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Isolation of the lysolipin gene cluster of *Streptomyces tendae* Tü 4042

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ABSTRACT

Streptomyces tendae Tü 4042 produces the aromatic polyketide antibiotic lysolipin. Lysolipin has strong antibacterial activity against a variety of multidrug-resistant pathogens. The complete lysolipin biosynthetic gene cluster was isolated and fully sequenced. Within a 42-kb genomic region, 42 genes were identified that code for a type II polyketide synthase (*llpF*, *E*, and *D*), cyclases (*llpCI–CIII*), methyltransferases (*llpMI–MVI*), a halogenase (*llpH*), an amidotransferase (*llpA*), a ferredoxin (*llpK*), a transporter (*llpN*) and regulatory proteins (*llpRI–RV*). In addition, 15 genes encoding enzymes involved in redox modifications of the polyketide precursor molecule (*llpOI–OVIII*, *ZI–ZIV*, *U*, *L*, and *S*) were present in the lysolipin biosynthetic gene cluster. With this high number of oxidoreductases, lysolipin is among the most highly modified aromatic polyketides known to date. The heterologous expression of the cluster in *Streptomyces albus* led to lysolipin production with a yield comparable to that of wild-type, indicating that all biosynthetic genes were successfully cloned.

1. Introduction

The occurrence of multidrug-resistant pathogens is a serious and constantly growing threat in human medicine (Nathan, 2004). To address this severe problem, new and more sophisticated approaches are needed to develop novel antimicrobials for clinical use. Alterations to these molecules can be achieved by chemical derivatizations (Khosla and Tang, 2005) or combinatorial biosynthesis (Floss, 2006; Rodriguez and McDaniel, 2001). Polyketides are an important family of compounds, comprising over 10,000 members. Many polyketides are commercially used as antibiotics (e.g., tetracyclines and erythromycin), immunosuppressants (FK506), anticancer substances (doxorubicin), and antifungals (nystatin). Polyketides are formed in a manner similar to fatty acid biosynthesis (for a review, see Hertweck, 2009). Modular polyketide synthase (PKS) enzymes (type I) resemble vertebrate fatty acid synthases (FAS), while PKS involved in the synthesis of aromatic compounds resemble bacterial or plant FAS. These type II PKS consist of a set of core enzymes called the "minimal PKS," consisting of ketosynthase α, (KS_α), ketosynthase β/chain-length factor (KS_β/CLF), and acyl carrier protein (ACP). The minimal PKS synthesizes a polyketide precursor, which is subsequently cyclized (for reviews, see Das and Khosla, 2009; Hertweck et al., 2007). This precursor finally is modified by tailoring enzymes, which can introduce, for example, oxygenations, halogenations, methylations, glycosylations, and structural rearrangements. These tailoring steps have important effects on the physicochemical properties and biological activity of the resulting molecule. Within these diverse tailoring reactions lies a huge potential for generating new substances with novel, and perhaps improved, properties by combining corresponding enzymes from different biosynthetic pathways (Floss, 2006; Pelzer et al., 2005).

The aromatic polyketide lysolipin (Fig. 1) was first isolated in 1975 from *Streptomyces violaceoniger* Tü 96 (Drautz et al., 1975), and later from *Streptomyces tendae* Tü 4042 (Blum, 1995). It is highly active at very low concentrations (MIC 0.001 μg mL^{-1}) against a variety of gram-positive bacteria; it also possesses tumorstatic activity (Pultar, 1988). Lysolipin displays a very high affinity for lipids. Drautz et al. (1975) suggested that its mode of antibacterial action may be based on an interaction with the C_{55}-lipid carrier bactoprenol, which is involved in cell-wall biosynthesis. The end product of biosynthesis is

Abbreviations: Acc., accession number; ACP, acyl carrier protein; CLF, chain-length factor; dNTP, deoxyribonucleoside triphosphate; FAD, flavin adenine dinucleotide; FAS, fatty acid synthase; HPLC, high-performance liquid chromatography; kb, kilobases; KS, keto synthase; MCAT, malonyl-CoA:ACP transacylase; MIC, minimal inhibitory concentration; MS, mass spectrometry; NADH, reduced nicotinamide-adenine dinucleotide; ORF, open reading frame; PCR, polymerase chain reaction; PKS, polyketide synthase; SAM, *S*-adenosylmethionine.

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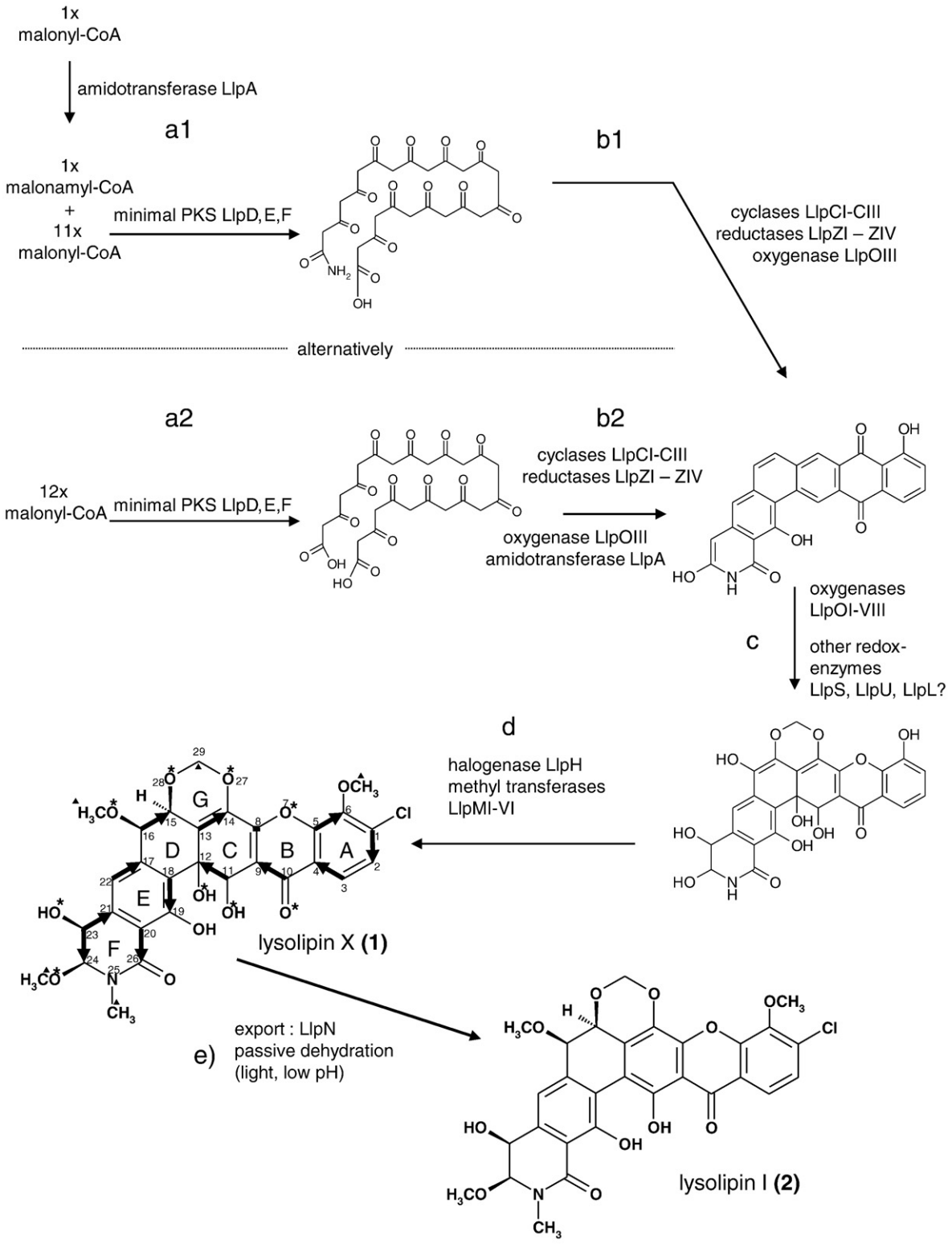

Fig. 1. Hypothetical pathway for lysolipin biosynthesis. (a1) Formation of the polyketide chain from malonamide starter and malonate extenders; (b1) cyclization and reduction of polyketide precursor; alternatively, the incorporation of the amino group might occur at a later stage of biosynthesis (a2, b2); (c, d) Oxidative modification and "tailoring" of the intermediate; (e) spontaneous reduction of lysolipin X to lysolipin I and export. $^*O_2$ from molecular oxygen; ▲ CH_3 from *S*-adenosylmethionine (SAM); ⟷ malonate unit; ➡ acetate unit; A–G: ring nomenclature; 1–29: atoms involved in forming the lysolipin backbone (according to Bockholt et al., 1994).

the precursor lysolipin X (**1**), which undergoes spontaneous dehydration at the C-12 position to form stable lysolipin I (**2**) (Fig. 1). The polycyclic and aromatic character of lysolipin implies that lysolipin biosynthesis is based on the type II PKS mechanism.

The 24-carbon polyketide backbone makes lysolipin one of the largest known aromatic polyketides. It has structural similarities to pradimicin, (Oki et al., 1988) griseorhodin (Eckardt et al., 1978; Li and Piel, 2002), rubromycin (Brockmann et al., 1966), fredericamycin