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Biosynthesis of 1-aminocyclopropane-1-carboxylic acid moiety on cytotrienin A in *Streptomyces* sp.

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ABSTRACT: Our recent research revealed that a soil bacteria *Streptomyces* sp. RK95-74 produced cytotrienin A, a new member of ansamycin derivatives containing a unique 1-aminocyclopropane-1-carboxylic acid (ACC) unit defined by the detailed 1D and 2D NMR techniques. Cytotrienin A is the first microbial metabolite with the ACC unit. We have investigated the biosynthesis of cytotrienin A with special attention given to the ACC unit biosynthetic pathway by stable isotope feeding experiments with cultures of *Streptomyces* sp. Sodium [1-^{13}C] acetate and [1-^{13}C] propionate were not incorporated into the ACC part but were incorporated into the 21-membered macrocyclic ring of the molecule. *L*-[U-^{13}C] methionine is efficiently and specifically incorporated into the ACC unit, suggesting that the ACC moiety at the 11-side chain of cytotrienin A is derived from *L*-methionine. This is the first report that a prokaryote synthesizes an ACC unit from *L*-methionine.

Recently we have isolated cytotrienin A from *Streptomyces sp.* RK-95-74, which induced apoptosis in human acute promyelocytic leukemia HL-60 cells with ED_{50} value of 7.7 nM[1,2]. Interestingly cytotrienin A possesses a unique 1-aminocyclopropane-1-carboxylic acid (ACC) unit. Although ACC is well known as a plant metabolites, cytotrienin A is the first example containing an ACC unit in bacterial metabolites. Here, we describe the results of our research on the biosynthesis of cytotrienin A, especially the ACC synthetic pathway in *Streptomyces* sp.

The incorporation pattern of isotopic labeled biosynthetic precursors was studied as described below. *Streptomyces* sp. RK95-74 was cultured in a 500-ml cylindric flask containing 70 ml of the fermentation medium consisting of 2.0% glucose, 2.5% soybean meal, 0.2% NaCl and 0.005% K_2HPO_4 (pH 7.3) on a rotary shaker at 28 °C. In each experiment, one of the following ^{13}C-labeled precursors, sodium [1-^{13}C] acetate (50.0 mg), sodium [1-^{13}C] propionate (5.0 mg), *L*-[methyl-^{13}C] methionine (5.0 mg), and *L*-[U-^{13}C] methionine (5.0 mg) was added to the fermentation broth at 24 hours after inoculation, and after a further 48 hours ^{13}C-labeled cytotrienin A was isolated from the whole broth.

In the ^{13}C NMR spectrum of the sodium [1-^{13}C] acetate labeled cytotrienin A, signal intensities of carbons 1, 3, 5, 7, 9 and 15 were increased by 4-10 fold, while sodium [1-^{13}C] propionate labeled cytotrienin A showed increased signal intensities for carbons 11 and 13 by ca. 4 fold as shown in Table 1. In addition, incorporation of *L*-[methyl-^{13}C] methionine confirmed the origin of methoxy group C-26 by transmethylation. The signal intensities and the ^{13}C-^{13}C coupling pattern in the ^{13}C NMR spectrum of the *L*-[U-^{13}C] methionine labeled cytotrienin A, as described in Table 1, at C-27 (171.64 d, J_{CC} = 84.3 Hz), C-28 (33.31 dt, J_{CC} = 84.3, 13.2 Hz), C-29, C-30 (16.68 t, 16.45 t, J_{CC} = 13.2 Hz) certified[3] that *L*-methionine was efficiently and specifically incorporated into the ACC unit at 11-side chain of cytotrienin A. This result established that the

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ACC unit at 11-side chain was biosynthesized from *L*-methionine similar to the biosynthetic pathway suggested for higher plants.

In mycotrienin, the m-C_7N unit of the ansa ring (hydroquinone moiety) is derived from 3-amino-5-hydroxybenzoic acid[4]. The cyclohexenecarboxylic acid moiety of cytotrienin A is presumably biosynthesized by the ω-cyclohexyl group of carboxylic acid formed from glucose, *i.e.* the same shikimate pathway as in the biosynthesis of mycotrienin[4,5].

Thus, the cytotrienin molecule is built up from six acetate, two propionate, and two methionine units as shown in Fig.1. Although the structure of cytotrienin A is considered to be a part of a series of related biogenetic analogues of mycotrienins, it is clearly different from the triene-ansamycin antibiotics possessing *D*-alanine such as mycotrienins or *L*-alanine such as ansatrienins[6,7].

It has been shown that ACC is a key intermediate in ethylene biosynthesis in higher plants and the formation of ACC from S-adenosyl-*L*-methionine is the rate-limiting reaction via a typical γ-elimination reaction from the starting material, *L*-methionine[8-10]. However, the *L*-methionine-ACC pathway has not been detected in microorganisms. This is the first report of a putative enzymatic system for constructing the ACC unit from *L*-methionine in bacteria.

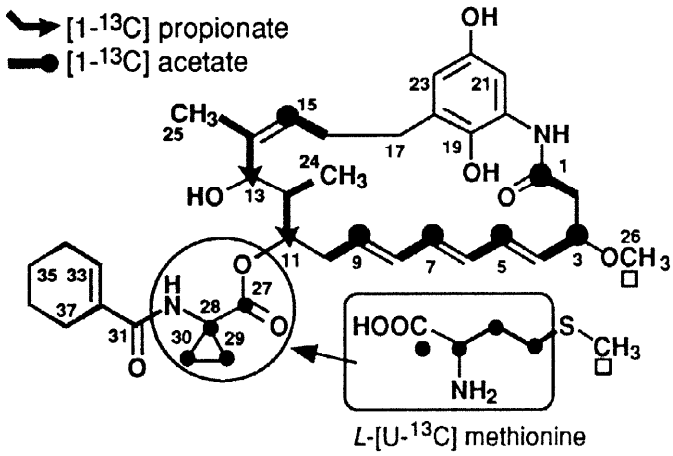

Fig. 1 Biosynthetic origin of cytotrienin A

Table 1. Incorporation of [1-^{13}C]acetate, [1-^{13}C]propionate, *L*-[methyl-^{13}C]methionine, and *L*-[U-^{13}C]methionine into cytotrienin A.

carbon	δ[a]	(^{13}C enriched fold[b])	$^1J_{CC}$ (Hz)
1	169.46 s	(A, 9)	
3	79.88 d	(A, 8)	
5	134.49 d	(A, 6)	
7	134.09 d	(A, 7)	
9	130.39 d	(A, 7)	
11	74.30 d	(P, 4)	
13	66.69 d	(P, 4)	
15	122.12 d	(A, 4)	
26	55.69 q	(M, 14)	
27	171.64 s	(uM, 6)	d, 84.3
28	33.31 s	(uM, 4)	dt, 84.3, 13.2
29	16.68 t	(uM, 7)	t, 13.2
30	16.45 t	(uM, 7)	t, 13.2

a) Shown in ppm with references to DMSO-d_6 as 39.70 ppm.
b) ^{13}C enrichment incorporated from: A, [1-^{13}C]acetate; P, [1-^{13}C]propionate; M, *L*-[methyl-^{13}C]methionine; uM, *L*-[U-^{13}C]methionine.

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