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BIOGENESIS OF BLASTICIDIN S

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Blasticidin S is an antibiotic produced by Streptomyces griseochromogenes[1] and the structure has been established as shown in FIG. 1.[2] It consists of a pyrimidine nucleoside and β-amino acid named cytosinine and blastidic acid, respectively.

Hitherto, several reports had been published on the biogenesis of purine nucleoside antibiotics such as cordycepin[3] and angustmycin C (psicofuranine),[4] but very few reports are concerned with pyrimidine nucleoside antibiotics. On the other hand, the biogenesis of β-alanine and α-methy-β-alanine are known, while these β-amino acids should be regarded as ω-amino acids rather than as β-amino acids from the point of view of their formation mechanisms.[5]

It is worth-while and of interest to account for the metabolic pathways of an unique nucleoside and an anomalous amino acid. This paper concerns the biogenetic aspect of blasticidin S.

For the incorporation tests of ^{14}C-compounds excepting ^{14}C-glucose into blasticidin S, the synthetic medium consisting of sucrose, glucose and mineral salts[6] was used. When ^{14}C-glucose was used, the medium consisted of 5 % sucrose, 2 % soybean meal and 0.5 % sodium chloride. Each ^{14}C-compound was added to the 48 hour old culture broths of St. griseochromogenes. After 72 hour cultivation, radioactive blasticidin S was isolated by cation exchange procedure.

The incorporation ratio of ^{14}C-compounds into the antibiotic are presented in TABLE 1. According to these results, it became evident that D-glucose-(U)-^{14}C, D-glucose-1-^{14}C, D-glucose-6-^{14}C, cytosine-2-^{14}C, cytidine-(U)-^{14}C, L-methionine-(methyl)-^{14}C, L-arginine-(guanidino)-^{14}C and L-arginine-(U)-^{14}C were incorporated into blasticidin S in higher yield as compared to other compounds.

TABLE 1

Incorporation Ratio of ^{14}C-labeled Compounds into Blasticidin S

	(%)		(%)
D-glucose-(U)-^{14}C	3.7	*L-aspartic acid-(U)-^{14}C	0.5
D-glucose-1-^{14}C	4.0	β-alanine-1-^{14}C	0.6
D-glucose-6-^{14}C	4.9	acetic acid-(U)-^{14}C	0.5
cytosine-2-^{14}C	95.1	glycine-(U)-^{14}C	1.1
cytidine-(U)-^{14}C	15.3	L-alanine-(U)-^{14}C	0.5
L-methionine-(methyl)-^{14}C	38.3		
L-arginine-(guanidino)-^{14}C	51.2		
L-arginine-(U)-^{14}C	30.3		

*Unlabeled cytosine was added simultaneously.

The isolated blasticidin S was degraded by the scheme presented in FIG. 1 and radioactivity of the degradation products was measured by liquid scintillation counter.

FIG. 1 Degradation Scheme of Blasticidin S

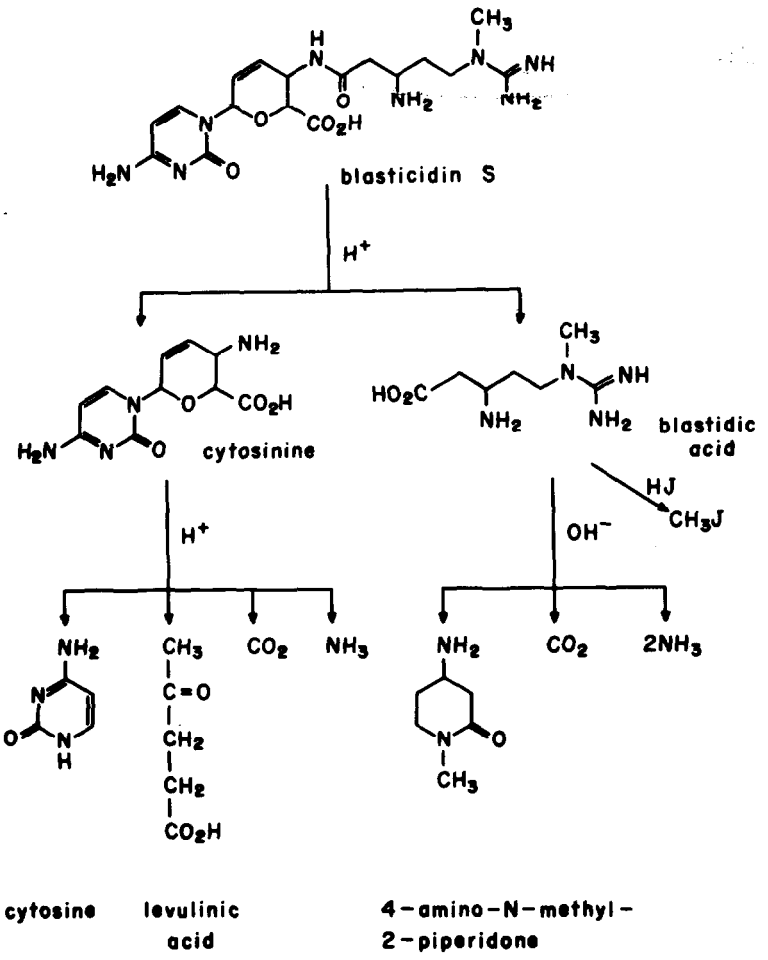


TABLE 2

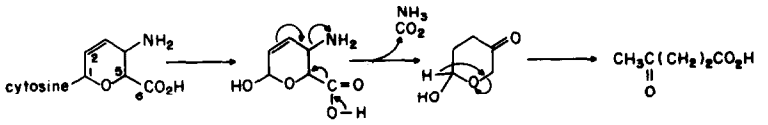
Percentage of Radioactivity in the Degraded Products of Blasticidin S

compound \ precursor	cytosine-2-^{14}C	cytidine-(U)-^{14}C	D-glucose-(U)-^{14}C	D-glucose-1-^{14}C	D-glucose-6-^{14}C	L-methionine-(methyl)-^{14}C	L-arginine-(U)-^{14}C	L-arginine-(guanidino)-^{14}C
blasticidin S	100	100	100	100	100	100	100	100
blastidic acid	0	0.5	16.9	14.8	26.6	98.3	98.0	97.9
CO_2	-	-	-	-	-	-	*24.5	99.5
4-amino-N-methyl-2-piperidone	-	-	-	-	-	-	*69.2	0
CH_3J	-	-	-	-	-	101.6	0	0
cytosinine	97.1	98.2	83.6	89.2	74.6	0.1	1.5	2.7
cytosine	96.1	97.9	22.8	21.6	23.8	0.1	1.1	2.6
levulinic acid	0	0.2	52.0	62.9	0	-	-	-
CO_2	0	0.3	11.3	6.7	45.7	-	-	-

* If L-arginine-(U)-^{14}C could be incorporated unmodified the radioactive ratio of CO_2 to 4-amino-N-methyl-2-piperidone must be 1:5, nevertheless, the value found in the experiment would approximately be 1:3. The deviation from the theoretical value is considered to be caused by transamidination between ^{14}C-labeled arginine and unlabeled ornithine which was produced during cultivation.

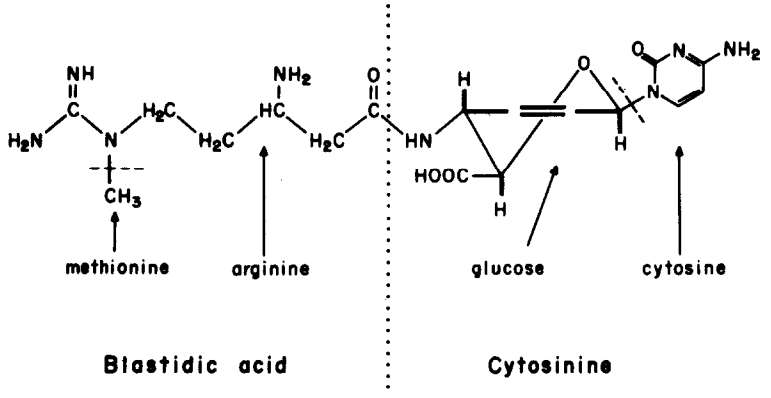
The results presented in TABLE 2 indicated the following facts: (i) Cytosine was incorporated intact into the cytosine nucleus of blasticidin S. (ii) The sugar part of cytosinine was derived from D-glucose. As explained previously[2] levulinic acid, ammonia and carbon dioxide were formed from the sugar part of cytosinine as shown in FIG. 2, and the formation mechanism was supported by the experiments used ^{14}C-labeled glucose.

FIG. 2



(iii) When cytidine-(U)-^{14}C was added, almost all of the radioactivity existed in the cytosine nucleus but was almost negligible in the sugar portion. Therefore, only the cytosine nucleus was incorporated into blasticidin S after cleavage of the C-N linkage of cytidine. (iv) N-Methyl group of blastidic acid was derived from methionine in a manner similar to the formation of creatine from guanidino acetic acid.[7] (v) The whole molecule of L-arginine, excepting the α-amino group, was incorporated into the skeleton of blastidic acid. The mechanism of β-amino acid formation from α-amino acid remains to be solved.

From the results mentioned above, the biogenesis of blasticidin S can be depicted as follows.



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