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The *mtaA* Gene of the Myxothiazol Biosynthetic Gene Cluster from *Stigmatella aurantiaca* DW4/3-1 Encodes a Phosphopantetheinyl Transferase that Activates Polyketide Synthases and Polypeptide Synthetases

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Myxothiazol is synthesized by the myxobacterium *Stigmatella aurantiaca* DW4/3-1 *via* a combined polyketide synthase/polypeptide synthetase. The biosynthesis of this secondary metabolite is also dependent on the gene product of *mtaA*. The deduced amino acid sequence of *mtaA* shows similarity to 4′-phosphopantetheinyl transferases (4′-PP transferase). This points to an enzyme activity that converts inactive forms of the acyl carrier protein domains of polyketide synthetases (PKSs) and/or the peptidyl carrier protein domains of nonribosomal polypeptide synthetases (NRPSs) of the myxothiazol synthetase complex to their corresponding holo-forms. Heterologous co-expression of MtaA with an acyl carrier protein domain of the myxothiazol synthetase was performed in *Escherichia coli*. The proposed function as a 4′-PP transferase was confirmed and emphasizes the significance of MtaA for the formation of a catalytically active myxothiazol synthetase complex. Additionally, it is shown that MtaA has a relaxed substrate specificity: it processes an aryl carrier protein domain derived from the enterobactin synthetase of *E. coli* (ArCP) as well as a peptidyl carrier protein domain from a polypeptide synthetase of yet unknown function from *Sorangium cellulosum*. Therefore, MtaA should be a useful tool for activating heterologously expressed PKS and NRPS systems.

Key words: myxothiazol, 4′-phosphopantetheinyl transferase, *Sorangium cellulosum*, *Stigmatella aurantiaca*, thiolation domain.

Most of the biologically active compounds used as agrochemicals or pharmaceuticals are produced as secondary metabolites by bacteria, fungi and plants. Based on their basic molecular structure, these metabolites can be classified into several groups, including the pharmaceutically important classes of polyketides and polypeptides. These include the well-known substances erythromycin, FK506, tetracycline, bleomycin or cyclosporin (for review see Ref. *1*). Polyketides and most low-molecular-weight polypeptides are synthesized by large multifunctional enzymes referred to as polyketide synthases (PKS) and nonribosomal polypeptide synthetases (NRPS) (*2*, *3*).

PKSs condense activated carbonic acids, such as acetate or propionate, with each other. The resulting 2-keto acid intermediates can be reduced stepwise, similar to fatty acid biosynthesis. Therefore, in analogy to the fatty acid synthases (FASs) of eukaryotes and prokaryotes, PKSs are divided into two classes. Type I PKSs contain all active centres as domains on one multifunctional protein. Each set of domains necessary for the attachment and reduction of an extender unit is termed a module, and several modules can be located on the same protein chain. Type II PKSs carry all catalytic sites on single individual proteins, which then form a multienzyme complex. In contrast to type I PKSs, which use each module only once during a synthesis cycle, type II PKSs act as iterative enzymes (*2*). NRPSs are also modularly composed enzymes and condense amino acids to polypeptides using the multiple carrier thiotemplate mechanism. Domains of NRPSs activate amino acids and join these to each other, resulting in peptide chains of variable length. Additional protein domains can modify the incorporated amino acids by, *e.g.*, methylation or epimerisation (*3*). The minimal set for a PKS or a NRPS module is comprised of domains with activities as ketosynthases, acyltransferases and acyl carrier proteins (ACPs) or enzymatic functions as condensation, adenylation and peptidyl carrier protein (PCP) domains, respectively (*4*).

Both PKSs and NRPSs are post-translationally modified to become catalytically active, similar to FAS. A 4′-phosphopantetheinyl (4′-PP) prosthetic group derived from coenzyme A is attached to the hydroxyl side chain of a conserved serine residue found in all of the PCP and ACP domains (thiolation domains). Thereby the enzymes are converted from the inactive apo-forms to the active holoforms (*5*). The first protein identified to be responsible for this type of modification was the holo-ACP synthase of *Escherichia coli* (*6*). With the increasing DNA sequence information available from various organisms, a 4′-PP

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Abbreviations: ACP, acyl carrier protein; ArCP, aryl carrier protein; ACPS, ACP synthase; FAS, fatty acid synthase; IPTG, isopropyl-thio-β-D-galactopyranoside; MALDI-TOF, matrix-assisted laser desorption/ionization-time-of-flight; NRPS, nonribosomal polypeptide synthetase; PCP, peptidyl carrier protein; PKS, polyketide synthetase; 4′-PP, 4′-phosphopantetheinyl.

transferase superfamily has been defined, which is characterized by the following amino acid sequence signature: $GKP(X)_{11-19}$ (F/G)(N/S)(h)(S/T)H$(X)_{14-30}$G(h)D(h)(E/h)$(X)_{36-46}$-(W/F)$(X)_2$KE$(X)_3$K (single letter code; h represents a hydrophobic amino acid) (*5*, *7*). Based on protein size and quaternary structure, the superfamily can be divided into two branches. Members of the first branch presumably act as monomers, comprise proteins with a size of >200 amino acids and are involved in various cellular processes. Members of the second branch, referred to as ACP synthase-(ACPS) type 4′-PP transferases, are characterized by a size of approx. 100 amino acids and the absence of the GKP-$(X)_{11-19}$(F/G)(N/S)(h)(S/T)H motif. Probably, they form homodimers (*7*). The majority of the latter class of enzymes activate type II FAS and are known to convert ACPs of various type II PKS systems (*8*, *9*). Several 4′-PP transferases have been analyzed in detail, amongst them EntD (*10*), ACPS and YhhU (*11*) from *E. coli*, Ppt1p from *Brevibacterium ammoniagenes* (*12*) and Ppt2 and Lys5 from *Saccharomyces cerevisiae* (*13*, *14*). Recently, the crystal structure of Sfp from *Bacillus subtilis* has been determined (*7*). Sfp and Gsp from *Bacillus brevis* activate the thiolation domains of the surfactin and the gramicidin polypeptide synthetases, respectively (*15*, *16*). Both enzymes accept additional substrates. For example, they are able to convert the apo-PCP domains involved in the synthesis of enterobactin and tyrocidin to their corresponding holo-forms (*16*, *17*). A discrete PCP protein participating in the bleomycin biosynthesis can be activated by Gsp (*18*).

The myxothiazol synthetase of *Stigmatella aurantiaca* DW4/3-1 consists of an unusual combination of PKS and NRPS modules. These direct the condensation and modification of a 3-methylbutyrate residue, three acetate, two propionate, and two cysteine residues along with an additional currently unknown amino acid (*19*). Six ACP and three PCP domains within the multifunctional protein complex are probably involved in tethering the growing polyketide chain. A putative 4′-PP transferase, encoded by the gene *mtaA* and identified as such by amino acid sequence comparison, is located directly upstream of the myxothiazol biosynthetic gene cluster. Gene inactivation experiments have shown that the production of the heteropolyketide compound myxothiazol depends on the *mtaA* gene (*19*). Hence, it is tempting to speculate that the phosphopantetheinyl group of both the ACP and the PCP domains is introduced by the same enzyme, suggesting a relaxed substrate specificity for MtaA.

The close association of genes encoding a 4′-PP transferase and genes encoding their cognate substrates prompted us to examine the substrate specificity of MtaA. The objective of the present work was to confirm that MtaA catalyses the transfer of a phosphopantetheinyl group to thiolation domains of the myxothiazol synthetase. Activation of other substrates, such as the ArCP domain of the enterobactin synthetase and a PCP domain of a peptide synthetase from *Sorangium cellulosum*, provide evidence for a broad substrate specificity of MtaA.

MATERIAL AND METHODS

Chemicals, Bacterial Strains, and Culture Conditions—Unless otherwise indicated, all chemicals were purchased from Sigma. *E. coli* XL1blue (Stratagene) was used for propagation of plasmids and for heterologous expression. *E. coli* was grown in LB medium (1% tryptone, 0.5% yeast extract, 0.5% sodium chloride). Plasmid maintenance was assured by the addition of ampicillin (100 μg ml^{-1}) and/or chloramphenicol (25 μg ml^{-1}). If not otherwise indicated, cultivation temperature was 37°C.

General Genetic Manipulation—Routine molecular genetic methods were carried out as described by Sambrook *et al.* (*20*). In general, all enzymes were purchased from New England Biolabs (Schwalbach, Germany). Oligonucleotides were synthesised by Gibco BRL Life Technologies (Karlsruhe, Germany).

Construction of Plasmids Containing mtaA or DNA Encoding Various Thiolation Domains—The oligonucleotides NGB13 (5′-TCGCTACGGGGT*CATATG*CCGACGTCC-3′), NBM4 (5′-*AGATCT*AGGAAACCGCCGCTCTAT-3′) and a Pwo/Taq DNA polymerase mixture (Expand™ high fidelity; Roche Diagnostics, Mannheim, Germany) were used for the amplification of *mtaA* from chromosomal DNA of *S. aurantiaca* DW4/3-1 (*21*). The following cycling profile was used: 30 s at 95°C, 30 s at 58°C, and 1 min at 72°C for 25 cycles. The PCR product was hydrolyzed with *Nde*I and *Bgl*II at restriction sites that were introduced by the oligonucleotides (shown in italics), then ligated with plasmid pET22b (Novagen, Madison, USA) that had been digested with *Nde*I and *Bam*HI. The resulting plasmid was named pMtaA-5. The fragment harboring the *mtaA* gene and the ribosomal binding site of pET22b was then excised with *Xba*I and *Xho*I and fused to the plasmid pSU18 (*22*), hydrolyzed with *Xba*I and *Sal*I. The expression plasmid created was named pSUMtaA. DNA fragments encoding the thiolation domains of (a) ArCP of EntB (*10*) from *E. coli* PBB7 (*23*), (b) PCPI of a peptide synthetase (RM and SB unpublished results) of *So. cellulosum* So ce90 (*24*) and (c) ACPf (MtaF) of the myxothiazol synthetase from *S. aurantiaca* DW4/3-1 (*19*) were amplified by PCR using the following oligonucleotide pairs: (a) primer 1 and primer 2 as described by Gehring *et al.* (*10*), (b) 5′-GTGGACCGCCGC-*CATATG*CCGGAGCCGGAGCAG-3′, 5′-GCGCTGCACCG-G*CTCGAG*CGGCGCGGGACCGGT-3′; (c) 5′-GCCACTTCC-*CATATG*AGTGCCTTC-3′, 5′-CCCGGCCGCCTGGCCACT-GTTTC-3′; Chromosomal DNAs of each of the strains were used as templates in PCR reactions using similar conditions to those described for the amplification of *mtaA*. The ArCP and PCPI PCR products were hydrolyzed with *Nde*I and *Xho*I and ligated with pCYB2 (New England Biolabs, Schwalbach, Germany), creating the plasmids pArCP and pPCPI. The applied cloning strategy slightly modifies the original DNA sequence encoding the respective thiolation domains (*cf.* Fig. 1): the PCR primers used generate an ATG start codon at the 5′-end and a CTC encoding a leucine residue at the 3′-end of the PCR fragments. Additionally, the primarily obtained amplification products were extended by nine base pairs derived from pCYB2 that encode the amino acids EPG (single letter code). The PCR product encoding ACPf was digested with *Nde*I and ligated into pCYB2 pre-cut with *Nde*I and *Sma*I. The resulting expression plasmid pACPf carries a DNA fragment that encodes an additional glycine residue at the 3′-terminus not existing in the original DNA sequence of *mtaF*. In all three plasmids, a translational fusion of the thiolation domains with an intein-chitin protein encoded by the vector was generated in order to facilitate the subsequent purification

of the gene products. All generated plasmids were checked for correctness of the insertion of fragments by DNA sequencing using a method adapted from Sanger *et al.* (*25*) and an ABI PRISM-System 377 Sequencer (Applied Biosystems, Weiterstadt, Germany).

Heterologous Expression of MtaA and Thiolation Domains—*E. coli* XL1blue cells carrying either one of the plasmids pArCP, pPCPI or pACPf, alone or in combination with plasmid pSUMtaA, were grown in LB medium containing 100 μg ml^{-1} ampicillin or 100 μg ml^{-1} ampicillin/25 μg ml^{-1} chloramphenicol, respectively. Seed cultures of 12 ml were started with 120 μl of an overnight culture. After 7 h, 2 ml of the seed culture was used to inoculate 100 ml of LB medium in 1-liter flasks. When the cells had grown to an optical density (OD_{600}) of 0.8 to 1.0, expression was induced with 0.5 mM IPTG. At the same time the incubation temperature was shifted from 37 to 27°C and the cultures were grown for another 12 to 14 h. Five parallel cultures of each expression experiment were combined and harvested by centrifugation. The cells were washed with a buffer containing 20 mM Tris/HCl (pH 8), 100 mM NaCl, and 0.1 mM EDTA, then resuspended in 10 ml of ice-cold cell lysis/column buffer [20 mM Na-HEPES (pH 8), 500 mM NaCl, 0.1 mM EDTA, 0.05% Triton-X-100]. All subsequent steps were carried out at 4°C. Cell disruption was performed with a French press (SLM Aminco, SLM Instruments, Inc.) using a normal cell chamber with the ratio selector level set at "high" and 1,300 PSIG. Cell-free extract was obtained by centrifugation of the cell-lysis extract at 20,000 ×*g*. Basically, the purification of the thiolation domains was carried out according to the instruction manual of the IMPACT I—One-Step Protein Purification System (New England Biolabs, Schwalbach, Germany). In brief, 10-ml columns (MoBiTec, Göttingen, Germany) were filled with 3 ml of chitin-beads, pre-equilibrated with lysis/column buffer, loaded with cell-free extract and flushed with 50 ml of lysis/column buffer. To cleave the target protein from the intein-chitin protein, the column was quickly flushed with cleavage buffer [20 mM Na-HEPES (pH 8), 50 mM NaCl, 0.1 mM EDTA, 20 mM DTT], locked and left overnight. Finally, the protein of interest was eluted with 9 ml of cleavage buffer without DTT and concentrated 150-fold by ultrafiltration using Centriplus RC YM-3 spin-columns (Millipore-Amicon, Eschborn, Germany) according to the recommendations of the supplier.

Protein Visualization, Protein Sequencing, and MALDI-TOF Analysis—Protein samples were separated using 10% SDS polyacrylamide gels or on 16% gels prepared as described by Laemmli (*26*) or Schägger and Jagow (*27*), respectively. Gels were stained with colloidal Brilliant BlueG (Sigma). Desalting of purified ACP and PCP domains was performed by dilution with de-ionised water (pH 7.5) followed by re-concentration of the probes using Microcon YM-3 spin-columns (Millipore-Amicon, Eschborn, Germany). N-terminal amino acid sequencing was performed by sequential Edman degradation. Molecular masses were determined by positive-ion matrix-assisted laser desorption/ionization (MALDI) analysis using a Bruker REFLEX time-of-flight (TOF) instrument. Protein samples were mixed with sinapinic acid as a matrix and dried at room temperature. Spectra were recorded in the linear mode. Protein masses of the deduced amino acid sequences were calculated with PAWS 8.1.1 (freeware edition, Proteometics). Amino acid sequence comparisons were carried out using Clustal W (*28*).

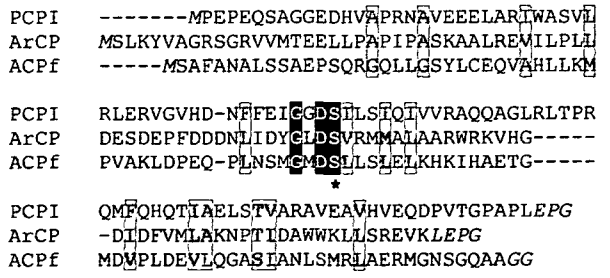

Fig. 1. **Amino acid sequence alignment of the heterologously expressed thiolation domains PCPI, ArCP, and PCPf.** Conserved and identical residues are shaded in grey or black, respectively. The invariant serine is indicated by an asterisk. Amino acids represented by italic letters were changed or added during the cloning process.

RESULTS AND DISCUSSION

Some 4′-PP-transferases such as EntD from *E. coli* or Sfp and Gsp from *Bacillus* were characterised in detail (see Introduction). The genes encoding these activating enzymes are located in close vicinity to the genes that code for the corresponding protein substrates. This chromosomal arrangement is indicative of a common transcriptional regulation of the genes and also of the specificity of each of the 4′-PP transferases for their respective acceptor molecules. On the other hand, examples have been described with no such correlations. Many polyketide biosynthetic gene clusters lack a gene encoding a 4′-PP transferase (*29*, *30*). Obviously, these PKSs are activated by enzymes that are encoded by genes localized elsewhere within the chromosome. In this respect, the myxothiazol biosynthetic gene cluster from *S. aurantiaca* DW4/3-1 represents an exception. The gene *mtaA* is located directly upstream of the gene cluster that encodes the proteins of a multifunctional enzyme complex containing both PKS and NRPS modules (*19*). We set up a series of experiments to prove that *mtaA* indeed encodes a 4′-PP transferase. A thiolation domain of the myxothiazol synthetase and cognate domains of other sources were used to test whether MtaA is able to phosphopantetheinylate both ACP and PCP domains.

Cloning Strategy and Expression of MtaA and Thiolation Domains—Fragments of approximately 300 bp encoding PCPI and ACPf from a peptide synthetase of *So. cellulosum* (RM and SB, unpublished results) and the myxothiazol synthetase (MtaF) from *S. aurantiaca* DW4/3-1 (*19*) were defined according to the recently expressed ArCP domain of the enterobactin synthetase from *E. coli* (*10*).

The expression of the thiolation domains as part of a PKS or a NRPS should result in peptides in which the conserved serine residue to which the 4′-PP is attached is flanked by approx. 50 amino acids (Fig. 1). We utilized a bivector system for *in vivo* production of non-pantetheinylated and pantetheinylated ACP and PCP domains. To achieve this, the *mtaA* gene was placed on the plasmid pSU18 (pSUMtaA), and the DNA fragments encoding the thiolation domains were introduced into the pCYB2 vector (pArCP, pPCPI, pACPf). These two types of plasmids are mutually compatible due to their different origins of repli-

cation (ColE1, P15A) and antibiotic selection markers (ampicillin, chloramphenicol). Transcription of *mtaA* was driven by the P_{lac} promoter/operator box, whereas the gene fragments for thiolation domains were placed under the control of the P_{tac} promoter/operator sequence. Due to the $lacI^q$ gene product encoded by pCYB2, the expression of MtaA (molecular mass of 31.6 kDa) and the carrier protein domains was inducible by IPTG.

No detectable amounts of expressed ACP or PCP domains were obtained in initial attempts using the pET-expression systems. Therefore, the strategy was changed in favor of the expression system based on the plasmid pCYB2. In this system, translation products encoded by the expression cassette result in the formation of a fusion protein containing an intein-chitin binding domain. The molecular mass of the polypeptide chains should therefore increase by 55 kDa, compared to the single thiolation domains. Although more reliable expression conditions were expected, only ArCP and PCPI were detectable as weak signals in Coomassie-stained polyacrylamide gels. Faint protein bands with a size of about 67 kDa were obtained, which were not visible in control cell extracts (exemplary shown for ArCP in Fig. 2A). Several ACP or PCP domains cloned from the myxothiazol synthetase genes could not be expressed in *E. coli*. An analysis of the expected gene products of various thiolation domains used during this study revealed that only those having a relatively low pI value could successfully be expressed in *E. coli*. For example, the second and the third ACP domains of the myxothiazol synthetase subunit MtaB have a calculated pI of about 10.5. In contrast, the thiolation domains ACPf (MtaF), PCPI (*So. cellulosum*) and ArCP (EntB, *E. coli*) are characterised by a pI of less than 6. By up-scaling the *E. coli* culture volumes to 500 ml, subsequent enrichment by affinity chromatography and concentration of the eluates, it was possible to recover the amount of thiolation domains necessary for further experiments (exemplary shown for PCPI in Fig. 2B). Nevertheless, the total amount of protein obtained was low. N-terminal protein sequencing confirmed the production of ArCP, ACP, and PCP domains and also showed the absence of the N-terminal amino acid methionine.

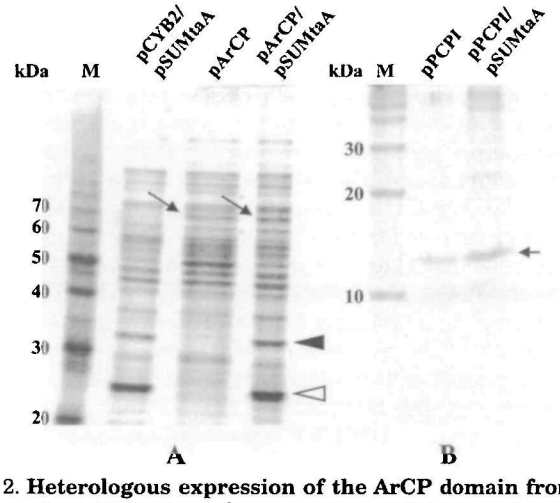

Fig. 2. **Heterologous expression of the ArCP domain from *E. coli* and of MtaA from *S. aurantiaca* DW4/3-1 and enrichment of the heterologously expressed PCPI domain of *So. cellulosum* So ce90.** A: Coomassie-stained polyacrylamide gel (10%). Cell-free extracts of recombinant *E. coli* XL1blue carrying the plasmids as indicated. Arrows indicate the ArCP-intein-chitin fusion protein (encoded by pArCP), the solid arrowhead indicates MtaA and the open arrowhead points at the chloramphenicol acetyl transferase (both encoded by pSUMtaA). B: Coomassie-stained polyacrylamide gel (16%). PCPI-domains enriched by chitin affinity chromatography from cell-free extracts of *E. coli* XL1blue carrying pPCPI and pPCPI and pSUMtaA. Lane M: standard proteins (10 kDa protein ladder, Gibco BRL). The PCPI-domains are indicated by an arrow.

Substrate Specificity of MtaA—The ACPf, ArCP, and PCPI domains that were heterologously expressed in *E. coli* span the amino acid region responsible for forming the active fold of the thiolation domains (*cf.* Fig. 1 and Ref. *31*). Therefore, a phosphopantetheinylating enzyme should be able to recognise these domains as an appendage of the intein-chitin fusion protein encoded by the vector pCYB2. To examine the modifying capacities of MtaA, mass spectrometry analyses were performed. The ACPf domain of the myxothiazol synthetase, and the ArCP and PCPI domains of *E. coli* and *So. cellulosum*, respectively, were heterologously produced in the presence and absence of MtaA. Subsequently, the molecular masses of the purified thiolation domains were determined by MALDI-TOF. This analysis routine has already been described by others (*16*, *18*, *32*). The formation of the holo-domain is indicated by a mass increase of 340 Da in respect to the apo-form. Masses differing by approximately 136 Da were also observed and are explainable by the purification procedure: the cleavage of the target protein from the intein-chitin tag is catalyzed by DTT, which is temporarily covalently attached to the C-terminal amino acid (see instruction manual of the purification kit).

Expected masses of all thiolation domains produced in *E. coli* in the absence of MtaA were observed, indicating a successful expression of the apo-forms of ArCP, PCPI and ACPf (Table I, Fig. 3). Interestingly, an additional signal was observed in all samples indicating a phosphothiolation

TABLE I. **Molecular mass data (kDa) of ArCP, PCPI, and ACPf heterologously produced in *E. coli*.**

	ArCP		PCPI		ACPf	
Calculated[a]						
apo	11,348		11,538		10,545	
holo	11,688		11,878		10,885	
	−MtaA	+MtaA	−MtaA	+MtaA	−MtaA	+MtaA
Determined[b]	11,352		11,536		10,541	
	11,693	11,695	11,877	11,880	10,887	10,884
	11,348		11,539		10,535	
	11,687	11,691	11,880	11,878	10,875	10,883

[a]Average isotopic mass minus the N-terminal methionine. [b]Detected mass −1, due to the charge of ionization; results of two independent expression experiments.

of the respective domains. The difference of approximately 340 Da agrees well with the masses of holo-thiolation domains. Observation of a 4'-PP transfer to heterologously expressed thiolation domains by endogenous *E. coli* enzymes has been previously reported for several other type I and type II ACP and PCP domains (*9*, *32*). It is not yet known which of the *E. coli* 4'-PP transferases described (*5*) is involved in this modification reaction: ACPS usually catalyses the transfer of 4'-PP to apo-ACP of the FAS complex. The biological purpose of YhhU (formerly o196) has not been elucidated to date. Both enzymes have restricted substrate specificity (*5*, *8*, *9*, *11*). EntD, which is involved in the activation of the enterobactin synthetase of *E. coli*, represents a putative candidate for the endogenous modification of the thiolation domains tested in this study. *In vitro* experiments demonstrated that EntD has a broader substrate specificity. In contrast to ACPS and YhhU, it is able not only to activate its specific substrate ArCP but also to convert a PCP domain of the tyrocidine synthetase into its corresponding holo-form (*5*). However, involvement of EntD in the phosphopantetheinylation of the ArCP, PCPI, and ACPf domains is questionable, since the transcription of *entD* in *E. coli* responds to the intracellular concentration of iron (*33*). Under normal culture conditions used in our experiments, iron is present in abundance and represses in concert with the transcriptional regulator Fur the expression of the enterobactin gene cluster including *entD*.

However, as shown for PCPI (Fig. 3a), the catalytic capacity of the endogenous 4'-PP transferring enzymes is obviously insufficient to guarantee the complete formation of the overexpressed holo-thiolation domains. ACPS and, perhaps to a lesser extent, YhhU are also probably synthesised to those levels required to convert the endogenous apo-ACPs. Obviously, any additional thiolation substrate will strain ACPS and YhhU. This possibly explains why heterologously expressed apo-ACP or apo-PCP domains of various systems (see above) are always only phosphopantetheinylated in small percentages, and it explains why a complete conversion can only be achieved by overexpression of a 4'-PP transferase.

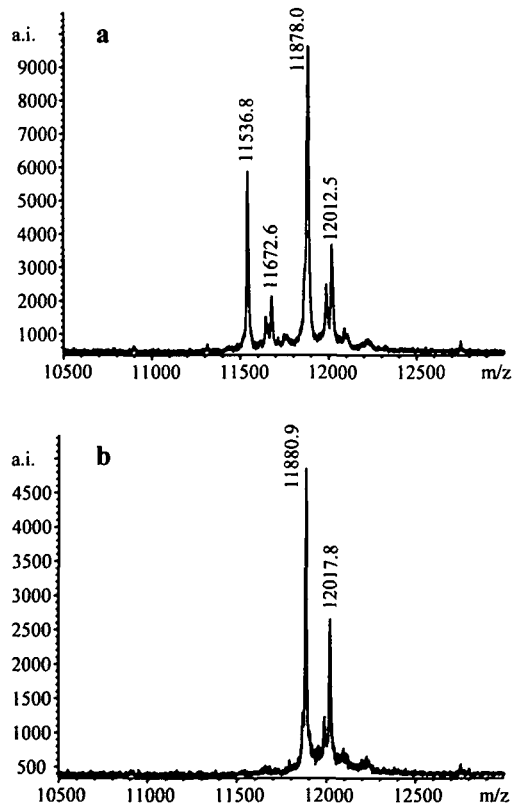

Fig. 3. **Phosphopantetheinylation of the PCPI-domain from *So. cellulosum* co-expressed in *E. coli* with and without MtaA from *S. aurantiaca*.** MALDI-TOF analysis of PCPI-domains purified from (a) *E. coli* XL1blue (pPCPI) and (b) *E. coli* XL1blue (pPCPI/pSUMtaA).

Thiolation domains co-expressed with the 4'-PP transferase MtaA of *S. aurantiaca* DW4/3-1 in *E. coli* (Fig. 2A) showed prominent signals representing only the 4'-PP form in the mass spectra (example shown for PCPI, Fig. 3b). No masses corresponding to the apo-forms of the domains were detected after co-expression (Table I). This demonstrates that MtaA does indeed completely convert the apo-form of its cognate substrate ACPf into the corresponding holo-form *in vivo*. Interestingly, MtaA does not seem to be able to discriminate between thiolation domains derived from the myxothiazol synthetase and those coming from different enzyme complexes even from different organisms, such as the enterobactin synthetase from *E. coli* or a peptide synthetase from *So. cellulosum*. The separately expressed thiolation domains ArCP and PCPI of those complex enzymes also served as substrates for MtaA (Table I, Fig. 3b).

This study corroborates the proposed function of the gene *mtaA* made by Silakowski *et al.* (*19*). *In vivo* experiments show that the gene product acts as a 4'-PP transferase and that it is able to activate not only the thiolation domains of the myxothiazol biosynthetic complex but also the ArCP and the PCP domains from different type I PKS or NRPS systems.

Heterologous production of secondary metabolites of the polyketide or the nonribosomal polypeptide type requires not only efficient expressions systems but also a posttranslational modification system that activates the biosynthetic enzymes produced. Since recombinant PKSs and NRPSs produced in *E. coli* are only partially phosphopantetheinylated (*34–37*), the co-expression of a 4'-PP transferase could circumvent this problem. The most commonly used gene for this purpose is *sfp* from *B. subtilis*. Several thiolation domains, especially those of the ArCP and PCP type, are recognised and converted to the holo-form by Sfp (*16*). MtaA from *S. aurantiaca* DW4/3-1 could be a further candidate for guaranteeing the production of catalytically active PKS, NRPS, or mixed PKS/NRPS systems in heterologous hosts. A major difference between the genes of *sfp* and *mtaA* is their G+C content. Sfp (Genbank accession number X63158) is encoded by a gene with a G+C content of 46.8 mol%, whereas the gene *mtaA* (Genbank accession number AF188287) consists of 66.1 mol% G+C. Therefore, MtaA could be a suitable alternative to Sfp, if co-expression of a 4'-PP transferase is necessary in organisms with a protein translation machinery adapted to G+C rich mRNAs, such as members of the genus *Pseudomonas*.

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