Experimental report

31/07/2018

Proposal: CRG-2487 **Council:** 4/2017

Title: Effect of pressure and osmolytes on the dynamics of Oligometric proteins

Research area:

This proposal is a new proposal

Main proposer: Judith PETERS

Experimental team: Samy AL AYOUBI
Paul Hendrik SCHUMMEL
Judith PETERS
Aline CISSE

Local contacts: Judith PETERS

Samples: Urea
alcohol dehydrogenase
TMAO

Instrument	Requested days	Allocated days	From	To
IN13	13	13	22/03/2018	04/04/2018

Abstract:

Exp. Report CRG-2487

The effect of pressure and osmolytes on the dynamics of oligomeric proteins

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

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

We investigated the internal dynamics of lactate dehydrogenase (LDH) at different degrees of oligomerization. To this end, we performed pressure dependent elastic incoherent neutron scattering (EINS) measurements on instrument IN13. These enabled us to gain information about how the dynamics of oligomers and monomers differ in the ps to ns timescale.

This enzyme catalyzes the interconversion of pyruvate to lactate during the anaerobic glycolysis. Pyruvate does not bind to the enzyme in the absence of the coenzyme NADH.[1,2] The pressure-induced dissociation of tetrameric LDH is well studied and occurs at approximately 1000-1200 bar, accompanied by a loss of enzymatic activity.[3–8] MD simulations revealed that the tetrameric conformation is necessary to maintain the geometry of the active site and to prevent the penetration of water molecules into this region.[9] In the apo-enzyme and the LDH-NADH-complex, the active site is open to the solvent, while in the presence of the substrate the active site is closed by a mobile loop.[10] To investigate the effect of substrate binding on the dynamic properties of the tetramer and the dimer of LDH, EINS measurements were also performed in the presence of the enzyme's cosubstrate NADH (molar ratio 1:5) and the substrate analogue oxamate + NADH (molar ratio: 1:5:5). Further, we performed EINS measurements in the presence of the naturally occurring osmolyte glycine, the main osmolyte in shallow water invertebrates.[11]

The experiment was undertaken on IN13 at constant temperature of 298 K. We used the high hydrostatic pressure equipment developed recently by J. Peters and the SANE group of ILL.[12,13] The measurements were performed in 25 mM Tris buffer (pD = 7.6). To prevent the oxidation of sulfhydryl groups at elevated pressure, the buffer contained 10 mM DTT and 1 mM EDTA.[3,4,14] Each pressure point was measured for at least 6 hours. The mean squared displacements (MSD), $\langle u^2 \rangle$, of the hydrogen atoms were determined using the Gaussian approximation.[15]

The pressure dependent development of the MSD of 90 mg mL^{-1} LDH is depicted in Figure 1. Upon compression, the MSD of the apo-enzyme remains essentially constant up to 1000 bar. A further increase of the applied pressure leads to increased MSD values. Above 2000 bar the MSD decreases drastically. The increase at 1200 bar is probably attributable to subunit dissociation. The dissociation leads to the hydration of the inter-subunit area and further induces the penetration of water into the active site of the enzyme.[9] This hydration change might explain the observed changes of the internal protein dynamics. The marked decrease of the MSD above 2000 bar is most likely attributable to the previously observed aggregation at 2000 bar.[4,7]

In the presence of NADH, a slight reduction of the MSD compared to the apo-enzyme is visible. Increased MSD values beyond 1000 bar, i.e. after the pressure-induced dissociation, are also observed in the complex of NADH and LDH. The dynamics of the ternary complex between the enzyme, NADH and oxamate are essentially unaffected by pressure application up to 3000 bar, where the MSD decreases. Again, slightly reduced MSD values compared to the apo-enzyme are implied. The binding of oxamate to the LDH-NADH-complex induces the closure of the active site via a rearrangement of a mobile loop region. In this closed conformation, the active site is, at least to some extent, shielded from the solvent and the MSD of the monomer and the dimer do not differ significantly. Further

substrate binding seems to stabilize the protein against pressure-induced aggregation, which is shifted up to 3000 bar in the presence of the substrate.

1 M glycine increases the MSD. Within the accuracy of the data, no pressure effect on the MSD up to 3000 bar was observed in the presence of glycine. The effect of glycine might be attributable to the direct interaction between glycine and the protein's backbone and side chains,[16] leading to an enhanced mobility. However, final data analysis and interpretation is still in process.

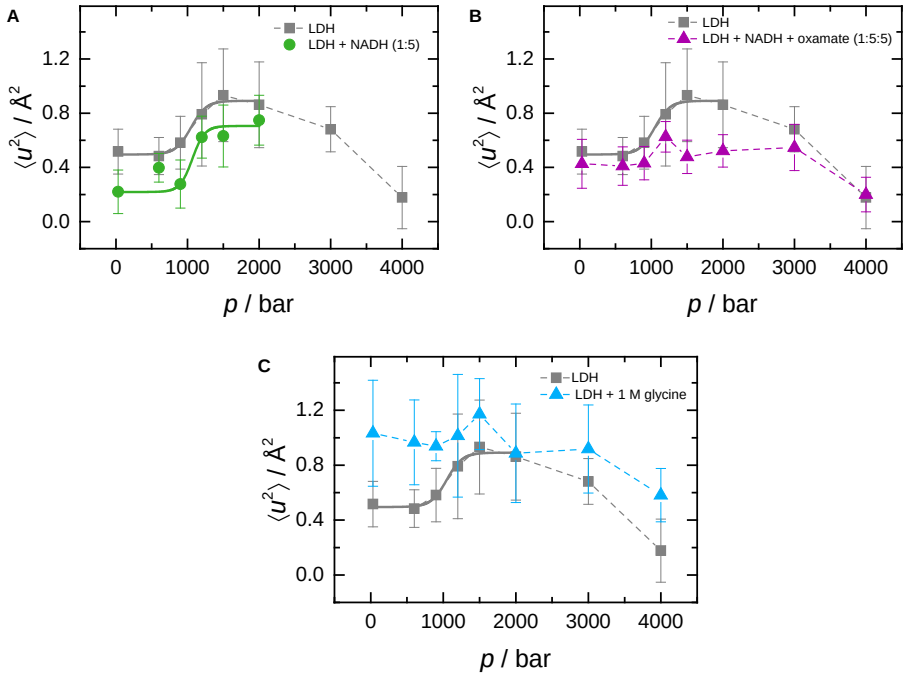

Figure 1: Pressure dependent development of the MSD of 90 mg mL^{-1} LDH and the complex between LDH and NADH (A), the complex between LDH, NADH and the substrate analogue oxamate (B) and LDH in the presence of 1 M glycine (C). The sigmoidal curves and dashed lines are shown as guide to the eyes only.

1 J. J. Holbrook and H. Gutfreund, *FEBS Lett.*, 1973, **31**, 157–169.
2 J. R. E. T. Pineda, R. Callender and S. D. Schwartz, *Biophys. J.*, 2007, **93**, 1474–1483.
3 B. C. Schade, R. Rudolph, H. D. Luedemann and R. Jaenicke, *Biochemistry*, 1980, **19**, 1121–1126.
4 G. Schmid, H. -D Lüdemann and R. Jaenicke, *Eur. J. Biochem.*, 1979, **97**, 407–413.
5 L. King and G. Weber, *Biochemistry*, 1986, **25**, 3637–3640.
6 L. King and G. Weber, *Biophys. J.*, 1986, **49**, 72–73.
7 T. Fujisawa, M. Kato and Y. Inoko, *Biochemistry*, 1999, **38**, 6411–6418.
8 R. Jaenicke, *Annu. Rev. Biophys. Bioeng.*, 1981, **10**, 1–67.
9 R. K. Schmidt and J. E. Gready, *J. Mol. Model.*, 1999, **5**, 153–168.
10 A. R. Clarke, T. Atkinson and J. J. Holbrook, *Trends Biochem Sci*, 1989, **14**, 101–105.
11 P. H. Yancey, W. R. Blake and J. Conley, *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.*, 2002, **133**, 667–676.
12 J. Peters, M. Trapp, D. Hughes, S. Rowe, B. Demé, J.-L. Laborier, C. Payre, J.-P. Gonzales, S. Baudoin, N. Belkhier and E. Lelièvre-Berna, *High Press. Res.*, 2012, **32**, 97–102.
13 E. Lelièvre-Berna, B. Demé, J. Gonthier, J.-P. Gonzales, J. Maurice, Y. Memphis, C. Payre, P. Oger, J. Peters and S. Vial, 2017, vol. 19.
14 G. Schmid, H. -D Lüdermann and R. Jaenicke, *Eur. J. Biochem.*, 1978, **86**, 219–224.
15 A. Rahman, K. S. Singwi and A. Sjölander, *Phys. Rev.*, 1962, **126**, 986–996.
16 L. Arns, V. Schuabb, S. Meichsner, M. Berghaus and R. Winter, *Zeitschrift fur Phys. Chemie*, 2017, **232**, 615–634.