Experimental report

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Proposal: 8-04-809 **Council:** 4/2017

Title: Effect of pressure and osmolytes on the dynamics of oligomeric proteins

Research area: Biology

This proposal is a new proposal

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Samples: Trehalose
Alcohol Dehydrogenase equine
Trimethylamine N-oxide
Alcohol Dehydrogenase from Saccharomyces cerevisiae

Instrument	Requested days	Allocated days	From	To
IN16B	6	2	18/06/2018	20/06/2018

Abstract:

We plan to investigate the effect of pressure on the global and internal dynamics of two different alcohol dehydrogenases consisting of dimeric and tetrameric complexes. The results will yield novel insights how hydrostatic pressure affects the dynamics of these enzymes and how the global and internal dynamics differ between the tetrameric, the dimeric and the monomeric states, the latter being obtained after pressure-induced dissociation. Further, we plan to investigate how the dynamical properties are affected by binding of the cofactor nicotinamide adenine dinucleotide and by the presence of the compatible osmolytes, which are known to alleviate environmental pressure stress imposed on proteins.

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Effect of pressure on the dynamics of oligomeric proteins

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Lactate dehydrogenase catalyzes the interconversion of pyruvate to lactate during the anaerobic glycolysis. This tetrameric enzyme dissociates at pressures above approximately 1000 bar.[1] We wanted to investigate here the global as well as the internal dynamics of LDH at different degrees of oligomerization, which would enable us to gain information about how the dynamics of oligomers and monomers differ on different timescales, and how they affect their activity. Until now our research was mainly focused on the influence of pressure, cosolvents and crowding on the structure, stability and intermolecular interactions of globular proteins like lysozyme or SNase, as well as on enzyme kinetics using the high-pressure stopped-flow methodology [2-4]. We additionally investigated the pressure-induced depolymerization of aggregate structures [5-8].

In a previous study we were able to explore the effect of high hydrostatic pressure on the internal dynamics of a globular monomeric protein [9] by incoherent neutron scattering on IN13. Now we went one step further and investigated how the dynamical behaviour of an oligomeric protein and different states of oligomerization affect their pressure stability and activity. For that, we needed the time window to have access to the nanosecond scale as available on IN16B. The pressure variable was applied to change the oligomeric state of the protein, but also because pressure is able to modulate the temperature stability and activity of the enzyme. In contrast to what we wrote in the proposal, we had not enough time given to the project to study also the sample in the presence of osmolytes under pressure.

The experiment was undertaken on IN16B at constant temperature of 298 K. We used the high hydrostatic pressure equipment developed recently by J. Peters and the SANE group of ILL [10-11]. The sample in solution (90 mg mL^{-1} LDH, 25mM Tris, 10 mM DTT, 1mM EDTA) was exposed to pressure values of 20, 400, 800, 1200, 1600, 2000, 3000 and 4000 bar and measured for 2 h by QENS. Additional elastic measurements were also performed.

Figure 1 shows the mean square displacements (MSD) extracted from the elastic measurements and examples of QENS curves, corrected for the buffer, for various pressure values. The MSD are rather similar up to 2000 bar, with eventually a local minimum at 1200 bar, and they decrease drastically at 3000 and 4000 bar. We measured them also under pressure release, but only up to 2000 bar to avoid effects due to denaturation.
The QENS curves were normalised to unity at the maximum. The vanadium curve is also shown to illustrate the instrumental resolution function. All sample curves present a clear broadening compared to the vanadium curve, indicating the presence of QENS. One should notice that the reference curve in red at ambient pressure (20 bar) is below the curves at 1200 and 1600 bar, the range where we expect the oligomeric dissociation under pressure which occurs at 1200 bar [1]. Apparently, it leads to a higher flexibility of the sample and counterbalances the compression due to pressure application. At higher pressures of 3000 and 4000 bar, likely the effect due to high pressure prevails and reduces drastically the sample mobility. EINS and QENS data lead to very similar conclusions with this respect.

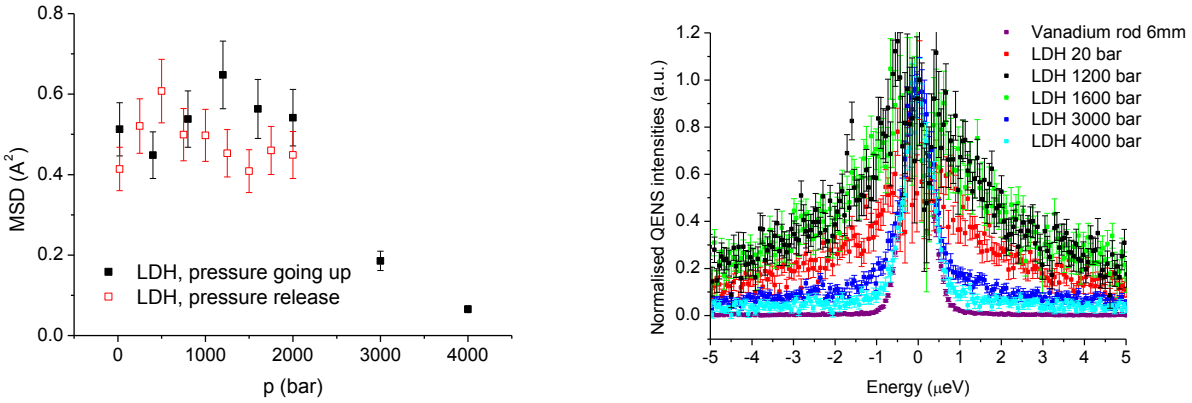

Figure 1: LDH in solution investigated at 298 K by EINS and QENS measurements on IN16B as a function of different hydrostatic pressures. The QENS intensities were corrected for the buffer and normalised to unity at the maximum.

More data analysis is actually under progress, especially concerning a precise analysis of the QENS to identify the motional processes present in the sample and to extract dynamical parameters such as diffusion coefficients, residence times etc.

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