Biochemical and Biophysical Research Communications 435 (2013) 28–33

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

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

Takeshi Hayashi [a], Yoshikazu Tanaka [b], Naoki Sakai [b], Nobuhisa Watanabe [b], Tomohiro Tamura [c], Isao Tanaka [b], Min Yao [b,*]

[a] Department of Food and Fermentation Science, Faculty of Food and Nutrition, Beppu University, Beppu 874-8501, Japan
[b] Faculty of Advanced Life Science, Hokkaido University, Sapporo 060-0810, Japan
[c] Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo 062-8517, Japan

ARTICLE INFO

Article history:
Received 9 April 2013
Available online 22 April 2013

Keywords:
SCO5550
Transcriptional activator
MerR family
gSELEX
X-ray crystallography

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.

* Corresponding author. Fax: +81 11 706 4481.
E-mail address: yao@castor.sci.hokudai.ac.jp (M. Yao).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.04.017

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

c = 39.0 Å, and an asymmetric unit contains one SCO5550 molecule, corresponding with the estimated value of the Matthews coefficient (V_M = 2.03 Å^3 Da^{-1}). The detail statistics of data collection are shown in Table 1.

The structure was solved by the Se-MAD method [15]. The five sites of six Se atoms were located and phases were calculated following phase-improving and model-building with the program SOLVE/RESOLVE [16,17]. Structure refinement was carried out automatically using LAFIRE [18] with the refinement program CNS (Crystallography & NMR System) [19]. The stereochemical quality of the final model was analyzed with the programs PROCHECK [20] and WHATIF [21]. The refinement statistics are summarized in Table 1.

2.4. Genomic systemic evolution of ligands by exponential enrichment (gSELEX)

The procedure for gSELEX was adopted with modifications from the protocol reported by Itou et al. [22]. Genomic DNA was digested with *Hae*III (Takara Bio Inc., Otsu, Shiga, Japan). The digested DNA fragments were cloned into *Eco*RV-digested pBR322 (New England Biolabs Inc., Boston, MA) using T4 DNA ligase (Takara Bio), and transformed into *Escherichia coli* XL-1 blue (Stratagene, La Jolla, CA). Over 2 × 10^5 clones with sufficient variety to cover the whole genome were collected for DNA library preparation. The DNA library for the gSELEX experiment was amplified by PCR as templates with *rTaq* DNA polymerase (Takara Bio) using primers corresponding to regions upstream and downstream of the *Eco*RV site in the pBR322 vector: gSF (5′-CTTGGTTATGCCGGTACTGC-3′) and gSR (5′-GCGATGCTGTCGGAATGGAC-3′), respectively. The PCR products (DNA pool) of genomic DNA library were mixed with Ni^{2+}-nitrilotriacetic acid resin (Novagen Inc., Madison, WI) preadsorbed with 25 μg of purified SCO5550 in binding buffer (20 mM Tris–HCl, pH8.0, 750 mM NaCl, 10 mM imidazole). After incubation for 5 min at room temperature, the mixture was washed with binding buffer, and the protein-DNA complex was eluted with elution buffer (20 mM Tris–HCl, pH 8.0, 500 mM NaCl, 500 mM imidazole). Extracted DNA fragments were amplified by PCR using the gSF and gSR primers described above, and the PCR products were used as a DNA pool for subsequent cycles of selection. After this process was repeated for two cycles, selected DNA fragments were cloned into the pGEM-T Easy Vector (Promega, Madison, WI), and their sequences were analyzed using a DNA sequencer (CEQ 2000; Beckman Coulter, Fullerton, CA).

2.5. Electrophoretic mobility shift assay (EMSA)

Fluorescein isothiocyanate (FITC)-labeled DNA fragments or oligonucleotides (Hokkaido System Science Co., Ltd., Sapporo, Japan) were mixed with 2 μM purified SCO5550 and 1 μg of poly(dI-dC)·poly(dI-dC) in a total volume of 15 μL of binding buffer (20 mM Tris–HCl, pH 8.0, 300 mM NaCl). After incubation for 10 min at 25 °C, 1.5 μL of loading solution [0.05% (w/v) bromophenol blue and 50% (v/v) glycerol] was added, and run in non-denaturing 7.5% acrylamide:*bis*-acrylamide gels (37.5: 1) with 40 mM Tris–HCl (pH 8.0), 20 mM acetic acid, and 1 mM EDTA at room temperature. The protein-DNA complexes within the gels were visualized directly using a fluorescence imager (Typhoon trio$^+$; Amersham Biosciences).

3. Results and discussion

3.1. Overall structure

The final model of SCO5550 includes 205 of 214 residues, two residues of linker between the protein and His-tag, and 94 water molecules in an asymmetric unit (Protein Data Bank code: 2DG6). Due to poor electrical density, the residues Arg30-Glu38 could not be built. The SCO5550 forms a dimer in which the two monomers are related by a crystallographic twofold axis (Fig. 1A, 1B).

The overall structure of SCO5550 is shown in Fig. 1. The monomer is composed of eleven α-helices (α1, Leu^3–Ser^{10}; α2, Thr^{14}–Glu^{23}; α3, Glu^{41}–Gly^{57}; α4, Val^{61}–Val^{71}; α5, Arg^{78}–Ala^{88}; α6, Pro^{100}–Leu^{116}; α7, Glu^{119}–Leu^{124}; α8, Pro^{129}–Arg^{143}; α9, Ala^{150}–His^{174}; α10, Lys^{180}–Leu^{190}; α11, Gln^{192}–Arg^{210}) and an unbuildable region (Arg30–Glu38), which can be divided into two domains: an N-terminal DNA-binding domain and a C-terminal dimerization domain (Fig. 1A). The DNA-binding domain consists of the two

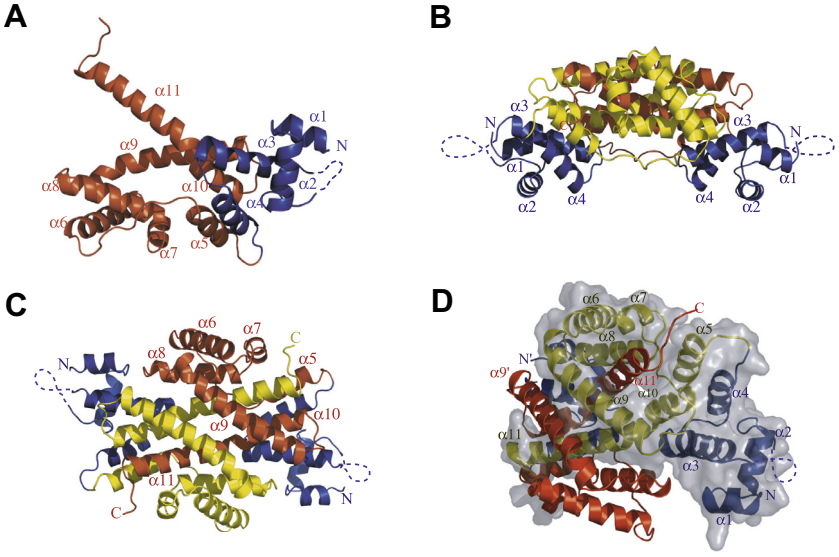

Fig. 1. Ribbon diagram of the SCO5550 structure. A: Ribbon diagram of the monomer structure of SCO5550. The DNA-binding and dimerization domains are shown in blue and yellow, respectively. Unbuildable residues between α2 and α3 are shown as broken lines. B: Ribbon diagram of the dimer structure of SCO5550. The DNA-binding domain is shown in blue, and the dimerization domains of the two molecules are shown in red and yellow, respectively. C: The dimer viewed from the top, colored as in B. D: The dimer viewed from B rotated 45°, colored as in B and C.

helix-turn-helix (HTH) motifs (α1–α2 and α3–α4) and the unbuildable region. The dimerization domain is formed by the seven α-helices (α5–α11), and the α9 and α11 mainly interact with the other molecule of the dimer (Fig. 1C and D). These two domains are connected by a loop between α4 and α5.

3.2. Comparison of the structures of SCO5550 with MerR family transcriptional regulators

Although the sequence of the dimerization domain of SCO5550 does not have any homologs except for an orthologous gene from *Streptomyces avermitilis* (Gene ID: SAV2689), the DNA-binding domain shows both sequential and structural similarity to those of MerR family transcriptional regulators such as MerR, CueR, MtaN, and ZntR (CueR (PDBID:1Q05): $Z = 10.4$: root mean square deviation (rmsd) = 1.4 Å for 62 C_α atoms; MtaN (PDBID:1JBG): $Z = 9.4$: rmsd = 1.2 Å for 60 C_α atoms; by A DALI search [23]) [7,8]. The DNA-binding domain of MerR family members generally consists of a HTH motif (α1–α2 helices) and two wing motifs, wing 1 (W1: an antiparallel β-sheet), and wing 2 (W2: a HTH). Structural superposition of the N-domain between SCO5550 onto that of MtaN-DAN complex showed that the 1st HTH motif (α1–α2) and 2nd HTH motif (α3–α4) of SCO5550 correspond to the HTH motif and the W2 of MerR family members, respectively (Fig. 2).

In addition, comparison of these structures suggested that the unbuildable region of SCO5550 (Arg30–Glu38) seems to form an antiparallel β-sheet that is similar to W1 (Met30–Thr38) of MtaN (Fig. 2). In several reports of the wHTH family members, the structures of W1 were disordered due to the flexible conformation [24–26], *e.g.*, the structure of Mu repressor (MuR) from bacteriophage Mu [27]. Furthermore, secondary structure prediction by the PredictProtein server (http://www.predictprotein.org/) showed that the unbuildable region of SCO5550 preferentially forms the β-sheet.

3.3. Speculation on the mechanism of operator DNA binding

Due to the high degree of structural similarity in the DNA-binding domains between SCO5550 and MtaN, the DNA-binding mechanism of SCO5550 was estimated by superposing the DNA-binding domains of SCO5550 and the MtaN-*mta* operator complex structure (PDB code: 1R8D) (Fig. 2B) [28]. The DNA-binding model suggests that the α2 helix of SCO5550 may be a key helix for DNA binding or specificity, and the wings (W1 and W2) of SCO5550 may recognize and bind the DNA by making contact with the flanking sites of the grooves of the operator DNA.

3.4. A novel fold of dimerization domain in the MerR family

The dimerization domain of SCO5550 is a α-helical domain, and these helices of two monomers wrap around each other. Among these helices, α9 and α11 stretch to the other molecule and form a stable dimer (Fig. 1C and D). The helix α9 associates with α9′ by hydrophobic interaction in an antiparallel orientation. The helix α11 is surrounded by an α-helical barrel formed by α5′, α6′, α7′, α8′, α9′, and α10′ of the other monomer (Fig. 1D), suggesting that α11 is a key helix for dimerization of SCO5550.

The similarity between SCO5550 and MerR family members is limited to the DNA-binding domain. The dimerization domain of SCO5550 differs substantially from that of MerR family members (Fig. 2). In the MerR family, the dimerization interface is composed mainly of a long α-helix (CueR; α5 helix), which forms an antiparallel coiled-coil with the equivalent helix of the other monomer [5]. This long helix of MerR family members corresponds to α5 of SCO5550, although the α5 of SCO5550 is much shorter than that of MerR members (Fig. 2). As a result, the dimerization domain of SCO5550 is markedly different from that of MerR family members (Fig. 2) [5]. Comparison of the dimerization domain of SCO5550 with all of the known structures showed that the topology is unique and is not similar to any other members of the wHTH protein family [10]. These results suggest that the dimerization domain of SCO5550 is a novel fold formed a new dimer shape, and may define a new subfamily of the MerR family.

3.5. The genomic DNA-binding region of SCO5550

As discussed in the previous section, the high degree of similarity of the DNA-binding domain between SCO5550 and MerR family transcriptional activators suggests that SCO5550 may be a transcriptional activator. These results raise the question of whether the protein has DNA-binding activity and acts as a transcriptional regulator. To address this question, a gSELEX experiment was carried out using a genomic library of *S. coelicolor* A3(2). A single band was observed after the second cycle under a high salt concentration of 750 mM NaCl, although recovered DNA fragments formed a smear on agarose gels in the first cycle of gSELEX (data not shown). The single band (SELEX fragment) was purified, sequenced, and its position on the genome map was identified using

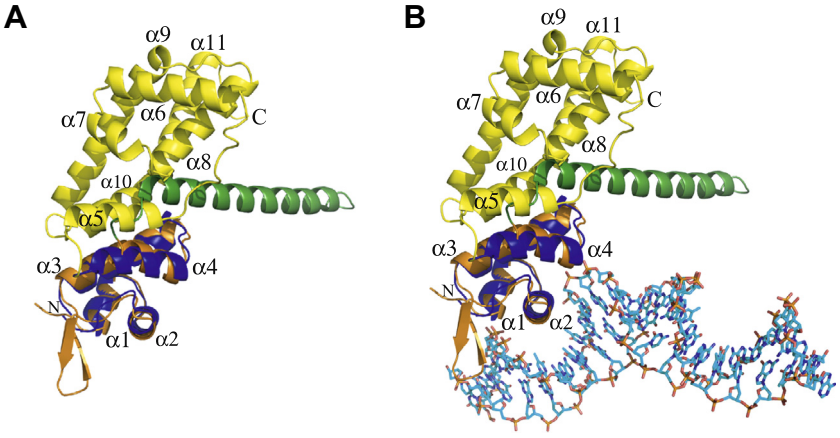

Fig. 2. Superposition of the SCO5550 and MtaN. Superposition of the monomer of SCO5550 onto MtaN (A), and MtaN-DNA complex (B). The DNA-binding and dimerization domain of SCO5550 are shown in blue and yellow, respectively, while orange and green color are applied to corresponding domains in MtaN-DNA complex. The DNA-binding and dimerization domain are shown in, respectively, and the DNA is shown in cyan (carbon), blue (nitrogen), red (oxygen), and orange (phosphate).

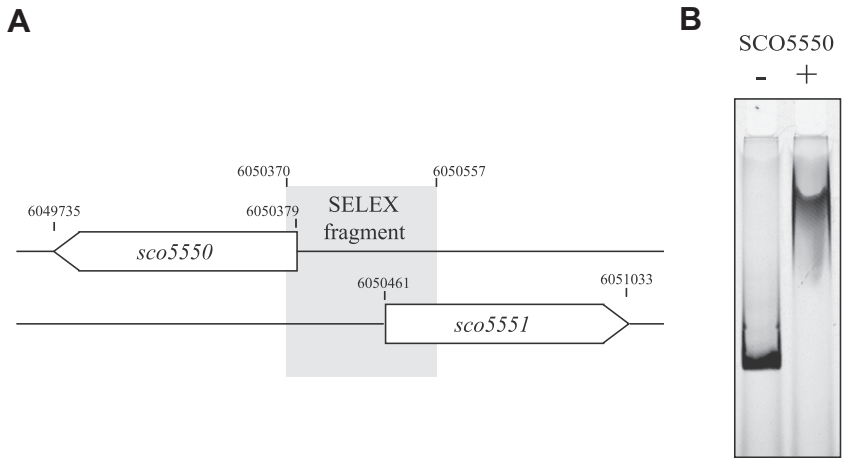

Fig. 3. The analysis of the genomic DNA-binding region. A. Schematic representation of the structures of the *sco5550*, *sco5551*, and *sco5550–sco5551* intergenic region. The DNA region identified by gSELEX (SELEX fragment) is indicated by the gray box. B: Electrophoretic mobility shift assay (EMSA) of DNA-binding activity of SCO5550. Lane 1, 50 nM FITC-labeled 188-bp SELEX fragment in the absence of SCO5550 (−). Lane 2, in the presence of 2 μM SCO5550 (+).

Fig. 4. Identification of binding site. A. DNA sequence of SELEX fragment. The positions of putative –35, –10 elements, and RBS of *sco5550* and *sco5551* are labeled. The opposing two arrows indicate the half-site of the imperfect inverted repeat sequence (iIR). *Symmetry center of the iIR sequence. The bent arrows indicate the translational start sites of *sco5550* and *sco5551*, and UR and DR fragments for EMSA are indicated by dashed arrows. B. EMSA for estimation of the DNA-binding site of SCO5550. The iIR sequence (15 bp), iIR sequence (25 bp), and UR fragment (23 bp) were synthesized as FITC-conjugated oligonucleotides, whereas FITC-labeled DR fragments (140 bp) were prepared by PCR using FITC-labeled primers. These DNA fragments (100 nM) were incubated with 2 μM SCO5550.

the genome database of *S. coelicolor* A3(2). The SELEX fragment is 188 bp in length, and located from position 6050370 to 6050557 on the genome map (Fig. 3A). This fragment mainly covers the intergenic region of *sco5550* and *sco5551*, and includes a fraction of the open reading frame (ORF) of the *sco5550* and *sco5551* encoding unknown functional proteins. Furthermore, the results of EMSA experiment with FITC-labeled SELEX fragment verified that SCO5550 has DNA-binding activity and binds specifically to the *sco5550–sco5551* intergenic region (Fig. 3B).

3.6. Identification of SCO5550 binding site by EMSA

To identify the binding site more precisely, an EMSA experiment was performed using FITC-labeled SELEX fragment. The DNA sequence of the SELEX fragment is presented in Fig. 4A. The translational start site, putative ribosomal binding site (RBS), putative –10, and –35 promoter elements of the *sco5550* and *sco5551* were found using the bacterial promoter predictor BPROM program (SoftBerry, Mt. Kisco, NY; http://linux1.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb). Moreover, it is remarkable that only an imperfect inverted repeat (iIR) [GATAGCGCCACTATC (15-bp)] exists in the *sco5550–sco5551* intergenic region (genome map position from 6050398 to 6050412), and it overlaps with the predicted binding sites (–10 element) of both genes *sco5550* and *sco5551* for RNA polymerase (Fig. 4A). Considering that the target DNA sequence of transcription factors generally shows a dyad symmetry reflecting the symmetrical structure of the dimeric protein [6] and MerR family members bind to the iIR sequences [5], EMSA was performed using synthetic 15-bp and 25-bp oligonucleotides including the iIR sequence, UR and DR fragments (Fig. 4A). As shown in Fig. 4B, it was clear that SCO5550 bound to 25-bp (genome map position from 6050393 to 6050417) but not to the 15-bp oligonucleotide (Fig. 4B), in accordance with previously studies indicating that the MerR family members interact with a region of approximately 25 bp including the IR sequence of operator sites [9,28]. These results indicated that SCO5550 may regulate both *sco5551* and its own gene expression as a transcription factor.

The transcriptional activators of the MerR family have a distinctive transcriptional activation mechanism. The activators bind to the operators located between –35 and –10 promoter elements that are separated by a longer spacer than normal (19 or 20 bp *vs.* 17 ± 1 bp) [5]. The activators markedly bend and twist the promoters, and activate the target genes by recognition of the –10 and –35 boxes for RNA polymerase. The identification of DNA-binding site (putative operator) suggests the similarity of operator binding mechanism between the MerR family members and SCO5550. The DNA-binding site of SCO5550 is between the promoter elements of the *sco5551*, and the distance between the putative –35 and –10 promoters of the *sco5551* is 20 bp in length (Fig. 4A). The results described above suggest that SCO5550 regulates the *sco5551* by a similar transcriptional mechanism to MerR family transcriptional activators.

In this study, we determined the structure of SCO5550 and its DNA-binding site on the genome. The similarities of DNA-binding domain between SCO5550 and MerR family transcriptional

activators imply that SCO5550 is a new member of the MerR family. The novel fold of the dimerization domain and new dimer shape of SCO5550 may define a new subfamily. The results of genomic DNA-binding site analysis suggest that the SCO5550 regulates expression of *sco5551* gene as a transcriptional activator. To further validation the regulatory role of SCO5550 on the expression of *sco5551*, effector-binding and gene disruption experiments of *sco5550* and *sco5551* are necessary.

Acknowledgments

We thank Ms. T. Onodera and K. Hatakeyama for help with protein expression and purification. We also thank the staff of beamline BL44B2, Spring-8, Japan, for their kind help with data collection. This was supported by National Project on Protein Structural and Functional Analyses from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

[1] G.L. Challis, D.A. Hopwood, Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species, Proc. Natl. Acad. Sci. USA 100 (2003) 14555–14561.
[2] S.D. Bentley, K.F. Chater, A.M. Cerdeno-Tarraga, G.L. Challis, N.R. Thomson, K.D. James, D.E. Harris, M.A. Quail, H. Kieser, D. Harper, A. Bateman, S. Brown, G. Chandra, C.W. Chen, M. Collins, A. Cronin, A. Fraser, A. Goble, J. Hidalgo, T. Hornsby, S. Howarth, C.H. Huang, T. Kieser, L. Larke, L. Murphy, K. Oliver, S. O'Neil, E. Rabbinowitsch, M.A. Rajandream, K. Rutherford, S. Rutter, K. Seeger, D. Saunders, S. Sharp, R. Squares, S. Squares, K. Taylor, T. Warren, A. Wietzorrek, J. Woodward, B.G. Barrell, J. Parkhill, D.A. Hopwood, Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2), Nature 417 (2002) 141–147.
[3] J. Davies, Inactivation of antibiotics and the dissemination of resistance genes, Science 264 (1994) 375–382.
[4] S. Donadio, M. Sosio, G. Lancini, Impact of the first *Streptomyces* genome sequence on the discovery and production of bioactive substances, Appl. Microbiol. Biotechnol. 60 (2002) 377–380.
[5] N.L. Brown, J.V. Stoyanov, S.P. Kidd, J.L. Hobman, The MerR family of transcriptional regulators, FEMS Microbiol. Rev. 27 (2003) 145–163.
[6] J.L. Huffman, R.G. Brennan, Prokaryotic transcription regulators: more than just the helix-turn-helix motif, Curr. Opin. Struct. Biol. 12 (2002) 98–106.
[7] M.H. Godsey, N.N. Baranova, A.A. Neyfakh, R.G. Brennan, Crystal structure of MtaN, a global multidrug transporter gene activator, J. Biol. Chem. 276 (2001) 47178–47184.
[8] A. Changela, K. Chen, Y. Xue, J. Holschen, C.E. Outten, T.V. O'Halloran, A. Mondragon, Molecular basis of metal-ion selectivity and zeptomolar sensitivity by CueR, Science 301 (2003) 1383–1387.
[9] E.E. Heldwein, R.G. Brennan, Crystal structure of the transcription activator BmrR bound to DNA and a drug, Nature 409 (2001) 378–382.
[10] K.S. Gajiwala, S.K. Burley, Winged helix proteins, Curr. Opin. Struct. Biol. 10 (2000) 110–116.
[11] N. Nakashima, T. Tamura, A novel system for expressing recombinant proteins over a wide temperature range from 4 to 35 C, Biotechnol. Bioeng. 86 (2004) 136–148.
[12] N. Nakashima, T. Tamura, Isolation and characterization of a rolling-cycle-type plasmid from *Rhodococcus erythropolis* and application of the plasmid to multiple-recombinant-protein expression, App. Microbiol. Biotechnol. 70 (2004) 5557–5568.
[13] Y. Mitani, X.Y. Meng, Y. Kamagata, T. Tamura, Characterization of LtsA from *Rhodococcus erythropolis*, an Enzyme with Glutamine Amdotransferase Activity, J. Bacteriol. 187 (2005) 2582–2591.
[14] Z. Otwinowski, W. Minor, Processing of X-ray diffraction data collected in oscillation mode, Methods Enzymol. 276 (1997) 307–326.
[15] W.A. Hendrickson, Determination of macromolecular structures from anomalous diffraction of synchrotron radiation, Science 254 (1991) 51–58.
[16] T.C. Terwilliger, J. Berendzen, Evaluation of macromolecular electron-density map quality using the correlation of local r.m.s. density, Acta Crystallogr. D Biol. Crystallogr. 55 (1999) 1872–1877.
[17] T.C. Terwilliger, Maximum-likelihood density modification, Acta Crystallogr. D Biol. Crystallogr. 56 (2000) 965–972.
[18] M. Yao, Y. Zhou, I. Tanaka, LAFIRE: software for automating the refinement process of protein-structure analysis, Acta Crystallogr. D Biol. Crystallogr. 62 (2006) 189–196.
[19] A.T. Brunger, P.D. Adams, G.M. Clore, W.L. DeLano, P. Gros, R.W. Grosse-Kunstleve, J.S. Jiang, J. Kuszewski, M. Nilges, N.S. Pannu, R.J. Read, L.M. Rice, T. Simonson, G.L. Warren, Crystallography & NMR system: A new software suite for macromolecular structure determination, Acta Crystallogr. D Biol. Crystallogr. 54 (1998) 905–921.
[20] R.A. Laskowski, M.W. Macarthur, D.S. Moss, J.M. Thornton, PROCHECK-a program to check the stereochemical quality of protein structures, J. Appl. Crystallogr. 26 (1993) 283–291.
[21] G. Vriend, WHAT IF: a molecular modeling and drug design program, J. Mol. Graph. 8 (1990) 52–56.
[22] H. Itou, U. Okada, H. Suzuki, M. Yao, M. Wachi, N. Watanabe, I. Tanaka, The CGL2612 protein from *Corynebacterium glutamicum* is a drug resistance-related transcriptional repressor, J. Biol. Chem. 280 (2005) 38711–38719.
[23] L. Holm, C. Sander, Mapping the protein universe, Science 273 (1996) 595–603.
[24] R.S. De Silva, G. Kovacikova, W. Lin, R.K. Taylor, K. Skorupski, F.J. Kull, Crystal structure of the virulence gene activator AphA from *Vibrio cholerae* reveals it is a novel member of the winged helix transcription factor superfamily, J. Biol. Chem. 280 (2005) 13779–13783.
[25] W.N. Lanzilotta, D.J. Schuller, M.V. Thorsteinsson, R.L. Kerby, G.P. Roberts, T.L. Poulos, Structure of the CO sensing transcription activator CooA, Nat. Struct. Biol. 7 (2000) 876–880.
[26] A.W. Schuttelkopf, D.H. Boxer, W.N. Hunter, Crystal structure of activated mode reveals conformational changes involving both oxyanion and DNA-binding domains, J. Mol. Biol. 326 (2003) 761–767.
[27] U. Ilangovan, J.M. Wojciak, K.M. Connolly, R.T. Clubb, NMR structure and functional studies of the Mu repressor DNA-binding domain, Biochemistry 38 (1999) 8367–8376.
[28] K.J. Newberry, R.G. Brennan, The structural mechanism for transcription activation by MerR family member multidrug transporter activation, N terminus, J. Biol. Chem. 279 (2004) 20356–20362.