Experimental report

21/06/2020

Proposal: 8-03-963 **Council:** 10/2018

Title: Investigating FICD mediated deAMPylation of BiP

Research area: Biology

This proposal is a new proposal

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Samples: PROTEIN

Instrument	Requested days	Allocated days	From	To
D11	0	1	19/07/2019	20/07/2019
D22	1	0		

Abstract:

The Hsp70 protein, BiP, is a major endoplasmic reticulum (ER) chaperone and is essential for maintaining protein folding homeostasis. The protein folding capacity of the ER is tightly linked to the amount and activity of BiP, through the unfolded protein response (UPR). This regulation is achieved at both a transcriptional and post-translational level, and deregulation is implicated in a number of disease states including type II diabetes. Covalent addition of an AMP moiety, onto Thr-518 of BiP, represents one such reversible post-translational modification. AMPylation inactivates BiP and, therefore, dynamically matches BiP activity to client protein load in the ER. The BiP AMPylation and deAMPylation reactions are both catalysed by the single active site of a bifunctional metazoan Fic protein, FICD. Mechanistic insight into the means of FICD-mediated eukaryotic deAMPylation is still lacking. Through analysis of selectively deuterated FICD/BiP complexes, by contrast variation SANS, we will be able determine the stoichiometry and internal arrangement of the deAMPylation complex. In so doing, we will further our understanding of eukaryotic deAMPylation and the post-translational UPR.

Experimental report for D11
16th-17th July 2019
Investigating FICD mediated deAMPylation of BiP
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Abstract

The Hsp70 protein, BiP, is a major endoplasmic reticulum (ER) chaperone and is essential for maintaining protein folding homeostasis. The protein folding capacity of the ER is tightly linked to the amount and activity of BiP, through the unfolded protein response (UPR). This regulation is achieved at both a transcriptional and post-translational level, and deregulation is implicated in a number of disease states including type II diabetes. Covalent addition of an AMP moiety, onto Thr518 of BiP, represents one such reversible post-translational modification. AMPylation inactivates BiP and, therefore, dynamically matches BiP activity to the burden of unfolded proteins in the ER. The BiP AMPylation and deAMPylation reactions are both catalysed by the single active site of a bifunctional metazoan Fic protein, FICD (Preissler *et al*, 2015, 2017). It has also been shown that these mutually antagonistic activities can be switched through the regulation of FICD's oligomeric state (Perera *et al*, 2019). A recent crystal structure of AMPylated BiP directly engaged with monomeric FICD (unpublished) has significantly increased our understanding of the mechanism of deAMPylation and the nature of the FICD-BiP interaction. Through contrast variation SANS analysis, we were able to confirm that the stoichiometry and quaternary structure of the complex formed between dimeric FICD and AMPylated BiP is very close to that predicted from the high-resolution heterodimeric crystal structure.

Experimental data collection

Deuterated proteins were produced in ILL's deuteration laboratory by Juliette Devos and Michael Härtlein as part of proposals DL-03-235 and DL-03-234 and protein purification was continued from the deuterated cell paste in Cambridge. After *in vitro* BiP AMPylation, complexes of BiP-AMP and dimeric FICD were co-purified by SEC in 0% D_2O buffer. The resulting complexes of deuterated BiP-AMP (dBiP) with non-deuterated FICD (hFICD) and non-deuterated BiP-AMP (hBiP) with deuterated FICD (dFICD) were transported to the ILL. Transfer of the semi-deuterated complexes into D_2O buffer was completed at the ILL by SEC. The elution profiles of the two complexes were largely identical in both 0 and 100% D_2O buffers. With the help of Sylvain Prévost, contrast variation SANS data were collected from a total of 17 samples at 12 ^oC on D11 with a range of final buffer D_2O concentrations (with a 5.5 Å wavelength neutron beam at distances of 1.4, 8 and 20.5 m). Data from relevant buffer-only controls were also collected with similar data collection times.

Results

Guinier analysis of the low Q data was informative. Comparison of the experimental I_0/c values across both data sets with the theoretical I_0/c values strongly suggest that the complex of BiP-AMP and dimeric FICD forms a heterotetramer (n = 2.02 ± 0.14 for dBiP:hFICD and n = 2.28 ± 0.29 for hBiP:dFICD; mean ± SD with respect to the theoretical values calculated from a 1:1 heterodimer). Uncomplexed BiP-AMP and FICD were also confirmed as being

monomeric and dimeric, respectively, which is consistent with previous SEC and SANS analysis.

Determination of experimental R_gs from Guinier analysis, in concert with the determination of the contrast match point facilitated a Stuhrmann analysis. The resulting R_ms for both complexes, as well as the R_g values derived for the BiPs alone, were consistent with the theoretical values of the predicted heterotetramer structure. The theoretical R_g of FICD alone also fell within the 95% confidence interval of the experimental R_g derived from the Stuhrmann analysis of dBiP:hFICD. The Stuhrmann curves also confirmed that the FICD molecules formed a dimer in the centre of the heterotetramer with the two BiP molecules towards the outside.

By using PEPSI-SANS software (Grudinin *et al*, 2017) we were able to interrogate all available SANS data over the entire q-range. Comparison of the theoretical scattering profiles, generated from the predicted heterotetramer structure, to the experimental scattering curves revealed remarkable similarities across both datasets. This was even the case at buffer H_2O/D_2O ratios close to the average contrast match points, where the neutron scattering is extremely sensitive to both the shape and stoichiometry of all molecules in solution. Through non-linear rigid block normal mode analysis-based flex-fitting of the theoretical heterotetramer structure to each scattering curve, marginally reduced χ^2 fits were achieved. When fitting the resultant flex-fit models to all of the scattering data, of the 12 output flex-fit structures, only 5 output models had a reduced average χ^2 and a significantly different and reduced χ^2 standard deviation relative to the input model. These 5 structures all possessed R_g parameters within the R_m and R_g values derived from the Stuhrmann analyses (of dBiP:hFICD and hBiP:dFICD). Of these 5 structures which have significantly reduced global χ^2 variability only 1 structure has a symmetrical quaternary structure (which is to be expected from the symmetry of the FICD dimer). As expected from the high degree of correlation between theoretical input model and the experimental scattering data, this best flex-fit model exhibited relatively little deviation from the input structure (7.1 Å RMSD overall). The majority of this divergence is observed in the BiP lid domains. This is a very plausible solution structure given that the Hsp70 α-helical lid has been observed, by various methods, to be dynamic in solution (reviewed in Mayer & Gierasch, 2019).

This work will form part of a manuscript which is currently in progress.

References

Grudinin S, Garkavenko M & Kazennov A (2017) *Pepsi-SAXS* : an adaptive method for rapid and accurate computation of small-angle X-ray scattering profiles. *Acta Crystallogr. Sect. D Struct. Biol.* **73:** 449–464

Mayer MP & Gierasch LM (2019) Recent advances in the structural and mechanistic aspects of Hsp70 molecular chaperones. *J. Biol. Chem.* **294:** 2085–2097

Perera LA, Rato C, Yan Y, Neidhardt L, McLaughlin SH, Read RJ, Preissler S & Ron D (2019) An oligomeric state-dependent switch in the ER enzyme FICD regulates AMP ylation and de AMP ylation of BiP. *EMBO J.* **38:**

Preissler S, Rato C, Chen R, Antrobus R, Ding S, Fearnley IM & Ron D (2015) AMPylation matches BiP activity to client protein load in the endoplasmic reticulum. *Elife* **4:** e12621

Preissler S, Rato C, Perera L, Saudek V & Ron D (2017) FICD acts bifunctionally to AMPylate and de-AMPylate the endoplasmic reticulum chaperone BiP. *Nat. Struct. Mol. Biol.* **24:** 23–29