| ESRF | **Experiment title:**<br>Crystallographic study of the structures of the 29kDa subunit of mitochondrial import stimulating factor **(MSF) and its complex with a signal peptide.** | **Experiment number**:<br>LS-950 |
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| **Beamline**:<br>ID 13 4 | **Date of experiment**:<br>from:26 June 1998 to:28 June 1998 | **Date of report**:<br>22 Febuary 1999 |
| **Shifts:**<br>6 | **Local contact(s)**:<br>WAKATSUKI, Soichi | *Received at ESRF:* |


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**Report:** Most mitochondrial proteins are synthesized on cytoplasmic ribosomes and post-translationally imported into mitochondria depending on the hydrolisys of cytoplasmic ATP, the electrochemical potential across the inner membrane, and ATP in the matrix, although the latter two are additionally required for the precursors crossing the inner membrane. Proteins produced by the free ribosomes usually process N-terminal target signal peptides which are recognized by cytosolic factors and brought to various destinations such as mitochondria or nucleus. Elucidation of detailed mechanisms of the signal peptide recognition by the cytosolic factors and the targeting of the carrier to the correct organelles will advance the understanding of protein transport in eukaryotic cells.

A cytosolic factor (MSF, mitochondrial import stimulation factor) stimulates precursor import into mitochondria. MSF is a heterodimer of large (32 kDa) and small (29 kDa) subunits that unfolds the aggregated, import-incompetent precursors with the dissipation of ATP and binds to the unfolded precursors to stabilize their import-competent states. Thus, MSF seems to possess the properties of a so- called molecular chaperone. Mechanisms of the signal recognition and how MSF binding is related to its role in this process be revealed by studying the three dimensional structure of the MSF and its complex with signal peptide.

**Experimental method.**

We have obtained crystals of native MSF small subunit under the following conditions:protein concentration 20mg/ml, in 50mM Tris-HCl, 2.8M ammonium sulfate, pH7.5 at 25 ˚C. Clear thin crystals (0.6 x 0.2 x 0.02 mm) were obtained. X-ray experiments of crystals were carried out at ID14 EH3. Frozen crystals by cryogenic stream with anti-cryo

solution were used during X-ray exposure. Total 28 crystals were used for X-ray experiments. Three crystals among them gave diffraction up to 5Å. Data acquisition condition was shown bellow: wavelength 0.933 Å, camera length 300 mm, MAR CCD detector, oscillation angle 1 ˚, exposure time 180 sec. Total 230 diffraction frames were recorded from three crystals, and processed by the program MOSFLM on the NICE ESRF computing system. Crystallographic cell dimensions and space group are a=70.52Å, b= 210.64Å, c=123.14Å, β=91.13˚, P21. MSF functions as a large and small subunit hetero-dimer in the cell, however, our MSF crystals are composed of MSF small subunits,therefore it is considered as a homo-dimer. Assumed the Vm value is 2.5 $Å^3$/Da, six MSF dimers are packing in the asymmetric unit. Molecular replacement method was used for the determination of phases of this diffraction data with the program AMORE on the NICE system. The amino acid sequence of the MSF is identical to that of 14-3-3 zeta protein. Cross rotation function with a dimer starting model was calculated using 6 Å resolution data and reasonably gave twelve and more solutions. Translational function was calculated by using and adding the solution of the rotation function. A consistent solution among some results of translational functions was obtained. However, the solution could not give a well-interpretative electron density map about the MSF dimer molecule in the asymmetric unit. A further calculation and crystallization of complex with signal peptide are now in progress.