ESRF	**Experiment title:** Eye lens proteins under pressure studied by SAXS and WAXS	**Experiment number**: SC1153
Beamline: ID2	**Date of experiment**: from: 19/07/2004 to: 21/09/2004	**Date of report**: 01/09/2004
Shifts: 8	**Local contact(s)**: S. Finet	*Received at ESRF:*

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Report:

The experiment SC1153 was scheduled for 8 shifts on ID2, from 2004/07/19 to 2004/07/21.

The aim of the proposal was to study the effect of the pressure and the temperature on the conformation, the stability and the interactions of the α- and γ-crystallins. α-, β- and γ-crystallins are the main components of mammalian eye lenses and their structural and associative properties are responsible for lens transparency. γ-crystallins are monomers (21 kDa), whereas α-crystallins are large hetero-oligomers of about 800kDa. The C-terminal domain of α-crystallins belongs to the ubiquitous superfamily of sHsps (small heat shock proteins): upon stress, they are able to incorporate the non-native proteins to prevent their aggregation. As far as secondary structure is concerned, all crystallins are essentially made of β-strands.

We used the high-pressure cell from ID2, with 2 diamond windows of 4mm diameter and 1mm thickness, to follow the changes in tertiary structure of the different γ-crystallins under pressure at different temperatures. The energy was set to 16.5 keV, corresponding to a wavelength of 0.75 Å, to improve the signal to noise ratio, limited by the absorption due to the diamond windows.

Previous high-pressure experiments performed with α-crystallins (SC727) have shown an increase of the radius of gyration with pressure, corresponding on a drastic change in size and conformation from 2 kb to 3 kb at room temperature. Preliminary results showed that the combination of temperature and pressure favours the denaturation of the α-crystallins. In this experiment, we were able to reproduce the pressure effect up to 2 kb as shown figure 1 (unfortunately, it was not possible to set the pressure above 2 kb because of linking of the cell). In order to complete the experiment with α-crystallins at various temperatures, we studied the effect of temperature from ambient to about 60°C at 2 kb. We report in the figures 1 and 2 the variation of the radius of gyration as a function of pressure and temperature for a solution at 30mg/ml.

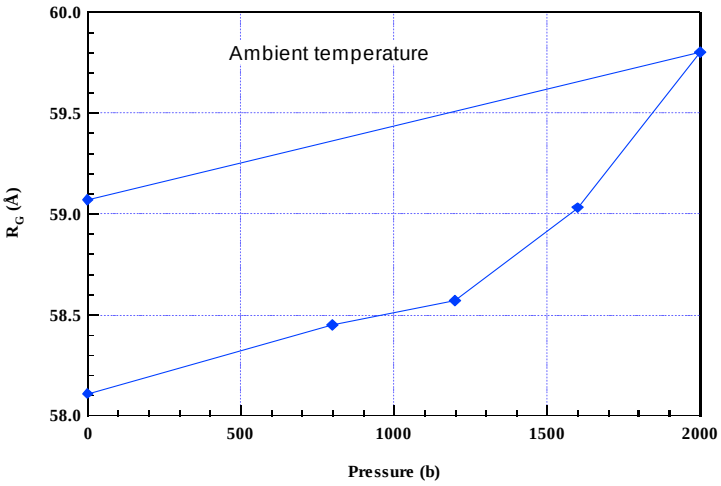

Figure 1

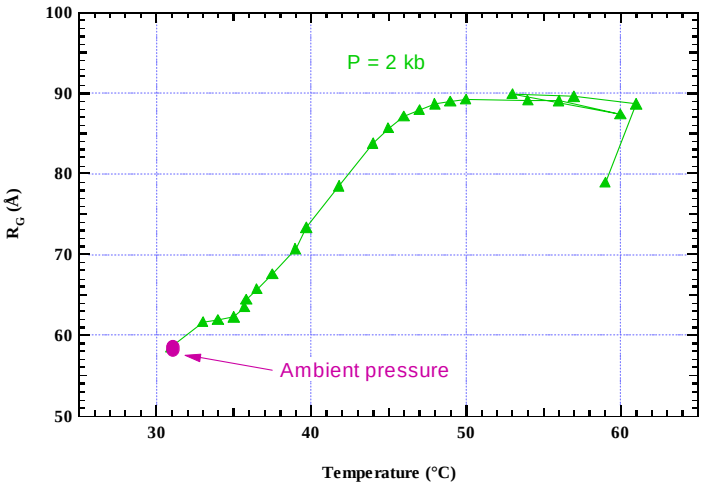

Figure 2

At ambient pressure and below 60°C, α-crystallins are known to be able to rapidly exchange their subunits while keeping their oligomeric form. Above 60°C, their size is double. At 2 kb, their size starts continuously increasing from 35°C to reach a plateau around 50°C.

At ambient temperature, the γ-crystallins are very stable even in extreme pressure conditions. The pressure available in this experiment (2kb) was not able to change their conformation. But the effect of pressure combined with temperature and pH depends upon the γ-crystallins itself. For example, the conformation of the gS at pH 6.1 was stable in the range of 2kb and 65°C available in this experiment, whereas at pH 6.1 and ambient pressure, the gE starts to denaturate above 45°C. At pH 2.5 and at 1.2kb, we observed the denaturation of the gS from 40°C (figure 3).

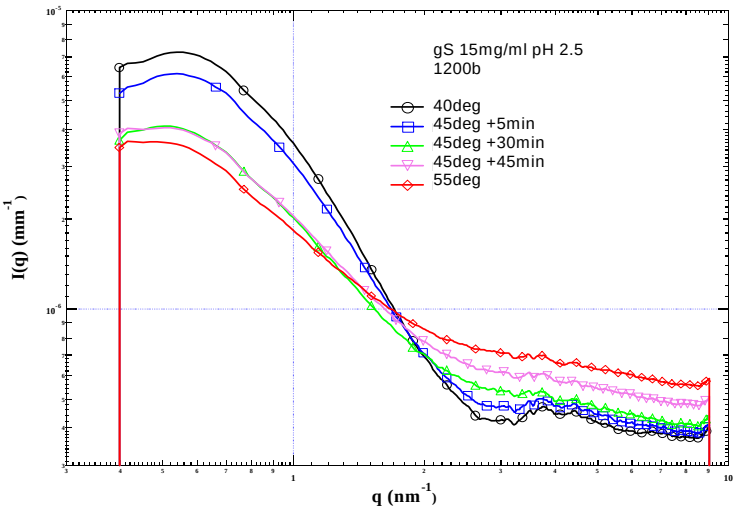

Figure 3: Evolution of the scattering intensity as a function of temperature for a solution of gS at 15mg/ml, at pH 2.5 and a pressure of 1.2kb. At pH 6.1, no change of conformation was observed.

This experiment has shown that these proteins (mainly beta strands) are very stable in pressure, as the combination of low pH, high pressure and temperature was needed to denaturate them. We showed that the pressure is very interesting to study the difference in stability of the different γ-crystallins, despite that the denaturation temperature of these proteins corresponds to the limit of the system (parafilm is used to close the sample chamber and is no longer tight). The study of the pressure alone would require a higher pressure range (5 kbars).