Cytochrome P450 oxidase SlgO1 catalyzes the biotransformation of tirandamycin C to a new tirandamycin derivative

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Received: 14 December 2018 / Accepted: 1 February 2019 / Published online: 9 February 2019
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Abstract

In the present study, an *Escherichia coli* whole cell system with overexpression of a cytochrome P450 oxidase SlgO1 involved in streptolydigin biosynthetic pathway, an *E. coli* flavodoxin NADP⁺ oxidoreductase (EcFLDR), and an *E. coli* flavodoxin A (EcFLDA) were constructed. Biotransformation experiments revealed that SlgO1 can convert tirandamycin C to tirandamycin F, indicating that it can introduce a hydroxyl group into the C-10 position of tirandamycin C. Subsequently, *slgO1* was cloned into pSET152AKE vector under the downstream of *ermE** promoter, which was, respectively, introduced into *Streptomyces* sp. SCSIO1666 (tirandamycin B producer), *Streptomyces* sp. Ju1008 (tirandamycin C producer), and *Streptomyces* sp. Ju1009 (tirandamycin E producer). A novel tirandamycin derivative tirandamycin L accumulated in the engineered strain *Streptomyces* sp. Ju1008::*slgO1* was isolated and its structure was determined on the basis of nuclear magnetic resonance (NMR) and mass spectrometry. Unlike most of the identified tirandamycins, tirandamycin L possessed a rare C-11–C-12 saturated bond as well as a C-10 ketone moiety. In addition, tirandamycin L showed weaker antibacterial activity. Based on the structure of tirandamycin L, SlgO1 was proposed to be responsible for multiple modifications toward tirandamycin C, including the formation of C-10 hydroxyl and C-11–C-12 saturated bond.

Keywords Tirandamycins · Streptolydigin · Cytochrome P450 oxidase SlgO1 · Combinatorial biosynthesis

Introduction

The cytochrome P450 oxidases (CYP450s) are heme-dependent enzymes which are ubiquitous in a wide range of organisms (Coon 2005; Munro et al. 2013). The enzymes of this family are involved in diverse reactions, and play important roles in steroid synthesis, xenobiotic catabolism, and biosynthesis of secondary metabolites (Lewis and Wiseman 2005; Munro et al. 2013; Alkhalaf et al. 2019). In biosynthetic pathways of microbial secondary metabolites,

the CYP450s often catalyze regio- and stereo-selectivity oxidations of precursors, leading to the structural diversification of the parental compounds as well as improved biological properties (Cochrane and Vederas 2014; Rudolf et al. 2017; Zhang and Li 2017). Up to now, a great number of CYP450s involved in biosynthetic pathways of natural products have been characterized, and many CYP450s catalyzing uncommon reactions have emerged (Rudolf et al. 2017; Zhang and Li 2017). Nowadays, CYP450s are important biocatalysts and making fundamental contributions to synthetic biology and biosynthesis of natural products (Bernhardt and Urlacher 2014; Girvan and Munro 2016; Zhou et al. 2019).

Tirandamycins, streptolydigin, and nocamycins belong to a small family of tetramic acid natural products featured with a bicyclic ketal moiety (Fig. 1) (Mo et al. 2014). Biosynthetically, the backbones of tirandamycin, streptolydigin, and nocamycins are assembled by hybrid type I polyketide synthase and non-ribosomal peptide synthetase (Olano et al. 2009; Carlson et al. 2010; Mo et al. 2011a, 2017). In tirandamycin biosynthetic pathway, the multifunctional CYP450 TamI catalyzes the formation of C-10 hydroxyl, C-11 and C-12 epoxy ring, and C-18 hydroxyl group (Carlson et al.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13205-019-1611-1>) contains supplementary material, which is available to authorized users.

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Fig. 1 The structures of tirandamycins, streptolydigin, and nocamycin I

2011). As for streptolydigin, two CYP450s, SlgO1 and SlgO2, are located within the biosynthetic gene cluster, and SlgO2 has been proposed to be involved in the formation of bicyclic ketal moiety on basis of gene disruption experiment (Gómez et al. 2012). For the high similarity between SlgO1 and TamI, Gómez et al. (2012) have suggested that SlgO1 might catalyze the formation of epoxy moiety during streptolydigin biosynthesis. By comparison of TrdI and SlgO1 sequences, it has been found that SlgO1 shows 70% identity/82% similarity to TamI/TrdI. Considering the high structural similarity in bicyclic ketal moiety, we assume that SlgO1 might modify the structure of tirandamycins.

In the current study, an *Escherichia coli* whole cell system with overexpression of SlgO1, an *E. coli* flavodoxin NADP⁺ oxidoreductase (EcFLDR), and an *E. coli* flavodoxin A (EcFLDA) were constructed, which can catalyze the

biotransformation of tirandamycin C to tirandamycin F. Moreover, *slgO1* gene was also introduced into various tirandamycin-producing strains, including tirandamycin B (1) producer *Streptomyces* sp. SCSIO1666 (Duan et al. 2010), tirandamycin C (3) producer *Streptomyces* sp. Ju1008 (Mo et al. 2011a), and tirandamycin E (5) producer *Streptomyces* sp. Ju1009 (Mo et al. 2011b). From the engineered strain *Streptomyces* sp. Ju1008::*slgO1*, we isolated an unexpected derivative tirandamycin L. By comparison with tirandamycin B, tirandamycin L showed inferior antibacterial activity.

Materials and methods

Bacteria, plasmids, primers, and culture conditions

The bacteria and plasmids used in this study are listed in Table 1. The primers used in this study are listed in

Table 2. The culture conditions used for all the *Streptomyces* in agar or liquid media were followed as the literature described previously (Mo et al. 2011a). All *E. coli* strains were cultured on Luria–Bertani (LB) medium with appropriate antibiotics (Mo et al. 2011a).

Table 1 Bacteria and plasmids used in this study

Strains or plasmids	Description	Reference or source
Strains		
<i>E. coli</i> DH5α	Host strain for general clone	Stratagene
<i>E. coli</i> ET12567/pUZ8002	Host strain for conjugation, Kan ^R , Cml ^R	Macneil et al. (1992)
<i>E. coli</i> BL21 (DE3)	Host strain for protein expression	Stratagene
<i>E. coli</i> FLDR/FLDA/slgO1	<i>E. coli</i> BL21 (DE3) carrying plasmids pACYCDuet-1/FLDR/FLDA and pET-28a/slgO1	This study
<i>E. coli</i> FLDR/FLDA/pET	<i>E. coli</i> BL21 (DE3) carrying pACYCDuet-1/FLDR/FLDA and empty pET-28a(+)	This study
<i>Streptomyces lydicus</i>	Streptolydigin producing strain, harboring <i>slgO1</i>	NRRL2433
<i>Streptomyces</i> sp. SCSIO1666	Tirandamycin B producing strain	Duan et al. (2010)
<i>Streptomyces</i> sp. Ju1008	Terminal product is tirandamycin C, <i>ΔtrdI</i> , Apr ^R	Mo et al. (2011a)
<i>Streptomyces</i> sp. Ju1009	Terminal product is tirandamycin E, <i>ΔtrdL</i> , Apr ^R	Mo et al. (2011b)
<i>Streptomyces</i> sp. SCSIO1666::slgO1	Heterologous expression of <i>slgO1</i> in <i>Streptomyces</i> sp. SCSIO1666, Apr ^R , Kan ^R	This study
<i>Streptomyces</i> sp. Ju1008::slgO1	Heterologous expression of <i>slgO1</i> in <i>Streptomyces</i> sp. Ju1008, Apr ^R , Kan ^R	This study
<i>Streptomyces</i> sp. Ju1009::slgO1	Heterologous expression of <i>slgO1</i> in <i>Streptomyces</i> sp. Ju1009, Apr ^R , Kan ^R	This study
<i>Bacillus subtilis</i>	Antibiotic sensitive strain, indicator for antibacterial assay	CCTCC AB2013121
<i>Bacillus thuringiensis</i>	Antibiotic sensitive strain, indicator for antibacterial assay	CCTCC AB2013119
<i>Staphylococcus aureus</i>	Antibiotic sensitive strain, indicator for antibacterial assay	ATCC 29213
Plasmids		
pCR TM 2.1	Vector for TA clone, Kan ^R , Amp ^R	ThermoFisher
pSET152AKE	<i>oriT</i> , integrase gene, intΦ31 site, Apr ^R , Kan ^R , <i>emrE</i> *	Ma et al. (2011)
pSET152AKE/slgO1	Derived from pSET152AKE, harboring <i>slgO1</i> gene	This study
pACYCDuet-1	Vector for protein co-expression, Cml ^R	Novagen
pACYCDuet-1/FLDR/FLDA	Co-expression plasmid carrying <i>E. coli</i> flavodoxin NADP ⁺ oxidoreductase and flavodoxin A gene	This study

Kan^R, kanamycin resistant; Apr^R, apramycin resistant; Cml^R, chloramphenicol resistant; Amp^R, ampicillin resistance

Table 2 The primers used in this study

Primer name	Sequence (5'–3') ^a	Restriction enzyme site
SlgO1-expF	GTACATACATATGATGTCAGAGAACCCGGTCCG	<i>NdeI</i>
SlgO1-expR1	ATCGGATCCTCACGCGGGTGCAGACGGA	<i>BamHI</i>
SlgO-expR2	TCACTAGTTTCACGCGGGTGCAGACGGA	<i>SpeI</i>
Fpr2-F	GGATCCTATGGCTGATTGGGTAACAGGCAAAG	<i>BamHI</i>
Fpr2-R	GCGGCGCGCTTACCAGTAATGCTCCGCTGTCATA	<i>NotI</i>
FldA2-F	GATATCCATGGCTATCACTGGCATCTTTTTCG	<i>EcoRV</i>
FldA2-R	CTCGAGTCAGGCATTGAGAATTTCTGTCGAG	<i>XhoI</i>
SlgO1-tF	ATGTCAGAGAACCCGGTCCG	
SlgO1-tR	TCACGCGCGGTGCAGACGGA	

^aUnderline is the restriction endonuclease cleavage site

Construction of an *E. coli* strain for co-expression of SlgO1, EcFLDR, and EcFLDA and in vivo biotransformation of tirandamycins

The *slgO1* gene fragment (accession No. CBA11578) was amplified from the *Streptomyces lydicus* genomic DNA with the primer pair SlgO1-expF and SlgO1-expR1. The primer pairs Fpr2-F/Fpr2-R and FldA2-F/FldA2-R were used to amplify *EcFLDR* gene and *EcFLDA* gene from *E. coli* genomic DNA, respectively. The PCR products with expected size were recovered, ligated into pCR™ 2.1 vector, and introduced into *E. coli* DH5 α , respectively. After confirming the inserted fragments by sequencing, the EcFLDR gene and EcFLDA gene fragments were excised by appropriate restriction enzymes and directly cloned into pACYCDuet-1 vector digested by the same enzymes to yield plasmid pACYCDuet-1/FLDR/FLDA. The *slgO1* gene was excised from pCR™ 2.1 vector and cloned into pET-28a to afford plasmid pET-28a/slgo1. Then, the plasmids pACYCDuet-1/FLDR/FLDA and pET-28a/slgo1 were co-transformed into *E. coli* BL21 (DE3) to afford *E. coli* FLDR/FLDA/slgo1. And the plasmid pACYCDuet-1/FLDR/FLDA and empty pET-28a(+) vector were introduced into *E. coli* BL21 (DE3) to yield *E. coli* FLDR/FLDA/pET as an negative control strain.

The procedures for biotransformation were described as follows: 30 μ L overnight cultures of *E. coli* FLDR/FLDA/slgo1 or *E. coli* FLDR/FLDA/pET were inoculated in 3 mL LB broth with the antibiotic chloramphenicol (25 μ g/mL) and kanamycin (50 μ g/mL). When *E. coli* BL21 were grown to OD₆₀₀ about 0.6, isopropyl β -D-1-thiogalactopyranoside (IPTG) and tirandamycin derivatives (tirandamycin C, tirandamycin D, tirandamycin A, and tirandamycin E) were added at a final concentration of 0.1 mM. After additional 36 h culture at 28 °C, 200 rpm, the broths were collected and extracted by ethyl acetate. The organic phase was evaporated into dryness and re-dissolved in 100 μ L methanol, and 40 μ L was subjected to HPLC for analysis.

Construction of the plasmid pSET152AKE/slgo1

The primer pair SlgO1-expF and SlgO-expR2 was used to amplify the *slgO1* gene fragment. The resultant PCR product was digested with *SpeI*–*NdeI* and cloned into the downstream of *ermE** promoter of the vector pSET152AKE. The plasmid with inserted DNA was sequenced for verification, and the plasmid with the correct sequences was designated as pSET152AKE/slgo1.

Construction, fermentation, and HPLC analysis of the engineered strains derived from *Streptomyces* sp. SCSIO1666

The plasmids pSET152AKE and pSET152AKE/slgo1 were independently introduced into *E. coli* ET12567/pUZ8002 to afford *E. coli* ET12567/pUZ8002/pSET152AKE and *E. coli* ET12567/pUZ8002/pSET152AKE/slgo1, which were used for further conjugation with *Streptomyces* sp. SCSIO1666, *Streptomyces* sp. Ju1008, and *Streptomyces* sp. Ju1009, respectively. The procedures for conjugation are followed the literature reported previously (Mo et al. 2011a). The exconjugants were first screened by the phenotype Kan^RApr^R, and their genotypes were further confirmed using PCR with the primer pair SlgO1-tF and SlgO1-tR targeted the *slgO1* gene. The expected PCR fragments were sequenced and the correct strains were designated as *Streptomyces* sp. SCSIO1666::slgo1, *Streptomyces* sp. Ju1008::slgo1, *Streptomyces* sp. Ju1009::slgo1, *Streptomyces* sp. SCSIO1666::pSET152AKE, *Streptomyces* sp. Ju1008::pSET152AKE, and *Streptomyces* sp. Ju1009::pSET152AKE, which were cultured using AM2 medium for 7 days and the broths were analyzed by HPLC as reported previously (Mo et al. 2011a).

Isolation and spectroscopic analyses of the newly produced compound from *Streptomyces* sp. Ju1008::slgo1

For structural characterization of the newly produced derivative, *Streptomyces* sp. Ju1008::slgo1 was fermented with AM2 medium in a large scale (6 L). After incubation, the culture broth was harvested and centrifuged. The supernatant was extracted by ethyl acetate and the mycelium was extracted by acetone, respectively. Subsequently, the entire organic phase was evaporated to dryness to yield crude extract. The crude extract was dissolved in a mixture of CH₃OH:CHCl₃ (1:1), mixed with appropriate amount of silica gel, which was applied on normal phase silica gel column chromatography and eluted with CHCl₃/CH₃OH (100/0, 98/2, 96/4, 94/6, 90/10, 85/15, 80/20, 70/30, 60/40, 50/50, v/v, each gradient was 300 mL) to give ten fractions (Fr.1–Fr.10). All fractions were analyzed by HPLC. Fractions 3 and 4 contained the targeted compound. The fractions 3 and 4 were combined and further purified on reverse phase C-18 silica gel eluted by a linear gradient from 20 to 90% CH₃CN in water. The 80–85% CH₃CN elution fraction which contained targeted compound was collected and further purified by preparative HPLC to afford compound 7.

The purified compound was subjected to high-resolution mass spectroscopy (HR-MS), ¹H, and ¹³C NMR spectra analysis. ¹H and ¹³C NMR spectra were recorded on Bruker AV

500 instrument at 25 °C. HR–MS spectra data were acquired on a Waters micro MS Q-ToF spectrometer.

Antibacterial assays of tirandamycin B and tirandamycin L

The antibacterial activities of tirandamycin B and tirandamycin L toward *Bacillus subtilis*, *Bacillus thuringiensis*, and *Staphylococcus aureus* were evaluated. The minimal inhibitory concentrations (MICs) were determined by the broth microdilution method (Xiao et al. 2013).

Results

Biotransformation of tirandamycins using *E. coli* whole cell system with overexpression of SlgO1, EcFLDR, and EcFLDA

For bacterial CYP450s, two redox partner proteins are required, which are not often adjacent to CYP450s. In both biosynthetic gene cluster of tirandamycin and streptolydigin, the ferredoxin and ferredoxin reductase are not clustered with the CYP450s. Since some CYP450s activities have been reconstructed using redox partner proteins EcFLDR and EcFLDA derived from *E. coli* (McIver et al. 1998), we here constructed an *E. coli* BL21 (DE3) whole cell system with co-expression of SlgO1, EcFLDR, and EcFLDA.

The biotransformation capability of tirandamycin C, D, E, and A was investigated using *E. coli* BL21 (DE3) whole cell system carrying SlgO1, EcFLDR, and EcFLDA. As shown in Fig. 2, when tirandamycin C was used as a substrate, a new polar peak with the characterized UV absorption spectra to tirandamycins appeared. The new peak showed the identical retention time to that of the authentic tirandamycin F. Furthermore, LC–MS analysis demonstrated that it had the same molecular mass to tirandamycin F. Therefore, these results demonstrated that SlgO1 can catalyze the hydroxylation in the C-10 position to yield tirandamycin F. No biotransformation of other tirandamycins was observed in the other samples.

Construction, fermentation, and HPLC analysis of strains derived from *Streptomyces* sp. SCSIO1666

It has been widely found that the substrate preference and catalysis efficiency of CYP450s are associated with the redox partner proteins (Munro et al. 2007). To use the redox partner proteins originated from tirandamycin-producing strains, the *slgO1* gene was cloned into pSET152AKE vector under the downstream of *ermE** promoter, which was then introduced into *Streptomyces* sp. SCSIO1666 and its derived strains such as *Streptomyces*

Fig. 2 HPLC analysis of in vivo biotransformation: (I) tirandamycin C (3) with control strain *E. coli* FLDR/FLDA/pET, (II) 3 with *E. coli* FLDR/FLDA/slglO1, (III) tirandamycin D (4) with *E. coli* FLDR/FLDA/pET, (IV) 4 with *E. coli* FLDR/FLDA/slglO1, (V) tirandamycin E (5) with control *E. coli* FLDR/FLDA/pET, (VI) 5 with *E. coli* FLDR/FLDA/slglO1, (VII) tirandamycin A (2) with control *E. coli* FLDR/FLDA/pET, (VIII) 2 with *E. coli* FLDR/FLDA/slglO1, and (IX) tirandamycin F (6) standard

sp. Ju1008 (tirandamycin C producer; deletion of CYP450 gene *trdI*) and *Streptomyces* sp. Ju1009 (tirandamycin E producer; deletion of FAD-dependent oxidase gene *trdL*). Subsequently, a series of *Streptomyces* sp. SCSIO1666 derivatives carrying *emrE**–*slgO1* cassette were constructed (Table 1, Figure S1). The fermentation broths of *Streptomyces* sp. 1666::*slgO1*, *Streptomyces* sp. Ju1008::*slgO1*, and *Streptomyces* sp. Ju1009::*slgO1* were analyzed by HPLC. By comparison of *Streptomyces* sp. Ju1008::pSET152AKE, a new polar peak with the characterized UV absorption spectra to tirandamycin was

Fig. 3 **a** Results of HPLC analysis of the extracts of the fermentation broth from (I) *Streptomyces* sp. SCSIO1666::slgO1, (II) *Streptomyces* sp. SCSIO1666::pSET152AKE, (III) *Streptomyces* sp. Ju1009::slgO1, (IV) *Streptomyces* sp. Ju1009::pSET152AKE, (V) *Streptomyces* sp. Ju1008::slgO1, (VI) *Streptomyces* sp.

Ju1008::pSET152AKE. 1: tirandamycin B, 2: tirandamycin A, 5: tirandamycin E, 3: tirandamycin C, and 7: tirandamycin L. **b** The HR-MS data of tirandamycin L (7). The molecular of 7 is C₂₂H₂₉NO₆ (*m/z* for [M+Na]⁺ 426.1890 and *m/z* for [2M+Na]⁺ 829.3876). **c** The structure of tirandamycin L (7)

detected in the extract of *Streptomyces* sp. Ju1008::slgO1 (Fig. 3a). However, no additional tirandamycin derivatives were detected in the extracts of the engineered strains *Streptomyces* sp. SCSIO1666::slgO1 and *Streptomyces* sp. Ju1009::slgO1 (Fig. 3a).

Isolation and structural characterization of the newly produced tirandamycin derivative

To elucidate the structure of the newly produced derivative, the *Streptomyces* sp. Ju1008::slgO1 was fermented in a 6-L scale, enabling us to isolate the newly produced compound 7

(6.1 mg). Next, the structure of compound **7** was determined by a series of spectroscopic analyses. Compound **7** was isolated as a white, amorphous powder. Its molecular formula was determined as $C_{22}H_{29}NO_6$ on basis of HR-MS at m/z 426.1890 $[M+Na]^+$ and m/z 829.3876 $[2M+Na]^+$ (Fig. 3b), indicating 9 degrees of unsaturation, which was one less than found for the known compound tirandamycin D. The 1H and ^{13}C NMR spectroscopic data of compound **7** disclosed three olefin proton signals (δ_H 7.61, 7.16, and 6.29) including a *trans* double bond on the basis of the coupling constants of 15.5 Hz, five methyl proton signals (δ_H 1.91, 1.43, 1.17, 1.11, and 0.72), two methylene proton signals (δ_H 2.13, 2.78, and 3.85), and five methine proton signals (δ_H 1.92, 2.37, 2.85, 3.7, and 4.0) (Figures S2, S3; Table 3). Full comparisons of the 1H and ^{13}C NMR spectroscopic data for compound **7** and tirandamycin D suggested that they possessed similar structures, except for the absence of a double bond between C-11 and C-12 in tirandamycin D (Figures S2, S3). In turn, a saturated C-11/C-12 bond was formed in compound **7**. This finding was confirmed by 1H – 1H COSY correlations of H-11/H-12, which was further validated by HMBC correlations from H-9, H-18 to C-11, and from H-11 to C-10, C-12 and C-13 (Figures S4–S7). Further 2D NMR data acquired

thereby allowed the full assignment of the other signals (Figures S3–S7). Consequently, the structure of compound **7** was elucidated and it was designated as tirandamycin L (Fig. 3c).

Antibacterial activity of tirandamycin B and tirandamycin L

Both tirandamycin B and tirandamycin L showed no inhibitory activity against *B. subtilis* and *S. aureus* ATCC 29213 at the concentration of $> 128 \mu\text{g/mL}$. Notably, tirandamycin B (MIC = $32 \mu\text{g/mL}$) showed much more potent antibacterial activity toward *B. thuringiensis* compared with tirandamycin L (MIC = $128 \mu\text{g/mL}$), indicating that the oxygenation level affected the antibacterial activity of tirandamycin derivatives.

Discussion

During the biosynthesis of microbial secondary metabolites, CYP450s often catalyze the oxidation reactions to diversify the parental compounds (Cochrane and Vederas 2014; Rudolf et al. 2017; Zhang and Li 2017). Nowadays, more and more CYP450s make fundamental contributions to engineering of natural products. The CYP450 SlgO1, derived from streptolydigin biosynthetic gene cluster, shows high similarity to the multifunctional CYP450 TrdI/TamI involved in tirandamycin biosynthetic pathway (Mo et al. 2011a; Gómez et al. 2012). In this study, we demonstrated that SlgO1 play a modification role in tirandamycin biosynthetic pathway. We introduced the heterologous CYP450 SlgO1 into various tirandamycin-producing strains and obtained a new derivative tirandamycin L, which possessed a C-11–C-12 saturated bond as well as a C-10 ketone moiety.

SlgO1 was initially proposed to be responsible for the formation of epoxy ring in bicyclic ketal moiety in streptolydigin biosynthetic pathway (Gómez et al. 2012). Therefore, a tirandamycin derivative with an epoxy ring in C-11–C-12 position or an epoxy ring in C-12–C-18 position was expected to be accumulated in our engineered strains capable of heterologously expressing SlgO1. Surprisingly, the new derivative tirandamycin L had no epoxy moiety and it possessed a saturated bond in C-11–C-12 position. For all the identified tirandamycin derivatives, only tirandamycin E' and pre-tirandamycin have a C-11–C-12 saturated bond as well as a C-11 hydroxyl group (Yu et al. 2011; Mo et al. 2012). In terms of biosynthesis, the elimination of C-11 hydroxyl results in a double bond in C-11–C-12 position. Pre-tirandamycin undergoes a dehydration step to generate tirandamycin C with a C-11–C-12 double bond, which is the direct precursor of the cytochrome P450 oxidase TrdI for further oxygenation modification (Carlson et al. 2011) (Fig. 4). For *Streptomyces* sp. SCSIO1666::slgO1 and

Table 3 1H and ^{13}C NMR spectroscopic data for tirandamycin L (**7**) recorded in $CDCl_3$

Position	δ_C type	δ_H mult. (J in Hz)
1	175.6	
2	116.5	7.16, d (15.5)
3	150.6	7.61, d (15.5)
4	134.7	
5	145.2	6.26, d (10.0)
6	35.5	2.85, m
7	78.5	3.7, m
8	32.6	1.92, m
9	80.5	4.0, d (6.0)
10	207.3	
11	45.9	2.13, d (10.6); 2.78, dd (8.8, 2.0)
12	39.2	2.37, m
13	97.7	
14	26.8	1.43, s
15	12.46	1.91, s
16	17.3	1.17, d (6.9)
17	11.9	0.72, d (7.0)
18	14.2	1.11, d (6.9)
1'(N)		
2'	176.7	
3'	Not observed	
4'	192.6	
5'	51.8	3.85, s

Fig. 4 The proposed modification steps introduced by SlgO1

Streptomyces sp. Ju1009::*slgO1*, the native P450 oxidase TrdI is ready to catalyze the formation of C-11–C-12 epoxy ring and C-10 hydroxyl group (Fig. 4) to afford tirandamycin A and tirandamycin E, respectively. Then, SlgO1 could not introduce further modifications in C-10, C-11, and C-12 positions. Consequently, it is rational that SlgO1 showed no other modifications toward tirandamycins to generate new derivatives in *Streptomyces* sp. SCSIO1666::*slgO1* and *Streptomyces* sp. Ju1009::*slgO1*. However, tirandamycin C with low oxygenation level is the terminal product of *trdI* deletion strain *Streptomyces* sp. Ju1008 (Mo et al. 2011a), SlgO1 could introduce further modifications toward tirandamycin C in C-11, C-12, and C-10 positions. For *Streptomyces* sp. Ju1008::*slgO1*, SlgO1 catalyzed the hydroxylation reaction to give rise to tirandamycin F, evidenced using the *E. coli* whole cell biotransformation experiments. Then, tirandamycin F undergoes a dehydrogenation step catalyzed by the FAD-dependent dehydrogenase TrdL to afford tirandamycin D with a C-10 ketone group (Mo et al. 2011b) (Fig. 4). The unique feature of tirandamycin L was its C-11–C-12 saturated bond, and SlgO1 was proposed to introduce a reduction reaction of C-11–C-12 double bond, giving rise to tirandamycin L (Fig. 4). The modifications introduced by SlgO1 were proposed to be a post-tailoring step. Otherwise, additional tirandamycin derivatives should also be detected in *Streptomyces* sp. SCSIO1666::*slgO1* and *Streptomyces* sp. Ju1009::*slgO1*. These results implied that SlgO1 could introduce multiple modifications toward tirandamycin C, although the exact role of SlgO1 in

streptolydigin biosynthesis remained largely unexplored. The uncommon reaction catalyzed by SlgO1 expanded the chemical space of tirandamycin and made it a potential candidate in synthetic biology.

Conclusion

In this study, an *E. coli* whole cell system with overexpression of SlgO1, EcFLDR, and EcFLDA was constructed. In vivo biotransformation experiments with this whole cell system demonstrated that SlgO1 could introduce a hydroxyl group in C-10 position of tirandamycin C, leading to form tirandamycin F. By introducing the *emerE**–*slgO1* cassette into a series of tirandamycins producing strains derived from *Streptomyces* sp. SCSIO1666, a novel derivative tirandamycin L accumulated in *Streptomyces* sp. Ju1008::*slgO1*. Tirandamycin L possessed an unusual C-11–C-12 saturated bond and a C-10 ketone moiety. On basis of structure of tirandamycin L, SlgO1 was proposed to be responsible for multiple modifications toward tirandamycin C. Compared to tirandamycin B, tirandamycin L displayed weak antibacterial activity, indicating that oxygenation modification was important in antibacterial activity of tirandamycins.

Acknowledgements We are very grateful to Professor Jianhua Ju for providing the tirandamycin-producing strains. Research in this work was supported by Natural Science Foundation of Shandong Province (No. ZR2013CL020) and Research Foundation for Advanced Talents of Qingdao Agricultural University (No. 631301).

Compliance with ethical standards

Conflict of interest All the authors declare no conflict of interest with respect to this paper.

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