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

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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 $a = b = c = 219.84$ Å. X-ray diffraction data were collected to a resolution of 3.4 Å.

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 Å 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*

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RNase G  5    LLVNVTPSET-RVAYIDGGILQEIHIEREARRGIVGNIYKGRVSRVLPGMQAAFVDIGLD  63
              +L+N T  E  RVA +DG  L ++ IE         NIYKG+++R+ P ++AAFVD G +
RNase E  4    MLINATQQEELRVALVDGQRLYDLDIESPGHEQKKANIYKGKITRIEPSLEAAFVDYGAE  63

RNase G  64   KAAFLHASDI----MPHTECVAGEEQKQFTVRDISELVRQGQDLMVQVVKDPLGTKGARL  119
              +  FL   +I     P      G          +I +++R+GQ+++VQ+ K+  G KGA L
RNase E  64   RHGFLPLKEIAREYFPANYSAHGRP-------NIKDVLREGQEVIVQIDKEERGNKGAAL  116

RNase G  120  TTDITLPSRYLVFMPGASHVG-VSQRIESESERERLKKVVAEYCDEQG-GFIIRTAAEGV  177
              TT I+L   YLV MP     G +S+RIE + +R  LK+ +A     +G G I+RTA  G
RNase E  117  TTFISLAGSYLVLMPNNPRAGGISRRIEGD-DRTELKEALASLELPEGMGLIVRTAGVGK  175

RNase G  178  GEAELASDAAYLKRVWTKVMERKKRPQTRYQLYGELALAQRVLRDFADAELDRIRVDSRL  237
                 L  D ++  + W  + +  +     + ++ E  +  R  RD+   ++  I +D+
RNase E  176  SAEALQWDLSFRLKHWEAIKKAAESRPAPFLIHQESNVIVRAFRDYLRQDIGEILIDN--  233

RNase G  238  TYEALLEFTSEYI-----PEMTSKLEHYTGRQPIFDLFDVENEIQRALERKVELKSGGYL  292
                 +LE   ++I     P+ +SK++ YTG  P+F  + +E++I+ A +R+V L SGG +
RNase E  234  --PKVLELARQHIAALGRPDFSSKIKLYTGEIPLFSHYQIESQIESAFQREVRLPSGGSI  291

RNase G  293  IIDQTEAMTTVDINTGAFVGHRNLDDTIFNTNIEATQAIARQLRLRNLGGIIIIDFIDMN  352
              +ID TEA+T +DIN+       ++++T FNTN+EA   IARQLRLR+LGG+I+IDFIDM
RNase E  292  VIDSTEALTAIDINSARATRGGDIEETAFNTNLEAADEIARQLRLRDLGGLIVIDFIDMT  351

RNase G  353  NEDHRRRVLHSLEQALSKDRVKTSVNGFSALGLVEMTRKRTRESIEHVLCNECPTCHGRG  412
                 H+R V + L +A+ +DR +  ++  S  GL+EM+R+R   S+     + CP C G G
RNase E  352  PVRHQRAVENRLREAVRQDRARIQISHISRFGLLEMSRQRLSPSLGESSHHVCPRCSGTG  411

RNase G  413  TVKTVETVCYEIMREI  428
              TV+  E++   I+R I
RNase E  412  TVRDNESLSLSILRLI  427
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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 (OD_{600}) of 0.8–1.0 and then overnight at 298 K in the presence of 0.1 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG).

The cells were harvested at 3910*g*, resuspended in 20 m*M* Tris–HCl pH 8.3 and 500 m*M* NaCl and lysed in a French pressure-cell press (Thermo) at 82.7 MPa. The insoluble material was removed by centrifugation at 25 500*g* 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 m*M* Tris–HCl pH 8.3, 500 m*M* NaCl and 25 m*M* imidazole. The RNase G was eluted with 20 m*M* Tris–HCl pH 8.3 and 500 m*M* imidazole. This elution fraction was further purified with Q High Performance (Amersham Biosciences); the RNase G was eluted with a 40–600 m*M* NaCl gradient in 20 m*M* Tris–HCl pH 8.3. Sodium dodecyl sulfate–polacrylamide gel electrophoresis (SDS–PAGE) was performed to examine the purified proteins at each step.

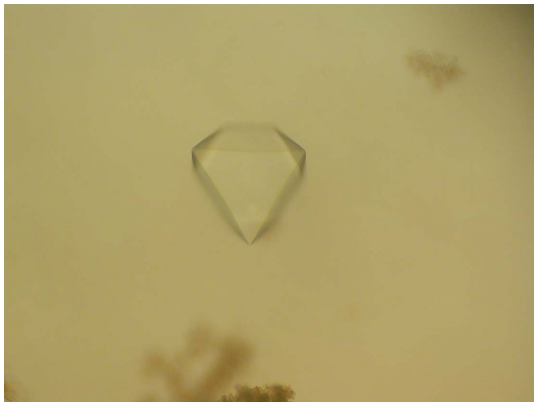

Figure 2
Crystal of RNase G protein. The crystal dimensions are about 0.2 × 0.2 × 0.2 mm.

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 $K\alpha$; 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 *CCP*4 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 Å^3 Da^{-1}, corresponding to a solvent content of 37.80%. Statistical data are given in Table 1.

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)
$R_{\rm merge}$†	0.146 (0.320)
Mean $I/\sigma(I)$	12.7 (5.5)
Space group	*F*432
Unit-cell parameters (Å)	$a = b = c = 219.84$
No. of observations	39999 (5699)
Unique reflections	6648 (952)
Molecules per ASU	1
$V_{\rm M}$ (Å^3 Da^{-1})	1.98
Solvent content (%)	37.80

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed 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 selenomethionine 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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