Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray diffraction analysis of an *Escherichia coli* tRNA^{Gly} acceptor-stem microhelix

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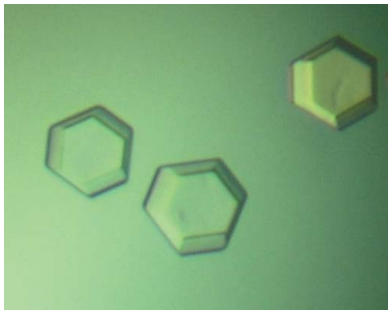
Received 7 November 2006
Accepted 6 December 2006



The tRNA^{Gly} and glycyl-tRNA synthetase (GlyRS) system is an evolutionary special case within the class II aminoacyl-tRNA synthetases because two divergent types of GlyRS exist: an archaebacterial/human type and an eubacterial type. The tRNA identity elements which determine the correct aminoacylation process are located in the aminoacyl domain of tRNA^{Gly}. To obtain further insight concerning structural investigation of the identity elements, the *Escherichia coli* seven-base-pair tRNA^{Gly} acceptor-stem helix was crystallized. Data were collected to 2.0 Å resolution using synchrotron radiation. Crystals belong to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 35.35$, $c = 130.82$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$ and two molecules in the asymmetric unit.

1. Introduction

tRNA identity elements ensure the correct aminoacylation of tRNAs with the cognate amino acids by aminoacyl-tRNA synthetases. These synthetases are unique and early enzymes in evolution and act in the translation of the genetic code, transferring its information into polypeptides. Owing to the redundancy of the genetic code (there are 64 codons for just 20 standard amino acids plus some special amino acids such as selenocysteine), multiple mRNA triplets exist for some amino acids and consequently multiple respective tRNA isoacceptors also exist varying in anticodon and sequence (Sprinzl & Vassilenko, 2005). However, there is only a single aminoacyl-tRNA synthetase for each amino acid, which has to mastermind the correct aminoacylation. In the *Escherichia coli* tRNA^{Gly} system, which is the focus of the present study, there are several isoacceptors that all are aminoacylated by the same glycine-tRNA synthetase (GlyRS). As GlyRS is a class II enzyme, it depends on rather simple and unique tRNA identity elements to ensure the correct aminoacylation (Eriani *et al.*, 1990). In the *E. coli* tRNA^{Gly} system, these determinants are located in the aminoacyl domain, base pair C2–G71 and the discriminator base U73 (McClain *et al.*, 1991).

Early studies showed that *E. coli* GlyRS consists of a heterodimeric $\alpha_2\beta_2$ structure (Ostrem & Berg, 1974; Webster *et al.*, 1983). Both protein subunits are required for the enzymatic activity of GlyRS (Ostrem & Berg, 1974). The α subunit is responsible for binding adenosine-5′-triphosphate (ATP) and glycine (Toth & Schimmel, 1990) and therefore serves in glycine activation. The β subunit is responsible for tRNA recognition and binding (Nagel *et al.*, 1984).

A variety of GlyRS sequences from different organisms have become available, including those from baker's yeast (Kern *et al.*, 1981), *Bombyx mori* (Nada *et al.*, 1993) and human (Shiba *et al.*, 1994). Surprisingly, two different species of GlyRS exist: a eukaryotic/archeabacterial type and another eubacterial type which can be further divided into subgroups. There are clear differences in the sequences (Shiba, 2005), even in the so-called motifs 1–3 that are known to be homologous for class II RS systems (Eriani *et al.*, 1990). The human and prokaryotic enzymes may have evolved from two different ancestors (Shiba, 2005).

Prokaryotic GlyRS normally have an $\alpha_2\beta_2$ subunit structure and their tRNA identity elements include the aminoacyl stem and the

discriminator base U73. In contrast, eukaryotic GlyRS have an α_2 structure and depend on recognizing the tRNA^{Gly} aminoacyl domain, with the discriminator base being A73. The crystal structure of *Thermus thermophilus* GlyRS has been solved to a resolution of 2.75 Å (Logan *et al.*, 1995). This enzyme is a special GlyRS because its subunit structure resembles that of the eukaryotic type, whereas its synthetase activity is dependent on the presence of a U in the discriminator position 73, thus resembling the eubacterial type of GlyRS.

Here, we present the crystallization and preliminary X-ray diffraction data of an *E. coli* tRNA^{Gly} acceptor-stem microhelix with the aim of attempting to compare the high-resolution structures of the respective prokaryotic and eukaryotic segments in detail. This study supplements a small but growing number of investigations aiming at determining the three-dimensional structures of tRNA acceptor-stem microhelices reflecting alternative amino-acid specificities (Mueller *et al.*, 1999; Ramos & Varani, 1997; Seetharaman *et al.*, 2003; Förster *et al.*, 1999, 2006).

2. Materials and methods

2.1. RNA hybridization and crystallization

The two complementary RNA oligonucleotides 5′-GCGGGAA-3′ and 5′-UUCCCGC-3′ were purchased from CureVac (Tübingen, Germany). The lyophilized RNA pellet was resuspended in H_2O and the exact amount was determined by alkaline hydrolysis and UV absorption of an RNA aliquot (Sproat *et al.*, 1995).

For the formation of the *E. coli* tRNA^{Gly} microhelix, the complementary RNA strands were hybridized in water at a concentration of 0.5 m*M* each. The sample was heated to 363 K, followed by cooling to room temperature for several hours. The resulting duplex was used for initial crystallization screening and for optimization of the crystallization conditions.

Screening experiments were performed using the sitting-drop vapour-diffusion technique, applying the Natrix Formulation and Nucleic Acid Mini Screen from Hampton Research (CA, USA). A 1 µl sample of RNA in H_2O was mixed with 1 µl reservoir solution and equilibrated against 80 µl reservoir solution at 294 K in CrystalQuick Lp Plates (Greiner Bio-One, Germany). Small crystals with dimensions of approximately 0.05 mm appeared after 2–3 d under various conditions using ammonium sulfate, lithium sulfate, 2-methyl-2,4-pentanediol (MPD) or polyethythylene glycol (PEG) 4000 as precipitant. The best crystals were obtained after optimizing the conditions using ammonium sulfate as precipitant. The final crystallization conditions consisted of 50 m*M* sodium cacodylate pH 6.0, 10 m*M* spermine, 10 m*M* magnesium sulfate, 2.0 *M* ammonium sulfate, applying the sitting-drop vapour-diffusion technique. 1 µl of 0.5 m*M* RNA duplex in H_2O was mixed with 1 µl reservoir solution and equilibrated against 1 ml reservoir solution at 294 K in 24-well Linbro Plates (ICN Biomedicals Inc., Ohio, USA). Crystals grew in about 2 d to approximate dimensions of 0.15 × 0.15 × 0.04 mm (Fig. 1).

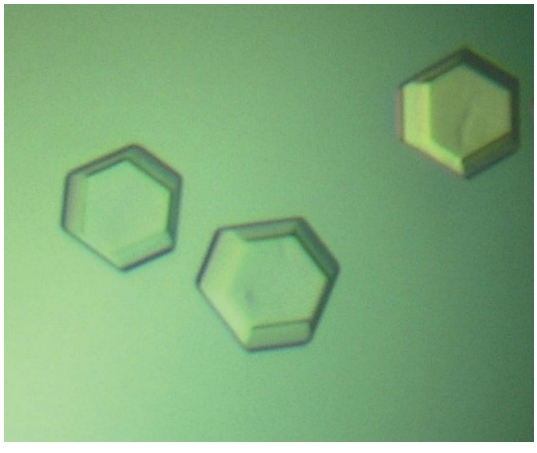


Figure 1
Hexagonal crystals of the *E. coli* tRNA^{Gly} acceptor-stem microhelix with approximate dimensions of 0.15 × 0.15 × 0.04 mm.

2.2. Crystallographic data collection and evaluation

X-ray diffraction data were collected at the DESY synchrotron in Hamburg on the X13 consortium beamline (DESY/HASYLAB, Hamburg). Prior to data collection, crystals were transferred to a cryoprotectant solution containing 50 m*M* sodium cacodylate pH 6.0, 10 m*M* spermine, 10 m*M* magnesium sulfate, 1.9 *M* ammonium sulfate and 18%(*v*/*v*) glycerol and were immediately flash-frozen in liquid nitrogen. A high-resolution data set (20–2.0 Å) was recorded at a wavelength of 0.8063 Å and at a temperature of 100 K. Data processing, space-group calculation and determination of unit-cell parameters were performed with the *HKL*-2000 package (Otwinowski & Minor, 1997).

3. Results and discussion

3.1. Crystallization

All three sequences of *E. coli* tRNA^{Gly} acceptor stems listed in the databank (Sprinzl & Vassilenko, 2005) were synthesized and screened for crystallization. Here, we present the crystallization of the duplex with sequence 5′-GCGGGAA-3′/5′-UUCCCGC-3′ (Fig. 2) derived from the *E. coli* tRNA^{Gly} G1661. The crystals shown in Fig. 1 appeared after 2 d at 294 K and represent the typical morphology resulting from the optimized crystallization conditions. These crystals

Figure 2
tRNA conformation (derived from tRNA^{Phe}; PDB code 1ehz) with the acceptor-stem region highlighted in yellow. The sequence of the *E. coli* tRNA^{Gly} acceptor-stem microhelix crystallized in this study is shown.

Table 1
Data-collection and processing statistics of the *E. coli* tRNA^{Gly} acceptor-stem microhelix.

Values in parentheses are for the highest resolution shell.

Beamline	DESY/HASYLAB X13
Wavelength (Å)	0.80630
Space group	$P3_121$ or $P3_221$
Unit-cell parameters (Å,°)	$a = b = 35.35$, $c = 130.82$, $\gamma = 120.00$
Matthews coefficient V_M (Å^3 Da^{-1})	2.68
RNA duplexes per ASU	2
Solvent content† (%)	65
Measured reflections	65153
Unique reflections	6908
Resolution range (Å)	20.0–2.0 (2.03–2.00)
Completeness (%)	99.1 (83.4)
Multiplicity (%)	9.4 (4.1)
R_{merge}‡ (%)	7.9 (29.3)
Average $I/\sigma(I)$	34.0 (5.8)

† Estimated using the average partial specific volume calculated for RNA by Voss & Gerstein (2005). ‡ $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ and $\langle I(hkl)\rangle$ are the observed individual and mean intensities of a reflection with the indices hkl, respectively, $\sum_i$ is the sum over the individual measurements of a reflection with indices hkl and $\sum_{hkl}$ is the sum over all reflections.

were subsequently transferred to the cryoprotecting solution, flash-frozen and used for data collection.

3.2. Crystallographic data

The *E. coli* tRNA^{Gly} acceptor-stem microhelix crystallizes in space group $P3_121$ or $P3_221$, with unit-cell parameters $a = 35.35$, $b = 35.35$, $c = 130.82$ Å, $\gamma = 120.00°$. We calculated a Matthews coefficient V_M (Matthews, 1968) of 2.68 Å^3 Da^{-1}, which corresponds to two RNA molecules per asymmetric unit, and estimated the water content to be 65% using the RNA parameters derived by Voss & Gerstein (2005) as summarized in Table 1.

X-ray diffraction data were collected from tRNA^{Gly} acceptor-stem crystals to 2.0 Å resolution using synchrotron radiation at a wavelength of 0.8063 Å and cryogenic cooling. The data were processed in the resolution range 20–2.0 Å with an overall completeness of 99.1% and a resulting overall R_{merge} of 7.9% (Table 1).

Crystal disorder and in particular crystal twinning are known to occur with short RNA helices (Rypniewski *et al.*, 2006; Mueller, Muller *et al.*, 1999; Mueller, Schübel *et al.*, 1999). Therefore, the diffraction data of the *E. coli* tRNA^{Gly} microhelix were analyzed for merohedral twinning by applying the algorithm described by Padilla & Yeates (2003; the web-server implementation at http://nihserver.mbi.ucla.edu/pystats/ was used). The results for our data set clearly correspond to those of a theoretical untwinned crystal (data not shown); thus, there is no indication of merohedral twinning.

Currently, molecular-replacement calculations are in progress to determine the structure of the tRNA^{Gly} acceptor-stem helix by trying both enantiomorphic space groups $P3_121$ and $P3_221$. As, to the best of our knowledge, there are currently no coordinates available for either tRNA^{Gly} microhelices or native tRNA^{Gly} molecules, various small-helical RNA structures are presently being tested as models for molecular-replacement calculations. Examples of models currently being explored include the tRNA^{Ala} microhelix (PDB code 434d; Mueller, Muller *et al.*, 1999; Mueller, Schübel *et al.*, 1999) and microhelices generated from native tRNAs such as that from tRNA^{Phe} (PDB code 1ehz; Shi & Moore, 2000).

A refined three-dimensional structure could provide the first view of an *E. coli* tRNA^{Gly} acceptor stem and, in conjunction with docking experiments to the crystal structure of *Th. thermophilus* glycyl-tRNA synthetase (Logan *et al.*, 1995), could also advance the understanding of the molecular-recognition process with a cognate synthetase. It is noteworthy that the *Th. thermophilus* GlyRS exhibits features of both the prokaryotic and eukaryotic systems. Further crystallographic investigations are in progress to examine the evolutionary divergence between the eukaryotic and the prokaryotic tRNA-recognition elements required by the two different GlyRS systems.

This work was funded within the RiNA network for RNA technologies by the Federal Ministry of Education and Research, the City of Berlin and the European Regional Development Fund. We thank the Fonds der Chemischen Industrie (Verband der Chemischen Industrie eV) and the National Foundation for Cancer Research, USA for additional support. We gratefully acknowledge the DESY synchrotron, Hamburg for providing beamtime and Norbert Krauss for helpful discussions.

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