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Review

Discovery and biosynthesis of thioviridamide-like compounds

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ABSTRACT

Thioviridamide-like compounds are a unique subfamily of ribosomally synthesized and post-translationally modified peptides and contain characteristic thioamide bonds and *S*-[(*Z*)-2-aminovinyl]-D-cysteine (AviCys). Members of this family are active against a number of cancer cell lines. The distribution, biosynthetic machinery and the mode of action of thioviridamide-like compounds remain largely unknown. In this review, we outlined recent advances in the discovery of thioviridamide-like peptide natural products and the effort in the elucidation of their biosynthetic origin.

1. Introduction

Facilitated by the advance of genome sequencing technology, unprecedented insights have been provided into the genetic capacity of microorganisms to generate bioactive secondary metabolites [1]. Four major classes of natural products have been identified in the past century, including alkaloids, polyketides, terpenoids, and nonribosomal peptides [2]. Peptide natural products are an area of increasing attention for providing leading compounds for current therapeutic challenges, such as "undruggable" protein-protein interactions and antibiotic-resistance of infectious bacteria [3]. Recently, ribosomally synthesized and post-translationally modified peptides (RiPPs) have emerged as a new family of peptide natural products with diverse bioactivities, such as lantibiotics and thiopeptides [2]. Typically, the biosynthesis of RiPPs requires a precursor peptide, which contains a core peptide fused to a leader peptide or a follower peptide that facilitates the installation of posttranslational modifications (PTMs) (Fig. 1). Upon completion of enzymatic modifications, the leader and/or follower peptide are removed to produce the final product [4]. The extensive modifications of the core peptide provide RiPPs improved resistance to proteolytic degradation and constrained conformation optimal for target binding. A number of RiPPs have advanced to clinical trials, including the thiopeptide LFF571 for the treatment of *Clostridium difficile* infection [5]. The studies of RiPPs have undergone a revolution in recent years due to the explosion of available genomic data and the genome mining technology that enables the identification of a biosynthetic gene cluster in silico to guide natural product discovery. RiPPs are particularly suited for genome mining because of the direct link between precursor gene and final product and the relatively short biosynthetic pathways, which lend them well for heterologous expression.

Thioviridamide-like compounds (Fig. 2) are a subfamily of RiPPs with potent antiproliferative and pro-apoptotic activities. Thioviridamide is first isolated in 2006 from the fermentation of *Streptomyces olivoviridis* NA005001 [6]. Comprehensive NMR analysis identified the presence of a N-terminal 2-hydroxy-2-methyl-4-oxopentanoyl group, five thioamide groups in the backbone, a β-hydroxy-*N*1,*N*3-dimethylhistidinium (hdmHis) residue, and a rare *S*-[(*Z*)-2-aminovinyl]-D-cysteine (AviCys) in the C-terminal macrocycle [7]. The biosynthetic machinery and the mode of action of thioviridamide and related compounds remain largely unknown. This review outlined recent advances in the discovery of thioviridamide-like peptide natural products and the elucidation of their biosynthetic origin.

2. Discovery of thioviridamide-like compounds and their bioactivities

In 2006, Hayakawa *et al.* reported the isolation and characterization of thioviridamide from *S. olivoviridis* when screening for antitumor antibiotics using 3Y1 rat fibroblasts transformed with adenovirus oncogenes [6]. In addition to the thioamide groups in the backbone, thioviridamide contains a C-terminal *S*-(2-aminovinyl) cysteine (AviCys) residue, which is also found in linaridins. For example, epidermin, microbisporicin and cypemycin all contain C-terminal AviCys motifs, which are proposed to derive from the cyclization between a Ser/Cys derived dehydroalanine

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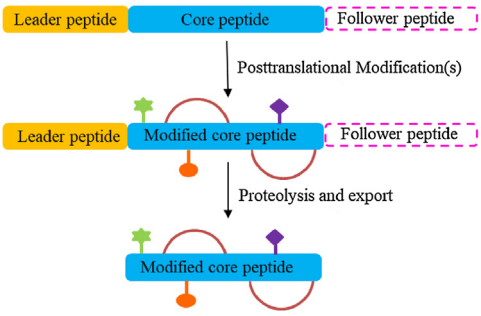

Fig. 1. Biosynthesis of *RiPPs*.

and a C-terminal Cys *via* oxidative decarboxylation. Therefore, thioviridamide was proposed to be synthesized following a RiPPs pathway, although the origin of thioamide backbone is not well understood.

Hayakawa group then sequenced the genomic DNA isolated form *S. olivoviridis* NA05001. Through gene analysis, they identified a *tva* gene containing 75 amino acids with a C-terminal sequence of VMAAAASIALHC, which is identical to that of thioviridamide (Fig. 3) [8]. Further analysis of the surrounding genomic region of the *tva* gene revealed a cluster containing 15 genes arranged as an operon [8]. To identify the essential genes for thioviridamide biosynthesis, *Streptomyces lividans* TK23 was transformed with the candidate genes for heterologous expression. Indeed, the *tva* gene cluster (tvaA to tvaO) produced matured thioviridamide in such expression system, conforming its role for thioviridamide production [8]. The antitumor activity of thioviridamide was then evaluated using a variety of cell lines, including 3Y1 rat normal fibroblasts and 3Y1 cells transformed with adenovirus type 12 (Ad12-3Y1), adenovirus E1A gene (E1A-3Y1), SV40 (SV-3Y1), v-src (SR-3Y1) or v-H-ras (HR-3Y1). Results showed that thioviridamide possessed potent cytotoxicity towards Ad12-3Y1 cells (IC_{50} = 3.9 ng/mL) and E1A-3Y1 cells (IC_{50} = 32 ng/mL). Recently, a second thioviridamide derivative, JBIR-140, was produced through heterologous expression of a bacterial artificial chromosome (BAC) clone prepared from *S. olivoviridis* OM13 containing the entire gene cluster for thioviridamide biosynthesis in *S. avermitilis* SUKA17 strain [9]. Thioviridamide and JBIR-140 were subjected to a number of cytotoxic activity assays, including SKOV-3 (human ovarian adenocarcinoma), Meso-1 (malignant pleural mesothelioma), and Jurkat (immortalized human T lymphocyte). Results showed that both compounds exhibited potent bioactivities against Jurkat, with IC_{50} values of 12.5 and 5.4 μmol/L, respectively (Table 1) [9].

In 2017, Truman and Müller groups independently reported their research on the discovery of thioviridamide-like molecules (TLMs) with the aid of genome mining technology [10]. Truman group used the YcaO-domain protein TvaH as a template for BLAST search in database and analyzed the genomic regions surrounding their respective genes using the *tva* BGC as the query [10b]. This strategy identified 14 closely related TLM-like BGCs in bacterial genomes. Interestingly, all the gene clusters are identified from actinobacteria, with the exception of the cyanobacterium Mastigocladus laminosus. Five strains were selected, including *A. alba* DSM 44262, *Streptomyces* sp. NRRL S-4, *Streptomyces* sp. NRRL S-15, *Streptomyces* sp. NRRL S-87, and *Nocardiopsis potens* DSM 45234, and subject to fermentation in multiple culture conditions. The fermentation extracts were subsequently screened by liquid chromatography-mass spectrometry (LC-MS) for TLM production based on their predicted mass. Four thioviridamide-like molecules (TLMs) were successfully identified using this methodology, including thioholgamide A, thioholgamide B [10a], thioalbamide and thiostreptamide S4 (Fig. 2) [10b]. Thioholgamides A and B were produced by *Streptomyces malaysiense* MUSC 13657 (DSM 100712), with amino acid substitutions including an N-terminal pyruvyl

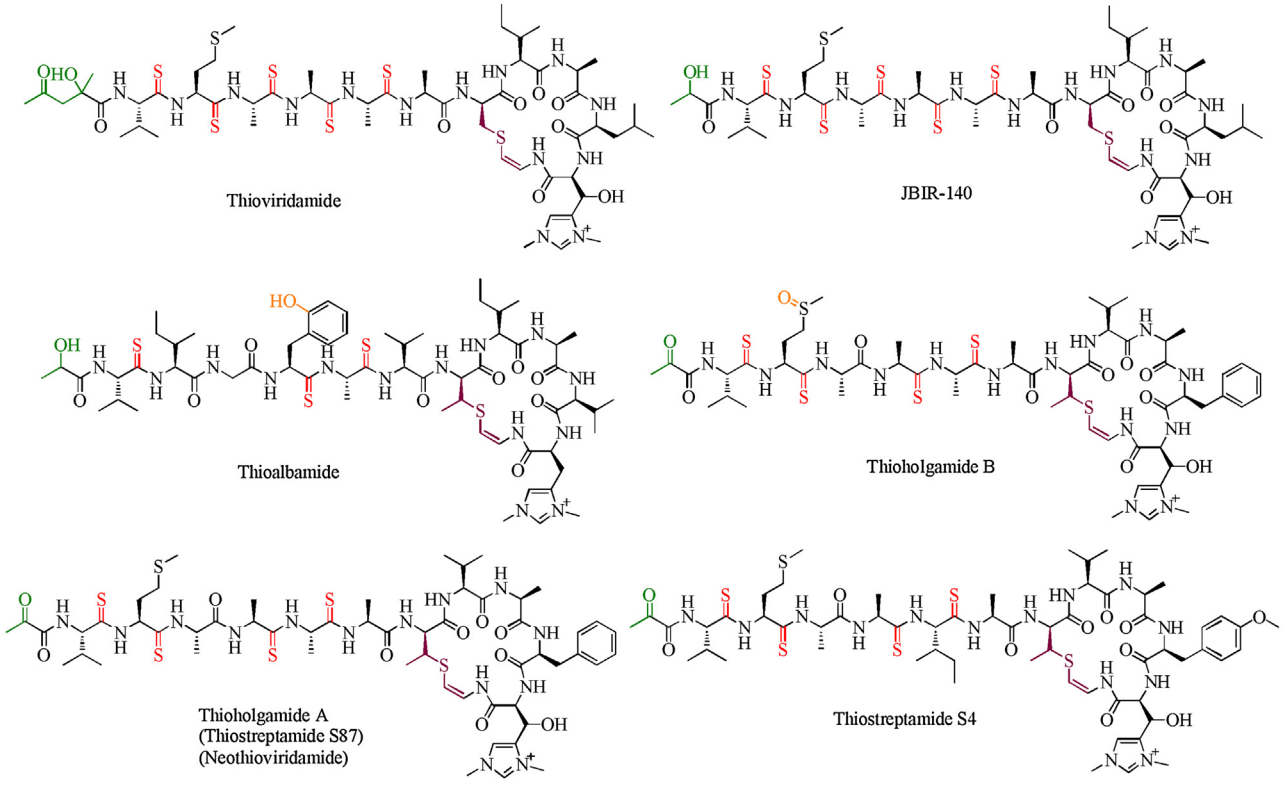

Fig. 2. Structures of thioviridamide-like molecules.

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