ROLE OF BRANCHED CHAIN FATTY ACID PRECURSORS IN REGULATING FACTOR PROFILE IN THE BIOSYNTHESIS OF A21978 C COMPLEX

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The lipopeptide antibiotic complex, A21978 C, is produced by *Streptomyces roseosporus* in liquid shake culture. The major antibiotics of this complex differ from each other in the type of fatty acyl unit attached to the *N*-terminal tryptophan. The cyclic peptide core of each antibiotic is the same (Fig. 1)[1]. Antibiotic activity of these compounds requires both the fatty acyl group and the cyclic peptide portion of the molecule[2]. During the study of the biosynthesis of these factors, it was found that the availability of branched chain fatty acid precursors determined the relative amount of each factor produced by the culture. This report describes that phenomenon and identifies two new factors produced by precursing the culture.

S. roseosporus was grown in complex vegetative medium for 2 days, then transferred to a complex fermentation medium[1] and grown for 5 to 7 days at 30°C. Additions to the culture were made aseptically after 2 days growth in the fermentation medium. As shown in Fig. 2a, the normal factor profile consists of approximately 1: 1.5: 1 of factors C1: C2: C3. When the culture was precursed with valine, the proportion of C2 increased dramatically when compared to the other factors (Fig. 2b). Likewise, when isoleucine was added, the amount of C1 and C3 was enhanced (Fig. 2c). These results indicate that the availability of these amino acids to serve as branched chain fatty acid primers determines the type and proportion of factors produced by this culture. No detectable amounts of iso-odd carbon chain branched fatty acyl antibiotic was made by the culture. However, when leucine was added to the fermentation, two new factors (X and Y, Fig. 2d) were produced. These two compounds were found to have biological activity as determined by stream splitting studies (data not shown). The biological activity was lost when these factors were incubated with the broth of *Actinoplanes utahensis* which possesses an enzyme that removes the fatty acyl moieties from the other C factors[3].

The two new factors were isolated by concentration on Diaion HP-20 followed by pre-

Fig. 1. Structures of the A21978 C complex antibiotics.

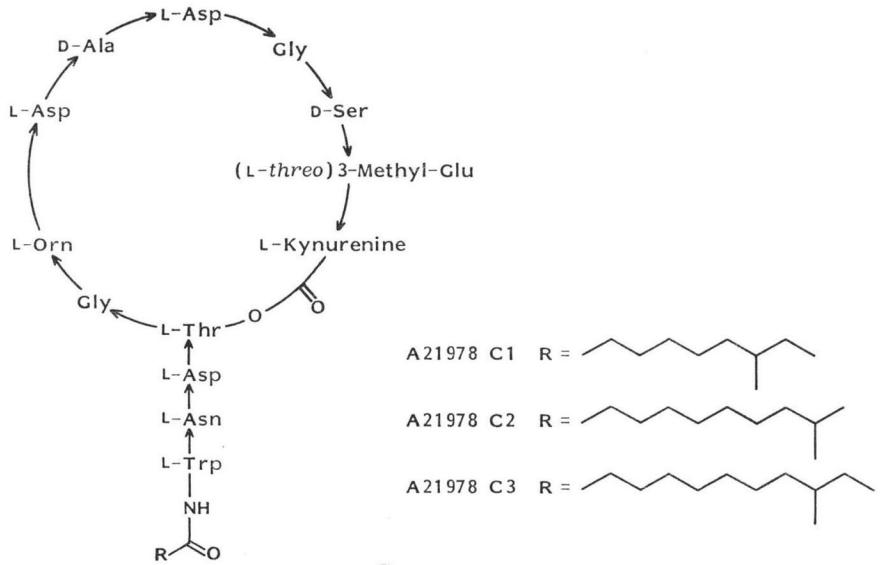

Fig. 2. HPLC tracing of *S. roseosporus* fermentation broth.

Natural distribution of factors (a). Fermentations precursed with valine (b), isoleucine (c) and leucine (d).

Column: Waters RCM Nova-18, 8×100 mm; Mobile phase: 37% acetonitrile, 1% ammonium phosphate, 1 ml/minute; Detection: 225 nm.

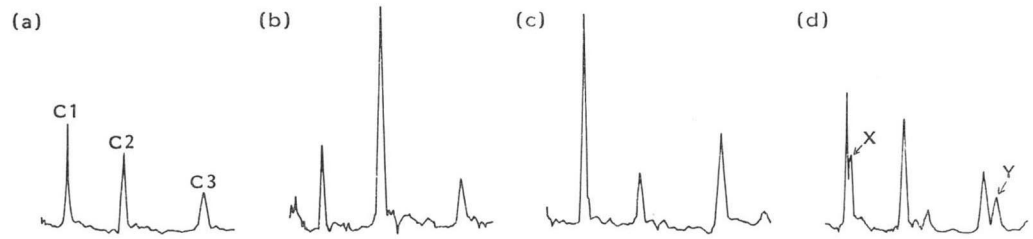

Table 1. Comparison of the amino acid composition of C3 and factor Y.

Amino acid	Mol A.A./Mol antibiotic*	
	C3	Factor Y
Asp+Asn	4.0	3.9
Thr	0.9	0.9
Ser	0.9	0.9
Gly	2.0	2.0
Ala	1.0	1.0
3-Methyl-Glu	0.8	0.8
Orn+Trp	2.0	1.6
Kynurenine	0.8	0.7
Val	0	0
Ile+Leu	0	0

* Amino acid analysis of the antibiotic was performed with an automatic amino acid analyzer[5].

parative reverse phase HPLC. The FAB mass spectrum of factor Y gave a molecular ion at 1,662 (M+H) indicating that this factor has the same molecular weight as C3. Amino acid analysis (Table 1) of the antibiotic gave an amino acid composition that was the same as C3, indicating that no leucine had been incorporated into the peptide core of the antibiotic. Factor Y was hydrolyzed with acid, and the fatty acid extracted into ether and then methylated with diazomethane. Gas chromatography of the fatty acid methyl ester gave a major peak (retention time, 11 minutes 10 seconds) which was more volatile than the C3 fatty acid methyl ester (11 minutes 13 seconds) but had the same molecular weight (288).

Taken together, this information suggests that factor Y is most likely the "iso" fatty acyl analog of C3 and that the carbons of leucine are incorporated into the fatty acid tail of the molecule. Not enough of factor X, in pure form, could be obtained for a detailed analysis. Based on its HPLC retention time on a reverse phase column, the finding that it has the same molecular weight as C1 (1,634, M+H), and that it is only produced in concert with factor Y, it is very likely that this factor is the "iso" fatty acyl analog of C1.

This study has shown that the type and proportion of C factors produced by *S. roseosporus* are determined by the availability of leucine, isoleucine, and valine to serve as primers for the synthesis of the fatty acyl portion of the antibiotic. These amino acids are then converted to their α-keto acids which are utilized as primers for branched chain fatty acid synthesis. The inability to find mutants that produce only the peptide core (ZMIJEWSKI, unpublished results) would suggest that a fatty acyl moiety initiates the synthesis of the peptide core. The biosynthesis of the A21978 C complex would, therefore, be similar to the biosynthesis of the polymyxins[4] where an octanoyl group is transferred from octanoyl Co-enzyme A to an enzyme-bound 2,4-diaminobutyl group. This is followed by peptidation to a decapeptide and then cyclization. In the case of A21978, the most abundant branched chain fatty acyl groups would be transferred to an enzyme-bound tryptophan and this would initiate peptide synthesis.

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