BIOSYNTHESIS OF KASUGAMYCIN. II

BIOSYNTHESIS OF THE TWO-CARBON-SIDE CHAIN OF KASUGAMYCIN

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The incorporation of various ^{14}C-labeled compounds into the two-carbon side chain of kasugamycin was studied. Among U-^{14}C-maltose, U-^{14}C-glucose, U-^{14}C-mannose, 1-^{14}C-glycerol, U-^{14}C-pyruvate, 1-^{14}C-acetate, U-^{14}C-myoinositol, 1-^{14}C-glycine, 2-^{14}C-glycine, U-^{14}C-glyoxylate, U-^{14}C-oxalate and U-^{14}C-serine tested, 1-^{14}C-glycine and 2-^{14}C-glycine were most highly incorporated into the side chain of kasugamycin. They were almost exclusively incorporated into two carbons of the side chain. U-^{14}C-Serine was incorporated at the rate 4 times less than 2-^{14}C-glycine. A dilution effect of glyoxylate, oxamate and oxalate on the incorporation of 2-^{14}C-glycine was not observed.

As reported in a previous paper[1], ^{14}C-glucose is incorporated into kasugamycin and the specific activity of the kasugamine moiety is higher than the residual parts. ^{14}C-Glycine and ^{14}C-myoinositol are taken into kasugamycin at a high rate, but not into the kasugamine moiety. The structure of kasugamycin suggests that glycine might be taken into the two-carbon side chain. In this paper, incorporation of both carbons of glycine into two carbons of the side chain is reported, together with the incorporation rate of other ^{14}C-labeled compounds.

Methods and Materilas

The methods of shaking culture, the procedure for addition of labeled compounds, the processes for isolating kasugamycin, the processes for degradation of kasugamycin into D-inositol, oxalate and kasuganobiosamine, and the measurement of radioactivity were described in a previous paper[1].

In an experiment testing the dilution effect of sodium glyoxylate, sodium oxamate, and ammonium oxalate on incorporation of glycine into kasugamycin, 2-^{14}C-glycine (5.0 μc) was added to the cultured broth (125 ml) of *Streptomyces kasugaensis* after 3 days of the shaking culture at 27°C together with 102 mg of sodium glyoxylate, 100 mg of sodium oxamate or 109 mg of ammonium oxalate and the shaking culture was continued further for 17 hours. Then, ^{14}C-kasugamycin was isolated and the incorporation rate was calculated. 2-^{14}C-Glycine was obtained from the Radiochemical Center and its specific activity was 8.0 mc/m mole. Therefore, 5.0 μc of 2-^{14}C-glycine corresponds to 47 mcg. The incorporations of U-^{14}C-glyoxylate (specific activity 4.71 mc/m mole), U-^{14}C-oxalate (specific activity 2.4 mc/m mole), and U-^{14}C-L-serine (107 mc/m mole) were studied. U-^{14}C-Glyoxalate was obtained from the Radiochemical Center. U-^{14}C-oxalate and U-^{14}C-L-serine were obtained from Daiichi Pure Chemicals Co., Ltd.

Results and Discussion

The two-carbon side chain (the amidine carboxylic acid moiety) of kasugamycin is obtained as barium oxalate by hydrolysis in barium hydroxide solution. The specific activity/μM of carbon of kasugamycin was calculated by dividing the specific activity/μM of kasugamycin to 14 carbons. The specific activity/μM of carbon of the side chain was calculated by dividing the specific activity/μM of oxalate to 2 carbons. The results are indicated in Table 1.

Kasugamycin has 14 carbons and the side chain 2 carbons. Therefore, if a labeled compound is exclusively incorporated into the side chain, then the ratio (relative incorporation) of the specific activity/μM of carbon of kasugamycin to the specific activity/μM of carbon of the side chain must be 7.0. This ratio in the case of maltose, glucose, mannose, or myoinositol is far less than 1.0. It is also less than 1.0 in the case of glycerol or pyruvate. It is higher than 1.0 in the case of 1-^{14}C-acetate. It is almost 7.0 in the case of both 1-^{14}C-glycine and 2-^{14}C-glycine. Thus, both carbons of the side chain of kasugamycin are almost exclusively derived from two carbons of glycine. The incorporation rate of U-^{14}C-glyoxylate, U-^{14}C-oxalate, U-^{14}C-serine and 2-^{14}C-glycine into kasugamycin is shown in Table 2. The incorporation of U-^{14}C-glyoxylate

Table 1. Distribution of ^{14}C in the two-carbon side chain of kasugamycin

^{14}C-Compound	^{14}C added (μc)	Incorp. rate into kasugamycin (%)	Specific activity of kasugamycin ($\times 10^3$ cpm/μM)	Specfic activity/μM of C		Relative incorp.* of side chain
				Kasugamycin	Side chain	
U-^{14}C-maltose	25.6	1.91	5.51	394 cpm	125 cpm	0.32
U-^{14}C-glucose	25.0	10.69	36.15	2,582	342	0.13
U-^{14}C-mannose	10.0	10.03	6.14	439	36	0.08
1-^{14}C-glycerol	7.5	1.41	0.96	57.9	32.5	0.56
U-^{14}C-pyruvate	10.0	1.28	0.97	69.3	58.5	0.84
1-^{14}C-acetate	6.3	0.13	0.06	4.3	7.0	1.63
U-^{14}C-myoinositol	6.0	60.29	36.74	2,634	88.0	0.03
1-^{14}C-glycine	25.0	18.52	48.27	3,448	24,007	6.96
2-^{14}C-glycine	10.0	22.39	22.74	1,624	11,322	6.97

* $\frac{\text{sp. act. of two-carbon side chain/}\mu\text{M of C}}{\text{sp. act. of kasugamycin/}\mu\text{M of C}}$

Table 2. Incorporation of U-^{14}C-glyoxylate, U-^{14}C-oxalate, and U-^{14}C-serine into kasugamycin

	U-^{14}C-glyoxylate	U-^{14}C-oxalate	U-^{14}C-serine	2-^{14}C-glycine
Specific activity	4.71 mc/mM	2.4 mc/mM	107 mc/mM	8.0 mc/mM
Total amount of ^{14}C added	10.0 μc	10.0 μc	8.5 μc	10.0 μc
^{14}C of kasugamycin recovered	3.3×10^4 cpm	0.12×10^4 cpm	1.98×10^5 cpm	9.709×10^5 cpm
Incorporation rate	0.30 %	0.01 %	2.12 %	8.84 %

Table 3. Dilution test on glycine, glyoxylate, oxamate and oxalate

Dilution compound	none	Glyoxylate	Oxamate	Oxalate
Specific activity of 2-^{14}C-glycine	10.0 mc/mM	10.0 mc/mM	10.0 mc/mM	10.0 mc/mM
Total amount of ^{14}C added	5.0 μc	5.0 μc	5.0 μc	5.0 μc
^{14}C of kasugamycin recovered	1.527×10^6 dpm	1.389×10^6 dym	1.109×10^6 dpm	1.329×10^6 dpm
Incorporation rate	13.89 %	12.64 %	10.08 %	12.09 %

and U-^{14}C-oxalate were far less than glycine and that of U-^{14}C-serine was about 4 or 5 times less than 2-^{14}C-glycine. As shown in Table 3, glyoxylate, oxamate and oxalate showed no dilution effect on the incorporation of 2-^{14}C-glycine into the side chain of kasugamycin.

The results described above indicate that glycine added is utilized for formation of the side chain, and that glyoxylate, oxamate and oxalate are not efficient precursors of kasugamycin.

As described in a previous paper[1], deoxysugars, for instance, tyvelose[2], are biosynthesized from glucose through a nucleotide glucose and it is considered that kasugamine, the diaminosugar moiety of kasugamycin is synthesized from glucose, probably through a nucleotide sugar. This course of biosynthesis of nucleotide kasugamine may be considered to take place in cells, and it may also be considered to react with glycine or its metabolized product forming side chain and to be carried to the cell surface where myoinositol exists as a constituent of lipid.

In studying the biosynthesis of antibiotics, it may be useful to compare with the biosynthetic pathway leading to other physiological products. Thus, the biosynthetic pathway of kasugamycin may resemble to that for cell wall synthesis.

The information that glycine is utilized for formation of the side chain of kasugamycin suggests the possible use of glycine to increase the yield of kasugamycin in the broth. However, it must be known that addition of glycine at the start of fermentation does not exhibit the precursor effect, because it is rapidly utilized. After 19 mcg of 1-^{14}C-glycine (2.0 μc) was added to the culture broth (10 ml) on the 4th day of the shaking culture, a sample was taken from the cultured broth at various time intervals. The filtrate was spotted and subjected to high-voltage electrophoresis and the radioactivity was measured by a scanner. Then, the glycine added disappeared 2 hours after addition, giving radioactive kasugamycin. This result indicates that glycine added disappears rapidly and in order to show the precursor effect of glycine, it must be added at least every two hours. The same thing should be considered in all cases, when a precursor effect of a nutrient is examined.

References

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