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Crystallization and preliminary X-ray characterization of the atypical glutaminyl-tRNA synthetase from *Deinococcus radiodurans*

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The glutaminyl-tRNA synthetase (GlnRS) from the radiation-resistant bacterium *Deinococcus radiodurans* differs from known GlnRSs and other tRNA synthetases by the presence of an additional C-terminal domain resembling the C-terminal region of the GatB subunit of tRNA-dependent amidotransferase (AdT). This atypical synthetase was overexpressed in *Escherichia coli*, purified and crystallized in the presence of PEG 3350. Orthorhombic crystals were obtained that belong to space group $P2_12_12_1$ and diffract to 2.3 Å resolution. The crystal structure was solved by molecular replacement using the structure of *E. coli* GlnRS as a search model.

1. Introduction

The protein biosynthesis machinery involves aminoacyl-tRNAs formed by aminoacyl-tRNA synthetases (aaRSs). Most organisms display 20 aaRSs, one to charge each family of isoaccepting tRNAs with the cognate amino acid (Ibba & Söll, 2000). However, various bacterial species, archaebacteria and eukaryotic organelles are deprived of glutaminyl-tRNA synthetase (GlnRS). Gln-tRNA^{Gln} is then formed by the amidation of Glu mischarged on tRNA^{Gln} by a GluRS of relaxed specificity (reviewed by Ibba & Söll, 2004). A similar indirect pathway forms Asn-tRNA^{Asn} in bacteria and archaebacteria deprived of asparaginyl-tRNA synthetase. In bacteria, conversion of Glu and Asp mischarged on tRNA^{Gln} and tRNA^{Asn}, respectively, is promoted by a tRNA-dependent amidotransferase (AdT), a trimeric enzyme formed by the GatA, GatB and GatC subunits (Curnow *et al.*, 1997).

The radiation-resistant bacterium *Deinococcus radiodurans* forms Asn-tRNA^{Asn} by an indirect route involving an aspartyl-tRNA synthetase that is able to charge Asp on tRNA^{Asn} and an AdT which converts Asp into Asn, whereas Gln-tRNA^{Gln} is formed directly by a GlnRS charging Gln on tRNA^{Gln} (Curnow *et al.*, 1998). This GlnRS comprises a catalytic core and an anticodon-binding domain like other known GlnRSs (Rould *et al.*, 1989), but sequence alignments have also revealed the presence of an additional C-terminal extension of 215 residues which exhibits 28% identity with the 168 final residues of the GatB subunit of *D. radiodurans* AdT. To date, GlnRS of *D. radiodurans* constitutes a unique example of an aaRS fused to a GatB-like extra domain. The function of this domain remains unknown. Establishment of the crystal structure of *D. radiodurans* GlnRS will provide information about the function of the appended GatB-like domain and help in understanding the role of the GatB subunit of AdT. Here, we describe the isolation of overexpressed *D. radiodurans* GlnRS, its crystallization and preliminary X-ray data.

2. Materials and methods

2.1. Expression of GlnRS of *D. radiodurans* in *E. coli* and purification of the protein

The open reading frame of GlnRS was amplified by PCR from *D. radiodurans* genomic DNA (strain R1) and inserted into expression vector pTYB11 (New England Biolabs) to produce the protein fused to intein and the chitin-binding domain. *Escherichia coli* strain ER2566 transformed with the recombined plasmid was grown at 291 K in 6 l LB medium containing ampicillin and expression of the protein was induced with IPTG. The cells harvested by centrifugation after 20 h of culture were suspended in 60 ml 20 m*M* Tris–HCl buffer pH 8.0 containing 0.5 *M* NaCl, 1 m*M* EDTA, 0.1 m*M* AEBSF (a serine-protease inhibitor, Uptima) and disrupted by ultrasonication. The cell debris was removed by centrifugation and the supernatant applied onto a 100 ml chitin affinity column (New England Biolabs) equilibrated with 20 m*M* Tris–HCl buffer pH 8.0 containing 0.5 *M* NaCl and 1 m*M* EDTA. The intein tag was cleaved on the column by 40 h treatment with 50 m*M* dithiothreitol. The protein was eluted with the equilibration buffer, dialyzed and adsorbed onto a 5 ml HiTrap Heparin column (Amersham Pharmacia) equilibrated with 50 m*M* Tris–HCl buffer pH 7.5 containing 0.5 m*M* EDTA and 5 m*M* 2-mercaptoethanol. GlnRS was eluted with a linear gradient from 0 to

0.3 *M* KCl. The active fractions were dialyzed, concentrated by ultrafiltration (Microcon device, Millipore, 50 kDa cutoff) to ∼20 mg ml^{-1} and stored at 277 K. The purity of the protein, analyzed by SDS–PAGE, was greater than 98%. GlnRS was identified by N-terminal sequencing and aminoacylation activity using standard conditions (Kern & Lapointe, 1979) and total tRNA extracted from *D. radiodurans*.

2.2. Crystallization of GlnRS

The homogeneity of the GlnRS samples was examined by dynamic light scattering (DLS). Measurements were performed at 293 K with a 1.7 mg ml^{-1} protein solution in 20 m*M* Tris–HCl buffer pH 7.5 using a dp-801 apparatus (Protein Solutions Inc., USA).

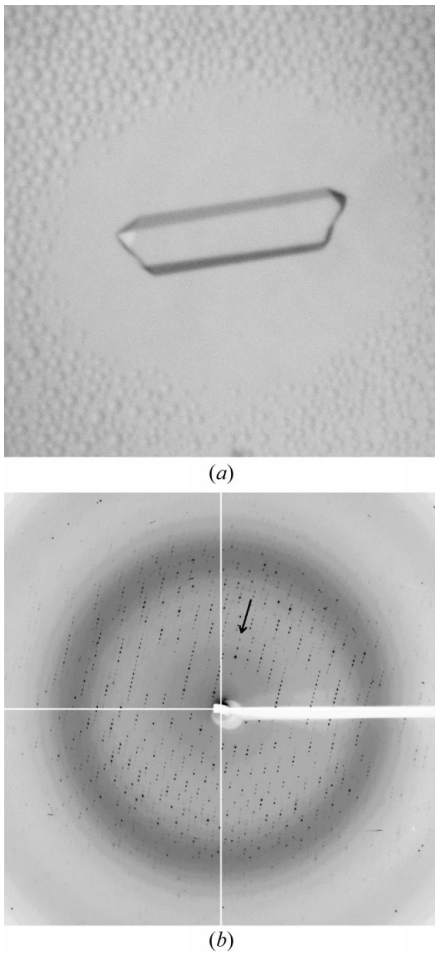

Figure 1
Typical *D. radiodurans* GlnRS crystal and diffraction pattern. (*a*) This GlnRS crystal (0.4 mm in length) was grown by microbatch under paraffin oil in 10% PEG 3350 containing 100 m*M* NaF. A phase separation occurs prior to nucleation and the crystal depletes the surroundings of the small 'oily' droplets during growth. (*b*) An oscillation image from the set collected on beamline ID14-1 (0.5° oscillation) shows the (00*l*) row with systematically absent reflections for $l = 2k + 1$ (see arrow). The resolution at the edges of the ADSC Quantum 4 CCD detector was 2.3 Å (crystal-to-detector distance 235 mm).

Table 1
X-ray analysis of *D. radiodurans* GlnRS crystals.

Values in parentheses are for the highest resolution shell.

Synchrotron beamline	X06SA (SLS)	ID14-4 (ESRF)
Wavelength (Å)	0.979	0.934
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)	a = 77.1, b = 95.7, c = 116.9	a = 74.1, b = 95.9, c = 115.7
Crystal mosaicity (°)	1.5	0.9
Resolution range (Å)	2.8–50 (2.8–2.88)	2.3–30 (2.3–2.36)
No. observations	121221	209589
No. unique reflections	19877	36991
Completeness (%)	90.3 (82.7)	98.1 (92.9)
$R_{\rm merge}$ (%)	7.4 (23.6)	6.2 (25.0)
$\langle I/\sigma(I)\rangle$	15.2 (4.1)	18.1 (5.5)
Matthews coefficient (Å^3 Da^{-1})	2.3	2.2
Solvent content (%)	46	44
Asymmetric unit content	1 monomer	1 monomer

Crystallization conditions were searched for using the vapour-diffusion method in Greiner microplates (Greiner BioOne). Sitting drops formed of 200 nl protein sample and 200 nl reservoir solution were set up using a Mosquito robot (TTP Labtech, UK). They were equilibrated against 100 µl reservoir solution at 293 K. A total of 576 conditions were tested using the Index (Hampton Research) and Wizard (DeCode Genetics) screens combined with three protein concentrations (20, 12 and 6 mg ml^{-1}). The favourable conditions were then optimized by testing 50 different PEG solutions using the microbatch method as described by Chayen *et al.* (1992). Crystallization drops were prepared in Nunc HLA plates (1 µl of each precipitant and protein solutions), overlaid with paraffin oil and stored at 293 K.

2.3. X-ray diffraction analysis

Crystals were mounted in cryoloops (Hampton Research) and flash-frozen in liquid propane after a brief soak in an appropriate cryoprotecting solution. Two data sets were collected at 100 K on beamlines X06SA (SLS, Switzerland) and ID14-EH1 (ESRF, France) on a MAR CCD and an ADSC Quantum 4 CCD detector, respectively. Data were processed using the *HKL* package (Otwinowski & Minor, 1997); statistics are given in Table 1. Analysis of the solvent content performed with the *CCP*4 package (Collaborative Computational Project, Number 4, 1994) gave a unique solution consisting of one polypeptide chain per asymmetric unit. Molecular-replacement (MR) trials were performed with the program *AMoRe* (Navaza & Saludjian, 1997) and the CaspR MR webservice (Claude *et al.*, 2004).

3. Results

The *D. radiodurans* GlnRS expression strategy and purification methodology permitted us to obtain 7 mg of highly pure protein from 1 l culture. The protein was stable and could be reproducibly crystallized after storage for several weeks at 277 K. DLS analysis showed GlnRS to be monodisperse in solution (polydispersity lower than 7%) and thus suitable for crystallization. A diffusion coefficient of 4.4 × 10^{-7} cm^2 s^{-1} was determined, which allowed the derivation of a hydrodynamic radius of 4.7 nm and an apparent MW of 128 kDa. This value exceeds the actual MW of the polypeptide chain (93.5 kDa) by 37% and suggests an elongated shape for the protein. These results are in agreement with a monomeric *D. radiodurans* GlnRS structure.

The first crystallization assays gave results varying between crystalline precipitate, thin needles, urchins and well faceted crystals. Large monocrystals were obtained in the presence of 25% PEG 1000 in 50 m*M* Tris–HCl buffer pH 8.0 (Wizard II No. 32) after 3–20 d with the highest and lowest GlnRS concentrations. The effects of PEG, salt and pH were further investigated using the microbatch method to speed up the crystallization process. Diffracting crystals were reproducibly obtained with solutions containing 4 mg ml^{-1} GlnRS, 10% PEG 3350 and 0.1 *M* NaF (final concentrations). The crystals grew to approximate dimensions of 0.5 × 0.2 × 0.2 mm within 5–7 d (Fig. 1).

Several cryocooling procedures were tested. The most effective was obtained by increasing the PEG concentration to 25%. Samples were quickly washed (<5 s) in 25% PEG 3350 and 0.1 *M* NaF in order to avoid cracking and were plunged in liquid propane. Slow evaporation through the paraffin oil covering the microbatch assays

over a period of two months (d'Arcy *et al.*, 1996) was also used to concentrate the growth media in order to reach cryoprotecting PEG levels. Nevertheless, this procedure led to a complete loss of diffraction which may result either from uncontrolled crystal dehydration or from protein aging.

Flash-frozen crystals were characterized using synchrotron radiation. They belong to the orthorhombic system and analysis of the systematic absences clearly indicates that they belong to space group $P2_12_12_1$. Complete data were collected to a resolution of 2.3 Å (Fig. 1*b*; Table 1). The size of the unit cell varies between the crystals by a few percent both in the *a* and *c* dimensions, but the existence of distinct crystal forms is not obvious. The asymmetric unit corresponds to one monomer of GlnRS with a solvent content of ∼45%.

Molecular-replacement trials were carried out using search models derived from the structures of *E. coli* GlnRS (44% sequence identity with *D. radiodurans* GlnRS) either in a free form (PDB code 1nyl; Sherlin & Perona, 2003) or in complex with the cognate tRNA (PDB codes 1o0b, 1o0c; Bullock *et al.*, 2003). Polyalanine models accounting for two-thirds of the monomer, *i.e.* the catalytic core and the anticodon-binding domain, produced a clear solution (∼30% higher than the second peak) for the rotation function in *AMoRe* with both data sets. The polyalanine version of 1o0b gave the best hit after the translation search, with a final correlation coefficient and *R* factor of 33.1 and 51.8%, respectively (resolution range 4–15 Å). The solution was confirmed by an automated MR search on the CaspR server (http://igs-server.cnrs-mrs.fr/Caspr), which led to *R* and free *R* values of 40.1 and 46.6%, respectively, after rigid-body refinement and least-squares minimization at 3 Å resolution. Electron density is observed in the first map that corresponds to the N- and C-terminal parts of the protein that are missing in MR models. Structure building and refinement are in progress.

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