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Crystallization and preliminary X-ray crystallographic analyses of Nur, a nickel-responsive transcription regulator from *Streptomyces coelicolor*

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Nickel ions serve in the correct folding and function of microbial enzymes implicated in metabolic processes. Although nickel ions are indispensable for the survival of cells, the intracellular level of nickel ions needs to be properly maintained as excessive levels of nickel ions are toxic. Nur, a nickel-uptake regulator belonging to the Fur family, is a nickel-responsive transcription factor that controls nickel homeostasis and antioxidative response in *Streptomyces coelicolor*. Nur was purified and crystallized at 295 K. A 2.4 Å native data set and a 3.0 Å Ni-MAD data set were collected using synchrotron radiation. The Nur crystals belong to space group $P3_1$, with unit-cell parameters $a = b = 78.17$, $c = 50.39$ Å. Assuming the presence of two molecules in the asymmetric unit, the solvent content is estimated to be about 54.7%.

1. Introduction

Nickel ions serve as an essential cofactor for several metabolic enzymes to maintain their structure and function. To date, nine enzymes are known to contain nickel ions: urease, hydrogenase, carbon monoxide dehydrogenase, acetyl-CoA decarbonylase/synthase, methyl-CoM reductase, glyoxylase I, acireductone dioxygenase, methylenediurease and superoxide dismutase (Mulrooney & Hausinger, 2003). Since accumulation of nickel ions is harmful to cells (Stohs & Bagchi, 1995), the intracellular level of nickel ions is tightly regulated. Nickel uptake through the nickel-transporter system controls the intracellular concentration of nickel ions (Navarro *et al.*, 1993). Thus, transcriptional repression of the transporter system is a prevalent mechanism to maintain nickel homeostasis when the *in vivo* nickel level becomes high. In *Escherichia coli*, for example, NikR serves as a nickel-responsive transcription factor that represses the transcription of the nickel-specific ABC transporter complex in the presence of excess nickel ions (De Pina *et al.*, 1999). Recently, we have identified a nickel-uptake regulator (Nur) in *Streptomyces coelicolor* that regulates nickel homeostasis (Ahn *et al.*, 2006). In the presence of nickel ions, nickel-bound Nur (Ni-Nur) represses the expression of a nickel-transporter gene cluster. This indicates that when nickel ions are overaccumulated, Ni-Nur allows cells to maintain nickel homeostasis by limiting nickel uptake.

Nur is composed of 145 amino acids and belongs to the Fur (ferric uptake regulator) family, the members of which are responsible for metal-responsive regulation of genes at the level of transcription in prokaryotes (Ratledge & Dover, 2000). The Fur-family regulators appear to be activated by metal binding. Only metal-bound forms of the family members form tight complexes with the promoter regions of their target genes, thus repressing their expression (Lee & Helmann, 2007). To date, structures are known of three Fur-family members: Fur from *Pseudomonas aeruginosa* (Pohl *et al.*, 2003), PerR from *Bacillus subtilis* (Traore *et al.*, 2006) and FurB from *Mycobacterium tuberculosis* (Lucarelli *et al.*, 2007). These three proteins have different physiological functions: Fur and FurB are involved in the control of iron and zinc homeostasis (Lucarelli *et al.*, 2007; Ratledge & Dover, 2000), respectively, and PerR is a peroxide sensor that regulates inducible peroxide-defence genes (Lee & Helmann, 2006; Mongkolsuk & Helmann, 2002). According to their crystal structures, the Fur-family members are homodimeric proteins that share the same topology. However, the local structures of metal-

binding sites and their dimeric conformations differ significantly from one another, making it difficult to interpret the Ni-Nur structural information based on the known structures of other Fur-family members. Here, we report the overexpression, crystallization and preliminary X-ray crystallographic analyses of Ni-Nur as a first step towards structure determination.

2. Materials and methods

2.1. Expression and purification of Nur

To express Nur in a soluble form, the *nur* gene was amplified by the polymerase chain reaction using the mutagenic forward primer 5′-GAC TCG T**CA TAT G**GT GAG CAC CGA-3′ (*Nde*I site in bold) and the reverse primer 5′-CAT AGC C**GG ATC C**TA CGA CTC GCT -3′ (*Bam*HI site in bold). The amplified gene was digested with *Nde*I and *Bam*HI and inserted downstream of the T7 promoter of the expression plasmid pET-3a (Novagen, Wisconsin, USA). The resulting construct expresses residues 1–145 of the Nur protein without additional residues. After verifying the DNA sequence, plasmid DNA was transformed into *E. coli* strain BL21 (DE3) for the overexpression of Nur.

The transformed cells were grown to an OD_{600} of 0.5 in Luria–Bertani medium (Merck) containing 0.1 mg ml^{-1} ampicillin and 0.034 mg ml^{-1} chloramphenicol at 310 K and expression of Nur was induced with 1 m*M* isopropyl β-D-1-thiogalactopyranoside. After 6 h induction at 303 K, the cells were harvested, resuspended in ice-cold 10 m*M* Tris–HCl pH 7.9 and disrupted by sonication. The crude lysate was centrifuged at 20 000*g* for 60 min at 277 K and the supernatant was loaded onto a nickel–nitrilotriacetic acid (Ni–NTA) column (Qiagen). The column was washed with a washing buffer containing 10 m*M* Tris–HCl pH 7.9 and 20 m*M* imidazole. Nur was eluted with the same buffer containing 200 m*M* imidazole. Although Nur was not expressed with a histidine tag, it did bind to the Ni–NTA column. In *E. coli*, nickel is a trace metal ion (Finney & O'Halloran, 2003) and thus the recombinant Nur appears not to contain nickel ions despite its intrinsic affinity for them. The empty nickel-binding sites explain why Nur expressed without a histidine tag is able to bind to the Ni–NTA column. Nur was further purified on a Superdex 75 HR 16/60 column (Amersham Biosciences) pre-equilibrated with 10 m*M* Tris–HCl pH 7.9. The purified Nur in 10 m*M* Tris–HCl pH 7.9 was then concentrated to ∼12 mg ml^{-1} for crystallization trials.

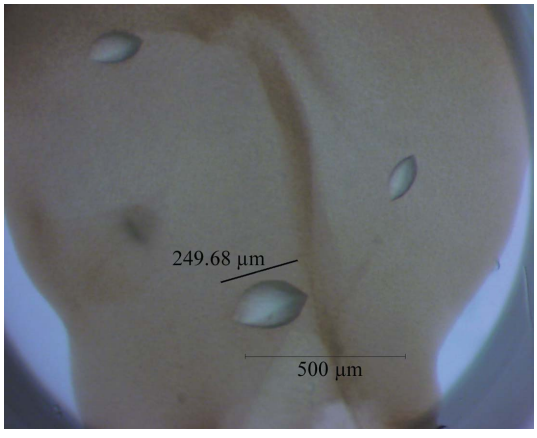

Figure 1
Crystals of Nur from *S. coelicolor*. Crystal dimensions are about 0.25 × 0.2 × 0.1 mm.

Table 1
Crystal information and data-collection statistics.

Values in parentheses are for the outer shell.

Data set	Peak	Inflection	Remote	Native
Space group	$P3_1$			
Unit-cell parameters (Å)	$a = b = 78.17$, $c = 50.39$			
Wavelength (Å)	1.48473	1.48578	1.45101	1.1271
Resolution (Å)	20–3.0	20–3.0	20–3.0	30–2.4
Completeness (%)	97.2 (86.3)	97 (85.3)	96.5 (83.3)	89.9 (70.9)
$R_{\rm merge}$† (%)	5.1 (13.6)	4.4 (12.8)	4.4 (13.7)	3.8 (35.4)
Average $I/\sigma(I)$	55.4 (6.4)	54.8 (6.5)	57.2 (6.3)	25.4 (1.5)
Unique reflections	156854	156388	156444	153163
Average redundancy	6.4 (3.7)	6.4 (3.6)	6.5 (3.6)	3.1 (1.7)
Mosaicity (°)	0.802	0.800	0.802	0.518
Figure of merit	0.64			

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

2.2. Microbatch crystallization and X-ray data collection

The same batch crystallization method was used for crystal screening and optimization at 295 K. Small drops composed of 1 µl protein solution and an equal volume of crystallization reagent were pipetted under a layer of a 1:1 mixture of silicon oil and paraffin oil in 72-well HLA plates (Nunc). Initial crystallization conditions were tested using all available screening kits from Hampton Research and Emerald Biostructures Inc. Microcrystals of Nur were grown using a precipitant containing 10%(*w*/*v*) polyethylene glycol 6000 (PEG 6K), 5%(*v*/*v*) MPD, 0.1 *M* HEPES pH 7.5 (condition No. 30 of Crystal Screen 2 from Hampton Research). The crystallization condition was then optimized to 5% PEG 6K, 5% MPD, 0.1 *M* Tris–HCl pH 8.0 and 0.6 m*M* $NiCl_2$, which produces larger single crystals suitable for data collection (Fig. 1).

Crystals were maintained at ∼100 K during data collection in order to minimize radiation damage. The addition of a cryoprotectant to the crystallization solution is a general method of maintaining crystals at low temperatures without ice formation and crystal damage. In the case of Nur, however, all the cryoprotectants tested including MPD caused severe crystal cracking when they were added to the crystallization solution. We therefore dehydrated Nur crystals in order to reduce their fragility. For dehydration, we transferred crystals in a crystallization drop to a 5 µl drop of the crystallization solution on a cover slip on the bench; this was then allowed to evaporate for 20 min at 295 K. The dehydrated crystals were used for data collection after a brief soak in a cryoprotectant solution consisting of 15% PEG 6K, 10% MPD, 0.1 *M* Tris–HCl pH 8.0. Native data were collected at 2.4 Å resolution using an ADSC Quantum 210 CCD at beamline NW12A of the Photon Factory, Japan (Table 1). Diffraction data were processed using *DENZO* and scaled using *SCALEPACK* from the *HKL*-2000 program suite (Otwinowski & Minor, 1997).

3. Results and discussion

Crystals of Nur belong to the trigonal space group $P3_1$ or $P3_2$, with unit-cell parameters $a = b = 78.17$, $c = 50.39$ Å. The calculated crystal volume per unit molecular weight ($V_{\rm M}$) is 2.7 Å^3 Da^{-1} with a solvent content of 54.7% by volume (Matthews, 1968) when the unit cell is assumed to contain six molecules. This corresponds to the presence of two molecules per asymmetric unit. The data-collection statistics are summarized in Table 1.

Molecular replacement was attempted with *MOLREP* (Vagin & Teplyakov, 2000) and *CNS* (Brünger *et al.*, 1998) to solve the crystal structure of Nur using the structure of Fur (PDB code 1mzb), PerR (PDB code 2fe3) or FurB (PDB code 2o03) as a search model.

However, all trials resulted in failure, pointing out that the Ni-responsive Nur would have different metal-binding sites and dimeric conformation compared with Zn-responsive or Fe-responsive Fur-family members. As an alternative, therefore, we decided to exploit the anomalous scattering from nickel ions in Nur for phase-angle determination. The high affinity of Nur for nickel ions and the existence of nickel ions in the optimized crystallization condition convinced us that our crystals are composed of Ni-Nur protein. Thus, in order to determine the structure of Nur by the MAD method, we collected a 3.0 Å resolution Ni-MAD data set at three wavelengths (peak, 1.48473 Å; edge, 1.48578 Å; remote, 1.45101 Å) using a Bruker Proteum 300 CCD on beamline 6B of Pohang Light Source, Korea (Table 1). Initial phases were calculated from the Ni-MAD data set using the *SOLVE* program (Terwilliger, 2004). The phase calculation made it clear that the space group of the Nur crystals was $P3_1$ and that two monomers with two nickel ions were present in the asymmetric unit. The initial phases were improved using the *RESOLVE* program (Terwilliger, 2004). The final electron-density map was interpretable. Model building is now in progress.

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