Experimental report

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Proposal: 8-04-795 **Council:** 10/2016

Title: Influence of Pressure on the Sub-Nanosecond Dynamics of Cytoskeletal Proteins

Research area: Other...

This proposal is a new proposal

Main proposer: Paul Hendrik SCHUMMEL

Experimental team: Charlotte NEITZEL
Samy AL AYOUBI
Paul Hendrik SCHUMMEL
Dominik ZELLER
Judith PETERS

Local contacts: Judith PETERS

Samples: Microtubules
G-actin
Tubulin
bundled microtubules
F-actin
B-actin

Instrument	Requested days	Allocated days	From	To
IN13	14	14	23/01/2017	06/02/2017

Abstract:

Actin and tubulin are the main components of the cytoskeleton in eukaryotic cells and can be found in two main states: tubulin, the monomeric state, and the polymeric state of microtubules, on the other hand, actin, the monomeric state with its globular shape (G-actin) and the polymeric filamentous actin (F-actin). Both, F-actin and microtubules can be assembled in highly organized, supramolecular structures, the so-called bundles. We will explore the sub-ns-dynamics of tubulin, microtubules and bundles thereof, as well as of G-actin, filamentous and bundled actin. The amplitudes of the local structural fluctuations, which determine their reactivity and are expected to be very sensitive to pressure, can be seen within this time window and will be explored up to pressures of 4 kbar. From these data we will reveal the effect of pressure on the conformational dynamics and population of conformational substates of these cytoskeletal proteins. These findings will be highly relevant for understanding the effect of pressure on the dynamics of intracellular movement and help explain the marked effect pressure has on the dynamics of the cytoskeleton as observed in vivo.

Exp. Report 8-04-795

Influence of Pressure on the Sub-Nanosecond Dynamics of Fibrillar and Cytoskeletal Proteins

Paul Hendrik Schummel[a], Samy Al-Ayoubi[a], Judith Peters[b], Roland Winter[a]

[a] Physical Chemistry I – Biophysical Chemistry, Faculty of Chemistry and Chemical Biology, TU Dortmund
[b] Laboratoire Interdisciplinaire de Physique, Université Grenoble Alpes, Institut Laue Langevin

The effect of pressure on the dynamical properties of proteinaceous polymer structures is still largely unexplored, but of high relevance to uncover the different stabilities of polymorphic protein structures and to reveal their stability under extreme environmental conditions. The aim of this work was to study the structure-dynamics relation of fibrillar and cytoskeletal proteins compared to monomeric proteins, and to explore the effect of solution conditions, such as the presence of cosolvents and crowding agents, on the sub-ns dynamics of the proteins. Before focussing on the extremely expensive cytoskeletal proteins actin or tubulin and to evaluate the accuracy of the method in a high-pressure sample environment, we decided to concentrate first on a synthetic homopolymer, polylysine, using L-lysine, D-lysine or mixtures thereof. Polylysine is an ideal model system for protein fibrillation studies. It undergoes an α-helix-to-β-sheet transition, the hallmark of protein aggregation, and forms amyloid-like fibrils.[1–3] At pH values higher than 11, increasing temperature induces the α-to-β-transition with subsequent aggregation to form extended poly-L-lysine (PLL) fibrils, and the same is observed for its enantiomer poly-D-lysine (PDL).[2,4] Dehydrating media and hydrophobic environments like lipid bilayers promote reversible formation of extended conformations in PLL.[5–7] Ordered β-sheet conformations at neutral pH are also observed in the presence of macromolecular crowding agents such as Ficoll 400.[8]

In a first experiment, we explored the effects of pH as well as the presence of the macromolecular crowder Ficoll on the pressure dependence of the internal dynamics of H-atoms of polylysine at a concentration of 100 mg mL^{-1}. The samples were dissolved in 25 mM BisTris D_2O buffer and the pD was adjusted to 4.5 and 7.6, respectively. The extracted summed intensities and mean-squared displacement (MSD) of atomic motions, $\langle u^2 \rangle$, from the elastic data collected on IN13 are shown in Figures 1 A and B.

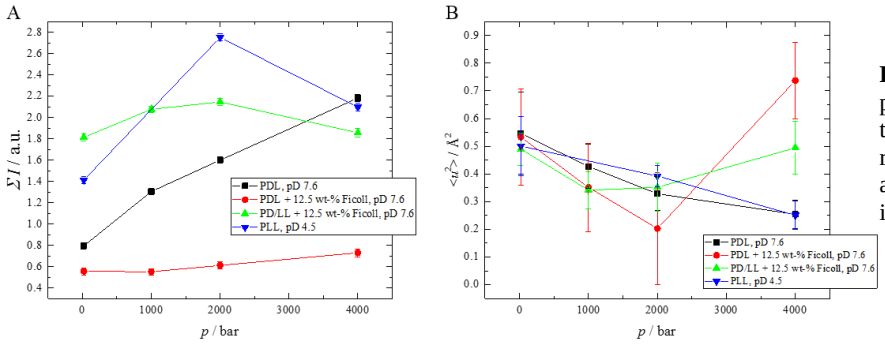

Figure 1: Summed EINS intensities of polylysine at different pD values as well as in the presence of 12.5 wt-% Ficoll 400 (A), and related mean-squared displacement $\langle u^2 \rangle$ (B) as a function of pressure at 300 K, evaluated in the *Q*-range from 1.32 to 5.2 Å^{-2}.

Figure 1A shows the summed EINS intensities of the different polymer samples at different pD values as well as in the absence and presence of the macrocrowder Ficoll. Overall, the summed intensities increase with pressure, reflecting the expected volume reduction upon compression via a decrease of motional amplitudes. Figure 1B depicts the MSD values for the various solution conditions. These data reveal a significant effect of both, the presence of the macrocrowder and of pressure on the internal H-atom dynamics of the polypeptide. The MSD of PDL and PLL in pure buffer solution decrease continuously with increasing pressure, while the MSD of PDL in the presence of 12.5 wt-% Ficoll decreases only until a pressure of 2 kbar is reached. At higher pressures, the MSD increases markedly, indicating a phase transition to a different aggregate structure of the polypeptide with a different dynamical signature. These EINS results will now be compared with in-house temperature and pressure dependent measurements of the secondary structure using FTIR spectroscopy to be able to construct - for the first time - a temperature-pressure stability/dynamics diagram of the polypeptide in its different enantiomeric forms, whose supramolecular structures feature different packing properties.

For comparison, we studied also the effect of osmolytes and crowders on the internal dynamics of a monomeric protein, i.e. lysozyme. In previous experiments on IN13, we measured the effects of TMAO and the destabilizing osmolyte urea on the internal H-atom dynamics of the globular protein lysozyme and observed marked effects.[9] While the pressure resistance of biomolecules in marine organisms is mainly enhanced by the synthesis of methylamines, especially TMAO, yeast and other organisms reveal an enhanced synthesis of the disaccharide trehalose to deal with pressure stress.[10] Hence, additional EINS data of lysozyme in sucrose and trehalose solution have been carried out to complete this study.

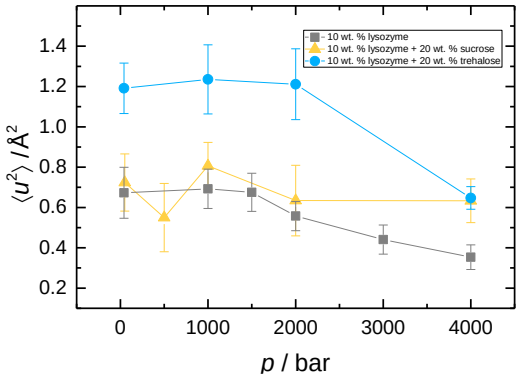

Figure 2: Pressure dependence of the mean-squared displacement (MSD), $<u^2>$, of 10 wt. % lysozyme as well as of 10 wt. % lysozyme in the presence of 20 wt. % sucrose and 20 wt. % trehalose, respectively. Error bars are from statistical analysis of EINS data recorded for at least 7 h.

As shown in Figure 2, sucrose does not significantly influence the internal protein dynamics under pressure. The MSD remains essentially constant over the pressure range covered in the presence of sucrose. Surprisingly, trehalose, on the other hand, leads to a distinct increase (~45%) of the MSD. In the presence of trehalose, the MSD remains essentially constant up to 2000 bar, followed by a distinct reduction upon compression. The dynamics in the probed time window is generally largely affected by the dynamics of the hydration shell of the protein. Trehalose, unlike sucrose which is largely excluded from the protein interface, exhibits a major effect on the dynamics of the hydration shell: it is well known to replace a certain amount of hydration shell water molecules by formation of hydrogen bonds with the protein.[11] Such water replacement scenario seems to enhance the protein's sub-ns dynamic properties. Sucrose, on the other hand, does not show such behavior and thus does not influence the MSD values significantly. Generally, the MSD values are larger compared to fibrillar proteins such as polylysine.

[1] M. Jackson, P. I. Haris, D. Chapman, *Biochem. Biophys. Acta* **1989**, *998*, 75–79.
[2] W. Dzwolak, R. Ravindra, C. Nicolini, R. Jansen, R. Winter, *J. Am. Chem. Soc.* **2004**, *126*, 3762–3768.
[3] M. Fändrich, C. M. Dobson, *EMBO J.* **2002**, *21*, 5682–5690.
[4] V. Smirnovas, R. Winter, T. Funck, W. Dzwolak, *J. Phys. Chem. B* **2005**, *109*, 19043–19045.
[5] D. Carrier, H. H. Mantsch, P. T. T. Wong, *Biochemistry* **1990**, *29*, 254–258.
[6] D. Carrier, H. H. Mantsch, P. T. T. Wong, *Biopolymers* **1990**, *29*, 837–844.
[7] J. S. Chiou, T. Tatara, S. Sawamura, Y. Kaminoh, H. Kamaya, A. Shibata, I. Ueda, *Biochim. Biophys. Acta* **1992**, *1119*, 211–217.
[8] K. Sankaranarayanan, N. Meenakshisundaram, *RSC Adv.* **2016**, *6*, 74009–74017.
[9] S. R. Al-Ayoubi, P. H. Schummel, M. Golub, J. Peters, R. Winter, *Phys. Chem. Chem. Phys.* **2017**, DOI 10.1039/c7cp00705a.
[10] Y. Dong, Q. Yang, S. Jia, C. Qiao, *Biochem. Eng. J.* **2007**, *37*, 226–230.
[11] M. V Fedorov, J. M. Goodman, D. Nerukh, S. Schumm, *Phys. Chem. Chem. Phys.* **2011**, *13*, 2294–2299.