Biosynthesis of Streptomycin

II. MYO-INOSITOL, A PRECURSOR OF THE STREPTIDINE MOIETY*

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Streptomycin, the antibiotic produced by Streptomyces griseus, contains as a part of its structure a diguanidinyl derivative of scylla-inositol called streptidine. Two major precursors of the streptidine portion of the molecule have now been defined, L-arginine and D-glucose (1, 2).

Arginine plays a role in the formation of the guanidine side chains, whereas glucose is involved in the synthesis of the cyclitol ring. However, the intermediates involved in streptidine synthesis remain unknown. The similarity in structure of myo-inositol and scylla-inositol suggested to a number of investigators the possibility that myo-inositol might be involved in the biogenesis of the streptidine moiety. Definite enhancement of streptomycin production by S. griseus has been noted when the medium was supplemented with myo-inositol, suggesting a role for this cyclitol in the synthesis of the antibiotic (3, 4). More definitive information supporting this hypothesis was obtained recently by Majumdar and Kutzner (5) with an isotope dilution technique. Equal quantities of uniformly labeled glucose-14C were administered under similar conditions to four S. griseus cultures; two of the cultures were supplemented with myo-inositol. When the 14C specific activities of the streptomycin synthesized by the cultures were compared, a marked reduction was found in the streptidine and streptobiosamine moieties, but the greatest decrease was noted in the streptidine. Such evidence strongly suggests a role for myo-inositol in streptidine synthesis.

In the present work, direct evidence is presented indicating that myo-inositol is a precursor of streptomycin. Uniformly labeled myo-inositol-14C was markedly incorporated into streptomycin synthesized by cultures of S. griseus. Degradation studies disclosed that essentially all of the incorporated isotope was concentrated in the streptidine moiety.

**EXPERIMENTAL PROCEDURE**

**Culture Techniques**—The organism used in these studies was Streptomyces griseus strain 3754. Procedures for maintenance of the culture, inoculation of media, and growth conditions were identical with those described previously (1). S. griseus was grown in a medium consisting of 1% glucose, 1% trypticase, 0.6% beef extract, and 1% NaCl in distilled water (6). Fifty-milliliter volumes of medium were used in all experiments. 14C compounds were administered 96 hours after spore inoculation. All cultures were harvested 24 hours after administration of the labeled compounds. The streptomycin synthesized was determined by the difference between the 96- and 120-hour production by the culture, as measured by the maltol procedure of St. John, Flick, and Tepe (7). Uniformly labeled myo-inositol-14C was obtained from the Nuclear-Chicago Corporation and uniformly labeled D-glucose-14C from the California Corporation for Biochemical Research.

**Purity of Myo-inositol-14C**—The myo-inositol-14C was chromatographed on Whatman No. 1 paper in a solvent system of isopropyl alcohol-pyridine-H2O-glacial acetic acid (80:80:40:10) (8). Bioautography of the chromatogram by the procedure of Lewin (9) with Saccharomyces cerevisiae as the test organism resulted in only one growth area, with an Rf of 0.23.

**Isolation and Degradation of Streptomycin**—Procedures employed for isolation and degradation of streptomycin were similar to those described in a previous publication (1). Streptomycin sulfate carrier (1 g) was added to each medium after removal of the mycelium by filtration. Then the streptomycin was isolated essentially by the method of Hunter, Herbert, and Hockenhull (10), which consists of removal of the streptomycin from the medium with Amberlite IRC-50 (Na+), elution with acid, and isolation of the streptomycin in the eluate as the triclinic derivative. The streptomycin trireineckate was recrystallized once prior to radioassay. Additional recrystallizations did not alter the specific activity. Control experiments indicated that streptomycin was completely separated from both myo-inositol-14C and glucose-14C at the resin absorption step of the isolation procedure.

For purposes of degradation the streptomycin trireineckate was first converted to the sulfate salt. Streptidine sulfate was obtained from streptomycin sulfate essentially by the procedure of Hunter and Hockenhull (2). Streptidine was assayed for radioactivity as the dipicrate dihydrate salt (11). Isotope incorporation into the guanidine groups of streptidine was determined by oxidizing the streptidine with KMnO4 and then isolating guanidine from the oxidation products as the picrate salt (12).
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TABLE I

Incorporation of myo-inositol-14C and D-glucose-14C into streptomycin

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity administered</th>
<th>Streptomycin synthesized</th>
<th>14C incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c.p.m. × 10^-4</td>
<td>µmoles × 10^-2</td>
<td>µmoles</td>
</tr>
<tr>
<td>Myo-inositol-14C</td>
<td>3.75</td>
<td>1.5</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>3.75</td>
<td>1.5</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td>7.50</td>
<td>3.0</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td>7.50</td>
<td>3.0</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>15.00</td>
<td>6.0</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>15.00</td>
<td>6.0</td>
<td>13.4</td>
</tr>
<tr>
<td>D-Glucose-14C</td>
<td>6.64</td>
<td>3.0</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>6.64</td>
<td>3.0</td>
<td>10.3</td>
</tr>
</tbody>
</table>

The purity of the streptomycin reineckate and of the picrates of streptidine and guanidine was ascertained by nitrogen analysis (Dumas). All analyzed samples were found to be pure within ±2% of theory.

Radioassay Procedures—All measurements were made with a Nuclear-Chicago gas flow detector. Solid sample counting techniques were used to determine the radioactivity of the reineckate and picrate derivatives.

RESULTS

Myo-inositol and D-Glucose Experiments—Myo-inositol-14C and D-glucose-14C were added to cultures of S. griseus 96 hours after spore inoculation of the culture. Streptomycin was isolated from the broths 24 hours later, and the amount synthesized and the 14C incorporated were measured. The results are summarized in Table I. In all experiments the quantity of streptomycin synthesized varied from 12.2 to 17.7 µmoles. The percentage of 14C incorporated following addition of labeled myo-inositol ranged from 14.4 to 29.5 and approximately paralleled the quantity of isotopic myo-inositol added. Incorporation of glucose that was added in amounts comparable to the myo-inositol was about 7 times less, i.e. 3.2 and 3.3%. The specific activity of the streptomycin indicates that considerable dilution of both labeled compounds occurred.

A portion of this reduction in specific activity probably reflects the dilution of the 14C compounds with unlabeled myo-inositol and glucose present in the medium. This was confirmed for the glucose-14C experiments, at the time labeled glucose was added to the cultures, the medium glucose was about 4.3 µmoles, thus representing a 143-fold dilution of the labeled glucose added (0.03 µmole).

Isotope Distribution Studies—The distribution of 14C in the isolated streptomycin is given in Table II. It can be seen in the myo-inositol experiments that essentially all of the isotope was concentrated in the streptidine moiety. The lack of labeling of the guanidine side chains showed that the label was confined exclusively to the scyllo-inositol ring of the streptidine. In the case of glucose experiments the pattern of labeling was more diffuse, with only 20 and 28% found in the streptidine portion, an observation first reported by Hunter and Hockenhull (2).

DISCUSSION

The isotope dilution experiments of Majumdar and Kutzner (5) suggest that myo-inositol or a derivative of myo-inositol may function as a precursor of streptomycin. The present work provides direct evidence that myo-inositol is a precursor of the antibiotic. Marked 14C incorporation into streptomycin was found following the addition of myo-inositol-14C to cultures of S. griseus (Table I).

In our experiments the 14C incorporation from labeled myo-inositol was confined solely to the streptidine moiety, whereas the distribution pattern from labeled D-glucose was more diffuse (Table II), being found in both the streptobiosamine and streptidine moieties, when the labeled compounds were added to S. griseus cultures under similar conditions. If significant conversion of myo-inositol to glucose had occurred prior to incorporation into streptomycin, the distribution of 14C in the antibiotic molecule would have resembled that of glucose.

Both glucose and myo-inositol are precursors of the streptidine ring structure. This suggests that myo-inositol and glucose may share a common pathway in streptidine biosynthesis, perhaps either by conversion of glucose to myo-inositol, a reaction known to occur in tissues (13), or by conversion of both to a common intermediate. The present data do not exclude either possibility. Only an identification of the intermediate reactions involved in their conversion to streptidine will clarify the role of each in the

1 Unpublished data.
synthesis of this moiety. Such studies are in progress in this laboratory.2

SUMMARY

Myo-inositol was shown to be a precursor of the streptidine moiety of streptomycin. Addition of uniformly labeled myo-inositol-14C to cultures of *Streptomyces griseus* resulted in 14.4 to 29.5% incorporation of 14C into the streptomycin synthesized by the organism.

Degradation of the isolated streptomycin disclosed that essentially all of the incorporated 14C was concentrated in the scyllo-inositol ring of the streptidine portion of the molecule.

A different label distribution pattern was obtained in the streptomycin synthesized following the administration of uniformly labeled myo-inositol-14C and glucose-14C to cultures under identical conditions. This finding excludes glucose as an intermediate in the pathway of myo-inositol incorporation into the antibiotic.

Acknowledgment—The author wishes to express his apprecia-

*Note—After submission of this manuscript, Heding (14) described the incorporation of myo-inositol into the streptidine moiety of streptomycin by *Streptomyces griseus*.
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