as function of time at the same points of 1, 2700 and 4000 bar.

During the experiment, we had problems with leakage of the high pressure cell. Anyway we analysed the QENS curves (see figure 1) to see if we were able to detect effects of pressure and in time. For that, the IN5 QENS spectra were fitted by sum of a Gaussian elastic peak, two Lorentz peaks and a flat background. The first Lorentz1 peak is rather sharp, with a width which remains in the range of 0.02 to 0.03 meV for all Q values. The second Lorentz2 peak is broad with a constant width of 0.244 meV for all Q values. We were unfortunately not able to see any influence due to pressure or time, probably due to the leakage problem.

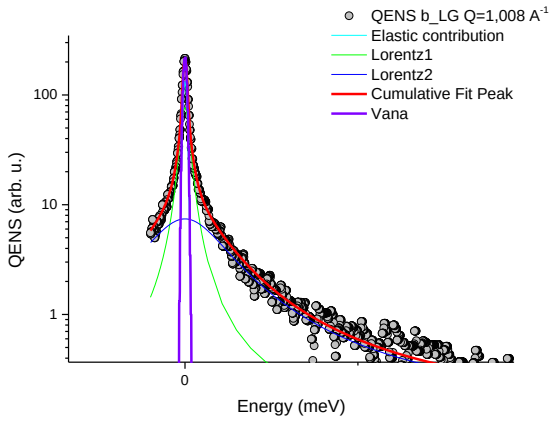

Figure 1. Fitting example of a QENS spectrum of βLG sample measured at Q=1.008 $Å^{-1}$ at 2700 bar.

References

(1) J. Yang et al., *J. Agric. Food Chem.* 2001, *49,* 3236-3243
(2) E.M. Dumay et al., *J. Agric. Food Chem.* 1994, *42,* 1861 -1868
(3) J. Peters et al., ILL LTP 8-4 report.