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Structural and genomic DNA analysis of the putative TetR transcriptional repressor SCO7518 from *Streptomyces coelicolor* A3(2)

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ABSTRACT

SCO7518 is a protein of unknown function from *Streptomyces coelicolor* A3(2) that has been classified into the TetR transcriptional regulator family. In this study, a crystal structure of SCO7518 was determined at 2.29 Å resolution. The structure is a homodimer of protomers that comprise an N-terminal DNA-binding domain and a C-terminal dimerization and regulatory domain, and possess a putative ligand-binding cavity. Genomic systematic evolution of ligands by exponential enrichment and electrophoretic mobility shift assays revealed that SCO7518 specifically binds to an operator sequence located upstream of the *sco7519* gene, which encodes a maltose O-acetyltransferase. These results suggest that SCO7518 is a transcriptional repressor of *sco7519* expression.

Structured summary of protein interactions:
SCO7518 and **SCO7518** bind by x-ray crystallography (View interaction)

1. Introduction

Streptomyces are ubiquitous soil bacteria that have the ability to produce a wide variety of beneficial secondary metabolites. More than 70% of clinically useful antibiotics, as well as many anticancer agents and other therapeutics, have been discovered from *Streptomyces* species [1]. The *Streptomyces* genus is extraordinarily important in a wide range of life sciences and medical fields. Its secondary metabolites are also useful in agricultural and industrial applications. The genetics of *Streptomyces coelicolor* A3(2) are among the best understood in the *Streptomyces* genus, so *S. coelicolor* is acknowledged as a model actinomycete. The complete genome sequence of the linear chromosome was determined in 2002 [2]. It contains a markedly larger number of putative genes (7825 predicted genes) than the genome of the simple eukaryote *Saccharomyces cerevisiae* (6607 predicted genes). In particular, an anomalous proportion of transcriptional regulator genes (965 predicted genes) were found in the *S. coelicolor* genome. Although these transcriptional regulators are expected to play important roles in adapting to environmental change and/or in controlling the production of antibiotics [3,4], the precise biological function of a large proportion of the regulators has not yet been identified.

An increasing number of transcription factors have been characterized in recent years, including a number of them from *S. coelicolor* A3(2). The technique known as genomic systematic evolution of ligands by exponential enrichment (gSELEX) enables the rapid identification of a DNA sequence specifically bound to a transcription factor [5]. The gSELEX method, like its archetype SELEX, involves iterative rounds of binding, selection, and amplification of genomic DNA fragments [6]. These two methods differ in the library used. The original SELEX uses synthetic DNA fragments of random sequence, whereas gSELEX uses a library derived from the genomic DNA of a selected organism. Therefore, the gSELEX method directly identifies binding site(s) on genomic DNA. When combined with structural analysis using X-ray crystallography,

Abbreviations: HTH, helix-turn-helix; PCR, polymerase chain reaction; gSELEX, genomic systematic evolution of ligands by exponential enrichment; FITC, fluorescein isothiocyanate; EMSA, electrophoretic mobility shift assay; IR, inverted repeat; N-DBD, N-terminal DNA-binding domain; C-DRD, C-terminal dimerization and regulatory domain

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gSELEX analysis works as an effective method for the identification of the biological function of uncharacterized transcription factors.

The gene *sco7518* encodes a putative TetR family transcriptional repressor; this family is widely distributed among bacteria [7]. TetR family members commonly comprise an N-terminal DNA-binding domain (N-DBD), which contains a helix-turn-helix motif (HTH), and a C-terminal dimerization and regulatory domain (C-DRD). Because each C-DRD specifically recognizes its own ligand molecule(s), the amino acid sequence conservation in C-DRD is lower than that in N-DBD. In the absence of the ligand molecule, N-DBD specifically binds to the operator sequence of the target gene. However, once the ligand molecule is recognized by the ligand-binding cavity of C-DRD, a marked conformational change results in a drastic decrease in the repressor's affinity for DNA. Consequently, the TetR family transcriptional repressor dissociates from the operator sequence, which activates gene transcription [7,8]. An allosteric mechanism was proposed to explain the change in DNA-binding affinity. N-DBD in the ligand-free form possesses enough flexibility to adopt a conformation suitable for DNA-binding. However, once a signal molecule binds, an allosteric transition alters the flexible N-DBD, causing it to become more rigid. Therefore, the DNA-binding helices of N-DBD are fixed in a conformation incapable of DNA binding [8,9]. The *S. coelicolor* A3(2) genome contains 122 genes encoding putative TetR family transcriptional regulators [2]. Most of their functions have not been elucidated, although the function of these uncharacterized transcriptional regulators may have great importance for medical, agricultural, and industrial development, as well as for elucidating *Streptomyces* transcriptional regulatory networks.

In this study, we determined the crystal structure of SCO7518, a putative TetR family transcriptional regulator, and determined the DNA binding properties using gSELEX. The results indicated that SCO7518 regulates the expression of *sco7519*, an adjacent gene, which encodes a maltose O-acetyltransferase.

2. Materials and methods

2.1. Protein preparation

Recombinant SCO7518 was prepared as previously described [10]. In brief, protein was overexpressed using the *Rhodococcus erythropolis* expression system, in which a His-tag was attached to the C-terminus. The expressed protein was purified using a Hi-Trap chelating HP column (GE Healthcare, Waukesha, WI), followed by a HiLoad 26/60 Superdex-200pg column (GE Healthcare).

2.2. Crystallization

SCO7518 was dialyzed overnight against 20 mM Tris–HCl, pH 8.0, at 277 K, and then concentrated to 8.7 mg ml^{-1} using an Amicon Ultra concentrator (Millipore Corp., MA, USA). Crystals were obtained with 300 mM di-ammonium hydrogen phosphate and 20% PEG 3350 using the hanging-drop vapor diffusion method. The crystals grew to approximately $0.4 \times 0.8 \times 0.2$ mm in 2 weeks at 293 K.

2.3. Data collection and structure determination

Single-wavelength anomalous diffraction (SAD) data were collected using a single crystal of SCO7518 under cryogenic condition (93 K) after soaking in a crystallization buffer containing 26% (v/v) glycerol. A Rigaku FR-E SuperBright source with a Cr target (wavelength 2.29 Å; 40 kV, 40 mA) and a Rigaku R-AXIS VII imaging plate detector were used for data collection. The crystal was mounted by the capillary-top crystal mounting method [11,12]. The crystal belonged to the space group $P2_1$ with cell dimensions a = 51.3 Å, b = 101.2 Å, and c = 76.9 Å. Each asymmetric unit contains four SCO7518 protomers. The diffraction data were indexed, integrated, and scaled using the HKL2000 program package [13].

The crystal structure of SCO7518 was determined by SAD phasing using two sulfur atoms derived from methionine as anomalous scatterers. The two sulfur sites were identified using the programs SELEXC and SHELXD [13,14] with a HKL2MAP interface [15]. Refinement of the sulfur sites and initial phase determination were performed using the program SOLVE [16]. Further phase improvements and automatic-model building were performed using RESOLVE [17,18] and REFMAC5 [19]. Structural refinement was performed automatically using the Crystallography & NMR System [20] (CNS) program implemented in the automatic refinement program LAFIRE [21]. The stereochemical quality of the final refined model was analyzed using the programs PROCHECK [22] and WHATIF [23]. The crystallographic parameters and refinement statistics are summarized in Table 1.

2.4. gSELEX

The gSELEX procedure was performed as previously described for SCO4008 and SCO5550 [10,24]. In brief, a DNA fragment library for genomic SELEX experiments was amplified by polymerase chain reaction (PCR). The amplified DNA library was mixed with Ni^{2+}-nitrilotriacetic acid resin (Novagen, Madison, WI) pre-adsorbed with 25 μg of purified SCO7518. After incubation for 5 min at room temperature, the resin was washed, and then the SCO7518–DNA complex was eluted. The DNA fragments were extracted from the protein–DNA complex, and then amplified by PCR. The PCR products were used as the DNA library for subsequent cycles of selection. This process was repeated three times. Selected DNA fragments were cloned into the pGEM-T Easy vector (Promega, Madison, WI), and the sequences of the inserted DNA fragment was analyzed using a DNA sequencer (CEQ 2000; Beckman Coulter, Brea, CA).

2.5. Electrophoretic mobility shift assays

Electrophoretic mobility shift assays (EMSAs) were performed as previously described [10,24]. Fluorescein isothiocyanate

Table 1
X-ray data collection and refinement statistics.

Data collection	
Resolution (Å)[a]	50–2.21 (2.29–2.21)
Wavelength (Å)	2.29
R_{sym} (%)[a,b]	6.3 (29.8)
Completeness (%)[a]	99.7 (99.1)
Unique reflections	38773
Averaged I/σ (I)	23.4 (6.24)
Average redundancy[a]	14.0 (12.1)
Refinement and model quality	
Resolution range (Å)	19.79–2.21
No. of reflections in working set	37953
R-factor[c]	0.218
R_{free}-factor[d]	0.247
Total protein atoms	5590
Total water atoms	149
Average B-factor (Å^2)	40.7
RMSD bond lengths (Å)	0.007
RMSD bond angles (°)	1.273

[a] The values in parentheses refer to data in the highest resolution shell.
[b] $R_{sym} = \sum_h \sum_i |I_{h,i} - \langle I_h \rangle| / \sum_h \sum_i |I_{h,i}|$, where $\langle I_h \rangle$ is the mean intensity of a set of equivalent reflections.
[c] R-factor = $\sum |F_{obs} - F_{calc}| / \sum F_{obs}$, 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.