STUDIES ON BIOSYNTHESIS OF KASUGAMYCIN. IV

BIOSYNTHESIS OF THE KASUGAMINE MOIETY FROM [1-^{14}C]-GLUCOSAMINE AND [1,2 or 6-^{14}C]-GLUCOSE

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1-^{14}C-Glucose, 2-^{14}C-glucose, 6-^{14}C-glucose and U-^{14}C-glucose were incorporated into kasugamycin in similar fashion, proving that the six carbons of glucose are incorporated into kasugamycin without fragmentation. 1-^{14}C-Glucosamine was incorporated into kasugamycin to a greater extent than glucose, and almost exclusively into the kasugamine moiety.

As reported in previous papers[1,2,3], [U-^{14}C]-glucose which is added during production of kasugamycin by *Streptomyces kasugaensis* is incorporated into the kasugamine (2,4-diamino-2,3,4,6-tetradeoxy-D-*arabino*-hexopyranose) moiety of kasugamycin to a greater extent than into the D-inositol moiety. Further studies presented in this paper made it clear that the entire carbon chain of glucose is incorporated into the kasugamine moiety without fragmentation. Moreover, D-glucosamine was found to be incorporated into kasugamycin to a greater extent than glucose, and almost exclusively into the kasugamine moiety.

Materials and Methods

1. Shaking culture of *S. kasugaensis*: The method was previously described[1]. The strain of *S. kasugaensis* employed produced 1,200 mcg/ml of kasugamycin when shake-cultured in a medium containing 1.5 % maltose, 1.5 % soybean meal, 0.1 % K_2HPO_4, 0.3 % NaCl, and 0.1 % $MgSO_4 \cdot 7H_2O$ after 5 days of shaking culture at 27°C.

2. Addition of labeled compounds: Ten μc of labeled compounds were dissolved in 0.1~0.2 ml of distilled water and added to 125 ml of the shake-cultured broth in a 500 ml shaking flask after 4 days growth of *S. kasugaensis*. It was further shake-cultured for 17~20 hours to yield ^{14}C-kasugamycin.

3. ^{14}C-Labeled compounds: [1-^{14}C]-Glucose (14.3 mc/mM), [6-^{14}C]-glucose (4.2 mc/mM) and [U-C-^{14}C]-glucose (6.3 mc/mM or 5.0 mc/mM) were purchased from Daiichi Pure Chemical Co., Ltd. in Tokyo, and [2-^{14}C]-glucose (3.24 mc/mM) and [1-^{14}C]-glucosamine·HCl (4.05 mc/mM) were obtained from Radiochemical Center. Each of these compounds showed one radioactive spot on a paper chromatogram using a solvent system of *n*-butanol - acetic acid - water (4 : 1 : 2).

4. Isolation and degradation of radioactive kasugamycin and their degradation products: The methods are similar to those described in previous papers[1,2,3]. That is, kasugamycin in the culture filtrate was determined by a cylinder plate method using *Pseudomonas fluorescens*. Kasugamycin was isolated from the filtrate by resin chromatography using XE-100 resin in NH_4^+ form and crystallized from aqueous ethanol with

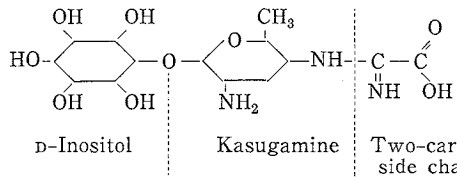
200 mg of cold kasugamycin. Before crystallization, the amount of kasugamycin in the aqueous solution was determined by the cylinder plate method.

Degradation in baryta gave barium oxalate derived from the two-carbon side chain and the residual part, kasuganobiosamine, which was isolated by the Amberlite CG-50 resin process as described previously[1]. The radioactivity of the kasugamine moiety was obtained by subtracting the radioactivity of D-inositol from that of kasuganobiosamine. The radioactivity was measured by the method described in a previous paper[1].

Results and Discussion

The amount of [1-^{14}C]-, [2-^{14}C]-, [6-^{14}C]-, and [U-^{14}C]- glucose incorporated into kasugamycin, that is, ratio of the radioactivity of kasugamycin produced to the radioactivity of the labeled compound added, was 5.93, 6.60, 5.56, and 7.91 % respectively. There was no essential difference in the incorporation of the precursors. The distribution of radioactivity in the three moieties of kasugamycin is shown in Table 1. In all cases, 63~70 % of the total radioactivity of kasugamycin was found in the kasugamine moiety and 27~34 % was found in the D-inositol fragment. The distribution of radioactivity in the two-carbon side chain was only 1.02~1.57 %. These results indicate that all the carbons of glucose are taken into the kasugamine and D-inositol moieties of kasugamycin.

Table 1. Distribution of radioactivity among D-inositol, kasugamine, and two carbon side chain in kasugamycin



^{14}C-compound added to the broth	D-Inositol	Kasugamine	Two-carbon side chain
1-^{14}C-Glucose	27.66 %	71.29 %	1.05 %
2-^{14}C-Glucose	29.19	69.79	1.02
6-^{14}C-Glucose	34.48	63.92	1.60
U-^{14}C-Glucose	31.21	67.22	1.57

At the end of 96-hour shaking culture of *S. kasugaensis* in a moltose-soybean medium at 27°C, 10.0 μc of the ^{14}C-glucose was added, and additional 20-hour shaking culture yielded ^{14}C-kasugamycin, which was isolated from the culture filtrate as described in a previous papar[1]. The incorporation into kasugamycin of 1-^{14}C-glucose, 2-^{14}C-glucose, 6-^{14}C-glucose and U-^{14}C-glucose was 5.93 %, 6.60 %, 5.56 % and 7.91 % respectively. ^{14}C-Kasugamycin was degraded to three subunits as described previously[1~3].

In another experiment, the incorporation of [1-^{14}C]-glucosamine into kasugamycin was compared with that of glucose. In this case, the labeled compounds were added after 3 days of the culture and the fermentation was stopped 17 hours thereafter. Then, 48.58 % of the radioactivity of glucosamine added was incorporated into kasugamycin. The incorporation of glucose was 17.02 %. The distribution of radioactivity in kasugamine, D-inositol and two carbon side chain was examined and the results are shown in Table 2. In radioactive kasugamycin obtained from the fermentation with [1-^{14}C]-glucosamine, 92.16 % of the radioactivity was found in the kasugamine moiety and 5.74 % in the D-inositol moiety, showing that glucosamine is

Table 2. Incorporation of 1-^{14}C-glucosamine and U-^{14}C-glucose into D-inositol, kasugamine and two-carbon side chain

	Incorporation rate into kasugamycin	Distribution of ^{14}C in kasugamycin		
		D-Inositol	Kasugamine	Two-carbon side chain
1-^{14}C-Glucosamine	48.58 %	5.74 %	92.16 %	2.10 %
U-^{14}C-Glucose	17.02	37.03	59.90	3.07

At the end of 96-hour shaking culture of *S. kasugaensis* in a maltose-soybean medium at 27°C, 10 μc of 1-^{14}C-glucosamine or U-^{14}C-glucose was added, and additional 17-hour shaking culture yielded ^{14}C-kasugamycin. The methods of isolation and degradation of ^{14}C-kasugamycin are described previously[1~3].

a more direct precursor for kasugamine than glucose.

Kasugamine (2,4-diamino-2,3,4,6-tetradeoxy-D-*arabino*-hexopyranose) is a deoxyhexose, and as discussed in a previous paper[1], it is speculated that a nucleoside diphosphate hexose may participate in its biosynthesis. Much higher incorporation of glucosamine than glucose into kasugamine suggests the participation of a nucleoside diphosphate, or N-acetyl derivative, of glucosamine in the biosynthesis of kasugamine. It may be noteworthy that 1- and 6-phosphates of glucosamine and N-acetylglucosamine, UDP-glucosamine, and UDP-N-acetylglucosamine have been found in nature[5,6,7].

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