**Report for experiment 30 01 592 « Crystallographic analysis of the SH2 and PIR domains of Grb7 family proteins »**
Recorded on BM30A beamline from 16/5 to 17/5 2003

The Grb7, Grb10 and Grb14 proteins belong to the new family of molecular adapters Grb7. These proteins are characterised by a multidomain structure. All Grb7 proteins are implicated in receptor tyrosine kinase (RTK) signalling. Recent studies propose a physiological role of Grb7 in the development of cancers and of Grb14 in insulin signal transduction as a direct inhibitor of the insulin receptor catalytic activity. The functional role of these Grbs proteins is mediated by the SH2 domain and a new adjacent interaction domain, PIR (Phosphorylated insulin receptor Interaction Region). PIR domain is responsible for the interaction and the inhibitory action of Grb14 with the insulin receptor, whereas the SH2 domain seems to play the predominant role in the interaction of these Grbs proteins with others RTKs. The relative importance of the two domains PIR and SH2 varies, considering the receptor and the Grb protein.

The SH2 domains of Grb7 and Grb14 display 67% amino acid identity, but they do not present the same interaction specificity. Recent studies showed that the SH2 domain of Grb7 has the same comportment as the one of Grb2, which was not predictable from the sequence analysis. It preferentially binds with peptides containing an asparagine at pY +2 position. In contrast the SH2 domain of Grb14 appeared to bind preferentially to peptides where the pY +3 position is occupied by a large hydrophobic residue.

We have cloned, expressed, purified and crystallised the SH2 domain of Grb14. We have obtained needle crystals of 40 x 40 x 300 $\mu m^3$ in size. Preliminary crystallographic analyses were performed on these native Grb14-SH2 crystals. However, using various structures of SH2 domains as search models, no molecular replacement solution was found with AmoRe. For this reason, we have produced and purified selenomethionyl-labeled Grb14-SH2 (2 selenium atoms for 105 residues). Crystals of selenomethionyl-labeled Grb14-SH2 were obtained in the same condition as crystals of native Grb14-SH2.

[SH2 Grb14] = 6,5 mg/ml
PEG 4000 25 % (w/v)
Glycérol 15 % (v/v)
Tris-HCl 0,1 M pH 8,5
$MgCl_2$ 0,2 M
18°C hanging drop 4$\mu$l+4$\mu$l

Data were collected at three wavelengths about the selenium K-edge on the beam line BM30 at the ESRF. All data were processed and scaled using MOSFLM/SCALA. Data collection results are summarised in the following table.

The determination of the Grb14-SH2 structure was unsuccessful because of the presence of a merohedral twinning in the crystals. Actually, subsequent analysis showed that both the native and the selenomethionine derivative crystals exhibited 42 and 48 % twin fraction respectively using Yeates statistics.

We have not been able to properly deconvolute the twinned intensities as the twin fraction is close to 50%. To overcome this problem, co-crystallisation attempts with inhibitor peptides are in progress at the moment.

| Data collection | Seleno-methionyl-labeled SH2 Grb14 | | |
|---|---|---|---|
| Beamline | BM30 (ESRF) (16-Bunch) | | |
| | λ1 (peak) | λ2 (edge) | λ3 (remote) |
| Wavelength (Å) | 0,979344 | 0,979501 | 0,977274 |
| Temperature (°K) | 100 | 100 | 100 |
| Resolution | 25-3,1 | 25-3,2 | 25-3,3 |
| Crystal to detector distance (mm) | 200 | 200 | 200 |
| No. images | 120 | 120 | 120 |
| Oscillation range (deg) | 1 | 1 | 1 |
| Time/image (sec) | 3 x 30 | 3 x 30 | 3 x 30 |
| No. observations | 184 012 | 171 496 | 172 066 |
| No. unique reflections | 3415 | 3118 | 2872 |
| Mosaicity (deg) | 1,3 | 1,3 | 1,3 |
| Redundancy | 13,2 | 12,8 | 13,1 |
| Completeness (%) | 98,0 (98,0) | 97,9 (97,9) | 98,0 (98,0) |
| Signal I/σI | 4,5 (1,7) | 4,2 (1,7) | 4,2 (2,6) |
| Rsym (%) | 15,6 (45,0) | 16,0 (45,1) | 17,1 (30,0) |
| Rano (%) | 5,9 (12,0) | 4,3 (11,6) | 4,6 (7,5) |
| | | | |
| Unit cell parameters (a, b, c ; α, β, γ) | 76,43 76,43 101,93 90° 90° 120° | 76,92 76,92 100,99 90° 90° 120° | 76,96 76,96 101,2 90° 90° 120° |
| Space group | $P6_422$ ou $P6_222$ | $P6_422$ ou $P6_222$ | $P6_422$ ou $P6_222$ |
| No. molecules per asymmetric unit | 1 or 2 with Vm = 3,55 $Å^3$/Da or Vm = 1,77 $Å^3$/Da | 1 or 2 with Vm = 3,55 $Å^3$/Da or Vm = 1,77 $Å^3$/Da | 1 or 2 with Vm = 3,55 $Å^3$/Da or Vm = 1,77 $Å^3$/Da |

Table : Statistics on data collection.