Experimental report

30/06/2021

Proposal: 8-04-886 **Council:** 4/2020

Title: Molecular bases of protein adaptation to high hydrostatic pressure inpiezophilic Archaea

Research area: Biology

This proposal is a new proposal

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Samples: proteins TERMP_2015; TK_2300

Instrument	Requested days	Allocated days	From	To
IN13	11	12	08/03/2021	22/03/2021

Abstract:

The most accepted scenarios for the origin of life imply an origin near deep-sea hydrothermal vents, e.g. an origin under both high temperature and high hydrostatic pressure (HHP). Understanding the basis of these adaptations is essential to strengthen origin of life scenarios as well as understand life in the most extreme environments. To date, genomic studies have failed to identify the basis of the adaptation to HHP. Based on recent results, we propose that HHP affects only the protein-water interface, mimicking the impact of self-crowding or organic osmolytes, explaining the failure of genomic approaches. To circumvent this problem, we propose to characterize in details the physical impact of HHP on a set of piezophile proteins, in order to determine the amino acids responsible for the adaptation to HHP and reconstruct the evolutionary path to HHP adaptation. Thus, this project will yield important data for protein folding and the understanding of the origins of life.

EXPERIMENTAL REPORT

8-04-886, IN13, 08-22/03/2021

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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 Genomic studies were not able to determine a clear adaptation pattern among the order of Thermococcales (unpublished data), and pressure adaptation is often considered as a *crossover adaptation*, that is, a concomitant process with another, more important, process [1] (e.g.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 Elastic Incoherent Neutron Scattering to unravel the dynamics of the *Ribosomal Protein 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. 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.

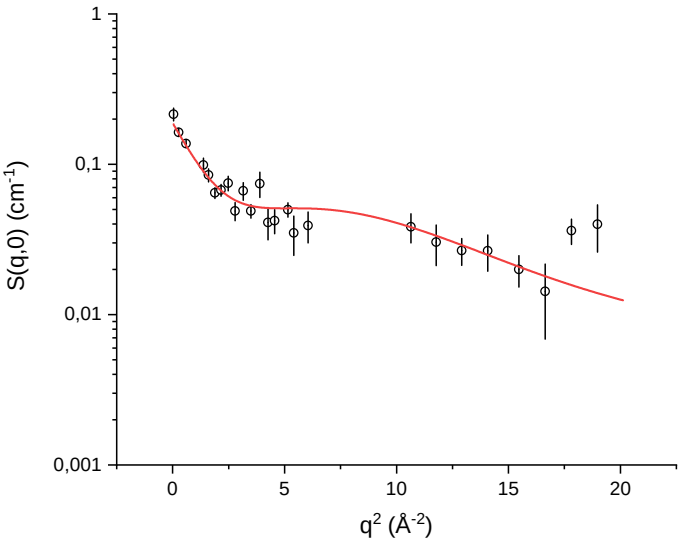


Figure 1: Fit example of Tba PMI at $1\ bar$ *and* $320\ K$.

The experiment was performed on IN13 at the elastic scattering position ($\lambda = 2.23$Å and $\sim 8\mu eV$ HWHM resolution, corresponding to a time window of $\sim\ 100\ ps$), in a temperature range of $283 - 363\ K$ at five pressure points (1, 200, 400, 600 and 800 bar) for both samples. The samples consisted of lyophilized protein powder dissolved in D_2O at a concentration of $120\ mg/ml$.

Spectra were acquired every $10\ min$ during a temperature ramp done at $0.05\ K/min$. Raw data were corrected for empty cell scattering, transmission, 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. 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 analyze it in the framework of the *two state model*[6]:

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

We performed a global fitting by assuming an Arrhenius behaviour for the transition probabilities ($p_1/p_2 = exp(-\Delta G/RT)$), which gives the T-dependent Δx_0^2 and the T-independent parameters d and ΔG (as $\Delta G = \Delta H - T\Delta S$). Figure 1 shows a fit example. Total *Mean Square Displacements* were then calculated according to $\Delta x_{tot}^2 = \Delta x_0^2 + p_1p_2d^2/3$.

Although we cannot exclude that global protein diffusion is contributing to the measured signal in our temperature range and time window, we must stress that both proteins have very similar molecular weight, and their diffusion coefficients (calculated with HYDROPRO[7]) are virtually identical. Thus, the effect of diffusion would be a systematic shift in some parameters and it would be the same for both samples, so that every different behaviour between the two proteins can be interpreted as a difference in their internal dynamics.

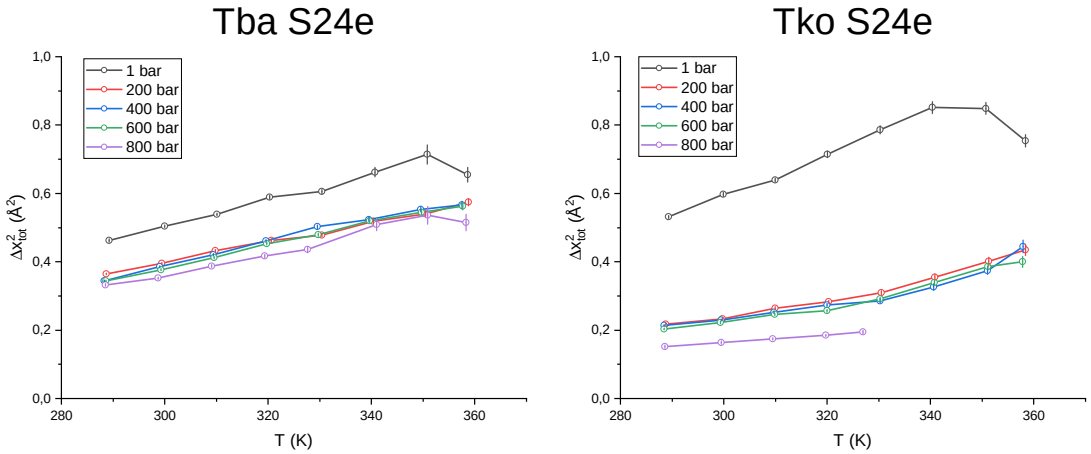


Figure 2: Total MSD for the two samples. Lines are plotted as a guide to the eye.

This is evident in the strong decrease of the total MSD from 1 to 150 bar in both samples (fig. 2). However, the different extent of this drop suggests distinctive pressure responses of the two proteins.

Pressure does not seem to affect the distance between the two wells for Tba S24e (fig. 3), showing that its energy landscape appears to be very stable. Moreover, the free energy difference between the two states is slightly reduced with increasing pressure, indicating that pressure might actually favour the protein's conformational changes.

On the other hand, Tko S24e displays a pressure dependence of d which resembles that of mesophilic proteins[8], monotonically decreasing with pressure (fig. 3), showing that transitions between states which bear bigger structural differences are hindered. The slight increase of ΔG also suggests that pressure limits the protein's conformational freedom.

Thus, the pressure stability of Tba PMI's energy landscape seems to be arising from smaller volume differences between the protein's functional substates.

These results are in line with our previous study on the *Phosphomannose Isomerases* from the same two organisms (Experiment 8-04-876), suggesting that the particular dynamical features of the piezophilic proteins (Tba PMI and Tba S24e) are strongly correlated with High-Pressure adaptation.

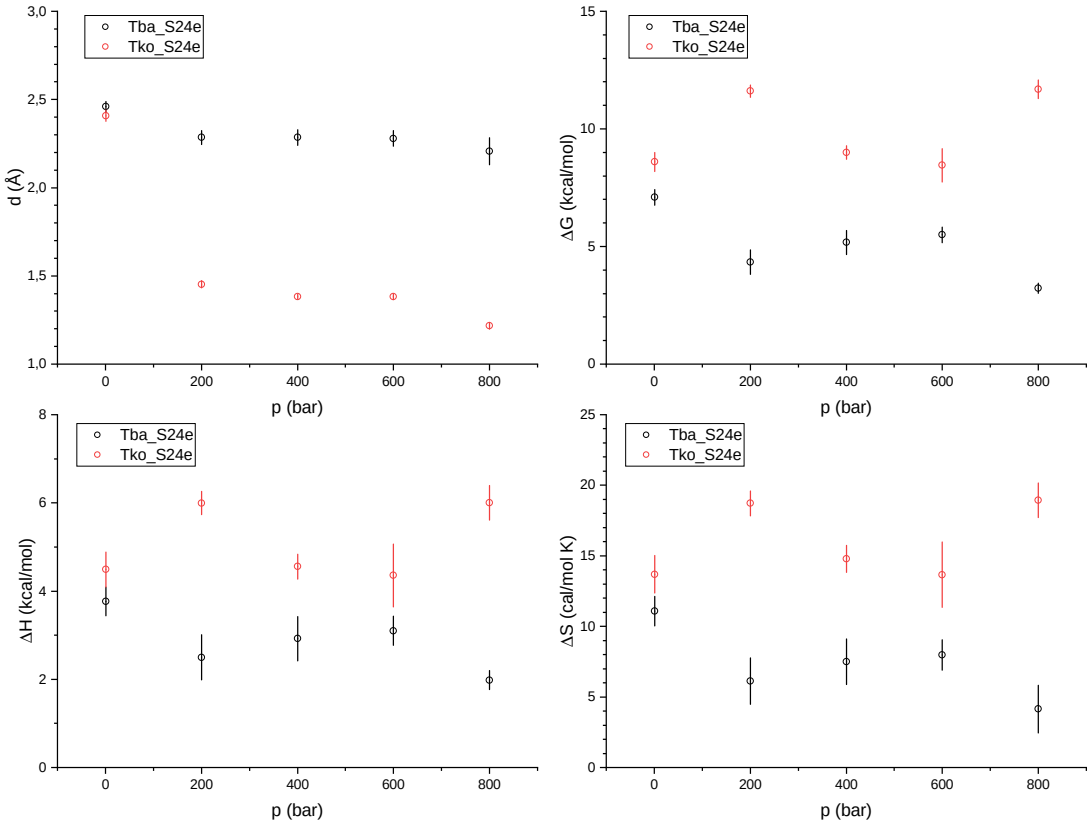


Figure 3: Values of d, ΔG, ΔH and ΔS as a function of pressure for the two samples.

References

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