Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Chemical Biology

Pictet-Spenglerase involved in tetrahydroisoquinoline antibiotic biosynthesis

Kento Koketsu[1], Atsushi Minami[1], Kenji Watanabe[2], Hiroki Oguri[1] and Hideaki Oikawa[1]

Nonribosomal peptide synthetase (NRPS) is a programmable modular machinery that produces a number of biologically active small-molecule peptides. Saframycin A is a potent antitumor antibiotic with a unique pentacyclic tetrahydroisoquinoline scaffold. We found that the nonribosomal peptide synthetase SfmC catalyzes a seven-step transformation of readily synthesized dipeptidyl substrates with long acyl chains into a complex saframycin scaffold. Based on a series of enzymatic reactions, we proposed a detailed mechanism involving the reduction of various peptidyl thioesters by a single R domain followed by iterative C domain-mediated Pictet-Spengler reactions. This shows that NRPSs possess a remarkable capability to acquire novel function for diversifying structures of peptide natural products.

Addresses
[1] Division of Chemistry, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan
[2] University of Shizuoka, School of Pharmaceutical Sciences, Shizuoka 422-8526, Japan

Corresponding author: Oikawa, Hideaki (hoik@sci.hokudai.ac.jp)

Current Opinion in Chemical Biology 2012, **16**:142–149

This review comes from a themed issue on
Biocatalysis and Biotransformation
Edited by Jon S Thorson and Ben Shen

Available online 10th March 2012

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2012.02.021

Introduction

Nonribosomal peptide synthetase (NRPS) is a well-known modular enzyme that catalyzes the biosynthesis of diverse small molecule-polypeptides [1]. Following the assembly line logic, Catalytic mechanism of this complex enzyme system has been elucidated in detail [1,2]. A set of minimal NRPS domains (condensation, adenylation, thiolation) compose a single module. Following the assembly-line logic, combined modules catalyze formation of a linear polypeptide that is eventually cleaved by domains for hydrolytic (thioesterase) or reductive (reduction) cleavage. The growing polypeptides are frequently modified by domains for epimerization, methylation and oxidation of heterocycle. Contrary to ribosomal polypeptide biosynthesis, this plasticity adapting various optional functions allows NRPSs to diversify the structure of natural polypeptides [3,4•].

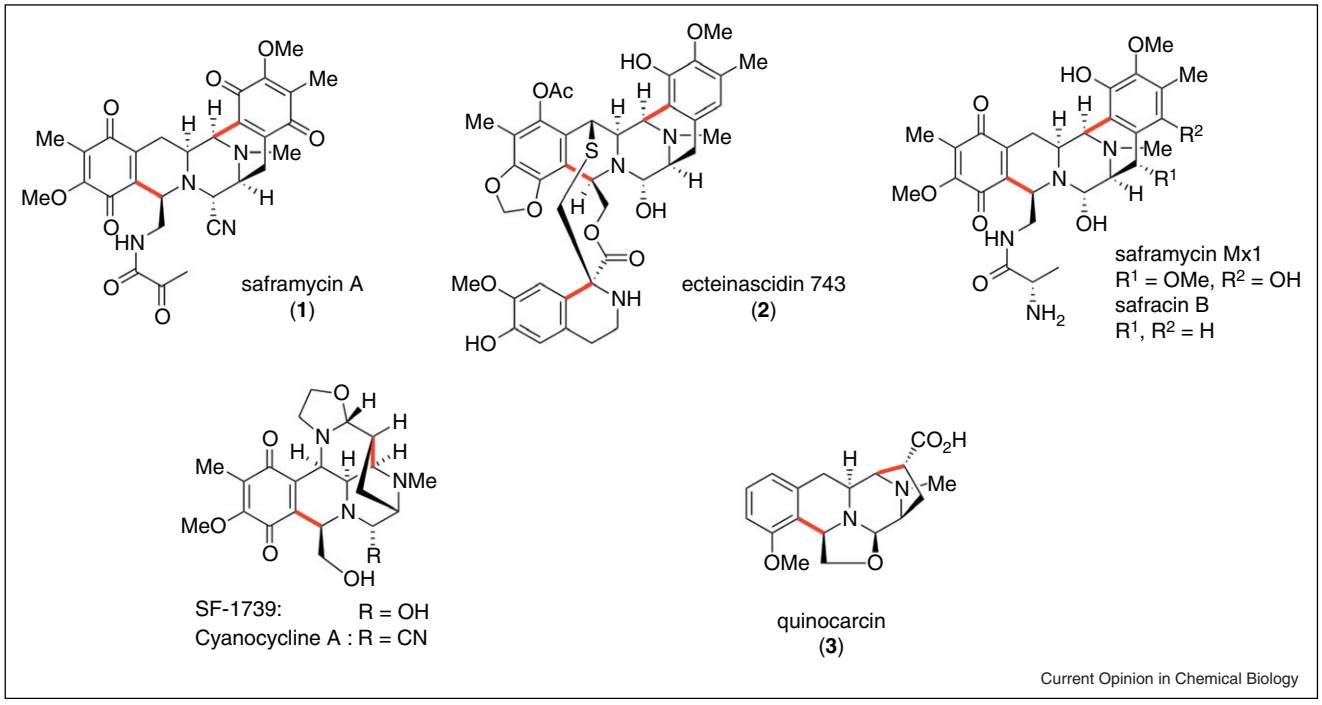
Tetrahydroisoquinoline (THIQ) antibiotics (Figure 1) from various soil bacteria and marine vertebrates such as sponges and ascidians [5••] reveal potent antitumor activity, which is originated from iminium ions via the carbinolamine moiety and its equivalents. Saframycin A (**1**) from *Strepomyces lavendulae* is a representative member of this family [6]. Among the saframycin analogs, ecteinascidin 743 (**2**, ET-743) [7] exhibited a remarkably potent antitumor activity against both common tumor cell lines and anthracycline-resistant tumor cell lines [8]. Recently, ET-743 **2** has been approved as an anticancer drug for use in patients with soft-tissue sarcoma [9]. During the biosynthetic studies of saframycin, we have found a remarkable reaction mechanism for a single NRPS module SfmC, which catalyzes seven chemical transformations via sophisticated communication of the individual domains [10••]. In this review, the novel functions of SfmC are discussed.

Unusual didomain for loading cryptic fatty acyl chain in NRPS for THIQ antibiotic biosynthesis

Saframycin A (**1**) has two oxidized THIQ systems whereas quinocarcin (**3**) [11] has a single THIQ and pyrrolidine ring system. In the total synthesis of saframycin B, Fukuyama and Sachleben incorporated intramolecular and intermolecular Pictet-Spengler reactions as a key step for the construction of the bisTHIQ core [12•]. Because of its efficiency, many syntheses adopted this strategy for constructing not only THIQ but also pyrrolidine skeleton [5••]. As described later, nature has adapted a similar Pictet-Spengler-based strategy in saframycin biosynthesis. Similar characteristic THIQ scaffolds are also found in the plant THIQ alkaloids, which are biosynthesized from *p*-hydroxyphenylacetaldehyde and dopamine by the 'Pictet-Spenglerase' norcoclaurine synthase [13].

A series of biosynthetic studies on saframycin A (**1**) established its biosynthetic precursors as L-alanine, glycine, and 2 mol of modified amino acid **5** derived from L-tyrosine [14]. Four biosynthetic gene clusters of THIQ antibiotics **1** [15•], safracin B [16], saframycin Mx1 [17] and **2** [18] have been identified and their bioinformatic analysis indicated that the saframycin backbone is constructed by a NRPS. In addition to these observations,

Figure 1



Chemical structures of tetrahydroisoquinoline antitumor antibiotics.

inspection of the saframycin structure suggested that characteristic amide bond is missing and key C–C bond formations probably occur at the positions of the amide carbonyl, strongly suggesting the involvement of a Pictet-Spengler-type reaction from precursors reminiscent of those employed in prior total synthesis [5••]. Recently, Wen and co-workers proposed a biosynthetic mechanism to proceed via a putative tetrapeptidyl intermediate **Y** (Figure 3) [15•]. This mechanism, however, requires novel iterative use of the last NRPS module SfmC, biochemically unusual amide reduction with the SfmC R domain and cyclization with unspecified enzyme(s) other than NRPSs. Although several groups have proposed alternative biosynthetic schemes [19,20], no experimental data on this intriguing transformation have yet been reported.

In addition to the NRPS-containing gene clusters mentioned above, six THIQ NRPSs including SF-1739 (cyanocycline) (T Hiratuka, K Koketsu *et al.*, unpublished data) and quinocarcin (**3**) (T Hiratuka, K Koketsu *et al.*, unpublished data) were aligned as shown in Figure 2. With the exception of the safracin cluster, all clusters showed similar domain organization and possessed the uncharacterized didomain (i.e. SfmA and its homologs). These domains showed homology to acyl CoA ligase-PCP didomains that are frequently found in the gene cluster of lipopeptides [21]. In addition, conserved substrate recognition residues from the crystal structure of long fatty acyl adenylation domain are found in the didomain [22]. *N*-terminal condensation (C) domain of the first module revealed modest similarity of 'starter C domain', which is responsible for initiating peptide biosynthesis by loading the first amino acid acylated with a cryptic long chain fatty acid [23].

Similar didomains have also be found in the gene clusters of bleomycin [24] and pyoverdine [25] although these natural products do not possess acyl chain. Recently, the cryptic role of the long acyl chain in biosynthesis of an iron chelating siderophore pyoverdine has been established by examination of the loading module and specific acylase PvdQ [26••]. This indicated that deacylation in the last step of the biosynthesis is important for bioactivity. Peptidases, which may cleave an installed acyl group at the late stage of biosynthesis, are common among THIQ gene clusters [15•,18]. Based on this circumstantial evidence, we speculated that the initial NRPS reactions proceed in the usual manner but to yield an *N*-acyldipeptidyl intermediate **I** (Figure 3) and the last module corresponding to SfmC catalyzes an intriguing transformation to the pentacyclic skeleton.

SfmC catalyzed reactions for construction of a pentacyclic THIQ core skeleton

To examine enzyme the activities of SfmC, several dipeptidyl-S-CoA esters (**4a**–**4e**) with various fatty acyl chains were synthesized. Each synthetic peptidyl-S-CoA