Experimental report

06/09/2024

Proposal: 8-04-956 **Council:** 4/2023

Title: Adaptation of proteins to high hydrostatic pressure conditions

Research area: Biology

This proposal is a new proposal

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Samples: Nicotinanidase from T. barophilus
Nicotinanidase from T. kodakarensis

Instrument	Requested days	Allocated days	From	To
IN5	4	2	13/09/2023	15/09/2023

Abstract:

The question of the adaptation mechanism of proteins to a high hydrostatic pressure (HHP) environment is tightly related to possible scenarios for the origin of life. They suggest that it appeared near hydrothermal vents in the depth of the Archaean ocean to prevent the deleterious effects of the young Sun. Thus, the first cells to appear on Earth and their constituents would have been adapted to both High Temperature (HT) and High Hydrostatic Pressure HHP. If the routes to the adaptation to HT are starting to be better understood, adaptive strategies to HHP still remain elusive, and an amino-acidic substitutional pattern has not been found yet. Deciphering these routes is essential to confront the putative deep-sea origins of life and will simultaneously give information on protein folding and adaptation strategies of modern piezophiles.

EXPERIMENTAL REPORT

8-04-956, IN5, 13-15/09/2023
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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 Genomic studies were not able to determine a clear adaptation pattern among the order of Thermococcales (unpublished data), and pressure adaptation is often considered as a *crossover adaptation*, that is, a concomitant process with another, more important, process[1] (e.g.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 *Ribosomal Protein S24e* (S24e) 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. These results, in addition to already performed 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.

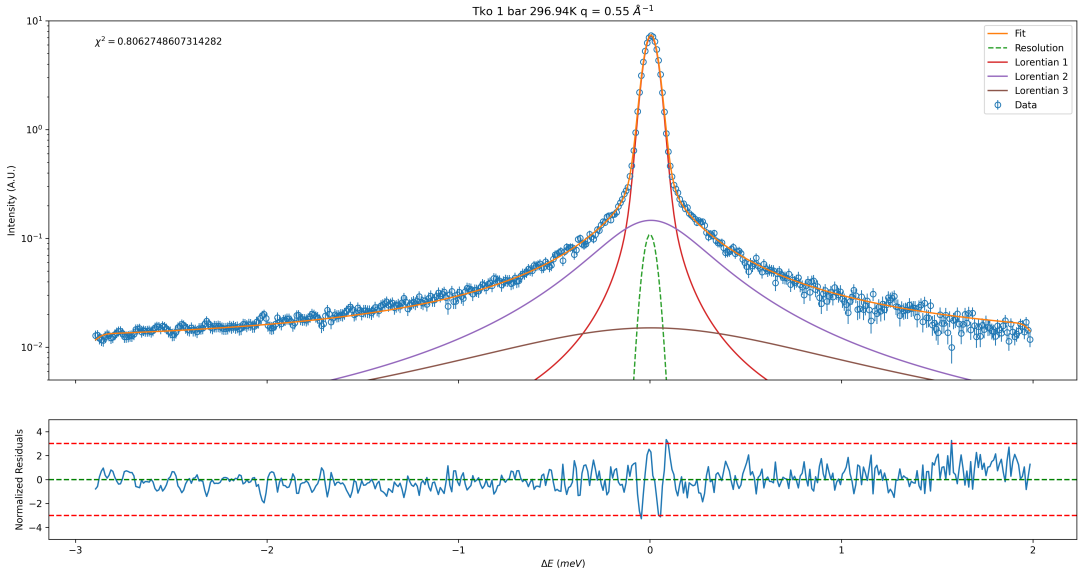

Figure 1: Example of a three-Lorentzian fitting.

The experiment was performed on IN5 at a wavelength of 5Å ($\sim 40\mu eV$ HWHM resolution), in a temperature range of $293 - 363\ K$ at three pressure points (1, 200 and 400 bar) for both samples.

Spectra were acquired every $5\ min$ during a temperature ramp done at $0.45\ K/min$. 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 re-binned in 22 q slices and evenly spaced energy slices ($dE = 0.01\ meV$). The reduction has been carried out using Mantid, the reduced data has then been analysed and fitted by Python scripts (fig. 1).

For the time being, we are in the process of analysing the data with a model-free approach (fig. 2), by employing three Lorentzian functions (as using only two was not giving satisfactory results), convoluted with the resolution function extracted from the Vanadium measurements. Compared to our last experiment on related proteins[7] (*Phosphomannose Isomerases*, PMI), we have an additional scattering component, seemingly due to the fact that S24e proteins are much smaller in size compared to the PMIs, therefore their global diffusion is clearly resolved in the instrument's time-window. This can be seen from the q-dependence of the first Lorentzian's HWHM (Gamma_1).

More in-depth analysis will be required to decipher the q-dependence of the other two components, in order to build a model for the global fitting of the data, which will help in the interpretation of the S24e's dynamical properties.

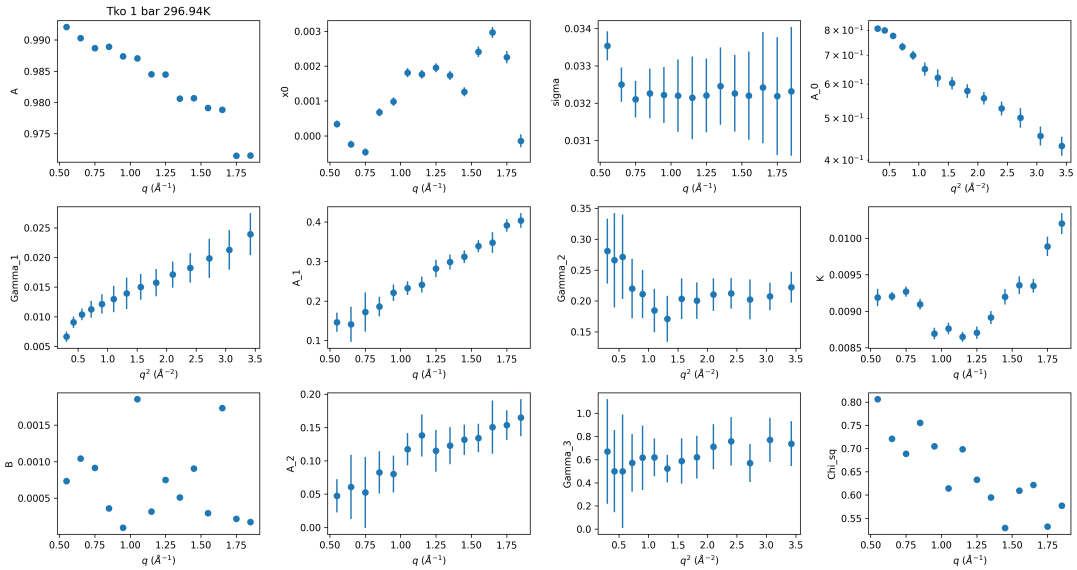

Figure 2: Parameters extracted from the model-free analysis for Tko S24e at 1 bar and 296K. A represents the amplitude of the resolution, x0 its center, and sigma its width. A_0 is the Elastic Incoherent Structure Factor and Gamma_1 the HWHM of the first Lorentzian, A_1 is the structure factor for the second Lorentzian, and Gamma_2 its HWHM. K is a multiplicative factor for the whole model, and B is a constant background. A_2 is the structure factor for the third Lorentzian, and Gamma_3 its HWHM. The Chi squared for each fit is also reported.

References

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