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Life at extreme conditions: Neutron scattering studies of biological molecules suggest that evolution selected dynamics

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Abstract. The short review concentrates on recent work performed at the neutrons in biology laboratories of the Institut Laue Langevin and Institut de Biologie Structurale in Grenoble. Extremophile organisms have been discovered that require extreme conditions of temperature, pressure or solvent environment for survival. The existence of such organisms poses a significant challenge in understanding the physical chemistry of their proteins, in view of the great sensitivity of protein structure and stability to the aqueous environment and to external conditions in general. Results of neutron scattering measurements on the dynamics of proteins from extremophile organisms, *in vitro* as well as *in vivo*, indicated remarkably how adaptation to extreme conditions involves forces and fluctuation amplitudes that have been selected specifically, suggesting that evolutionary macromolecular selection proceeded via dynamics. The experiments were performed on a halophilic protein, and membrane adapted to high salt, a thermophilic enzyme adapted to high temperature and its mesophilic (adapted to 37°C) homologue; and *in vivo* for psychrophilic, mesophilic, thermophilic and hyperthermophilic bacteria, adapted respectively to temperatures of 4°C, 37°C, 75°C and 85°C. Further work demonstrated the existence of a water component of exceptionally low mobility in an extreme halophile from the Dead Sea, which is not present in mesophile bacterial cells.

Keywords. Extremophile bacteria; molecular adaptation; halophile; water dynamics; protein dynamics.

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1. Introduction

The elastic temperature scan on a neutron spectrometer to observe incoherent scattering provides a window in space-time for atomic and molecular fluctuations, on a scale that depends on the scattering vector range and energy resolution of the instrument [1]. The IN13 Italian–French Collaborating Research Group spectrometer at the Institut Laue Langevin (ILL) permits measurements of fluctuations on the Ångstrom length scale taking place within the 100 ps time scale. The instrument is very well suited to the study of *internal* motions in biological macromolecules in solution; water diffusion is too fast to contribute significantly to the data so that H_2O solutions can be used, despite the large incoherent cross-section of H; global

macromolecular diffusion is too slow to contribute significantly to the scattering data.

It has been established by calorimetric measurements that the active structures of biological macromolecules are stabilized by weak forces associated to energies of the order of thermal energy [2]. The two parameters, calculated from the neutron scattering elastic temperature scans, are very useful for experimental characterization of the macromolecular stabilization forces. They are: the absolute mean square value of the fluctuations in the sample, $\langle u^2 \rangle$ (dominated by the motions of H because of its large incoherent scattering cross-section), and, from the temperature dependence of $\langle u^2 \rangle$, an effective mean elastic constant (in a quasi-harmonic approximation) for the macromolecular structures, $\langle k' \rangle$ [1]. As discussed below, neutron scattering measurements on different systems have established the significance of these parameters for biological function.

2. Biological systems studied

Biological macromolecules have evolved over billions of years to achieve dynamic structures adapted to their function. They are stable in a restricted temperature range and solvent composition range with a free energy of stabilization that is close to thermal energy. Organisms living under extreme conditions of salinity (for example, halophiles in the Dead Sea), or temperature (for example, psychrophiles are adapted to low glacier and arctic water temperatures, thermophiles and hyperthermophiles to high or very high water temperatures found in hot volcanic springs) are excellent models for the study of dynamics–function relationships. Data were collected on the following systems: the purple membrane from the halophile, *Halobacterium salinarum*, live cells of the halophile, *Haloarcula marismortui*, live cells from psychrophile (living at 4°C), mesophile (37°C), thermophile (65°C) and hyperthermophile (85°C) bacteria, and two pure protein samples of homologous enzymes, extracted from a mesophile and a hyperthermophile, respectively.

3. Results and discussion on the evolution of the dynamics–function relation

The purple membrane of *H. salinarum* functions as a light activated proton pump that participates in the bioenergetics of the halophilic organism. The only protein it contains has been named bacteriorhodopsin (BR) because it binds one molecule of retinal (like rhodopsin in the retina of animal eyes). The proton pump activity of BR is associated with a photocycle of changes in the light absorption properties of the retinal, which has become a precious spectroscopic tool for the study of activity. Neutron scattering has contributed significantly to our current understanding of BR structure–function and dynamics–function relationships; work up to the end of the twentieth century is reviewed by Zaccai [1]. In particular, it has been shown in early experiments that a certain level of hydration was required for BR to access the anharmonic motions necessary for its biological activity. Recent experiments have permitted a refinement of this result and established the extent of dynamical

coupling between the membrane protein and the membrane hydration water [3] and compared it to that in a soluble protein [4]. The experiments took advantage of the fact that the purple membrane lends itself very well to *in vivo* isotope labelling. Neutron experiments on samples in which specific groups were specifically H-labelled in fully deuterated (^{2}H, D) membrane, led to a very good definition of the motions of these groups. We recall that the incoherent scattering cross-section of D is about 40 times smaller than that of H. Thus, the active core of BR was shown, quantitatively, to be stiffer or more resilient than the protein globally, which would give better control of retinal orientation that acts as a valve in the proton pump mechanism [5,6]. In another set of experiments, the dynamics of individual amino acid types in BR was studied using H-D labelling [3]. The experimental results were combined with molecular dynamics simulations to reveal the dynamical heterogeneity of amino acid motions in the protein – the dynamics of a given group depending not only on its type but also on its location in the structure. By using deuterium labelling and reconstitution procedures, the lipids and protein were labelled separately in purple membrane, and their motions and dynamical couplings were measured [7]. The results indicated that the lipid environment of the membrane was softer or less resilient than BR, and that the dynamical transition in the protein appeared to be coupled to that in the lipid environment.

The dynamic state of water in biological cells is the object of intense study because it might have specific properties related to confinement in the crowded macromolecular environment of the cytoplasm. Quasi-elastic neutron scattering experiments in a large range of time and length scales (on the back-scattering, IN16, and time of flight, IN6, spectrometers at ILL) on the Dead Sea organism, *H. marismortui*, have shown the existence of a very slow (compared to bulk water) component in these cells, by using live samples of fully deuterated cells suspended in H_2O [8]. This appears, however, to be related to the specific environment inside the cytoplasm of extreme halophiles, rather than to a general property of biological cells; similar experiments on *E. coli* showed that cytoplasmic water behaved very similarly to pure water beyond the first macromolecular hydration shell [9].

Incoherent neutron scattering experiments on live cells have a great advantage that they yield information on the mean internal dynamics of all macromolecular structures in the cells. The two measured parameters (the mean square fluctuation, $\langle u^2 \rangle$, which through the flexibility of a structure is related to biological activity, and the effective mean elastic constant, $\langle k' \rangle$, which is related to the stability of the structures) are good indicators of global evolutionary adaptation mechanisms. In an important set of experiments, published in a biological journal in 2004, the two parameters were measured for bacterial cells, adapted to live in different temperatures [10]. It was shown how the two parameters were sensitive to whether or not the cells were alive: both $\langle u^2 \rangle$ and $\langle k' \rangle$ increased sharply when the cells died. Evolutionary adaptation through macromolecular dynamics was strikingly established by the results. The thermophile and hyperthermophile cells displayed the highest effective elastic constant, followed by the mesophiles as expected for stability at different temperatures. The psychrophiles had the smallest $\langle k' \rangle$ value, still sufficient for macromolecular stability at low temperature but with effectively soft structures to permit functional fluctuations. If the psychrophile structures were as resilient as the mesophiles, they would of course be stable at low temperature,

but their $\langle u^2 \rangle$ values would be too low for activity. Another important result from these experiments was the observation that the rms value, $\sqrt{\langle u^2 \rangle}$, extrapolated to the physiological temperature, in each case, converged quite sharply between 1.1 and 1.3 Å.

The results on the live bacteria were reproduced in experiments on two homologous enzymes, lactate dehydrogenase from rabbit (a mesophile, optimal enzymatic activity at 37°C) and malate dehydrogenase from *Methanocaldococcus jannaschii* (a hyperthermophile, optimal enzymatic activity at 90°C). The resilience of the hyperthermophile protein was significantly higher, and the rms fluctuation values were 1.5 Å for both proteins, at their respective physiological temperature. The fluctuation value is higher than the mean for the bacterial cells, but this is not unexpected because the malate and lactate dehydrogenase activities involve larger than average conformational fluctuations [11].

In conclusion, it should be emphasized that protein dynamics is strongly environment-dependent, as has been shown by neutron scattering measurements on malate dehydrogenase (*Hmm* MalDH) from the extreme halophile, *H. marismortui*. Previous experiments had shown the protein to be stabilized by solvent interactions involving hydrated ions, and this was reflected in its dynamics. *Hmm* MalDH was found to be more resilient in molar solutions of NaCl than of KCl, as a result of the stronger hydration interactions of the Na^+ ion. Since the cytoplasmic environment in which the enzyme is active is highly concentrated in KCl, it would suggest that low resilience were necessary for biological activity [12].

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