ESRF	**Experiment title:** Crystal structure of *T. thermophilus* leucyl-tRNA synthetase and substrate complexes.	**Experiment number:** LS1386, LS1535
Beamline: ID14-EH4 ID14-EH2	**Date of experiment:** from: 3/6/99 to: 4/6/99 27/11/99 to: 28/11/99	**Date of report:** 1/3/00
Shifts: 6	**Local contact(s):** Sean McSweeney, Ed Mitchell	*Received at ESRF:*

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Report:

Leucyl-, isoleucyl- and valyl-tRNA synthetases are closely related large (~100 KDa) monomeric class I synthetases each containing a homologous insertion domain of about 200 residues. This domain is thought to be responsible for the ability of these enzymes to hydrolyse ('edit') cognate tRNA that has been mischarged with a chemically similar but non-cognate amino acid. Here we describe the first crystal structure of a leucyl-tRNA synthetase, from the hyperthermophile *T. thermophilus*, at 2.0Å resolution and its complex with leucine and a non-hydrolysable leucyl-adenylate analogue. The overall architecture of the enzyme is similar to that of isoleucyl-tRNA synthetase except that the putative editing domain is inserted at a significantly different position in the primary structure. This feature is unique to prokaryote-like leucyl-tRNA synthetases as is the presence of a novel additional flexibly inserted domain. Comparison of the three states of the enzyme shows that binding of particularly the adenosine moiety of leucyl-adenylate causes significant conformational changes in the active site which are necessary for amino acid activation and tight binding of the adenylate. These changes are also propagated to more distant regions of the enzyme leading to a significantly more ordered structure which may be a prerequisite for the subsequent amino-acylation and/or editing steps.

Data collections

Components co-crystallised	**LeuRS + LeuAMS + norvaline**	**LeuRS:SeMet +Leu Peak**	**LeuRS:SeMet +Leu Inflection**	**LeuRS:SeMet +Leu Remote**
Beamline/detector	ID14-EH4/ADSC	ID14-EH4/ADSC	ID14-EH4/ADSC	ID14-EH4/ADSC
Wavelength	0.979Å	0.9794Å	0.9795Å	0.9301Å
Exposure/image	5s/0.5deg	7s/0.5 deg	7s/0.5deg	2s/0.5deg
Cell dimensions (Å)	a=102.4 b=155.6 c=176.3	a=102.2 b=154.0 c=174.6	a=102.2 b=154.0 c=174.6	a=102.2 b=154.0 c=174.6
Resolution	20-2.0Å	20-3.2Å	20-3.2Å	20-2.7Å
Unique reflections	88328	22864	22846	37780
Average redundancy	4.1	3.3	3.5	3.4
Completeness (%) (highest bin)	90.4 (60.2)	99.0 (99.1)	99.0 (99.7)	99.0 (99.5)
R-merge (highest bi	0.077 (0.39)	0.075 (0.227)	0.069 (0.205)	0.074 (0.310)

Structure determination and refinement.

The structure was solved initially at 3.5Å resolution using data from a three wavelength MAD experiment on a single crystal of seleno-methionated LeuRSTT. Fifteen out of the 24 possible selenium sites were found by SOLVE giving a mean figure of merit <m>=0.59 and Z-score=32.8 (Terwilliger *et al.*, 1999). Phases from the MAD experiment were used to locate heavy atom sites in the gold (2 sites), platinum (4 sites) and mercury (1 site) derivatives and three additional selenium sites. Improved phases were then calculated to 2.7Å resolution with MLPHARE using the three heavy atom derivatives and the peak and remote selenium data giving <m>=0.51 for 37781 reflections. A partial model (651 residues) was built into the electron density map after solvent flattening with DM and then transferred by molecular replacement into the non-isomorphous crystal form co-crystallised with the Leu-AMS. The higher resolution (2.0Å) enabled WarpNtrace to automatically build and refine 750/814 visible residues including side-chain positions (Perrakis *et al.*, 1999). The C-terminal residues 815-878 are invisible in the electron density. The current state of refinement for the LeuRSTT+Leu-AMS data is shown in the table.

Resolution (Å)	20.0-2.0	<b> protein	29.9
Work reflections	83980	<b> solvent	40.1
Test reflections	4402 (4.6%)	<b> substrate	20.0
R-free	0.229	RMS bonds (Å)	0.0065
R-work	0.209	RMS angles (degrees)	1.30
# protein atoms	6597 atoms	Ramachandran plot Favourable %	93.1
# substrate atoms	31 (LeuAMS)	Additional %	6.3
# solvent molecules	518 water, 2 sulphate	Generous %	0.4
# metal atoms	2 Zn	Disallowed %	0.1 (1, Ala 8)