Experimental report

08/04/2020

Proposal: 8-05-440 **Council:** 10/2018

Title: The influence of crowding, cosolvents and high pressure on protein dynamics

Research area: Biology

This proposal is a new proposal

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Samples: human butyrylcholinesterase
human butyrylcholinesterase + ammonium sulfate
juman butyrylcholinesterase + lithium thiocyanate

Instrument	Requested days	Allocated days	From	To
IN5	5	0		
IN13	7	7	10/09/2019	17/09/2019

Abstract:

The enzyme human Butyrylcholinesterase (hBChE) will be investigated in the presence or not of salts and as function of high hydrostatic pressure. The exact effects of lyotropic salts on the structure and dynamics of biological systems is a highly debated question, especially their potential to counteract environmental stresses as pressure. Therefore, we would like to study hBChE under various salt conditions on IN5 and IN13 and conduct molecular dynamics simulations in parallel in order to shed light on the effects of salts of the Hofmeister series on enzymes, water and water-ions interactions. Earlier experiments showed that significant effects can be expected for this enzyme.

Exp. Report 8-05-440

The influence of crowding, cosolvents and high pressure on protein dynamics

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For over a century, since Hofmeister's experiments concerning protein precipitation by the addition of salt to solutions [Hofmeister 1888], scientists have aimed to understand how cosolutes interact with biomolecules and affect their structure, dynamics and function. Indeed, cells are highly crowded environments (up to 400 g/L) with high content of macromolecules as proteins, nucleic acids, lipids and carbohydrates, but also 5 to 40% of cosolutes as metabolites or osmolytes. Almost all organisms accumulate such cosolvents inside their cells to prevent osmotic catastrophes from high salinity, dehydration, high hydrostatic pressure or the presence of a denaturant. For instance, the intracellular osmolytes in marine animals can reach total concentrations above 0.6 M to counter the osmotic pressure of ocean water. These osmolytes also protect cellular proteins against denaturation induced by extreme physical conditions. Crowders and cosolvents can thus be efficient means to counteract environmental stresses. This property has been exploited to prevent protein unfolding during lyophilisation, for example. A recent proof was given through a neutron scattering study on IN13 where cosolvents and crowding had a strong effect on dynamics and folding stability of lysozyme under high pressure [Al-Ayoubi 2017].

Salts are composed of ions bound through their charge interaction. It is generally accepted that ions affect the structure and dynamics of water on one side, but also protein's secondary, tertiary and quaternary structure and ion-water interactions. Although there is much experimental evidence about strong effects due to such cosolutes, no theory is able so far to describe completely the variety of the effects in a satisfactory way [Timasheff 1969, Xie 2013] and more investigations and molecular dynamics simulations are needed to better understand molecular details on how salts, small molecules and water interact with proteins and affect their structure and dynamics under different external conditions.

The protein we would like to investigate under various salt conditions is human Butyrylcholinesterase (BChE) [Chatonnet, 1989], because results by P. Masson et al. indicate clear enzymatic differences under several salt and high hydrostatic pressure (HHP) conditions [Masson, 2002 and 2013]. Moreover, we did recently measurements by high-pressure absorption spectroscopy together with A. Freiberg's group at Tartu University (Estonia) [Kangur 2019] on the native sample and samples in the presence of a chaotropic (LiSCN) and a kosmotropic (ammonium sulfate) salt, the latter in two different concentrations (see Figure 1). The full width half maxima (FWHM) of the spectra clearly demonstrate that the half-transition points (middle of the slope which indicates denaturation) is shifted in the presence of the salts: to a 1 kbar lower pressure by the destabilizing chaotropic salt LiSCN and to a 0.4 and 2.3 kbar higher pressure by the stabilizing kosmotropic salt ammonium sulfate at two concentrations. LiSCN could not be used at the higher concentration because it then denatured the enzyme immediately. These findings indicate a strong correlation between the presence of salt and the response to high pressure.

In the present experiment, we investigated how such salts at 0.1 M concentration can affect molecular dynamics of BChE by elastic incoherent neutron scattering (EINS) (see figure 2).

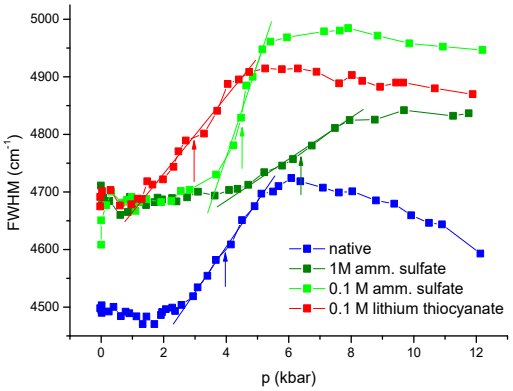

Figure 1 : FWHM of high-pressure absorption spectra of native BChE in Tris (50 mM, pH 7.5) buffer and BChE in the presence of salts at various concentrations. The arrows show the half-transition points.

The error bars are high as the HHP cell is particularly thick to withstand these extreme conditions. However, clear tendencies are evidenced: The native sample is almost insensitive to the external HHP conditions, probably due to the self-crowding, which has a protective effect [Al-Ayoubi 2017]. In the presence of 0.1 M ammonium sulfate the MSD are first slightly increasing up to 3 kbar indicating a stabilizing effect which permits enhanced mobility of the atoms despite the HHP and a decrease in mobility only after the denaturation sets in. In contrast, LiSCN destabilizes the protein already much earlier so that the MSD are starting to decrease immediately when pressure goes up.

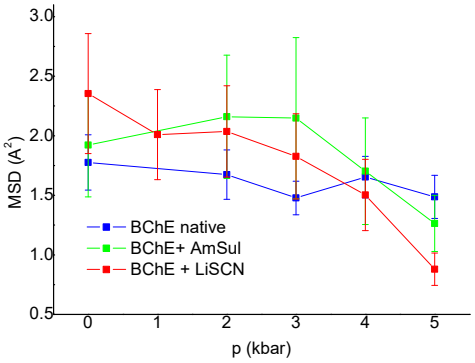

Figure 2: Mean square displacements (MSD) of the three samples measured in Tris buffer by EINS under HHP.

To identify more precisely which kind of motions are concerned by these effects, we underwent also quasi-elastic neutron scattering measurements on IN5 under HHP on the same kind of samples (Exp. 8-05-452). Data analysis is under progress and should permit to shed more light on the impact of these salts on molecular dynamics.

Literature

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