Experimental report

06/12/2017

Proposal: INTER-349 **Council:** 4/2016

Title: Internal time on IN13

Research area:

This proposal is a new proposal

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Samples: polyglutamic acid (PGA)
Tryptophane cage (Trp-cage) miniprotein
Poly-L-lysine (PLL)

Instrument	Requested days	Allocated days	From	To
IN13	2	2	28/11/2016	30/11/2016

Abstract:

Experimental report for Experiments Inter 346 and Inter 349

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We performed experiments on IN13 in September and November 2016. The investigated samples were Polyglutamic Acid (PGA), Myoglobin (MBO) and Poly-L-Lysine (PLL). The experiments are a continuation of our previous project where we have elaborated a thermodynamic theory that could coherently interpret the diverse effects of Hofmeister ions on proteins, based on a single physical parameter, the protein-water interfacial tension [1]. This theory, implying a "liquid drop model", predicts changes in protein conformational fluctuations upon addition of Hofmeister salts (containing either kosmotropic or chaotropic anions) to the medium. Subsequently, we reported experimental tests of this prediction using a complex approach by applying methods, including neutron scattering experiments performed at the ILL, that are especially suited for the detection of protein fluctuation changes [2]. It was demonstrated that Hofmeister salts, via setting the hydrophobic/hydrophilic properties of the protein-water interface, control conformational fluctuations even in the interior of the typical membrane transport protein bacteriorhodopsin. With the present experiments we were addressing this point from another aspect. Similarly to salt ions, surface-exposed amino acids are also expected to control the structure of the adjacent water layer. The present neutron scattering experiments are designed to monitor the water dynamics in the vicinity of target protein or peptide molecules. Poly-glutamic acid, as a counterpart of the kosmotropic acetate, is expected to lower rotational mobility of adjacent water molecules, contrary to Poly-L-lysine, that is expected to exert a chaotropic effect on water. Myoglobin, on the other hand, is expected to contain both kosmotropic and chaotropic groups, with an emphasis on the latter.

For the experiments samples were purchased from Sygma Aldrich. Samples were dissolved in water applied to the surface of standard rectangular IN13 sample holders, dried in vacuum, rehydrated above saturated H_2O or D_2O solution of KNO_3 and hermetically closed. Hence 6 samples were produced (3 samples hydrated in H_2O and in D_2O, respectively).

On IN13 elastic runs were performed in the 306 K – 20 K temperature range with continuous temperature ramping. The ramping speed was c.a. -0.15 K/min between 306 K and 180 K and -0.4 K/min – -0.5 K/min between 180 K and 20 K. Experimental data were used to calculate mean square displacement (MSD) values for the samples. For this the data were normalized by Vanadium measurements and empty cell subtraction was also applied. In the case of PGA samples hydrated in D_2O the Vanadium normalization provided negative MSD values for low temperature, therefore for this sample the normalization was performed with respect to the same sample in the 20 K – 40 K temperature range, where the motions are purely elastic.

MSD values were calculated for each sample at a lower ($0.1\ Å^{-2} < Q^2 < 3\ Å^{-2}$) and higher ($6.5\ Å^{-2} < Q^2 < 15\ Å^{-2}$) momentum transfer range, where the Gaussian model could be best applied.

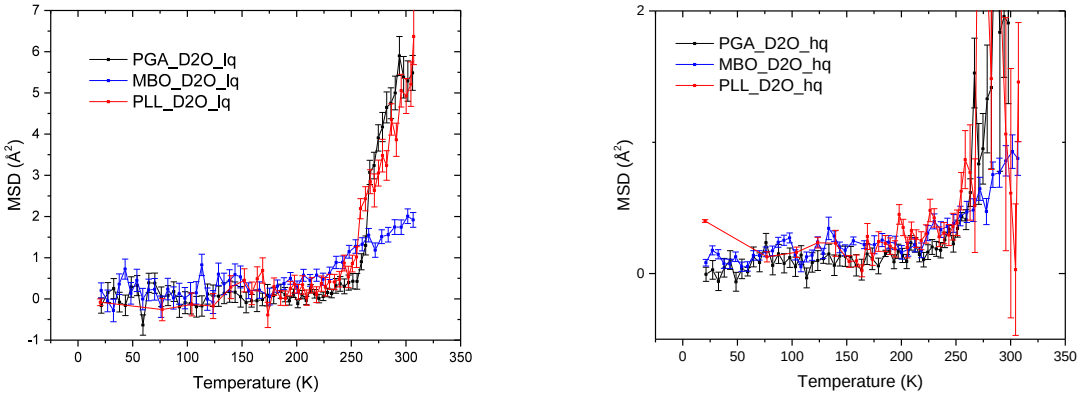

Figure 1 MSD values of the sample hydrated with D_2O (empty cell subtraction was applied). Values were obtained from the ln(I) vs Q^2 curves in the low momentum transfer range (left, 0.1 Å^{-2}< Q^2<3 Å^{-2}) and the high momentum transfer range (right, 6.5 Å^{-2}< Q^2<15 Å^{-2}).

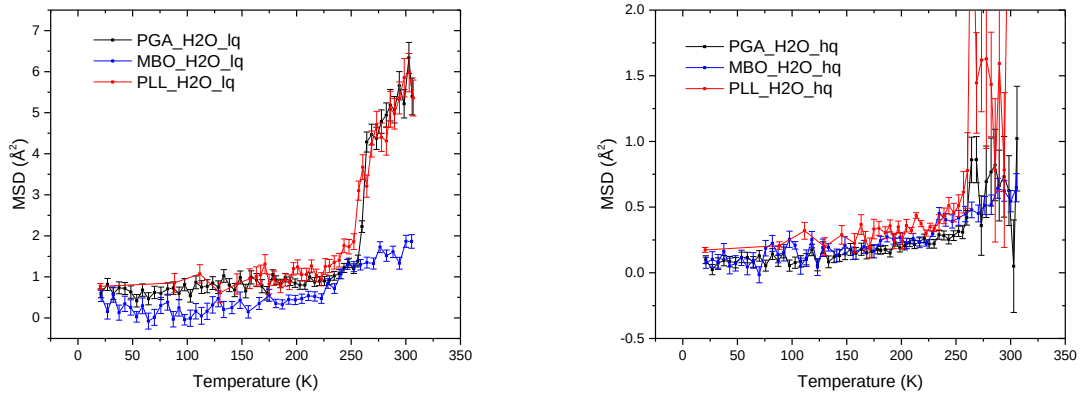

Figure 2 MSD values of the sample hydrated with H_2O (empty cell subtraction was applied). Values were obtained from the ln(I) vs Q^2 curves in the low momentum transfer range (left, 0.1 Å^{-2}< Q^2<3 Å^{-2}) and the high momentum transfer range (right, 6.5 Å^{-2}< Q^2<15 Å^{-2}).

The figures reveal that both in the case of H_2O and in the case of D_2O hydration the MBO samples exhibit an onset of increased of mobility at a lower temperature of 200 – 220 K as opposed to the 230 – 250 K in the case of PGA and PLL samples. However at higher temperatures (T > 250 K) the flexibilities of PGA and PLL are rapidly increasing and exhibit several times higher MSD values than the MBO sample. The MSD of the MBO samples follows similar trends as observed earlier (see e.g. [3], measured under similar r.h. conditions), though the magnitude of MSD values calculated from the low momentumtransfer region is approximately a factor of 2 higher than presented in [3].

A possible explanation for the latter differences can be the fact that despite identical r.h. values of the investigated samples, the water (or heavy water) content of the PGA and PLL samples is above 1 mg H_2O (D_2O) / 1 mg dry sample, while in the case of MBO this ratio is c.a. 0.4 g H_2O (D_2O) / 1 mg dry sample.

We also evaluated the differences of the dynamics of the surface bound and bulk water for the 3 different types of samples. For this we subtracted the Vanadium normalized, empty cell corrected, weight corrected scattering signal of the D_2O hydrated samples from the corresponding H_2O hydrated samples. Unfortunately, probably due to the insufficient neutron count of the performed measurements, MSD values calculated on such curves are very noisy. We are presently working on alternative data treatment protocols to overcome this obstacle.

References: [1] Dér, A., et al., *J. Phys. Chem. B.* **111** (2007) 5344-5350. [2] Szalontai, B., et al., *BBA Gen. Subj.* **1830** (2013) 4564-4572. [3] Doster, W., et al., *Nature* **337** (1989) 754-756