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Crystallization and preliminary crystallographic analysis of the nickel-responsive regulator NikR from *Pyrococcus horikoshii*

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The nickel-responsive repressor from *Pyrococcus horikoshii* OT3 (*Ph*NikR) has been crystallized in the apo form (*Ph*NikR-apo) and two nickel-bound forms (*Ph*NikR-Ni-1 and *Ph*NikR-Ni-2). The *Ph*NikR-apo crystals belong to space group $P2_1$, with unit-cell parameters $a = 75.78$, $b = 54.32$, $c = 77.28$ Å, $\beta = 116.07^\circ$, and diffract to 2.2 Å. The *Ph*NikR-Ni-1 crystals belong to space group $P4_12_12$, with unit-cell parameters $a = b = 99.89$, $c = 97.98$ Å, and diffract to 3.0 Å and the *Ph*NikR-Ni-2 crystals belong to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 109.95$, $c = 79.0$ Å, and diffract to 2.1 Å. The crystals obtained were suitable for detailed structural studies.

1. Introduction

Nickel is an essential nutrient for microorganisms and is critical for survival under anaerobic conditions (Maroney, 1999). Two types of nickel permeases, single-component and multi-component, have been discovered. Single-component permeases related to HoxN from *Ralstonia eutropha* have been identified in Gram-negative and Gram-positive bacteria, while high-affinity multi-component permeases related to the ATP-dependent NikABCDE permease from *Escherichia coli* (Navarro *et al.*, 1993) have been identified in both bacteria and archaea (Eitinger & Mandrand-Berthelot, 2000). The transcription of the *nikABCDE* operon is repressed by the nickel-regulatory protein NikR at high intracellular nickel concentrations (De Pina *et al.*, 1999).

NikR belongs to the ribbon–helix–helix family of transcription factors and functions as a homotetramer (Chivers & Sauer, 1999). The crystal structure of apo-form *E. coli* NikR (*Ec*NikR) shows that the molecule consists of a central tetrameric domain (TD) flanked by dimeric DNA-binding domains (DBD; Schreiter *et al.*, 2003). The TD binds Ni^{2+} ions avidly with a K_d of 7 p*M* (Chivers & Sauer, 2002). Four similar metal-binding sites are located at the tetramerization interface and the coordination of nickel at these sites is square planar (Schreiter *et al.*, 2003). Full occupation of these sites results in binding to the operator site of the *nikABCDE* promoter with 30 n*M* affinity; however, with a 20–50 µ*M* excess of nickel the DNA-binding affinity increases dramatically to a K_d of 15 p*M*, pointing to the presence of a second low-affinity nickel-binding site (Chivers & Sauer, 2000, 2002).

The most intriguing questions of how NikR senses nickel at low concentrations, how the nickel coordination responds to DNA binding and what the role of nickel is in DNA recognition remain unanswered. In order to resolve some of the remaining questions further structural studies are required. Here, we report the crystallization and preliminary crystallographic studies of a product of the *Pyrococcus horikoshii* OT3 gene *PH0601* (*Ph*NikR) in the apo form (*Ph*NikR-apo) and two nickel-bound forms (*Ph*NikR-Ni-1 and *Ph*NikR-Ni-2) prepared with a protein:nickel ratio of 1:1. Crystals soaked in solution with high nickel content (*Ph*NikR-Ni-2h) and with high nickel content and phosphate ions (*Ph*NikR-Ni-2h-PO_4) were also prepared and characterized.

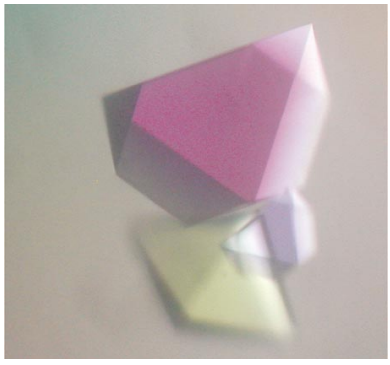


Table 1
Crystal parameters and data-collection statistics.

Values in parentheses are for the last shell.

Crystal	*Ph*NikR-apo	*Ph*NikR-Ni-1	*Ph*NikR-Ni-2	*Ph*NikR-Ni-2h		*Ph*NikR-Ni-2h-PO_4
Crystal parameters						
Unit-cell parameters						
a (Å)	75.78	99.89	109.95	110.21		108.54
b (Å)	54.32	99.89	109.95	110.21		108.54
c (Å)	77.28	97.98	79.00	78.60		78.82
β (°)	116.07					
Space group	$P2_1$	$P4_12_12$	$P3_121$ or $P3_221$	$P3_121$ or $P3_221$		$P3_121$ or $P3_221$
Z	8	16	12	12		12
Solvent content (%)	62.4	68.2	71.8	71.8		70.9
Data collection						
Temperature (K)	100	100	100	100	100	100
X-ray source	BL26B1	BL26B1	BL26B1	BL26B1	BL26B1	Rigaku FR-D
Detector	R-AXIS V	R-AXIS V	R-AXIS V	R-AXIS V	R-AXIS V	R-AXIS V
Wavelength (Å)	1.00	1.00	1.00	1.00	1.4851	1.54178
Resolution (Å)	30–2.2 (2.28–2.2)	30–3.0 (3.11–3.0)	30–2.1 (2.18–2.1)	30–2.1 (2.18–2.1)	30–2.4 (2.49–2.4)	30–3.0 (3.11–3.0)
Unique reflections	28283	18601	32158	32178	21834	10353
Redundancy	3.1 (2.5)	9.4 (5.6)	8.3 (5.9)	5.2 (3.5)	6.6 (6.1)	2.6 (2.0)
Completeness (%)	97.8 (94.7)	99.0 (98.8)	99.3 (99.2)	98.6 (96.1)	99.9 (99.9)	93.3 (84.3)
R_{sym} (%)	5.3 (28.2)	6.5 (29.2)	6.3 (28.2)	4.2 (29.7)	5.6 (33.7)	6.5 (36.3)
$\langle I/\sigma(I)\rangle$	21.9 (4.5)	20.8 (4.8)	24.8 (5.5)	23.1 (3.5)	18.5 (5.8)	26.1 (3.8)

2. Materials and methods

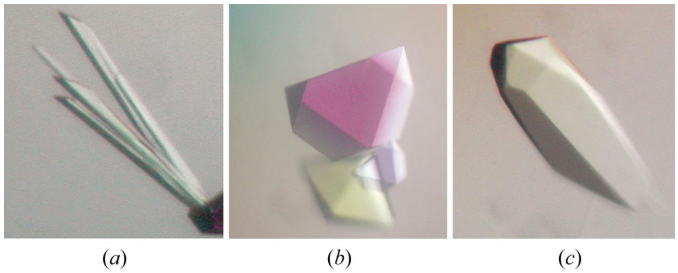
2.1. Expression and purification

The protocols used for the expression and purification of *Ph*NikR were similar to those described for phosphopantetheine adenylyltransferase by Takahashi *et al.* (2004).

Dynamic light-scattering measurements of the purified protein were performed at 291 K using DynaPro MS/X (Protein Solution) and the data were analyzed using *DYNAMICS* software (Protein Solutions).

2.2. Crystallization

Initial crystallization conditions were obtained by screening at a temperature of 298 K in 96-well plates using the sitting-drop vapour-diffusion method with Crystal Screens I and II (Hampton Research; Jancarik & Kim, 1991; Cudney *et al.*, 1994). 1 µl drops of protein solution (10 mg ml^{-1} *Ph*NikR, 49 m*M* NaCl, 20 m*M* Tris–HCl pH 8.0, with or without 0.65 m*M* $NiCl_2$) mixed with 1 µl reservoir solution were equilibrated against 100 µl reservoir solution. Some conditions produced small crystals. Based on the conditions producing these crystals, grid screens with variations in precipitant concentrations, salt types and concentrations and pH were prepared and tested. Finally, we obtained one type of diffraction-quality crystals for apo protein and two types of crystals for nickel-bound protein. The *Ph*NikR-apo crystals of thin elongated tetragonal prism shape (Fig. 1*a*) grew to dimensions of 0.01 × 0.02 × 0.5 mm in 2–3 d using reservoir solution containing 21%(*v*/*v*) PEG 400, 200 m*M* magnesium chloride hexahydrate and 100 m*M* HEPES buffer pH 7.5. *Ph*NikR-Ni-1 crystals of tetragonal bipyramid shape (Fig. 1*b*) appeared in 2–3 d and grew to dimensions of 0.4 × 0.4 × 0.4 mm in two weeks using a reservoir containing 30 m*M* lithium sulfate monohydrate and 0.6%(*w*/*v*) PEG 8000. Finally, *Ph*NikR-Ni-2 crystals of hexagonal prism shape (Fig. 1*c*) appeared after 1–2 weeks and grew to dimensions of 0.15 × 0.15 × 0.4 mm in one month using reservoir solution containing 4%(*w*/*v*) PEG 8000, 4%(*v*/*v*) ethylene glycol and 50 m*M* HEPES buffer pH 7.5.



(*a*) (*b*) (*c*)

Figure 1
Photomicrographs of (*a*) *Ph*NikR-apo, (*b*) *Ph*NikR-Ni-1 and (*c*) *Ph*NikR-Ni-2 crystals.

2.3. Crystal soaking

Previous studies have indicated that the *Ec*NikR molecule contains two types of nickel-binding sites: low-affinity and high-affinity sites (Chivers & Sauer, 2000, 2002). The crystal structure of the nickel-bound C-terminal tetramerization domain of *Ec*NikR has confirmed the presence of four high-affinity sites, one per subunit (Schreiter *et al.*, 2003). The position of the low-affinity site remains unknown. As an increase of nickel concentration resulted in heavy precipitation of *Ph*NikR, we were only able to obtain *Ph*NikR-Ni-1 and *Ph*NikR-Ni-2 crystals at a protein:nickel ratio of 1:1. In order to locate the low-affinity nickel-binding sites, a *Ph*NikR-Ni-2 crystal was soaked for 1.5 h in reservoir solution with the addition of 20 m*M* $NiCl_2$. The X-ray diffraction pattern of the soaked crystal (*Ph*NikR-Ni-2h) was comparable to that of the *Ph*NikR-Ni-2 crystal (Table 1).

K-edge X-ray absorption spectroscopy (XAS) of Ni-bound *Ec*NikR in the presence and absence of bound DNA shows that the nickel coordination is DNA-dependent (Carrington *et al.*, 2003). It is most likely that the nickel coordination is influenced by the phosphate groups of bound DNA. In an attempt to simulate the possible effect of DNA phosphates, the *Ph*NikR-Ni-2 crystals were soaked in a reservoir solution with the addition of 20 m*M* nickel chloride and 5 m*M* sodium phosphate. Unexpectedly, after 30 min soaking the crystals had completely dissolved. This phenomenon also occurred at increased concentrations of precipitant. In order to reduce the crystal damage, the concentrations of nickel chloride and sodium phosphate were reduced to 5 m*M* and 1 m*M*, respectively. The crystal was soaked for only 10 min and was immediately cryoprotected and flash-cooled. The resulting crystal (*Ph*NikR-Ni-2h-PO_4) diffracted to 3 Å.

2.4. Data collection

For data collection, the crystals were soaked in cryoprotectant for a few seconds, mounted in nylon-fibre loops and flash-cooled in a dry nitrogen stream at 100 K. 36%(*v*/*v*) PEG 400 was used as a cryoprotectant for all crystals, although in the case of the nickel-containing crystals 0.65, 5 or 20 m*M* $NiCl_2$ was added to the solution. Complete data sets were collected at 100 K using synchrotron radiation at SPring-8 beamline BL26B1 or using Cu $K\alpha$ radiation from a Rigaku FR-D rotating-anode generator (Table 1). All intensity data were indexed, integrated and scaled with *DENZO* and *SCALEPACK* from the *HKL*2000 program package (Otwinowski, 1993; Otwinowski & Minor, 1997). The crystal parameters and data-processing statistics are summarized in Table 1.

3. Results and discussion

Dynamic light-scattering measurements show that both apo and nickel-bound molecules of *Ph*NikR are monodisperse with a molecular weight of 62 kDa. This suggests that *Ph*NikR subunits may form tetramers both in solution and in crystals, similar to the *Ec*NikR molecule (Schreiter *et al.*, 2003), which shares 33.8% amino-acid sequence identity with *Ph*NikR. Solvent-content calculations (Matthews, 1968) show that if four subunits (a possible homotetramer) of *Ph*NikR-apo are assigned to the asymmetric unit of the crystal, the solvent content of the crystal will be 62.4%. In the case of the *Ph*NikR-Ni-1 and *Ph*NikR-Ni-2 crystals, assuming the presence of two protein subunits in the asymmetric unit (half a possible homotetramer) gives solvent contents of 68.2 and 71.8%, respectively. The crystals obtained are suitable for detailed structural studies of *Ph*NikR.

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