Experimental report

17/01/2017

Proposal: 8-04-709 **Council:** 4/2014

Title: The correlation between protein folding and dynamics investigated using high-resolution neutron TOF spectroscopy.

Research area: Biology

This proposal is a continuation of 8-04-688

Main proposer: Andreas STADLER

Experimental team: Andreas STADLER

Local contacts: Jacques OLLIVIER

Samples: apomyoglobin, D2O

Instrument	Requested days	Allocated days	From	To
IN5	4	5	19/09/2014	24/09/2014

Abstract:

The aim of the proposal is the investigation of the underlying correlation between protein dynamics and protein folding in apomyoglobin (apoMb). We will measure three samples of apoMb: the unfolded state of apoMb at pH2, one folding intermediate stabilized by NaCl with 28% helical content as an example of a folding intermediate and the fully folded state of apoMb (55% helical content). From high-resolution QENS measurements on IN5 of protein solutions we can separate internal dynamics and global diffusion. Good statistics of the quasielastic signal will allow us to interpret the internal dynamics with analytical theories such as the model for Brownian diffusion in a harmonic potential or the model for fractional Brownian dynamics in a harmonic potential. From temperature dependent measurements we will determine the evolution of the entropic stabilisation Delta S with temperature, and gain information about forces within the unfolded, partially folded and fully folded structures, which are related to the enthalpic stabilisation Delta H.

Myoglobin can be trapped in fully folded structures, partially folded molten globules, and unfolded states under stabile equilibrium conditions. Here, we report an experimental study on conformational dynamics of different folded conformational states of apo- and holomyoglobin in solution. Global protein diffusion and internal molecular motions were probed by neutron time-of-flight and neutron backscattering spectroscopy on the picosecond and nanosecond time scales. Global protein diffusion was found to depend on the α-helical content of the protein suggesting that charges of the macromolecule increase protein short-time diffusion. Concerning the molten globules a gel-like phase due to protein entanglement and interactions with neighbouring macromolecules was visible due to a reduction of the global diffusion coefficients on the nanosecond time scale. Diffusion coefficients and residence times of internal protein dynamics and root mean square displacements of localised internal motions were determined for the investigated structural states. The difference in conformational entropy ΔS_{conf} of the protein between the unfolded and the partially or fully folded conformations was extracted from the measured root mean square displacements. Using thermodynamic parameters from the literature and the experimentally determined ΔS_{conf} values we could identify the entropic contribution of the hydration shell ΔS_{hyd} of the different folded states. Our results point out the relevance of conformational entropy of the protein and the hydration shell for stability and folding of myoglobin.

Stadler, Demmel, Ollivier, Seydel. Picosecond to Nanosecond Dynamics Provide a Source of Conformational Entropy for Protein Folding, 2016, Phys Chem Chem Phys, 18, 21527-21538

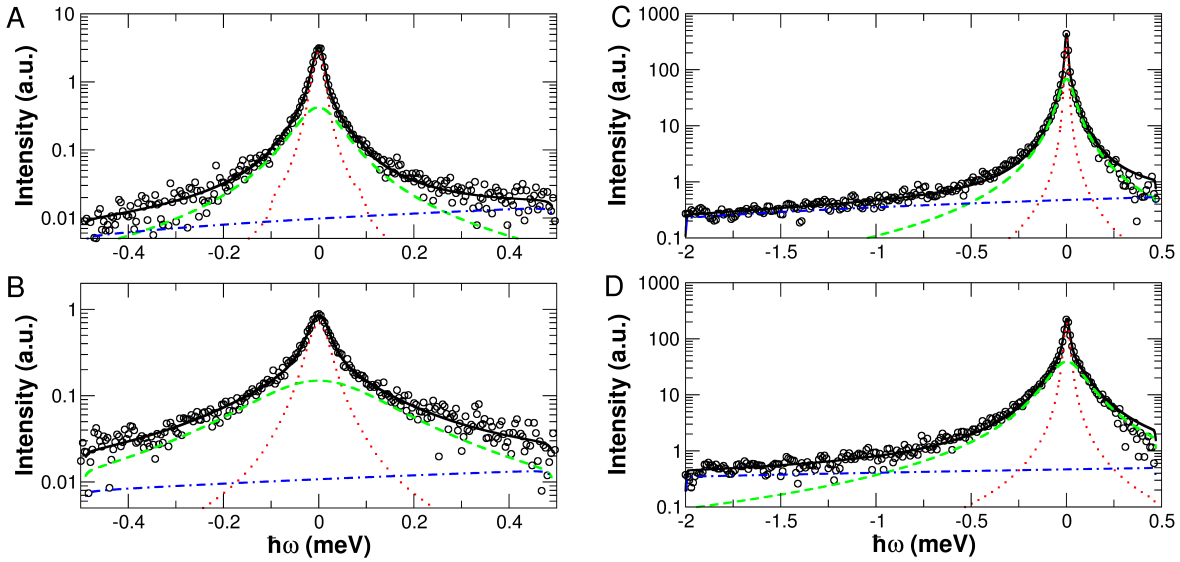

Figure 1: QENS spectra of unfolded apo-Mb at pD 2 (6 % α-helical content) measured on IRIS (A) at q=0.50 Å^{-1} and (B) q=1.44 Å^{-1}, and on IN5 (C) at q=0.54 Å^{-1} and (D) q=1.10 Å^{-1}. The sample temperatures are 303 K and 295 K for the shown IRIS and IN5 data, respectively. Empty symbols are the experimental data. For clarity only every forth data point is shown for IRIS spectra and every fifth point for data measured on IN5. The solid black line represents the total fit to the QENS spectra, the red dotted line is the narrow Lorentzian accounting for global protein diffusion, the green dashed line is the broad Lorentzian caused by the combination of global diffusion and internal protein dynamics, and the blue dashed-dotted line accounts for the linear background. All curves are convoluted with the instrumental resolution functions.

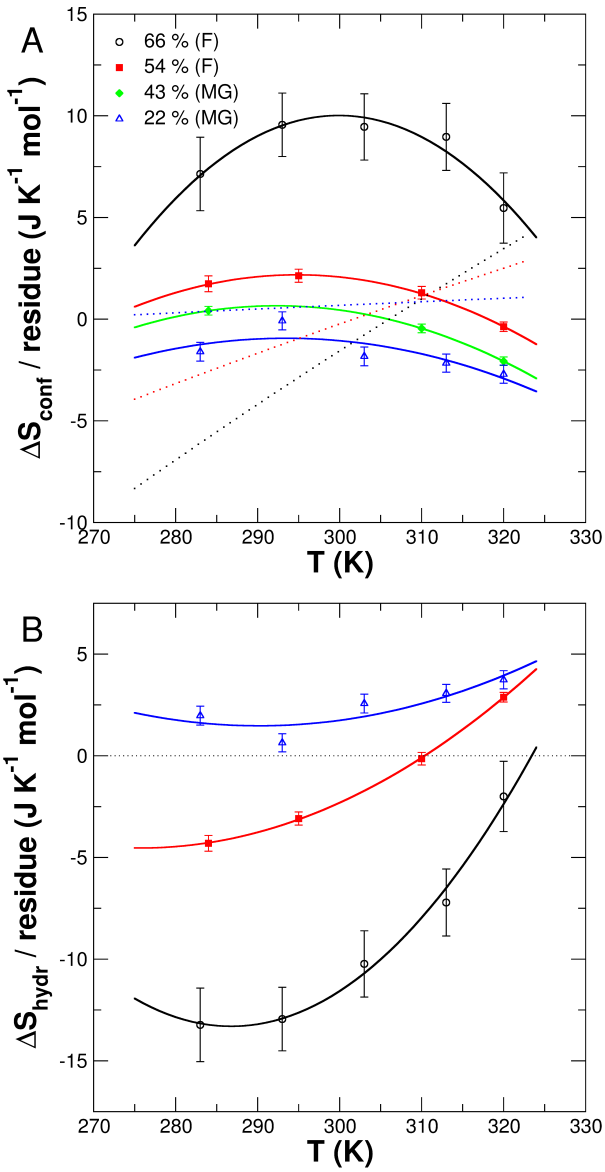

Figure 2: (A) Difference in conformational entropy ΔS_{conf} between the acid denatured state of apo-Mb and the protein in the investigated partially folded and folded. The α-helical content of the native folded (F), partially folded molten globule (MG), and unfolded (U) states is given in the legend (empty symbols IRIS, filled symbols IN5 data). The dotted lines are $\Delta S = \Delta S_{conf} + \Delta S_{hydr}$ calculated from the thermodynamic parameters T_m, ΔC_p and ΔH. (B) Difference in conformational entropy $\Delta S_{hydr} = \Delta S - \Delta S_{conf}$ related to water molecules of the hydration shell and protein motions that are out of the time window of the neutron spectrometers. The solid lines in (A) and (B) are polynomial fits of second order and serve as a guide to the eye.