ESRF

Experiment title:

crystal strutures of aminoacyl-tRNA synthetases and their substrate complexes.

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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 lysyl- and prolyl-tRNA synthetases. The experiment was divided into two periods in which different detectors were employed.

(1) 17- 18/4/96 in which the ESRF image-intensifier/CCD detector was employed with a wavelength of 0.9Å and crystal-detector distance of 197 mm. Calibration of the detector was first done for spatial distortion and non-uniformity, FIT2D being used for calculation and subsequently applying these corrections. The following data collections were made:

(a) Complex of *T thermophilus* lysyl-tRNA synthetase (LysRS) with *T thermophilus* $tRNA^{lys}$(34-CUU) made by *in vitro* transcription. The aim was to compare the anticodon conformation with 34-Cytosine in the wobble position with the already known complex structure with *E. coli* $tRNA^{lys}$ which has a modified uracil (mnm^5s^2U) in the wobble position: LysRS charges both tRNAs. Crystals are of space-group $P4_12_12$ with cell-dimensions a=b=232.32A, c= 11 6.76A. The crystal was frozen in 29% glycerol, but diffracted weakly necessitating long exposures (6x30s passes for 0.5 degrees). This together with the long unit cell edge is not ideal for the CCD. Data were integrated with MOSFLM to 3.5A resolution. Of 147399 measured reflections, 40098 independent reflections were derived (98.5% complete, redundancy 3.7), with R-merge 0.11 (0.28 in highest bin). Despite the modest quality and resolution of the data, a map calculate using phases derived from the LysRS complex with *E. coli* $tRNA^{lys}$ showed reasonable density for the tRNA. See below for improved data collection.

(b) *T. thermophilus* prolyl-tRNA synthetase (ProRS) crystallises in space-group $P2_12_12$ with probably two dimers (each of about 100Kdaltons) in the asymmetric unit. No crystal structure is yet available for ProRS. Data were collected on a native crystal and one soaked with tri-methyl lead acetate, a putative derivative, both crystals being frozen using 35% PEG 400 as cryoprotectant. Half degree images were taken with typically 3x20s passes. Integration statistics are as follows:

	Native	Trimethyl-lead
Cell dimensions	131.8189 .9125.0	131.8190 .3125.4
Resolution	15-3.3 Å	15-3.8Å
Total reflections	212613	100044
Unique reflections	44275	28823
Average redundancy	3.4	3.5
Completeness	98.7	97.6
R-merge (highest bin)	0.087 (0.208)	0.105 (0.203)

Again the results are only of modest quality due to the weakness of the data from the small crystals used (about 150 microns). Analysis of the derivative data did not lead to any clear heavy atom sites.

(2) 30-3 1/5/96 in which the EMBL 300mm Mar-detector was used. On this occasion, much better results were obtained for these relatively large unit cell crystals using the image-plate and using somwhat larger crystals.

(c) *T. thermophilus* prolyl-tRNA synthetase. Three data collections were made, native at high resolution and gold and platinum putative derivatives at low resolution only. Crystals were frozen in 35% ethylene glycol this time and were somewhat larger (250 microns maximum dimension).

	Native	Pt	Au
Detector	300mm Mar	150mm Mar	150mm Mar
Distance	420mm	420mm	420mm
Wavelength	0.984Å	0.912A	0.912Å
Exposure/image	140s/0.8 deg	80s/ldeg	60s/ldeg
Cell dimensions (Å)	132.6191 .6125.3	131.6189 .8125.1	132.4190 .9125.5
Resolution	18-2.7Å	18-4.4Å	18-4.35Å
Total reflections	312470	71650	74441
Unique reflections	86899	19972	18664
Average redundancy		3.6	4.0
Completeness	98.6	97.6	87.6
R-merge (highest bin)	0.040 (0.105)	0.049 (0.072)	0.071 (0.148)

The native data is of *excellent* quality. Collection of even higher resolution data is clearly possible although spot-overlap becomes a problem. Unfortunately no heavy atom sites could be found from the derivatives. Nor can a molecular replacement solution be found using other class IIa synthetases (e.g. SerRS, HisRS, GlyRS) as search models. The derivative search continues.

(d) Complex of *T thermophilus* lysyl-tRNA synthetase (LysRS) with *T thermophilus* $tRNA^{lys}$(34-CUU) made by *in vitro* transcription. Repeat of (a) above but with a thicker crystal. Data collection and integration statistics:

Detector	300mm Mar	Distance	420mm
Wavelength	0.984Å	Exposure/image	120s/0.7 deg
Cell dimensions (Å)	232.6232 .6117.2	Resolution	18-2.9Å
Total reflections	272770	Unique reflections	69586
Average redundancy		Completeness	97.5
R-merge (highest bin)	0.047 (0.22)		

With this excellent data a model has been refined to an R-free of 24.9%. It shows that the wobble base C-34 binds in the same way as previously seen for mnm^5S^2U-34 there being a hydrogen bond between the N3 of C34 and the main-chain NH of Lys- 115 of the synthetase. These results support the idea that in mnm^5s^2U-34 the N3 is most probably de-protonated in this environment and makes the same interaction. All the work on the LysRS-$tRNA^{lys}$ complex is in press in EMBO J. with the title "The crystal structures of *T. thermophilus* lysyl-tRNA synthetase complexed with *E, coli* $tRNA^{lys}$ and a *T. thermophilus* $tRNA^{lys}$ transcript: anti-codon recognition and confirmational changes upon binding of a lysyl-adenylate analogue" by S. Cusack, A. Yaremchuk and M. Tukalo.