Experimental report

28/08/2022

Proposal: 8-04-903 **Council:** 10/2020

Title: The relation of protein folding and dynamics investigated with wide-angle NSE

Research area: Biology

This proposal is a resubmission of 8-04-892

Main proposer: Andreas STADLER

Experimental team: Andreas STADLER

Local contacts: Peter FALUS

Samples: Apomyoglobin

Instrument	Requested days	Allocated days	From	To
WASP	6	6	29/06/2021	05/07/2021

Abstract:

We suggest to investigate the correlation between protein folding and dynamics for apomyoglobin in native folded conformations, partially folded intermediates and denatured states. We propose to use wide-angle neutron spin-echo spectroscopy (NSE) on WASP to measure average protein dynamics in the incoherent scattering range. The experiment on WASP would allow us to access a time range from the ps to several ns. This broad time window would enable us to measure both global diffusion and internal protein dynamics. Our guess is that in the fully unfolded state at high concentration of the denaturant guanidinium hydrochloride internal protein dynamics should show a pure polymer-like behavior, whereas as the secondary structure content is increased more complex dynamics will emerge for the partially folded intermediates. Finally, the fully folded Mb would be most-likely more rigid as the secondary structure elements are permanently folded. The experiment on WASP would allow us to identify fast motional patters that sit on top of slow collective motions that were identified recently by NSE experiments using coherent neutron scattering (Balacescu et al. Scientific Reports 2020, 10:1570).

The initial aim of the experiment on WASP was to investigate protein dynamics using NSE and incoherent scattering at high q-vectors. The primary goal was to explore the overall feasibility to study protein dynamics using incoherent NSE on WASP. For that purpose, different structural states of myoglobin were intended to be measured on WASP: two folded states with and without heme group, one partially folded molten globule at pH 4 and two denatured states (pH 2 and 3 M GndCl). The partially and unfolded structural state are only stabile as 30 mg/mL solutions. Higher protein concentrations result in highly viscous solutions and the protein aggregates rather rapidly. Our initial guess was that 12h beam time would be required per protein and per buffer solution for the 30 mg/mL solutions.

The WASP instrument was set to an incident wavelength of 7 Å which allowed us to cover a q-range of 0.5 to 2 $Å^{-1}$ and a time range of a few ps to 5.5 ns.

First experiments using 50 mg/mL myoglobin solutions demonstrated that at least 1 day of beam time on WASP is needed for a protein solution with such a concentration. The error bars of the data were, however, still comparatively high. Hence, we have abandoned the initial plan to measure 30 mg/mL solutions of the partially and unfolded myoglobin samples as they would not have scattered strongly enough and went for 200 mg/mL myoglobin solutions to increase the scattered signal.

During the experiment we measured myoglobin at concentrations of 50 and 200 mg/mL in D2O buffer and 70% deuterated glycerol buffers and the corresponding buffers with sufficient statistics. The instrumental resolution and the empty cell were measured as well.

The experiment on WASP demonstrated that proteins concentrations with 200 mg/mL and a beam time of 1 day per sample are needed to be able to measure incoherent scattering of proteins in solution with WASP. Sample volume needs to be 1 mL under those conditions.

WASP data are currently still in analysis and the results will give insights how internal dynamics of myoglobin depend on the viscosity of the solvent.