Experimental Report

Proposal: **8-04-688** **Council:** 10/2012

Title: The contribution of thermal fluctuations to protein folding

This proposal is continuation of: 8-04-641

Researh Area: Biology

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Samples: apomyoglobin, D2O

Instrument	Req. Days	All. Days	From	To
IN6	3	3	19/03/2013	22/03/2013
IN16	7	6	06/05/2013	12/05/2013

Abstract:

Folding of a protein into its functional structure can be described in thermodynamic terms by the difference in Gibb's free energy dG between the unfolded and the folded state dG=dH-T*dS. Protein conformational fluctuations and disordered water molecules in the hydration shell account for the entropic contribution T*dS. The aim of this neutron scattering study is to quantify the contribution of protein conformational fluctuations to entropic stability of different folded states of apomyoglobin by quasielastic neutron scattering in the ps and ns time scale. The proposal covers two aspects: i) The continuation on IN6 would allow us to complete our first IN6 experiment by measuring the fully unfolded state, and characterize another folding intermediate with 28% structure and finally the fully folded state of myoglobin with 66% secondary structure. ii) The IN16 neutron spectrometer would allow us to investigate the role of conformational fluctuations in a different time windows of several ns. By combining the IN6 and IN16 results we could deduce if fluctuations in the ps or the ns time scale are the driving force for conformational stabilization in protein folding.

Protein solutions of the different folding intermediates of apomyoglobin were prepared in D_2O buffer at concentrations of around 50 mg/ml. Concentrated protein solutions were needed to allow for pH and salt effects and to induce the different folding levels. At even higher concentrations the partially folded intermediates aggregate strongly. All measurements were performed in 1mm thick flat Al holders and between 1.5 and 2 ml protein solutions were used. Sample temperature was maintained at 16°C for all measurements. On IN6 the protein solution and each individual buffers could be measured, while on IN16 due time limitations and as D_2O diffusion is mostly out of the energy window from -15 to 15 μeV only the empty cell was subtracted.

On IN6 we could complete our investigations of a broad range of unfolded, partially folded and folded conformations of apo- and holomyoglobin (see also the previous experimental report 8-04-641 for previous results). From the QENS spectra the Elastic Incoherent Structure Factor (EISF) was determined, see figure 1, and interpreted within the Gaussian approximation. Mean square displacements and the fraction of protons, which appear immobile on the picosecond time scale were obtained from the EISF, see figure 2.

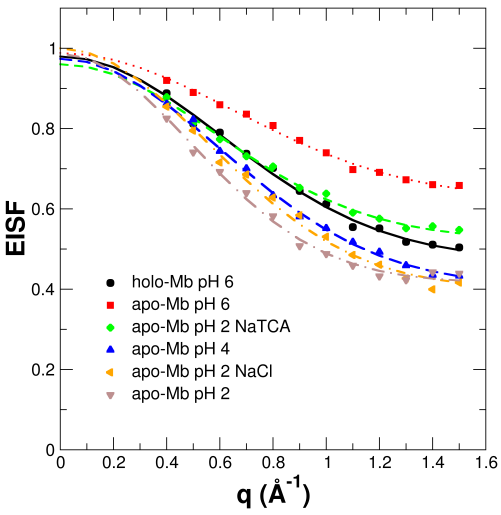

Figure 1: EISF from all investigated samples on IN6.

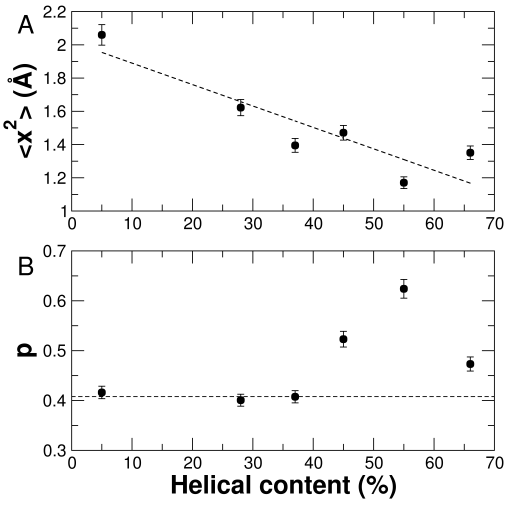

Figure 2: Mean square displacements and immobile fraction.

We found a clear difference in the MSDs and immobile fractions between the different folded states. From the MSDs we determined the entropy difference Δ*S* between the partially folded and fully folded conformations compared to the unfolded state at pH2, see figure 3.

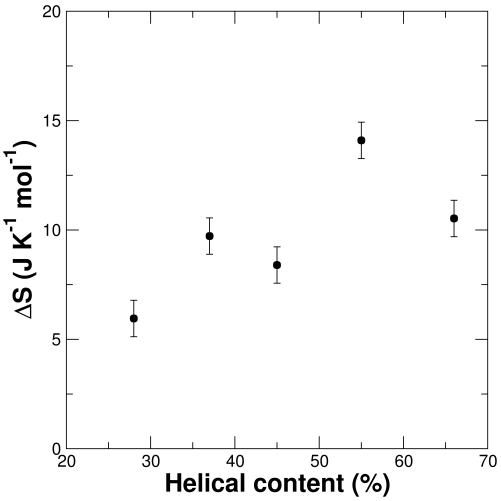

Figure 3: Entropy difference between the partially folded states and the unfolded state a pH2 determined from the experiment on IN6.

Slow dynamics of different folded states of apomyglobin were measured on IN16. Typical measured QENS spectra of apomyoglobin are given in figure 4.

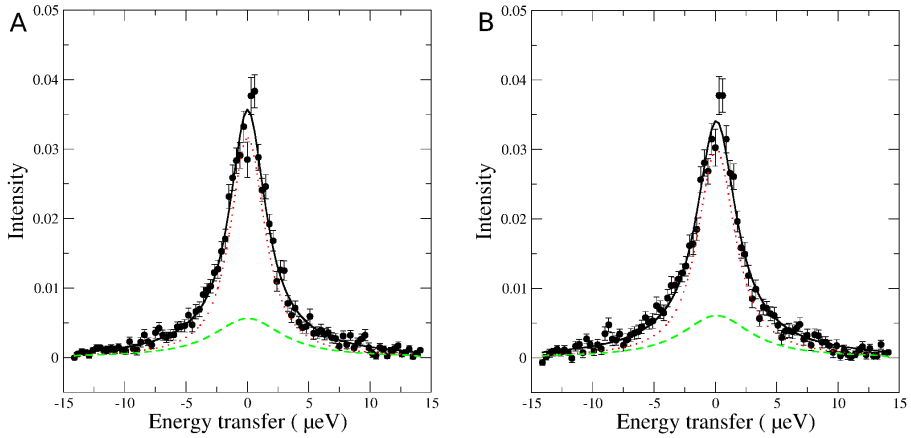

Figure 4: Measured spectra of (A) apomyoglobin at pH 6 and (B) apomyoglobin at pH2 at the scattering vector of q=0.65 Å^{-1}.

The amplitude of motion in the nanosecond timescale was found to be too large to be measured accurately with IN16. The drop in the EISF due to the large amplitudes of motion would be seen clearly at q<0.25 Å^{-1}, but this q-range is not well covered. The errors of the EISF at large q-values are also large and the data points drift away. The higher neutron flux on the new IN16B would allow to perform these measurements of weakly scattering protein solutions more precisely.

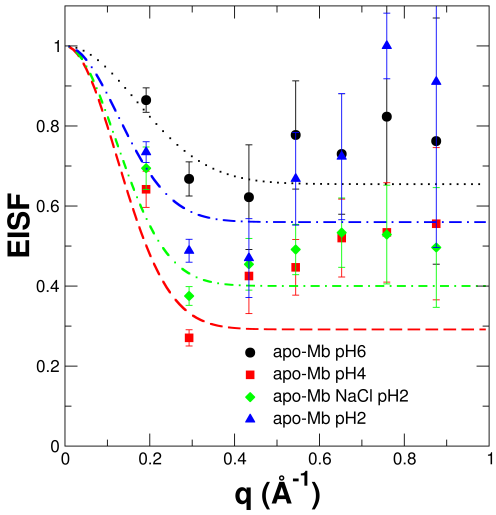

Figure 5: EISF measured on IN16 with Gaussian model with immobile fraction.

From the quasielastic line-broadening we could determine the effective diffusion coefficients of global protein diffusion, see figure 6, and a q-independent line-width caused by internal protein motions, given in figure 7. Both folding intermediates show a reduced diffusion coefficient, which is caused by the formation of a gel-like network. The fully unfolded state has a higher diffusivity due to strongly charged proteins similar to the fully folded state of apomyoglobin. The rate of internal motions was found to be independent of the degree of folding.

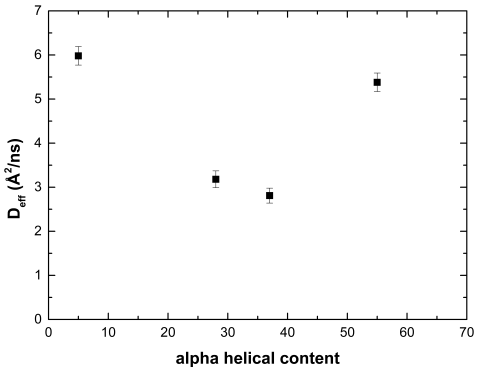

Figure 6: Effective diffusion coefficients of apomyoglobin.

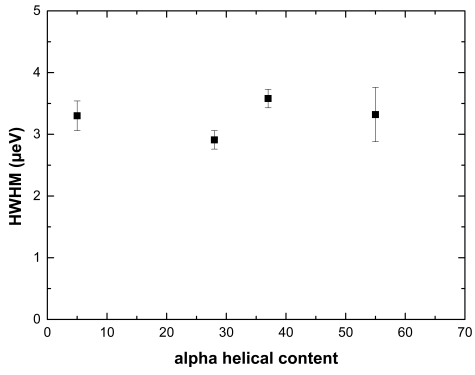

Figure 7: Line-widths of the quasielastic broadening of the internal modes.