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Expression and Characterization of Polyketide Synthase Module Involved in the Late Step of Cephabacin Biosynthesis from *Lysobacter lactamgenus*

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The cephabacins produced by *Lysobacter lactamgenus* are β-lactam antibiotics composed of a cephem nucleus, an acetate residue, and an oligopeptide side chain. In order to understand the precise implication of the polyketide synthase (PKS) module in the biosynthesis of cephabacin, the genes for its core domains, β-ketoacyl synthase (KS), acyltransferase (AT), and acyl carrier protein (ACP), were amplified and cloned into the pET-32b(+) expression vector. The *sfp* gene encoding a protein that can modify apo-ACP to its active holo-form was also amplified. The recombinant KS, AT, apo-ACP, and Sfp overproduced in the form of His_6-tagged fusion proteins in *E. coli* BL21(DE3) were purified by nickel-affinity chromatography. Formation of stable peptidyl-S-KS was observed by *in vitro* acylation of the KS domain with the substrate [L-Ala-L-Ala-L-Ala-L-^{3}H-Arg] tetrapeptide-*S*-*N*-acetylcysteamine, which is the evidence for the selective recognition of tetrapeptide produced by nonribosomal peptide synthetase (NRPS) in the NRPS/PKS hybrid. In order to confirm whether malonyl CoA is the extender unit for acetylation of the peptidyl moiety, the AT domain, ACP domain, and Sfp protein were treated with ^{14}C-malonyl-CoA. The results clearly show that the AT domain is able to recognize the extender unit and decarboxylatively acetylated for the elongation of the tetrapeptide. However, the transfer of the activated acetyl group to the ACP domain was not observed, probably attributed to the improper capability of Sfp to activate apo-ACP to the holo-ACP form.

Keywords: Polyketide synthase, cephabacin, β-ketoacyl synthase, acyltransferase, acyl carrier protein

Gram-negative bacterium *Lysobacter lactamgenus* produces cephabacins consisting of a cephem nucleus, an acetate residue, and an oligopeptide side chain incorporated at the C-3 position of the cephem ring [17]. The peptidyl moiety could be a dipeptide (L-Arg-L-Ala), tripeptide (L-Arg-L-Ala-L-Ala), or tetrapeptide (L-Arg-L-Ala-L-Ala-L-Ala) and represents the metabolic diversity of cephabacin biosynthesis. Based on the presence or absence of the formylamino group (-NHCHO) at the C-7 position from the thiazolidine ring, cephabacins are categorized into F (presence of -NHCHO) and H (absence of -NHCHO) group antibiotics. Cephabacin antibiotics show antibacterial activity against a wide range of resistant bacterial strains, including β-lactamase-producing clinical isolates and anaerobic bacteria [12].

The cephabacins are assembled by connecting the cephem nucleus synthesized by the gene products of *pcbAB*, *pcbC*, *cefE*, *cefF*, and *cefD* [6, 7, 14] and the peptide moiety synthesized by the gene product of four nonribosomal peptide synthetase (NRPS) modules in the *cpbI* (ORF9) and *cpbK* (ORF11) genes [16]. Even though the *cpbJ* gene for an ABC transporter is located between these two genes [13], the *cpbI* gene (ORF10) encodes three NRPS modules and one polyketide synthase (PKS) module composing a giant multifunctional NRPS-PKS hybrid, whereas the *cpbK* encodes only one NRPS module. The substrate specificity of each NRPS module from *cpbI* and *cpbK* genes was already characterized in the assembling of the (L-Ala-L-Ala-L-Ala-L-Arg) tetrapeptide [3], but the function of the PKS module in *cpbI* gene still remains unclear. The biochemical characterization of the PKS module was conducted in this work to understand the final condensation step of the oligopeptide with cephem compound.

Materials and Methods

Bacterial Strains, Vectors, and Cultivation

Escherichia coli XL1-Blue was used for cloning and sequencing purposes, and *E. coli* BL21(DE3) was used for the overexpression of N- or C-terminal His_6-tagged recombinant proteins. pGEM-T

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easy plasmid (Promega, WI, U.S.A.) and pET-32b(+) plasmid (Novagen, WI, U.S.A.) were used as a cloning and expression vector, respectively. *E. coli* strains were grown in Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract, 1% NaCl, and 1.5% agar) or 2×YT medium (1.6% tryptone, 1% yeast extract, and 0.5% NaCl) at 37°C. For selection of clones, ampicillin was added in a final concentration of 100 μg/ml.

Gene Amplification and DNA Manipulation

The pPTS5-1 cosmid [16] and pUC8-sfp plasmid [11] were used as templates for gene amplification. The polymerase chain reaction (PCR) mixture (50 μl) was composed of 5 μl of 10×EF-*Taq* buffer, 1.5 μl of 10 mM dNTP mix, 1 μl of forward primer (20 pmol/μl), 1 μl of reverse primer (20 pmol/μl), 15 ng of template DNA, 10 μl of 5×Band doctor, and 1.25 unit of EF-*Taq* polymerase. Amplification of target DNA fragments was done under the following PCR conditions: initial denaturation at 95°C for 3–5 min; 30–35 cycles of denaturation at 95°C at 1 min, annealing at 55–60°C (depending on G+C content and/or length of the primers) for 0.5–2 min, and extension at 68°C for 1–2 min; and final extension at 68°C for 7 min, by using the GeneAmp PCR system 2400 (Perkin-Elmer, CT, U.S.A.). The primers designed were KS-F (5'-ATACACCATGG-CCGGCCGCTTCCC-3') and KS-R (5'-ATGTTAAGCTTGCTTCT-TCCAGCACCACA-3') for the β-ketoacyl synthase (KS) domain, AT-F (5'-ATACACCATGGGCGCGAACGTCTCG-3') and AT-R (5'-ATGTTAAGCTTACGCCGGCGGG CGA-3') for the acyltransferase (AT) domain, ACP-F (5'-ATAAACCATGGACAAGTGGGTC GA-AAAG-3') and ACP-R (5'-TGCATAAGCTTGAGCTCGTCGATCT-GCAG-3') for the acyl carrier protein (ACP) domain, and Sfp-F (5'-AGCAGTAGATCTGATGAAGATTTACGG AATTTATA-3') and Sfp-R (5'-AATATTGTCGACTTATAAAAGCTCTTCG TACGA-3') for the *sfp* gene. The PCR products were purified using a PCR purification kit (Qiagen, CA, U.S.A.), firstly cloned into pGEM-T easy vector, and then subcloned into pET-32b(+) expression vector. Other gene manipulations, including isolation of gene fragment from agarose gel, plasmid DNA isolation and identification, and *E. coli* transformation, were performed according to standard methods [15].

Gene Expression and Protein Identification

E. coli strain BL21(DE3) harboring the PKS gene modules or *sfp* gene in pET-32b(+) was grown on LB agar plates containing ampicillin (100 μg/ml). The cells were grown at 37°C to mid-log phase (OD_{600}=0.6–0.8) and induced with isopropyl-β-D-thiogalactose (IPTG) in a final concentration of 0.3 mM. Cultivation was allowed to proceed for an additional 2–4 h at lower temperature (25–28°C). The cells were harvested by centrifugation (10,000 ×*g*, 20 min), and resuspended in 20 mM Tris-HCl (pH 7.9) containing 300 mM NaCl, 5 mM imidazole, 1 mM phenylmethanesulfonyl fluoride (PMSF), 0.6% Sarcosyl, and 1 mg/ml of lysozyme. After incubation on ice for 30 min, the cells were sonicated with an Ultrasonic processor (Heat Systems, Inc., NY, U.S.A.) and the cellular debris was removed from the lysate by centrifugation at 13,000 ×*g* for 20 min. The presence of desired recombinant protein in the sample was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The Western blot analysis was also undertaken for identification of His-tagged protein using anti-(His-tag) mouse monoclonal antibody (IG Therapy, Chuncheon, Korea) and goat anti-(mouse IgG)-HRP conjugate (Santa Cruz Biotechnology, CA, U.S.A.). The recombinant His-tagged proteins were identified on X-ray film using a chemiluminescence detection kit (Pierce, CA, U.S.A.).

Purification of Recombinant Proteins

The recombinant proteins were purified under native conditions by nickel-nitrilotriacetic acid (NTA) column chromatography using His·Bind resin (Novagen, WI, U.S.A.). After loading and washing the NTA column, the recombinant proteins were eluted with elution buffer (20 mM Tris-HCl, 300 mM NaCl, 100–200 mM imidazole, and 10% glycerol, pH 7.9) at a flow rate of 1 ml/min. Fractions (2 ml) containing desired protein (>90% pure) were pooled and dialyzed against storage buffer (20 mM Tris-HCl, 300 mM NaCl, 1 mM EDTA, 10% glycerol, pH 7.5). After dialysis, dithiothreitol (DTT) was added at a concentration of 5 mM and stored at −70°C. Protein concentration was determined by Bradford assay [1] using dye concentrate reagent from Sigma-Aldrich (MO, U.S.A.). Bovine serum albumin (BSA) was used as the standard.

Preparation and Radiolabeling of Tetrapeptide-*S*-*N*-Acetylcysteamine (Tetrapeptide-*S*-NAC)

The (L-Ala-L-Ala-L-Ala-L-Arg) tetrapeptide supplied from Peptron (Daejon, Korea) (1.2 equiv.) was dissolved in dimethyl sulfoxide and reacted with *N*-acetylcysteamine (HS-NAC) (4 equiv.) in the presence of dicyclohexylcarbodiimide (DCC) (7.8 equiv.) and hydroxybenzotriazole (2.2 equiv.) [8]. The reaction product was isolated by high-performance liquid chromatography (HPLC) (Shimadzu, Japan) using a reverse phase C_{18} column (VP-ODS, 4.6×250 mm) with flow rate of 1 ml/min. The solvent system used was a gradient from 30–90% of 0.1% trifluoroacetic acid (TFA)/acetonitrile in 0.1% TFA/water. The identity of the product was confirmed by liquid chromatography/mass spectrometry (LC/MS). The radiolabeling of purified tetrapeptide-*S*-NAC product was carried out at 4°C by proton exchange with 20 μl of L-[2,3,4,5-^{3}H]-arginine monohydrochloride (250 μCi/mol; Amersham, IL, U.S.A.). Aliquots were withdrawn at periodical intervals and the radioactivity was quantified with an LS-6800 liquid scintillation counter (LSC) (Beckman, CA, U.S.A.), and purified by HPLC using the same conditions as above.

***In Vitro* Assay of KS Activity**

The assay of KS activity was performed using [L-Ala-L-Ala-L-Ala-L-2,3,4,5-^{3}H-Arg] tetrapeptide-*S*-NAC in 20 μl of incubation buffer [100 mM Tris-HCl (pH 7.2), 1 mM EDTA, 5 mM DTT, 10% glycerol] [18]. The concentration of KS was adjusted to 10 μM and the concentration of L-[2,3,4,5-^{3}H]-tetrapeptide-*S*-NAC was varied from 1 to 10 mM. The reactions were incubated at 22°C and aliquots were withdrawn at periodic intervals from 10 to 360 s. The quenched mixtures in ice-cold acetone were kept at −70°C for 90 min and subsequently centrifuge for 30 min at 14,000 rpm (4°C) to separate the precipitated proteins. After washing with 0.8 ml of ice-cold acetone, the protein pellet was allowed to dry and dissolved in 15 μl of 20 mM Tris-HCl (pH 7.5). Ten μl from each sample was used for the determination of its radioactivity by LSC, and the remaining 5 μl was run on SDS-PAGE. The protein gel was stained, destained, soaked in 2-propanol/acetic acid/water (25:15:60, v/v/v) for 30 min, and subsequently soaked in amplifier solution (Amersham, IL, U.S.A.) for an additional 30 min. The gels were dried and exposed on X-ray film at −70°C for 2 weeks.

In Vitro Assay of AT Activity with ACP in the Presence of Sfp Protein

AT-catalyzed loading of the malonyl group from malonyl CoA to ACP was assayed in a two-step reaction [2]. Firstly, apo-ACP was activated to holo-form by phosphopantetheinylation with Sfp phosphopantetheinyl transferase (PPTase) and CoA-SH. The reaction mixture containing 100 mM Tris-HCl (pH 7.5), 12.5 mM $MgCl_2$, 2.5 mM DTT, 33.3 μM CoA, 5 μM ACP, and 2 μM Sfp, in a final volume of 75 μl, was incubated at 20°C for 60 min. Secondly, a mixture of 2 μM AT and [2-^{14}C]malonyl CoA (200 mM, 52 mCi/mmol) (Amersham, U.K.) in 20 μl was added to initiate the reaction. The reaction was incubated at 25°C and quenched with ice-cold acetone at various time points. The quenched mixture was kept at −70°C for 90 min and subsequently centrifuged for 30 min at 14,000 rpm (4°C) to separate the precipitated proteins. After washing with 1 ml of ice-cold acetone, the protein pellet was allowed to dry and dissolved in 15 μl of 20 mM Tris-HCl (pH 7.5). Three μl from each sample was checked for the radioactivity by LSC, and the remaining 12 μl was run on SDS-PAGE. After staining and destaining, the gel was exposed to X-ray film for 6 days.

RESULTS

Construction of Expression Vectors for KS, AT, apo-ACP, and Sfp Genes

From the PKS module in the *chpI* gene (ORF9) of the cephabacin gene cluster of *L. lactamgenus*, KS, AT, and apo-ACP domain genes were separately amplified by PCR. The *sfp* gene, a part of the surfactin biosynthetic gene cluster of *Bacillus subtilis*, was also amplified from the pUC8-sfp plasmid in *E. coli* MV1190, because ACP domains of PKS should be modified by PPTase from inactive apoenzyme to active holo-form by transferring the 4'-phosphopantetheinyl moiety of CoA to a conserved serine residue of the protein. The PCR products with size of 1,270 bp for the KS domain, 890 bp for the AT domain, 290 bp for the ACP domain, and 675 bp for the Sfp protein (Fig. 1) were purified, cloned into pGEM-T easy vector, and transformed into *E. coli* XL1-Blue. In order to construct expression vectors, the KS, AT, and ACP genes in pGEM-T easy vector were digested with NcoI and HindIII, and the *sfp* gene by BglII and SalI digestion. The genes isolated were subcloned into pET-32b (+) vector for a His-tag fused protein, and transformed into *E. coli* BL21(DE3).

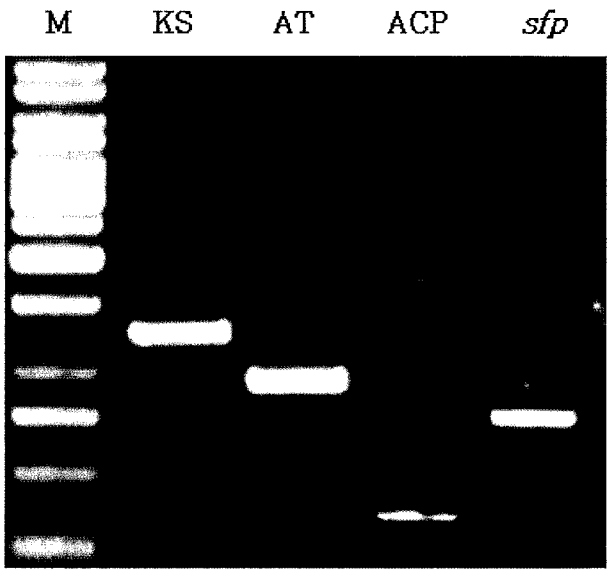

Fig. 1. Confirmation of the amplified KS, AT, AC, and *sfp* genes. The KS, AT, and ACP genes were amplified using *L. lactamgenus* chromosomal DNA as a template, and the *sfp* gene was amplified using pUC8-sfp plasmid in *E. coli* MV1190 as a template.

Gene Expression and Purification of Recombinant Proteins

The high-level expression of foreign fusion protein in *E. coli* often results in the formation of a dense insoluble precipitated aggregate, termed an inclusion body.

In order to obtain soluble and active forms of recombinant proteins, several factors were verified: growth temperature, cell density at time of induction, and length of induction. When the growth temperature was reduced to 25–28°C, the yield of intact soluble fusion protein was considerably greater than at 37°C. Gene induction at low cell density (OD_{600}=0.5) resulted in greater yields of the fusion protein in a soluble form when induced by 0.3–0.4 mM IPTG. The optimal gene induction time was 4 h for the KS domain, 3 h for the AT and ACP domains, and 2 h for the Sfp protein. Another approach to enhance the solubilization of recombinant proteins was tried by treatment with a combination of Triton X-100 and Sarcosyl during the lysis stage after cell harvest. Aliquots of each protein fraction were subjected to SDS-PAGE and Western blotting analysis in order to confirm each recombinant protein (Fig. 2).

The recombinant proteins were purified by affinity chromatography on Ni-NTA resin under native conditions, because those were produced as fused forms with C- and N-terminal His_6 (hexahistidine) tag. The cell lysates were diluted with binding buffer before loading onto NTA-affinity column. Binding of non-tagged proteins was inhibited by using a low concentration of imidazole in the binding and washing buffers, 5 mM and 40 mM, respectively. Elution of His-tagged proteins from the column was achieved by high concentration of imidazole above 100 mM. Fractions containing the His-tagged fusion protein were pooled and dialyzed against storage buffer. DTT in a final concentration of 5 mM was also added to stabilize the proteins. Dialyzed proteins were concentrated, consequently flash frozen and stored at −70°C (Fig. 3).

In Vitro Peptidylation of KS Domain

As a substrate for KS domain, the (L-Ala-L-Ala-L-Ala-L-Arg) tetrapeptide-*S*-NAC was synthesized by condensing

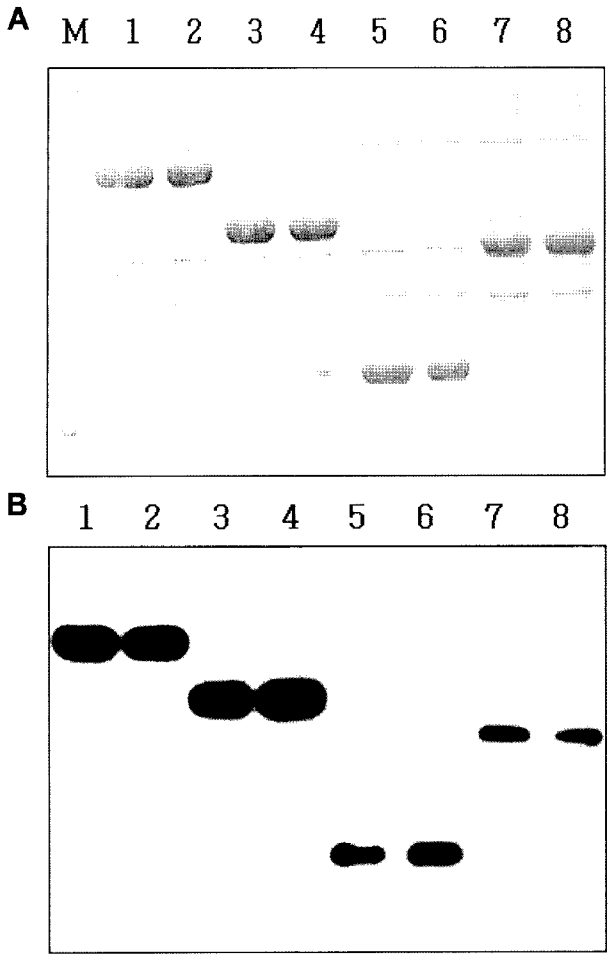

Fig. 2. Gene expression of recombinant KS, AT, apo-ACP, and Sfp proteins in *E. coli* BL21(DE3).
Samples were analyzed by 11% SDS-PAGE, followed by Coomassie blue staining (**A**) or Western immunoblot with an anti-His-tag antibody (**B**). Ten μl of protein fraction was loaded in each lane. Lane 1, total cell protein of *E. coli* harboring pET-32b-KS; lane 2, soluble supernatant after disruption of *E. coli* harboring pET-32b-KS; lane 3, total cell protein of *E. coli* harboring pET-32b-AT; lane 4, soluble supernatant after disruption of *E. coli* harboring pET-32b-AT; lane 5, total cell protein of *E. coli* harboring pET-32b-ACP; lane 6, soluble supernatant after disruption of *E. coli* harboring pET-32b-ACP; lane 7, total cell protein of *E. coli* harboring pET-32b-Sfp; lane 8, soluble supernatant after disruption of *E. coli* harboring pET-32b-Sfp; lane 9, protein molecular marker.

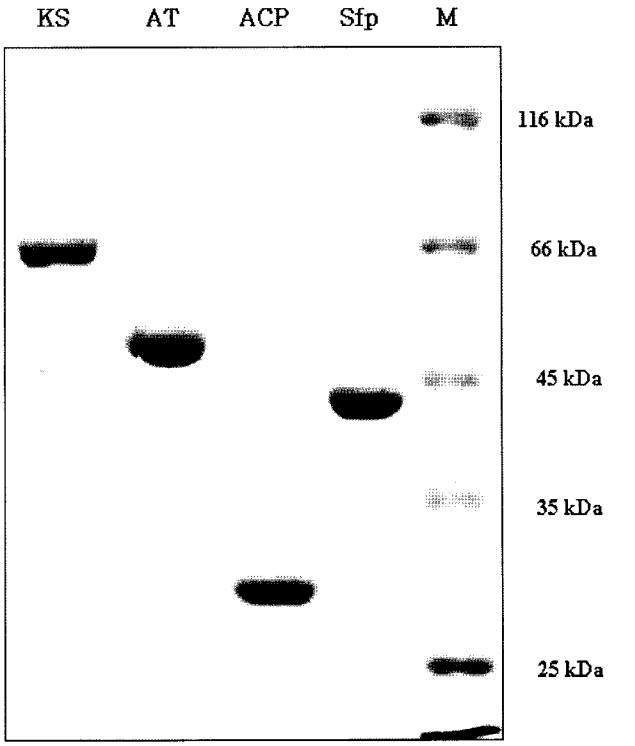

Fig. 3. The purified recombinant KS, AT, and apo-ACP domains and Sfp protein.
The recombinant KS, AT, and ACP domains, and Sfp protein purified by Ni-NTA affinity chromatography were analyzed on 11% SDS-PAGE. The proteins were dialyzed against storage buffer and their concentrations were determined by Bradford assay.

with *S*-NAC in the presence of DCC. When the reaction mixture was loaded on HPLC, tetrapeptide-*S*-NAC was separated through a reverse-phase C_{18} column. Using an electrospray-ionization mass detector, the molecular mass peak (*m/z*) of 472.1 corresponding to [M+H] of the isolated tetrapeptide-*S*-NAC was confirmed in the mass spectrum (data not shown).

To investigate the acylation of the KS domain by its natural peptidyl intermediate, the recombinant KS domain was assayed with (L-Ala-L-Ala-L-Ala-L-Arg) tetrapeptide-*S*-NAC as a mimic of the tetrapeptide-*S*-peptidyl carrier protein (PCP) of NRPS. Firstly, chemically synthesized tetrapeptide-*S*-NAC was radiolabeled with ^{3}H by proton exchange with L-[2,3,4,5-^{3}H] arginine. Activity assay was performed with 10 μM KS and variable concentrations of tetrapeptide-*S*-NAC in order to optimize the molar ratio (Fig. 4). Aliquots of the reaction mixture were withdrawn after periodical intervals up to a total incubation time of 8 min and the reactions were quenched with ice-cold acetone. The LSC analysis of each aliquot suggested that the KS domain can be acylated with tetrapeptide-*S*-NAC within 2 min. When the precipitated protein was subjected to 10% SDS-PAGE and subsequently exposed on X-ray film for 2 weeks, it was also confirmed that the KS domain was covalently labeled by L-Ala-L-Ala-L-Ala-L-[2,3,4,5-^{3}H]Arg-*S*-NAC.

In Vitro Assay of AT with ACP Domains

To investigate the acetylation of AT and ACP domains, a radioactive assay was applied, in which the incorporation degree of ^{14}C-labeled malonyl CoA was counted. Because most ACPs produced in *E. coli* are in the nonfunctional apo-form, ACP was incubated with CoA and Sfp PPTase to ensure that apo-ACP is converted into functional holo-form. Subsequently, holo-ACP was incubated with [2-^{14}C]malonyl CoA and the AT domain directly to test loading of the malonyl CoA extender unit. The reaction was quenched and the precipitated proteins were pelleted, washed, and counted for radioactivity. Alternatively, the precipitated samples

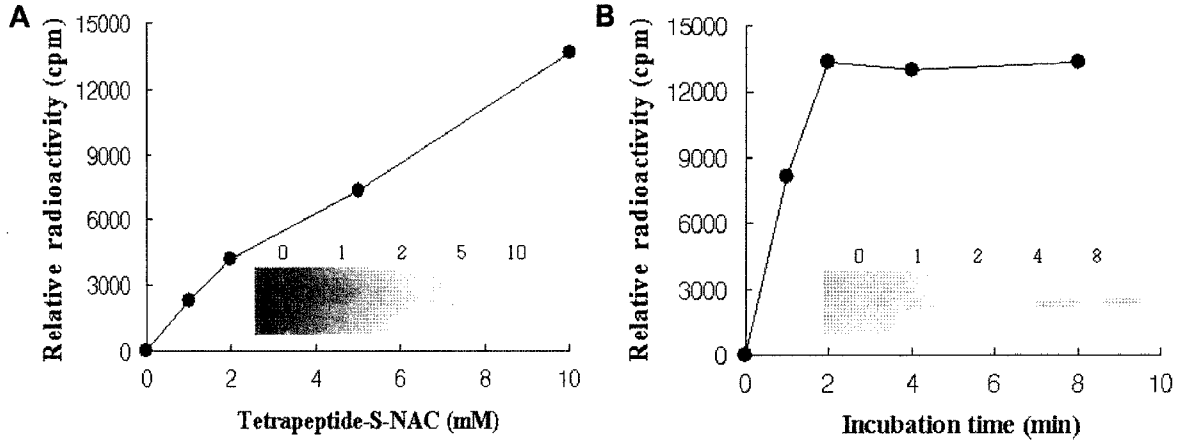

Fig. 4. *In vitro* peptidylation of KS with tetrapeptide-*S*-NAC.
A. *In vitro* acylation of KS was carried out with 10 μM KS domain and different concentrations of [L-Ala-L-Ala-L-Ala-L-Ala-L-(^{3}H-Arg)]-*S*-NAC at 22°C for a total incubation time of 8 min. **B.** *In vitro* acylation of KS domain was carried out with 10 μM KS domain and 10 mM of [L-Ala-L-Ala-L-Ala-L-Ala-L-(^{3}H-Arg)]-*S*-NAC at 22°C for 0, 1, 2, 4, and 8 min. The autoradiographic results of SDS-PAGE after the *in vitro* acylation reaction of KS domain are shown in films.

were subjected to 11% SDS-PAGE and exposed on X-ray film to detect [2-^{14}C]malonyl group specifically loaded onto the phosphopantetheinyl group of ACP. The results confirmed the covalent labeling of the AT domain, but labeling of the ACP domain was not observed (Fig. 5). It might be thought that the Sfp protein could not effectively activate the apo-ACP to holo-ACP by phosphopantetheinylation.

DISCUSSION

Cephem antibiotics are synthesized through the biosynthetic pathway of sulfur-containing β-lactams that includes two early steps for penicillin N formation, three intermediate steps for cephalosporin C biosynthesis, and late decoration steps [9, 10]. In the case of cephabacin biosynthesis, the final decoration steps involve the introduction of an oligopeptide moiety at the C-3 position and formylamino group at the C-7 position of the cephem nucleus.

At the upstream region of the *pcbAB* gene in *L. lactamgenus*, two ORFs comprising four NRPS modules and one PKS module (*cpbI* and *cpbK* genes) were found [16]. Even though the *cpbJ* gene encoding an ABC transporter [14] was located between *cpbI* and *cpbK*, a simultaneous expression of *cpbI* and *cpbK* leads to formation of a huge multifunctional NRPS-PKS hybrid.

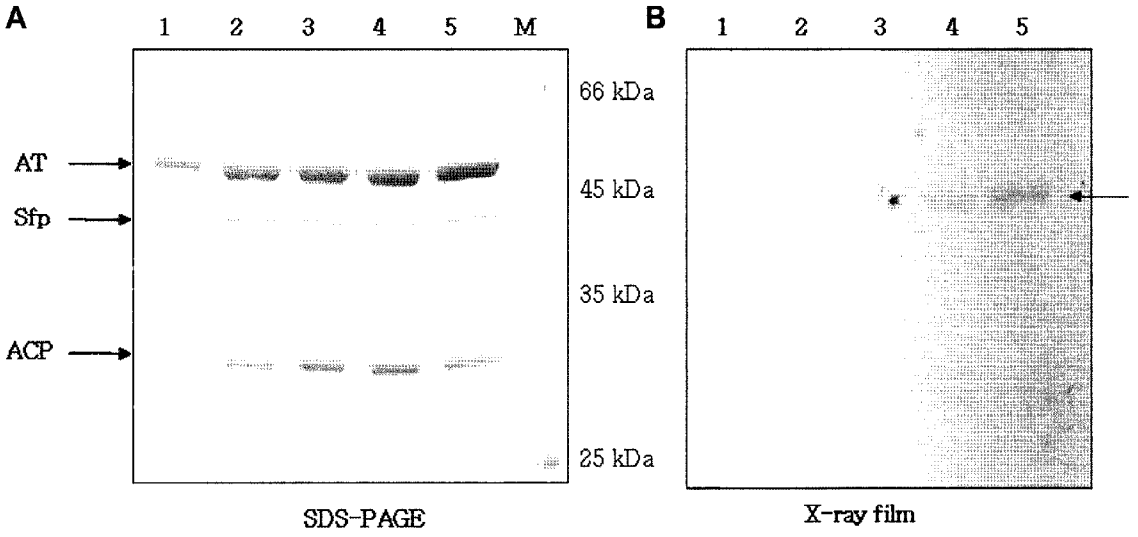

Fig. 5. *In vitro* acylation of AT and ACP domains with [2-^{14}C]malonyl CoA.
In vitro assay of AT-catalyzed loading of malonyl CoA to the holo-ACP was performed with 2 μM of AT domain and [2-^{14}C]malonyl CoA at 25°C for different reaction times, after 5 μM of apo-ACP was phosphopantetheinylated by Sfp protein. Acylation of KS and ACP domains was confirmed on X-ray film (**B**) by autoradiography after 11% SDS-PAGE (**A**). Lane 1, negative control with denatured protein; lane 2, incubation for 1 min; lane 3, incubation for 5 min; lane 4, incubation for 20 min; lane 5, incubation for 50 min; lane M, standard protein marker.

According to the hybrid NRPS/PKS model in cephabacin production, it could be easily envisaged that the biosynthesis of the tetrapeptide moiety and its acetylation occur in two stages: NRPS-mediated assembling of (L-Ala-L-Ala-L-Ala-L-Arg) tetrapeptide and PKS-mediated acetylation of the peptidyl moiety through decarboxylative condensation with malonyl CoA. The substrate specificity of the adenylation domains in each NRPS module from *cpbI* and *cpbK* genes was evidence for involvement of these mega-size proteins in the assembling of the (L-Ala-L-Ala-L-Ala-L-Arg) tetrapeptide [3]. However, the final condensation step of the oligopeptide with cephem compound has not been characterized yet.

Based on the fact that the KS domain in all known NRPS-PKS hybrids contains highly conserved catalytic residues, we proposed that the transfer of the tetrapeptide from the PCP domain of the first upstream NRPS module is catalyzed by the KS domain in a similar mechanism as in a normal PKS. To biochemically confirm this statement, the tetrapeptide-*S*-NAC was synthesized as a surrogate donor of the peptidyl intermediate for *in vitro* studying of the KS domain, confirmed by LC/MS, and radiolabeled by proton exchange with ^{3}H-arginine, in order to measure the amount of the incorporated tetrapeptide into the active site cystein of KS. When 10 μM recombinant KS domain was treated with variable concentrations of tetrapeptide-*S*-NAC, the proportional increase of radiolabeled KS domain was observed depending on the substrate concentration, even though the exact stereochiometry of the tetrapeptide bound to KS domain was not examined owing to the limited amount of tetrapeptide-*S*-NAC. The time-course assay showed that the time required for the acylation of the KS domain was achieved within 2 min. Autoradiographic analysis also confirmed covalent labeling of the KS domain by ^{3}H-labeled tetrapeptide-*S*-NAC.

For another confirmation of the involvement of AT and ACP domains in acetylation of the peptide moiety using malonyl CoA as the extender unit, those domains were reacted with ^{14}C-malonyl CoA. Because most recombinant ACPs expressed in *E. coli* are in nonfunctional apo-form [4, 5], the serine residue at the active site of the ACP domain was post-translationally primed with a 4'-phosphopantetheine moiety derived from CoA-SH to introduce the thiol group by Sfp PPTase. In the activity assay of the KS domain and the activated ACP domain by ^{14}C-malonyl CoA, the result showed the covalent radiolabeling of the AT domain with ^{14}C-malonyl CoA, which was evidence that the AT domain could be decarboxylatively acetylated with malonyl CoA. However, the acetyl group of the AT domain was not transferred into the activated phosphopantetheinyl group in holo-form of ACP, differently from expectation. Thus, it was assumed that this phenomenon is attributed to the improper capability of Sfp protein to activate apo-ACP to holo-ACP by phosphopantetheinylation, or to inefficient communication between the AT domain and ACP domain because of their recombinant nature. It is yet to be determined whether Sfp PPTase with the broad carrier protein specificity is a general solution to provide a functional ACP domain and whether there is any *cis* or *trans* physiological recognition problem of cognate ACP by AT.

Acknowledgments

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