	Experiment title: Bacillus subtilis uridine/cytidine kinase	**Experiment number**: ls1770
Beamline: ID-14-4	**Date of experiment**: from: 20/9-2000 to: 22/9-2000	**Date of report**: 11/4-2002
Shifts: 6	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

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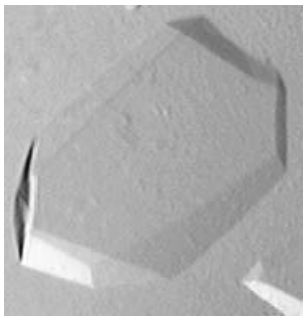
Report:

Uridine-cytidine kinase (UCK; EC 2.7.1.48) is a pyrimidine salvage pathway enzyme catalyzing the phosphorylation of uridine and cytidine to the corresponding ribonucleoside 5'-phosphates, using nucleoside triphosphates as phosphate donors. In addition, the enzyme phosphorylates various antitumoral, antibacterial and antiviral pyrimidine nucleoside analogs, and is therefore required for the pharmacological activation of these analogs (Cihak and Rada, 1976; Neuhard, 1983).

Currently no structure is known for any uridine-cytidine kinase. Initially we produced crystals of the unliganded enzyme, which grew to about 200 μm but with miserable diffraction qualities. Results from stabilization studies suggested crystallization of the enzyme in the presence of GTP, CTP or the analogs βγ-mGTP, βγ-imido-GTP and we were able to reproduce crystals of SeMet substituted UCK in complex with GTP (McGuire *et al.*, 2002). Such a crystal, which grew to a maximum size of about 800 μm is shown below.



A data set of a crystal of UCK in complex with the substrate GTP, and a three wavelength MAD data set measured on SeMet-substituted UCK in complex with GTP was measured on ID14-4. Due to the very large cell, fine slicing was necessary to avoid overlap in high resolution shells and 90° was collected with 0.2° oscillation. All intensity data were processed independently with the HKL program suite (Otwinowski and Minor, 1997). The X-ray fluorescence spectra on the SeMet substituted sample showed a good quality of the anomalous signal (f'=-10.28 and f''=6.87).

The space group of all crystals of enzyme complexes was determined to be *R32* with cell dimensions *a* = 280.6 Å, *c* = 249.3 Å. The diffraction limit of the native enzyme-GTP complex was 2.8 Å on ID14-4 and of the SeMet-substituted enzyme-GTP complex 3.6 Å at ID14-4.

We have attempted to solve the structure from SAD, MAD and MIR phasing using the program SOLVE (Terwilliger and Berendzen, 1999), but without success. We are now planning to get data from new heavy atom derivatives to get enough phase information.

References

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