



Engineering of avermectin biosynthetic genes to improve production of ivermectin in *Streptomyces avermitilis*

Meng Li, Zhi Chen, Xiuping Lin, Xuan Zhang, Yuan Song, Ying Wen, Jilun Li*

State Key Laboratories for Agrobiotechnology and College of Biological Sciences, Beijing 100193, PR China

ARTICLE INFO

Article history:

Received 30 May 2008

Revised 10 September 2008

Accepted 16 September 2008

Available online 19 September 2008

Keywords:

Ivermectin

Combinatorial biosynthesis

Polyketide synthase

Avermectin

aveC

Streptomyces avermitilis

ABSTRACT

Two new recombinants of avermectin polyketide synthases were constructed by domain and module swapping in *Streptomyces avermitilis* 73-12. However, only the strain, *S. avermitilis* OI-31, formed by domain substitution could produce ivermectin. Analysis of the ivermectin synthesized gene cluster showed that decreased amount of *aveC* transcripts was one of the factors causing low yield of ivermectin. Overexpression of *aveC* could improve ivermectin yield.

© 2008 Elsevier Ltd. All rights reserved.

Avermectins, a group of antiparasitic macrolides produced by *Streptomyces avermitilis*, are composed of eight structurally related polyketide compounds (Fig. 1).¹ Among those compounds, avermectin B1a has the strongest antiparasitic activity. Ivermectins (Fig. 1) (22, 23-dihydroavermectin B1), which are derived from avermectin B1, show lesser toxic side effects than avermectin B1, and are used worldwide in livestock production and in health care of animals. They have also been applied in human medicine, particularly treatment of onchocerciasis and strongyloidiasis.² The previously established industrial process for producing ivermectin involves extracting a mixture of avermectins from fermentation broth of *S. avermitilis*, isolating avermectin B1 from the mixture, and chemically reducing the double bond between C²² and C²³ of avermectin B1 using rhodium chloride as a special catalyst for region-specific hydrogenation.³ This process is expensive, and causes heavy metal pollution. A preferred alternative is to produce genetically engineered strains of *S. avermitilis* which produce ivermectins directly.

The complete nucleotide sequence of the linear chromosome of *S. avermitilis* was published, and sequence analysis revealed the organization of the avermectin biosynthetic genes and their deduced functions.⁴ The responsible avermectin synthase (AVES) was a typical modular polyketide synthase (PKS). The avermectin PKS were consisted of four giant multifunctional polypeptides (AVES1, AVES2, AVES3, and AVES4), which were encoded by four

large open reading frames (*aveA1-aveA2* and *aveA3-aveA4*).^{4b} The unsaturated bond of C²²–C²³ is determined by a putative partially active dehydratase (DH) and ketoreductase (KR) in module 2 without an enoylreductase (ER). In order to directly synthesize ivermectins and avoid any chemical steps, several attempts have been made to replace the DH2 domain of AVES1 with completely active DH and ER domain from other modular PKSs. In a previous study, we replaced *aveDH2* with the DNA fragment encoding DH4-ER4 from erythromycin PKS. However, neither ivermectins nor avermectins were detected from the resulting mutants. Next, *aveDH2-KR2* was replaced by the DNA fragment encoding DH4-ER4-KR4 from pikromycin PKS. The mutants had the ability to produce ivermectins, but the yield was only 1–4 µg/ml.⁵ When the DNA fragment encoding DH13-ER13-KR13 from rapamycin PKS was used to replace the domains of *AveDH2-KR2*, the estimated total amount of avermectins and 22, 23-dihydroavermectins produced in the mutant was decreased approximately eightfold compared to the yield of avermectins of wild-type control.⁶ Therefore, the yield of ivermectins produced by hybrid PKSs needs to be improved.

Oligomycins, strongly toxic compounds that specifically inhibit the oxidative-phosphorylation reaction in mammalian cells, are another family of macrocyclic lactones synthesized by *S. avermitilis*.⁷ The oligomycin biosynthetic gene cluster (*olm*) contains 18 ORFs spanning a distance of 104 kb. The *olm* gene was deleted from the chromosome of *S. avermitilis* in our previous study,⁸ and the *aveD* which encodes C5 O-methyltransferase in avermectin biosynthetic gene cluster was also deleted.⁹ The resulting mutant strain *S.*

* Corresponding author.

E-mail address: jilunli@gmail.com (J. Li).

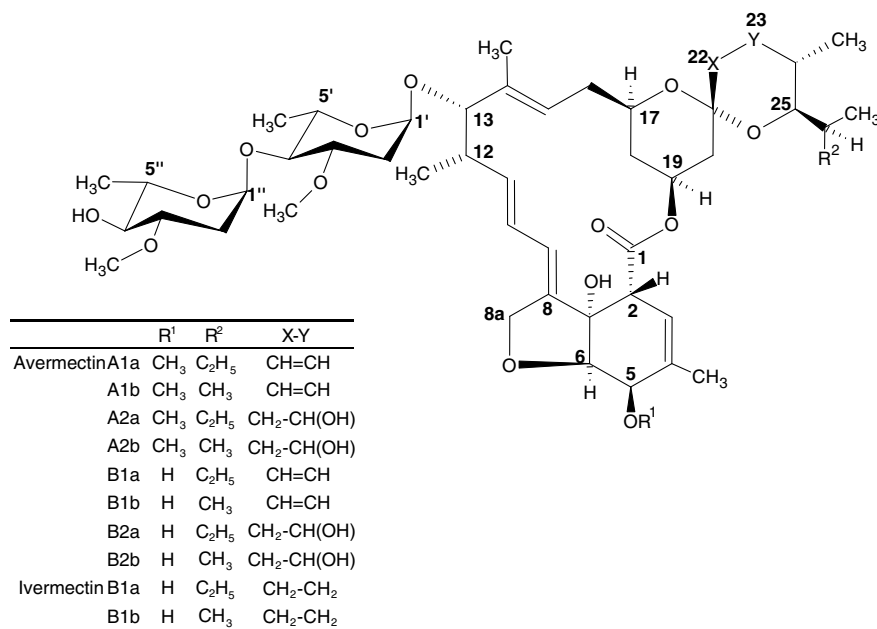


Figure 1. Structures of avermectins and ivermectins.

avermitilis Olm73-12 produces only avermectins B, and no oligomycin. And it was reported that by employing naturally occurring subunits from different PKSs, the yield of a new compound can be improved over 100-fold.¹⁰ The goal of the present study was to substitute AVES1 module 2 or DH2-KR2 domain (Scheme 1A) by module or domains in oligomycin synthase (OLMS), to increase the yield of ivermectins.

The OLMS was analyzed by the database SEARCHPKS (<http://www.nii.res.in/pksdb.html>),¹¹ and found to consist of 17 modules carrying 79 catalytic domains. Among these, modules 3, 7, and 9 contain DH-ER-KR domains. It was reported that relative substrate tolerance of diverse modules is essential for the rational design of hybrid multimodular PKS. Especially the substrate specificity of the KS domains is the critical determinant for generating functional hybrid PKS systems.¹² The native substrates of AVES module 2 contain sp³ hybridized carbons (α -methyl- β -hydroxy) would only process sp³ hybridized substrates. In these three OLMS modules, only module 3 contains sp³ hybridized carbons. Native substrates of modules 7 and 9 contain sp² hybridized carbons (β -keto or α,β -unsaturated) may not process the sp³ hybridized substrates of avermectin module 2. To improve the yield of ivermectins, we chose OLMS module 3 to substitute the AVES1 module 2, to generate hybrid PKSs that produce ivermectins (Scheme 1B). The putative substituted mutants were named OM-9. The double-crossover recombination event in the mutant was confirmed by PCR analysis. Yields of avermectins and ivermectins in Olm73-12 and OM-9 were determined by fermentation experiments and HPLC analysis. HPLC results showed that the module swapping strain produced no avermectins and ivermectins, indicating that substituting the AVES1 module 2 inactivated the avermectin PKS.

Next, OlmDH3-ER3-KR3 was used to substitute AveDH2-KR2, to construct a functional hybrid PKS with reduction of the double bond between C²² and C²³ of avermectins B1 (Scheme 1C). The putative substitution mutant was named OI-31. The double-crossover recombination event in these mutants was confirmed by PCR analysis. Cultures of the substituted mutant OI-31 and the parental strain *S. avermitilis* Olm73-12 were extracted with methanol and the extracts were analyzed by RP-HPLC/UV. A new compound

has the same retention time of the authentic sample 22, 23-dihydroavermectin B1a yield in OI-31, which was not detected in Olm73-12, as monitored by RP-HPLC with UV detection at 246 nm (Fig. 2).

Structure of the new compound was determined by LC/MS and NMR analyses. The mass of the compound was observed at m/z 897 ($[M+Na]^+$), which corresponds to the mass of 22, 23-dihydroavermectin B1a, and the mass fragmentation pattern was fully consistent with 22, 23-dihydroavermectin B1a. Results of ¹H and ¹³C NMR analysis were also consistent with 22, 23-dihydroavermectin B1a. These findings confirmed that the new compound produced by OI-31 is ivermectin B1a. The ivermectin yield of OI-31 was 15.6 μ g/ml, which was about fivefold higher than that of Ive12-4, an ivermectin-producing strain constructed by replacement of AveDH2-KR2 with DH4-ER4-KR4 domains from pikromycin PKS in our previous study.⁵ To evaluate transcription of exogenous domain ER in the substitution mutant OI-31, total RNA was isolated from exponentially growing cultures. A 542-bp fragment inside *olmER3* was detected by RT-PCR. When primer pairs ER1/ER2 were used for PCR analysis of the mutant OI-31, a 0.5-kb band appeared, whereas no such band was detected when total RNA of Olm73-12 was used as template. Thus, *olmER3* could be transcribed in the substituted mutant.

Oligomycin is another secondary metabolite synthesized by type-I PKS of *S. avermitilis* besides avermectins. And the spatial relationships among domains within its modules may approximate those of avermectin. It may improve the ability of mutual recognition of modules in hybrid PKS and recognized to substrate. Our results indicate that domains from oligomycin PKS may be more suitable for swapping AveDH2-KR2 than domains from pikromycin PKS or erythromycin PKS. However, the yield of ivermectins is still too low for large-scale production and it still produces avermectin B, indicating that the β -processing steps at C²²-C²³ are incomplete. The module-substituted mutants produce no avermectins or ivermectins. It is likely that module swapping disrupts interactions between protein modules in the assembly line.

Although genetic engineering of modular PKSs has been reported frequently, and many kinds of new polyketides have been produced, how to investigate the hybrid modules inter identified

Download English Version:

<https://daneshyari.com/en/article/1364734>

Download Persian Version:

<https://daneshyari.com/article/1364734>

[Daneshyari.com](https://daneshyari.com)