Experimental report

01/02/2021

Proposal: 8-05-458 **Council:** 10/2019

Title: Molecular bases of proteome adaptation to high pressure in extremophilic Archaea

Research area: Biology

This proposal is a new proposal

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Samples: peptidyl-tRNA hydrolase
mannose-6-phosphate isomerase

Instrument	Requested days	Allocated days	From	To
IN5	4	2	21/09/2020	23/09/2020
IN6-SHARP	4	0		

Abstract:

In the last years, we studied successfully prokaryotes from the deep sea under high hydrostatic pressure conditions and were able to identify first routes to pressure adaptation in such systems. Now we want to extend the study to proteins extracted from these organisms, which are sufficiently sensitive to pressure to provide answers on how they are thriving with extreme conditions. Dynamics of surrounding water molecules will be included in the study to get an as complete picture as possible about piezophilic proteins, their environment and the effects of temperature and pressure variation on them. We hope to gain more insights on their functionalities and their specific capacities of adaptation, as structural modifications were ruled out to explain them.

EXPERIMENTAL REPORT

8-05-458, IN5, 21-23/09/2020

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Proteome adaptation to high-pressure in Archaea is still an open debate. Genomic studies were not able to determine a clear adaptation pattern among the order of Thermococcales (unpublished data), and pressure adaptation is often considered as a *crossover adaptation*, that is, a concomitant process with another, more important, process [1] (e.g. the adaptation to high-temperature).

However, recent studies on whole cells [2,3] highlighted the differences in the proteome dynamics between *Thermococcus barophilus* (Tba) and *Thermococcus kodakarensis* (Tko), two closely related species which grow at the same optimal temperature ($85°C$) but differ only for the optimum pressure ($400\ bar$ for Tba, $1\ bar$ for Tko)[4]. Such choice of organisms permits to focus only on pressure adaptation. The observed results could arise from two causes: genomic differences, which would bring about the difference in dynamics of the two proteomes and their different interaction with intracellular water, or the existence of a protective mechanism put into operation by the cell itself, e.g. the production of organic osmolytes [5].

To investigate the first hypothesis, we performed Quasielastic Neutron Scattering to unravel the dynamics of the protein *Phosphomannose Isomerase* (PMI) from the two organisms. This approach permits to characterize in detail the dynamics of the two proteins without the complications of a whole-cell environment. Further NMR studies will enable us to pinpoint the residues which are responsible for the different dynamics, and thus to reveal the pressure adaptation strategy on a genomic level.

The experiment was performed on IN5 at a wavelength of 5Å ($\sim\ 40\mu eV$ HWHM resolution), in a temperature range of $283 - 363\ K$ at three pressure points (1, 150 and 300 bar) for both samples.

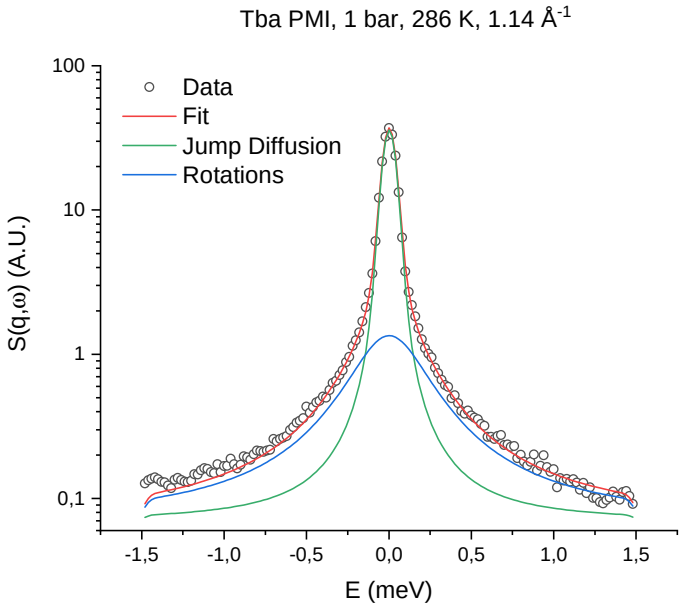

Figure 1: Fit example of Tba PMI at 1 *bar and* 286 K*, at a q value of* $1.14\ \mathring{A}^{-1}$.

Spectra were acquired every $5\ min$ during a temperature ramp done at $0.4\ K/min$. Raw data were corrected for empty cell scattering, normalized to a vanadium standard and binned in $10\ K$ steps. The same treatment has been done on the D_2O measurement as well, in order to subtract water contribution according to the procedure described in [6]. TOF corrections were then applied and the resulting $S(q,\omega)$ were rebinned in 20 q slices and evenly spaced energy slices ($dE = 0.02\ meV$). The reduction has been carried out on LAMP, the reduced data has then been analyzed and fitted by GNU Octave scripts.

Thanks to the very good quality of the data we were able to develop a detailed model for the analysis: we started from a scattering func-

tion for rotational motions[7] (elastic contribution plus a Lorentzian with a q-independent width Γ_{rot}) convoluted by a Lorentzian describing the jump-diffusion process of protein residues, here described by means of the Hall-Ross model[8] ($\Gamma_j(q) = \frac{\hbar}{\tau}\left(1 - \exp\left(-\frac{q^2\langle l\rangle^2}{2}\right)\right)$).

Such model function is then convoluted with the resolution function (derived from the vanadium measurement), multiplied by a scale factor, and then fitted to the data using a global fitting approach, which gives the q-dependent *Elastic Incoherent Structure Factor* ($A_0(q)$) and the q-independent parameters Γ_{rot}, τ and $\langle l\rangle$. Figure 1 shows a fit example, in which the two components are highlighted.

Figure 2 shows the behaviour of the fitting parameters. The pressure independence of the parameters concerning Tba PMI is consistent with the fact that this organism is not an obligate piezophile[4] (i.e. it can grow at ambient pressure despite its optimum being at $400\ bar$), thus the protein is able to retain its dynamics (and likely its function) over the whole pressure range studied. On the other hand, Tko PMI is apparently destabilized by the application of pressure, consistently with the fact that Tko's growth is inhibited by increasing pressure.

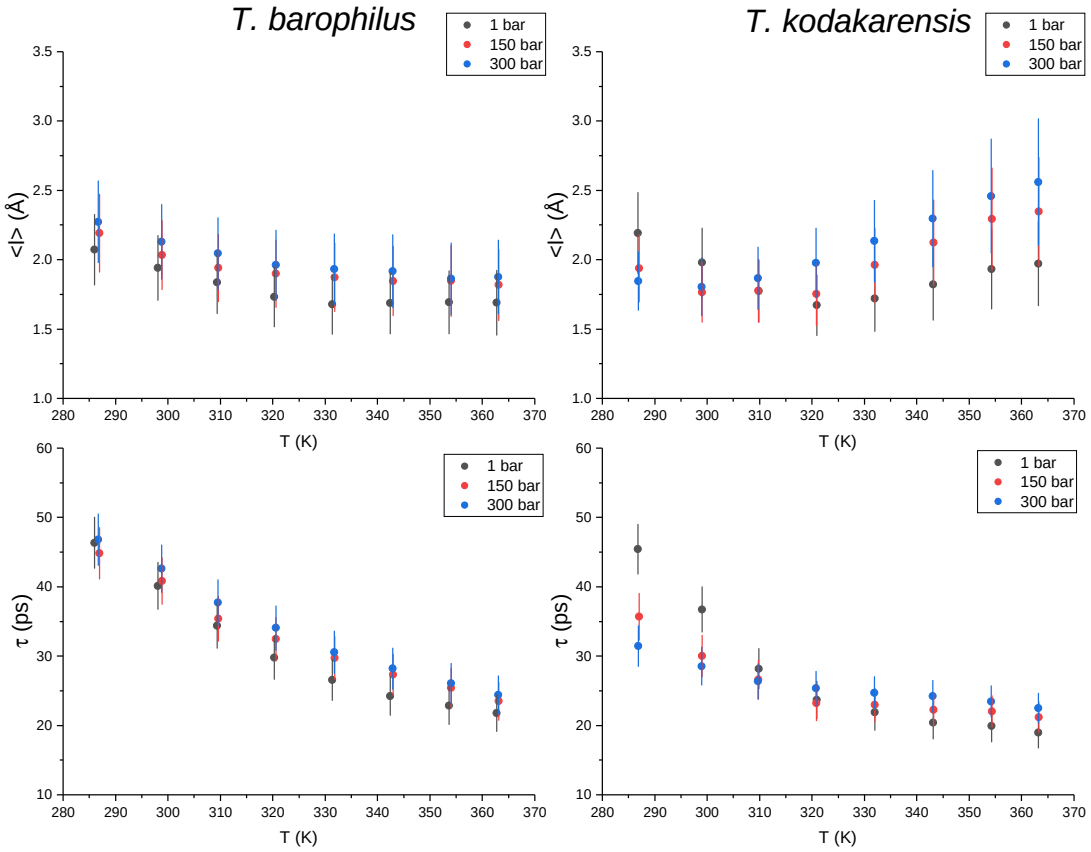

Figure 2: q-independent parameters extracted from the global fit at different temperatures and pressures.

The EISF has then been analyzed taking into account methyl rotation ($A_{3-j} = \frac{1}{3}\left[1 + 2j_0(qa_M)\right]$, $a_M = 1.715$ Å[9]) and restricted jump-diffusion of protein residues ($A_j(q) = j_0^2\left(\frac{qR}{2}\right)$, from the Hall-Ross model).

Again, parameters appear largely pressure-independent for Tba PMI, while for Tko PMI we highlight a sizeable increase of the confinement radius with pressure (fig. 3). This result could appear counterintuitive at first, but it can be rationalized by thinking of R as a measure of the protein's solvent-accessible cavities: higher pressure forces water into them, increasing their volume. This volume increase could also explain the enhanced mean jump length and the faster jump rate displayed by the protein's residues at high pressure (fig. 2).

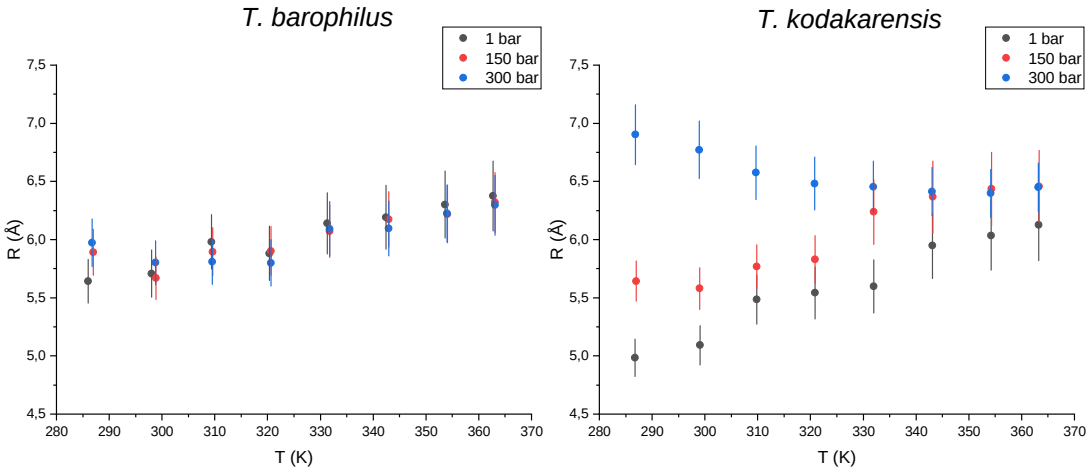

Figure 3: Values of confinement radius R for the two proteins.

Thus, reduced water penetration into the cavities seems to be a key feature for the pressure stability of Tba PMI.

References

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