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

Crystallization and preliminary X-ray analysis of a class II release factor RF3 from a sulfate-reducing bacterium

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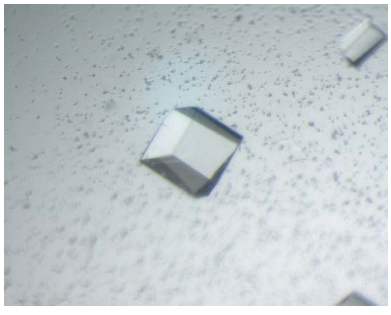
Received 7 May 2008
Accepted 23 May 2008



Class II release factor 3 (RF3) from the sulfate-reducing bacterium *Desulfovibrio vulgaris* Miyazaki F, which promotes rapid dissociation of a class I release factor, has been overexpressed, purified and crystallized in complex with GDP at 293 K using the sitting-drop vapour-diffusion method. A data set was collected to 1.8 Å resolution from a single crystal at 100 K using synchrotron radiation. The crystal belongs to space group $P1$, with unit-cell parameters a = 47.39, b = 82.80, c = 148.29 Å, α = 104.21, β = 89.78, γ = 89.63°. The asymmetric unit contains four molecules of the RF3–GDP complex. The Matthews coefficient was calculated to be 2.3 Å^3 Da^{-1} and the solvent content was estimated to be 46.6%.

1. Introduction

Translation of an mRNA is terminated when a stop codon is encountered. Since tRNAs do not recognize stop codons, this event is performed by proteins called release factors. Prokaryotes have two class I release factors (RFs), RF1 and RF2 (Scolnick *et al.*, 1968), and one class II release factor, the G protein RF3 (Milman *et al.*, 1969; Grentzmann *et al.*, 1994; Mikuni *et al.*, 1994). RF1 and RF2 hydrolyze and release the completed polypeptide from the peptidyl-tRNA at the ribosomal P-site in response to a stop codon. RF1 recognizes the stop codons UAA and UAG, whereas RF2 recognizes UAA and UGA. RF3 catalyses the removal of RF1 or RF2 from the A-site in a GTP-dependent manner (Freistroffer *et al.*, 1997; Zavialov *et al.*, 2001, 2002).

When an RF3–GDP complex associates with the ribosome in complex with a class I RF, the GDP on RF3 can rapidly dissociate and be exchanged for GTP (Zavialov *et al.*, 2001, 2002). RF3 in the GTP-bound form has a high affinity for the ribosome in the absence of a class I RF and its binding to the ribosome destabilizes the interaction between the ribosome and RF1 or RF2. The formation of the RF3–GTP complex therefore leads to rapid dissociation of RF1 or RF2 from the ribosome (Zavialov *et al.*, 2001). Subsequent hydrolysis of GTP leads to dissociation of RF3 in the GDP conformation from the ribosome (Zavialov *et al.*, 2001, 2002). Recently, the X-ray structure of RF3 in the GDP-bound state from *Escherichia coli* has been determined at 2.8 Å resolution (Gao *et al.*, 2007). The polypeptide chain of RF3 in complex with GDP is folded into three distinct domains. Domain I is composed of a classic GTPase domain (G domain) and an 'EF-G-like' G′ subdomain (Laurberg *et al.*, 2000; Czworkowski *et al.*, 1994). Domain II forms a β-barrel structure as observed in EF-Tu (Song *et al.*, 1999), eRF3 (Kong *et al.*, 2004) and EF-G (Czworkowski *et al.*, 1994). Domain III is composed of a central β-barrel flanked by two α-helices. Klaholz *et al.* (2004) and Gao *et al.* (2007) have reported cryo-EM density maps of RF3-bound ribosome in complex with GDPNP. These reports suggest that the binding of RF3–GTP leads to large conformational changes of the ribosome. In the X-ray crystal structure of the RF3–GDP complex, however, the switch 1 region, which is a consensus element of the GTPases that participates in translation, was disordered. In addition, an Mg^{2+} ion,

Table 1
Crystal parameters and X-ray diffraction data-collection statistics.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.0000
Space group	*P*1
Unit-cell parameters (Å, °)	a = 47.39, b = 82.80, c = 148.29, α = 104.21, β = 89.78, γ = 89.63
Matthews coefficient (Å^3 Da^{-1})	2.3
Solvent content (%)	46.6
No. of molecules in the ASU	4
Resolution (Å)	50.0–1.80 (1.86–1.80)
No. of measured reflections	363159
No. of unique reflections	187413
$R_{\rm merge}$† (%)	4.3 (32.6)
Completeness (%)	92.8 (89.0)
Mean $I/\sigma(I)$	9.8 (2.4)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I(hkl)$ is the intensity of reflection hkl, $\sum_{hkl}$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection hkl.

which is considered to be an essential cofactor for GTP hydrolysis in all G proteins, was not observed in the crystal structure of the RF3–GDP complex, even though the crystallization buffer contained 5 m*M* $MgCl_2$. Thus, the real mechanism of RF3 still remains unclear. In order to elucidate the detailed reaction mechanism in the final stage of translation of mRNA carried out on the ribosome on the basis of the structure at atomic level, we have initiated the high-resolution crystal structure analysis of RF3. In this paper, we report the crystallization and preliminary X-ray analysis of RF3 from *Desulfovibrio vulgaris* Miyazaki F.

2. Materials and methods

2.1. Cloning and expression

The plasmid pHin67 was obtained by cloning the *fur-rbr-rlp* operon and contained an approximately 8.6 kbp *Hin*dIII–*Hin*dIII fragment of *D. vulgaris* Miyazaki F. There is also a gene encoding RF3 (*prfC*) in this fragment; *prf*C was amplified by PCR with pHin67 using primers 5′-TACCGTCGACCATATGAGCAGCAGACTGGA-3′ and 5′-CCGGATCCAAACTGATTTCGCGCG-3′. The amplified fragment was cloned into the plasmid vector pUC18 previously digested with *Sal*I and *Bam*HI and the plasmid obtained was named pRFC-101. After confirmation of the nucleotide sequence, the plasmid pRFC-101 was digested with *Nde*I and *Bam*HI and ligated into pUT7 previously digested with the same restriction enzymes. The resulting plasmid for overexpression of *prf*C with an N-terminal six-histidine tag was designated pUTRF3.

Escherichia coli strain BL21 (DE3) cells transformed with the plasmid pUTRF3 were grown at 298 K in LB medium containing 50 mg ml^{-1} ampicillin. When the absorbance of the culture reached 0.5 at 595 nm, protein expression was induced by the addition of isopropyl β-D-1-thiogalactopyranoside to a final concentration of 0.1 m*M*. After 18 h induction, the cells were harvested and disrupted by sonication in buffer *A* (20 m*M* Tris pH 7.5 and 300 m*M* NaCl) with 1 m*M* PMSF.

2.2. Protein purification

The disrupted cells were centrifuged at 180 000*g* at 277 K for 30 min. The supernatant was loaded onto a 5 ml HisTrap HP column (GE Healthcare) equilibrated with buffer *A*. The unbound proteins were flushed with buffer *A*. RF3 with an N-terminal histidine tag was eluted with a stepwise gradient of imidazole. The eluted fractions containing RF3 were dialyzed against buffer *B* (20 m*M* Na HEPES pH 7.6 and 120 m*M* NaCl) and further purified using a Toyopearl DEAE-650S column (TOSOH) equilibrated with buffer *B*. The product homogeneity of the purified preparation was judged by SDS–PAGE and native PAGE.

2.3. Crystallization

Prior to crystallization, the purified RF3 was transferred to a buffer containing 20 m*M* Na HEPES pH 7.6, 300 m*M* NaCl, 5 m*M* $MgCl_2$ and 2 m*M* GDP and concentrated to 8 mg ml^{-1} using a Vivaspin 20 (30 000 MWCO PES, Vivascience). The initial crystallization screening was performed with Crystal Screen, Crystal Screen 2 and Crystal Screen Lite (Hampton Research) in Crystal Clear Strips 96-well sitting-drop plates (Hampton Research) at 293 K. The drop size was 2 µl, with a 1:1 protein:reservoir ratio. Bundles of rod-shaped crystals were obtained using Crystal Screen Lite condition No. 46 (9% PEG 8000, 0.1 *M* sodium cacodylate pH 6.5 and 0.2 *M* calcium acetate). By optimizing the conditions and seeding technique, single crystals suitable for X-ray diffraction experiments, which had dimensions of about 0.3 × 0.3 × 0.15 mm, were obtained by mixing an equal volume of protein solution and a reservoir solution consisting of 6–12%(*w*/*v*) PEG 8000, 0.1 *M* sodium cacodylate pH 7.0 and 0.2 *M* calcium acetate (Fig. 1).

2.4. Data collection

Prior to data collection, the crystal was soaked in a reservoir solution containing 20%(*w*/*v*) MPD for a few seconds and then flash-cooled in the nitrogen stream at 100 K. X-ray diffraction data were collected to 1.8 Å resolution on an ADSC Quantum315 detector using synchrotron radiation of wavelength 1.0000 Å on the BL41XU beamline, SPring-8. Diffraction images covering a total oscillation range of 180° with a step size of 0.3° and an exposure time of 1 s were processed using the *HKL*-2000 program package (Otwinowski & Minor, 1997) and the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

The crystal belongs to space group *P*1, with unit-cell parameters a = 47.39, b = 82.80, c = 148.29 Å, α = 104.21, β = 89.78, γ = 89.63°. Assuming the presence of four molecules per asymmetric unit, the calculated Matthews coefficient $V_{\rm M}$ was 2.3 Å^3 Da^{-1} (Matthews, 1968). The solvent content of the crystal was therefore calculated to be 46.6%. Details of the statistics of data collection are shown in

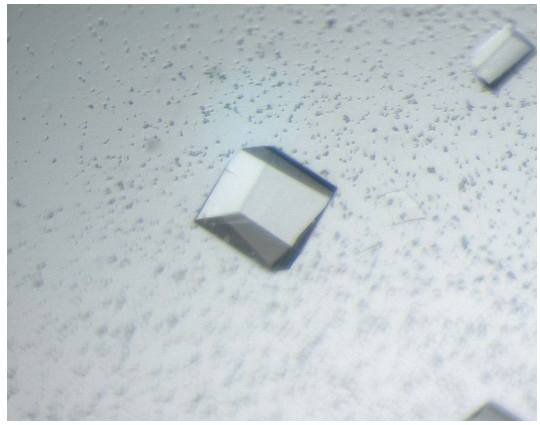


Figure 1
A crystal of RF3 in complex with GDP. The crystal dimensions are 0.3 × 0.3 × 0.15 mm.

Table 1. Structure determination was performed by molecular replacement using the program *Phaser* v.1.3.1 (McCoy *et al.*, 2007) from the *CCP*4 v.6.0.0 software suite, using the coordinates of RF3 from *E. coli* (PDB code 2h5e) as a search model. The results of molecular replacement showed a clear solution and suggested that the crystal contains four monomers per asymmetric unit. Structure analysis and refinement are now in progress.

This work was supported in part by Grants-in-Aid from The GCOE Programs (YH) and The Japanese Aerospace Exploration Agency Project (YH). We are grateful to Drs N. Shimizu, M. Kawamoto and M. Yamamoto at SPring-8 for help during data collection at synchrotron beamline BL41XU. We also thank P. K. R. Kumar and S. Nishikawa at AIST, Tsukuba, Japan for useful discussions and advice.

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