ESRF	**Experiment title:** Crystal structures of T. thermophilus aminoacyl-tRNA synthetases and their substrate complexes.	**Experiment number:** LS-493
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Shifts: 6	**Local contact(s):** Andrew Thompson	*Received at ESRF :* 04 MAR 1997

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Report:

The aim of this experiment was to continue measurements on crystals of a number of aminoacyl-tRNA synthetase/substrate complexes in particular the prolyl- and asparaginyl-tRNA synthetases. These two synthetase structures have now been solved.

(1) *T. thermophilus* prolyl-tRNA synthetase (ProRSTT). ProRSTT is a dimeric class IIa synthetase with 477 residues/subunit. Comparison of the primary sequence with all other known prolyl-tRNA synthetases shows significantly more similarity to eukaryote cytoplasmic ProRS than to other eubacterial prolyl-tRNA synthetases. This highlights the fact that there are two distinct structural forms of ProRS: (a) 'eukaryote/archae-like' including ProRS from *T. thermophilus*, characterised by the absence of an insertion domain between motifs 2 and 3 and an extra C-terminal domain beyond the normal class IIa anti-codon binding domain; (2) 'prokaryote-like' including ProRS from mitochondria of eukaryotes, *E. coli* and *C. trachomatis*, which are larger enzymes with a very large insertion between motifs 2 and 3 and no extra C-terminal domain. ProRSTT crystals are of space-group $P2_12_12$ with cell dimesions a=132.6 b=191.6 c=125.3Å and were measured frozen in 35% ethylene glycol.

Beamline	**ProRS Native BM14**	**Mercury aniline BM14**	**ProRS+proline Swiss-Norwegian**
Detector	300mm Mar	15Omm Mar	300mm Mar
Wavelength	0.984Å	1.00Å (LIII edge)	0.873Å
Exposure/image	14Os/O.8 deg	70s/1deg	600s/1deg
Resolution	18-2.7Å	18-4.1Å	15-2.9Å
Total reflections	312470	93054	284962
Unique reflections	86899	25110	71146
Average redundancy	3.6	3.7	4.0
Completeness	98.6	97.6	99.0
R-merge (highest bin)	0.040 (0.105)	0.031 (0.036)	0.068 (0.223)

The crystal structure of ProRS was solved to 5Å resolution by SAD (single wavelength anomalous diffraction) using *only* the optimised anomalous scattering from the mercury aniline derivative. The low resolution phases were improved by four-fold averaging and phase extension to 2.7Å using the native data and the fact that there are two ProRS dimers in the asymmetric unit. The final experimental map was of excellent quality and a model has been refined to R_{work}=0.234, R_{free}=0.264 with excellent geometry, but no added water yet. The same initial, poor quality, 5Å SAD phases, followed by 4-fold averaging and phase extension, were applied to the 2.9Å data on ProRS soaked with proline. This again gave rise to an excellent *experimenta l*map (not difference map!) which clearly shows the proline bound in the active site together with the significant conformational changes in the enzyme associated with its binding.

ProRSTT shows two remarkable and unpredicted features. The unique extra C-terminal domain is in fact a zinc-binding domain situated in the three-dimensional position normally occupied by the insertion domain between motifs 2 and 3 in other class II synthetases. The zinc is tetrahedrally co-ordinated by four cysteines and appears to have a structural role. The extreme C-terminus, a tyrosine, absolutely conserved in the 'eukaryote/archae-like' ProRS, doubles back into the active site strongly suggesting that the exposed carboxyl-group plays an important role in activity.

***(2) T. thermophilus* asparginyl-tRNA synthetase (AsnRSTT).**

Asparaginyl-tRNA synthetase is a class IIb synthetase, closely related to the two other members of this sub-group, aspartyl- and lysyl-tRNA synthetases. The particular interests in AsnRS are: how it discriminates asparagine from aspartic acid, how it discriminates $tRNA^{asp}$(G/QUC anti-codon) from $tRNA^{asn}$(G/QUU anticodon) and the evolutionary relationship between AspRS and AsnRS.

AsnRSTT is a dimeric enzyme with 438 residues/subunit. The best crystals belong to space group $P6_422$ space groups with unit cell parameters a = b = 124.5Å, c = 122.6Å and the monomer in the asymmetric unit. Molecular replacement using the coordinates from yeast AspRS (18% identity) allowed a first determination of the phases but several regions which differ from the model were not well defined. An independent structure solution was therefore found by MIR using three derivatives containing Pb, U and Sm.

	AsnRS Native	**SmN03**	**Uac**	**Asn-adenylate**
Beamline	**BM14**	**BM14**	**BM14**	**BM14**
Detector	300mm Mar	300mm Mar	300mm Mar	300mm Mar
Distance	420mm	420mm	420mm	410 mm
Wavelength	0.93Å	0.93Å	0.88Å	1.002 Å
Exposure/image	12Os/l deg	120s/0.8deg	150s/ldeg	360s/deg
Resolution	31-2.8Å	31-2.8Å	43-2.6Å	27-3.2A
Total reflections	73000	160045	70630	46376
Unique reflections	14619	14864	17567	9777
Average redundancy	5.0	10.8		4.7
Completeness	98.9	100	96.3	99.8
R-merge (highest bin)	0.067 (0.250)	0.065 (0.157)	0.033 (0.075)	0.081 (0.27)

The MIR map permitted a complete model to be obtained which has been refined satisfactorally at 2.7Å resolution to an Rwork = 23.4% and Rfree = 30.0%.

As expected from sequence alignments the structure of AsnRSTT is remarkably similar to that of yeast AspRS, although the orientation of the N-terminal, anti-codon binding P-barrel domain differs by about 12 degrees between the two enzymes. The AsnRS insertion domain has the same topology as that of yeast AspRS, which is distinct from that of LysRS. The active sites of AsnRS and yeast AspRS are extremely similar, differing only in two residues. In order to understand exactly how these changes specify asparagine instead of aspartic acid, we have co-crystallised AsnRSTT with ATP-Mg^{2+} and asparagine which enzymatically give the stable intermediate, asparaginyl-adenylate in the crystal. The crystals are small and we have only managed to collect data to 3.2A resolution on BM14. Neverthless, good difference density is observed for the Asn-AMP although the resolution is too poor to be sure about the interactions of the asparagine side-chain. As with the corresponding seryl-tRNA synthetase case (Belrhali *et* al., 1995), there is a residual magnesium ion bound between the a-phosphate of the adenylate and Glu-361 and Asp-352 of AsnRSTT.