ESRF	Experiment title: Crystal structure of *T. thermophilus* aminoacyl-tRNA synthetases and their complexes with ATP, cognate amino acid and cognate tRNA	**Experiment number :** LS910
Beamline : **ID2**	Date of experiment: 5/4/98-6/4/98 and 25/6/98-26/6/98	**Date of report:** 30/8/98
Shifts: 6	**Local contact(s) :** Jonathan Grimes	*Received at ESRF.* 01 SEP. 1998

Names and affiliations of applicants (* indicates experimentalists):

Stephen CUSACK* EMBL Grenoble Outstation
Anya YAREMCHUK* c/o ILL
Michael TUKALO 156X, 38042 Grenoble Cedex 09
FRANCE

Report: We have a major on-going and long term programme of molecular biological, biochemical and structural studies on the aminoacyl-tRNA synthetases from various organisms and their substrate complexes with the aim of understanding their substrate specificity and mechansim of action. Systems currently being studied are the synthetases for proline, asparagine, lysine, histidine, leucine and tyrosine. The experiments reported here concern *T. thermophilus* histidyl-tRNA synthetase and prolyl-tRNA synthetase.

Histidyl-tRNA synthetase.

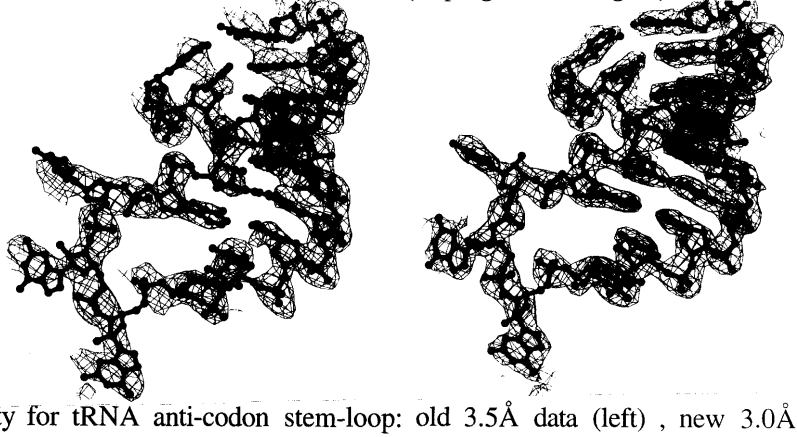
We have previously determined the crystal structure of *T. thermophilus* histidyl-tRNA synthetase complexed with histidine and histidyl-adenylate which enabled us to give the structural basis of histidine recognition and the activation of histidine with ATP to form histidyl-adenylate (Åberg, A. Yaremchuk, M. Tukalo, B. Rasmussen and S. Cusack. Biochemistry, 36, 3084-3094 (1997)). We have now measured for the first time data on a crystal of substrate-free histidyl-tRNA synthetase.

Two related crystal forms of substrate-free histidyl-tRNA synthetase have been obtained. Form 1 is monoclinic ($P2_1$) with cell dimensions a=99.3Å b=103.3Å c=57.1Å β=117.2^o (with half dimer in the asymmteric unit). This crystal form is highly reproducible but the diffraction is very aniosotropic (2.4Å in one direction, 3.3Å in the perpendicular

direction). Crystal form 2 is orthorhombic ($C222_1$) with cell dimensions a=111.1Å b=102.1Å c=91.5Å and also a half dimer in the asymmteric unit. Only one crystal of this type has ever been found and measured! Data obtained on ID2 at 100K are 86.7% complete to 2.4Å with R-merge 0.074 (0.14 at high resolution). The structure was readily solved by molecular replacement and has been refined with XPLOR to an R-factor of 0.224 (R-free 0.272) for all data between 2.4-12Å (with solvent correction) and now represents the highest resolution structure of histidyl-tRNA synthetase available. The new crystal structure shows that in the absence of histidine the active site is opened up notably by a significant movement of the characteristic histidine binding loop as well as the disordering of another active site loop. Furthermore the insertion domain (proposed to be involved in tRNA binding, Aberg et al., 1997) has undergone a substantial rotation. Put the other way, histidine binding causes significant induced fit conformational changes which are probably a pre-requisitie for correct tRNA binding.

Prolyl-tRNA synthetase.

We have previously determined the crystal structure of *T. thermophilus* prolyl-tRNA synthetase at 2.4Å resolution, the complex with proline at 2.9Å resolution and the complex with cognate $tRNA^{pro}$ at 3.5Å resolution (Cusack, S., Yaremchuk, A, Krikliviy, I. and Tukalo, M. *Structure* 6, 101-108 (1998). The original tRNA complex crystals diffract with diffculty to 3.1Å resolution and are unstable to radiation damage even when frozen (see report to LS611). We have recently altered the crystallisation conditions of the tRNA complex and obtained a modified form in which the c-axis dimension increases from about 230Å to 236Å. These crystals diffract to about 2.8Å resolution and are more stable in the beam. Preliminary data has been measured on these crystals on ID2 giving a native data set to 3Å resolution (94% complete, R-merge 0.10). This permits for the first time a proper model of the tRNA complex to be built and refined (in progress, see figure).



Electron density for tRNA anti-codon stem-loop: old 3.5Å data (left) , new 3.0Å data