Note

A Possible Biosynthetic Precursor of 3-Hydroxypicolinic Acid in Etamycin

Nobuo NAGATO, Yasushi OKUMURA,*
Rokuro OKAMOTO and Tomoyuki ISHIKURA

Central Research Laboratories,
Sanraku-Ocean Co., Ltd.,
9-1 Johnan 4-chome,
Fujisawa 251, Japan

Received April 11, 1984

3-Hydroxypicolinic acid (3-HPA) is found in the virginiamycin B group¹⁾ and in pyridomycin.²⁾ For biosynthetic precursors of the 3-HPA moiety in both antibiotics, two precursors have been proposed. Ogawara *et al.* reported that ¹⁴C-labeled L-aspartic acid and glycerol were incorporated into the 3-HPA moiety in pyridomycin,³⁾ while Hook and Vining reported that ¹⁴C-labeled L-lysine but not L-aspartic acid was incorporated into the 3-HPA moiety in etamycin, one of the virginiamycin B group.⁴⁾ In the case of etamycin, as the incorporation of ¹⁴C-labeled L-lysine was extremely low (approx. 0.15%) and the incorporation time was very long (approx. 72 hr), it has not been confirmed that L-lysine is the precursor of the 3-HPA moiety in etamycin. As a consequence, we have attempted to determine the biosynthetic origin of 3-HPA in etamycin in a short term of incubation.

Streptomyces griseoviridis P8648, one of etamycin-producing micro-organisms, was employed in this study. Cultivation was carried out as described previously.⁵⁾ L-[¹⁴C-(U)]lysine (300 mCi/mmol) and L-[¹⁴C-(U)]aspartic acid (200 mCi/mmol) were added to the culture at 72 hr after inoculation at the concentration of 0.04 μ Ci/ml each. At an appropriate time, 2 ml of the broth was sampled and subjected to analyses of the antibiotic production, incorporation of the labeled amino acids, and distribution of the radioactivities by the methods described previously.⁵⁾ For determination of the biosynthetic pathway of 3-HPA from L-lysine, various compounds listed in Table II were added to the culture at 1 hr before the addition of the labeled L-lysine, and their effects on etamycin production and the incorporation of labeled L-lysine into the antibiotic were determined at 24 hr after the addition of the labeled L-lysine.

The incorporation of ¹⁴C-labeled L-lysine into the antibiotic was compared with that of ¹⁴C-labeled L-aspartic

acid (Table I). Time course of the incorporation of the labeled L-lysine is shown in Fig. 1. In addition, there was no radioactive residue except 3-HPA detected in etamycin (data not shown). These results sustain the idea that L-lysine but not L-aspartic acid is one of the precursors of 3-HPA in etamycin.

We have tried to elucidate the biosynthetic pathway of 3-HPA in etamycin from L-lysine. The results are shown in Table II. S-2-Amino-ethyl-L-cysteine, one of the L-lysine analogs, caused a great reduction of not only antibiotic

FIG. 1. Time Course of L-[¹⁴C-(U)] Lysine Incorporation into Etamycin.

S. griseoviridis P8648 was cultivated in a 250-ml Erlenmeyer flask containing 25 ml of the medium described previously.⁵⁾

TABLE I. COMPARISON OF THE INCORPORATION RATIO AND ETAMYCIN PRODUCTION WITH L-[¹⁴C-(U)]LYSINE AND WITH L-[¹⁴C-(U)]ASPARTIC ACID

S. griseoviridis P8648 was cultured in a 250-ml Erlenmeyer flask containing 25 ml of the medium described previously.⁵⁾ With labeled L-aspartic acid, L-glutamine was used as a nitrogen source instead of L-asparagine.

Labeled compound	Antibiotic production (μ g/ml)	% incorporation
L-[¹⁴ C-(U)]Lys	62	18.0
L-[¹⁴ C-(U)]Asp	60	0.1

* To whom all correspondence should be addressed.

TABLE II. INFLUENCES OF VARIOUS COMPOUNDS
UPON THE INCORPORATION OF LABELED
L-LYSINE INTO ETAMYCIN AND
THE ANTIBIOTIC PRODUCTION

Compound	Concn. (mM)	Etamycin production (% control)	Labeled L-lysine incorporation (% control)
Control	—	100	100
S-2-AEC	1	15	18
DL-DAP	2	100	100
DL-AAA	2	100	95
L-Pip	1	100	90
L-Saccharopine	1	95	90
DL-Hylys	2	100	53
L-Hypip	1	105	24
3-HPA	1	120	10
PA	1	100	100
2,3-PDA	1	100	100
L-Try	1	95	100

Abbreviations: S-2-AEC, S-2-aminoethyl-L-cysteine; DL-DAP, DL-diaminopimelic acid; DL-AAA, DL-2-aminoadipic acid; L-Pip, L-pipecolic acid; DL-Hylys, 5-hydroxyl-DL-lysine; L-Hypip, 5-hydroxy-L-pipecolic acid; 3-HPA, 3-hydroxypicolinic acid; PA, picolinic acid; 2,3-PDA, 2,3-pyridine dicarboxylic acid.

production but also lysine incorporation. The other compounds except 3-HPA scarcely affected the antibiotic production. 3-HPA slightly enhanced the antibiotic production. On the other hand, 3-HPA, 5-hydroxy-L-pipecolic acid, and 5-hydroxy-DL-lysine significantly reduced the incorporation of labeled L-lysine into the antibiotic. This suggests that a free form of 3-HPA derived from L-lysine is a direct precursor of the 3-HPA moiety in etamycin, and that 5-hydroxy-L-pipecolic acid and 5-hydroxy-DL-lysine may also be precursors of 3-HPA in etamycin. Furthermore, since picolinic acid is not a precursor of 3-HPA in etamycin, the hydroxy group at the C-3 position of 3-HPA may be introduced into the pyridine ring of picolinic acid before the pyridine ring formation.

REFERENCES

- 1) Y. Okumura, "Biochemistry and Genetic Regulation of Commercially Important Antibiotics," ed. by L. C. Vining, Addison-Wesley Publishing Co., Massachusetts, 1983, pp. 147~178.
- 2) K. Maeda, H. Kosada, Y. Okami and H. Umezawa, *J. Antibiot.*, **6A**, 140 (1953).
- 3) H. Ogawara, K. Maeda and H. Umezawa, *Biochemistry*, **7**, 3296 (1968).
- 4) D. J. Hook and L. C. Vining, *Can. J. Biochem.*, **51**, 1630 (1973).
- 5) Y. Okumura, M. Onishi, R. Okamoto and T. Ishikura, *Agric. Biol. Chem.*, **46**, 3063 (1982).