J Ind Microbiol Biotechnol (2003) 30: 510–515
DOI 10.1007/s10295-003-0075-8

ORIGINAL PAPER

Miho Izumikawa · Paul R. Shipley
Jörn N. Hopke · Thomas O'Hare · Longkuan Xiang
Joseph P. Noel · Bradley S. Moore

Expression and characterization of the type III polyketide synthase 1,3,6,8-tetrahydroxynaphthalene synthase from *Streptomyces coelicolor* A3(2)

Received: 13 December 2002 / Accepted: 18 April 2003 / Published online: 7 August 2003

Abstract Sequence analysis of the metabolically rich 8.7-Mbp genome of the model actinomycete *Streptomyces coelicolor* A3(2) revealed three genes encoding predicted type III polyketide synthases (PKSs). We report the inactivation, expression, and characterization of the type III PKS homologous SCO1206 gene product as 1,3,6,8-tetrahydroxynaphthalene synthase (THNS). Incubation of recombinant THNS with malonyl-CoA showed THN production, as demonstrated by UV and HPLC analyses. The K_m value for malonyl-CoA and the k_{cat} value for THN synthesis were determined spectrophotometrically to be 3.58 ± 0.85 μM and 0.48 ± 0.03 min^{-1}, respectively. The C-terminal region of *S. coelicolor* THNS, which is longer than most other bacterial and plant type III PKSs, was shortened by 25 amino acid residues and the resulting mutant was shown to be slightly more active ($K_m = 1.97 \pm 0.19$ μM, $k_{cat} = 0.75 \pm 0.04$ min^{-1}) than the wild-type enzyme.

Keywords Chalcone synthase · Polyketide synthase · Tetrahydroxynaphthalene

M. Izumikawa · P. R. Shipley · J. N. Hopke · T. O'Hare
L. Xiang · B. S. Moore (✉)
College of Pharmacy, University of Arizona,
1703 E. Mabel, Tucson,
AZ 85721-0207, USA
E-mail: moore@pharmacy.arizona.edu
Tel.: +1-520-6266931
Fax: +1-520-6262466

J. P. Noel
Structural Biology Laboratory,
The Salk Institute for Biological Studies,
10010 North Torrey Pines Road,
La Jolla, CA 92037, USA

B. S. Moore
Department of Chemistry,
University of Arizona, Tucson, AZ 85721, USA

Introduction

A newly discovered polyketide biosynthetic pathway for the assembly of small aromatic metabolites was recently characterized in bacteria [20]. This pathway utilizes novel bacterial polyketide synthases (PKSs) termed type III PKSs, which are most closely related to the chalcone synthase (CHS)/stilbene synthase (STS) superfamily of plant PKSs [1, 24]. Type III PKSs are structurally and mechanistically distinct from the type I and type II PKSs and use coenzyme A (CoA)-linked thioester substrates rather than 4′-phosphopantetheine residues on acyl carrier proteins. Members of the CHS/STS superfamily of condensing enzymes are relatively modest-sized proteins of 40–47 kDa that function as homodimers. Although several bacterial type III PKSs have been identified through DNA sequence analysis, to date only two have been biochemically characterized (Fig. 1): 1,3,6,8-tetrahydroxynaphthalene synthase (THNS) from several actinomycetes [6, 10, 11, 12] and 3,5-dihydroxyphenylacetic acid synthase from two *Amycolatopsis* strains in the vancomycin group [5, 18, 22]. A third bacterial type III PKS, PhlD from *Pseudomonas fluorescens*, has been implicated through genetic studies to be involved in 2,4-diacetylphloroglucinol biosynthesis [2, 19].

Different groups of type III PKSs form monophyletic clusters, as shown by strong bootstrap support values that are suggestive of distinct biosynthetic specifications (Fig. 2). The largest group by far comprises seven actinomycete sequences that include the first characterized bacterial type III PKS protein RppA from *Streptomyces griseus* [12], which functions as a THNS. Analysis of the complete 8,667,507 base pair (bp) genome of the model actinomycete *S. coelicolor* A3(2) revealed three putative type III PKS genes of unknown functions [3]. *S. coelicolor* A3(2) gene SCO1206 (accession number CAC01488) appears to encode a *S. griseus* RppA-like THNS whose THN product may be further modified by tailoring enzymes encoded by neighboring genes. This

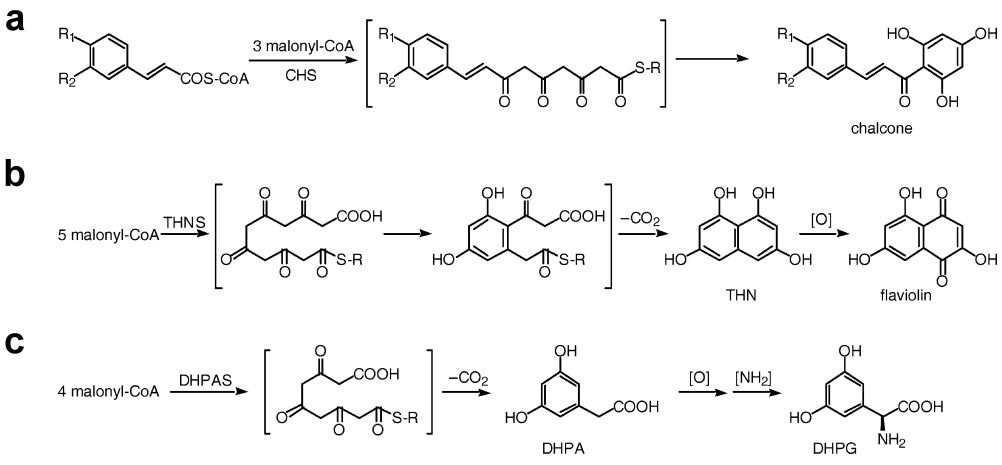

Fig. 1 Biosynthesis of type III polyketide synthase (PKS) products: **a** chalcone in higher plants, **b** 1,3,6,8-tetrahydroxynaphthalene (*THN*) in *Streptomyces griseus* and *S. coelicolor*, and **c** 3,5-dihydroxyphenylacetic acid (*DHPA*) in *Amycolatopsis mediterranei* and *A. orientalis*

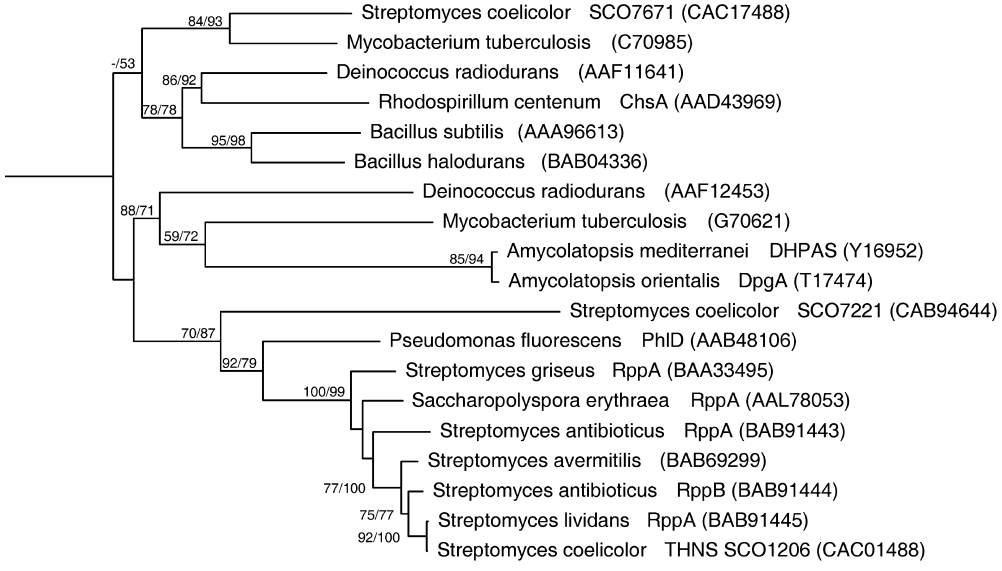

Fig. 2 Phylogenetic tree of bacterial type III PKSs. Sequences were retrieved from GenBank (accession numbers given in parentheses). The tree was rooted with a bacterial fatty acid synthase sequence as outgroup (data not shown). *Numbers at branching points* are quartet puzzling/ProtDist bootstrap support values. Only values > 50% are shown

type III PKS pathway leading to red-brown pigments, which was first documented in *S. griseus* [25], appears to be commonly associated with actinomycetes. The remaining *S. coelicolor* A3(2) type III PKS genes, SCO7221 and SCO7671, (Fig. 2) are less sequence homologous and may encode functionally novel type III PKSs.

S. coelicolor A3(2) harbors more than 20 secondary metabolic gene clusters, several of which are involved in the synthesis of pigmented compounds [3]. These include *act* (the blue polyketide antibiotic actinorhodin) [7], *red* (red oligopyrrole prodiginine antibiotics) [4], and *whiE* (grey spore pigment of unknown polyketide structure) [27]. Even though these three aforementioned gene clusters were chromosomally deleted in *S. coelicolor* YU105 [26], the mutant strain still produced a light orange pigment, which may be attributable to a type III PKS-derived product. A recent proteomic analysis of *S. coelicolor* A3(2), however, did not identify any representative proteins associated with any of the three type III PKS-containing gene clusters [13]. We thus set out to determine the in vivo and in vitro function of the RppA homologous SCO1206 gene in *S. coelicolor* A3(2). Our results demonstrate that, although this gene indeed codes for THNS, it does not contribute toward pigmentation in *S. coelicolor* A3(2) much like it does in other actinomycetes.

Materials and methods

Bacterial strains, culture conditions, and chemicals

S. coelicolor A3(2) and YU105 were obtained from Prof. D.A. Hopwood (John Innes Centre, Norwich, UK) and Dr. T.-W. Yu (University of Washington, Seattle, Wash.), respectively. *Escherichia coli* strains BL21(DE3)pLysS (Invitrogen) and XL1-Blue were used for expression and subcloning, respectively, and were grown on LB plates or in LB or TB liquid medium (1.2% Bacto tryptone, 2.4% Bacto yeast extract, 0.4% glycerol). All chemicals were from Sigma (St. Louis, Mo.) unless otherwise indicated.

DNA manipulations

S. coelicolor A3(2) total genomic DNA was isolated as previously described [16]. Recombinant DNA procedures were performed by standard techniques [16, 23]. Biotin-labeling and detection of chemiluminescent positives were performed with the DNADetector HPR Southern blotting kit (KPL, Gaithersburg, Md.). Oligonucleotides were obtained from Sigma Genosys (The Woodlands, Tex.). PCR was carried out on a PTC-2000 thermal cycler (MJ Research, Waltham, Mass.) with Taq (Gibco BRL, Gaithersburg, Md.) or PfuTurbo (Stratagene, La Jolla, Calif.) DNA polymerase. DNA sequencing by BigDye terminator cycle sequencing reaction using an ABI 377 sequencer (ABI, Toronto, Canada) was performed at the Laboratory of Molecular Systematics and Evolution (University of Arizona, Tucson, Ariz.).

Gene inactivation of SCO1206

The SCO1206 disruption vector was constructed as follows. A 760-bp internal fragment of SCO1206 was PCR-amplified from *S. coelicolor* A3(2) genomic DNA with the primers 5′-G*GA ATTC*CCGCACCCGGCACATC-3′ and 5′-AA*CTGCAG* GTTGCCGTACTCGGTG-3′, digested with *Eco*RI and *Pst*I (the introduced restriction sites are indicated by italics), and cloned into the appropriate sites of the suicide vector pDH5 [14]. This plasmid was then introduced via transformation into *S. coelicolor* A3(2) and YU105 as previously described [16]. The SCO1206 single-crossover mutants were selected after propagating transformants on R2YE plates with 50 μg thiostrepton ml^{-1} at 30 °C; and thiostrepton-resistant colonies were confirmed by Southern hybridization with biotinylated SCO1206. Spores of both mutants were spread on culture plates of R2YE [10.3% sucrose, 0.025% K_2SO_4, 1.012% $MgCl_2{\cdot}6H_2O$, 1% glucose, 0.01% Difco casamino acids, 0.5% Difco yeast extract, 0.005% KH_2PO_4, 0.2944% $CaCl_2{\cdot}2H_2O$, 0.3% L-proline, 0.573% *N*-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer (pH 7.2), 0.2% trace element solution (0.00004% $ZnCl_2$, 0.0002% $FeCl_3{\cdot}6H_2O$, 0.00001% $CuCl_3{\cdot}2H_2O$, 0.00001% $MnCl_2{\cdot}4H_2O$, 0.00001% $Na_2B_4O_7{\cdot}10H_2O$, 0.00001% $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$), 0.5% 1N NaOH, 2.2% Difco Bacto agar], TSB (3% Oxoid tryptone soya broth powder), ISP-4 (3.7% Bacto ISP medium 4) and 2CM [1% potato starch, 1% NaCl, 0.2% $(NH_4)_2SO_4$, 0.1% K_2HPO_4, 0.2% $CaCl_2$, 0.2% $MgSO_4{\cdot}7H_2O$, 0.2% Bacto tryptone, 0.1% inorganic solution (0.1% $FeSO_4{\cdot}7H_2O$, 0.1% $MgCl_2{\cdot}6H_2O$, 0.1% $ZnSO_4{\cdot}7H_2O$), 2% Bacto agar] containing 50 μg thiostrepton ml^{-1} and incubated at 30 °C for 6 days. The agar culture plates were extracted with 95:5 ethyl acetate:methanol and the resulting organic extract was dried, re-dissolved in methanol, and analyzed by reversed-phase HPLC.

Expression and purification of recombinant THNS

Open reading frame (ORF) SCO1206 was PCR-amplified from *S. coelicolor* A3(2) genomic DNA with the primers 5′-GC*GA ATTC*GGCACCCCTGAAAAGGTGCAC-3′ and 5′-GG*TCT AGA*TCATGCCTGCCTCACCCTCCG-3′, digested with *Eco*RI and *Xba*I (the introduced *Eco*RI and *Xba*I restriction sites are in italics), ligated into *Eco*RI/*Xba*I-digested pMAL-c2x (New England Biolabs, Beverly, Mass.), and its sequence verified. The SCO1206-containing *Eco*RI/*Hin*dIII fragment was excised from pMAL-c2x-THNS, cloned into the pHIS8 expression plasmid [15], and overexpressed as a N-terminal octahistidyl-tagged protein in *E. coli* BL21(DE3)pLysS. Transformed *E. coli* was grown at 37 °C in 150 ml TB containing 50 μg kanamycin ml^{-1} (Gibco BRL) and 37 μg chloramphenicol ml^{-1} until the optical density at 600 nm reached 0.7. After induction with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG), growth was allowed to continue for an additional 20 h at 28 °C and the protein was purified as previously described for CHS [15]. The cells were harvested by centrifugation for 20 min at 2,700 *g* and the pellet was resuspended in 15 ml of lysis buffer (50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 20 mM imidazole, pH 8.0, 20 mM β-mercaptoethanol, 10% glycerol, 1% Tween-20). The solution was then sonicated and the soluble proteins were collected by centrifugation for 30 min at 17,900 *g* and 4 °C. The supernatant was passed over a Hi-Trap metal chelate column (Pharmacia–Amersham, Piscataway, N.J.) and the column was washed with 10 bed-volumes of lysis buffer and 10 bed-volumes of lysis buffer minus Tween-20. The His8-tagged protein was eluted with lysis buffer minus Tween-20 but containing 250 mM imidazole (pH 8.0). Individual fractions were analyzed by SDS-PAGE, pooled, concentrated to a volume of 2.5 ml, and, after the addition of EDTA (final concentration 0.1 mM), subjected to buffer exchange into 10 mM Tris-HCl (pH 7.5), 25 mM NaCl, 5 mM dithiothreitol, using a PD-10 desalting column (Pharmacia–Amersham). The protein was concentrated to 10 mg ml^{-1}, using a Microcon-30 filtration device (Millipore, New York, N.Y.), and stored at −80 °C in 100-μl aliquots until needed.

Construction of C-terminal truncated mutant

The C-terminal truncated THNS mutant was amplified by PCR from pHIS8(THNS) with the primers 5′-GGCC*GAATTC*GCGAC TTTGTGCAGACCCTCG-3′ and 5′-CCGC*AAGCTT*GTCAGT CCGCCGTCTGCCAGC-3′, digested with *Eco*RI and *Hin*dIII (the introduced *Eco*RI and *Hin*dIII restriction sites are in italics), ligated into *Eco*RI/*Hin*dIII-digested pHIS8, and sequence-verified. Protein expression and purification were carried out in a manner analogous to that described for wild-type THNS.

THN synthase assay

THNS activity was determined by measuring the conversion of malonyl-CoA into THN. The standard assay conditions contained 100 mM Tris-HCl buffer (pH 7.5), 0.43 μM THNS enzyme, and 0.2 mM malonyl-CoA. UV absorbance was monitored at 340 nm ($\epsilon = 18{,}000$ for THN). The reaction was additionally followed by HPLC. It was carried out in 100 mM Tris-HCl (pH 7.5) containing 1 mM malonyl-CoA and 20 μM enzyme in 100 μl total volume and was conducted at room temperature for 16 h in 1.5-ml tubes. Following incubation, the sample was quenched with 5 μl of concentrated HCl. Then, 10 μl of the sample was directly analyzed by HPLC. Separations were achieved using a YMC ODS-AQ 4.6×150 mm reversed-phase column with a linear solvent gradient of 0.15% trifluoroacetic acid in water to methanol over 30 min, at a flow rate of 0.5 ml min^{-1}. Alternatively, the sample was extracted with 3×100 μl of ethyl acetate, dried, and re-dissolved in 50 μl of methanol for HPLC analysis.

Kinetic analysis

Kinetic constants were determined from initial velocity measurements, in which product formation was linear over the time periods monitored, using the standard assay conditions with 1.07 μM THNS and varied malonyl-CoA concentrations (3–20 μM).

Results

Inactivation of the THNS-encoding gene SCO1206

To test whether the RppA-homologous THNS contributes to *S. coelicolor* mycelial pigmentation, we disrupted SCO1206 by single-crossover homologous recombination. A pDH5-based suicide plasmid carrying a 760-bp internal fragment of SCO1206 was introduced into

wild-type *S. coelicolor* A3(2) and the slightly pigmented mutant strain YU105. No phenotypic differences were detected in the wild-type strain, due to intense pigmentation by the *act* and *red* metabolites. Furthermore, the transformed YU105 strain did not noticeably differ from non-transformed YU105 when grown on various solid media, suggesting that the unknown light orange pigment originates from another pathway or that the SCO1206-containing locus is silent.

Expression of THNS in *E. coli*

The putative THNS-encoding gene SCO1206, which has an identity/similarity score of 70/83 in relation to *S. griseus* RppA (THNS) [12], was next cloned and expressed. The 1.2-kbp PCR product was initially cloned into pMAL-c2x, introducing a maltose-binding protein affinity tag at the N-terminus. Unfortunately, the activity of the recombinant protein was considerably affected upon amylose affinity purification and removal of the affinity tag. Consequently, the THNS-containing fragment was excised from pMAL-c2x-THNS, cloned into the pHIS8 expression plasmid [15], and overexpressed as a N-terminal octahistidyl-tagged protein in *E. coli* BL21(DE3)/pLysS. Upon induction with IPTG, the *E. coli* transformant produced a dark red pigment, which was easily extracted into ethyl acetate upon acidification. Reversed-phase HPLC-mass spectrometric analysis of the crude organic extract verified the presence of the pigment flaviolin, a known auto-oxidation product of THN [9]. Recombinant *S. coelicolor* THNS was purified as previously described for CHS [15] in greater than 80% yield, following Ni^{2+}-affinity chromatography over a Hi-Trap metal chelate column, as determined by SDS-PAGE. Its mobility upon SDS-PAGE corresponded to a mass of 43 kDa, in close agreement with the value of 43,364 calculated for the native protein. Approximately 5–6 mg of the recombinant His_8-tagged protein was isolated per 300 ml of culture.

Characterization of recombinant THNS

To confirm that the purified synthase was indeed generating THN, the recombinant protein was incubated with malonyl-CoA and the resulting products analyzed by UV spectroscopy and HPLC. Enzymatic activity was assayed spectrophotometrically at 30 °C by monitoring the increase in absorbance at 340 nm accompanying the formation of THN. The reaction mixture was directly analyzed by reversed-phase HPLC. Within the first hour, malonyl-CoA was completely consumed, resulting in the maximum production of THN (Fig. 3). Over time, the amount of THN decreased with a concomitant increase in its auto-oxidized product flaviolin. The identities of CoA, malonyl-CoA, acetyl-CoA, THN, and flaviolin were confirmed by HPLC-mass spectrometry and by comparison with authentic standards.

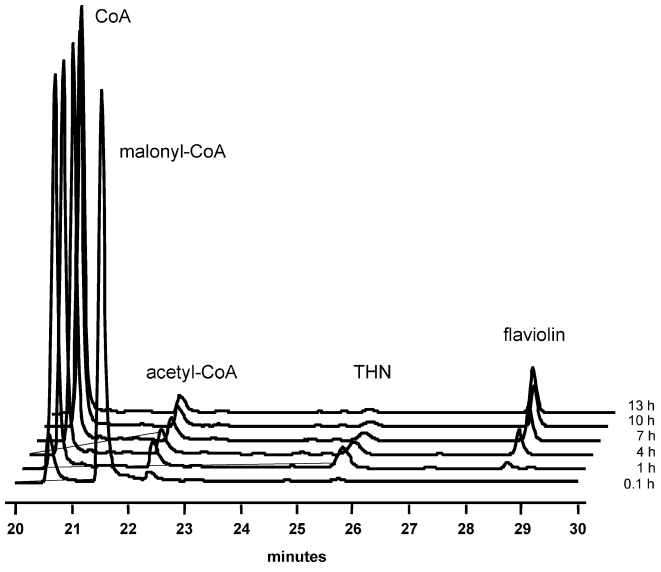

Fig. 3 Reaction profile of the conversion of malonyl-CoA to THN and CoA by *S. coelicolor* THNS, as monitored by reversed-phase HPLC at 254 nm. Although the enzymatic reaction is complete within 1 h, as shown by the total consumption of malonyl-CoA, THN is modified by auto-oxidation, ultimately forming flaviolin over longer periods of time. This non-enzymatic oxidative conversion can be inhibited when run under an inert atmosphere in oxygen-free buffers

Kinetic parameters for THN formation

The steady-state kinetic parameters of recombinant *S. coelicolor* THNS were determined spectrophotometrically. Kinetic constants were calculated from initial velocity measurements at 340 nm ($\epsilon = 18{,}000$ for THN), in which product formation was linear over the time periods monitored. The kinetic experiments showed that the recombinant *S. coelicolor* THNS was approximately six times less active ($k_{cat} = 0.48 \pm 0.03$ min^{-1}, $K_m = 3.58 \pm 0.85$ μM) than the *S. griseus* recombinant RppA, which was analyzed using a radiometric assay with [2-^{14}C]malonyl-CoA [12]. While k_{cat} for the two enzymes is similar, K_m is considerably lower in the *S. coelicolor* enzyme, suggesting a difference in malonyl-CoA binding efficiencies in the two enzymes.

Analysis of the C-terminal region

S. coelicolor THNS has the longest C-terminal region amongst most other bacterial and plant type III PKSs, including THNSs (Fig. 4). To evaluate the function of this region towards catalysis, we prepared a C-terminal truncated THNS mutant that was shortened by 25 amino acid residues, beginning at position 350. The kinetic parameters ($k_{cat} = 0.75 \pm 0.04$ min^{-1}, $K_m = 1.97 \pm 0.19$ μM) showed that the mutant was slightly more active than the wild-type enzyme, suggesting that the C-terminal addition common amongst bacterial type III PKSs in relation to plant enzymes is not necessary for

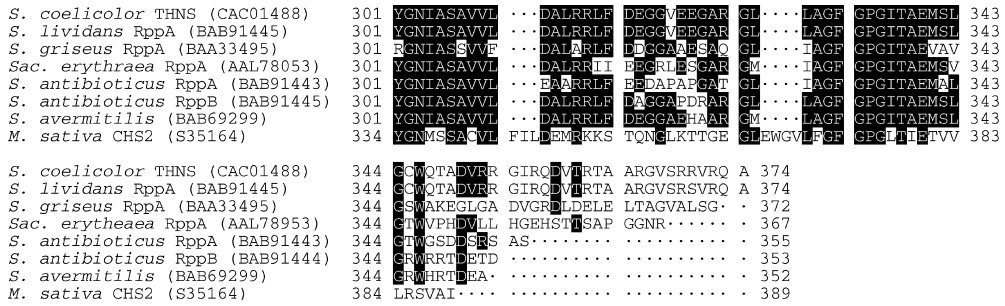

Fig. 4 Sequence alignment of the C-termini of bacterial THNSs and CHS2 from *Medicago sativa* (alfalfa). Sequences were retrieved from GenBank (accession numbers given in parentheses)

enzymatic activity, but may rather be involved in protein–protein interactions with downstream processing enzymes.

Discussion

The model actinomycete *S. coelicolor* A3(2) harbors three type III PKS homologous genes, one of which, SCO1206, was shown in this study to encode a RppA-like THNS. Disruption of the ORF SCO1206, however, failed to alter the light-orange phenotype of the *act*/*red*/*whiE*-deficient mutant *S. coelicolor* YU105 when grown on several different agars, suggesting that the *S. coelicolor rppA* gene is not expressed under normal growth conditions and may in fact be silent. This notion is supported by a recent proteomic analysis of *S. coelicolor* A3(2), in which none of the three Rpp proteins associated with the operon SCO1206–SCO1208 were detected [3]. Hence, the unidentified light-orange pigment of *S. coelicolor* YU105 may rather be the carotenoid isorenieratine [17] associated with the uncharacterized carotenoid biosynthesis genes SCO0185–SCO0191 [3].

In vivo and in vitro expression of the *S. coelicolor* SCO1206 gene product did indeed demonstrate that this enzyme functions as a THNS. The protein belongs to a growing family of pentaketide-forming enzymes responsible for pigment biosynthesis in actinomycetes (Fig. 2). Seven bacterial THNSs, all of which come from actinomycetes, have been characterized to date and share many features with the plant PKSs. Although *S. coelicolor* THNS and *Medicago sativa* (alfalfa) CHS2 [8] share only 25% amino acid identity, there are a number of conserved features, including the catalytic triad residues [11], which allow the construction of a three-dimensional homology model of THNS based upon the known CHS structure [21]. In addition to the conserved catalytic cysteine residue at CHS position 164, the bacterial THNSs harbor three to four additional cysteine residues in their active sites, which is suggestive of novel biochemistry. The large number of active site cysteine residues may explain why the *S. coelicolor* THNS can be stabilized with EDTA to prevent metal poisoning. The bacterial THNSs furthermore share an abbreviated N-terminus, which often begins at CHS residue 15, and a variably extended C-terminus. For instance, while *S. griseus* THNS is two amino acids shorter than the *S. coelicolor* and *S. lividans* THNSs, the *S. antibioticus* and *S. avermitilis* homologues are considerably shorter, much like CHS2 from *M. sativa* (Fig. 4). Our constructed C-terminal truncated mutant, which was shortened by 25 amino acid residues from position 350 to coincide with the terminal amino acid residue in CHS2, showed slightly higher activity than the wild-type enzyme, based on kinetic analysis. This observation suggests that the bacterial C-terminal addition is not necessary for the activity, but may rather be involved in protein–protein interactions with downstream processing enzymes involved in pigment biosynthesis.

While type III PKSs, including THNS, are architecturally simple, they arguably represent the most sophisticated PKSs mechanistically, since embodied within their homodimeric architecture is the catalytic machinery necessary for starter molecule recognition and loading, malonyl-CoA decarboxylation, polyketide extension, and multiple pathways for termination. Recent mutagenesis studies on the *S. griseus* THNS enzyme focused on many active site residues and showed that Tyr-224 is essential for starter unit selection, while Ala-305 is important for chain-length recognition [11]. We recently purified to homogeneity and solved the three-dimensional crystal structure of the *S. coelicolor* THNS protein, which will be reported in due course.

Acknowledgements We are grateful to D.A. Hopwood, John Innes Centre, Norwich, UK, for *S. coelicolor* A3(2), to T.-W. Yu, University of Washington, Seattle, Wash., for *S. coelicolor* YU105, and to I. Fujii and Y. Ebizuka, University of Tokyo, Tokyo, Japan, for authentic samples of THN and flaviolin. This work was supported in part by grants from the Petroleum Research Foundation administered by the American Chemical Society (34265-G4 to B.S.M.), the National Institutes of Health (AI52443 to B.S.M., J.P.N.), and the National Science Foundation (MCB9982586 to J.P.N.).

References

1. Austin MB, Noel JP (2002) The chalcone synthase superfamily of type III polyketide synthases. Nat Prod Rep 20:79–110
2. Bangera MG, Thomashow LS (1999) Identification and characterization of a gene cluster for the synthesis of the polyketide antibiotic 2,4-diacetylphloroglucinol from *Pseudomonas fluorescens* Q2–87. J Bacteriol 181:3155–3163
3. Bentley SD, Chater KF, Cerdeño-Tárraga A-M, Challis GL, Thomson NR, James KD, Harris DE, Quail MA, Kieser H, Harper D, et al (2002) Complete genome sequence of the model

actinomycete *Streptomyces coelicolor* A3(2). Nature 417:141–147
4. Cerdeno AM, Bibb MJ, Challis GL (2001) Analysis of the prodiginine biosynthesis gene cluster of *Streptomyces coelicolor* A3(2): new mechanisms for chain initiation and termination in modular multienzymes. Chem Biol 8:817–829
5. Chen HW, Tseng CC, Hubbard BK, Walsh CT (2001) Glycopeptide antibiotic biosynthesis: enzymatic assembly of the dedicated amino acid monomer (*S*)-3,5-dihydroxyphenylglycine. Proc Natl Acad Sci USA 98:14901–14906
6. Cortés J, Velasco J, Foster G, Blackaby AP, Rudd BAM, Wilkinson B (2002) Identification and cloning of a type III polyketide synthase required for diffusible pigment biosynthesis in *Saccharopolyspora erythraea*. Mol Microbiol 44:1213–1224
7. Fernández-Moreno MA, Martinez E, Boto L, Hopwood DA, Malpartida F (1992) Nucleotide sequence and deduced functions of a set of cotranscribed genes of *Streptomyces coelicolor* A3(2) including the polyketide synthase for the antibiotic actinorhodin. J Biol Chem 267:19278–19290
8. Ferrer J-L, Jez JM, Bowman ME, Dixon RA, Noel JP (1999) Structure of chalcone synthase and the molecular basis of plant polyketide synthesis. Nat Struct Biol 6:775–784
9. Fujii I, Mori Y, Watanabe A, Kubo Y, Tsuji G, Ebizuka Y (1999) Heterologous expression and product identification of *Colletotrichum lagenarium* polyketide synthase encoded by the *PKS1* gene involved in melanin biosynthesis. Biosci Biotechnol Biochem 63:1445–1452
10. Funa N, Ohnishi Y, Fujii I, Shibuya M, Ebizuka Y, Horinouchi S (1999) A new pathway for polyketide synthesis in microorganisms. Nature 400:897–899
11. Funa N, Ohnishi Y, Ebizuka Y, Horinouchi S (2002a) Alteration of reaction and substrate specificity of a bacterial type III polyketide synthase by site-directed mutagenesis. Biochem J 367:781–789
12. Funa N, Ohnishi Y, Ebizuka Y, Horinouchi S (2002b) Properties and substrate specificity of RppA, a chalcone synthase-related polyketide synthase in *Streptomyces griseus*. J Biol Chem 277:4628–4635
13. Hesketh AR, Chandra G, Shaw AD, Rowland JJ, Kell DB, Bibb MJ, Chater KF (2002) Primary and secondary metabolism, post-translational protein modifications, as portrayed by proteomic analysis of *Streptomyces coelicolor*. Mol Microbiol 46:917–932
14. Hillemann D, Pühler A, Wohlleben W (1991) Gene disruption and gene replacement in *Streptomyces* via single stranded DNA transformation of integration vectors. Nucleic Acids Res 19:727–731
15. Jez JM, Ferrer J-L, Bowman ME, Dixon RA, Noel JP (2000) Dissection of malonyl-coenzyme A decarboxylation from polyketide formation in the reaction mechanism of a plant polyketide synthase. Biochemistry 39:890–902
16. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA (2000) Practical *Streptomyces* genetics. The John Innes Foundation, Norwich
17. Krügel H, Krubasik P, Weber K, Saluz HP, Sandmann G (1999) Functional analysis of genes from *Streptomyces griseus* involved in the synthesis of isorenieratene, a carotenoid with aromatic end groups, revealed a novel type of carotenoid desaturase. Biochim Biophys Acta 1439:57–64
18. Li T-L, Choroba OW, Hong H, Williams DH, Spencer JB (2001) Biosynthesis of the vancomycin group of antibiotics: characterisation of a type III polyketide synthase in the pathway to (*S*)-3,5-dihydroxyphenylglycine. Chem Commun 2156–2157
19. Mavrodi OV, Gardener BBM, Mavrodi DV, Bonsall RF, Weller DM, Thomashow LS (2001) Genetic diversity of *phlD* from 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. Phytopathology 91:35–43
20. Moore BS, Hopke JN (2001) Discovery of a new bacterial polyketide biosynthetic pathway. Chem Biochem 2:35–38
21. Moore BS, Hertweck C, Hopke JN, Izumikawa M, Kalaitzis JA, Nilsen G, O'Hare T, Piel J, Shipley PR, Xiang L, Austin MB, Noel JP (2002) Plant-like biosynthetic pathways in bacteria: from benzoic acid to chalcone. J Nat Prod 65:1956–1962
22. Pfeifer V, Nicholson GJ, Ries J, Recktenwald J, Schefer AB, Shawky RM, Schröder J, Wohlleben W, Pelzer S (2001) A polyketide synthase in glycopeptide biosynthesis—the biosynthesis of the non-proteinogenic amino acid (*S*)-3,5-dihydroxyphenylglycine. J Biol Chem 276:38370–38377
23. Sambrook J, Russell DW (2001) Molecular cloning, a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
24. Schröder J (1999) The chalcone/stilbene synthase-type family of condensing enzymes. In: Sankawa U (ed) Comprehensive natural products chemistry, vol 1. Elsevier, Amsterdam, p 749–771
25. Ueda K, Kim K-M, Beppu T, Horinouchi S (1995) Overexpression of a gene cluster encoding a chalcone synthase-like protein confers redbrown pigment production in *Streptomyces griseus*. J Antibiot 48:638–646
26. Yu T-W, Hopwood (1995) Ectopic expression of the *Streptomyces coelicolor whiE* genes for polyketide spore pigment synthesis and their interaction. Microbiology 141:2779–2791
27. Yu T-W, Shen Y, McDaniel R, Floss HG, Khosla C, Hopwood DA, Moore BS (1998) Engineered biosynthesis of novel polyketides from *Streptomyces* spore pigment polyketide synthases. J Am Chem Soc 120:7749–7759