Experimental report

29/06/2020

Proposal: 8-03-966 **Council:** 10/2018

Title: Observing unfolding and proteolysis of a model protein in real-time: a time-resolved SANS study

Research area: Biology

This proposal is a continuation of 8-03-927

Main proposer: Frank GABEL

Experimental team: Frank GABEL
Jacques COVES
Emilie MAHIEU

Local contacts: Anne MARTEL

Samples: hydrogenated and deuterated proteins in H2O/D2O solvents

Instrument	Requested days	Allocated days	From	To
D22	1	1	03/08/2019	04/08/2019

Abstract:

The specific recognition, unfolding and degradation of faulty proteins is an essential process in any living cell in order to guarantee protein homeostasis and keep the proteome in a healthy and functional state. Dysfunction of this process can lead to accumulation of misfolded proteins that are potentially toxic and lead to multiple diseases. Despite the crucial importance of understanding this process, detailed structural insights, at a molecular level, are limited, mainly due to specific flexibility and conformational changes involved.
Here, we propose to study the unfolding and proteolysis of a specifically tagged model protein, GFPssrA, by SANS in solution, in the presence of the archaeal unfoldase complex PAN and the proteolytic core particle 20S. Using alternate deuteration of the different partners will allow us to describe both the specific conformation of GFP and PAN during the process. Using archaeal (hyperthermophilic partners) will allow a temperature control and fine-tuning of the rate of the process. In addition, an online fluorescence device on D22 will monitor the folded state of GFP.

Experimental Report: Experiment No. 8-03-966

Observing unfolding and proteolysis of a model protein in real-time: a time-resolved SANS study

Frank Gabel[1,2]

[1] Institut Laue-Langevin (ILL), Grenoble, France
[2] Institut de Biologie Structurale (IBS), Grenoble, France

Background and Summary

This SANS experiment was the central part of Emilie Mahieu's PhD thesis at IBS with the aim to follow regulated protein degradation by the archaeal PAN-20S proteasome system *in vitro* by combining perdeuteration of a substrate (Green Fluorescent Protein, GFP) with SANS and solvent contrast variation (H2O/D2O exchange).

Regulated protein degradation by the proteasome is an essential process in all kingdoms of Life and guarantees protein homeostasis in living cells. Misfolded or dysfunctional proteins that would otherwise be harmful to the function of the cell are thus eliminated in a controlled way. A malfunction of this pathway can lead to protein aggregation, which has been connected to a number of diseases in humans.

While there has been a lot of progress in recent years on the structural determination of the proteasome (e.g. by cryo-EM), very little is known on the dynamics of substrate degradation and states in solution. Our project had the aim to follow the GFP substrate states during the active unfolding and hydrolysis by the PAN-20S complex in solution. SANS, together with deuteration and contrast variation was essential in order to mask the signal of the very large PAN-20S complex (> 1 MDa) with respect to the relatively small substrate GFP (28 kDa). The separation of these signals would have been completely impossible by using X-rays (SAXS) where the overall signal would have been very largely dominated by the PAN-20S complex.

Methods

Our ILL collaborator in this project, Anne Martel, has developed a very innovative sample environment on D22 with an online light spectroscopy that allowed to measure the fluorescence signal of GFP (which is a measure for its folding state), in parallel to the scattered neutrons [1]. Perdeuteration of GFP (carried out at the ILL DLAB in collaboration with Martine Moulin and Michael Haertlein) allowed us to obtain a good signal-to-noise at 42% D2O (match-point of hydrogenated PAN and 20S) at mg/ml concentrations. The unfolding and hydrolysis of GFP was followed by a series of individual 30 second exposure frames. Temperature activation at 55 degrees Celsius allowed us to fine-tune the speed of

the reaction of this thermophilic molecular system in order to cover the interesting reaction rates over a period of 45 minutes.

Results

This innovate time-resolved (TR) SANS approach, coupled to online fluorescence, allowed us to follow the native GFP population and the reaction products in real-time, with a resolution of 30 seconds. The degradation of GFP was following a bi-exponential process, strongly correlated with ATP consumption by the PAN-20S complex (measured off-line under identical conditions). Furthermore, by comparing isolated PAN (the particle that unfolds GFP) with the fully assembled PAN-20S complex, we were able to draw conclusions on the processing pathway of GFP through the complex and compare our solution results with recent structures obtained by cryo-EM on substrate-engaged eukaryotic proteasomes.

The results have been published in *Biophysical Journal* [2]. Our paper has also been awarded a rare honor by being selected and accompanied by a "New & Notable" note in the same issue of *Biophysical Journal* [3]. In this note, the author praises the innovate methodological character of the SANS experiment and the perspectives of our approach for a large number of similar biomacromolecular systems.

References

[1] Ibrahim, Z., Martel, A., Moulin, M., Kim, H.S., Härtlein, M., Franzetti, B. and Gabel, F. (2017) ***Sci. Rep.*** 7, 40948.

[2] Mahieu, E., Covès, J., Krüger, G., Martel, A., Moulin, M., Carl, N., Härtlein, M., Carlomagno, T., Franzetti, B. and Gabel, F. (2020) Observing protein degradation by the PAN-20S proteasome by time-resolved neutron scattering. ***Biophys. J.*** [https://doi.org/10.1016/j.bpj.2020.06.015].

[3] Gupta, K. (2020) New and Notable: Hiding the Elephant in the Room with Experimental Neutrons ***Biophys. J.*** [https://doi.org/10.1016/j.bpj.2020.05.038].