# BIOSYNTHESIS OF 2-DEOXYSTREPTAMINE

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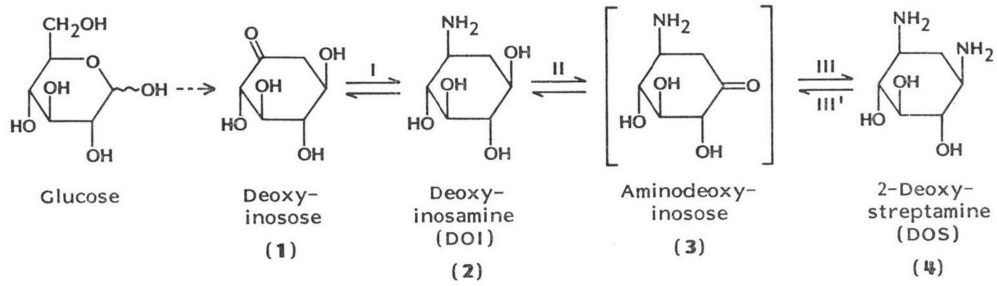
Extracts of *Streptomyces fradiae* 75-078, a producer of an antibiotic neomycin, were found to catalyze three reactions which are included in the proposed biosynthetic pathway to 2-deoxystreptamine; amination of deoxyinososе yielding deoxyinosamine, conversion of deoxyinosamine to 2-deoxystreptamine and deamination of 2-deoxystreptamine. Glutamine was effective as an amino-donor for both transamination reactions; conversions of deoxyinososе to deoxyinosamine and of aminodeoxyinososе to 2-deoxystreptamine. Conversion of deoxyinosamine to 2-deoxystreptamine, presumably including successive dehydrogenation and transamination at position 1, was stimulated by $NAD^+$. On DEAE-Sepharose CL-6B ion-exchange chromatography, the enzyme activity catalyzing amination of deoxyinososе and deamination of 2-deoxystreptamine was eluted as an entity (aminotransferase), while the one converting deoxyinosamine to 2-deoxystreptamine, only if the aminotransferase is supplemented, can be eluted as a separate peak (deoxyinosamine dehydrogenase). The molecular weight of the aminotransferase was estimated to be 130,000 daltons by chromatography on Sepharose CL-6B.

Enzymatic synthesis of 2-deoxystreptamine from deoxyinososе was demonstrated by the cell free extracts.

2-Deoxystreptamine is a component of some clinically important aminocyclitol antibiotics, such as neomycin, kanamycin, gentamicin and sisomicin[1]. Isotopic competition techniques with $^{14}C$-, $^{13}C$- and $^{2}H$-labeled putative precursors[2,3], and production of antibiotics by idiotrophic mutants[2,4] suggested the biosynthetic pathway to 2-deoxystreptamine, as shown in Fig. 1. However, little was known of the properties of the enzyme reaction including the pathway except that the two amino groups of 2-deoxystreptamine were possibly derived from L-glutamine[5]. Among enzyme reactions involved in biosyntheses of aminocyclitols, only those of the streptidine biosynthesis have been investigated[6]. Studies on the enzymes would provide direct clues to prove proposed pathways. We examined the enzyme reactions included in the proposed pathway (Fig. 1); amination of deoxyinososе (**1**) yielding deoxyinosamine (**2**) (Reaction I), conversion of deoxyinosamine to 2-deoxystreptamine (**4**) (Reaction II+III), deamination of 2-deoxystreptamine yielding probably aminodeoxyinososе (**3**) (Reaction III'). It is interesting to note that the four or more enzymes responsible for biosynthesis of 2-deoxystreptamine exist as a multi-enzyme, as in the case of gramicidin and some other peptide antibiotics[7]. Because 2-deoxystreptamine is a common structural unit of many aminocyclitol antibiotics, we tested this possibility, also. The present communication describes some properties of the enzymes responsible for the above reactions. Enzymatic synthesis of 2-deoxystreptamine from deoxyinososе was successful and the proposed reaction sequence was proven. It is unlikely that all these enzymes exist as a single entity because DEAE-Sepharose CL-6B chromatography separated the amino-

Fig. 1. Proposed pathway to 2-deoxystreptamine.




transferase and the putative dehydrogenase.

## Materials and Methods

### Sources of Chemicals

$NAD^+$, NADH, ATP and pyridoxal phosphate were purchased from the Sigma Chemical Co., and ferritin, catalase and aldolase (Calibration proteins, Combithek Kit Size II) were purchased from the Boehringer Mannheim GmbH.

### Preparations of 2-Deoxy[$^{14}$C]streptamine, Deoxy[$^{14}$C]inosamine and Deoxy[$^{14}$C]inosose

One hundred $\mu$Ci of D-[*U*-$^{14}$C]glucose (360 mCi/mmol) was added to a 100-ml culture of *Streptomyces fradiae* three days after inoculating the seeds and 24-hour incubation was continued using a rotary shaker at 30°C. The culture broth was harvested and the pH was adjusted to 2.0. After centrifugation, the supernatant was neutralized with 2 N $NH_4OH$, applied to an Amberlite IRC50 ($NH_4^+$ and $H^+$, 7: 3) column and eluted with 1 N $NH_4OH$. The eluate was again applied to an Amberlite CG50 ($NH_4^+$ and $H^+$, 7: 3, the same follows) column and [$^{14}$C]neomycin fractions which were eluted with 0.4~0.7 N $NH_4OH$ were collected.

[$^{14}$C]Neomycin was hydrolyzed in 6 N HCl at 120°C for 24 hours in a sealed tube. The hydrolysate was decolorized with charcoal and filtered. The filtrate was neutralized and applied to an Amberlite CG50 column and eluted with 0.1 N $NH_4OH$ and fractions containing 2-deoxy[$^{14}$C]streptamine were collected and concentrated.

To a solution of 2-deoxy[$^{14}$C]streptamine in a mixture of $H_2O$ and dioxane an equimolar amount of *p*-nitrophenyl formate was added and the solution was stirred overnight at room temp. After filtration, the filtrate was applied to an Amberlite CG50 column. Mono-*N*-formyl-2-deoxy[$^{14}$C]-streptamine was eluted with 0.15 N $NH_4OH$. The mono-*N*-formyl-2-deoxy[$^{14}$C]streptamine was dissolved in $H_2O$ and a solution of 1.1 equivalents of 3,5-di-*tert*-butyl-1,2-benzoquinone in MeOH was added dropwise to the aqueous solution under nitrogen atmosphere. After stirring overnight, an equimolar amount of oxalic acid was added and the stirring was continued for 2 hours. The reaction mixture was washed with a solution of BuOH and $H_2O$ and mono-*N*-formyl-keto derivative was extracted in a $H_2O$ layer. After neutralization with 1 N NaOH, $NaBH_4$ (2 equiv) was added to the aqueous solution and incubated for 3 hours with stirring at room temp. The mixture was adjusted to pH 6 and concentrated to dryness. After heating the product with 1 N HCl at 100°C for 2 hours with stirring, the mixture was neutralized and applied to an Amberlite CG50 column and epimeric mixture of deoxy[$^{14}$C]inosamine was eluted with 0.2 N $NH_4OH$. A subsequent chromatography of epimers on silica gel with $CHCl_3$ - MeOH - 3.5% $NH_4OH$ (1: 5: 3) gave a pure deoxy-isomer.

To an aqueous solution of deoxy[$^{14}$C]inosamine was added a solution of 1.1 equivalent of 3,5-di-*tert*-butyl-1,2-benzoquinone in MeOH with stirring under nitrogen atmosphere at room temp. After an addition of oxalic acid, the mixture was treated with a mixture of BuOH - $H_2O$. The aqueous layer was neutralized with Amberlite IRA-45 ($OH^-$), filtered and lyophilized to give a colorless solid of deoxy[$^{14}$C]inosose.

During the course of the synthesis, [$^{14}$C] products were developed by TLC (silica gel) and an automatic TLC linear analyzer (Belthold, model 3500) was used for the analysis.

The standard sample of 2-deoxy-*scyllo*-inosamine was supplied from Dr. H. KASE, Kyowa Hakko Kogyo Co., Ltd., Tokyo.

Enzyme Reactions

Transamination of Deoxy[$^{14}$C]inosose with Glutamine (Reaction I): A reaction mixture for amination of deoxyinosose consisted of 50 mM Tris-HCl, pH 9.0, 4.3 mM deoxy[$^{14}$C]inosose (10 $\mu$Ci/mmol), 25 mM glutamine, 0.5 mM pyridoxal phosphate and an indicated amount of the enzyme in a total volume of 50 $\mu$l. The mixture was incubated at 37°C for 1 hour and the reaction was terminated by chilling. The formation of deoxyinosamine was determined by paper electrophoresis, as will be described below.

Formation of 2-Deoxystreptamine from Deoxyinosamine (Reaction II+III): A reaction mixture for conversion of deoxyinosamine to 2-deoxystreptamine consisted of 50 mM Tris-HCl, pH 8.0, 4.3 mM deoxy[$^{14}$C]inosamine (10 $\mu$Ci/mmol), 25 mM glutamine, 5 mM $NAD^+$, 0.5 mM pyridoxal phosphate and an indicated amount of the enzyme in a total volume of 50 $\mu$l. After incubation for 3 hours at 37°C, the reaction was terminated by chilling. 2-Deoxystreptamine was identified by paper electrophoresis.

Deamination of 2-Deoxystreptamine (Reaction III′): A reaction mixture for deamination of 2-deoxystreptamine consisted of 50 mM Tris-HCl, pH 8.0, 4.3 mM 2-deoxy[$^{14}$C]streptamine (10 $\mu$Ci/mmol), 2 mM *scyllo*-inosose, 0.5 mM pyridoxal phosphate and an indicated amount of the enzyme in a total volume of 50 $\mu$l. The mixture was incubated at 37°C for 3 hours and the reaction was terminated by chilling. The bracketed putative reaction product was identified by paper electrophoresis as the same mobility as deoxyinosamine.

The reaction conditions and the components of the reaction mixture were modified in specific experiments (see legends).

Identification of Reaction Products

For paper electrophoresis, 30 $\mu$l of the reaction mixture were applied to a filter paper (Toyo No. 514A, 2×40 cm) and electrophoresed at 800 volts for 50 minutes with formate - acetate - $H_2O$ (1 : 3 : 36), pH 1.9. Relative mobilities toward the negative electrode were 0.03 (deoxyinosose), 1.00 (alanine), 1.04 (deoxyinosamine) and 1.83 (2-deoxystreptamine). Alanine, deoxyinosamine and 2-deoxystreptamine were detected by the ninhydrin color reaction while deoxyinosose by the 2,4-dinitrophenylhydrazine color reaction. Under these conditions, alanine moved toward the cathode about 8 cm.

Preparation of Enzyme

Preparation of a Crude Fraction: The neomycin-producing strain *Streptomyces fradiae* 75-078 obtained from the Nippon Kayaku Co., Ltd. (Tokyo) was cultured at 30°C in a medium (15% millet honey (Asadaame Co., Ltd., Tokyo), 3.5% Prorich (Ajinomoto Co., Ltd., Tokyo), 1% Pharmamedia (Traders Protein, U.S.A.), 1% $CaCO_3$, pH 7.0) for 3, 4 or 5 days. Mycelia were harvested, washed three times with 50 mM Tris-HCl, pH 8.0 (at 0°C), and stored at −80°C until use. Purification of the enzyme was performed below 10°C. In one experiment, 8 g mycelia were suspended in 18 ml of 0.1 M phosphate buffer, pH 7.0 and sonicated with a Branson sonifier for 90 seconds in 30 seconds segments separated by recooling periods. The sonicates were centrifuged at 2,500×$g$ for 20 minutes and the supernatant was mixed with 5 $\mu$g/ml deoxyribonuclease. The mixture was centrifuged at 9,000×$g$ for 20 minutes. The supernatant (S9 fraction, 6 ml) was centrifuged at 18,000×$g$ for 60 minutes, and the supernatant (S18 fraction, 4 ml) was taken.

Sepharose CL-6B Gel Filtration: Four milliliter of S18 fraction was mixed with 0.2 ml of 1 M phosphate buffer, pH 7.0, 0.2 ml of 100 mM pyridoxal phosphate and 0.27 ml of 150 mM EDTA, and dialyzed against $H_2O$ for 18 hours (dialysate fraction). A 2-ml aliquot of the dialyzed preparation was applied to a Sepharose CL-6B column (1.5×85 cm) equilibrated with a mixture of 1 mM phosphate buffer, pH 7.0, 0.4 mM pyridoxal phosphate and 0.3 mM EDTA, and the column was eluted with the same mixture. Active fractions were pooled as Sepharose CL-6B fraction.

Table 1. Specificity for amino donor.

Each 25 mM amino acid was added to reaction mixture instead of glutamine. S18 fraction (49 μg/reaction mixture for Reaction I, 369 μg/reaction mixture for Reaction II+III) for the reaction converting deoxyinosamine (DOI) to 2-deoxystreptamine (DOS) was used as the enzyme. Other conditions are described under Materials and Methods.

| Amino donor | Reaction I: Deoxyinosose→ DOI | Reaction II+III: DOI→DOS |
|---|---|---|
| Glu·$NH_2$ | 1,588 | 50 |
| Glu | 7 | 46 |
| Ala | 18 | 18 |
| Asp·$NH_2$ | 27 | 0 |
| Asp | 0 | 14 |
| None | 0 | 21 |

nmol/mg protein.

DEAE-Sepharose CL-6B Column Chromatography: A sample of 1.5 ml of S18 fraction was applied to a DEAE-Sepharose CL-6B column (1 ml) equilibrated with 20 mM Tris-HCl, pH 8.0. The column was washed with 8 ml of the same buffer and developed with a 30-ml linear gradient of 0~0.5 M NaCl in the same buffer. Active fractions were pooled as DEAE-Sepharose CL-6B fraction.

Table 2. Neomycin production and enzyme activity.

*Streptomyces fradiae* 75-078 was cultured in the medium including 15% or 2% millet honey as the carbon source. The amount of neomycin in the cultured broth was determined by the cylinder-plate assay against *Bacillus subtilis* PCI219. In Reaction II+III glutamine or glutamic acid was used as the amino-donor, and S18 fraction of neomycin high-producing mycelium (316 μg/reaction mixture) or that of neomycin low-producing mycelium (75 μg/reaction mixture) was used as the enzyme. Other conditions are given under Materials and Methods.

| Concentration of millet honey in growth medium (%) | Neomycin productivity (mg neomycin/ml broth) | Reaction II+III: DOI→DOS | |
|---|---|---|---|
| | | Amino-donor | |
| | | Glu·$NH_2$ | Glu |
| 15 | 2.4 | 215 (100) | 129 (100) |
| 2 | 0.1 | 5.9 (2.8) | 32 (24.4) |

nmol/mg protein and (% activity).

## Results

### Amino-donor Specificities

The aminotransferase which catalyzed Reaction I (**1**→**2**) utilized glutamine as most potential amino donor to deoxyinosose (**1**), while the enzymes catalyzed Reaction II+III (**2**→**4**) utilized glutamic acid as well as glutamine as shown in Table 1. To test whether a single or more than two kinds of aminotransferases are present in Reaction II+III, optimum pH for this reaction in the presence of either glutamine or glutamic acid was compared. Optimum pH of Reaction II+III in the presence of glutamine as an amino-donor was 8, while pH 7 was optimum when glutamic acid was used as an amino-donor. The observation suggested that two different aminotransferases are present for this reaction, one is preferring glutamine and the other glutamic acid as an amino-donor.

In order to determine which aminotransferase was involved in real 2-deoxystreptamine (**4**) biosynthesis, the activities of the enzymes extracted from the cells producing high (70 mg neomycin per gram mycelia) and low (22 mg) amount of neomycin were examined by Reaction II+III with glutamine or glutamic acid as an amino-donor (Table 2). We thought the enzyme activities of the aminotransferase and the dehydrogenase which are involved in 2-deoxystreptamine biosynthesis might be reduced in neomycin low-producing cells, therefore the activity of Reaction II+III should be lower than that in neomycin high-producing cells. As shown in Table 2, the activity of the enzyme reaction with glutamine as an amino-donor was lower than that with glutamic acid in neomycin low-producing cells. These results suggested that an enzyme which utilized glutamine as an amino-donor is pre-

Fig. 2. Sepharose CL-6B column chromatography.

*Streptomyces fradiae* 75-078 was cultured in the medium including 15% (●, ▲) or 2% (■) millet honey as the carbon source. Other components of the medium are described under Materials and Methods.

Dialysate fractions of both mycelia were applied to a Sepharose CL-6B column respectively. The activities of aminotransferases were determined by Reaction I (**1**→**2**) (▲, ■) and Reaction III′ (**4**→possibly **3**) (●). Other conditions are given under Materials and Methods.

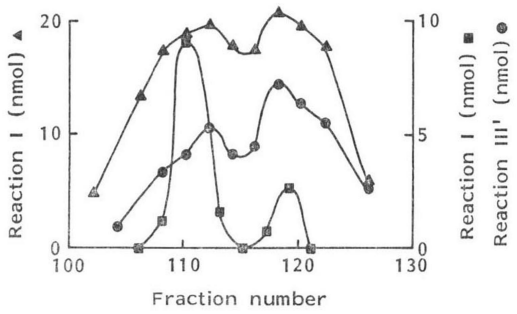



Table 3. Cofactors for the reaction converting deoxyinosamine to 2-deoxystreptamine.

$NAD^+$ and $NADP^+$ were tested at 5 mM, and ATP and $Mg(OAc)_2$ at 4 mM. Undialyzed S9 fraction (198 μg/reaction mixture) was used as the enzyme. Other conditions are described under Materials and Methods.

| Reaction mixtures | [$^{14}$C]DOS formed (nmol/mg protein) |
|---|---|
| [$^{14}$C]DOI+Glu·$NH_2$+enzyme | |
| +$NAD^+$+pyridoxal phosphate | 138 |
| +$NAD^+$+pyridoxal phosphate +ATP+$Mg^{++}$ | 114 |
| +$NAD^+$ | 95 |
| +Pyridoxal phosphate | 19 |
| +$NADP^+$+pyridoxal phosphate | 17 |

ferentially responsible for the biosynthesis of 2-deoxystreptamine. The enzyme activity of exchange transamination of Reaction III′ (**4**→possibly **3**) was inhibited by 50% of control by the addition of glutamine but not glutamic acid (data not shown), supported the above results.

## Sepharose CL-6B Column Chromatography

In order to determine whether aminotransferases which catalyzed Reaction I and Reaction III′ were separated or not, Sepharose CL-6B gel filtration was conducted. Both activities showed the similar elution profiles which contained two main peaks as shown in Fig. 2. The extract from neomycin low-producing cells was also applied to the same gel filtration and the fractions were determined for the activity of Reaction I. However, the activity of the enzyme which eluted in smaller molecular weight fractions was decreased remarkably, suggested that this enzyme was responsible for the biosynthesis of the two amino groups of 2-deoxystreptamine in *S. fradiae*. The molecular weight of the enzyme was estimated to be about 130,000 daltons.

## Properties of Dehydrogenase

Reaction II+III was stimulated by $NAD^+$ remarkably, but not by $NADP^+$ as shown in Table 3. Pyridoxal phosphate stimulated the reaction further extent. ATP and $Mg^{++}$ inhibited the reaction to same extent. These results suggested that 2-deoxystreptamine was formed by oxidation and amination *via* aminodeoxyinosose (**3**) as shown in Fig. 1.

## Separation of Aminotransferase and Dehydrogenase

Crude cell free extracts were applied to DEAE-Sepharose CL-6B column. The column was developed with a linear gradient of 0 to 0.5 M NaCl. The activities of the aminotransferase catalyzing Reaction I and Reaction III′ were detected in the same fractions eluted at the NaCl concentrations between 0.20 to 0.27 M as shown in Fig. 3. However the activity of Reaction II+III was not found in any fractions. Another attempt had to be made. A small column was used and developed with

Fig. 3. DEAE-Sepharose CL-6B column chromatography.

The experiment was conducted as described under Materials and Methods. The activity of the aminotransferase was determined based on Reaction I (**1**→**2**) (●) and Reaction III′ (**4**→possibly **3**) (○).

Specific activities, which were determined by Reaction I, in S18 fraction and DEAE fraction (No. 22) were 0.46 μmol and 1.19 μmol deoxyinosamine formed per mg protein, respectively.

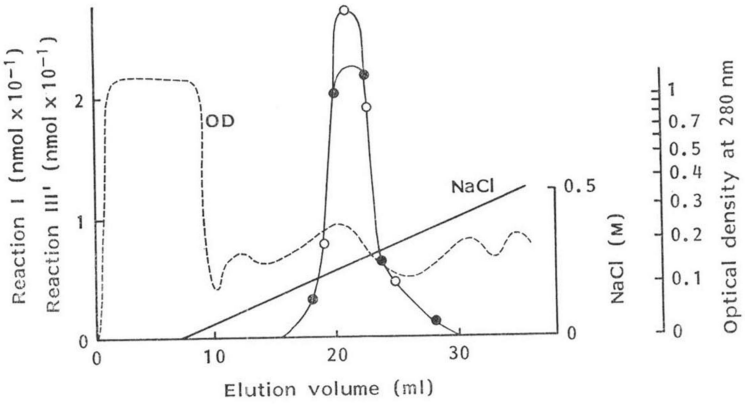
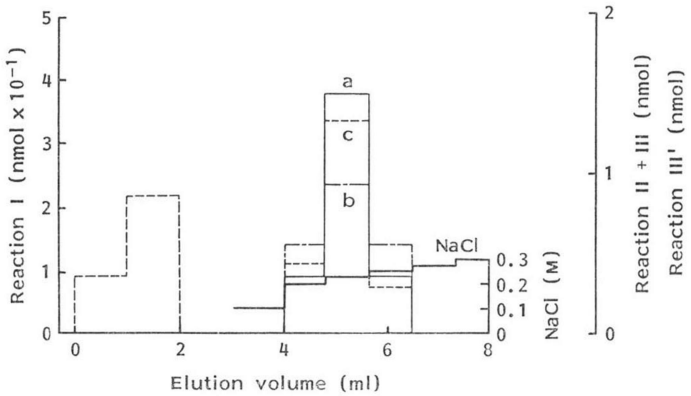



Fig. 4. DEAE-Sepharose CL-6B column chromatography.

One milliliter of undialyzed S18 fraction was applied to 0.5 ml of DEAE-Sepharose CL-6B column equilibrated with 20 mM Tris-HCl buffer, pH 8.0. The column was washed with 2 ml of the same buffer and developed with stepwise gradient of 0.1～0.3 M NaCl.

A part of each fraction was utilized as the enzyme in Reaction I (**1**→**2**) (a) and Reaction III′ (**4**→possibly **3**) (b). In Reaction II+III (**2**→**4**) a portion of each fraction was utilized with (c) or without S18 fraction (133 μg/reaction mixture) as the enzyme. Without S18 fraction the activity of Reaction II+III was not detected in any fractions of the chromatography (data not shown).




stepwise gradient of 0.1 to 0.3 M NaCl. The activities of Reaction I, Reaction II+III and Reaction III′ were all detected in the same fractions except with an additional peak for Reaction II+III as shown in Fig. 4. The highest activities of Reaction I and Reaction III′ were detected in 0.225 M NaCl fraction. The activity of Reaction II+III in each fraction was determined with or without crude fraction (S18) which included both the aminotransferase and dehydrogenase activities. No activity was detected in any fractions without S18. However, by adding small amounts of S18 to each fraction, the activity of the Reaction II+III was detected in separate two fractions, the flow through and 0.225 M

NaCl fractions (Fig. 4). This demonstrated that the aminotransferase was eluted in 0.225 M NaCl fraction, while the dehydrogenase did not bind to DEAE-Sepharose CL-6B column.

Enzymatic Synthesis of 2-Deoxystreptamine

2-Deoxystreptamine was formed from deoxyinosose in the presence of glutamine, $NAD^+$, pyridoxal phosphate and S18 enzyme fraction, whereas only deoxyinosamine was formed in the reaction mixture without $NAD^+$ (Table 4).

Table 4. Enzymatic synthesis of 2-deoxystreptamine.

A reaction mixture for Reaction I consisted of 50 mM Tris - HCl, pH 8.0, 8.6 mM deoxy[$^{14}$C]inosose (10 $\mu$Ci/mmol), 25 mM glutamine, 0.5 mM pyridoxal phosphate and 48 $\mu$g S18 enzyme fraction in a total volume of 50 $\mu$l. The mixture was incubated at 37°C for 3 hours. Radioactive products were analyzed as Materials and Methods. Conditions for Reaction II were as Reaction I except that 5 mM $NAD^+$ was added and 300 $\mu$g S18 enzyme fraction was used.

| Reaction | Amount of products (nmol) | |
|---|---|---|
| | DOI | DOS |
| 1. Deoxyinosose +Glu·$NH_2$+pyridoxal phosphate+enzyme | 40.1 | neg. |
| 2. Deoxyinosose +Glu·$NH_2$+$NAD^+$ +pyridoxal phosphate +enzyme | 70.6 | 3.4 |

neg.: Negligible.

## Discussion

The route of 2-deoxystreptamine biosynthesis from glucose has been suggested to be as shown in Fig. 1 from the results of; 1) incorporation of isotopically labeled precursors into 2-deoxystreptamine (**4**)[2,3], 2) feeding putative biosynthetic intermediates such as deoxyinosose (**1**) and deoxyinosamine (**2**) to 2-deoxystreptamine-less idiotrophs[2,4] and 3) cell free enzyme studies[5]. Although WALKER *et al.* speculated that a single aminotransferase catalyzed both aminations of 2-deoxystreptamine, there has not been any experimental data to prove this.

The results presented here suggest that a single aminotransferase utilizing glutamine as an amino-donor might participate in biosynthesis of both amino groups of 2-deoxystreptamine and demonstrate that the dehydrogenation reaction on the route of 2-deoxystreptamine biosynthesis in cell free systems for the first time. Glutamine was suggested to be the most effective amino-donor for the aminotransferase reaction involved in Reaction I and Reaction II+III (Table 1).

The transamination enzymes catalyzing both Reaction I and Reaction III′ could not been separated either by Sepharose CL-6B gel filtration or DEAE-Sepharose column chromatography (Figs. 2 and 3). There is a possibility, however, that the enzymes catalyzed two transamination reactions could be separated by further purification procedures. The aminotransferase activity which was eluted in larger molecular weight fractions (MW 200,000) may be glutamine $\alpha$-keto acid aminotransferase, because high aminotransfer activity of glutamine to pyruvate was observed (data not shown).

Nothing has yet been reported concerning the dehydrogenation reaction on the route of 2-deoxystreptamine biosynthesis. Our present paper demonstrated this enzymic activity in the coupled reaction with the transaminase (**2**→**3**→**4**) in cell extracts of *S. fradiae* and showed that $NAD^+$, but not $NADP^+$ was required for the activity. The biosynthesis of 2-deoxystreptamine seems to proceed *via* free form of intermediates instead of modified ones, such as phosphorylated, as the biosynthesis of streptidine moiety of streptomycin[6], because ATP and $Mg^{++}$ did not stimulate Reaction II+III (Table 3). Formation of 2-deoxystreptamine from radioactive deoxyinosose with S18 enzyme fraction (Table 4) suggested that the carbon atom corresponding to C-5 of glucose[2] underwent the first oxidation and amination and that corresponding to C-1 of glucose underwent the second oxidation and amination to form 2-deoxystreptamine.

The stability of enzymes have been examined at various purification stages. The aminotransferase activities assayed by Reaction I and Reaction III′ lost only 10% activity during storage in S18 fraction for 50 days, however, 10% was lost in Sepharose CL-6B chromatography fraction after 24 hours and about 50% in DEAE-Sepharose CL-6B column chromatography fraction after 2 days at −20°C. The enzymes which catalyzed Reaction II+III lost about 60% and 85% activities in S18 fraction during

storage for 1 day at −20°C and 4°C, respectively, and almost all activity was lost after dialysis at 4°C for 18 hours. Addition of Tween 80, Brij 58, β-mercaptoethanol or a mixture of protease inhibitors such as leupeptin, chymostatin and phosphoramidon did not stabilize any enzyme activities. To find a way to stabilize the enzymes will be necessary for further studies on the enzyme reactions involved in 2-deoxystreptamine biosynthesis.

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