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Crystallization and preliminary X-ray diffraction studies of NusG, a protein shared by the transcription and translation machines

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N-utilization factor G (NusG) from *Aquifex aeolicus* (*Aa*) was overexpressed in *Escherichia coli*, purified and crystallized using the hanging-drop vapor-diffusion technique. The drops consisted of 2.5 µl protein solution (~30 mg ml^{-1} in 20 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl, 2 m*M* EDTA and 10 m*M* DTT) and 2.5 µl reservoir solution (0.085 *M* Na HEPES pH 7.5, 15% glycerol, 11% 2-propanol and 20% PEG 4000) derived from condition number 41 of the Hampton Cryo Screen. The crystals grew at 291 ± 1 K and reached dimensions of 0.2 × 0.1 × 0.05 mm in 5–7 d. The crystals, which diffracted to 2.45 Å resolution, belonged to space group $C222_1$, with unit-cell parameters $a = 65.95$, $b = 124.58$, $c = 83.60$ Å. One *Aa*NusG molecule is present in the asymmetric unit, corresponding to a solvent content of 59.80% (Matthews coefficient = 3.06 Å^3 Da^{-1}). Crystal structure determination is in progress.

1. Introduction

The involvement of one protein in two crucial cellular activities is an indication of the importance of that protein. N-utilization factor G (NusG) is an essential protein in *Escherichia coli* and participates in both transcription and translation, the two major activities leading to gene expression (Squires & Zaporojets, 2000). On one hand, NusG is a regulator of Rho-dependent transcription termination (Burns & Richardson, 1995; Burova & Gottesman, 1995; Burova *et al.*, 1995; Burns *et al.*, 1998, 1999; Pasman & von Hippel, 2000), phage HK022 Nun termination (Sullivan & Gottesman, 1992; Burova *et al.*, 1999) and rRNA transcription antitermination (Downing *et al.*, 1990; Li *et al.*, 1992, 1993; Sullivan & Gottesman, 1992; Sullivan *et al.*, 1992; Kyrpides *et al.*, 1996; Zellars & Squires, 1999; Squires & Zaporojets, 2000). On the other hand, it shares a KOW motif, a 27-residue sequence, with ribosomal protein L24 (Kyrpides *et al.*, 1996) and may play a role in translation (Zellars & Squires, 1999; Squires & Zaporojets, 2000). To date, there is no evidence that any bacterial NusG forms oligomers in solution.

NusG is present in all prokaryotes and its homolog proteins have been identified in yeast (Hartzog *et al.*, 1998) and humans (Wada *et al.*, 1998). To understand the roles of NusG in transcription and translation, a three-dimensional structure is essential. NusG from *E. coli* (*Ec*NusG; SWISS-PROT P16921) contains 181 amino-acid residues (molecular weight 20.5 kDa). However, we were not able to crystallize *Ec*NusG. The NusG protein from *Aquifex aeolicus* (*Aa*NusG; SWISS-PROT O67757) contains 248 residues (molecular weight 28.0 kDa) with an ~70-residue insertion that is not present in *Ec*NusG. Here, we report the expression, purification, crystallization and preliminary X-ray diffraction studies of *Aa*NusG.

2. Materials and methods

2.1. Cloning and expression

The expression vector was constructed by polymerase chain reaction (PCR) amplification of the open reading frame (ORF) encoding *Aa*NusG from pKM631 (Kapust *et al.*, 2002). The *Aa*NusG ORF was moved from pKM699 by recombinational cloning into the destination vector pKM596 (Fox & Waugh, 2002) to create pKM702. With pKM702, *Aa*NusG was overproduced in *E. coli* BL21(DE3) cells as a fusion to the C-terminus of *E. coli* maltose-binding protein (MBP). Because the majority of the fusion protein failed to bind to amylose resin, it was cleaved *in vivo* by TEV protease (Kapust & Waugh, 2000) to separate the *Aa*NusG from MBP.

The cells were lysed by homogenization. The cell lysate was treated with polyethyleneimine (PEI) and centrifuged at 277 K. After being dialyzed with 1 µg ml^{-1} benzonase (Merck catalog No. 1695), the crude extract was heated at 343 K for 20 min, cooled on ice for 10 min and centrifuged at 277 K for 10 min. The supernatant was filtered and dialyzed overnight in 20 m*M* sodium citrate pH 5.5 and 25 m*M* sodium chloride at 277 K.

2.2. Purification and crystallization

A BioCAD Sprint workstation (Applied Biosystems) was used to carry out the chromatographic purification in three steps: cation-exchange chromatography, hydrophobic interaction chromatography and gel-filtration chromatography. A stirred-cell concentration unit (Amicon) was used to concentrate the protein to $\sim$30 mg ml^{-1}.

Crystals were obtained by the hanging-drop vapor-diffusion method. The drops consisted of 2.5 µl protein solution ($\sim$30 mg ml^{-1} in 20 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl, 2 m*M* EDTA and 10 m*M* DTT) and 2.5 µl reservoir solution (0.085 *M* Na HEPES pH 7.5, 15% glycerol, 11% 2-propanol and 20% PEG 4000) derived from condition No. 41 of the Hampton Cryo Screen. The drops were equilibrated against 500 µl reservoir solution. The crystals grew at 291 $\pm$ 1 K and reached dimensions of 0.2 $\times$ 0.1 $\times$ 0.05 mm in 5–7 d.

2.3. Data collection and processing

The X-ray diffraction data of the *Aa*NusG crystal were collected from a single crystal using a MAR345 image-plate system mounted on a Rigaku rotating-anode generator (50 kV, 100 mA) with Cu $K\alpha$ radiation. The cryoprotectant contained 0.5 *M* NaCl, 6% propanol, 12% PEG 4000 and 15% glycerol. The crystal was flash-frozen and maintained at 100 K with an Oxford cryosystem during data collection. The oscillation range was 1.0° and the crystal-to-detector distance was 170 mm.

The crystal of *Aa*NusG, which diffracted to 2.45 Å resolution, belonged to space group $C222_1$, with unit-cell parameters a = 65.95, b = 124.58, c = 83.60 Å. One *Aa*NusG molecule is present in the asymmetric unit, corresponding to a solvent content of 59.80% (Matthews coefficient 3.06 Å^3 Da^{-1}). The raw data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). Crystal structure determination is in progress.

Table 1
Data statistics for *Aa*NusG crystals in space group $C222_1$.

Values in parentheses are for the last resolution shell (3.73–3.60 Å for the His-tagged crystal and 2.54–2.45 Å for the non-His-tagged crystal, respectively).

	His-tagged	Non-His-tagged
Unit-cell parameters (Å)		
a	69.50	65.95
b	130.21	124.58
c	80.43	83.60
Unit-cell volume (Å^3)	727858.93	686861.86
Resolution range (Å)	40.00–3.60	40.00–2.45
Total unique reflections	4413	12763
Reflections with $I \geq 2\sigma(I)$	3486	10388
Redundancy	3.47	4.36
Completeness (%)	99.0 (96.4)	98.2 (96.7)
$I/\sigma(I)$	21.65 (2.54)	33.99 (3.75)
$R_{\rm scaling}$†	0.054 (0.455)	0.053 (0.415)

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

3. Results and discussion

*Aa*NusG was first produced with MBP and a His tag fused to its N- and C-termini, respectively (Kapust *et al.*, 2002). The protein was purified in four steps: affinity chromatography with an Ni–NTA column (Qiagen), affinity chromatography with amylose resin (New England Biolabs), cleavage of the fusion protein by TEV protease and affinity chromatography with amylose resin. The protein was concentrated using Amicon stirred cells to $\sim$30 mg ml^{-1}. Crystals of the His-tagged *Aa*NusG protein were also grown by the hanging-drop vapor-diffusion method under different conditions (40% ammonium sulfate, 25 m*M* imidazole pH 8.0 and 5% MPD) at 277 K. Although the His-tagged crystals had similar morphology to non-His-tagged crystals, they failed to diffract to better than 3.6 Å.

The *Aa*NusG protein was then cloned without the His tag, purified and crystallized as described above. These crystals diffracted to 2.45 Å. The X-ray data statistics for both His-tagged and non-His-tagged crystals are summarized in Table 1. The unit cell of the non-His-tagged crystal is smaller than that of the His-tagged crystals by $\sim$6% (Table 1). The reason for the different behavior of His-tagged and non-His-tagged proteins may become clearer when the structure is solved.

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References

Burns, C. M., Nowatzke, W. L. & Richardson, J. P. (1999). *J. Biol. Chem.* **274**, 5245–5251.
Burns, C. M. & Richardson, J. P. (1995). *Proc. Natl Acad. Sci. USA*, **92**, 4738–4742.
Burns, C. M., Richardson, L. V. & Richardson, J. P. (1998). *J. Mol. Biol.* **278**, 307–316.
Burova, E. & Gottesman, M. E. (1995). *Mol. Microbiol.* **17**, 633–641.
Burova, E., Hung, S. C., Chen, J., Court, D. L., Zhou, J. G., Mogilnitskiy, G. & Gottesman, M. E. (1999). *Mol. Microbiol.* **31**, 1783–1793.
Burova, E., Hung, S. C., Sagitov, V., Stitt, B. L. & Gottesman, M. E. (1995). *J. Bacteriol.* **177**, 1388–1392.
Downing, W. L., Sullivan, S. L., Gottesman, M. E. & Dennis, P. P. (1990). *J. Bacteriol.* **172**, 1621–1627.
Fox, J. D. & Waugh, D. S. (2002). In the press.
Hartzog, G. A., Wada, T., Handa, H. & Winston, F. (1998). *Genes Dev.* **12**, 357–369.
Kapust, R. B., Tözsér, J., Copeland, T. D. & Waugh, D. S. (2002). *Biochem. Biophys. Res. Commun.* **294**, 949–955.
Kapust, R. B. & Waugh, D. S. (2000). *Protein Expr. Purif.* **19**, 312–318.
Kyrpides, N. C., Woese, C. R. & Ouzounis, C. A. (1996). *Trends Biochem. Sci.* **21**, 425–426.
Li, J., Horwitz, R., McCracken, S. & Greenblatt, J. (1992). *J. Biol. Chem.* **267**, 6012–6019.
Li, J., Mason, S. W. & Greenblatt, J. (1993). *Genes Dev.* **7**, 161–172.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pasman, Z. & von Hippel, P. H. (2000). *Biochemistry*, **39**, 5573–5585.
Squires, C. L. & Zaporojets, D. (2000). *Annu. Rev. Microbiol.* **54**, 775–798.
Sullivan, S. L. & Gottesman, M. E. (1992). *Cell*, **68**, 989–994.
Sullivan, S. L., Ward, D. F. & Gottesman, M. E. (1992). *J. Bacteriol.* **174**, 1339–1344.
Wada, T., Takagi, T., Yamaguchi, Y., Ferdous, A., Imai, T., Hirose, S., Sugimoto, S., Yano, K., Hartzog, G. A., Winston, F., Buratowski, S. & Handa, H. (1998). *Genes Dev.* **12**, 343–356.
Zellars, M. & Squires, C. L. (1999). *Mol. Microbiol.* **32**, 1296–1304.