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Structural and genomic DNA analysis of a putative transcription factor SCO5550 from *Streptomyces coelicolor* A3(2): Regulating the expression of gene *sco5551* as a transcriptional activator with a novel dimer shape

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ABSTRACT

SCO5550 from the model actinomycete *Streptomyces coelicolor* A3(2) was identified as a putative transcriptional regulator, and classified into the MerR family by sequence analysis. Recombined SCO5550 was successfully produced in *Rhodococcus erythropolis*, which can be used to stably express recombinant protein by optimizing the temperature over a wide range (4–35 °C). Crystal structure analysis showed that the dimerization domain (C-terminal domain) of SCO5550 has a novel fold and forms a new dimer shape, whereas the DNA-binding domain (N-terminal domain) is very similar to those of MerR family members. Such the new dimer form suggests that SCO5550 may define a new subfamily as a new member of the MerR family. Binding DNA sequence analysis of SCO5550 using the genomic systematic evolution of ligands by exponential enrichment (gSELEX) and electrophoretic mobility shift assay (EMSA) indicated that SCO5550 regulates the expression of the immediately upstream gene *sco5551* encoding a putative protein, probably as a transcriptional activator.

1. Introduction

The *Streptomyces* genus is of major pharmaceutical importance because over 70% of commercially important antibiotics for human disease are derived from *Streptomyces* species [1]. As a model actinomycete, *Streptomyces coelicolor* A3(2) is genetically the best known representative of the *Streptomyces* genus. The complete nucleotide sequence of the linear chromosome was determined at the Sanger Institute in 2002 [2]. The genome information revealed that *S. coelicolor* A3(2) has an enormous coding sequence (7825 genes), containing an unprecedented proportion of transcriptional regulator genes (965 genes). These transcriptional regulators predominantly play important roles in adaptation of the actinomycete to environmental variations and in controlling production of antibiotics [3,4]. Therefore, detailed knowledge regarding these transcriptional regulators will be indispensable to gain a further understanding of the transcriptional regulation mechanism in *S. coelicolor* A3(2) and consequently in the construction of new strains that can overproduce useful antibiotics.

The *sco5550* is annotated as encoding a putative transcriptional regulator SCO5550 in the *S. coelicolor* A3(2) genome database (http://www.sanger.ac.uk/Projects/S_coelicolor/). The N-terminal region of SCO5550 shows sequence similarity to the DNA-binding domain of MerR family members, most of which are transcriptional activators activated by various effectors, such as the heavy metals, oxygen radicals, or cytotoxic compounds [5]. These activators bend and twist the promoter DNA of target genes between the –35 and –10 regions with a longer than usual spacer of 19/20 bp (normally 17 ± 1 bp), and cause RNA polymerase to initiate transcription [5].

MerR family proteins are dimeric and belong to the winged helix-turn-helix (wHTH) family, which is a large and specific family of HTH DNA binding proteins [6]. Several crystal structures of MerR family members have been determined [7–9]. Each of these proteins contains a conserved N-terminal DNA-binding domain (MerR-type HTH domain) composed of an HTH motif and two wings, an HTH and a β-hairpin (antiparallel β-sheet) [5]. These wings show considerable flexibility in their utilization for DNA recognition and binding [10]. In the C-terminal domain of the MerR

Abbreviations: wHTH, winged helix-turn-helix; HTH, helix-turn-helix; PCR, polymerase chain reaction; Se-Met, selenomethionine; gSELEX, genomic systematic evolution of ligands by exponential enrichment; FITC, fluorescein isothiocyanate; EMSA, electrophoretic mobility shift assay; ORF, open reading frame; IR, inverted repeat.

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Table 1
X-ray data collection and refinement statistics.

Data collection	Peak	Edge	Remote
Resolution (Å)[a]	50.0–2.10 (2.18–2.10)	50.0–2.10 (2.18–2.10)	50.0–2.10 (2.18–2.10)
Wavelength (Å)	0.9788	0.9795	0.9000
R_{sym} (%)[a,b]	0.072 (0.224)	0.065 (0.230)	0.070 (0.234)
Completeness (%)[a]	98.3 (88.8)	98.4 (89.8)	98.5 (90.7)
Unique reflections	11793	11812	11816
Averaged I/σ (I)	11.8	12.1	11.0
Average redundancy[a]	6.9 (6.3)	6.9 (6.4)	7.0 (6.5)
Refinement and model quality			
Resolution range (Å)			19.95–2.20
No. of reflections in working set			10362
No. of reflections in test set			1008
R-factor[c]			23.1
R_{free}-factor[d]			27.6
Total protein atoms			1624
Total water atoms			94
Average B-factor (Å^2)			28.0
RMSD bond lengths (Å)			0.004
RMSD bond angles (°)			1.10

[a] The values in parentheses refer to data in the highest resolution shell.
[b] $R_{sym} = \Sigma_h \Sigma_i |I_{h,i} - \langle I_h \rangle| / \Sigma_h \Sigma_i |I_{h,i}|$, where $\langle I_h \rangle$ is the mean intensity of a set of equivalent reflections.
[c] R-factor = $\Sigma|F_{obs} - F_{calc}|/\Sigma$ Fobs, where F_{obs} and F_{calc} are observed and calculated structure factor amplitudes, respectively.
[d] R_{free}-factor was calculated for R-factor, with a random 10% subset from all reflections.

family members, the effector binding region shows various sequences, lengths, and structures corresponding to diverse effectors, while the dimerization region shows a high degree of structural similarity [5].

Here, we report the crystal structure and genomic DNA-binding site analysis of SCO5550 from *S. coelicolor* A3(2) as a putative transcriptional regulator that is involved in actinomycete gene regulation. The structure and results of DNA-binding analysis using the genomic systematic evolution of ligands by the exponential enrichment (gSELEX) method indicted that SCO5550 is a new member of the MerR family and it regulates the expression of immediately upstream gene *sco5551* encoding an unknown functional protein, probably as a translational activator.

2. Materials and methods

2.1. Protein preparation

Recombinant SCO5550 with a C-terminal His-tag was produced using *Rhodococcus erythropolis* [11] as a host and pTip-QC2 as an expression vector [12]. *R. erythropolis* can be used to stably express recombinant protein by optimizing the temperature over a wider range (4–35 °C). The *sco5550* was amplified by PCR using *S. coelicolor* A3(2) genomic DNA as a template. The amplified gene was digested with *Nde*I/*Xho*I, and the digested product was cloned into the corresponding sites of the pTip-QC2 vector, yielding a C-terminal His-tagged protein. The recombinant protein was expressed in *R. erythropolis* strain L-88 [13]. Cells were grown at 30 °C in Luria–Bertani (LB) medium containing 34 μg mL^{-1} chloramphenicol. The expression of SCO5550 was induced by addition of 1 μg mL^{-1} thiostrepton into LB broth. After adding thiostrepton, the medium was cultured at 30 °C for 18 h with shaking. The cells were harvested by centrifugation at 4 °C and 4500*g* for 10 min, washed with buffer A (50 mM sodium phosphate, pH 8.0, 300 mM NaCl), and then disrupted with a sonicator followed by centrifugation at 8000*g* for 30 min at 4 °C. The supernatant was loaded onto a Hi-Trap chelating HP column (Amersham Biosciences Inc., Piscataway, NJ) charged with $NiSO_4$ and previously equilibrated with buffer B (50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 10 mM imidazole). SCO5550 was eluted by the mixture of buffer B and buffer C (50 mM sodium phosphate, pH 8.0, 300 mM NaCl, 400 mM imidazole) with a linear gradient of 0–100% (v/v) buffer C. The fractions containing SCO5550 were desalted with a HiPrep desalting 26/10 column (Amersham Biosciences) against buffer D (20 mM Tris–HCl, pH 8.0, 300 mM NaCl) and then applied to a HiLoad 26/60 Superdex-200 pg column (Amersham Biosciences) equilibrated with buffer D. The SCO5550 was collected as a single peak, then dialyzed overnight at 4 °C against 20 mM Tris–HCl (pH 8.0) and concentrated to 5.0 mg mL^{-1} using Amicon Ultra (Millipore Corp., Billerica, MA). For production of the selenomethionine-substituted (Se-Met) SCO5550, the cells were grown in M9 medium supplied with 1 mM Se-Met. The procedure for purification of Se-Met SCO5550 was the same as that of the native protein.

2.2. Crystallization

Crystallization trials were carried out by the sitting-drop vapor diffusion method using Crystal Screen kits, PEG/Ion Screen kits, Index kits (Hampton Research Inc., North CA), and Wizard kits (Emerald BioSystems Inc., Bainbridge Island, WA), and initial crystals appeared at No. 30 of Index kit. After optimization by adjustment of salt and precipitant contents, diffraction quality crystals were obtained under conditions of 100 mM Bis-Tris (pH 6.5), 1.25 M ammonium sulfate, and 150 mM sodium chloride using the hanging-drop vapor diffusion method by mixing aliquots of 1.0 μL of protein solution with 1.0 μL of reservoir solution. The crystals were grown to 0.1 × 0.1 × 0.2 mm in 2 months at 20 °C. The crystals of Se-Met SCO5550 were obtained under the same conditions as the native protein.

2.3. Data collection and structure determination

The X-ray diffraction experiment for Se-Met SCO5550 was carried out at beamline BL44B2 of Spring-8 (Harima, Japan). Based on the fluorescence spectrum at the Se *K* edge, multiple-wavelength anomalous diffraction (MAD) data were collected to 2.1 Å resolution using three wavelengths (0.9788, 0.9795, and 0.9000 Å) from a single crystal under cryogenic conditions (100 K) after soaking in cryoprotectant solution containing 26% (v/v) glycerol. The diffraction data were processed using the HKL2000 program package [14]. The Se-Met SCO5550 crystal belongs to the space group $P2_12_12$ with cell dimensions of a = 49.7 Å, b = 100.4 Å, and