Experimental Report

Proposal: **8-04-707** **Council:** 10/2012

Title: Acetylcholinesterase investigated under osmotic pressure

This proposal is a new proposal

Researh Area: Biology

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Samples: Acetylcholinesterase in presence of glycerol or saccharose

Instrument	Req. Days	All. Days	From	To
IN16	4	4	17/07/2013	26/07/2013
IN13	6	4	25/07/2013	29/07/2013
IN6	6	4	05/07/2013	09/07/2013

Abstract:

The enzyme acetylcholinesterase (AChE) plays an important role in the nervous system of animals. By rapid hydrolysis of the neurotransmitter acetylcholine, AChE terminates neurotransmission at cholinergic synapses. We already studied the influence of an inhibitor on the dynamics of the enzyme on several spectrometers of the ILL (IN6, IN13 and IN16) [1] and we are currently investigating the effect of high hydrostatic pressure on the dynamics and the structure of this protein [2]. However, up to now all studies were based on the assumption that the solvent was an ideal dilute substance treated as a heat bath. But enzymes found in organisms adapted to very low (psychrophiles) and very high (thermophiles) temperatures are also subjected to variable solute concentrations and viscosities [3]. We now wish to explore the effect of osmotic pressure, which can be obtained by using water-cosolvent mixtures to solvate the protein. This could be very useful for determining the thermodynamics of enzymes catalyzing reactions at temperature extremes in the presence of substrate solutions of different compositions and viscosities.

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Acetylcholinesterase investigated under osmotic pressure

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Many studies are actually devoted to Acetylcholinesterase (AChE), to the effects of inhibitors and/or ligands on it and to the relation with enzymatic activity. The enzyme is the object of so many studies as it is supposed to be implicated in several diseases, among them Alzheimer's disease, and as it has some very specific characteristics. Indeed the first crystallographic structure of AChE revealed a number of surprising features [1]. A long, narrow gorge leads from the surface of the enzyme to the chamber containing the active site. Along the passageway, the channel is so narrow that substrate would have no access to the active site if the enzyme were too rigid. In earlier experiments, we found in fact very high dynamics for human AChE (hAChE) and evidence for a correlation between the dynamics and enzymatic activity in hAChE, butyrylcholinesterase (BChE) and mouse AChE (mAChE) [2].

Recently, we have also measured the dynamics of AChE under high hydrostatic pressure, and our data suggests that this enzyme enters an intermediate state at 1.5 kbar, before being fully denaturated at 3 kbar [3]. This intermediate state (molden globule) is of great interest as enzymatic assays performed after the experiment showed an increase in activity. Osmotic pressure is believed to have different effects, as already shown for butyrylcholinesterase [4] and discussed theoretically [5]. By using water/cosolvent mixtures, one modifies the hydration and thus the molecular dynamics and the transfer velocity of the ligands and substrates. An osmolyte added to the solvent can either modify the viscosity, only, as for instance the smaller molecule of glycerol (molar mass of 92 g/mol), or have a real osmotic effect, as for instance the bigger molecule of saccharose (molar mass of 342.3 g/mol). Saccharose will not be able to penetrate into the gorge of the active site of AChE and thus play the role of a semi permeable membrane by pumping the water molecules from inside the enzyme. Comparing the dynamics of AChE in presence or not of saccharose, one could thus have an idea about the dynamics of water inside the gorge, because the two samples will essentially differ close to the active site, and this information is extremely difficult to get by any other technique ! Both effects can possibly have a great influence on the internal dynamics and global diffusion of proteins and induce variations of the enzymatic activity. Experiments with enzymes in presence of an osmolyte would thus allow us to complete our understanding on how the external conditions influence AChE dynamics and the water dynamics inside the gorge.

We prepared 4 samples of mouse AChE (mAChE) with 0%, 5%, 10% and 15% of deuterated saccharose and hydrated them over D_2O. We have chosen deuterated sucrose to avoid a significant contribution from it to the incoherent scattering signal. All samples

were measured on IN6, IN13 and IN16, as proposed, and we extracted intensities summed over the available Q-range and mean square displacements from the elastic data (see figure 1).

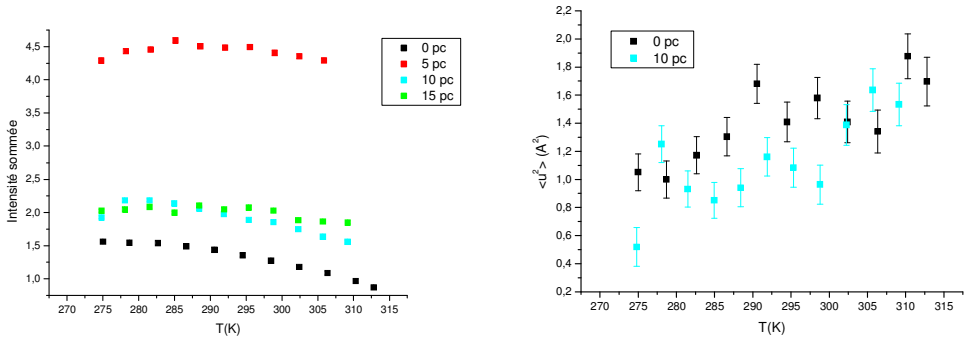

Figure 1 : a) Summed intensities and b) mean square displacements extracted from elastic data taken on IN16.

Unfortunately, we then realized that there was a problem with the 5% saccharose sample, as the summed intensities were much higher than for the other samples. We expected in contrary an almost linear decrease of intensity with increasing mAChE amount. All samples were still conserved in the sample holders and we opened the corresponding sample holder. In fact, the sample in presence of 5% of saccharose was completely dry, what means that the sample holder was not closed in a tight way. Therefore it was not possible to use these data to compare them to the other ones and figure 1b shows the MSD for 0 and 10 % saccharose, only.

We submitted now a CRG proposal for beamtime on IN13 and hope to be able to repeat the measurement at least on this instrument.

[1] J. Sussman et al., Science, 253, 872-879, 1991
[2] J. Peters et al., Phys. Chem. Chem. Phys., 14, 6764 - 6770, 2012.
M. Trovaslet et al., Chem Biol Int 203 (2013) 14 – 18.
[3] J. Marion et al., submitted to BBA Prot. Proteomics.
[4] P. Masson et al., Biochem. J., 343, 361 – 369, 1999.
[5] S.N. Timasheff, Biophys. Chem., 101 – 102, 99 – 111, 2002.
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