ESRF	Experiment title: Structural analysis of prokaryotic Threonyl-tRNA synthetase from *Thermus thermophilus*	**Experiment number:** LS71
Beamline: BL4	**Date of experiment:** 2-3 Sept. 1994 and l-2 Oct. 1994	**Date of report:** 21 oct. 1996
Shifts:	**Local contact(s):**	*Received at ESRF:* 28 OCT 1996

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Report:

The determination of the crystal structure of Threonyl-tRNA synthetase (ThrRS) from *Thermus thermophilus* is part of a long-range effort aimed at an understanding at atomic level of the mechanisms involved in the recognition of the substrates (tRNA, aminoacid, ATP) and the catalysis of the aminoacylation of tRNA by the aminoacyl-tRNA synthetases, essentially those of class II.

Diffraction data from native crystals were collected at 4°C. The wavelength was 0.95Å. The space group was at that time believed to be tetragonal($P4_22$) but was later recognized to be only pseudo-tetragonal, in reality orthorhombic ($C222_1$). Two crystal forms were identified, which are not isomorphous and could not be scaled together: they have essentially the same a and b parameters (a=1 19, b=120 A) but differ by the c parameter, which is 3 14 Å for one form and 318 Å for the other. The crystals suffer from severe radiation damage. The best crystals diffract initially to 2.8 A, but, due to radiation damage, the effective limit of resolution is not better than 3.5 Å. Even so, a reasonably complete data set of the first crystal form could be collected from 5 crystals. After scaling, data reduction and averaging, 14000 unique reflections were obtained. The R-factor on the intensities was 6.9 %.

Two heavy-atom derivatives were used in an attempt to solve the structure by MIR. One was a mercury derivative (PCMBS), the other contained lead (TMLA). The diffraction limit of the mercury crystals was insufficient and their life-time too short for the measured intensities to be of any use (better measurements have been obtained later). In the case of the lead derivative, 4 crystals were used for collecting difraction data. The diffraction limit was about 3.5 A, and the R-factor after data reduction and merging 10.5 %. But the completeness of the data set was only 57 %. No unambiguous positions of the lead atoms could be identified.

The crystals of ThrRS from *Thermus thermophilus* used so far are very fragile, sensitive to radiation damage and very difficult to freeze at cryogenic temperatures without partial or complete loss of diffraction power. The measured reflections could never be phased, even with better mercury derivative data than the ones obtained in this experiment. For these reasons, this form of ThrRS has been discarded, at least temporarily, and our effort has been put on another prokaryotic ThrRS, that of *E. coli*. Two crystal forms of *E. coli* ThrRS-tRNA complex have recently been obtained, one orthorhombic and one hexagonal. In both cases, the diffraction limit still needs to be improved and shock-freezing attempted.