ESRF	**Experiment title:** **Time dependent small angle scattering studies on the refolding process of substrate protein mediated by the E. *coli* chaperonin system GroEL-GroES**	**Experiment number:** **LS-717**
Beamline: **ID2**	**Date of experiment:** from: 6.09.97 to: 8.09.97	**Date of report:** 16.02.97
Shifts: **6**	**Local contact(s):** 0. Diat, T. Narayanan	*Received at ESRF:* - 3 MAR. 1998

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Report:

Chaperonins like the GroEL-GroES system of E. *coli* are proteins which assists denatured proteins to get into their native conformation. The aim of our work is to investigate the kinetics of the interaction of substrate protein with the chaperonin system GroEL (MW: 800kDa) and GroES (MW: 70kDa) on the folding pathway.
Following measurements have been carried out:

- Scattering behaviour of the chaperonins depending on different exposure times with the aim to monitor possible radiation damage
- Time dependent small angle scattering (SAS) studies with the aim to analyse the binding interactions and kinetics of GroES with GroEL (molar ratio 1: 1)

Using the new CCD camera we could take advantage of the maximal flux of photons the ID2 provides avoiding saturation effects of the detector.
Figure 1. shows a comparison of SAS-curves of GroEL determined at ESRF with a small angle neutron (SANS) measurement from ILL D22 beamline. Both curves agree within the experimental errors except at a q-range higher 0.08 $Å^{-1}$. This is due to an error in the CCD analysis software which is now removed (T. Narayanan 2/98).
The radii of gyration (Rg) of the GroEL were determined using different exposure times in order to analyse a possible radiation damage (fig. 2). The decrease of the Rg indicates dissociation of GroEL into subunits and subfragments. The data indicates that the protein is stable only the first 10s-20s exposure. It is interesting to note that GroEL dissociates upon radiation and do not aggregate to larger particles like most other proteins. For the interpretation of future kinetics it is important to note that the damaging leads to smaller radii of gyration and I(0) (data not shown).

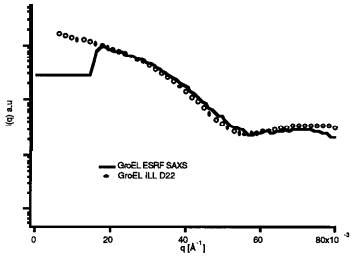

Fig.1
comparison of ESRF SAXS and ILL SANS measurements show a good agreement of the data

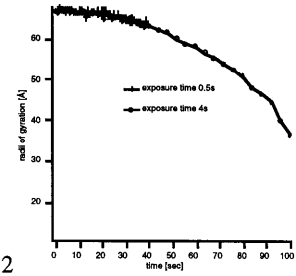

Fig.2
time dependence of the Rg (GroEL 14mer) at different exposure times

In order to observe the kinetics of the formation of the asymmetric (1: 1) GroEL-GroES complex the fastest possible data acquisition time was chosen. Therefore the CCD camera was switched to binning mode in order to reach a readout time of 400ms. The measurements was repeated several times to improve the statistic. The result is shown in fig.3.

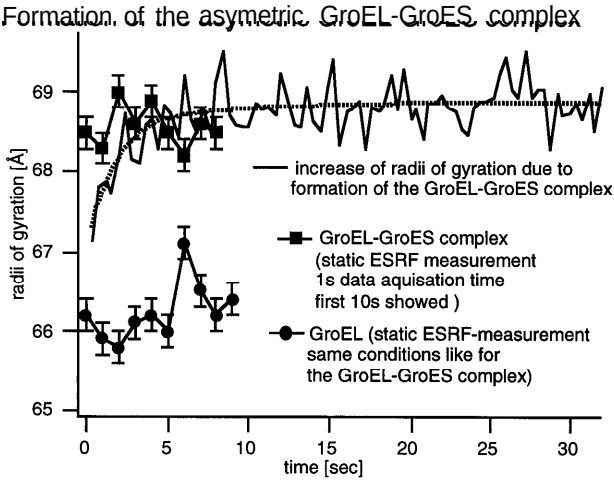

Fig. 3.: Time dependent interaction of GroES and GroEL
It is plotted the radii of gyration versus the time interval after mixing of GroEL and GroES in a 1: 1 molar ratio in the presents of ADP using a stopped flow apparatus. The increase of the Rg indicates the formation of the asymmetric GroEL-GroES (1:1) complex. This is conclusion is in line with previous SANS studies (R. Stegmann et al, JSB, (1998)). Analysis of the I(0) (data not shown) at different time interval support our conclusion as well as biochemical studies.

The plot Rg versus time ((——) in fig. 3.) shows a periodic modulation of Rg within in the first interval of 10s. This observation is reproducible. We suggest that this is due to a periodic binding of a second GroES chaperonin on the opposite side of GroEL. The data have to be improved in order to verify this conclusions.

Measurements in the next period are aimed to achieve this by decreasing the readout time of the CCD camera to 0.1s. This shorter readout time along with short time slices of 0.1 s will give us the opportunity to observe this periodic interaction of GroEL and GroES. We believe that this rather unexpected behaviour reflects a possible highly co-operative feature of the GroEL-GroES chaperonin system.