Experimental report

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Title: Influence of Cosolvents, Pressure, and Self-Crowding on the Sub-Nanosecond Dynamics of Globular Proteins

Research area: Biology

This proposal is a new proposal

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Samples: Lysozyme

Instrument	Requested days	Allocated days	From	To
IN13	12	8	03/07/2016	11/07/2016

Abstract:

Recently, the influence of crowding and cosolvents was studied on the temperature and pressure dependent structure, intermolecular interaction potential and phase behavior of concentrated lysozyme solutions. Moreover, first data on the effect of self-crowding and pressure on the internal sub-nanosecond dynamics of highly concentrated lysozyme was studied by elastic incoherent neutron scattering (EINS) on the backscattering spectrometer IN13. Here we propose to extend these studies and explore the effects of chaotropic and cosmotropic osmolytes on the temperature and pressure dependence of the internal dynamics of the protein lysozyme over a range of protein concentrations, including cell-like crowding conditions. The proposed experiments will yield valuable information how various types of cosolvents are able to modulate the temperature and pressure dependent dynamic properties and hence function of biologically relevant dense protein solutions.

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Influence of Cosolvents, Pressure and Self-Crowding on the Sub-Nanosecond Dynamics of Globular Proteins

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In nature, living organisms use osmolytes to rescue proteins from denaturation and to be able to compensate for extreme environmental conditions. Thus, organic osmolytes are accumulated under anhydrobiotic, thermal, and pressure stresses.[1] Among those are amino acids, sugars, methylamines such as trimethylamine-N-oxide (TMAO), and urea. TMAO has been found to enhance protein folding and ligand binding most efficiently. On the other hand, urea, a highly concentrated metabolic waste product in mammalian kidneys, is a perturbant. Interestingly, TMAO has been found to counteract perturbations imposed by urea and hydrostatic pressure in deep-sea animals, most effectively at a 2:1 urea:TMAO ratio. In the deep sea, hydrostatic pressures up to the 1 kbar range are encountered, and living organisms have to cope with such extreme environmental conditions. TMAO has also been shown to largely offset these deleterious pressure effects. In fact, it was found that the amount of TMAO in the cells of a series of marine organisms increases linearly with the depth of the ocean.[2] For that reason, TMAO is thought to serve as pressure counteractant, and the term "piezolyte" has been coined for such kind of cosolute. To yield a deeper understanding of this phenomenon, we determined the intermolecular interaction of dense protein solutions in the absence and presence of cosolvent mixtures of TMAO and urea also under high pressure conditions and found a compensating effect of these two osmolytes on $V(r)$.[1] The underlying mechanism of this "chemical chaperon", in particular its influence on the dynamical properties of the proteins, are still largely unknown, however, and are hence in the focus of this proposal.

We obtained first data on IN13 on the effect of self-crowding on the internal sub-nanosecond dynamics of concentrated lysozyme by elastic incoherent neutron scattering (EINS), recently, and a marked influence of both, protein concentration and pressure on the mean-squared displacement (MSD) of the internal H-atom dynamics was found.[3] With increasing pressure, the internal dynamics of H-atoms of lysozyme are reduced, which suggests a loss in protein mobility. Interestingly, the amplitude of the protein fluctuations depends drastically on the protein concentration, and protein structural and interaction parameters as well as the dynamical properties are affected by pressure in a nonlinear way at medium protein concentrations. At high protein concentrations (e.g., 160 mg mL^{-1}), i.e. under strong self-crowding conditions, as they are also encountered in the biological cell, strong restriction of the dynamics of protein motions takes place, reducing the MSD of H-atoms by 60%, and rendering its pressure dependence almost negligible.[3]

Logically, the aim of the present proposal was thus to extend our MSD studies and explore the effects of the osmolytes TMAO and urea as well as of mixtures thereof on the pressure dependence of the internal dynamics of H-atoms of the protein lysozyme at protein concentrations of 80 and 160 mg mL^{-1}. The sample preparation and the experimental procedures were similar to the previous ones.[3] We extracted summed intensities (Figure 1) and MSD of

atomic motions, $<u^2>$, from the elastic data collected on IN13 in the Q^2-range of 0.27-4.56 Å^{-2} (Figure 2).

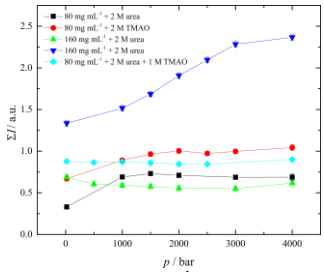

Figure 1: Summed EINS intensities of 80 and 160 mg mL^{-1} lysozyme samples in the presence of TMAO, urea and mixtures thereof as a function of pressure at room temperature, evaluated in the Q^2-range of 0.27-4.56 Å^{-2}.

Figure 1 shows the EINS intensities of the different protein samples containing different cosolvents. Overall, the summed intensities increase with pressure, reflecting the expected volume reduction upon compression via a decrease of motional amplitudes. Figure 2 depicts the extracted MSD values determined at two different concentrations.

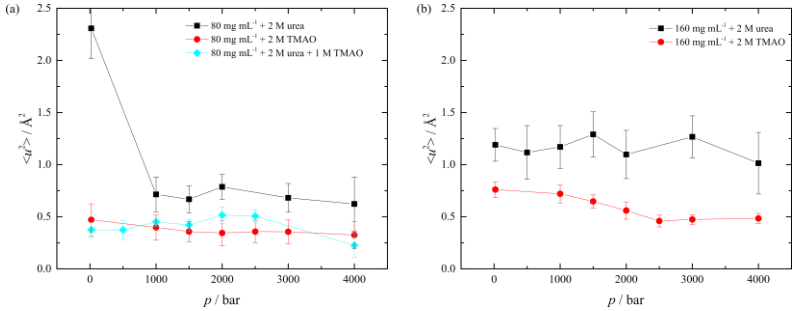

Figure 2: MSD extracted from elastic data collected on IN13 of lysozyme solutions at (a) 80 mg mL^{-1} and (b) 160 mg mL^{-1}, in the presence of TMAO, urea and a mixture thereof as a function of pressure at room temperature, evaluated in the Q^2-range of 0.27-4.56 Å^{-2}.

For both protein concentrations, the determined MSD values of lysozyme in the presence of 2 M urea are higher than the ones of lysozyme in the presence of 2 M TMAO, indicating a higher sub-ns dynamics of the urea-solvated protein. TMAO leads to a reduced flexibility, which seems to be in accord with its marked volume-exclusion capacity. Only at the lower lysozyme concentration in the presence of 2 M urea, the MSD decreases drastically with increasing pressure up to 1 kbar at a rate of -1.6 x 10^{-2} Å^2 kbar^{-1}. For all other samples, pressure has a minor effect on the MSD, only. Clearly, TMAO and urea seem to compensate each other to some extent regarding their effect on the internal sub-ns dynamics of the protein, suggesting some kind of "slaving" effect the cosolvent imposes on the dynamical properties of the protein. A more detailed analysis of the data is in progress. These EINS results will then also be compared with thermal and pressure stability measurements carried out in house using spectroscopic methodologies to be able to identify putative correlations between the global stability and dynamics of the protein bathing in various cosolvents.

References

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