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Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of hypothetical protein SCO4226 from *Streptomyces coelicolor* A3(2)

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A non-Pfam hypothetical protein SCO4226 of molecular weight 9 kDa from *Streptomyces coelicolor* A3(2) was overexpressed in *Escherichia coli* and the purified recombinant protein was crystallized using the sitting-drop vapour-diffusion method. An X-ray diffraction data set was collected to 2.0 Å resolution. The crystal belonged to space group $P2_1$, with unit-cell parameters a = 29.67, b = 67.00, c = 34.43 Å, $\alpha = \gamma = 90.00$, $\beta = 94.26^\circ$.

1. Introduction

The *Streptomyces coelicolor* A3(2) open reading frame (ORF) *SCO4226* encodes an 82-residue protein of unknown function with a theoretical molecular weight of 9049 Da (Redenbach *et al.*, 1996). The results of searches using *BLASTP* and *PSI-BLAST* (http://www.ncbi.nlm.nih.gov; Altschul *et al.*, 1997) suggested that SCO4226 is similar in primary sequence to the N-termini of three proteins: a guanylate cyclase (YP_001978230; 40% identity), an AraC-family member (YP_860235; 39% identity) and a putative two-component system (PhoR/PhoP system) regulator protein (ZP_01106696; 35% identity) (Sola-Landa *et al.*, 2005; Fig. 1). To date, the structures of these proteins have not been reported.

Guanylate cyclases catalyze the cyclization of GTP and produce secondary messenger cGMP (Schulz *et al.*, 2008; Schmidt *et al.*, 2004). It has been proposed that the expression of soluble guanylate cyclase (sGC) might be alternated by increased superoxide production (Schulz *et al.*, 2008). Lineage analysis of sGC suggests that sGC was originally an O_2 sensor in primordial eukaryotes and then evolved to specifically recognize NO for more effective local cell-to-cell signalling (Fitzpatrick *et al.*, 2006).

Most AraC-family members are transcription regulators and share a DNA-binding helix–turn–helix motif within the homologous C-terminus (Gallegos *et al.*, 1993). However, the N-terminal regions of these regulators are highly variable and may be responsible for specific binding to different activator molecules (Gallegos *et al.*, 1993; Martin & Rosner, 2001). One member of the AraC family, *Escherichia coli* SoxS, the counterpart of which in *S. coelicolor* is located downstream of *SCO4226*, has been reported to be involved in the regulation of responses to oxidative stress (Wu & Weiss, 1991; Li & Demple, 1994).

The PhoR/PhoP system, which lies upstream of *SCO4226*, also regulates the expression of oxidative stress-related proteins (Ghorbel *et al.*, 2006; Sola-Landa *et al.*, 2008). The membrane-bound protein PhoR senses the phosphate-limitation signal and undergoes self-phosphorylation. Phosphorylated PhoR then transfers the phosphate group to PhoP, resulting in the subsequent expression of more than 30 genes including *Pho* regulons (Sola-Landa *et al.*, 2003). This system also plays an important role in the regulation of secondary metabolism and internal oxidative stress (Ghorbel *et al.*, 2006). The primary sequence homology between SCO4226, the AraC-family member and the PhoR/PhoP system regulator protein implies a possible relation of their functions, which needs to be proved by structure-based

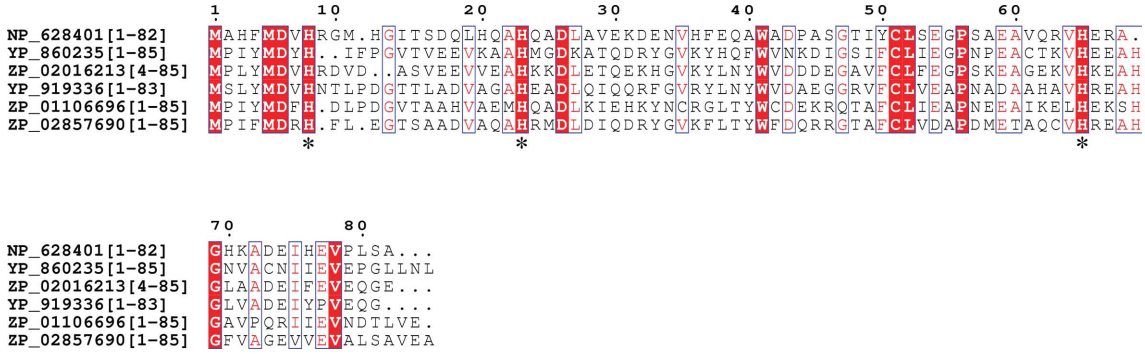


Figure 1
Sequence alignment of SCO4226 using the programs *MULTALIN* (Corpet, 1988) and *ESPript* (Gouet *et al.*, 2003) based on the results of *BLASTP* and *PSI-BLAST* searches (http://www.ncbi.nlm.nih.gov). NP_628401.1, SCO4226 from *S. coelicolor*; YP_860235.1, AraC-family transcriptional regulator protein from *Gramella forsetii*; ZP_01106696.1, putative two-component system sensor kinase from *Flavobacteriales bacterium*; ZP_02857690.1, adenylate/guanylate cyclase from *Rhizobium leguminosarum*; ZP_02016213.1, putative cyclase/kinase from *Halorubrum lacusprofundi*; YP_919336.1, putative guanylate cyclase protein from *Nocardioides* sp. JS614. Conserved residues are marked in red and conserved histidine residues are marked by asterisks.

functional interpretation and/or biochemical/biophysical investigations.

2. Materials and methods

2.1. Cloning and protein expression

The ORF encoding SCO4226 was amplified by PCR using the cosmid DNA of *S. coelicolor* A3(2) as a template (provided by Dr Marie-Joelle Virolle at the Institute of Genetics and Microbiology, University Paris-Sud XI). Primers for amplification were designed based on the sequence available from http://streptomyces.org.uk (*SCO4226*, 2SCD46.40c). *Nde*I and *Xho*I restriction sites were incorporated into the sequences of the sense and antisense primers 5′-CCACATATGGCTCACTTCATGGACGTACAC-3′ and 5′-ATTCTCGAGCTATGCCGACAGGGGG-3′, respectively. After digestion with *Nde*I and *Xho*I restriction enzymes, the PCR product was cloned into the expression vector pET22b. *E. coli* Top10 cells were transformed with the ligation mixture. The sequence of the construct, which had no tags or extra residues, was confirmed by DNA sequencing. *E. coli* Rosetta (DE3) cells were transformed with plasmid pET22b-*SCO4226* and grown on agar plates containing 50 µg ml^{-1} ampicillin. A single colony was verified and cultured in LB broth with 50 µg ml^{-1} ampicillin and 30 µg ml^{-1} chloramphenicol. Expression of the target protein was induced for 5 h at 310 K by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.2 m*M* when the OD_{600} reached 0.6. Cells were harvested by centrifugation at 7330*g* for 10 min and then resuspended in cold lysis buffer (20 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl). The suspension was sonicated and clarified by centrifugation at 29 300*g* for 25 min at 277 K.

2.2. Protein purification

The high histidine-residue content of SCO4226 enabled us to purify the protein by affinity chromatography. The supernatant was loaded onto a nickel-chelating Sepharose Fast Flow column (Amersham Biosciences) pre-equilibrated with buffer containing 20 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl. After the column had been washed with 20 ml of the same buffer, the binding protein was eluted by a gradual increase in imidazole concentration from 10 to 200 m*M*. The fraction that was eluted by binding buffer containing 200 m*M* imidazole was then further purified on a HiLoad 16/60 Superdex 75 column (Amersham Biosciences) equilibrated with buffer containing 20 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl. The fractions containing the target protein were verified by SDS–PAGE.

2.3. Crystallization and X-ray data collection

Crystals of SCO4226 were obtained by the sitting-drop vapour-diffusion method at 289 K using screening kits from Hampton Research. Each sitting drop consisted of 1.0 µl 10 mg ml^{-1} protein

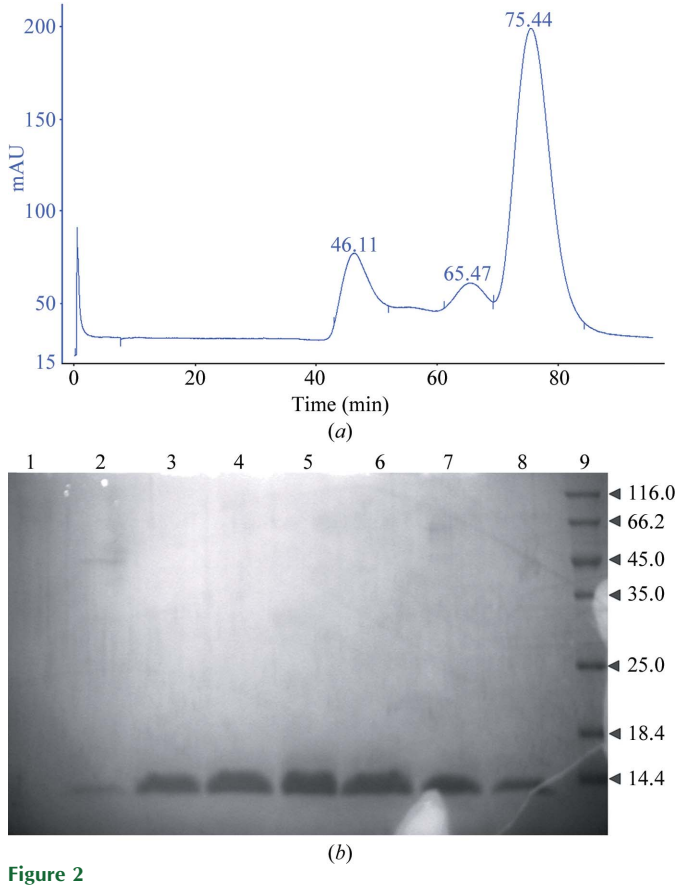


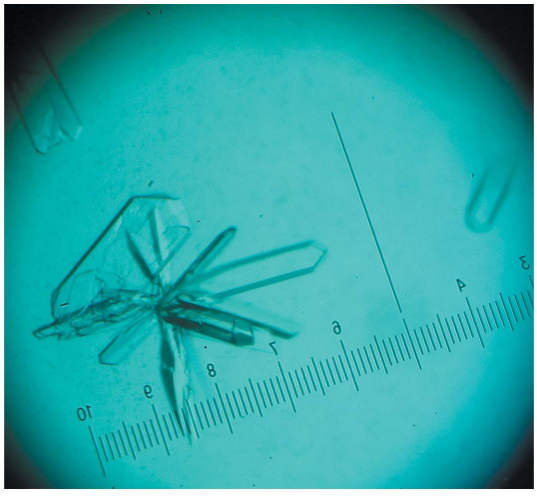
Figure 2
(*a*) Gel filtration of SCO4226 using a HiLoad 16/60 Superdex 75 column. The flow rate was 1 ml min^{-1}. Three main peaks were detected: peak I (46.11 min), peak II (65.47 min) and peak III (75.44 min). (*b*) 15% SDS–PAGE analysis of fractions from gel filtration. Lanes 1 and 2 correspond to the fractions in peaks I and II, respectively; lanes 3–8 correspond to fractions from peak III; lane 9 contains low-molecular-weight markers (kDa).

Table 1
Data-collection statistics.

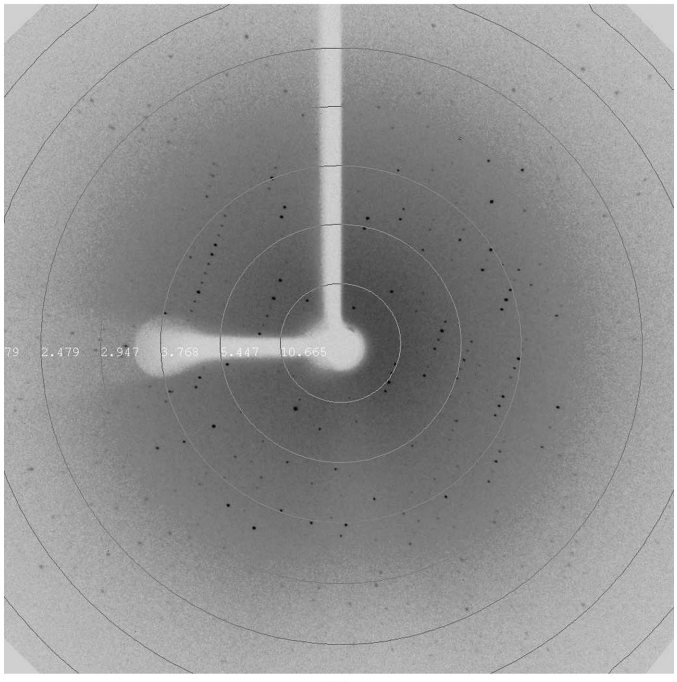
Values in parentheses are for the highest resolution shell.

Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 29.67, b = 67.00, c = 34.43, $\alpha = \gamma$ = 90.00, β = 94.26
Temperature (K)	100
Wavelength (Å)	1.54056
Resolution range (Å)	19.12–2.00 (2.11–2.00)
Total No. of observations	28975 (2570)
No. of unique reflections	9105 (1305)
Mean $I/\sigma(I)$	15.6 (3.7)
Completeness (%)	99.5 (98.3)
Redundancy	3.2 (2.0)
$R_{\rm merge}$ (%)	0.082 (0.196)
$R_{\rm p.i.m.}$†	0.053 (0.171)

† $R_{\rm p.i.m.} = \sum_{hkl}[1/(N-1)]^{1/2}\sum_i |I_i(hkl) - \langle I(hkl)\rangle|/\sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ are the observed intensities, $\langle I(hkl)\rangle$ are the average intensities and N is the multiplicity of reflection hkl. Calculated according to Weiss (2001) and Evans (2006).



(*a*)



(*b*)

Figure 3
(*a*) Crystals of SCO4226. (*b*) Diffraction pattern of a SCO4226 crystal.

solution (20 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl) and 1 µl reservoir solution and was equilibrated against 100 µl reservoir solution. Crystals suitable for X-ray diffraction appeared within 3 d using a reservoir consisting of 1.6 *M* sodium citrate dehydrate pH 6.5. Crystals were flash-frozen in liquid nitrogen using a cryoprotectant consisting of the reservoir solution plus 25% glycerol. Diffraction data were collected to 2.00 Å resolution at 100 K using a Gemini R Ultra mounted with a 135 mm diagonal Ruby CCD detector and co-mounted Enhance (Mo) and Enhance Ultra (Cu) X-ray sources (Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences). The data were indexed and integrated with *CrysAlisPro* processing software (Oxford Diffraction) and scaled using *SCALA* (Evans, 1997) from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

Despite lacking a His tag, SCO4226 showed a moderate affinity for the nickel-chelating Sepharose column. We therefore speculated that it may possess potential metal-binding ability. Atomic absorption spectra indicated that the purified protein contains a traceable amount of nickel, with an occupancy of 11%. Inspection of the primary sequences revealed nine histidine residues, three of which are well conserved among different species (Fig. 1). It is likely that the function of SCO4226 involves metal-ion ligation and the conserved histidine residues might be clustered in the tertiary structure in order to coordinate a metal ion.

Gel filtration indicated that SCO4226 eluted at a volume corresponding to about 20 kDa, which is approximately twice its theoretical molecular weight of 9 049 Da (Fig. 2*a*). The purity was checked by SDS–PAGE (Fig. 2*b*). Liquid-chromatography mass spectrometry (LTQ, Thermo Fisher) was used to further ensure that the collected fractions contained the target protein. A detected peptide (AGHK-ADEIHEVPLSA) matched the last 15 residues of SCO4226 exactly, whereas no matching fragment was detected in the *E. coli* protein database.

Plate-like crystals appeared in a drop consisting of 1.6 *M* sodium citrate dehydrate pH 6.5 in 3 d (Fig. 3*a*). A set of diffraction data was collected from a single crystal to 2.00 Å resolution (Fig. 3*b*). The crystal belonged to space group $P2_1$, with unit-cell parameters $a = 29.67$, $b = 67.00$, $c = 34.43$ Å, $\alpha = \gamma = 90.00$, $\beta = 94.26^\circ$. Based on the Matthews coefficient calculation, it was possible that each asymmetric unit contained one molecule, with a Matthews coefficient of 3.77 Å^3 Da^{-1} and a solvent content of 67.39%, or two molecules, with a Matthews coefficient of 1.89 Å^3 Da^{-1} and a solvent content of 34.8% (Matthews, 1968). However, the observation of a twofold noncrystallographic axis might better support the notion of two molecules per asymmetric unit. The data-collection statistics are summarized in Table 1.

The gene cluster including *SCO4226* was proposed to be involved in regulation of phosphate metabolism and/or response to oxidative stress. An increasing number of studies of these genes and their encoded proteins have been reported (Ghorbel *et al.*, 2006; Sola-Landa *et al.*, 2005, 2008; Blanco *et al.*, 2002), but the function of SCO4226 still remains unknown. The structure-based functional interpretation will provide us with new information about this protein.

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