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Expression, purification, crystallization and preliminary X-ray analysis of the extracellular sensory domain of DraK histidine kinase from *Streptomyces coelicolor*

The bacterium *Streptomyces coelicolor* produces useful antibiotics from its secondary metabolites. DraK is a sensory histidine kinase involved in the differential regulation of antibiotics in *S. coelicolor* through the DraR/DraK two-component system. Here, the extracellular sensory domain of DraK was overexpressed in *Escherichia coli*, purified and crystallized using the sitting-drop vapour-diffusion method. The crystal diffracted to 2.2 Å resolution and belonged to space group C222₁, with unit-cell parameters $a = 41.91$, $b = 174.50$, $c = 145.25$ Å, $\alpha = \beta = \gamma = 90^\circ$.

1. Introduction

Because the cell surface directly contacts the surrounding environment, its ability to immediately monitor environmental conditions is critical for cell survival. The bacterial two-component system (TCS) performs signal transduction in response to various external stimuli. The TCS consists of histidine kinase (HK) and a cognate response regulator (RR). HKs sense specific stimuli from the environment of the cell and transfer the signals to the RRs, which control the gene expression for the regulation of target proteins to adapt to changes in the environment. Owing to their role in bacterial metabolism and signal specificity, HKs are frequently attractive drug targets (Gotoh *et al.*, 2010; Fields *et al.*, 1989). A typical HK is composed of three major domains: an N-terminal sensory domain, which senses external signals, a dimeric transmembrane domain, which spans across the membrane and transfers the signal through a conformational change, and a cytoplasmic domain, which receives the signal through the phosphorylation of a conserved histidine and transfers the signal through phosphotransfer to a conserved aspartate on a cognate RR (Mascher *et al.*, 2006; Casino *et al.*, 2010). The activated RR controls the expression of the target genes for adaptation to external stimuli. Despite high structural similarity among the sensor domains of HKs, the low sequence homology of the sensor domain of HKs enables them to sense specific signals (Sevvana *et al.*, 2008; Pappalardo *et al.*, 2003; Cheung *et al.*, 2008). Therefore, the structure of the sensory domain of an HK is critical to understanding the sensing mechanism of its specific signal transduction system.

The *Streptomyces* genus is attractive for the production of medically and industrially useful antibiotics from its multiple secondary metabolites (Challis & Hopwood, 2003). *S. coelicolor* is well known as a soil bacterium and is the best known representative of *Streptomyces*. Because *S. coelicolor* is a soil-dwelling organism, its environmental stimuli are highly variable. However, little is known about the structural and functional properties of the HKs in *S. coelicolor*. Therefore, structural studies of the HKs from *S. coelicolor* may provide useful information for understanding the synthetic mechanisms of valuable secondary metabolites, including antibiotics and anticancer agents, under various conditions and for understanding and influencing the mechanisms of regulation to improve the production of these compounds.

Recently, Yu and coworkers reported that the DraR/DraK TCS is involved in the regulation of differential antibiotic production in *S. coelicolor* and that it works in minimal medium with a high concentration of nitrogen sources (Yu *et al.*, 2012). A domain-analysis

service (*SMART*) determined that DraK consists of an extracellular domain flanked by two transmembrane helices (Leu5–Val27 and Leu126–Ile145), a sensory domain (Glu28–Thr125), a HAMP (histidine kinases, adenyl cyclases, methyl-accepting chemotaxis proteins and phosphatases) domain (Asn149–Thr201) and a kinase domain (Ala202–Pro410) composed of His KA and CA domains. We have characterized the biochemical and biophysical properties of the extracellular sensory domain of DraK in the solution state (Yeo *et al.*, 2013). However, the structure and sensing mechanism of the extracellular domain of DraK remain unknown. Here, we report the cloning, purification, crystallization and preliminary X-ray analysis of the extracellular domain of DraK.

2. Materials and methods

2.1. Cloning and overexpression

The DNA sequence of the extracellular sensory domain of DraK consisting of 88 residues (Glu28–Arg115) was amplified from the genomic DNA of *S. coelicolor* A3(2) (ATCC BAA471D) with the primers 5'-CGCCATATGGAGACCCGGACCATCAGC-3' and 5'-CGCCTCGAGTTAGCGGGGCTCCTCGACG-3' by polymerase chain reaction (PCR) and was subsequently cloned into pET-28a(+) (Novagen) using *NdeI* and *XhoI* as the restriction enzymes and T4 ligase for the plasmid ligation. To improve the stability of the sensory domain of DraK, its C-terminus (Ser116–Thr125) was eliminated. Following confirmation by DNA sequencing, the recombinant plasmid was transformed into *Escherichia coli* strain BL21 (DE3). After cell growth reached an OD₆₀₀ of ~0.6 at 310 K in Luria–Bertani (LB) broth containing 50 µg ml⁻¹ kanamycin, the protein was expressed by adding 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 291 K. The cells were grown for 18–20 h at 291 K following the addition of IPTG. The cells were harvested by centrifugation at 3000g for 30 min at 277 K and were stored at 193 K until purification.

2.2. Purification

The harvested cells were lysed by sonication in a binding buffer consisting of 20 mM Tris pH 8.0, 300 mM NaCl, 20 mM imidazole containing 2 mM phenylmethylsulfonyl fluoride (PMSF). Following centrifugation at 100 000g for 30 min at 277 K, the supernatant was loaded onto Ni-NTA resin equilibrated with the binding buffer and the resin was washed with ten column volumes of the binding buffer. The target protein was eluted using an elution buffer consisting of 20 mM Tris pH 8.0, 300 mM NaCl, 500 mM imidazole. To cleave the

N-terminal His₆ tag, the eluted protein was dialysed at 277 K overnight in 20 mM Tris pH 8.0, 100 mM NaCl following the addition of thrombin protease. Finally, the target protein containing the artificial sequence Gly-Ser-His-Met arising from the cloning at its N-terminus was purified by size-exclusion chromatography using a Superdex 75 column equilibrated with a buffer consisting of 10 mM sodium acetate pH 4.5, 100 mM NaCl. The fractions containing the target protein were collected and concentrated to 12 mg ml⁻¹ using an Amicon Ultra-15 filter (Millipore). Since the sensory domain of DraK at low pH is more structured than at high pH, the final sample was obtained at pH 4.5 (Yeo *et al.*, 2013). The concentration of protein was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific). The extinction coefficient of the protein is 1490 M⁻¹ cm⁻¹ at 280 nm.

2.3. Crystallization

The crystallization of the extracellular sensory domain of DraK was set up using the sitting-drop vapour-diffusion method at 293 K. Initial screening was performed using the screening kits Crystal Screen 2 (Hampton Research), Index (Hampton Research), Wizard I and II (Emerald BioSystems), PEG/Ion (Hampton Research), SaltRx (Hampton Research) and PACT (Qiagen). 0.2 µl drops of protein solution at 12 mg ml⁻¹ were mixed with the same volume of reservoir solution and equilibrated against 70 µl reservoir solution. Crystals of the protein were grown in various conditions. After incubation for several months, a single crystal was used for X-ray diffraction.

2.4. X-ray diffraction analysis

A suitable crystal from the drop was transferred to a cryoprotectant buffer consisting of reservoir solution with 15% ethylene glycol. For the X-ray diffraction tests, a MicroMax-007 HF microfocus X-ray generator and an R-Axis IV⁺⁺ imaging-plate area detector (Rigaku, Japan) were used. The cryoprotected crystal was flash-cooled and

Figure 1
Crystals of the native extracellular sensory domain of DraK.

Figure 2
X-ray diffraction image obtained from a crystal of the native extracellular sensory domain of DraK. The edge of the detector corresponds to a resolution of 2.2 Å.

Table 1

X-ray data-collection statistics.

Values in parentheses are for the outermost resolution shell.

Beamline	BL17A, PF
Temperature (K)	100
Wavelength (Å)	1.000
Oscillation range (°)	1
Resolution range (Å)	50–2.2
Unit-cell parameters (Å, °)	$a = 41.91$, $b = 174.50$, $c = 145.25$, $\alpha = \beta = \gamma = 90$
Space group	$C222_1$
Reflections (unique/total)	27750/286779
Mean $I/\sigma(I)$	10.2
Multiplicity	10.3 (10.1)
Completeness (%)	98.7 (98.3)
$R_{\text{merge}}^{\dagger}$	0.112 (0.567)
$R_{\text{r.i.m.}}^{\ddagger}$	0.143 (0.461)
$R_{\text{p.i.m.}}^{\S}$	0.047 (0.160)

$$\dagger R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}, \quad \ddagger R_{\text{r.i.m.}} = \frac{\sum_{hkl} \{N(hkl) / [N(hkl) - 1]\}^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}, \quad \S R_{\text{p.i.m.}} = \frac{\sum_{hkl} \{1 / [N(hkl) - 1]\}^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}.$$

stored in liquid nitrogen until the final diffraction data collection. The final diffraction data were collected using an ADSC Quantum 270 CCD detector on the BL-17A beamline of the Photon Factory (PF), Tsukuba, Japan. The wavelength of the X-ray beam was 1.00 Å and the crystal-to-detector distance was 259 mm. The diffraction images were integrated and scaled using *HKL-2000* (Otwinowski & Minor, 1997).

3. Results and discussion

The recombinant extracellular sensory domain of DraK was over-expressed from *E. coli* strain BL21 (DE3) in a soluble form. The protein was purified using Ni-NTA affinity resin and the final protein was obtained by gel filtration. The best crystals were obtained from a solution consisting of 0.2 M potassium/sodium tartrate, 20% PEG 3350 (Fig. 1). A suitable crystal was used for X-ray diffraction experiments and diffracted to 2.2 Å resolution (Fig. 2) under cryogenic conditions (100 K). The crystal belonged to the *C*-centred orthorhombic space group $C222_1$, with unit-cell parameters $a = 41.91$, $b = 174.50$, $c = 145.25$ Å, $\alpha = \beta = \gamma = 90^\circ$. The data-collection statistics are summarized in Table 1. Because the scaled data showed high intensity values for even-numbered *l*-index reflections, space group $C222_1$ was assigned. This space group gives a calculated Matthews coefficient (V_M) of $2.47 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 50.2%,

assuming the presence of six molecules in the asymmetric unit (Matthews, 1968). Although there is low sequence homology among sensory domains of HKs, they have similar structures. We attempted molecular replacement (MR) with the program *Phaser* (McCoy *et al.*, 2005) using several crystal structures of other sensory domains of HKs, such as CitA (PDB entry 1p0z; Reinelt *et al.*, 2003), DcuS (PDB entry 3by8; Cheung *et al.*, 2008) and PhoR (PDB entry 3cwj; Chang *et al.*, 2010), which have 14.73, 14.08 and 17.36% sequence identity, respectively, but no solution could be found. The crystal structure determination of DraK is currently in progress by the multi-wavelength anomalous dispersion (MAD) method using selenomethionine as an anomalous scatterer (Terwilliger, 1994).

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