Experimental Report

Proposal: **6-05-911** **Council:** 4/2012

Title: Effect of glassy matrices on the dynamics of embedded proteins: studyof the bioprotectant properties ofmodified monosaccharides.

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Researh Area: Soft condensed matter

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Samples: glucose + lysozyme + D2O and levoglucosan + lysozyme + D2O

Instrument	Req. Days	All. Days	From	To
IN4	4	3	19/11/2012	22/11/2012
IN6	6	4	31/10/2012	05/11/2012

Abstract:

Bioprotectant glassy matrices have been proven not only to induce a noticeable retardation of protein molecular movements but also
to reduce their extent, thus preventing thermal protein degradations. Recently some of us studied the slow dynamic properties of
supercooled and glassy levoglucosan (LG) and compared with those of D-glucose (G), a common bioprotectant. The more rigid
structure of LG molecule has great impact on the molecular mobility: alpha-relaxation becomes very sensitive to temperature and
the secondary relaxation, responsible for the mobility in the glassy state, is suppressed. Quite stikingly, despite its lower Tg (glass
transition temperature), LG has been reported to preserve and stabilize freeze-dried proteins better than usual excipients. To
investigate how this is related to fast (ps) dynamics, we propose an incoherent neutron scattering investigation vs. T of

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The experiments have been performed on the IN6 and IN4 spectrometers. In more detail, on IN4 we have performed inelastic scans at 150K on glassy glucose (G) and levoglucosan (LG), Lysozyme in glucose, lysozyme in glucose plus D2O (h=0.4 grams of D2O/grams of protein), Lysozyme in levoglucosan, lysozyme in levoglucosan plus D2O (h=0.4 grams of D2O/grams of protein). The scans were performed at the different incident wavelengths λ=0.9 Å, 1.1 Å, 2.2 Å, so as to cover a quite wide dynamic range with variable energy resolution. On IN6 we have measured the same samples as IN4, but in the temperature range from 100K to 300K. The incident wavelength was 5.1 Å, corresponding to an energy resolution of about 90 μeV. In such a way, we could also measure the quasielastic part of the signal.

In Fig.s 1 and 2 we show some of the preliminary results measured on IN4.

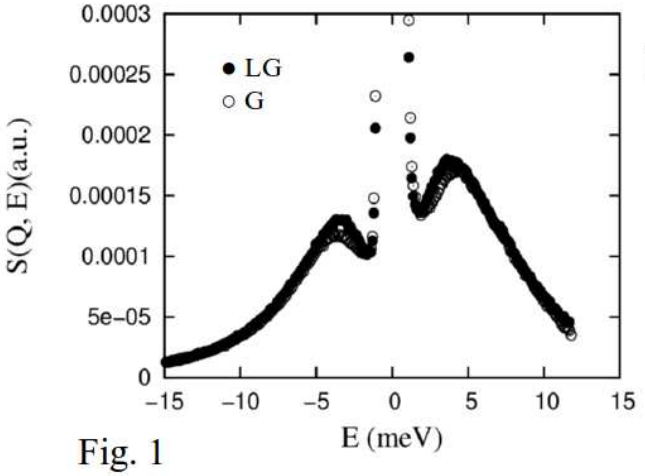

Fig. 1

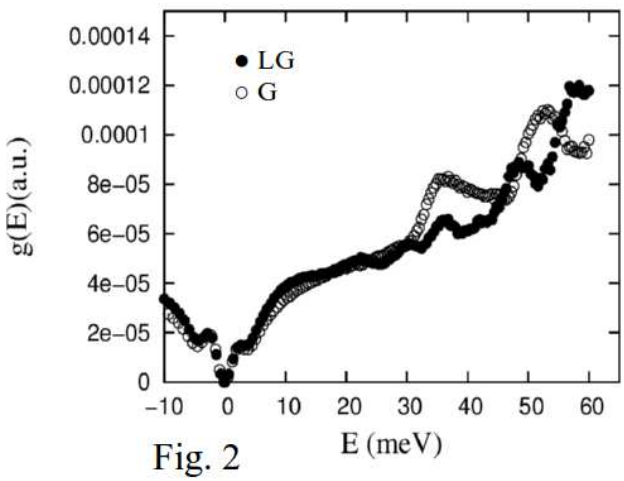

Fig. 2

The inelastic features of LG and G in the glassy phase are significantly different in both the low-energy regime of the Boson peak (see Fig. 1, incident λ=2.2 Å, 150K) and the high-energy regime above 30 meV (see Fig. 2, incident λ=1.1 Å, 150K). These findings strongly suggest that the phonon-like collective dynamics of the two sugars may as well

show marked differences. Quite interestingly, the low-frequency vibrations of LG show a softer character, that can be put in relationship with its antiplasticizing nature. The analysis of data from IN6 has begun and shows consistent results with findings from IN4 experiment.