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Crystallization and preliminary X-ray analysis of *Escherichia coli* RNase G

The homologous RNases RNase E and RNase G are widely distributed in bacteria and function in many important physiological processes, including mRNA degradation, rRNA maturation and so on. In this study, the crystallization and preliminary X-ray analysis of RNase G from *Escherichia coli* is described. Purified recombinant *E. coli* RNase G, which has 497 amino acids, was crystallized in the cubic space group *F*432, with unit-cell parameters $a = b = c = 219.84 \text{ \AA}$. X-ray diffraction data were collected to a resolution of $3.4 \text{ \AA}$.

1. Introduction

Escherichia coli RNase G was previously called CafA protein because of its ability to form cytoplasmic axial filaments when overexpressed. Cytoplasmic axial filaments are a kind of singular intracellular structure and when this structure appears the cell morphology often undergoes a series of transformations, such as the formation of chained cells and minicells. Therefore, it has been inferred that this protein plays a role in the formation of the cytoskeleton and can enhance the rate of cell division and/or inhibit chromosome partitioning after replication (Okada *et al.*, 1994).

A strong resemblance has been identified between CafA and the N-terminal portion of *E. coli* RNase E (McDowall *et al.*, 1993). They share 57% sequence similarity (35% identity) in an overlap of 436 amino acids (Fig. 1). They are both required for the 5' maturation of 16S ribosomal RNA (Wachi *et al.*, 1999; Li *et al.*, 1999) and they even recognize each other's recognition sequence to a certain degree (Wachi *et al.*, 1999). CafA protein has been renamed 'RNase G' because of its ribonucleic activity. RNase G has been recognized to be important in the degradation of *AdhE* and *eno* mRNAs (Umitsuki *et al.*, 2001; Kaga *et al.*, 2002). It was subsequently found that RNase E and other degradosome components are organized into helical filamentous structures that coil around the length of the cell (Taghbalout & Rothfield, 2006). This interesting discovery confirmed that the RNase E/G family participates not only in RNA incision but also in the cytoskeleton.

The homologous RNases E and G are widely distributed in bacteria, but only the β and γ subdivisions of the Proteobacteria express both RNase E and RNase G. In most cases, a bacterial chromosome encodes only a single RNase E/G-family member which is similar to either RNase G or RNase E (Condon & Putzer, 2002).

In this large family, only the crystal structure of *E. coli* RNase E has been determined ($2.9 \text{ \AA}$ resolution; Callaghan *et al.*, 2005). To learn more about the molecular structure of this family and to compare the efficiencies of the RNase E and RNase G catalytic capacities, RNase G was crystallized in order to determine its molecular structure by X-ray analysis. Here, we describe the crystallization and preliminary X-ray analysis of *E. coli* RNase G.

2. Materials and methods

2.1. Protein expression and purification

The complete cDNA encoding RNase G (accession No. NP_417713) was amplified by PCR from the genomic DNA of *E. coli*

<i>RNase G</i>	5	LLVNVTPSET-RVAYIDGGILQEIHIEREARRGIVGNIYKGRVSRVLPQMGAFFVDIGLD	63
		+L+N T E RVA +DG L ++ IE	
<i>RNase E</i>	4	MLINATQQEELRVALVDGQRLYDLDDIESPGHEQKKANIYKGIKTRIEPSLEAAAFVDYGAE	63
<i>RNase G</i>	64	KAAPLHASDI----MPHTECVAGEEQKQFTVRDISELVRQGGDLMVQVVKDPLGTGKARL	119
		+ FL +I P G +I +++R+GQ+++VQ+ K+ G KGA L	
<i>RNase E</i>	64	RHGFLPLKEIAREYFPANYSAGRPF-----NIKDVLRGQEVIVQIDKEERGNKGAAL	116
<i>RNase G</i>	120	TTDITLPSRYLVFMPGASHVG-VSQRIESESERERLKKVVAEYCDEQG-GFIIRTAAGV	177
		TT I+L YLV MP G +S+RIE + +R LK+ +A +G G I+RTA G	
<i>RNase E</i>	117	TTFISLAGSYLVMPNPNPRAGGISRRIEGD-DRTELKEALASLELPEGMGLIVRTAGVGK	175
<i>RNase G</i>	178	GEAELASDAAYLKRVWTKVMERKKRPQTRYQLYGELALAQVLRDFADAELDRIRVDSRL	237
		L D ++ + W + + + + ++ E + R RD+ ++ I +D+	
<i>RNase E</i>	176	SAEALQWDLDFRLKHWEAIIKKAESRPAPFLIHQESNVIVRAFRDYLRQDIGELIDN--	233
<i>RNase G</i>	238	TYEALLEFTSEYI-----PMTSKLEHYTGROPIFDLFDVENEIQRALERKVELKSGGYL	292
		+LE ++I P+ +SK++ YTG P+F +E++I+ A +R+V L SGG +	
<i>RNase E</i>	234	--PKVLELARQHIAALGRPDFSSIKLYTGEIPLFSHYQIESQIESAFQREVRLPSSGSI	291
<i>RNase G</i>	293	IIDQTEAMTTVDINTGAFVGHRLDDTIFNTNIEATQAIARQLRLRLGGIIIDFIDMN	352
		+ID TEA+T +DIN+ +++++T FNTN+EA IARQLRLRL+LGG+I+IDFIDM	
<i>RNase E</i>	292	VIDSTEALTAIDINSARATRGGDIETAFNTNLEAADEIARQLRLRLDGLGIVDFIDMT	351
<i>RNase G</i>	353	NEDHRRRLVLSLEQALS KDRVKTSVNGFSALGLVEMTRKRTRESIEHVLNCECPTCHGRG	412
		H+R V + L +A+ +DR + ++ S GL+EM+R+R S+ + CP C G G	
<i>RNase E</i>	352	PVRHQRAVENRLREAVRQDRARIQISHISRFGLLEMSRQLSPSLGESSHHVCPRCSTG	411
<i>RNase G</i>	413	TVKTVETVCYEIMREI	428
		TV+ E++ I+R I	
<i>RNase E</i>	412	TVRDNESLSLSILRLI	427

Figure 1

Sequence alignment of *E. coli* RNase G and RNase E using the BLASTP suite of NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

strain K12. This amplified DNA fragment was then cloned into pET22b (Novagen) that had been digested with *Nde*I and *Xho*I to generate plasmid pRNG. As a consequence of these manipulations, the RNase G enzyme encoded by pRNG contains an -LEHHHHHH tag at its C-terminus. The G→A mutation, which causes an Arg206His amino-acid substitution in the RNase G protein, was verified by DNA sequencing (Sangon). pRNG was subsequently transformed into *E. coli* BL21 (DE3) to produce strain sRNG. Cultures of sRNG were grown at 310 K in LB broth containing 100 µg ml⁻¹ ampicillin to an optical density (OD₆₀₀) of 0.8–1.0 and then overnight at 298 K in the presence of 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG).

The cells were harvested at 3910g, resuspended in 20 mM Tris-HCl pH 8.3 and 500 mM NaCl and lysed in a French pressure-cell press (Thermo) at 82.7 MPa. The insoluble material was removed by centrifugation at 25 500g and the resulting supernatant was passed through an appropriate volume of Ni Sepharose High Performance (Amersham Biosciences). The column was washed with six column volumes of 20 mM Tris-HCl pH 8.3, 500 mM NaCl and 25 mM

imidazole. The RNase G was eluted with 20 mM Tris-HCl pH 8.3 and 500 mM imidazole. This elution fraction was further purified with Q High Performance (Amersham Biosciences); the RNase G was eluted with a 40–600 mM NaCl gradient in 20 mM Tris-HCl pH 8.3. Sodium dodecyl sulfate–polacrylamide gel electrophoresis (SDS–PAGE) was performed to examine the purified proteins at each step.

2.2. Crystallization

Purified RNase G was concentrated to about 15 mg ml⁻¹ in distilled water using an Amicon ultrafilter (Millipore) before the crystallization trials. Crystallization experiments were performed at 298 K using the hanging-drop vapour-diffusion method. The initial crystallization conditions were screened using the commercially available Crystal Screens I and II (Hampton Research) with drops formed by mixing 6 µl protein solution and 1 µl precipitant solution. Crystals of RNase G suitable for X-ray diffraction experiments were grown from drops containing 1.2 M magnesium sulfate and 0.1 M MES pH 6.5 (Fig. 2).

2.3. Data collection

A RNase G crystal was mounted in a nylon loop and then transferred to cryoprotectant solution composed of 1.2 M magnesium sulfate and 0.1 M MES pH 6.5 supplemented with 25% glycerol before being flash-frozen in liquid nitrogen at 100 K. The reflection data were collected using a rotating-anode X-ray source (Cu Kα; Rigaku Micro007) and an imaging plate (MAR Research dtb345), with a crystal-to-detector distance of 250 mm. The diffraction data were processed using the CCP4 software suite. The crystal belonged to space group *F*432, with unit-cell parameters *a* = *b* = *c* = 219.84 Å, and diffracted to a resolution of 3.4 Å. Assuming the presence of one protein molecule per asymmetric unit, the Matthews coefficient is 1.98 Å³ Da⁻¹, corresponding to a solvent content of 37.80%. Statistical data are given in Table 1.

Figure 2

Crystal of RNase G protein. The crystal dimensions are about 0.2 × 0.2 × 0.2 mm.

Table 1

Statistical data for the RNase G crystal.

Values in parentheses are for the highest resolution shell.

Resolution (Å)	40–3.4 (3.58–3.40)
Completeness (%)	99.5 (100.0)
$R_{\text{merge}}^{\dagger}$	0.146 (0.320)
Mean $I/\sigma(I)$	12.7 (5.5)
Space group	$F432$
Unit-cell parameters (Å)	$a = b = c = 219.84$
No. of observations	39999 (5699)
Unique reflections	6648 (952)
Molecules per ASU	1
V_M (Å ³ Da ^{−1})	1.98
Solvent content (%)	37.80

$^{\dagger} R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed reflection intensity and $\langle I(hkl) \rangle$ is the mean intensity of the reflection.

3. Results and discussion

RNase G migrated as a major band with a molecular weight of 56 kDa on SDS–PAGE together with a minor band with a molecular weight of 112 kDa. If 2-mercaptoethanol was added to the sample before electrophoresis the minor band disappeared, indicating that RNase G can form a dimer *via* disulfide bonds. Because these two forms are in equilibrium (Briant *et al.*, 2003), we did not add any 2-mercaptoethanol to the protein solution during the crystallization experiments. The crystals were in fact smaller when 2-mercaptoethanol was added.

It was difficult to determine the structure of RNase G by directly using the structure of RNase E (PDB code 2bx2; Callaghan *et al.*, 2005) as a molecular-replacement model. Optimization of the model is still in progress. We are also attempting to use the selenomethio-

nine single-wavelength anomalous dispersion (SAD) method, but the selenomethionine-derivative crystals that we have produced so far are too small to allow the collection of diffraction data.

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References

- Briant, D. J., Hankins, J. S., Cook, M. A. & Mackie, G. A. (2003). *Mol. Microbiol.* **50**, 1381–1390.
- Callaghan, A. J., Marcaida, M. J., Stead, J. A., McDowall, K. J., Scott, W. G. & Luisi, B. F. (2005). *Nature (London)*, **437**, 1187–1191.
- Condon, C. & Putzer, H. (2002). *Nucleic Acids Res.* **30**, 5339–5346.
- Kaga, N., Umitsuki, G., Nagai, K. & Wachi, M. (2002). *Biosci. Biotechnol. Biochem.* **66**, 2216–2220.
- Li, Z., Pandit, S. & Deutscher, M. P. (1999). *EMBO J.* **18**, 2878–2885.
- McDowall, K. J., Hernandez, R. G., Lin-Chao, S. & Cohen, S. N. (1993). *J. Bacteriol.* **175**, 4245–4249.
- Okada, Y., Wachi, M., Hirata, A., Suzuki, K., Nagai, K. & Matsushashi, M. (1994). *J. Bacteriol.* **176**, 917–922.
- Taghbalout, A. & Rothfield, L. (2006). *Proc. Natl Acad. Sci. USA*, **104**, 1667–1672.
- Umitsuki, G., Wachi, M., Takada, A., Hikichi, T. & Nagai, K. (2001). *Genes Cells*, **6**, 403–410.
- Wachi, M., Umitsuki, G., Shimizu, M., Takada, A. & Nagai, K. (1999). *Biochem. Biophys. Res. Commun.* **259**, 483–488.