Experimental report

03/10/2015

Proposal: 9-13-563 **Council:** 4/2014

Title: Life in extreme environments: The role of intrinsically disordered proteins under conditions of abiotic stress

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Alexander ROUTH

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Local contacts: Isabelle GRILLO

Samples: D-LEA protein
Citrate synthase
H-LEA protein

Instrument	Requested days	Allocated days	From	To
D22	2	0		
D11	2	0		
D33	2	2	30/09/2014	02/10/2014

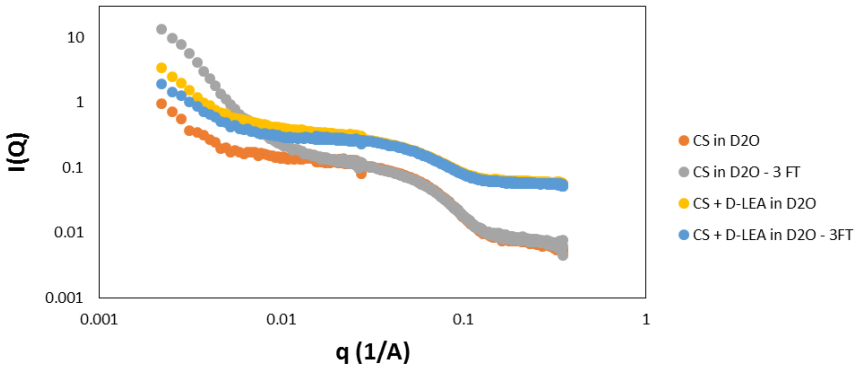
Abstract:

Extremophile organisms have been found in environments once thought unable to sustain life. There has been substantial interest in researching the mechanisms and unlocking the mystery behind these abilities in order to apply new technologies to the preservation of biological materials. Intrinsically disordered proteins (IDPs) are thought to play a key role in stress tolerance because some extremophiles have been found to upregulate these proteins as the organism gains cold and desiccation resistance. Since IDPs are unfolded in their native state, they cannot be denatured by environmental stresses and are hypothesized to interact and stabilize folded cellular proteins under conditions of abiotic stress. Our previous SANS results, at a limited Q range, did not align with the conventional models in literature. The aim of this proposal is to use the D33 SANS instrument with an enhanced Q-range to provide unambiguous data in order to assess a hypothesis of interactions between IDPs and their client proteins in conditions of freeze-stress.

ILL Experimental Report

		Experiment Number:	9-13-563
Title of Experiment:	The role of intrinsically disordered proteins under conditions of abiotic stresses	**Local Contact:**	Isabelle Grillo
Principal Proposer:	Dr Alex Routh	**Instrument:**	D33
Affiliation:	University of Cambridge		
Experimental Team:	Fanny Yuen, Alex Routh, Matthew Watson	**Date of Experiment:**	30/09/2014 – 01/10/2014

(a)



(b)

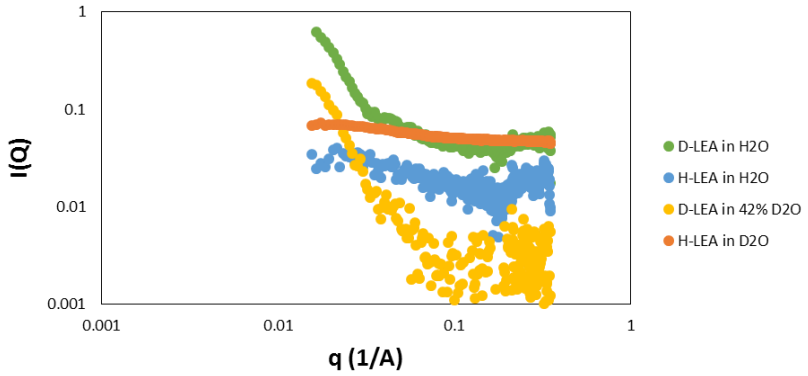


Figure 1: SANS results for (a) CS and CS + D-LEA in D_2O native and after three cycles of freeze-thaw, (b) D-LEA and H-LEA in various solvents.

Disordered LEA proteins are found to be upregulated as extremophiles gain cold and desiccation tolerance. Leading models of protection include chaperone- and shield-like interactions between LEA protein and its folded client protein. Some initial experiments were performed at ISIS, UK, but the results were unexpected because they contradict the leading hypotheses, and indicate that there may be no interaction between the proteins. The aim of this experiment was to investigate the structure conformations formed by the LEA protein when protecting a model globular protein (citrate synthase, CS) from freeze stress at a lower Q-range than those provided by the SANS-2D instrument, to attain further information on this protein system.

The bulk solution properties of the CS+LEA protein system were investigated using the D33 instrument. Deuterated LEA protein was synthesized by growing genetically modified bacteria in deuterated media. By contrast matching the solvent to each of the proteins separately, each component could be monitored individually over multiple cycles of freeze-thaw.

Despite some experimental difficulties, a number of interesting observations resulted and these allowed us to confirm some of our previous SANS results. An extensive data range was collected and the most striking results are shown above.

Comparing the red and orange lines in figure 1A one can conclude that there was no structural change to CS after mixing with D-LEA. This indicates no interaction between the two components and so directly contradicts the steric stabilisation argument ofter postulated for intrinsically disordered proteins.

Multiple cycles of freeze-thaw of CS alone showed increased conformational changes at low Q-values. This is shown in the grey line of Figure 1a. There was no change at higher Q-values indicating that the monomeric structure of CS was not greatly affected by freeze-thaw. The addition of LEA showed a definite decrease in aggregation. This is shown in the blue line on Figure 1a, which matched the red line corresponding to the same mixture with no freeze-thaw stress.

Most strikingly, it was evident that the hydrogenated and deuterated forms of our LEA protein, presented different structures in solution. This is shown in Figure 1b. This potentially indicates that D-LEA formed dimers and tetramers while H-LEA remained mainly monomeric. Since the clustering propensity of polymers increases with increasing backbone and solvent deuteration, it is reasonable that D-LEA would exhibit a more pronounced oligomerised structure in comparison to H-LEA. The effectiveness of D-LEA as a freeze-thaw protectant for CS was compared against H-LEA in both H_2O and D_2O. Both deuterated and non-deuterated H-LEA protected CS from multiples cycles of freeze-thaw in both solvents. Thus, although the bulk structure of H-LEA may be affected by backbone or solvent deuteration, its ability to protect CS remained unaffected.

Understanding the structural differences between the hydrogenated and deuterated forms of our LEA protein is extremely significant, since it was previously assumed that both forms were identical. The SANS results directly contradict this.

Unfortunately, the experiment encountered numerous issues, both equipment and sample related. Since the instrument was set to 7°C, there was difficulty removing the condensation from the cells, causing a few spurious results. A bigger issue arose because the measurements required protein concentrations beyond their solubility limit. Therefore, a number of stability issues were experienced. This finding was surprising since we have previously used these proteins, at higher concentrations, without issue. There are two possible explanations for this difficulty. The first is simply biological variability and any protein experiment is subject to some variability. The more feasible explanation concerns the age of the sample. We had run an experiment on Figaro immediately before the D33 experiment. Consequently the proteins were 3 or 4 days older than samples previously investigated. Whatever the reason for the stability issue, we had to use protein concentrations lower than ideal and consequently the scattering was lower than intended.

Due to the neutron work at ISIS and ILL, on SANS and reflectivity, we are now in a position to make some radical hypotheses concerning the mechanism of action of these proteins. The SANS results demonstrate a lack of interaction between the LEA and the globular protein – despite a clear protection from freeze thaw stress. The structure of the LEA does not play a role in its protectivity since HLEA and DLEA both protect CS from freeze-thaw. The reflectivity results demonstrate the propensity of the LEA for any interface and consequently we hypothesise that competitive adsorption at interfaces is the reason for the LEA proteins protecting the globular protein from denaturation.