Experimental report

20/06/2022

Proposal: CRG-2876 **Council:** 4/2021

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

Research area:

This proposal is a new proposal

Main proposer: Judith PETERS

Experimental team: Judith PETERS
Antonino CALIO

Local contacts: Alessio DE FRANCESCO

Samples: peptidyl-tRNA hydrolases (TERMP_749 and TK_2300)

Instrument	Requested days	Allocated days	From	To
IN13	12	12	01/09/2021	13/09/2021

Abstract:

EXPERIMENTAL REPORT

CRG-2876, IN13, 01-11/09/2021

Antonino Caliò, Alessio De Francesco, Philippe Oger, Judith Peters

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 Genomic studies were not able to determine a clear adaptation pattern among the order of Thermococcales (unpublished data), and pressure adaptation is often considered as a *crossover adaptation*, that is, a concomitant process with another, more important, process[1] (e.g.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 proteome dynamics between *Thermococcus barophilus* (Tba) and *Thermococcus kodakarensis* (Tko), two closely related species that 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]. After investigating the first hypothesis[6], we focused here on the second. We performed Elastic Incoherent Neutron Scattering to study the dynamics of the protein *Phosphomannose Isomerase* (PMI) from the two organisms in presence of *trimethylamine-N-oxide* (TMAO). This approach permits to characterize in detail the dynamics of the two proteins without the complications of a whole-cell environment.

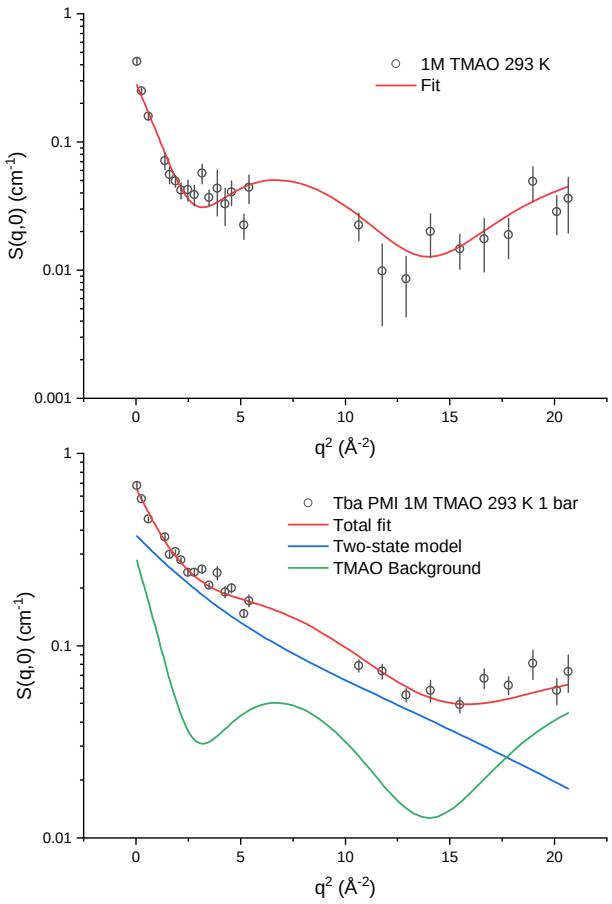

Figure 1: Fit examples for TMAO at 293 K (upper panel), and for Tba PMI with 1 M TMAO at 1 bar and 293 K (lower panel). The different fit components are highlighted.

The experiment was performed on IN13 at the elastic scattering position ($\lambda = 2.23$Å and $\sim 8\mu eV$ FWHM resolution, corresponding to a time window of $\sim 100\ ps$), in a temperature range of $283 - 363\ K$ at two pressure points (1 and $400\ bar$) for the protein alone, and three pressure points (1, 150 and $400\ bar$) after addition of TMAO. The samples consisted of lyophilized protein powder dissolved in D_2O at a concentration of $120\ mg/ml$, and the total sample volume was $1\ ml$. For the measurements with the osmolyte, $100\ \mu l$ of a $10\ M$ TMAO solution were added directly to the sample cell, giving a final concentration of $1\ M$ within a 10% error. Since hydrogenated TMAO was used, a temperature scan of a $1\ M$ TMAO solution in D_2O was also performed to estimate

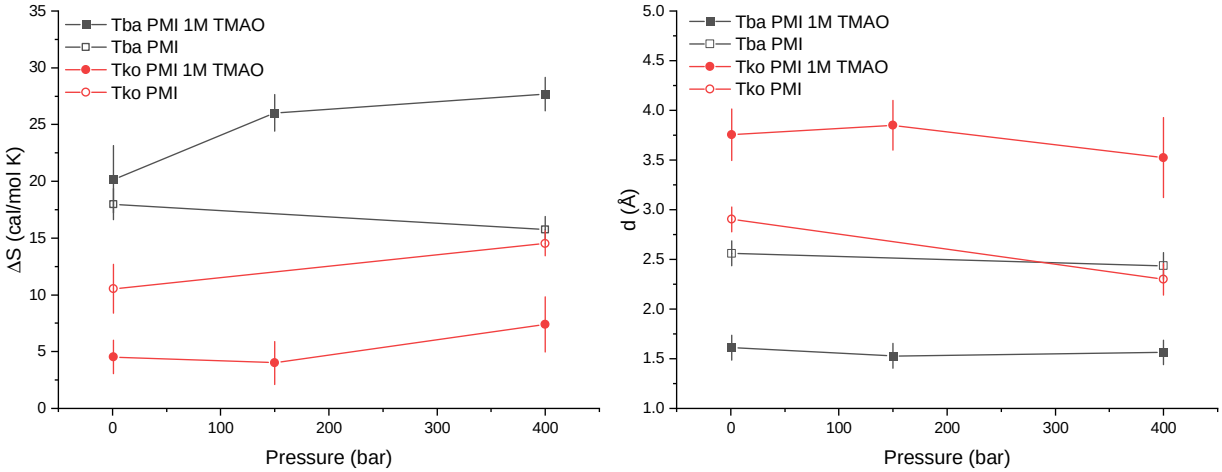

Figure 2: Distance between potential wells (right panel) and entropy difference (left panel) for the two samples with (full symbols) and without (empty symbols) TMAO.

its contribution on the protein measurements. Spectra were acquired every $10\ min$ during a temperature ramp done at $0.09\ K/min$. Raw data were corrected for empty cell scattering, transmission, normalized to a vanadium standard and binned in $20\ K$ steps. The same treatment has been done on the D_2O and TMAO measurements as well, in order to subtract their contribution. The reduction has been carried out on LAMP, the reduced data has then been analysed and fitted by GNU Octave scripts. The TMAO scattering curves were interpreted as a sum of two three-site jump contributions[7] (fig. 1):

$$S(q,0) = \frac{e^{-\Delta x^2 q^2}}{9}\left[3 + 2j_0(qr_1\sqrt{3}) + 4j_0(qr_2\sqrt{3})\right] \tag{1}$$

where $e^{-\Delta x^2 q^2}$ is the Debye-Waller factor, j_0 is the zeroth-order spherical Bessel function, and r_1 and r_2 are the radii of the two motions. It is well known that TMAO has a structuring effect on the surrounding water molecules[8], and this likely leads to the emergence of a potential that hinders the free rotation of the molecule, and only allows three-site jumps. In fact, we find that the value for r_1 is consistent with rotation of the methyl groups of the molecule as expected (0.9 ± 0.1Å), while r_2 (1.7 ± 0.1Å) is consistent with $120°$ jumps of the whole molecule about its C_3 axis (passing through the N-O bond). The fitted curves for TMAO alone at each temperature were then used as a fixed background for the protein measurements, analysed in terms of the *two state model*[9] (fig. 1):

$$S(q,0) = e^{-\Delta x_0^2 q^2}\left[1 - 2p_1p_2\left(1 - \frac{\sin(qd)}{qd}\right)\right] \tag{2}$$

We performed a global fitting by assuming an Arrhenius behaviour for the transition probabilities ($p_1/p_2 = exp(-\Delta H/RT + \Delta S/R)$), which gives the T-dependent Δx_0^2 and the T-independent parameters d, ΔH and ΔS. Figure 1 shows a fit example and highlights the two components. Figure 2 reports two of the extracted parameters. The addition of TMAO has an opposite effect on the two proteins: it increases d and reduces ΔS for Tko PMI, while the opposite is true for Tba PMI. In previous work[6], we have shown that the decrease in d and increase in ΔS under high pressure for Tko PMI correspond to a general stiffening of the protein and to the loss of structural stability, therefore, TMAO seemingly helps the recovery of flexibility for Tko PMI, consistently with the hypothesis that the osmolyte could preserve protein function under pressure.

The opposite effect it has on Tba PMI is probably due to the very different surface charge arrangement of this protein from its piezosensitive counterpart, as it is known that TMAO does not interact directly with proteins, but its effect is mediated by water[8].

References

[1] C. Brininger, S. Spradlin, L. Cobani, and C. Evilia, *Seminars in Cell and Developmental Biology*, vol. 84, pp. 158 – 169, 2018.

[2] J. Peters, N. Martinez, G. Michoud, A. Cario, B. Franzetti, P. Oger, and M. Jebbar, *Zeitschrift für Physikalische Chemie*, vol. 228, no. 10-12, pp. 1121 – 1133, 28 Dec. 2014.

[3] N. Martinez, G. Michoud, A. Cario, J. Olliver, B. Franzetti, M. Jebbar, P. Oger, and J. Peters, *Scientific Reports*, vol. 6, 2016.

[4] V. T. Marteinsson, J. Birrien, A. Reysenbach, M. Vernet, D. Marie, A. Gambacorta, P. Messner, U. B. Sleytr, and D. Prieur, *International Journal of Systematic and Evolutionary Microbiology*, vol. 49, no. 2, pp. 351–359, 1999.

[5] M. Salvador-Castell, M. Golub, N. Martinez, J. Ollivier, J. Peters, and P. Oger, *Soft Matter*, vol. 15, pp. 8381–8391, 2019.

[6] A. Caliò, M. M. Koza, S. Fontanay, P. Oger, and J. Peters, *bioRxiv*, 2022.

[7] M. Bée. Adam Hilger, 1988.

[8] Q. Zou, B. J. Bennion, V. Daggett, and K. P. Murphy, *Journal of the American Chemical Society*, vol. 124, no. 7, pp. 1192–1202, 2002.

[9] W. Doster, S. Cusack, and W. Petry, *Nature*, vol. 337, pp. 754 – 756, 1989.