FUNGIMYCIN, BIOGENESIS OF ITS AROMATIC MOIETY

Sir:

Fungimycin (syn: perimycin), an antifungal heptaene macrolide produced by *Streptomyces coelicolor* var. *aminophilus* was found to contain a novel amino sugar, perosamine[1~3]. At the same time it was shown to yield an aromatic ketone upon alkaline hydrolysis[1]. The chemical structure of the aromatic moiety has been elucidated as N-methyl-*p*-aminoacetophenone[4]. In this communication, we wish to report the biogenetic origin of the N-methyl-*p*-aminophenyl moiety of fungimycin.

Fungimycin fermentation was carried out in shake flasks with a medium containing (in grams per liter): corn-steep liquor, 60; Cerelose (technical glucose), 30; and Staley 4-S soybean meal, 20[5]. The pH of the medium was adjusted with NaOH to 7.6 before sterilization. The culture was harvested after 6 days of fermentation in an incubator-shaker at 28°C. Provisions were taken to trap the metabolic CO_2. Labeling of fungimycin was carried out by adding various ^{14}C-precursors to the culture medium during fermentation. The antibiotic was isolated and purified by the following procedure: Fermentation broth was acidified with 0.1 N H_2SO_4 to pH 4.5 and filtered with the aid of 6% (w/v) of Hyflo Super-Cel. The mycelium pad was then washed several times with acidified distilled water (pH 4.5), and extracted with a mixture of chloroform and 95% ethanol (1:1, v/v). The extract was then purified by chromatography on silica gel thin-layer plates using $CHCl_3$-EtOH-H_2O (1:1:0.16, v/v/v) as the solvent system. One major and one minor component of the fungimycin complex could be extracted from fresh mycelium cake and detected by the thin-layer chromatography system. The Rf values were respectively, 0.72 and 0.98. The component with Rf of 0.72 was extracted from TLC plates and used for the analysis of radioactivity incorporation. N-Methyl-*p*-aminoacetophenone was obtained from fungimycin by alkaline retro-aldolization[1] and purified by thin-layer chromatography using fluorescent silica gel plates (Mallinckrodt, Silicar TLC 7GF) and benzene-acetone (4:1, v/v) as the solvent system. The Rf value of the aromatic compound was 0.68. Radioactivity measurements were carried out as described previously[6]. Table 1 summarizes the results obtained from these incorporation studies.

The N-methyl group of the aromatic

Fig. 1. Possible routes for the incorporation of N-methyl group into fungimycin.

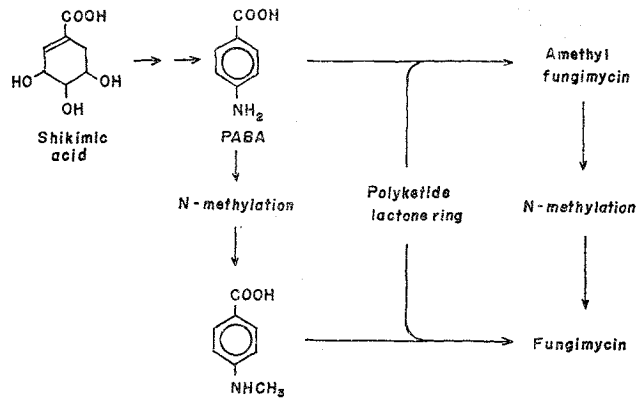

Table 1. Incorporation of ^{14}C-precursors into the aromatic moiety of fungimycin

Precursor	Amount of precursor added	Specific activity of fungimycin (cpm/μmole)	Specific activity of N-methyl-*p*-aminoacetophenone (cpm/μmole)	Percent radioactivity in the aromatic moiety
Acetate-1-^{14}C	30 μc	1.91×10^3	no significant activity	—
Glucose-(UL)-^{14}C	50 μc	5.17×10^3	3.59×10^2	6.95
Methionine-methyl-^{14}C	116 μc	1.44×10^5	1.48×10^5	102.0
Shikimic acid-(G)-^{14}C	50 μc	4.76×10^3	5.0×10^3	105.0
p-Aminobenzoic acid-(ring UL)-^{14}C	48 μc	4.72×10^6	5.5×10^6	117.0*

* Calculated values were sometimes much higher than 100%. The reason of this higher value is not known.

ketone from fungimycin is evidently derived from the methyl group of L-methionine. Like candicidin[6], the aromatic ring in fungimycin is the product of the shikimic acid pathway with *p*-aminobenzoic acid (PABA) as the direct terminal intermediate to be incorporated into this moiety of fungimycin. Possible routes for the incorporation of the N-methyl group into fungimycin are depicted in Fig. 1. Studies are now underway in an attempt to differentiate these possibilities. The low incorporation of acetate-^{14}C into the aromatic ketone rules out the possibility that the aromatic moiety is a product of polyketide synthesis. The high efficiency of PABA incorporation into fungimycin (38 %) made it possible to produce ^{14}C-labeled fungimycin with high specific activity. From these results together with the studies of candicidin biosynthesis[6], it is inferrable that the other phenyl moieties of the aromatic heptaene macrolides such as trichomycin might have a similar biogenetic origin.

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