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

Crystallization and preliminary X-ray diffraction analysis of the tRNA-modification enzyme GidA from *Aquifex aeolicus*

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Received 17 February 2009
Accepted 10 April 2009

The 5-carboxymethylaminomethyl modification of uridine at the first position of the tRNA anticodon is crucial for accurate protein synthesis by stabilizing the correct codon–anticodon pairing on the ribosome. Two conserved enzymes, GidA and MnmE, are involved in this modification process. *Aquifex aeolicus* GidA was crystallized in two different crystal forms: forms I and II. These crystals diffracted to 3.2 and 2.3 Å resolution, respectively, using synchrotron radiation at the Photon Factory. These crystals belonged to space groups $I2_12_12_1$ and $P2_1$ with unit-cell parameters a = 101.6, b = 213.3, c = 231.7 Å and a = 119.4, b = 98.0, c = 129.6 Å, β = 90.002°, respectively. The asymmetric units of these crystals are expected to contain two and four molecules, respectively.

1. Introduction

Transfer RNA (tRNA) contains a variety of modified nucleosides which are post-transcriptionally introduced in all organisms (Björk, 1995). In *Escherichia coli*, ∼1% of the genome is devoted to encoding tRNA-modification enzymes (Grosjean *et al.*, 1995). Many of the modified nucleosides in tRNA are observed at the first position of the anticodon (the wobble position) and immediately 3′-adjacent to the anticodon. In particular, modifications at the wobble position are indispensable for precise decoding of the genetic code (Björk, 1995; Urbonavičius *et al.*, 2001; Yokoyama & Nishimura, 1995). In eubacteria, 5-carboxymethylaminomethyluridine (cmnm^{5}U) or 5-methylaminomethyluridine (mnm^{5}U) modifications are commonly found at the wobble position of $tRNA^{Glu}_{UUC}$, $tRNA^{Lys}_{UUU}$, $tRNA^{Gln}_{UUG}$, $tRNA^{Leu}_{UAA}$ and $tRNA^{Arg}_{UCU}$ and are responsible for deciphering the purine-ending split codon boxes (NNR) (Björk, 1995; Yokoyama & Nishimura, 1995). The cmnm^{5}U and mnm^{5}U modifications stabilize the correct codon–anticodon pairing on the ribosome (Yarian *et al.*, 2000; Urbonavičius *et al.*, 2001; Murphy *et al.*, 2004), which enables the tRNAs for Glu, Lys, Gln, Leu and Arg to discriminate purines from pyrimidines in order to prevent the incorrect decoding of the NNY codon for translational fidelity.

In eubacteria, two conserved enzymes, GidA and MnmE, are involved in the incorporation of the cmnm^5 group at the 5-position of the wobble uridine (Elseviers *et al.*, 1984; Brégeon *et al.*, 2001). In some eubacteria, such as *E. coli*, the cmnm^5 group is then converted into the final mnm^5 group by MnmC (Bujnicki *et al.*, 2004). GidA and MnmE are strictly conserved in eukarya, in which MSS1 and MTO1 have been identified as their respective homologues (Decoster *et al.*, 1993; Colby *et al.*, 1998).

Biochemical studies have revealed that GidA interacts with MnmE to form a functional complex, suggesting that these two proteins collaborate to modify the 5-position of the wobble uridine in an interdependent manner (Yim *et al.*, 2006). GidA contains a consensus dinucleotide-binding motif, G*X*G*XX*G, a portion of the Rossmann-fold domain which is present in a large number of flavin adenine dinucleotide (FAD) binding proteins (Dym & Eisenberg, 2001). In fact, GidA binds FAD, which has been proposed to be involved in the cmnm^{5}U modification process (Yim *et al.*, 2006). However, the precise function of GidA during the modification process remains

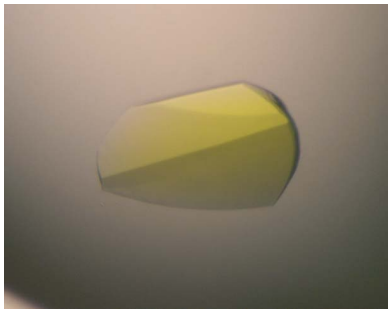


Table 1
Data-collection statistics.

Values in parentheses are for the last shell.

	Form I	Form II
Wavelength (Å)	0.97928	1.00
Space group	$I2_12_12_1$	$P2_1$
Unit-cell parameters (Å, °)	a = 101.6, b = 213.3, c = 231.7	a = 119.4, b = 98.0, c = 129.6, β = 90.002
Resolution (Å)	50–3.20 (3.26–3.20)	50–2.30 (2.33–2.30)
Measured reflections	333405 (10435)	436815 (13035)
Unique reflections	41584 (2046)	123831 (5214)
Redundancy	8.0 (5.1)	3.7 (2.5)
Completeness (%)	99.5 (99.3)	92.2 (78.5)
$\langle I/\sigma(I)\rangle$	11.5 (2.2)	12.2 (2.0)
$R_{\rm merge}$†	0.126 (0.398)	0.083 (0.248)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity and $\langle I(hkl)\rangle$ is the average intensity over symmetry-equivalent measurements.

elusive. To gain insight into the structure–function relationships of GidA, we cloned and overproduced GidA from *Aquifex aeolicus*, which is referred to as AaGidA. In the present paper, we report the crystallization and preliminary X-ray diffraction analysis of AaGidA.

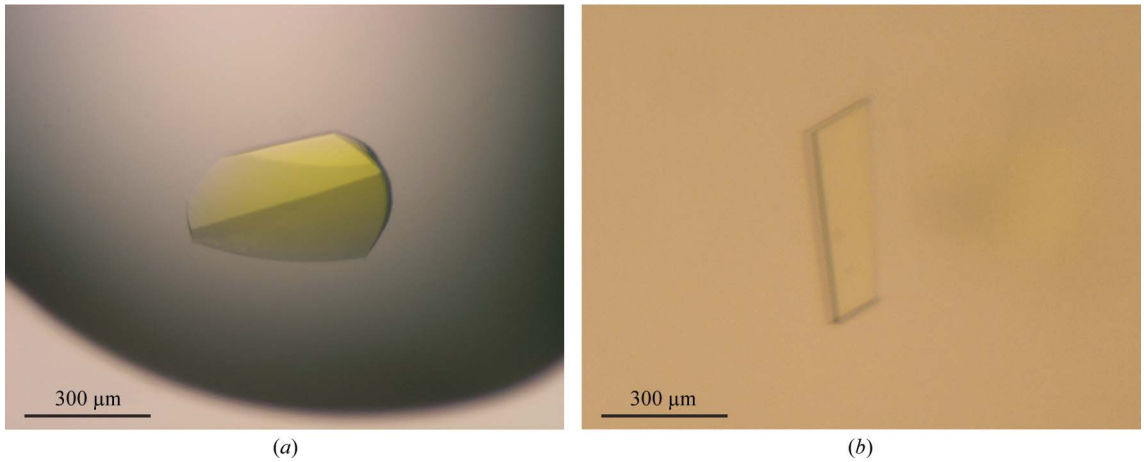
2. Materials and methods

2.1. Protein preparation

A DNA fragment encoding AaGidA was produced by PCR amplification and cloned into the expression vector pET28b (Novagen). The recombinant AaGidA protein with a His tag attached to the N-terminus was produced in *E. coli* strain BL21-CodonPlus (DE3)-RIL and was purified as follows. After sonication of the *E. coli* cells in 20 m*M* Tris–HCl buffer pH 8.0 containing 300 m*M* NaCl, 10 m*M* imidazole, 7 m*M* β-mercaptoethanol, 1 m*M* PMSF and 1 m*M* benzamidine, the clarified lysate was incubated at 338 K for 20 min. The heat-treated lysate was centrifuged at 8000 rev min^{-1} for 30 min and the supernatant was loaded onto an Ni–NTA column (Qiagen) equilibrated with buffer *A* (20 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl, 10 m*M* imidazole and 7 m*M* β-mercaptoethanol). AaGidA was eluted with buffer *B* (as buffer *A* but containing 250 m*M* imidazole). The eluate from the Ni–NTA column was dialyzed against buffer *C* (10 m*M* potassium phosphate pH 6.0, 200 m*M* NaCl and 7 m*M* β-mercaptoethanol). The protein solution was then loaded onto a HiTrap Heparin column (16 × 25 mm) previously equilibrated with buffer *C*. A linear gradient was developed from 200 to 1000 m*M* NaCl in buffer *C*. The fractions rich in the target protein were combined and dialyzed against buffer *D* (20 m*M* potassium phosphate pH 6.0, 100 m*M* NaCl and 7 m*M* β-mercaptoethanol). After the concentration of NaCl in the protein solution had been reduced to 50 m*M* by mixing with an equal volume of buffer *E* (20 m*M* potassium phosphate pH 6.0 and 7 m*M* β-mercaptoethanol), the protein solution was further purified on a Resource S column (16 × 30 mm) previously equilibrated with buffer *F* (20 m*M* potassium phosphate pH 6.0, 50 m*M* NaCl and 7 m*M* β-mercaptoethanol). GidA was eluted with a linear gradient of 50–1000 m*M* NaCl. The fractions enriched in AaGidA were combined and dialyzed against buffer *G* (20 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl and 7 m*M* β-mercaptoethanol) and the solution was concentrated to 5–10 mg ml^{-1}. Selenomethionine-labelled AaGidA was produced in the methionine auxotroph *E. coli* strain B834-CodonPlus (DE3)-RIL and was purified using the same procedure as used for the native protein.

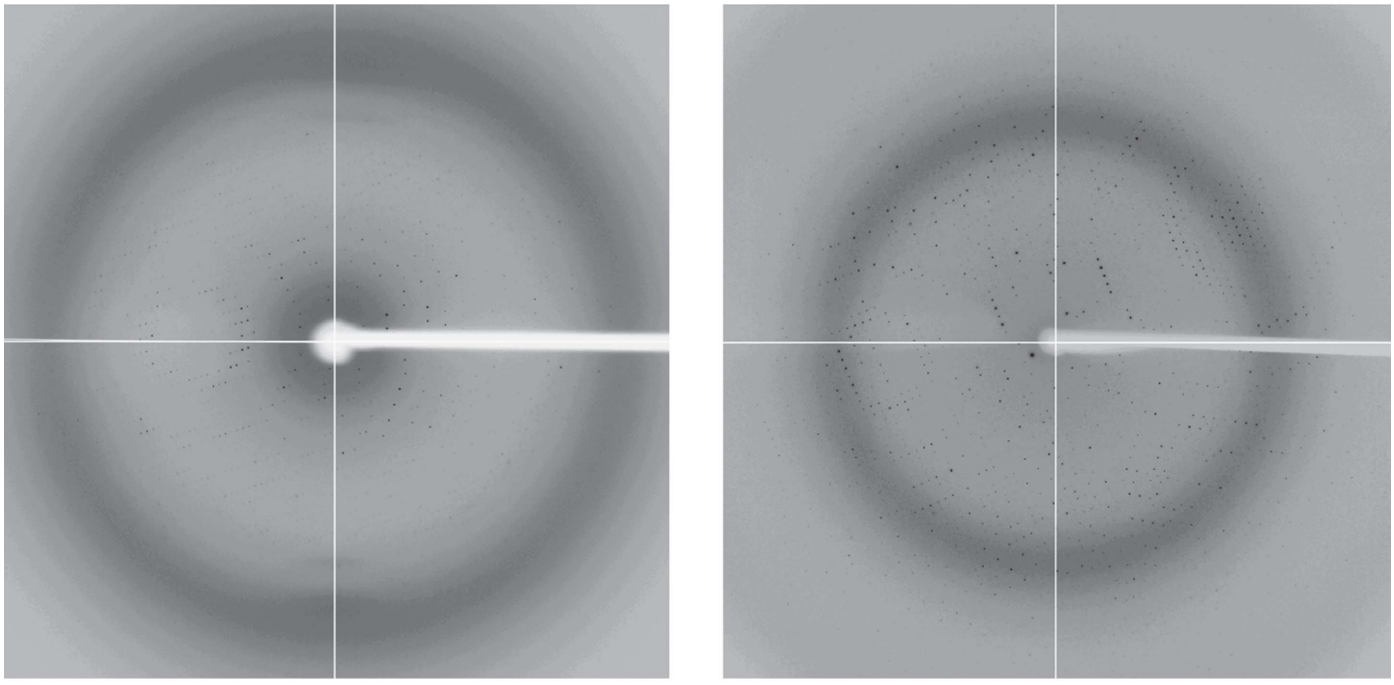
2.2. Crystallization

Crystallization conditions were screened by the sitting-drop vapour-diffusion method at 293 K using the following commercial crystallization screening kits: Crystal Screen, Crystal Screen 2, Crystal Screen Lite, Crystal Screen Cryo, Natrix, PEG/Ion, Index and SaltRx (Hampton Research). Sitting drops were prepared by mixing 1 µl reservoir solution with 1 µl His-tagged AaGidA solution (5 mg ml^{-1}) and were equilibrated against 100 µl reservoir solution. AaGidA was crystallized in two different crystal forms: forms I and II. Form I crystals were obtained within 3–4 d using reservoir solution containing 1.0–1.2 *M* sodium/potassium phosphate pH 6.9–7.2. Form II crystals grew within two weeks in 200 m*M* ammonium chloride and 10–20% PEG 3350. The form II crystals prepared as described above were polycrystals that were unsuitable for X-ray diffraction experiments. Therefore, we improved the crystallization conditions for the form II crystal by using a pH Buffer kit and an Additive Screen kit (Hampton Research). Finally, large single crystals that were suitable for X-ray diffraction experiments were obtained using a reservoir solution containing 160 m*M* ammonium chloride, 100 m*M* HEPES pH 7.2, 10 m*M* sodium citrate pH 5.6, 200 m*M* 1,6-hexanediol and 10–20% PEG 3350.



(*a*) (*b*)

Figure 1
Two crystal forms of *A. aeolicus* GidA. (*a*) Form I crystals belonging to space group $I2_12_12_1$. (*b*) Form II crystals belonging to space group $P2_1$.



(*a*) (*b*)

Figure 2
Diffraction patterns of form I crystals (*a*) and form II crystals (*b*) of *A. aeolicus* GidA.

2.3. X-ray data collection

For data collection, the AaGidA form I and II crystals were cryoprotected in reservoir solution containing 20% ethylene glycol and 20% glycerol, respectively. The crystals were mounted in a cryoloop and were flash-cooled in a nitrogen stream at 100 K. X-ray diffraction data were collected on the NW12A and BL-17A beamlines of KEK (Ibaraki, Japan) using ADSC Q210r and ADSC Q270 CCD detectors, respectively. Diffraction data were processed with the program *HKL*-2000 (Otwinowski & Minor, 1997). The processing statistics are summarized in Table 1.

3. Results and discussion

The His-tagged AaGidA protein was overexpessed in *E. coli* cells using the pET28b expression vector and was purified to homogeneity by several chromatography steps. The purified protein was coloured yellow, indicating that the cofactor FAD was co-purified with AaGidA from the *E. coli* cell lysate. This is consistent with the presence of the FAD-binding motif G*X*G*XX*G (Gly14, Gly16 and Gly19 in AaGidA), which is conserved among the GidAs. The His-tagged AaGidA was crystallized in two different crystal forms: forms I and II (Fig. 1). The form I crystals, which grew to maximum dimensions of 300 × 150 × 50 µm, diffracted to a resolution of 3.2 Å (Fig. 2*a*) and belonged to the orthorhombic space group $I2_12_12_1$, with unit-cell parameters a = 101.6, b = 213.3, c = 231.7 Å. On the basis of the molecular mass of AaGidA (70 kDa), the form I crystals are expected to contain two molecules per asymmetric unit, which corresponds to a solvent content of 71.9% and a Matthews coefficient of 4.37 Å^3 Da^{-1}. The form II crystals grew to dimensions of 300 × 200 × 10 µm within two weeks and diffracted to 2.3 Å resolution (Fig. 2*b*). The form II crystals belonged to the monoclinic space group $P2_1$, with unit-cell parameters a = 119.4, b = 98.0, c = 129.6 Å, β = 90.002°. The calculated Matthews coefficient was approximately 2.64 Å^3 Da^{-1}, assuming the presence of four molecules in the asymmetric unit, with a solvent content of 53.5%. Both AaGidA crystals have a deep yellow colour (Fig. 1), suggesting that the crystals contain the FAD cofactor. Since FAD binding to GidA is required for the synthesis of cmnm^{5}U (Yim *et al.*, 2006), structural analysis of AaGidA complexed with FAD could reveal the catalytic site of GidA and provide structural insight into the reaction mechanism of the cmnm^{5}U modification process. Attempts to solve the AaGidA structure complexed with FAD by the SAD method, with selenium as the anomalous scattering atom, are in progress.

We thank H. Hori for donating the *A. aeolicus* genomic DNA. We also thank the beamline staff at NW12A, BL-5A and BL-17A of KEK (Ibaraki, Japan) for technical assistance during data collection. This work was supported by a PRESTO Program grant from JST (Japan Science and Technology), a Grant-in-Aid for Young Scientists from JSPS and grants from the Sumitomo Foundation, the Kurata Memorial Hitachi Science and Technology Foundation and the Kato Memorial Bioscience Foundation to TN.

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