BIOSYNTHESIS OF AKLAVINONE AND ACLACINOMYCINS

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Anthracycline antibiotics aclacinomycins (ACMs) are aklavinone glycosides with variation in the trisaccharide moiety and were produced by *Streptomyces galilaeus* MA144-M1. Their production[1] and structural elucidation[2] were previously reported. By the isolation of various mutants[3] and the conversion of hypothetical precursors fed in the cultural medium[4], we found that ACMs were formed by a step-wise glycosidation of aklavinone followed by enzymatic modification of the terminal sugar[5]. We report here the ^{13}C NMR study of the biosynthesis of aklavinone by the incorporation of ^{13}C-acetate and ^{13}C-propionate and the radioisotopic analysis of ACM labeled with ^{14}C-precursors.

For the incorporation experiments, a blocked mutant strain 3AR-33, which accumulated aklavinone by the genetic loss of glycosidation ability[3], and an aclacinomycin A (ACM-A) producing strain 6U-21[3] were inoculated into a 500-ml Erlenmeyer flask containing 50 ml of productive medium consisting of 1.5% potato starch, 1.0 % glucose, 3.0 % soybean meal (Meat, Ajinomoto Co.), 0.1 % yeast extract, 0.3 % NaCl, 0.1 % K_2HPO_4, 0.1 % $MgSO_4 \cdot 7H_2O$, 0.0007 % $CuSO_4 \cdot 5H_2O$, 0.0008 % $MnCl_2 \cdot 4H_2O$, 0.0002 % $ZnSO_4 \cdot 5H_2O$ and 0.0001 % $FeSO_4 \cdot 7H_2O$, pH 7.4[6]. During a rapid productive phase of antibiotic (around 20~35-hour cultivation), each ^{13}C- or ^{14}C-labeled compound was added to the culture and the cultivation at 28°C on a rotary shaker (220 rpm) was continued for one to 20 hours. The cultured broth was then harvested and centrifuged. The crude antibiotic was extracted from the mycelium with acetone and from the supernatant fluid with chloroform. The isolation and purification of aklavinone and ACM-A from the extracts were performed by preparative layer chromatography using silica gel 60 PF_{254} (E. Merck) according to the method previously described[2]. Solvent systems used were $CHCl_3$ - MeOH (50: 1) for aklavinone and $CHCl_3$ - MeOH (20: 1) for ACM-A. ^{13}C NMR spectra of the antibiotics were recorded in $CDCl_3$ on a Varian model XL-100 with tetramethyl silane as an internal standard, and the radioactivity was measured by a liquid scintillation spectrometer (Aloka LSC-653) or by a radiochromatoscanner (Aloka JTC-203).

^{13}C-Labeled aklavinone was obtained from the culture (1.5 liters) of strain 3AR-33 supplemented with [1-^{13}C]acetate (75 mg), [2-^{13}C]acetate (75 mg), [1,2-^{13}C]acetate (100 mg), or [1-^{13}C]propionate (75 mg); all were purchased from Merck Sharp & Dohme Canada Ltd. The ^{13}C-enriched carbons were determined by comparison of ^{13}C NMR spectra between the labeled and unlabeled antibiotics. The results are shown in Table 1. The incorporation of [1-^{13}C]acetate resulted in enrichment of C-2, 4, 5, 6, 7, 10a, 11a, 12a, and 15, while that of [2-^{13}C]acetate enriched C-1, 3, 4a, 5a, 6a, 8, 10, 11 and 12. These indicated the consecutive incorporation of intact acetate in the aklavinone molecule, further confirmed by the fact that all resonances, except those of C-9, 13, 14 and 16, in ^{13}C NMR spectrum of aklavinone enriched with [1,2-^{13}C]acetate were accompanied by two satellite signals due to spin ^{13}C-^{13}C coupling as shown in Table 2, while [1-^{13}C]propionate enriched only one signal at C-9. These results show that ring carbon of aklavinone was made up of nine acetates (or acetyl-CoA) and one propionate (or propionyl-CoA)

Table 1. Incorporation of ^{13}C-labeled precursors into aklavinone.

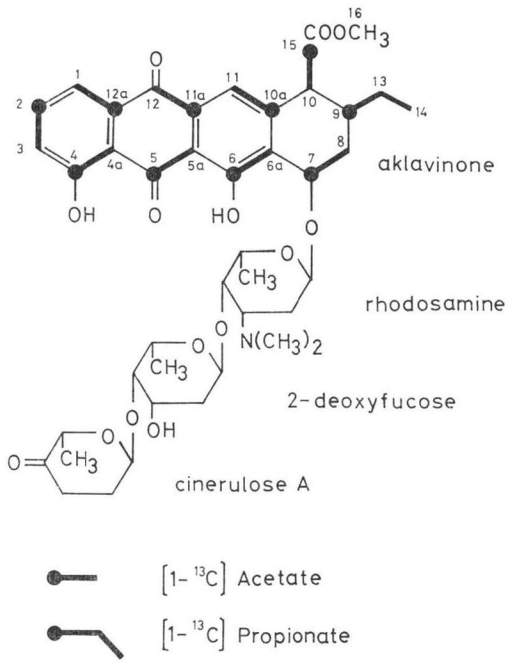
^{13}C-Precursor	Enriched carbon*
[1-^{13}C] Acetate	C-2 (1.94), C-4 (1.68), C-5 (1.59), C-6 (1.54), C-7 (1.72), C-10a (1.48), C-11a (1.63), C-12a (1.91), C-15 (1.69)
[2-^{13}C] Acetate	C-1 (1.52), C-3 (1.47), C-4a (2.13), C-5a (1.68), C-6a (1.55), C-8 (2.00), C-10 (1.92), C-11 (1.56), C-12 (2.00)
[1-^{13}C] Propionate	C-9 (1.95)

* Value in parenthesis indicates a ratio for the level of ^{13}C at each carbon with reference to the natural abundance level. The carbon-13 signal assignment of aklavinone is described in Reference 2.

Table 2. ^{13}C-^{13}C coupling constants for [1,2-^{13}C]-acetate-enriched aklavinone.

Coupled carbons	J (Hz)	Coupled carbons	J (Hz)
C(1)-C(12a)	60.4	C(6a)-C(6)	66.9
C(12a)-C(1)	61.8	C(7)-C(8)	38.0
C(2)-C(3)	58.2	C(8)-C(7)	37.5
C(3)-C(2)	58.5	C(10)-C(15)	55.0
C(4)-C(4a)	62.3	C(15)-C(10)	55.3
C(4a)-C(4)	63.2	C(10a)-C(11)	57.1
C(5)-C(5a)	56.1	C(11)-C(10a)	57.4
C(5a)-C(5)	56.0	C(11a)-C(12)	54.0
C(6)-C(6a)	67.5	C(12)-C(11a)	53.8

Fig. 1. Biosynthesis of aclacinomycin A from nine acetates and one propionate.



as shown in Fig. 1, where the propionate unit was incorporated as a starter in the condensation for the ring formation.

For the ^{14}C-incorporation study, ^{14}C-labeled ACM-A was also obtained from a 1.5-liter culture of strain 6U-21 supplemented with 150 μCi of [1-^{14}C]acetate (57.0 Ci/mole), [1-^{14}C]propionate (14.0 Ci/mole), [U-^{14}C]-D-glucose (213 Ci/mole), [U-^{14}C]glycerol (131 Ci/mole), [U-^{14}C]-L-leucine (320 Ci/mole) or [methyl-^{14}C]-L-methionine (40.6 Ci/mole). When ^{14}C-labeled acetate, propionate, L-methionine and D-glucose were added to the culture, ACM-A was labeled with the incorporation efficiency of 1.8 %, 4.4 %, 9.4 % and 0.3 %, respectively. However, no significant incorporation was observed with ^{14}C-glycerol and ^{14}C-L-leucine. Each labeled ACM-A was hydrolyzed with 0.1 N HCl at 70°C for 60 minutes to obtain aklavinone and sugar moieties. Aklavinone was extracted from the hydrolysate with $CHCl_3$ and purified by thin-layer chromatography as described above. The sugar component in the aqueous phase were separated into rhodosamine, 2-deoxyfucose and cinerulose on a silica gel F_{254} plate (E. Merck) with a solvent of *n*-BuOH - AcOH - water (4: 1: 1) as previously described[2]. Most radioactivity derived from ^{14}C-acetate and ^{14}C-propionate was found in aklavinone and that from ^{14}C-methionine was found in both aklavinone and rhodosamine. The aklavinone ^{14}C-labeled with ^{14}C-methionine, but not that with ^{14}C-acetate or ^{14}C-propionate, lost the radioactivity by demethoxycarbonylation by alkaline treatment[7], whereas the radioactivity from ^{14}C-D-glucose was present in the sugar moiety where the radioactivity was equally distributed among the three component sugars.

The results described above showed that the biogenesis of aklavinone ring was same as that of daunomycinone, which was also proved to be built up from nine acetate units and one propionate unit[8]. This coincided with our previous evidence obtained by the biotransformation studies that aklavinone was precursor in the biosynthesis of daunomycinone[9]. An esterified methyl (C-16) on aklavinone ring and *N,N*-dimethyl group in the aminosugar were derived from methionine. ^{14}C-Incorporation study suggested that sugars in ACM were originated from D-glucose as in the case of daunomycin[8].

References

1) OKI, T.; I. KITAMURA, A. YOSHIMOTO, Y. MATSUZAWA, N. SHIBAMOTO, T. OGASAWARA, T. INUI, A. TAKAMATSU, T. TAKEUCHI, T. MASUDA, M. HAMADA, H. SUDA, M. ISHIZUKA, T. SAWA & H. UMEZAWA: Antitumor anthracycline antibiotics, aclacinomycin A and analogues. I. Taxonomy, production, isolation and physicochemical properties. J. Antibiotics 32: 791~800, 1979

2) OKI, T.; I. KITAMURA, Y. MATSUZAWA, N. SHIBAMOTO, T. OGASAWARA, A. YOSHIMOTO, T. INUI, H. NAGANAWA, T. TAKEUCHI & H. UMEZAWA: Antitumor anthracycline antibiotics, aclacinomycin A and analogues. II. Structural

determination. J. Antibiotics 32: 801~819, 1979

3) YOSHIMOTO, A.; Y. MATSUZAWA, T. OKI, T. TAKEUCHI & H. UMEZAWA: New anthracycline metabolites from mutant strains of *Streptomyces galilaeus* MA144-M1. I. Isolation and characterization of various blocked mutants. J. Antibiotics 34: 951~958, 1981
4) OKI, T.; A. YOSHIMOTO, Y. MATSUZAWA, T. TAKEUCHI & H. UMEZAWA: Biosynthesis of anthracycline antibiotics by *Streptomyces galilaeus*. I. Glycosidation of various anthracyclines by an aclacinomycin-negative mutant and biosynthesis of aclacinomycins from aklavinone. J. Antibiotics 33: 1331~1340, 1980
5) YOSHIMOTO, A.; T. OGASAWARA, I. KITAMURA, T. OKI, T. INUI, T. TAKEUCHI & H. UMEZAWA: Enzymatic conversion of aclacinomycin A to Y by a specific oxidoreductase in *Streptomyces*. J. Antibiotics 32: 472~481, 1979
6) OKI, T.; Y. MATSUZAWA, A. YOSHIMOTO, K. NUMATA, I. KITAMURA, S. HORI, A. TAKAMATSU, H. UMEZAWA, M. ISHIZUKA, H. NAGANAWA, H. SUDA, H. HAMADA & T. TAKEUCHI: New antitumor antibiotics, aclacinomycins A and B. J. Antibiotics 28: 830~834, 1975
7) TANAKA, H.; T. YOSHIOKA, Y. SHIMAUCHI, Y. MATSUZAWA, T. OKI & T. INUI: Chemical modification of anthracycline antibiotics. I. Demethoxycarbonylation, 10-epimerization and 4-O-methylation of aclacinomycin A. J. Antibiotics 33: 1323~1330, 1980
8) CASEY, M.L.; R. C. PAULICK & H.W. WHITLOCK: Carbon-13 nuclear magnetic resonance study of the biosynthesis of daunomycin and islandicin. J. Org. Chem. 43: 1627~1634, 1978
9) YOSHIMOTO, A.; T. OKI & H. UMEZAWA: Biosynthesis of daunomycinone from aklavinone and ε-rhodomycinone. J. Antibiotics 33: 1199~1201, 1980