ESRF	**Experiment title:** Three dimensional structure of the signal recognition particle protein SRP9	**Experiment number:** 1s263
Beamline: BM14	**Date of experiment:** from: 10/1 11995 to: 12/1 11995	**Date of report:** 28 feb 1996
Shifts: 9	**Local contact(s):** Andy Thompson	*Received at ESRF:* 29 FEB 1996

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Report:

Signal recognition particle (SRP) is a cytoplasmic ribonucleoprotein that plays an essential role in sorting proteins to the endoplasmic reticulum. SRP recognizes and binds to the signal sequence of the nascent chain as it emerges from the ribosome. This interaction triggers a pause in the synthesis of the polypeptide chain and the ribosome-nascent chain-SRP complex is then targeted to the endoplasmic reticulum (ER) via the interaction of SRP with its receptor. Protein synthesis is resumed and the nascent chain engages in the actual translocation process.

Canine SRP, the first example to be discovered and by far the most studied, is composed of an RNA molecule (SRP RNA), two heterodimeric protein subunits (SRP68/72 and SRP9/14) and two monomeric polypeptides (SRP54 and SRP19). The two polypeptides SRP9/14 bind to the sequences at the 5' end of SRP RNA that are homologous to the Alu family of repetitive sequences.

SRP9/14 is absolutely required to mediate the the pause in the translation of secretory proteins.

We have undertaken the crystallographic analysis of SRP9/14 and SRP9 in collaboration with Dr. K. Strub, Université de Genève. Mouse SRP9 and SRP9/14 were cloned and overexpressed in *E. coli*. It is of particular interest that all SRP proteins lack apparent structural similarities to already characterized RNA binding motifs. Two crystal forms of SRP9/14 and one crystal form of SRP9 have been obtained so far, using incomplete factorial experiment designs.

Native data on the SRP9 crystals collected on ID9, ESRF, is 95% complete to 2.3 Å with an $\mathbf{R}_{sym}$ on intensities of 4.4%. A search to find heavy atom derivatives has so far been unsucessfull.

In order to get a derivative to be used either as an isomporphous derivative or to used for MAD phaisng we have produced the selenomethionyl-SRP9 protein. SRP9 has 4 methionines for a total number of 85 residues. Selenomethionine incorporation was checked using mass spectroscopy. A difference in molecular weight of 188.5 between the native and selenomethionyl proteins was measured corresponding to full incorporation of 4 selenium atoms.

The selenomethionyl SRP9 crystals belong to the same space group as native $\mathbf{P3_{(n)}21}$ with cell dimensions a=b=64Å c=l 11 Å. However they are not isomorphous with the native crystals. Complete data sets at four different wavelengths (0.9797 Å 0.9799 Å 0.9463 Å and 1.1000 Å) was collected on BM14 from a single flash-frozen crystal of the selenomethionyl SRP9 protein using a MAR Research image plate as detector. The data is complete to 2.5 Å with an $\mathbf{R}_{sym}$ on intensities of 4%. An energy scan clearly showed an absorption edge corresponding to the selenium K-edge. So far we have not been able to locate the selenium sites from anomalous Patterson maps.