BIOSYNTHETIC INCORPORATION OF METHYL GROUPS INTO FORTIMICINS

SHUZO OKUMURA, TAKASHI DEGUCHI and HIROFUTO MARUMO

Pharmaceuticals Research Laboratory,
Kyowa Hakko Co., Ltd.
1188 Shimotogari, Nagaizumi-machi,
Suntogun, Shizuoka, Japan

(Received for publication July 10, 1981)

Fortimicins are unique aminoglycoside antibiotics produced by *Micromonospora olivoasterospora*.[1,2], the structures of which were shown in Fig. 1. For the preparation of labeled aminoglycoside antibiotics, biosynthetic labeling has been mainly studied. In the previous studies, the methyl groups of micronomicin (sagamicin)[3] and gentamicin[4] were demonstrated to be derived from methionine, which was used as a labeled precursor.

In the present paper we wish to report the biosynthetic origin of methyl groups in fortimicins by means of the preparation of ^{13}C- or ^{14}C-labeled fortimicins and some analytical results on the ^{13}C distribution in the labeled antibiotic.

^{14}C Labeled fortimicins were prepared by means of fermentation or biotransformation. In fermentation, L-methionine, [methyl-^{14}C]-, a radioactive precursor, was added at 48 hours after inoculation. After the fermentation at 30°C for 7 days ^{14}C labeled fortimicins were isolated according to the method reported previously[2]. Subsequently about 6% of radioactivity of L-methionine, [methyl-^{14}C]-, was incorporated into fortimicin A, and about 2% into fortimicin B.

Since intact cell system is useful for obtaining labeled antibiotics as observed in ^{14}C labeled micronomicin[3], the preparation of ^{14}C labeled fortimicins was carried out by means of the biotransformation with the intact cells. Each flask contained 5 μCi of a radioactive precursor, glycine, [^{14}C(U)]-, sucrose, [^{14}C(U)]-, L-methionine, [methyl-^{14}C]-, or methylcobalamin, [methyl-^{3}H]-. After incubation at 30°C for 24 hours the reaction mixture was purified by ion exchanger and the resulting fraction of antibiotics was submitted to tlc separation. The radioactivities in fortimicin A and fortimicin B fraction were summarized in Table 1 for each radioactive precursor. Alkaline hydrolysis of fortimicin A derived from ^{14}C-glycine afforded radioactive glycine (97%) and fortimicin B (0.3%) moieties. However, L-methionine, [methyl-^{14}C]-, was incorporated into fortimicin A by approximately 5%, and much less into fortimicin B (0.7%).

Methylcobalamin, [methyl-^{3}H]-, showed high incorporation into fortimicin A, but lower into fortimicin B. This result indicated that cobalamin was required in the biosynthesis of fortimicin A and besides methyl group of methylcobalamin was directly incorporated into fortimicins, particularly, fortimicin A. YAMAMOTO[7] reported that cobalamin showed a stimulatory effect on fortimicin A production and had much less effect on the fortimicin B production. It was also observed that in micronomicin biosynthesis methylcobalamin showed more incorporation of radioactivity into micronomicin (0.07%) than L-methionine (0.02%). TESTA *et al.*[8] also reported the involvement of cobalt dependent methylation for

Fig. 1. Structures of fortimicins.

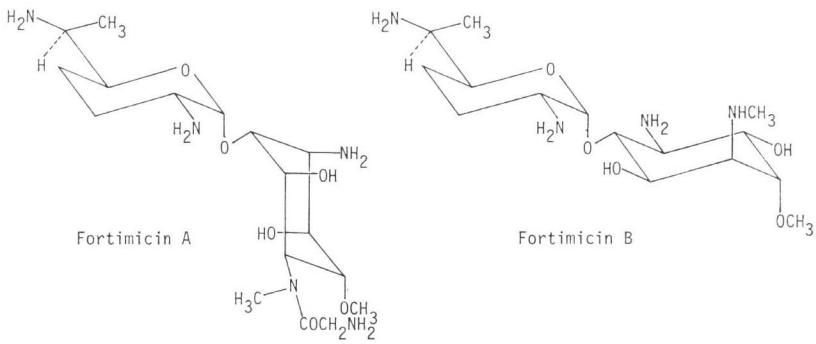

Table 1. Incorporation of ^{14}C-labeled precursors into fortimicin A.

Precursor	Specific activity (mCi/mmole)	Incorporation of radioactivity into fortimicin A (%)[b]		Specific activity of fortimicin A (μCi/mg)
		Fermentation	Intact cell system[c]	
Glycine, [^{14}C-(U)]-	112	0.16	0.47	0.128
Sucrose, [^{14}C-(U)]-	277	0.004	0.002	0.0006
Methylcobalamin, [methyl-^{3}H]-[a]	56.3	0.06	2.67	0.72
L-Methionine, [methyl-^{14}C]-	53.7	5.72	5.61	1.57

a) Hydroxocobalamin was reduced with sodium borohydride and subsequently reacted with methyliodide, [^{3}H]- according to SCHRAUZER[5,6].

b) Determined by radioactivities in silica gel tlc fractions (solvent; isopropanol - chloroform - 29% ammonium hydroxide, 2: 1: 1, v/v).

c) The mycelium at 3 days after inoculation was used, when the best incorporation of radioactivity into fortimicins from L-methionine, [methyl-^{14}C]- was observed.

gentamicin fermentation.

In micronomicin all the *C*- and *N*-methyl groups were derived from methionine and they were labeled to an equal degree[3]. In the case of fortimicin A it was interested whether methionine could label three kinds of methyl groups. ^{14}C-Fortimicin A was prepared by a large scale incubation and subsequent isolation procedure. About 6% of radioactivity of L-methionine, [methyl-^{14}C]-, was incorporated into fortimicin A. The specific activity and radiochemical purity of ^{14}C-fortimicin A were 1.84 μCi/mg and 96.0%, respectively (by fluorometrical analysis).

In order to determine the distribution of ^{14}C in the labeled fortimicin A, an incorporation experiment was carried out in intact cell system, using L-methionine, [methyl-^{13}C]-, as a labeled precursor. When a large amount of L-methionine, [methyl-^{13}C]-, was used, fortimicin A production was inhibited. In the experiment where less than 0.05 g/liter of L-methionine, [methyl-^{13}C]-, was used, a higher rate of incorporation into fortimicin A was observed. From the latter incubation mixture, ^{13}C-fortimicin A was isolated according to the same method as mentioned above. ^{13}C-FT NMR spectra were taken with a JNM-FX-100 spectrometer. Samples were dissolved in D_2O containing dioxane as the internal reference (67.4 ppm). ^{13}C NMR spectra of ^{13}C-fortimicin A indicated that all the *C*-, *N*- and *O*-methyl peaks were enriched by ^{13}C, which appeared at 15.1, 32.0 and 56.8 ppm (relative intensities 1.06: 0.90: 1.00), respectively. Therefore all methyl groups of fortimicin A were estimated to be derived from methionine.

The radioactive fortimicins have been found to be useful for studying the metabolic behaviors of the antibiotics in animals[9], as well as the radioimmunoassay of fortimicin A[10].

Acknowledgement

We wish to thank Mrs. M. YOSHIDA of Tokyo Research Laboratory, Kyowa Hakko Co., for ^{13}C NMR spectra.

References

1) NARA, T.; M. YAMAMOTO, I. KAWAMOTO, K. TAKAYAMA, R. OKACHI, S. TAKASAWA, T. SATO & S. SATO: Fortimicins A and B, new aminoglycoside antibiotics. I. Producing organism, fermentation and biological properties of fortimicins. J. Antibiotics 30: 533~540, 1977

2) OKACHI, R.; S. TAKASAWA, T. SATO, S. SATO, M. YAMAMOTO, I. KAWAMOTO & T. NARA: Fortimicins A and B, new aminoglycoside antibiotics. II. Isolation, physico-chemical and chromatographic properties. J. Antibiotics 30: 541~551, 1977

3) DEGUCHI, T.; S. OKUMURA, A. ISHII & M. TANAKA: Synthesis of carbon-14 and tritium labeled sagamicin. J. Antibiotics 30: 993~998, 1977

4) DANIELS, P. J. L.; A. YEHASKEL & J. B. MORTON: The biosynthetic origin of the methyl groups of the gentamicin antibiotics. 16th Intersci. Conf. on Antimicr. Agents & Chemoth., Chicago, Oct. 1976

5) JOHNSON, A. W.; L. MERVYN, N. SHAW & E. L. SMITH: A partial synthesis of the vitamin B_{12} coenzyme and some of its analogues. J. Chem.

Soc. 1963: 4146~4156, 1963

6) SCHRAUZER, G. N.: The chemistry of Co(I) derivatives of vitamin B_{12} and of related chelates. Ann. N. Y. Acad. Sci. 158: 526~539, 1969

7) YAMAMOTO, M.; R. OKACHI, I. KAWAMOTO & T. NARA: Fortimicin A production by *Micromonospora olivoasterospora* in a chemically defined medium. J. Antibiotics 30: 1064~1072, 1977

8) TESTA, R. T. & L. KAMNITZER: Gentamicin production in a synthetic medium. Abst. Papers No. E-107, p. 18, 47th Annual Meeting of Am. Soc. Microbiol., Chicago, May 12~17, 1974

9) OKUMURA, S.; T. DEGUCHI & H. MARUMO: Radioimmunoassays of ^{3}H fortimicins. Jap. J. Antibiotics 33: 1125~1128, 1980

10) INOUE, A.; S. OKUMURA, T. DEGUCHI & H. MARUMO: The physiological disposition of ^{14}C-KW-1070. Chemotherapy, in preparation.