Isolation of Streptomycin-Nonproducing Mutants Deficient in Biosynthesis of the Streptidine Moiety or Linkage Between Streptidine 6-Phosphate and Dihydrostreptose

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Eight streptidine idiotrophic mutants (SD20, SD81, SD141, SD189, SD245, SD261, SD263, and SD274) which required streptidine to produce streptomycin were derived from Streptomyces griseus ATCC 10137 by UV mutagenesis. By both the characterization of intermediates accumulated by the idiotrophs and the assay of enzymes involved in streptidine biosynthesis, the biochemical lesions of the mutants were deduced as follows: SD20 and SD263, transamination; SD81, SD261, and SD274, phosphorylation; SD141, transamination; SD189, dehydrogenation; SD245, linkage between streptidine 6-phosphate and dihydrostreptose. An accumulation of streptidine 6-phosphate was found in SD245 to impair its aminotransferase activity. This finding suggests that aminotransferase activity might have been negatively controlled by the end product, streptidine 6-phosphate, of the streptidine biosynthetic pathway.

The antibiotic streptomycin, N-methyl-α-D-glucosamine (1′→2)α-D-streptose (1′→4) streptidine (Fig. 1), is produced by certain strains of Streptomyces. The biosynthesis of streptomycin has been studied extensively by a number of investigators, and a majority of the enzymatic reactions dedicated to streptomycin biosynthesis have been identified (19). Particularly, the Walkers’ elegant studies with conversion of myo-inositol to streptidine using cell-free extracts have revealed an intriguing biosynthetic pathway to streptidine (17; Fig. 2). It involves two analogous sequences of five enzymatic reactions to convert a hydroxy group to a guanidino group: dehydrogenation (C, H), transamination (D, I), phosphorylation (E, J), transaminationation (F, K), and dephosphorylation (G, dephosphorylation of streptomycin 6-phosphate). Streptidine 6-phosphate, not streptidine, is incorporated into the streptidine biosynthetic pathway as follows (7, 8, 10): (i) transfer of dihydrostreptose to streptidine 6-phosphate, (ii) transfer of N-methyl-D-glucosamine to O-α-D-dihydrostreptose (1′→4)streptidine 6-phosphate, (iii) oxidation of dihydrostreptomycin 6-phosphate, (iv) dephosphorylation of streptidine 6-phosphate to streptomycin.

We have been interested in the structure and regulation of the genes involved in the streptomycin biosynthesis and have developed a cloning system in Streptomyces griseus ATCC 10137 (12). Despite an extensive knowledge of the enzymological aspects of the biosynthesis, there have been few genetic studies (3). Only one well-characterized mutant has been isolated by Nagaoka and Demain [11]. The mutant that produces no streptomycin unless streptidine is added to the culture, i.e., a streptidine idiotroph, is reported to be deficient in a dehydrogenation step (H) of the streptidine biosynthesis (19). Therefore, we attempted to isolate a number of streptomycin-nonproducing strains with clearly defined mutations. The purpose of this paper is to describe the isolation and characterization of streptidine idiotrophic mutants and thereby to reassess Walkers’ streptidine biosynthetic pathway.

MATERIALS AND METHODS

Media. Nutrient agar and antibiotic medium no. 5 were purchased from Difco Laboratories, Detroit, Mich. GMP medium was described elsewhere (4). ISP2M medium for maintenance and sporulation contained 15 g of malt extract, 5 g of yeast extract, 5 g of soluble starch, 3 g of CaCO3, and 20 g of agar per liter (pH 7.5).

Bacterial strains. S. griseus ATCC 10137 was used as a parental strain in an attempt to isolate Waksman’s original streptomycin-producing strain by cloning (13). Bacillus subtilis ATCC 6633 was used as an indicator in the bioassay of streptomycin.

Chemicals. Reagents were obtained from the following sources: dihydrostreptomycin sulfate, pyridoxal phosphate, and scylo-inosose from Sigma Chemical Co., St. Louis, Mo.; myo-inositol from P-L Biochemicals Inc., Milwaukee, Wis.; [U-14C]myo-inositol (253 Ci/mol) from New England Nuclear Corp., Boston, Mass.; hydroxyguanidine hemisulfate from Kanto Chemical Co., Tokyo, Japan; pentacyanoaminoferrate from Tokyo Kasei Industrial Co., Tokyo. All other reagents were from Wako Chemical Industries, Osaka, Japan.

Streptidine sulfate was prepared from dihydrostreptomycin sulfate by a mild acid hydrolysis following two recrystallizations as described elsewhere (14).

Isolation of streptomycin-nonproducing mutants. Spores of S. griseus ATCC 10137 were irradiated with UV rays to give approximately 1% survival ratio, spread on ISP2M medium, and incubated at 28°C for 4 days. The colonies were transferred by a toothpick onto cylindrical plugs (5 mm diameter) of nutrient agar (Difco) and incubated at 28°C for 3 days. After the incubation, the agar plugs were transferred onto antibiotic medium no. 5 (Difco) seeded with spores of B. subtilis and incubated at 33°C for 18 h. Streptomycin-nonproducing mutants were detected by the lack of inhibitory zone against B. subtilis and purified from the agar plugs by a single colony isolation.

Isolation of the compounds accumulated by streptidine idiotrophs. Spores of the streptidine idiotrophs were inoculated to 20 ml of GMP medium and incubated at 28°C for 24

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FIG. 1. Structure of streptomycin.

h on a rotary shaker. The preculture (5 ml) was transferred to 100 ml of GMP medium and incubated at 28°C for 22 h. To the culture, 5 μCi of [14C]-L-arginine was added, and after a 2-h incubation, mycelia were harvested by filtration. To the washed wet pad (2 to 3 g) was added 2 ml of water, and the mixture was heated in a boiling water bath for 7 min. After centrifugation, the supernatant was absorbed on a Dowex 50W - X8 (H+)-cation-exchanged resin (200 to 400 mesh) column (0.7 by 20 cm). The column was washed with 50 ml of water, and successive stepwise elutions were performed, employing in sequence 50 ml of 0.5, 1.0, 2.0, 2.5, and 5.0 N HCl. Column fractions were ca. 1.7 ml each. A 50-μl sample from each tube was spotted on a Whatman GF/C filter and, after drying, its radioactivity was counted with a liquid scintillation system. Pooled fractions from a peak were dried in a vacuum desiccator, dissolved in a small volume (ca. 500 μl) of water, and neutralized with KOH.

Characterization of the compounds accumulated by streptidine idiophots. The radioisotopically labeled compounds isolated from the streptidine idiophots were characterized by both paper chromatography and paper electrophoresis as described by Walker (14). Ascending paper chromatograms (Whatman no. 1 paper) were developed for a distance of 23 cm with a solvent system of 80% (vol/vol) phenol and an NH3 atmosphere. Paper electrophoresis was performed at 30 V/cm for 90 min, using a refrigerated horizontal apparatus and Whatman no. 1 paper (46 cm in effective path). For electrophoresis, glycine-NaOH buffer (pH 10.4, I 0.2) and ammonium formate buffer (pH 3.6, I 0.2) were used. At pH 10.4, the guanidine group has one positive charge, the phosphate group has two negative charges, and the amino group is uncharged. At pH 3.6, both guanidino and amino groups have one positive charge, whereas the phosphate group has one negative charge. Picric acid was used as the reference compound. In both procedures, strips were cut at 1-cm intervals and counted with the liquid scintillation system.

Assay of aminotransferase and amidinotransferase activities. From cultures of the parental strain and the streptidine idiophots in GMP medium as described above, cell-free extracts were prepared by sonication (14).

Aminotransferase activity was detected as transamination from labeled scyllo-inosamine (V) to cold scyllo-inosose (IV), yielding labeled scyllo-inosose. The incubation mixture contained 5 μl (700 cpm) of [14C]scyllo-inosamine prepared from SD141, 5 μl of 100 mM potassium phosphate buffer containing 13 mM EDTA and 8 mM pyridoxal phosphate (pH 7.4), and 10 μl of cell extract (300 to 400 μg of protein). After a 90-min incubation at 35°C, the mixture was separated by paper electrophoresis at pH 3.6 and counted with the liquid scintillation system.

For assay of amidotransferase activity, transamination from L-arginine to hydroxyamine, yielding hydroxyguanidine, was measured. The incubation mixture contained 0.1 ml of 1 M L-arginine, 0.1 ml of 1 M potassium phosphate buffer (pH 7.4), 0.3 ml of 2 M hydroxyamine, and 0.5 ml of cell extract (15 to 20 mg of protein). After a 90-min incubation, the hydroxyguanidine formed was measured as its colored complex with pentacyanoaminoferrate by the method of Walker (14). One unit of amidotransferase is defined as the amount that catalyzes the formation of 1 μmol of hydroxyguanidine per h at 37°C. The concentration of protein was determined by the method of Lowry et al. (9).

Cosynthesis test between the streptidine idiophots. Cosynthesis of streptomycin was tested both on nutrient agar and in GMP liquid medium. On nutrient agar, the two streptidine idiophots to be tested were inoculated at an interval of 1 cm. After 3 days of incubation at 28°C, antibiotic medium no. 5 containing spores of B. subtilis was overlaid on the plate for detection of streptomycin. In GMP medium, two of each fully grown culture (5 ml) of the idiophots were mixed and further incubated at 28°C for 48 h. After centrifugation, 50 μl of the broth was spotted on a paper disk and its bioactivity was assayed.

RESULTS

Isolation of streptidine idiophots. S. griseus ATCC 10137 produced streptomycin (100 to 200 μg/ml in GMP medium). By UV mutagenesis, we isolated 226 streptomycin-non-producing mutants from ca. 20,000 colonies tested (ca. 1%). Among these mutants, 190 strains could not sporulate on nutrient agar; they might be blocked in the biosynthesis of A-factor in view of the report from Hara and Beppu (4). That A-factor-deficient mutants of S. griseus lose their ability to produce streptomycin as well as to sporulate. On the other hand, the other 36 mutants, exhibiting normal sporulation, appeared to be blocked somewhere in the biosynthetic pathway to streptomycin.

When the sporeforming mutants were cultivated on nutrient agar supplemented with streptidine sulfate (100 μg/ml), eight mutants produced streptomycin (Fig. 3). They were named SD20, SD81, SD141, SD189, SD245, SD261, SD263, and SD274. Usually, extracellular streptidine can be incorporated into the biosynthetic pathway of streptomycin after phosphorylation by streptomycin 6-phosphotransferase (Fig. 2, part L) (16). Therefore, the eight streptidine idiophots that require streptidine to produce streptomycin presumably carry a defective gene(s) for an enzyme or its regulation involved in the streptidine biosynthesis, whereas
FIG. 2. Biosynthetic pathway of streptidine moiety. Compounds: (I), glucose 6-phosphate; (II), myo-inositol 1-phosphate; (III), myo-inositol; (IV), scyllo-inosose; (V), scyllo-inosamine; (VI), scyllo-inosamine 4-phosphate; (VII), amidino-scyllo-inosamine 4-phosphate; (VIII), amidino-scyllo-inosamine; (IX), amidino-3-keto-scyllo-inosamine; (X), amidinostreptamine; (XI), amidinostreptamine 6-phosphate; (XII), streptidine 6-phosphate; (XIII), streptidine; KGAM, α-ketoglutaramate; Orn, ornithine; Pyr, pyruvate.

the biosynthesis of other moieties of streptomycin may have been unaltered. We selected these streptidine idiotrophs for further studies because of the unique features of the streptidine biosynthetic pathway and ease of the enzymatic assays (14).

Streptomycin productivity of the streptidine idiotrophs, except for SD245 on nutrient agar containing 100 μg of streptidine sulfate per ml, was comparable to that of the parental strain, whereas the streptidine supplementation recovered the productivity of SD245 to a lesser extent (Fig. 3). SD20 and SD263 produced streptomycin slightly on nutrient agar and thus seemed to be leaky mutants. For all of the streptidine idiotrophs, streptidine-dependent production of streptomycin was confirmed by the use of a liquid culture (GMP medium).

Supplementation (400 μg/ml) with myo-inositol (III) or scyllo-inosose (IV), both of which are precursors of streptidine, did not restore the streptomycin productivity of any of the streptidine idiotrophs (Fig. 3). Since S. griseus appeared to present no permeability barrier to scyllo-inosose (5), it was conceivable that all of the streptidine idiotrophs might be blocked at some steps beyond the first dehydrogenation (C) in the sequence of reactions shown in Fig. 2.

Characterization of intermediates accumulated by streptidine idiotrophs. To investigate the blocked steps of the streptidine idiotrophs, we isolated and characterized the compounds accumulated by the mutants as well as the parental strain. The metabolites were labeled in the cyclohexose ring with [U-14C]myo-inositol as a precursor. The labeled compounds were extracted in hot water and separated by column chromatography with a cation-exchange resin. Elution patterns for the parental strain and the idiotrophs are shown in Fig. 4. SD20 and SD263 accumulated little of the labeled compounds, whereas the other idiotrophs contained a substantial amount of the labeled compounds, which exhibited characteristic elution profiles (Fig. 4).
FIG. 3. Streptidine-dependent streptomycin production of the streptidine idiotrophs. The idiotrophs were cultured on agar plugs of nutrient agar (Difco) supplemented with: N, none; N + (XIII), streptidine sulfate (100 μg/ml); N + (III), myo-inositol (400 μg/ml); N + (IV), scy1lo-inosose (400 μg/ml). The plugs were transferred to the bioassay plate seeded with spores of B. subtilis. SD20 and SD263, both of which appeared to be leaky mutants, slightly recovered their productivity when supplemented with myo-inositol; the reason was not known. In the experiment shown here, it is noted that SD20 and SD245 produced less streptidine than other streptidine idiotrophs on nutrient agar containing streptidine. It was confirmed from repeated runs (not shown here) that SD20 produced nearly the same amount of streptomycin as the other idiotrophs on N + (XIII), albeit the recovery in this particular run was poor. On the other hand, SD245 produced the lesser amount throughout.

After concentration, each peak was analyzed by paper chromatography and paper electrophoresis (Table 1). A comparison of our data with those previously reported by Walker (14) permitted the identification of the accumulated compounds, which are shown in the last column of Table 1. However, one peak of SD141, which was eluted with water (Fig. 4C), could not be assigned an identity. The compound was indistinguishable from scy1lo-inosamine 4-phosphate (VI) by the analyses with paper chromatography and electrophoresis (Table 1). Furthermore, it could be amidinated by amidinotransferase prepared from the parental strain and dephosphorylated by Escherichia coli alkaline phosphatase, like scy1lo-inosamine 4-phosphate (data not shown). Although this compound proved to be closely related to scy1lo-inosamine 4-phosphate, its structure remains to be determined.

It was revealed that each streptidine idiotroph accumulated the following intermediates: SD20 and SD263, none; SD81, SD261, and SD274, scy1lo-inosamine (V); SD141, scy1lo-inosamine and scy1lo-inosose 4-phosphate (VI) (and its unidentified analog); SD189, amidino-scyllo-inosamine (VIII); SD245, amidinostreptose 6-phosphate (XI) and streptidine 6-phosphate (XII) (Fig. 4 and Table 1). On the basis of the streptidine biosynthetic pathway proposed by Walker (Fig. 2), the most probable biochemical lesions were deduced as follows: SD20 and SD263, transamination (D); SD81, SD261, and SD274, phosphorylation (E); SD141, amidinotransamidination (F); SD189, dehydrogenation (H); SD245, linkage between streptidine 6-phosphate and dihydrostreptose.

It seemed strange that SD245 exhibited streptidine-dependent production of streptomycin despite its ability to synthesize streptidine 6-phosphate. This behavior of SD245 might be attributed to a change of substrate specificity of dihydrostreptosyltransferase. The enzyme, which naturally transfers a dihydrostreptose moiety to streptidine 6-phosphate but not to streptidine (8), would be altered to accept only streptidine as the acceptor and form ω-α-I-dihydrostreptose (1-4) streptidine, a dephosphorylated form of the normal intermediate. The product would then be incorporated into the streptomycin biosynthetic pathway after phosphorylation by streptomycin 6-phosphotransferase (16). The fact that streptomycin productivity of SD245 was incompletely restored by streptidine sulfate supplementation (Fig. 3) would reflect a limited catalytic capacity of the mutated dihydrostreptosyltransferase.

Aminotransferase and amidinotransferase activities in streptidine idiotrophs. Transamination (D) of scyllo-inosose (IV) is the first step unique to the biosynthesis of streptidine and was presumed to be lacking in SD20 and SD263. For verification of this assumption, the activity of aminotransferase was assayed with scyllo-inosamine (V) as an amino donor (scyllo-inosose + [14C]scy1lo-inosamine → scy1lo-inosamine + [14C]scyllo-inosose). Labeled scyllo-inosose formed was separated from the labeled substrate, scy1lo-inosamine, by paper electrophoresis. An extract from the parental strain gave labeled scy1lo-inosose, whereas those from SD20 and SD263 did not at all (Fig. 5). In both SD20 and SD263, the enzymatic activity was impaired to an extent of several percent of that in the parental strain (Table 2). Consequently, these streptidine idiotrophs were confirmed to be deficient in aminotransferase in reaction sequence (D).

When the activity of aminotransferase was measured for the remaining streptidine idiotrophs, an intriguing result was obtained with SD245. The specific activity of the enzyme detected in SD245 was only ca. 20% of that in the parental strain (Fig. 5D and Table 2). SD245 accumulated a substantial amount of streptidine 6-phosphate and, therefore, was assumed to have a mutated dihydrostreptosyltransferase. The partial defect in the aminotransferase observed in SD245 suggested that the aminotransferase activity would be negatively controlled by streptidine 6-phosphate. The negative control system would prevent incorporation of excess scyllo-inosose into the streptidine biosynthetic pathway and, in SD245, would result in a marked decrease of the accumulation of scyllo-inosamine as compared with the parental strain (Fig. 4A and F). The possibility that SD245 might have a leaky mutation in the structural or regulatory gene for the aminotransferase in addition to the mutation in the dihydrostreptosyltransferase gene would be minimized, since this possibility could not account for the scarcity of scyllo-inosamine in SD245.

The transamidination step (F) was presumably blocked in SD141, judging from the accumulation of scyllo-inosamine 4-phosphate. The amidinotransferase assay employed here involves the reaction of the enzyme-amidine ("active urea") intermediate with hydroxyamine and measurement of the hydroxyguanidine formed. This assay works well with the enzyme(s) catalyzing reaction steps of (F) and (K) (14). SD141 completely lost this enzymatic activity (Table 2). Therefore, SD141 was deficient in the two transamidination steps.

Cosynthesis tests between the streptidine idiotrophs. In some antibiotic producers, the cosynthesis test is effective for analyzing and ordering the sequence of the nonproducing mutants (I). The cosynthesis of streptomycin was examined in the liquid culture and on the agar plate as described above. By the two culture methods, all of the possible combinations between the streptidine idiotrophs failed to cosynthesize streptomycin.

DISCUSSION

We derived eight streptidine idiotrophic mutants from S. griseus ATCC 10137 by UV mutagenesis. The streptidine
idiotrophs accumulated the compounds assigned to scylo-inosamine (V) (SD81, SD141, SD261, and SD274), scylo-inosamine 4-phosphate (VI) (SD141), amidino-scyllinoamine (VIII) (SD189), amidinostreptamine 6-phosphate (XI) (SD245) and streptidine 6-phosphate (XII) (SD245). Walker and Walker have proposed that all of the compounds described above are the intermediates of streptidine biosynthesis in the course of an in vitro conversion study, using cell-free extracts from a streptomycin-producing strain of Streptomyces bikiniensis (17). However, one compound accumulated by SD141 could not be identified, and this compound was also detected in the parental strain of S. griseus (Fig. 4 and Table 1). The compound seemed to be closely related, but not identical, to scylo-inosamine 4-phosphate. Walker and Walker have ruled out the possibility that any isomers of scylo-inosamine 4-phosphate might be intermediates in streptidine biosynthesis in S. bikiniensis (18). An answer to the problem of whether this compound has any function in the streptidine biosynthesis in S. griseus or is only a shunt product must await further in vitro interconversion studies and elucidation of its structure.

As a whole, our study with the mutants would justify the in vivo validity of the Walkers' streptidine biosynthetic pathway. From the biosynthetic scheme shown in Fig. 2, an analysis of the accumulated compounds implied the biochemical lesions of the streptidine idiotrophs as follows: SD20 and SD263, transamination (D); SD81, SD261, and SD274, phosphorylation (E); SD141, transamidination (F); SD189, dehydrogenation (H); SD245, linkage between streptidine 6-phosphate and dihydrostreptose. By the use of enzymatic assays of cell-free extracts, it was directly asserted that SD20 and SD263 were deficient in the transamination step.
TABLE 1. Characterization of the compounds accumulated by the streptidine idiotrophs

<table>
<thead>
<tr>
<th>Strain</th>
<th>Fraction no.</th>
<th>HCl elution from Dowex 50 column</th>
<th>Rf</th>
<th>Electrophoretic mobility relative to picric acida</th>
<th>Assignment of the compoundb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pH 3.6</td>
<td>pH 10.4</td>
</tr>
<tr>
<td>Parent</td>
<td>17 - 21</td>
<td>H2O</td>
<td>0.08</td>
<td>-0.06</td>
<td>+1.06</td>
</tr>
<tr>
<td></td>
<td>41 - 45</td>
<td>0.5 N</td>
<td>0.34</td>
<td>-0.86</td>
<td>-0.13</td>
</tr>
<tr>
<td>SD81</td>
<td>39 - 44</td>
<td>0.5 N</td>
<td>0.37</td>
<td>-0.86</td>
<td>-0.13</td>
</tr>
<tr>
<td>SD141</td>
<td>17 - 20</td>
<td>H2O</td>
<td>0.09</td>
<td>-0.01</td>
<td>+0.97</td>
</tr>
<tr>
<td></td>
<td>31 - 33</td>
<td>0.5 N</td>
<td>0.09</td>
<td>-0.06</td>
<td>+1.03</td>
</tr>
<tr>
<td></td>
<td>36 - 38</td>
<td>0.5 N</td>
<td>0.36</td>
<td>-0.84</td>
<td>-0.11</td>
</tr>
<tr>
<td>SD189</td>
<td>52 - 56</td>
<td>0.5 N</td>
<td>0.51</td>
<td>-0.84</td>
<td>-0.80</td>
</tr>
<tr>
<td>SD245</td>
<td>95 - 97</td>
<td>2.0 N</td>
<td>0.30</td>
<td>-0.61</td>
<td>+0.55</td>
</tr>
<tr>
<td></td>
<td>102 - 106</td>
<td>2.0 N</td>
<td>0.35</td>
<td>-0.55</td>
<td>-0.13</td>
</tr>
<tr>
<td>SD261</td>
<td>40 - 44</td>
<td>0.5 N</td>
<td>0.35</td>
<td>-0.85</td>
<td>-0.13</td>
</tr>
<tr>
<td>SD274</td>
<td>39 - 42</td>
<td>0.5 N</td>
<td>0.36</td>
<td>-0.88</td>
<td>-0.13</td>
</tr>
</tbody>
</table>

a Rf values are for paper chromatograms. 
b Migration toward the negative pole; +, migration toward the positive pole.
c Abbreviations for the compounds are the same as in Fig. 2.
d The compound was separated from VI on cation-exchange column but proved to be closely related to VI (see the text).

(D) and that SD141 contained a lesion in both transaminidation steps (F) and (K). Streptidine biosynthesis involves two analogous sequences of five reactions (dehydrogenation, transamination, phosphorylation, transamidination, and dephosphorylation). Because of the unusual nature of this biosynthetic scheme, it is important to ask whether the analogous steps in the two sequences are catalyzed by the same enzyme. Walker et al. have observed that S. bikiniensis contained different enzymes catalyzing the two dehydrogenations (C, H), the two transaminations (D, I), and the two dephosphorylations (G, dephosphorylation of streptomycin 6-phosphate) (14, 18). Whether the two phosphorylation reactions (E, J) and the two transaminidations (F, K) are catalyzed by single enzymes has not been established yet. However, Walker et al. favored a single enzyme, judging from their data on partially purified enzymes (15). In this study of S. griseus, it is inferred that two different enzymes might catalyze each dehydrogenation step, because SD189 lacks only one step (H). In contrast, a single amidotransferase seems to catalyze the two transaminidation steps, since SD141 simultaneously lost both transaminidation activities (F, K).

Based on the premise that the unphosphorylated intermediates such as amidino-scyllo-inosamine (VIII) were excreted into the medium, the results of a cosynthesis test would provide additional data for understanding whether one or two enzyme systems are involved. Particularly, the absence of cosynthesis between SD189 and the other idiotrophs is inconsistent with the idea that the streptidine idiotrophs (SD20, SD81, SD261, SD263, and SD274) are blocked only at a specific step (D or E) before the dehydrogenation (H) step; otherwise, these strains would have converted amidino-scyllo-inosamine of SD189 to streptomycin. Consequently, these idiotrophs must be blocked in certain steps beyond the reaction (H) in streptidine biosynthesis besides (D) or (E). This observation might give rise to the inference that the two steps of transamination (D, I) and phosphorylation (E, J) as well as transamination (F, K) are catalyzed by single enzymes. Accordingly, SD20 and SD263 would be deficient in both transamination steps, and SD81, SD261, and SD274 would be deficient in both phosphorylation steps. The inference might be in our favor, although there was no direct evidence on the excretion of amidino-scyllo-inosamine accumulated by SD189. For verification of the inference from the cosynthesis test, further biochemical and genetic studies are now in progress in our laboratory.

TABLE 2. Specific activities of amidotransferase and amidotransferase in the streptidine idiotrophs

<table>
<thead>
<tr>
<th>Strain</th>
<th>Aminotransferasea (cpm of [1-3]scyllo-inosose formed per mg of protein per h)</th>
<th>Amidotransferaseb (U/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent</td>
<td>616</td>
<td>0.157</td>
</tr>
<tr>
<td>SD20</td>
<td>35</td>
<td>0.120</td>
</tr>
<tr>
<td>SD81</td>
<td>268</td>
<td>0.110</td>
</tr>
<tr>
<td>SD141</td>
<td>511</td>
<td>0.001</td>
</tr>
<tr>
<td>SD189</td>
<td>572</td>
<td>0.136</td>
</tr>
<tr>
<td>SD245</td>
<td>103</td>
<td>0.095</td>
</tr>
<tr>
<td>SD261</td>
<td>386</td>
<td>0.104</td>
</tr>
<tr>
<td>SD263</td>
<td>18</td>
<td>0.103</td>
</tr>
<tr>
<td>SD274</td>
<td>667</td>
<td>0.117</td>
</tr>
</tbody>
</table>

a Aminotransferase activity was assayed for transamination step (D) (14). Actual counts per minute were corrected for background (45 cpm). The values are means of two experiments.
b Amidotransferase activity was assayed for transamidination steps (F) and (K) (14).
The involvement of one enzyme in the two transamination steps contradicts the Walkers' results (18). In this context, it is worthwhile to note that, in Streptomyces fradiae, Micromonospora purpurea, and Micromonospora sagamiensis, which are producers of aminocyclitol antibiotics, neomycin, gentamicin, and sagamicin, the two transamination steps involved in 2-deoxystreptamine biosynthesis appear to be catalyzed by a single enzyme (2, 6, 15). The possibility exists that this contradiction would be attributable to strain variations between streptidine biosynthetic enzymes in S. griseus in this study and the Walkers' S. bikiniensis.

There have been no reports on regulation of the streptidine biosynthesis (3). Mutant SD245 appears to be a good candidate with which to begin studies on regulation. This streptidine idiotype accumulates streptidine 6-phosphate and probably has a mutated dihydrostreptosyltrasf erase that uses streptidine as an acceptor. Aminotransferase activity in SD245 is only ca. 20% of that in the parental strain, and the lesser activity results in a decrease of scyllo-inosamine accumulation. These results suggest that the aminotransferase activity is negatively controlled by streptidine 6-phosphate. The transamination is the first reaction step unique to the streptidine biosynthesis (14), and streptidine 6-phosphate is the end product of the pathway. It is an attractive idea to surmise that streptidine biosynthesis involves a feedback regulation. This regulation system (repression or inhibition) of aminotransferase activity deserves further study.

A final deduction of the biochemical lesions of the streptidine idiopath is summarized in Fig. 6. Isolation of genes for streptidine biosynthesis is promising when the series of the mutants characterized here is used, since a molecular cloning system is now available in S. griseus ATCC 10137 (12). Cloning and gene manipulation would provide additional materials and information useful for revealing the molecular mechanisms of streptidine biosynthesis, and for articulating the mutated gene(s) of the streptidine idiopaths.

ACKNOWLEDGMENTS

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