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Adaptation to extreme environments: Macromolecular dynamics in complex systems

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Abstract

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Minireview

Adaptation to extreme environments: Macromolecular dynamics in complex systems

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Abstract

What we previously thought of as insurmountable physical and chemical barriers to life, we now see as yet another niche harbouring 'extremophiles'. Extremophiles and their macromolecules had to develop molecular mechanisms of adaptation to extreme physico–chemical conditions. Using neutron spectroscopy, we have demonstrated that molecular dynamics represents one of these molecular mechanisms of adaptation. To which extent do hyper-saline conditions and extreme temperatures influence molecular dynamics? First, molecular dynamics were analysed for halophilic malate dehydrogenase from *Haloarcula marismortui* (Hm MalDH) under different molar solvent salt concentration conditions influencing its stability. Secondly, mean macromolecular motions were measured in-vivo in psychrophile (*Aquaspirillum arcticum*), mesophile (*Escherichia coli* and *Proteus mirabilis*), thermophile (*Thermus thermophilus*), and hyperthermophile (*Aquifex pyrofilus*) bacteria. The mean constant force of Hm MalDH increases progessively with increasing stability. The results show that the molecular adaptation of Hm MalDH to hyper-saline conditions is achieved through an increasing resilience of its structure dominated by enthalpic mechanisms. The study of bacteria has provided tools to quantify the macromolecular adaptation to extreme temperatures in the naturally crowded environment of the cell. The macromolecular resilience of bacteria increases with adaptation to high temperatures.
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Keywords: Neutron scattering; Dynamics; Macromolecular adaptation; Extreme condition; Halophilic malate dehydrogenase; Bacterial adaptation

1. Introduction

The cell can be considered as the elementary unit of life. Genetic analysis of organisms leads to grouping them in three distinct kingdoms: the eukaryotes (Eucarya) and two groups of prokaryotes, the eubacteria (Bacteria) and the archaebacteria (Archaea).

The cell is a complex system, its cytoplasm a crowded environment of different macromolecules, of which proteins are the main type, in terms of quantity and variety of function. They constitute more than 50% of the dry weight of the cell. Their extraordinary variety makes possible the diverse structural and functional cellular activities. Protein enzymes catalyse the extraordinary range of biochemical reactions. A consensus has arisen that, to carry out their role in and around the cell, enzymes adopt a specific tridimensional structure and also specific atomic and molecular motions adapted to their biological function. Thus, they were selected by evolution in relation to these properties.

The concept of dynamics, from the Greek Δυναμισ, strength, pertains to forces. The forces that maintain biological molecular structure and govern atomic motions in macromolecules are "weak" forces (hydrogen bonds, ionic bonds, van der Waals, and hydrophobic interactions) because their associated energies are similar to thermal energy at usual temperatures. Fast atomic thermal motions on the picosecond to nanosecond timescale allow proteins to achieve the stability and motions, and, therefore, the necessary rigidity and flexibility to perform their biological

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functions (enzymatic activity, ion pump activity, . . .) [1, 2]. Neutron spectroscopy is particularly adapted to the study of these motions, because neutron wavelengths ($\approx$Å) and energies ($\approx$meV) match, respectively, the amplitudes and frequencies of molecular motions [1,3,4]. Furthermore, neutron absorption is low for protein atoms and the radiation penetrates deeply into the sample with negligible radiation damage. Important isotope effects, in particular for hydrogen (H) and deuterium (D), make neutron scattering a very powerful technique in the study of water and complex systems that can be selectively deuterium-labelled.

Organisms can thrive in what we call extreme environments on Earth and perhaps elsewhere in the Solar System. Macelroy [5] named these lovers ('philos' to Greeks) of extreme environments 'extremophiles'. They had to adapt to one or several physico–chemical extreme parameters: high temperatures for thermophiles and hyperthermophiles that live above 60 °C near geysers and hydrothermal vents; psychrophiles grow at temperatures below 15 °C, in glacier water and polar seas; halophiles thrive in hyper-saline environments like the Lac Rose in Senegal (Fig. 1). Other physico–chemical extreme parameters are, for example, high pressure, high radiation activity, high and low pH, etc. The three kingdoms are represented by extremophile organisms. Extremophiles and their enzymes have an important economic potential in multiple areas, either by direct applications for catalysis under extreme conditions or by tapping them as sources of ideas to modify mesophile enzymes (adapted to 'normal' physico–chemical conditions), with the aim of

improving their properties and stability at high temperature, for example. Evaporite minerals (Jarosite, Kieserite) have been identified in the Meridiani region on Mars, which suggests that at one time, there was a shallow "sea" or lake at that location [6,7]. Its chemistry was that of slightly oxidising, strongly acidic water. On Earth, fossils are often found in evaporite deposits associated with lake beds [8,9]. If traces of life exist in Martian Jarosite evaporite, the corresponding organisms will be likely to be adapted to an acidic environment and will fall into the extremophile category. Moreover, it is believed that on Mars, the process of lake and sea evaporation was prolonged enough to allow a cellular life form to evolve in hyper-saline conditions. For such reasons, the study of the extremophile adaptation has broad implication for exobiology.

This mini review concerns work from our laboratory on (i) the enzyme malate dehydrogenase from the extreme halophilic Archaea *Haloarcula marismortui* (Hm MalDH) that were discovered in the Dead Sea and (ii) psychrophile, mesophile, thermophile, and hyperthermophile bacterial cells. Using neutron spectroscopy, we have demonstrated that molecular dynamics represents one of the molecular mechanisms of adaptation to extreme physico–chemical conditions. We present results for Hm MalDH in H_2O and D_2O hyper-saline solvent conditions influencing its stability. The neutron results combined with Circular Dichroism data in corresponding conditions established the correlation between dynamics and stability. We discuss these observations in terms of entropy or enthalpy dominated mechanisms for the stabilization of the protein in a given solvent environment.

The study of the molecular adaptation of psychrophile, mesophile, thermophile, and hyperthermophile bacteria to extreme temperature provided tools to characterize and compare mean macromolecule dynamics in vivo, in bacterial cytoplasm, and established how adaptation at the cellular level occurred through dynamics acting to optimise protein stability and flexibility.

2. The halophilic malate dehydrogenase from *H. marismortui* (Hm MalDH)

Malate dehydrogenase from *H. marismortui* is certainly the most extensively studied halophilic protein [10,11]. The crystal structure of Hm MalDH shows intersubunit salt-bridge clusters, similar to hyperthermophilic protein (Fig. 2). It was among the first halophilic proteins whose structures were solved by cristallography [12–14]. The enzyme is a homo-tetramer of the lactate dehydrogenase (LDH)-like MalDH family [15,16]. The surface of the enzyme is coated with the acidic residues that are characteristic of halophilic proteins: Hm MalDH has an excess of 156 negative charges that play a role in the solvent ion binding properties of the protein fundamental to protein stability and solubility at high salt [17,18].
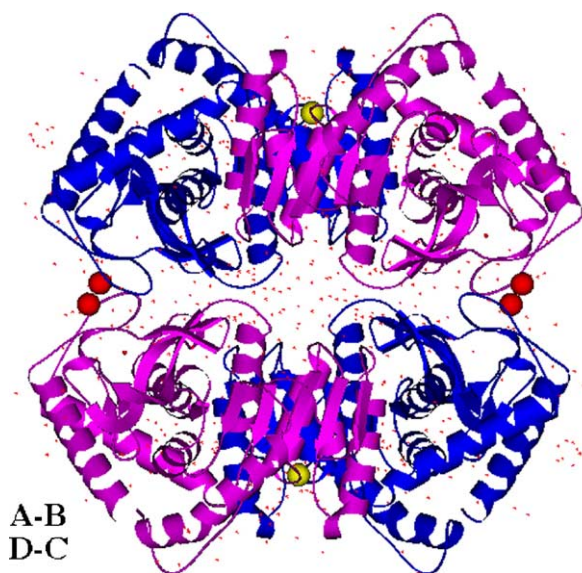

Fig. 1. Lac Rose in Senegal.

Fig. 2. The three-dimensional structure of Hm MalDH. Representation of the four protein subunits A–D: the two bound sodium (yellow balls) ions participate to salt bridges between monomers of the two dimers A–B and D–C; and the four bound chloride (red balls) ions participate to salt bridges between the two dimers. Water molecules are shown as red points [13].

Hm MalDH requires molar salt solvent concentrations for stability and solubility. Low salt solvent-induced inactivation of the protein is due to concomitant dissociation of the tetramer and unfolding of monomers. When increasing the salt concentration, the folded tetrameric form is stabilized. The stability of Hm MalDH has been studied as a function of salt type and concentration in H_2O and heavy water (D_2O) solutions [19,20]. In molar NaCl or KCl in H_2O, the stabilization of Hm MalDH is dominated by enthalpic terms. The protein is more stable in NaCl than in KCl, which was interpreted as caused by the higher hydration and binding energies of Na^+ compared with K^+. Hm MalDH is also more stable in D_2O than in H_2O [19,20].

2.1. Light water, heavy water

Heavy water is often used as a solvent for proteins in NMR, neutron-scattering, and spectroscopic studies. However, it is known to affect protein stabilization and has been used as probe in work on protein folding. H_2O and D_2O are molecules of almost identical dipole moment, shape, size, and bond lengths. However, the different masses (D has twice the mass of H), reduced masses, and moments of inertia make their vibrational and librational frequencies substantially different [21,22]. The origin of the different properties of H_2O and D_2O with respect to ionic solvation also lies in how the presence of ions affects the frequencies of these modes. Zero-point frequencies of the modes in the bulk solvents and at ions differ by an isotope factor of about $\sqrt{2}$ (the exact value depends on the mode), with corresponding differences in zero-point energy. A smaller isotope effect is observed

in the intermolecular vibration along the hydrogen bond, where the frequency is reduced by a factor of the square root of the molecular mass ratio $[(16+2)/(16+4)]^{1/2} = 0.948$. The fundamental OH– –O and OD– –O hydrogen bond stretch modes, however, show large frequency differences with correspondingly large differences in zero-point energy of about 1.3 kcal/mol, which are the principal factors determining the different properties of H_2O and D_2O. D_2O has a greater degree of structure than H_2O at a given temperature and displays a higher temperature of maximum density, greater viscosity, and larger heat of vaporization and sublimation when compared with H_2O. For proteins, this difference in properties between H_2O and D_2O leads to stronger hydration-bond interactions in D_2O, as well as to the solubility of apolar groups being lower in D_2O than in H_2O, which favors the hydrophobic interaction [21].

3. Macromolecular dynamics

3.1. Force fields and neutron scattering

As we wrote in the Introduction, atoms are maintained in their average positions in a macromolecular structure by weak forces, arising from hydrogen bonds, etc. In terms of a force field, the width of the potential well in which an atom moves is a measure of its flexibility in terms of a fluctuation amplitude ($\sqrt{<u^2>}$), whereas the detailed shape of the well reflects the resilience of the structure, in terms of an effective force constant ($<k>$). In this picture, the stability would be given by the depth of the well [20]. Two limiting situations are easy to describe. If the atom motion takes place in an infinite square well, the flexibility will present a temperature-independent value, while the effective force constant is infinitely high. If, on the other hand, the confining potential can be approximated by a harmonic potential, $V(u) = 1/2<k> u^2$ and the atomic mean square fluctuation is related to the force constant by $<u^2(T)> = k_B T / <k>$ [23]; a less rigid harmonic structure is indeed more flexible and vice versa. Flexibility and rigidity therefore characterize independent parameters, related by temperature dependence. Because of the nature of the weak forces that maintain active biological structures and govern atomic motions in macromolecules, incoherent neutron scattering spectroscopy is strongly suited to the flexibility and the rigidity. Results described in this review are from experiments performed on the backscattering spectrometer IN13 at the Institute Laue Langevin Grenoble, France (information on the Institute and the instrument is available on the web at: http://www.ill.fr). The instrument allows one to examine atomic motions in the space and time window of about 1 Å in 0.1 ns. All motions outside the window, such as the diffusion of bulk water (≈ 10 Å in 0.1 ns), small peptides, or the smaller membrane components, for example, do not contribute to the scattering signal, so that experiments could be performed in the H_2O

solvent (we recall that many of the previous neutron scattering experiments were performed in heavy water). In this space-time window and according to a Gaussian approximation, the incoherent elastic scattered intensity can be analysed as [4]:

$$I(Q, 0 \pm \Delta\omega) = \text{constant} \cdot \exp\left\{(1/6)\left(-<u^2>Q^2\right)\right\} \qquad (1)$$

where Q is $4\pi\sin\theta/\lambda$, 2θ is the scattering angle, and λ is the incident neutron wavelength; $<u^2>$ values include all contributions to motions in the accessible space and time windows, from vibrational fluctuations (usually expressed as a Debye-Waller factor) as well as from diffusional motions. The validity of the Gaussian approximation for the mean square fluctuation $<u^2>$ and its analogy to the Guinier formalism for small angle scattering by particles in solution have been discussed by Réat et al. (1997) [24] and more recently by Gabel (2005) [25]. In the Guinier formalism, a radius of gyration R_g^2 of a particle in solution is calculated [26]. The particle equivalent is the volume swept out by a single proton during the time scale of the experiment (~100 ps). The analogy holds if the motion is localised well within the space-time window defined by the Q and energy transfer ranges, respectively. The Guinier approximation is valid if $\sqrt{R_g^2 * Q^2} \approx 1$. Following our definition of $<u^2>$, $R_g^2 = 1/2 * <u^2>$. As a consequence, the Gaussian approximation is valid in the domain where $\sqrt{<u^2> * Q^2} \approx \sqrt{2}$. The mean square fluctuations $<u^2>$ at a given temperature T were calculated according to the Gaussian approximation as (Fig. 3):

$$\text{Ln}[I(Q, 0 \pm \Delta\omega)] = \text{constant} + A * Q^2 \qquad (2)$$

The mean square fluctuations were therefore calculated as:

$$<u^2> = -6A \qquad (3)$$

Elastic incoherent scattering data were collected in a scattering vector range of $1.2 \ \text{Å}^{-1} \leq Q \leq 2.2 \ \text{Å}^{-1}$. The
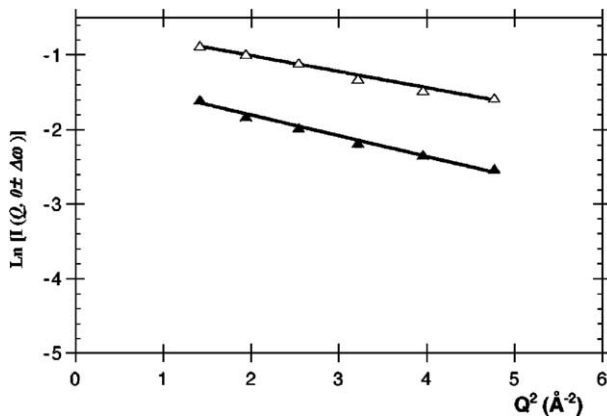

Fig. 3. Variation of Ln[I(Q,0±Δω)] as a function of Q^2 for the *E. coli* sample at 277 °K (△) and 310 °K (▲), from which mean square fluctuations $<u^2>$ were calculated by using Eqs. (2) and (3). The range of the fit corresponds to $1, 34 \leq \sqrt{<u^2> Q^2} \leq 2, 5$.
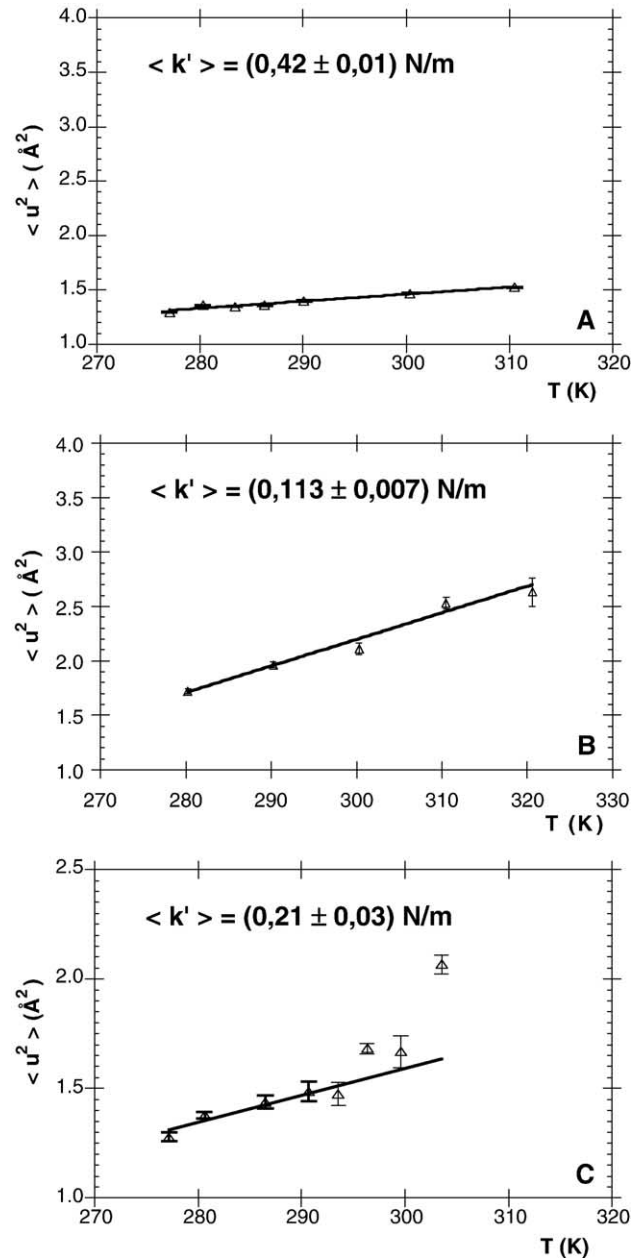

Fig. 4. Mean square fluctuations $<u^2>$ were plotted against absolute temperature T for *E. coli* (A), Hm MalDH in 2 M NaCl_H$_2$O (B), and *A. arcticum* (C). Effective mean force constants $<k'>$, describing mean macromolecular resilience, was calculated from the slopes of the straight-line fits by using Eq. (4), in the temperature region where the bacteria proteins and Hm MalDH are stable [20,33].

$<u^2>$ values were then plotted as a function of absolute temperature T (Fig. 4). The value of the root mean square fluctuation $\sqrt{<u^2>}$ in absolute Å units quantifies the global flexibility of the system studied. An effective mean force constant $<k'>$, defining mean resilience, can be calculated from the derivative of $<u^2>$ plotted versus temperature, T [23,27] (Fig. 4):

$$<k'> = 0.00276/\left(\text{d} <u^2> /\text{d}T\right) \qquad (4)$$

The numerical constants are chosen to express $<k'>$ in Newtons/meter (N/m) when $<u^2>$ is in Å^2 and T in Kelvin.

3.2. Hm MalDH dynamics under various solvent conditions influencing its stability

Solvent interactions provide a complex contribution to protein structure stabilization through hydration, van der Waals interactions, hydrogen bonds, ion binding, and the hydrophobic effect. Because the same forces control thermal fluctuations, a relation among solvent interactions, protein stabilization, and dynamics is expected intuitively, in which a softer, more flexible protein structure would be less stable. Stability, however, need not necessarily be associated with lower flexibility. The stability of a structure is quantified by the value of the free energy of stabilization, $\Delta G_{Stab} = \Delta H_{stab} - T\Delta S_{stab}$, the difference between the free energies of the folded and unfolded states. The enthalpic term, ΔH_{stab}, relates to bonding energy, and the entropic term, $T\Delta S_{stab}$, to the free energy arising from conformational substate distribution. A more flexible protein that can sample different conformations could be more stable if its free energy of stabilization is dominated by the entropic term with an effective force constant $<k'>$, which is smaller. If the enthalpic term dominates, a protein will be more stable with a larger $<k'>$ value. It is interesting to note that neutron scattering samples were close to 200 mg/ml, similar to cytoplasmic crowding conditions in bacteria.

Circular dichroism data combined with neutron results in corresponding solvent conditions established a correlation between stability and dynamics for Hm MalDH. Three solvent conditions were examined, in which Hm MalDH is progressively more stable: 2 M NaCl_H$_2$O, 2 M KCl_D$_2$O and 2 M NaCl_D$_2$O. The effective mean force constant $<k'>$ of Hm MalDH increases progressively with increasing stability, with $<k'>$ values varying from (0.113 ± 0.007) N/m in 2 M NaCl_H$_2$O through (0.205 ± 0.04) N/m in 2 M KCl_D$_2$O to (0.505 ± 0.049) N/m in 2 M NaCl_D$_2$O. Moreover, we showed that the isotope effect of D$_2$O in molar-salt solutions and the effect of ions on Hm MalDH are dominated by the stronger D-bond in the hydration shell and by the larger hydration shell energy of Na$^+$ than that of K$^+$. This suggests that the enthalpic contribution of the hydration shell dominates the stability and the dynamics behaviour of the halophilic protein in 2 molar-salt solutions [20].

3.3. Macromolecular dynamics measured in vivo in bacteria adapted at different temperatures

The instrument space-time window essentially selected motions of atoms that are anchored to macromolecules (proteins, nucleic acids, and their complexes) and was not sensitive to cytoplasmic bulk water, small peptides, or the smaller membrane components, for example, which diffuse out of the window during the timescale of the experiment.

Strongly bound water will contribute as an internal part of the macromolecules.

We proposed a novel neutron scattering approach that allows the characterization of the mean motions of the entire cellular macromolecular population in vivo, in order to compare the macromolecular dynamics on whole live bacteria: the psychrophile *Aquaspirillum arcticum*, the mesophiles *Escherichia coli* and *Proteus mirabilis*, the thermophile *Thermus thermophilus*, and the hyperthermophile *Aquifex pyrofilus*. The aim of these neutron scattering experiments was to specify how the macromolecular dynamics in vivo is affected by the adaptation to extreme temperatures. The $<u^2>$ values of *A. arcticum* showed a striking transition above 20 °C, reflecting macromolecular denaturation (Fig. 4C). We note that 17 °C is the maximum temperature at which *A. arcticum* can maintain net growth. A hypothesis has been formulated that thermo-adaptation is associated with protein dynamics [28], in the sense that psychrophile proteins have adapted to achieve increased structural flexibility, necessary for activity at low temperature [29,30], and that the enhanced thermal stability of thermophile proteins is associated with increased rigidity [31]. Thermophile enzymes are also characterized by a higher temperature of maximum activity [28,32]. The more rigid hyperthermophilic enzyme would then require higher temperatures in order to achieve the requisite conformational flexibility for activity. We found that the flexibilities are in fact maintained within narrow limits around 1.2 Å, independent of physiological temperature for all cells in their functional state. Mean effective fore constant values, $<k'>$, of 0.21 ± 0.03, 0.42 ± 0.01, 0.39 ± 0.01, 0.67 ± 0.011, and 0.60 ± 0.01 N/m were found for *A. arcticum*, *E. coli*, *P. mirabilis*, *T. thermophilus*, and *A. pyrofilus* cells, respectively. Therefore, in the cells measured, thermophile and mesophile macromolecules are, respectively, three times and twice as resilient as those in psychrophiles (Fig. 5). Thus, we showed, in vivo, a strong correlation between resilience and adaptation to a physiological temperature. Therefore,
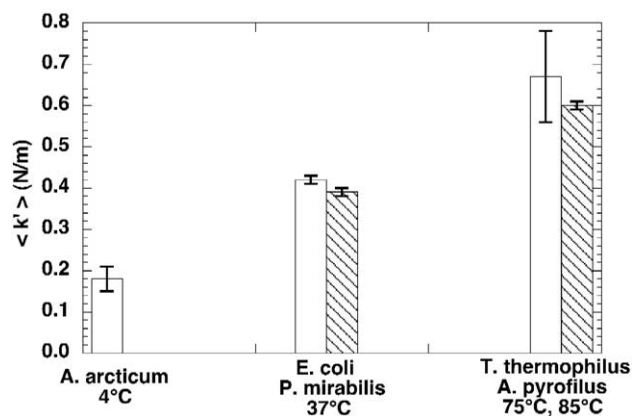

Fig. 5. Mean macromolecular force constant values $<k'>$ measured in cellulo for each bacterial type, plotted versus adaptation temperature: 4 °C for *A. arcticum* (blank), 37 °C for *E. coli* (blank) and *P. mirabilis* (hatched), 75 °C for *T. thermophilus* (blank), and 85 °C for *A. pyrofilus* (hatched) [33].

the macromolecular resilience of bacteria increases with adaptation to high temperatures. Macromolecules in hyper-thermophile and thermophile cells are, on average, the most resilient, followed by those in mesophile, while those in psychrophiles present the softest structures [33]. The overall macromolecular composition of the bacteria cells examined is not expected to vary significantly from one cell type to another [34]. Macromolecules constitute 96% of the total dry weight of an *E. coli* cell. DNA represents 3%, and lipids and polysaccharides about 17%; the majority, more than 75% of the dry weight, consists of proteins and ribosomes, themselves made up of about 50% protein and 50% RNA by mass [35,36]. Within a given bacterium, differences in protein expression due to metabolic modifications in unstressed cells affect a few hundred proteins out of about 5000 [37]. It is reasonable to assume, therefore, that the neutron scattering data described here are dominated by the dynamics of the proteins, making up the cellular proteome, in association with their natural environment. The resilience values, which increased with stabilization temperature, indicated the dominance of enthalpy terms in the stabilization free energy differences. For proteins in which entropy terms (such as the hydrophobic interaction) are dominant, a more flexible and less resilient macromolecule will be more stable [20,30,38–40]. Haney et al. (1999) [41] have published properties which are strongly correlated with thermal adaptation by comparison of protein sequences from mesophilic and extremely thermophilic *Methanococcus* species. The observed replacements decrease the content of uncharged polar residues, increase the content of charged residues, increase residue hydrophobicity, and increase residue volume in the extremely thermophilic proteins. Cambillau and Claverie (2000) [42] have published a correlation between adaptation to high temperature and the average charged minus polar amino-acid percentage (Ch-Pol) in protein structures. The average percentage (Ch-Pol) can be increased in two ways: namely, by increasing the content of charged residues or decreasing the number of polar residues. An increase in charged residues favors hydrogen bonds, hydration interactions [15], and salt bridges, and, in particular, Lys residues can also contribute to an increase in local hydrophobic interactions. Furthermore, charged residues in the thermophile proteins contribute 56% of residue volume increase [41], which reduces the entropic freedom of the unfolded protein backbone [43]. Uncharged-polar residue losses involve replacement by either charged or by bulkier hydrophobic residues. In addition to stabilizing the fold, decreasing polar residues helps avoid deamination reactions and backbone cleavages [44, 45]. In general, therefore, although an increase in the Ch-Pol percentage could lead to both enthalpic (H-bonds, hydration interactions, salt bridges) and entropic (increase in bulkier hydrophobic residues) contributions to the free energy landscape, the correlation between the temperature stabilization and the increase in resilience values suggest the dominance of enthalpic terms in thermo-adaptation. A number of studies have been published analysing structural differences among homologous psychrophile, mesophile, and thermophile proteins [46–48]; common trends include a decrease in the number of salt bridges and of surface-exposed side chains in the psychrophiles as well as decreased protein–protein and interdomain interactions within proteins. All these effects would contribute to the decrease in resilience observed in the data presented for the psychrophile bacteria. Protein dynamics is strongly affected by solvent effects [20], however, and adaptation of cytoplasm composition (e.g., through the presence of salt or compatible solutes) may also contribute to the observed macromolecular resilience differences.

Acknowledgements

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