Experimental report

25/05/2020

Proposal: 8-05-452 **Council:** 4/2019

Title: The effect of co-solutes to counteract environmental stress conditions on proteins

Research area: Biology

This proposal is a new proposal

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Samples: hBChE + ammonium sulfate
hBChE + LiSCN
human Butyrylcholinesterase (hBChE)

Instrument	Requested days	Allocated days	From	To
IN5	3	2	01/08/2019	03/08/2019

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 by quasi-elastic neutron scattering 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.

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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. 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. 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 full width half maxima (FWHM) of the spectra clearly demonstrated that the half-transition points (middle of the slope which indicates denaturation) were shifted in the presence of the salts: to a 1 kbar lower pressure by the destabilizing chaotropic salt LiSCN and to a 0.4 kbar higher pressure by the stabilizing kosmotropic salt ammonium sulfate. 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 (pD 6.6) can affect molecular dynamics of BChE by quasi-elastic neutron scattering (QENS). To analyse the spectra, we used the following equation including local and global motions:

$$S(Q,\omega) = [A_0(Q)\delta(\omega) + \{1 - A_0(Q)\}L_{\text{local}}(Q,\omega)] \otimes L_{\text{global}}(Q,\omega) \otimes RF(Q) + BG,$$

where RF refers to the resolution function and BG to a background. The data treatment permitted to extract the following motional parameters of all samples: the apparent translational diffusion coefficient D_{app} at different pressures (see fig. 1), the residence time τ, the fraction of immobile atoms p and the radius a of a sphere delimiting the motions.

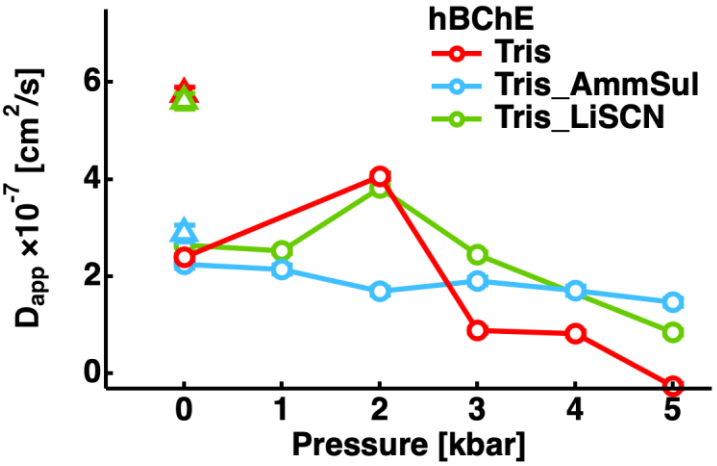

Figure 1: Translational diffusion coefficient of the three samples under HHP. The triangles correspond to return measurements after pressure release.

The diffusion decreases for both the native sample and the sample containing LiSCN above 2 kbar, but AmmSul weakens considerably the effect of pressure and has thus a protective effect. The triangles corresponds to data after pressure release. The diffusion coefficient is increased for the native enzyme and in the presence of LiSCN, but the original value is retrieved in the presence of AmmSul. Likely the enzyme is partially denatured after pressure application except in the presence of AmmSul which has a protective effect.

Concerning the fraction of immobile atoms p and the radius a, the two salts have opposite effects: AmmSul decreasing the immobile fraction and increasing the radius of the motions, LiSCN provoking in contrary more immobile atoms and less degrees of freedom for the motions (results not shown). We would like to complement these results with DSC and/or FTIR measurements before their publication.

Literature

[Hofmeister, 1888] F. Hofmeister, Arch. Exp. Pathol. Pharmakol. 24 (1888) 247.
[Al-Ayoubi, 2017] S. R. Al-Ayoubi, et al., Phys.Chem.Chem.Phys., 2017, 19, 14230.
[Timasheff, 1969] S.N. Timasheff, G.D. Fasman, *Structure and Stability of Biological Macromolecules*, Dekker, New York, 1969.
[Xie, 2013] W.J. Xie, Y.Q. Gao, Phys. Chem. Lett. 4 (2013) 4247.
[Chatonnet 1989] A. Chatonnet and O. Lockridge, Biochem J. 1989 Jun 15; 260(3): 625–634.
[Masson, 2002] P. Masson et al., BBA 1597 (2002) 229.
[Masson, 2013] P. Masson et al, 2013, Biochem. J, 454 (2013) 387-399.
[Kangur, 2017] L. Kangur, K. Timpmann, D. Zeller, P. Masson, J. Peters and A. Freiberg, BBA – Proteins and Proteomics **1867** (2019), 107 – 113.