Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic studies of ribosome recycling factor from *Escherichia coli*

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Received 16 August 1999
Accepted 27 October 1999

Ribosome recycling factor (RRF) catalyzes the disassembly of a termination complex during the final stage of protein synthesis. RRF from *Escherichia coli* has been crystallized with PEG 400 as precipitant at 287 K. The crystal belongs to the trigonal space group $P3_121$ (or $P3_221$), with unit-cell parameters $a = b = 48.08$, $c = 141.67$ Å. Native data were collected from a frozen crystal to a resolution of 3.0 Å on a Cu $K\alpha$ rotating-anode X-ray source.

1. Introduction

Ribosome recycling factor (RRF) recycles the components of the termination complex by catalyzing the release of mRNA and tRNA from ribosomes with elongation factor G (EF-G) or release factor 3 (RF-3; Kaji *et al.*, 1998; Grentzmann *et al.*, 1998). RRF is also involved in preventing errors during peptide elongation (Janosi *et al.*, 1996). In *E. coli*, it is found that the absence of RRF causes an increase in translation errors (Janosi *et al.*, 1996) and unscheduled reinitiation (Ryoji *et al.*, 1981; Janosi *et al.*, 1998). Although RRF has a major role in translation and is essential for bacterial growth (Janosi *et al.*, 1994), the molecular mechanism of the RRF action in ribosome recycling and translation-error prevention is not clear. It was proposed that RRF can bind to the A-site of the ribosome and competes for the binding site with release factor 1 (RF-1) in *E. coli* (Pavlov *et al.*, 1997). Considering its binding affinity to the ribosome, it may have the same structural motif as other translation factors which bind to the A-site of the ribosome. The tRNA-mimicry domain postulated in several translation factors might be the structural motif shared between translation factors (Nakamura *et al.*, 1996; Brock *et al.*, 1998).

The gene encoding RRF is widely distributed in prokaryotes with high sequence homology (Janosi *et al.*, 1996). From sequence analyses, several genomic sequences from eukaryotes have been identified as the gene encoding the RRF homologue which appears to be present in organelles (Janosi *et al.*, 1996). However, it is known that the RRF homologue in yeast is not essential for cell growth (Kaji *et al.*, 1998) and spinach RRF homologue in chloroplasts is incompatible with *E. coli* RRF (Rolland *et al.*, 1999). Therefore, eukaryotic RRFs might not be essential for cell viability and inhibition of eukaryotic RRFs present in organelles could not influence the protein synthesis of cytoplasmic proteins. Considering the bactericidal effect of removal of RRF as well as the non-essential role of RRF in eukaryotes, RRF is considered to be a potential target for developing new antibacterial agents (Kaji *et al.*, 1998).

In order to investigate the structural relevance of RRF to other translation factors and to understand the mechanism of ribosome recycling for future application in antibacterial drug development, a structural study is indispensable. As the first step in the structure determination, we have overexpressed, purified and crystallized the *E. coli* RRF.

2. Bacterial expression and purification

The *E. coli* RRF gene was amplified by PCR using *E. coli* genomic DNA as a template. The PCR product was restricted and ligated into an *Nde*I/*Xho*I-restricted pET22b vector (Novagen). The *E. coli* cells B834(DE3) transformed with pET22b–RRF plasmid were grown at 310 K in LB medium containing 50 µg ml^{-1} ampicillin. The *E. coli* RRF was induced with 0.5 m*M* IPTG for 4 h. The cells were harvested at 277 K by centrifugation (5000*g* for 6 min) and resuspended in buffer *A* (20 m*M* Tris–HCl pH 8.0, 10 m*M* $MgCl_2$, 1 m*M* PMSF) following sonication. After centrifugation for 30 min at 15 000*g*, the supernatant was applied onto a 5 ml HiTrap Q column (Pharmacia) equilibrated with buffer *A*. The flowthrough containing RRF was dialyzed against buffer *B* (20 m*M* NaH_2PO_4 pH 6.0, 10 m*M* $MgCl_2$) and loaded onto a 5 ml HiTrap SP column (Pharmacia) equilibrated with buffer *B*. The protein identified in the flowthrough was concentrated by ultrafiltration (Amicon, YM 10) and loaded onto a Superdex 200 gel-filtration column (HiLoad 16/60, Pharmacia) equilibrated with buffer *A* containing 150 m*M* NaCl. The fractions containing RRF were pooled and concentrated for crystallization.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution bin.

Space group	$P3_121$ or $P3_221$
Unit-cell parameters (Å, °)	$a = b = 48.08$, $c = 141.67$; $\alpha = \beta = 90$, $\gamma = 120$
Resolution (Å)	30.0–3.00 (3.16–3.00)
Number of unique reflections	3952 (535)
Redundancy	3.22 (3.19)
$R_{\rm merge}$† (%)	9.5 (36.7)
Completeness (%)	95.7 (95.5)
$I/\sigma(I)$	11.3 (3.7)

† $R_{\rm merge} = \sum |I - \langle I \rangle| / \sum I$.

The purity of RRF was checked by SDS gel electrophoresis.

3. Crystallization

Protein was prepared at a concentration of 100 mg ml^{-1} in buffer *A* containing 150 m*M* NaCl. Initial crystallization conditions were tested using the Hampton Research Crystal Screens I and II, MemFac and detergent screen kits using the hanging-drop vapour-diffusion method with the drops equilibrated against 500 µl of reservoir solution at 287 and 295 K. Several microcrystals were obtained from a few conditions in the detergent screen. Rod-shaped crystals with trigonal faces were grown with polyethyleneglycol (PEG) 400 and 350 monomethyl ether (MME) as precipitants and decyl-β-D-maltopyranoside as a detergent additive (Fig. 1). Further refinement of this condition gave suitably sized crystals for X-ray experiments. The best crystals were obtained from drops prepared by mixing 2 µl of protein solution and 2 µl reservoir solution with an additional 0.4 µl of 18 m*M* detergent solution. Crystals were clustered and grew to dimensions of 0.15 × 0.15 × 1.0 mm within a week under the optimized reservoir conditions 0.1 *M* MES–NaOH (pH 6.5), 10% PEG 350 MME, 12% PEG 400 at 287 K (Fig. 1). The crystals were detached from the cluster and a single crystal was used for data collection.

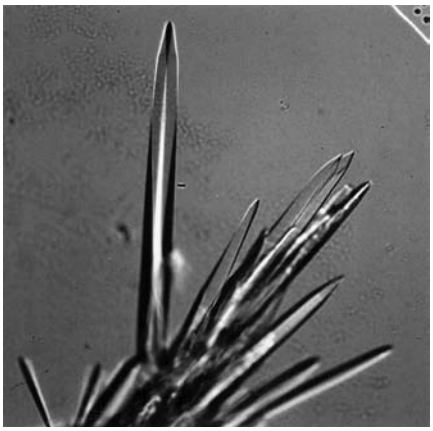

Figure 1
Trigonal crystals of the *E. coli* ribosome recycling factor (RRF) grown in the presence of PEG 400 as precipitant. The approximate dimensions of the largest crystal in the centre of the cluster are 0.15 × 0.15 × 1.0 mm.

4. Data collection and processing

X-ray data were collected on a MacScience 2030b area detector with mirror-focused Cu $K\alpha$ X-rays from a MacScience M18XHF rotating-anode generator operated at 50 kV and 90 m*M*. The crystal mounted in a glass capillary diffracted to a resolution limit of 3.0 Å at room temperature. However, cryo-cooling was required to collect the complete data set, owing to the radiation sensitivity of the crystal.

The concentration of PEG 400 in the reservoir solution was increased in steps of 2% until the reservoir acted as a cryoprotectant. In each step, the crystals in the drop were equilibrated against the new conditions for more than 48 h. Individual crystals equilibrated against 20% PEG 400 were scooped up in cryo-loops and frozen in liquid nitrogen before being mounted on the goniometer in the nitrogen stream at 100 K.

A total of 33 frames of 2° oscillations were measured from a frozen crystal at 100 K using a crystal-to-detector distance of 200 mm. Data were processed and integrated using *DENZO* and scaled using *SCALEPACK* from the *HKL* program suite (Otwinowski & Minor, 1997). The crystals belong to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 48.08$, $c = 141.67$ Å. Assuming a molecular weight of 20 639 Da and one molecule in the asymmetric unit, the value of the crystal-packing parameter V_M is 2.29 Å^3 Da^{-1}, which is within the range commonly observed for protein crystals (Matthews, 1968). A native data set was collected to 3.0 Å with an $R_{\rm merge}$ of 9.5%; data statistics are summarized in Table 1. The crystal structure determination of the *E. coli* RRF by multiple isomorphous replacement is now under way.

We thank M. J. Cho and J.-C. Hong for providing research facilities. This work was supported by grants from Korea Science and Engineering Foundation through the Plant Molecular Biology and Biotechnology Research Center at Gyeongsang National University and Center for Molecular Catalysis at Seoul National University.

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