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Biosynthesis of Streptothricin Antibiotics. VI.[1] Mechanisms of β-Lysine and Its Peptide Formation[2]

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1. *Streptomyces lavendulae* ISP-5069 produces racemomycin-A which yields β-lysine as a component on hydrolysis. β-Lysine was derived from an intermediate α,ε-diaminopimelic acid *via* lysine pathway. Exogenous lysine acted as a donor of β-lysine to form racemomycin-C and -B by *S. lavendulae* NT-1008 by means of incorporation of ^{14}C-labeled lysine.

2. Two taxonomically different strains, *S. lavendulae* NT-1008 and *S. albidoflavus* S-0003, were selected as the strains producing racemomycin-A, -C, and -B as a mixture. These antibiotics were present both in the intracellular pool and in the broth.

3. Addition of ^{14}C-racemomycin-A to the culture medium resulted in the incorporation of ^{14}C into racemomycin-C and -B. Majority of the radioactivity was present in the extracellular racemomycin-C and -B, but a significant portion was present in the intracellular antibiotics. These data indicate that racemomycin-C and -B were transformed stepwisely from racemomycin-A and β-lysine, suggesting an endogenous formation.

Keywords——racemomycins; lysine; β-lysine; α,ε-diaminopimelic acid; *Streptomyces lavendulae*; *Streptomyces albidoflavus*; incorporation; intracellular content; extracellular excretion; precursor

Since the discovery of streptothricin by Waksman and Woodruff[4] in 1942 from the culture broth of *Streptomyces lavendulae*, many antibiotics of this family have been found in several number of the genus *Streptomyces*.[5] It was also shown that these antibiotics had a closely related structure. The antimicrobial activity and acute toxicity of these antibiotics in mice were related directly to the number of β-lysine units in their molecule.[6,7] On the basis of these facts more detailed experiments were carried out to elucidate the biosynthetic mechanism of β-lysine moiety, especially the formation of β-lysine peptide, though a few investigations have been undertaken on the β-lysine synthesis in various unrelated species.[8–10]

From the biosynthetic studies of β-lysine moiety in racemomycin-A, a representative streptothricin produced by *S. lavendulae* ISP-5069, it was found[11] that β-lysine in the anti-

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2) This work was presented at the 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1977.
3) Location: *1–14, Bunkyo-machi, Nagasaki 852, Japan.*
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biotic was specifically produced from lysine.[12] Lysine biosynthetic process involves at least three distinct pathways: (a) α-aminoadipic acid pathway (higher fungi), (b) α,ε-diaminopimelic acid pathway (bacteria, lower fungi, and higher plants), and (c) *p*-aminobenzoic acid pathway (*S. noursei*).[13] The phylogenetic position of the *Streptomyces* and the presence of α,ε-diaminopimelic acid in streptomycetes cell wall[14] suggested that the α,ε-diaminopimelic acid pathway would be the main one in this genus. It soon became apparent that β-lysine was synthesized *via* lysine from α,ε-diaminopimelic acid in *S. lavendulae* ISP-5069. In this pathway, lysine seemed to play an important role in the antibiotic synthesis. Before the beginning of the biosynthetic study, it was necessary to establish a basis for related metabolism.

Materials and Methods

Organism——*S. lavendulae* ISP-5069, *S. lavendulae* NT-1008, and *S. albidoflavus* KCC S-0003 were used as strains producing streptothricins. *Bacillus subtilis* PCI-219 was used as an indicator strain for the antibacterial test.

Culture Condition——To prepare the seed culture, a loopful of spores of the strain grown on an agar slant (0.4% yeast extract, 1% malt extract, 0.4% glucose, 1.7% agar, pH 7.3) was transferred to a 500 ml flask containing 80 ml of the precultivation medium (1% glucose, 1% yeast extract, 1% peptone, 0.2% NaCl, 0.01% $(NH_4)_2SO_4$, 0.01% KH_2PO_4, 0.01% K_2HPO_4, 0.01% $MgSO_4 \cdot 7H_2O$, pH 7.2) and this flask was incubated at 27° with reciprocal shaking at 120 rpm for 24 hr. An aliquot of 1 ml of the seed culture was inoculated in a flask containing 100 ml of the fermentation medium; A: 1% maltose, 1% peptone, 0.5% meat extract, 0.25% yeast extract, 0.5% NaCl, 0.05% $MgSO_4 \cdot 7H_2O$, pH 7.0. B: 2% soybean meal, 0.5% Ebios (yeast extract), 2.5% starch, 0.35% $CaCO_3$, 0.007% $CuSO_4$, 0.001% $FeSO_4$, 0.008% $MnCl_2$, 0.002% $ZnSO_4$, pH 7.4. C: 2% maltose, 0.5% peptone, 0.5% meat extract, 0.3% yeast extract, 0.3% NaCl, 0.1% $MgSO_4 \cdot 7H_2O$, 0.01% KH_2PO_4, 0.01% K_2HPO_4, 0.01% $(NH_4)_2SO_4$, pH 7.4. The flask was then cultured under the same condition.

Assay of Antibiotic Activity——Antimicrobial activity was determined by the standard disk assay method, using a plate (heart infusion agar, pH 7.0) seeded with *B. subtilis* (10^6 spores /ml). The test plate was cultured at 27° for 16—18 hr.

Determination of Mycelial Weight——The cultured broth (100 ml) was centrifuged at 0° to separate mycelial cells. The cells in the weighed tube were washed twice with saline and reweighed.

Determination of Lysine——Lysine content was measured by the colorimetric method.[15] The assay involves ninhydrin reaction under acidic condition (pH 5.5). Optical density was recorded at 570 nm with a Hitachi spectrophotometer, Model 124.

Assay of Radioactivity——Radioactivity was measured with a liquid scintillation counter using H_2O-soluble scintillator as previously reported.[11]

Degradation of ^{14}C-Labeled Racemomycins and Radioactivity of Their Hydrolysis Products——^{14}C-Labeled racemomycins were each hydrolyzed with 6 N HCl at 110—120° for 24 hr. The hydrolysate was evaporated to dryness and the residue was submitted to paper chromatography. Radioactivity in each component was measured in the same manner as reported previously.[11]

Paper Chromatography——Paper chromatography was carried out with a solvent system (A) of BuOH–pyridine–AcOH–H_2O–*tert*-BuOH (15: 10: 3: 12: 4) on Toyo Roshi No. 51 UH paper, unless otherwise noted. Separation of acid hydrolysis products of the antibiotic was carried out with a solvent system (B) of BuOH–pyridine–AcOH–H_2O–*tert*-BuOH (75: 50: 191: 236: 548) using Toyo Roshi No. 51 paper.

Isolation of Lysine from Cells——The cells collected by centrifugation from the culture broth (100 ml) were washed twice with saline, and the sediment (*ca.* 11 g) was suspended in 0.1 M sodium phosphate buffer (20 ml, pH 7.5) and disrupted in a cell disruptor (Bronson Sonic Power Co., New York) at 75 W for 10 min in an ice bath. Debris was removed by centrifugation at 0°, the supernatant was passed through a column (2×5 cm) of Amberlite IRC-50 (Na^+). After washing with H_2O (20 ml), the column was eluted with 0.3 N HCl. Fractions positive to ninhydrin were collected and evaporated to dryness. The residue was suspended in 3 ml of solvent system A and then applied to a column (2×27 cm) of cellulose (Whatman Co. England). The column was eluted with the same solvent. Fractions containing lysine were monitored by paper chro-

12) This refers to L-α-lysine, unless otherwise noted.
13) U. Gräfe, H. Bocker, G. Reinhardt, and H. Thrum, *Z. Allg. Mikrobiol.*, **14**, 659 (1974); H. Bocker and H. Trum, *Antibiot.*; *Adv. Res. Prod. Clin. Use. Proc. Congr.*, 1966, 584.
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matography, fractions containing lysine were collected and evaporated to dryness. The residue was dissolved in 20 ml of H_2O and the lysine content was assayed.

Isolation of Lysine from Broth——Filtered broth (*ca.* 100 ml) was applied to a column (2×10 cm) of Amberlite IRC-50 (Na^+). After washing with H_2O (40 ml), the column was eluted with 0.3 N HCl. Fractions positive to ninhydrin were collected, pH adjusted to 8.0, and passed through a column (4×5 cm) of activated carbon. The column was eluted with H_2O. The eluate did not contain racemomycin-A (bioassay) and only a single spot corresponding to lysine was detected on paper chromatogram (ninhydrin). The eluate was evaporated to dryness, the residue was dissolved in H_2O (20 ml), and its lysine content was assayed.

Identification of Lysine——Lysine was identified by nuclear magnetic resonance (NMR) spectrum of its hydrochloride in D_2O. Its configuration was determined by circular dichroism curve of its copper complex in H_2O, as reported previously,[16] and a negative Cotton effect was indicated, proving the L-configuration.

Incorporation of ^{14}C-labeled Compounds into Racemomycin-A and Lysine——*S. lavendulae* ISP-5069 was grown in theifermentation medium A. After 6 hr, ^{14}C-labeled compound (Table I, 0.05 mCi) in 1 ml of sterile H_2O was separated aseptically into five flasks containing the same medium (100 ml). Cultivation was continued for further 40 hr and cultured broth (potency, *ca.* 150 μg/ml) was obtained.

Isolation of ^{14}C-Labeled Racemomycin-A and Lysine——The culture broth (500 ml) was centrifuged to remove the cells and the supernatant fluid was applied to a column (2×20 cm) of Amberlite IRC-50 (Na^+). After washing the column with H_2O (40 ml), racemomycin-A was eluted from the column with 0.3 N HCl. The fractions containing the antibiotic were pooled, neutralized with 1 N NaOH, and evaporated to dryness. The residue was extracted twice with MeOH. The MeOH solution was evaporated to dryness and the residue was dissolved in H_2O (20 ml). This solution was applied to a column (2×5 cm) of activated carbon, and the column was washed with H_2O (50 ml). The antibiotic was eluted from the column with H_2O–acetone (1: 1, pH 2.0 with HCl). The fractions containing racemomycin-A were pooled, concentrated to 5 ml, and then applied to a column (2×140 cm) of Sephadex LH-20. The column was eluted with H_2O. Fractions showing a single spot on a paper chromatogram (detected with ninhydrin and Rydon-Smith reagents) were collected and lyophilized to give racemomycin-A as a white powder, in a yield of 35 mg.

The cells (*ca.* 40 g) in the tube were washed twice with saline and disrupted in 0.1 M sodium phosphate buffer (pH 7.5). ^{14}C-Lysine was isolated by chromatography in a similar manner as described above. Radioactive lysine was obtained 7 mg yield.

Incorporation of ^{14}C-Lysine into Racemomycins by *S. lavendulae* NT-1008——The fermentation medium B was used. At 6 hr, ^{14}C-lysine (70 mg, 32327 dpm/mg) in sterile H_2O (5 ml) was added to the fermentation broth (100 ml). After shaking the flask for 45 hr, antibiotics in the broth were isolated according to the procedures described below.

Incorporation of ^{14}C-Racemomycin-A into Racemomycin-C and -B——Medium B was used for *S. lavendulae* NT-1008 and C for *S. albidoflavus* S-0003. After shaking the cultures at 27° for 20 hr, ^{14}C-racemomycin-A (23999 dpm/mg) was added to the culture. The medium was further cultivated for 30 hr. The culture (100 ml) was diluted with a cold medium (400 ml). The broth (500 ml) was centrifuged to separate the cells. The supernatant fluid was adsorbed on a column (2×15—20 cm) of Amberlite IRC-50 (Na^+). After washing with H_2O, (40 ml) the column was eluted with 0.3 N HCl. Active fractions against *B. subtilis* were collected, neutralized with 1 N NaOH, and evaporated to dryness. The residue was extracted with MeOH, solution was concentrated to 5 ml, and applied to a column (3×140 cm) of Sephadex LH-20. The column was eluted with H_2O. Fractions containing the antibiotics were pooled and evaporated to dryness. The residue was suspended in the solvent system A (5 ml), applied to a column (2×40 cm) of cellulose, and the column was eluted with the same solvent system. Fractions containing racemomycin-C or -B were evaporated to dryness. The residue was dissolved in 0.3 N HCl and acetone was added to this solution to precipitate the antibiotic. The precipitated antibiotic was collected by centrifugation. The fractions containing racemomycin-A were evaporated to dryness, the residue was dissolved in H_2O (50 ml), adjusted to pH 8.0 with 1 N NaOH, and applied to a column (2×4 cm) of activated carbon. After washing with H_2O (50 ml), racemomycin-A was eluted from the column with H_2O–acetone (1: 1, pH 2 with HCl). The eluate was concentrated to a small volume and acetone was added to this solution to give a white precipitation. Racemomycin-A was collected by centrifugation and dried.

The cells in the tube were disrupted and the antibiotics were isolated in a similar manner. In *S. lavendulae* NT-1008, a mixture of cells and insoluble nutrients was sonicated together.

Chemicals——Heart infusion agar was purchased from the Eiken Kagaku Co., Tokyo, Ebios (yeast extract) from the Ebios Co., Tokyo, activated carbon from the Wako Pure Chemical Ind., Osaka, α,ε-diaminopimelic acid [1-^{14}C] (specific activity, 32 mCi/mM), DL-α-aminoadipic acid [6-^{14}C] (specific activity, 46.9 mCi/mM), and *p*-aminobenzoic acid [carboxyl-^{14}C] (specific activity, 50 mCi/mM) from the Cea Ire Sorin, and lysine [U-^{14}C] (specific activity, 280 mCi/mM) from New England Nuclear Co. Boston. ^{14}C-Racemomycin-A was obtained from the fermentation broth of *S. lavendulae* ISP-5069 by means of ^{14}C-glucose addition as reported previously.[11]

16) H. Taniyama, Y. Sawada, and T. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 2631 (1971).

Results

Antibiotic and Lysine Production

Fig. 1 indicates the fermentation pattern of *S. lavendulae.* ISP-5069 which produces racemomycin-A as a single antibiotic. The antibiotic production started at an early log phase of mycelial growth. This strain accumulated lysine in its amino acid pool accompanying mycelial growth, and a maximum lysine accumulation in the cells was observed at the concentration of about 0.16 mg/g of wet cells when the organism was cultured for 28 hr, and the lysine accumulation decreased slightly as the cultivation prolonged. On the other hand, extracellular lysine increased with increase of antibiotic production. These patterns suggested that lysine production may exert some control on the antibiotic production.

Incorporation of ^{14}C-Labeled Compounds into Racemomycin-A

Uptake of α,ε-diaminopimelic acid[1-^{14}C], α-aminoadipic acid[6-^{14}C], *p*-aminobenzoic acid [carboxyl-^{14}C], and lysine [U-^{14}C] into racemomycin-A by *S. lavendulae* ISP-5069 was tested. As shown in Table I, racemomycin-A was labeled with ^{14}C from α,ε-diaminopimelic acid. ^{14}C-Labeled lysine was also incorporated into racemomycin-A approximately 2-fold over that with α,ε-diaminopimelic acid, while α-aminoadipic acid and *p*-aminobenzoic acid were practically not incorporated. Lysine in the amino acid pool was also labeled by α,ε-diaminopimelic

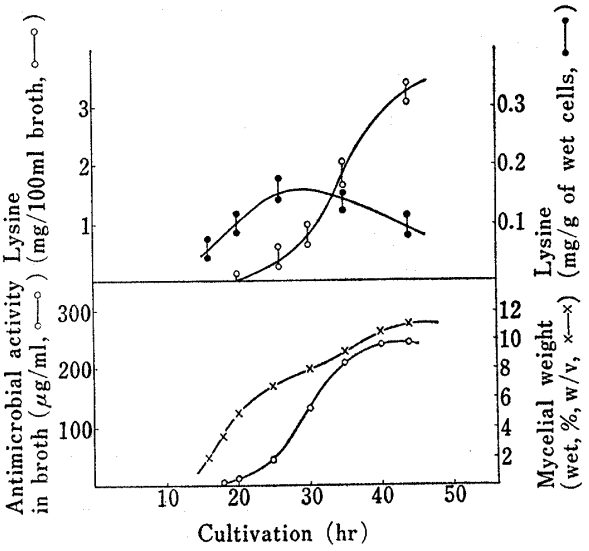

Fig. 1. Fermentation Pattern of *S. lavendulae* ISP-5069 (Medium A)

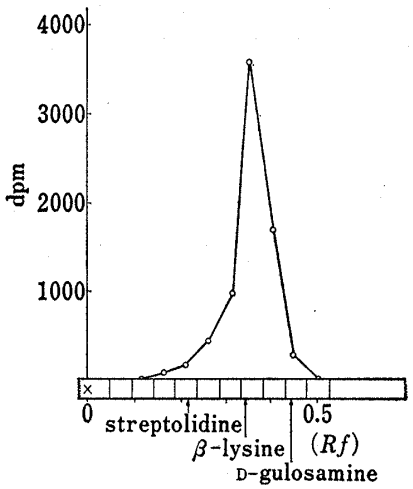

Fig. 2. Radiochromatogram of Racemomycin-A Components indicating the Conversion of ^{14}C-Lysine to β-Lysine

Table I. Incorporation of ^{14}C-Compounds into Racemomycin-A and Lysine in the Cells of *S. lavendulae* ISP-5069

Origin	Radioactivity (dpm/mg)			
	α,ε-Diaminopimelic acid [1-^{14}C]	α-Aminoadipic acid [6-^{14}C]	*p*-Aminobenzoic acid [carboxyl-^{14}C]	Lysine [U-^{14}C]
Lysine from cells[a)]	170200(1.05)[b)]	45	30	—
Racemomycin-A[c)]	4080(0.54)	43	15	8100(1.10)

a) Hydrochloride, total content was determined by the ninhydrin method.
b) Figures in parentheses show the incorporation rate (%), calculated from the amount of racemomycin-A by disk assay of the culture broth.
c) Hydrochloride.

acid[1-^{14}C]. These data indicate that lysine in this strain was synthesized *via* α,ε-diaminopimelic acid pathway. This pathway is normally seen in *Escherichia coli*[17] and *Streptomyces*.[18]

Incorporation of ^{14}C-labeled Diaminopimelic Acid into β-Lysine Moiety in Racemomycin-A

Radioactive racemomycin-A from ^{14}C-labeled α,ε-diaminopimelic acid was acid-hydrolyzed and three fractions of streptolidine, β-lysine, and D-gulosamine were separated from this hydrolysate. An autoradiogram made after paper chromatography revealed that a considerable amount of radioactivity was located only in the fraction corresponding to β-lysine. Fig. 2 illustrates a presentative scan. Recovery of radioactivity was 84%. Thus, the direct conversion of α,ε-diaminopimelic acid into β-lysine was established and utilization of β-lysine for racemomycin-A formation by *S. lavendulae* ISP-5069 was implicated.

Incorporation of ^{14}C-Lysine into Racemomycin-C and -B

The incorporation of exogenous lysine into racemomycins with longer β-lysine chains was examined by using a strain of *S. lavendulae* NT-1008. This strain was cultured in the presence of ^{14}C-lysine racemomycin-A, -C, and -B were each isolated in a pure form from the culture broth. All the antibiotics were highly labeled with ^{14}C. Degree of radioactivity increased with an increase in their β-lysine content (Table II). Total recovery of radioactivity was 25%. The isotope localization in the components of racemomycin-C and -B was examined and, as shown in Fig. 3, β-lysine moiety of both antibiotics showed a high radioactivity, as in racemomycin-A from *S. lavendulae* ISP-5069 (Fig. 2). Recovery of radioactivity from racemomycin-C was 67% and that from -B was 91%. From these results, β-lysine chains in racemomycin-C and -B seemed to be elongated stepwisely, from lysine as a precursor.

Table II. Incorporation of Lysine [U-^{14}C] into Racemomycin-A, -C, and -B by *S. lavendulae* NT-1008

Antibiotics[a]	Radioactivity[b] (dpm/μM)
Racemomycin-A	23878
Racemomycin-C	32146
Racemomycin-B	40519

a) Hydrochloride.
b) Molecular weight of -A (682), -C (870), and -B (1058) was used for the calculation of specific radioactivity.

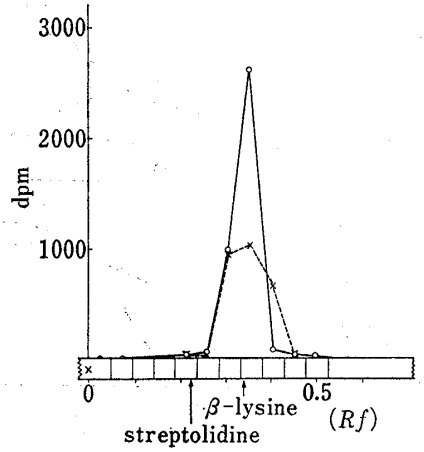

Fig. 3. Radiochromatogram of Racemomycin-C and -B Components
×—×, racemomycin-C.
○—○, racemomycin-B.

Change of Streptothricin Composition as a Function of the Age of Culture

The following two pathways were presumed for the formation of β-lysine peptide: Racemomycins with a longer peptide chain originated from racemomycin-A *via* the antibiotics with a shorter chain or individual racemomycins were produced independently. The former speculation was confirmed on the basis of experiments described below.

17) W. Farkas and C. Gilvarg, *J. Biol. Chem.*, **240**, 4717 (1965); S.H. Kindler and C. Gilvarg, *J. Biol. Chem.*, **235**, 3532 (1960); B. Peterkofsky and C. Gilvarg, *J. Biol. Chem.*, **236**, 1432 (1961); Y. Yugari and C. Gilvarg, *J. Biol. Chem.*, **240**, 4710 (1965).
18) J.K. Kirkpatrick, L.E. Doolin, and O.W. Godfrey, *Antimicrob. Agents Chemother.*, **4**, 542 (1973).

Two taxonomically unrelated strains *S. lavendulae* NT-1008 and *S. albidoflavus* S-0003 were used for this purpose. First of all, the proportion of individual antibiotics isolated from the cultures of three life stages of these strains was analyzed. Through the stages of *S. lavendulae* NT-1008, the relative amount of racemomycin-B produced was in parallel with that of racemomycin-A, whereas the formation of racemomycin-C decreased to some extent at the last stage (Fig. 4). In this case, racemomycin-C seemed to be supplied for the production of racemomycin-B. In *S. albidoflavus* S-0003, the relative amount of racemomycin-C or -B rather increased (Fig. 5). This fact strongly suggested that racemomycin-A was first formed and was further bioconverted to racemomycin-C and -B.

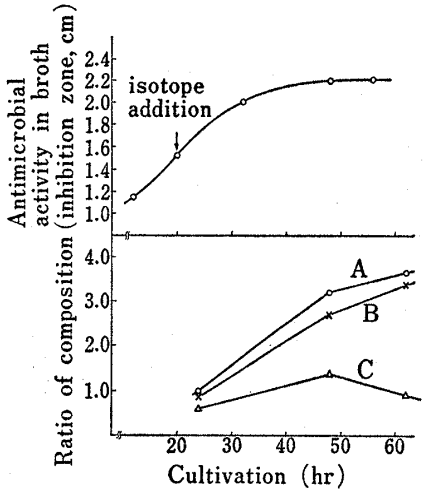

Fig. 4. Fermentation Pattern of *S. lavendulae* NT-1008 (Medium B)

Amount of racemomycin-A at 24 hr was taken as 1.0.

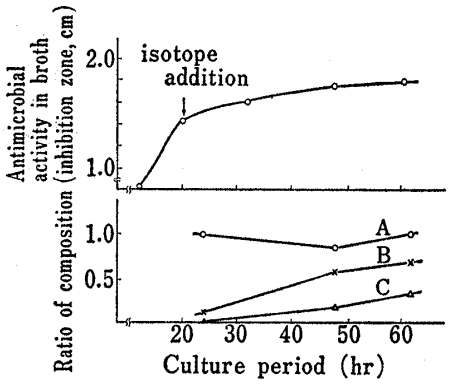

Fig. 5. Fermentation Pattern of *S. albidoflavus* S-0003 (Medium C)

Incorporation of ^{14}C-Racemomycin-A into Racemomycin-C and -B

A large amount of the antibiotics was found in the cells of *S. lavendulae* NT-1008 and *S. albidoflavus* S-0003 through the fermentation experiments. Therefore, composition of the antibiotic mixture in the cells and broth after 60 hr of cultivation of the two strains was examined in detail. As shown in Table III, a large proportion of the antibiotics was observed in the cells of *S. lavendulae* NT-1008 and *S. albidoflavus* S-0003, contrary to the cells of *S. lavendulae* ISP-5069 in which racemomycin-A was absent. After being washed, the cells of *S. lavendulae* NT-1008 were incubated in saline at 27°. The antibiotics in these cells were excreted gradually into saline (data not shown). Similar observations were made in the cells of *S. albidoflavus* S-0003, From these results, racemomycins seemed to be produced in the cells and then excreted into the broth in both strains.

Table III. Relation of Streptothricin Production in Broth and in Cells by Two Strains of *Streptomyces* (60-hr cultivation)

Source	NT-1008 Yield (mg)				S-0003 Yield (mg)			
	-A	-C	-B	Total	-A	-C	-B	Total
Broth	37.8	26.6	13.7	78.1	14.5	9.7	7.5	31.7
Cells	6.3	27.8	5.5	39.6	5.3	2.5	4.9	12.7

-A, -C, and -B indicate the corresponding racemomycins.

Therefore, uptake of ^{14}C-racemomycin-A was tested with both strains. The time chosen for the addition of the labeled antibiotic was the log phase of the antibiotic production (20-hr cultivation, in Fig. 4 and 5). No growth inhibition was observed preliminarily by the exogenous addition of racemomycin-A at the same concentration. ^{14}C-Racemomycin-C and -B were each isolated in a pure form. As shown in Table IV, racemomycin-C and -B from the broths of two strains was heavily labeled. Radioactivity of Racemomycin-B was higher than that of racemomycin-C in both strains. The antibiotics in the cells were also labeled to a relatively high extent.

Putting these results together, we may assume that ^{14}C-racemomycin-A is transformed into racemomycins with longer chains, implicating an endogeneous formation.

Table IV. Incorporation of ^{14}C-Racemomycin-A into Racemomycin-C and -B by Two Strains of *Streptomyces*

Strain	Source	Amount added ^{14}C-racemomycin-A (total dpm)	Specific activity (dpm/μM) -A	-C	-B	Total dpm	Recovery (%)
NT-1008	Broth	143995	356	322	429	43664	30.3
	Cells		447	90	128	9327	6.5
S-0003	Broth	239991	730	504	1023	28468	11.9
	Cells		301	376	341	5269	2.2

Discussion

It was obvious that β-lysine moiety in racemomycins was synthesized from α,ε-diaminopimelic acid *via* lysine pathway by the *Streptomyces* species. This pathway may be the main one in this genus and in *Clostridium*.[10] We have made a great attempt to find the conversion enzyme in streptomycetes. However, no conversion of lysine to β-lysine could be detected. This enzyme seemed to be unstable or to be present in a very low level.

As for *S. lavendulae* ISP-5069, lysine accumulated in its pool before the antibiotic production and was excreted with the antibiotic excretion into the broth. Although data are not presented here, this finding is consistent with the results obtained from the two strains of *S. lavendulae* NT-1008 and *S. albidoflavus* S-0003. Thus, lysine production seemed to be closely related to the antibiotic production, suggesting that lysine metabolism might pull the trigger for the production. Exogenous addition of lysine at a concentration of 1000 μg/ml did not inhibit the production. However, threonine, one of the asparagine family, at concentrations up to 500 μg/ml partially inhibited the antibiotic production by *S. lavendulae* NT-1008, and also inhibited the mycelial growth. Details on the control of lysine or its catabolites over the antibiotic production still remain to be studied.

Although Voronina and Khokhlov[9] found β-lysine itself in the broth of *Actinimyces polymycini*, which produces streptothricin group antibiotic, polymycins, we could not find it in the extracts from the cells or culture fluids of *S. lavendulae* ISP-5069 and NT-1008 by the use of NMR spectra and amino acid analyses. This fact suggests that a limited amount of β-lysine required for the antibiotic production may be formed, by a very minor pathway.

It is worth noting that a large quantity of racemomycins has been found in the cells of *Streptomyces* strains used, except for *S. lavendulae* ISP-5069, and that these antibiotics did not inhibit protein synthesis.[19] This endogenous formation is different from that of

19) T.K. Misra and R.K. Sinha; *Experientia*, **27**, 642 (1971); G.N. Telesnina, I.D. Ryabova, K.I. Shutova, and A.S. Khokhlov, *Dokl. Acad. Nauk SSSR*, **213**, 743 (1973).

streptomycin[20] but similar to formycin formation.[21] Racemomycin-A would be converted to racemomycin-C or -B *in vivo* by the action of a novel enzyme(s). In the uptake experiments of ^{14}C-racemomycin-A, racemomycin-C and -B in the cells were heavily labeled, especially in *S. albidoflavus* S-0003. This fact strongly endorsed the foregoing speculation. Although there was no racemomycin-A in the cells of *S. lavendulae* ISP-5069, if the antibiotic is present in the cells, racemomycins with longer chains would be produced. The biosynthetic mode of streptothricins seemed to depend on the regulation, that is, intracellular content (or extracellular excretion) of racemomycin-A as a precursor.

^{14}C-Racemomycin-A was incorporated into racemomycin-C and -B. However, the recovery of radioactivity was poor as shown in Table IV. Therefore, there is a possibility that some of racemomycin-A added may be degraded into inactive compounds at the stage of back-pass as reported by our group[22] and others.[23] Once the antibiotic was excreted in the broth, it seemed to difficult for the cells to re-absorb it. It is also considered that the recovery of radioactivity, that is, re-absorption of ^{14}C-racemomycin-A, may depend on the time of the addition of ^{14}C-racemomycin-A into the growth culture. By the well-timed addition of the isotope, more radioactivity would be recovered. It is interesting to speculate that, if other antibiotics possessing β-lysine in their molecule such as viomycin, capreomycin, and tuberactinomycin, are incubated with *S. lavendulae* NT-1008, formation of new analogs of β-lysine derivative with a longer peptide chain may be anticipated. Viomycin was tentatively used for this purpose. However, new active spots were not found in the antibiotic mixture. From these result it seemed likely that viomycin itself was not incorporated into the cells.

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