Biotechnology of Antibiotics

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Lipopeptide Antibiotics Produced by *Streptomyces roseosporus* and *Streptomyces fradiae*

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I. INTRODUCTION

Streptomyces roseosporus NRRL11379 produces A21978C, a complex of acidic lipopeptide antibiotics (1). The cyclic depsipeptide portion (Figure 1a) contains 13 amino acids cyclized to form a 10-amino-acid ring. The A21978C factors contain different 10-, 11-, 12-, or 13-carbon fatty acids attached to the amino-group of the terminal L-Trp (1). The fatty acid side chains are readily removed by incubation with *Actinoplanes utahensis* (2), and the cyclic peptide can be reacylated at the amino-terminus of Trp to produce semisynthetic acyl, aroyl, and extended peptide derivatives (3). The *n*-decanoyl analog of A21978C, LY146032 or daptomycin, is a potent antibiotic active against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis*, vancomycin-resistant enterococci, and penicillin-resistant *Streptococcus pneumoniae* (see Section IV). *Streptomyces fradiae* A54145 also produces a complex of cyclic lipopeptides containing 13 amino acids with a 10-amino-acid ring (4,5). The A54145 complex is similar to daptomycin in that different factors contain various long-chain fatty acids attached to the *N*-terminal Trp (Figure 1b). A54145 differs from daptomycin in that the cyclic peptide is variable in positions 12 and 13, and all four possible peptides are observed in normal fermentations (4,5).

In addition to the *N*-terminal Trp, A54145 contains some amino acids identical or similar to those in daptomycin in other positions, including the position 4 Thr, which participates in the ester linkage that closes the ring. A54145 factors have in vitro antibacterial properties similar to those of daptomycin (see Section IV). The fatty acyl side chains of A54145 can be removed by incubation with *A. utahensis* (6), and the *N*-terminal Trp can be reacylated (6), just as with the daptomycin nucleus (2,3).

The similarities in the structures of these two lipopeptides suggest that the biosynthetic pathways may have evolved from a common ancestral pathway. Thus, the study of the structural organization and physical map locations of the biosynthetic genes may provide insights into the evolution of these complex lipopeptide pathways. Further, the diversity in amino acid sequence in these related molecules suggests that further modifi-

cation of the peptide portion of these cyclic peptides may generate as yet unknown, but related antibiotics—some of which may prove to be superior to either of the parent molecules. In this chapter, I summarize what is known about the genetics, biosynthesis, mode of action, and antibacterial activity of these antibiotics. I also speculate on how the activities of these molecules may be further altered by modifying the genes encoding the multienzyme peptide synthetases involved in determining the amino acid sequences of the peptide portion of the molecules.

II. BIOSYNTHESIS OF LIPOPEPTIDES

A. Daptomycin Biosynthesis

S. roseosporus produces A21978C, a complex of lipopeptide antibiotics highly active against Gram-positive bacteria (1). The A21978C complex was separated into three major factors, C_1, C_2, and C_3, and three minor factors, C_4, C_5, and C_0 (Table 1). All six factors contain an identical 13-amino-acid core cyclic peptide, containing 11 common L– or D–amino acids and 2 unusual amino acids, 3-methyl glutamic acid (3mGlu), and the L-tryptophan metabolite L-kynurenine (L-Kyn). Enzymatic studies employing glutamine synthetase and L-glutamic acid decarboxylase established that the stereochemistry of the glutamate analog as L-*threo*-3-methyl glutamic acid (1). The linear sequence of the peptide core was established as L-Trp–L-Asn–L-Arg–L-Thr–Gly–L-Orn–L-Asp–D-Ala–L-Asp–Gly–D-Ser–L-3mGlu–L-Kyn. The cyclic depsipeptide is formed by an ester linkage between L-Kyn and the L-Thr hydroxyl-group (Figure 1). The factors C_1, C_2, and C_3 were shown to contain *anteiso*-undecanoyl (C11), *iso*-dodecanoyl (C12), and *anteiso*-tridecanoyl (C13) side chains, respectively. The minor components C_0, C_4, and C_5 contain C10, C12, and C12 fatty acids (1). The ratios of factors C_1, C_2, and C_3, which are normally about 1:1.5:1, can be modulated by adding different branched-chain amino acids to the fermentation medium. Factor C_2 can be increased by adding valine to the fermentation, whereas factors C_1 and C_3 can be increased by adding isoleucine (7). The authors concluded that the branched-chain amino acids served as precursors to provide the branched-chain fatty acid primers for the biosynthesis of the fatty acid side chains of the A21978C factors.

Daptomycin, which contains the C10 fatty acid decanoic acid, is normally produced by *S. roseosporus* in trace amounts. Huber et al. (8) have shown that decanoic acid mixed 1:1 (v:v) in methyl oleate can be fed continuously to fermenters at rates that avoid the accumulation of decanoic acid, which is normally toxic to *S. roseosporus*. Under these conditions, *S. roseosporus* A21978.65 produced over 900 μg/ml of daptomycin directly, and less than 400 μg/ml of factors containing other fatty acid side chains. The process was modified for large-scale production, and daptomycin yields of >1000 μg/ml representing 77% of total A21978C factors have been reported (9). High yields of daptomycin can also be produced by feeding caproic acid (10). This process is much simpler and less costly than the process that combines enzymatic removal of the fatty acid side chains and chemical reacylation with decanoic acid (see Section III).

B. A54145 Biosynthesis

The cyclic lipopeptide antibiotic complex A54145 is produced by *S. fradiae* A54145 (NRRL 18158). The complex has eight factors composed of four different cyclic peptides

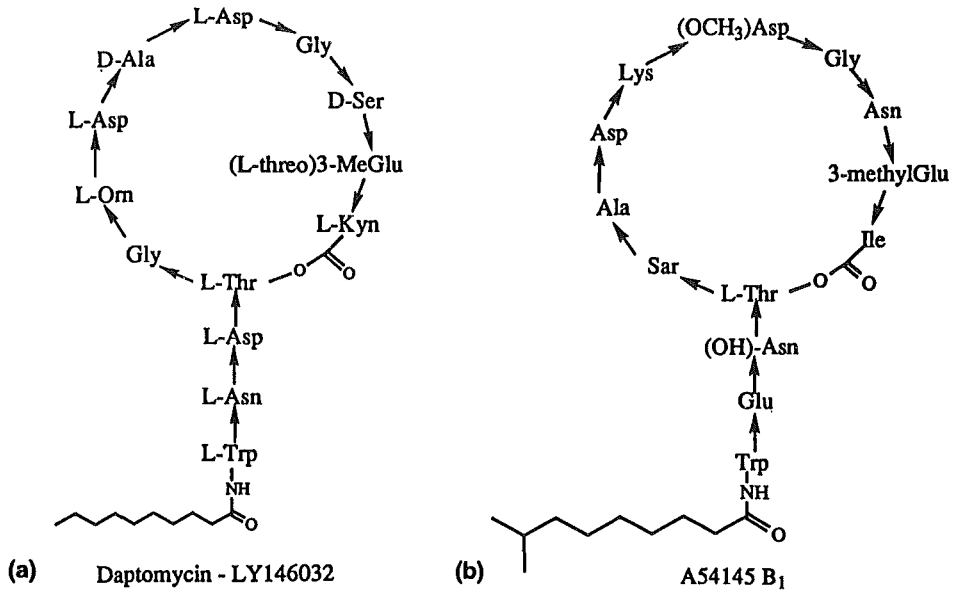

Figure 1 Structures of daptomycin and A54145B_1. Daptomycin (a) contains an *N*-decanoyl side chain. The A21978C factors produced in standard fermentations contain the fatty acyl side chains shown in Table 1. A54145B_1 (b) is the most abundant factor produced in standard fermentations. The other A54145 factors are shown in Table 2.

Table 1 Lipopeptide A21978C Factors Produced by *S. roseosporus*

Factor	Fatty acid
C_0	Unidentified (10 carbon)
C_1	anteiso-Undecanoyl
C_2	iso-Dodecanoyl
C_3	anteiso-Tridecanoyl
C_4	Unidentified (12 carbon)
C_5	Unidentified (12 carbon)

Data from Ref. 1.

and three different lipid side chains (4,5). The major peptide nucleus has the sequence Trp–Glu–hAsn–Thr–Sar–Ala–Asp–Lys–OmAsp–Gly–Asp–3mGlu–Ile (where hAsn = hydroxy Asn, OmAsp = hydroxymethyl Asp, and 3mGlu = 3-methyl Glu). The peptide is cyclized by an ester linkage between the carboxy-group of Ile and the Thr hydroxy-group. The four different peptide structures are identical in the first 11 amino acids, but have 3mGlu or Glu at position 12 and Ile or Val at position 13. The different peptide structures coupled with three different possible lipid side chains account for the eight factors identified in fermentation broths (Table 2). (Presumably, the four missing factors were present in such low amounts that they were not observed by the high-performance liquid chromatography [HPLC] system used.)

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