Experimental report

20/04/2020

Proposal: CRG-2653 **Council:** 4/2019

Title: The role of internal dynamics and resilience on protein unfolding investigated by means of pressure modulation

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: MYOGLOBIN

Instrument	Requested days	Allocated days	From	To
IN13	8	8	28/01/2020	04/02/2020

Abstract:

In order to get insight on the role of internal dynamics on the protein folding/unfolding transition, we propose here to study the internal motions of myoglobin in the temperature range 273-363 K, at different pressure values, from 0 to 5 kbar. Our aim is to investigate the relation between the dynamics/softness of the protein and its thermal/pressure stability. In fact, measurement of elastic scans in the temperature range 273-363 K and at various pressure values will enable us to put in relation the internal dynamics of the protein, as reflected in the Mean Square Displacements (MSD) of non-exchangeable hydrogen atoms, and its pressure-dependent unfolding temperature (as obtained from a high temperature abrupt change in the MSD behavior). The obtained results will give important information on the correlation between protein internal dynamics and unfolding and in particular on the existence of a unique dynamical regime in the proximity of the unfolding process, as recently suggested. In addition, thermodynamic quantities as free energy, enthalpy and entropy will be extracted and compared to values obtained by calorimetry and densitometry.

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Exposure of proteins to high pressure conditions leads to the so-called cold denaturation. In spite of the very large number of studies dedicated to it, this phenomenon (of extreme biophysical and biotechnological relevance) is not yet thoroughly understood (for discussions on the effects of pressure on protein denaturation see refs. [1] and [2] and references therein).

We recently studied the effect of pressure on the dynamical properties of myoglobin and measured the mean square displacements (MSD) of the hydrogen atoms of the protein as a function of both temperature (from cryogenic to room temperature) and pressure (0 to 5 kbar) [3]. This enabled us to evidence pressure effects on the energy landscape of the protein. Based on these findings, our working hypothesis about the pressure effects on protein denaturation is that a relevant role might be played by protein internal dynamics. In fact, recent experiments suggest the existence of a unique dynamical regime for protein internal motions in the proximity of thermal unfolding, even when the unfolding occurs at completely different temperature values, as it is for instance when proteins are embedded in different solvents [4]. To investigate the relationship between protein internal dynamics and unfolding, we performed EINS measurements on a D_2O highly hydrated myoglobin sample to ensure homogeneous pressure transmission (h=1.0 grams of water per grams of protein), in the temperature interval 273-363 K and in the pressure range 0-4 kbar. Indeed, the temperature behavior of the MSD will give information on the internal dynamics of the protein, but also on the unfolding temperature, which can be estimated by the abrupt change in the MSD vs. T dependence [3].

Fig. 1 (a-e) reports the MSD as a function of temperature at the various pressure values, as obtained in the framework of the Gaussian approximation [5], i.e. by considering $S(Q,\omega=0) \approx \exp[-(\langle \Delta r^2 \rangle Q^2)/3]$. Panel (a) also reports MSD values relative to hydrated Mb powder, adapted from ref. [6]. The comparison suggests that at high temperature our MSD values may be affected by some contribution from overall translational and rotational diffusion of protein molecules, most likely enhanced by the lowering of solution viscosity at increasing temperature values. In this scenario, the MSD reduction observed at high temperature can be ascribed to unfolding and aggregation of protein molecules, as in ref. [7]. For this reason, we performed a preliminary analysis of our data by using eq. 9 of ref. [7], thus obtaining for each pressure value from 0 to 3 kbar an unfolding temperature interval. The corresponding values are reported in a P-T diagram in Fig. 1f and show the typical elliptical shape predicted by Hawley in 1971 [8]. No MSD reduction can be clearly noticed at 4 kbar, suggesting that, according to previous data [3], the protein undergoes unfolding even at low

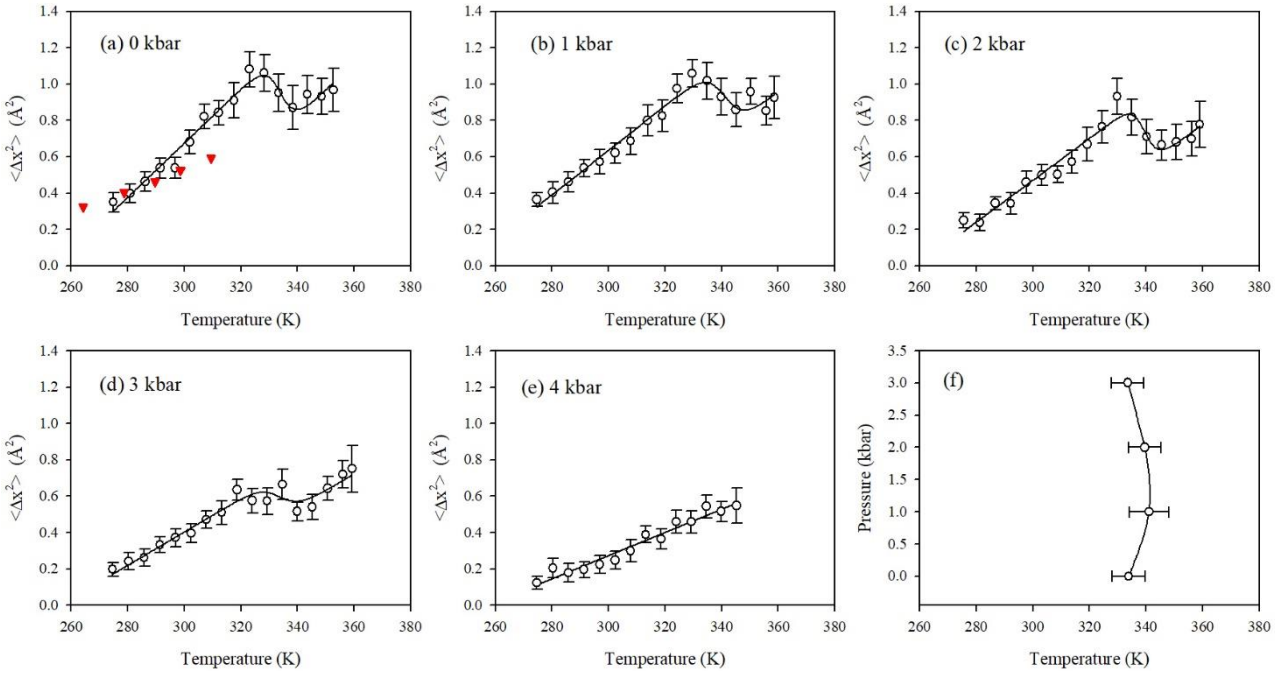

Fig. 1: (a-e) MSD as a function of temperature at the various pressure values, from 0 to 4 kbar. In panel a red triangles refer to hydrated Mb powder, adapted from ref. [6]. (f) Pressure-Temperature diagram for the unfolding/aggregation process. The solid line is a guide to the eye.

temperature. Work is in progress to separate the diffusional contribution to the MSD from those ascribable to protein internal dynamics.

References.

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