FORMATION OF 8-AZAGUANINE FROM GUANINE BY *STREPTOMYCES ALBUS*

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For many years, 8-azaguanine (**1**) has received much attention because of its remarkable biological activity[1]. In 1961, this compound was found to be a metabolite of *Streptomyces albus* var. *pathocidicus* and named pathocidin[2]. The same strain also produces a cytosine nucleoside antibiotic, blasticidin S. Because it is an example of an unusual nucleobase occurring in nature, we became interested in studying its biosynthesis.

In vivo feeding experiment was performed with growing cells of *S. albus* in a medium containing 2% glucose, 1% starch, 2.5% soybean meal, 0.1% meat extract, 0.5% dry yeast, 0.2% NaCl, and 0.005% K_2HPO_4. After 45-hour fermentation in shake flasks, ^{14}C-labeled compounds were added to the culture. After 45-hour additional incubation, 8-azaguanine was isolated from the culture filtrate by a procedure modified from that of originally described utilizing Amberlite IR-45. Complete separation from guanine was achieved by preparative thin-layer chromatography on cellulose (Merck cellulose F) with the solvent: *n*-propanol - 1 N NH_4OH (7:3) and paper chromatography with water. 8-Azaguanine was finally recrystallized from water. Results of the incorporation of ^{14}C-labeled compounds are summarized in Table 1. [2-^{14}C] Guanine was very efficiently incorporated (Exp. 1). In contrast, [8-^{14}C] guanine was not incorporated (Exp. 1). [2-^{14}C] Adenine was incorporated with an efficiency comparable to [2-^{14}C] guanine (Exp. 2). From these results, it appears that 8-azaguanine is formed from guanine by the replacement of C-8 of guanine with nitrogen.

GTP-formylhydrolase which catalyzes the hydrolytic cleavage of C-8 of GTP, is regarded as a key enzyme for the biosynthesis of pterins, lumazines, flavins, pyrrolopyrimidine antibiotics *etc*[3]. Similar hydrolytic cleavage of guanine may be probable in the biosynthesis of 8-azaguanine. However, addition of 6-hydroxy-2, 4, 5-triaminopyrimidine (**2**, R=H), a possible intermediate, at a concentration of 5×10^{-3} M resulted neither in decrease of the specific activity of 8-azaguanine nor in increased yield (Exp. 3). This may suggest that the biosynthesis of 8-azaguanine from guanine proceeds at the nucleoside or nucleotide level, but not at the base

Scheme 1. Possible pathway for the biosynthesis of 8-azaguanine

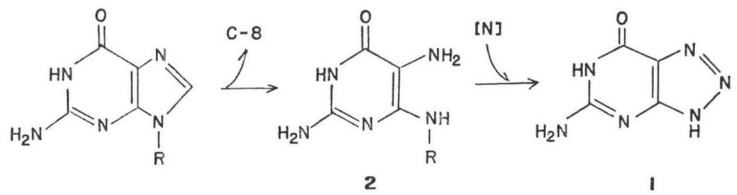

Table 1. Incorporation of ^{14}C-labeled compounds into 8-azaguanine

Exp. No.	Compound added		8-Azaguanine isolated	
		Sp Act. (Ci/mol)	Sp Act. (mCi/mol)	Dilution*
1	[2-^{14}C] Guanine	1.88	23.3	81
	[8-^{14}C] Guanine	1.11	0.09	12,000
2	[2-^{14}C] Adenine	1.60	13.4	120
3	[2-^{14}C] Guanine	20.0	13.1	1,530
	[2-^{14}C] Guanine plus HTAP**	20.0	16.5	1,210

* Sp Act. of compound added÷Sp Act. of 8-azaguanine isolated

** 6-Hydroxy-2,5,5-triaminopyrimidine was added (5×10^{-3} M) 15 minutes prior to addition of the labeled compound.

level. Determination of 8-formylhydrolase activity in the cell-free extract of *S. albus* using [8-^{14}C] guanine, -guanosine, -GMP, or -GTP as substrate has so far been unsuccessful.

References

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