Experimental Report

Proposal: **8-05-408** **Council:** 10/2011

Title: Role of amorphous/amorphous and crystal/amorphous water interfaces incold destabilization of proteins

This proposal is a new proposal

Researh Area: Materials

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Samples:
70 wt% d-sorbitol – 30 wt% D2O
70 wt% d-sorbitol – 30 wt% D2O- sodium citrate
70 wt% d-sorbitol - 30 wt% D2O- sodium citrate - lysozyme
70 wt% d-sorbitol - 30 wt% D2O- sodium citrate - hGH (protein)
60 wt% d-sorbitol - 40 wt% D2O-sodium citrate – lysozyme
60 wt% d-sorbitol - 40 wt% D2O-sodium citrate – hGH
60 wt% deuturated glucose - 40 wt% D2O-sodium citrate – hGH

Instrument	Req. Days	All. Days	From	To
D22	3	2	10/07/2012	11/07/2012
			16/11/2012	17/11/2012

Abstract:

Sugars and other polyhydroxycompounds are well-known cryo- and lyo-protectors which minimize destabilization of proteins and other biological systems during freeze-drying process. However, freeze-destabilization of proteins is commonly observed even in presence of sugars. There are several mechanisms proposed for freeze-destabilization of proteins, including different freeze-concentration effects, cold-denaturation of protein molecules , and destabilization of proteins due to interfaces between ice crystals and remaining unfrozen solution. In particular, formation of ice per se was shown to have destabilizing effect on protein molecules during freezing. However, details of such destabilizing effect of ice water interfaces have not been studied.

In this proposed study, we will investigate water distribution in biologically-relevant glasses, in particular carbohydrate-sugar systems, and its impact on stability of proteins. We also will study the role of water interfaces, by creating different types of the interfaces, and monitoring changes in protein molecules interaction using small angle neutron scattering technique.

Role of amorphous/amorphous and crystal/amorphous water interfaces in cold destabilization of proteins

Sugars and other polyhydroxy compounds are well-known cryo- and lyo-protectors, which minimize destabilization of proteins and other biological systems during the freeze-drying process. However, freeze-destabilization of proteins is commonly observed even in presence of sugars. There are several mechanisms proposed for freeze destabilization of proteins, including different freeze-concentration effects [1], cold-denaturation of protein molecules [2], and destabilization of proteins due to interfaces between ice crystals and the remaining unfrozen solution [3]. In particular, formation of ice *per se* was shown to have a destabilizing effect on protein molecules during freezing. However, details of such a destabilizing effect have not been fully understood.

By this work, we initiated our investigation on the impact of different water-polyhydroxy-protein interfaces on the stability of proteins. In our recent X-ray and neutron scattering studies of concentrated sorbitol-water solutions and glasses, water clusters and interfaces between water-rich and sorbitol-rich areas were observed under certain conditions, with the system retaining amorphous nature [4]. By the current experiments, we extended these studies to protein-water-polyhydroxy systems, which we hope will allow us to understand the role of different interfaces in protein destabilization. We are addressing a key question about the impact of the type of the interface, i.e., amorphous/amorphous (water-rich and sugar-rich) on the stability of the native protein molecules during the freeze-drying process.

We performed SANS experiment at ILL on two protein-water-sorbitol systems using lysozyme as a model protein in the temperature range of 100°K to 298°K. The different weight ratios of the components in the two samples measured are indicated in Table 1. Deuterated sorbitol (by exchanging exchangeable hydrogen atoms to deuterium) and D_2O was used to reduce the incoherent scattering coming from the solvent and only focus on the protein interaction peak (q_{peak})[5]. The interaction peak reflects the average distance between two protein molecules and can be used as a rough indication of the degree of packing $d_{interaction} \sim 2\pi/q_{peak}$). The experiments were performed at D22 using instrument configurations that allowed us to access the q-range of 0.002 -0.32 $Å^{-1}$.

Sample	D2O (w/w)	Deuterated sorbitol (w/w)	Lysozyme (w/w)
80% water	80% (1.6 g)	15% (0.3 g)	5% (0.1 g)
30% water	30% (0.6 g)	52.5% (1.05 g)	17.5% (0.35 g)

Table 1- Sample Specifications

We followed the changes in scattering intensities for both samples during both cooling and heating cycles. We saw significant differences in scattering intensities of both samples as they crossed their corresponding T_g values during cooling. However these changes are reversible, as the scattering profiles are completely reproducible after the system heated back to the initial temperature of 298°K. We are still investigating the connection between our observations, the type of formed interfaces and the stability of the protein during the process. Also our observations suggest that these systems are very sensitive to temperature changes and annealing. Therefore, sample environment, temperature stability and allowing the system to equilibrate are essential to have reproducible results.

References:

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