STUDIES ON THE BIOCHEMISTRY OF THE STREPTOMYCES

II. Fixation of C\textsuperscript{14}O\textsubscript{2} by Intact Cells of *Streptomyces griseus*

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It has been demonstrated that in *Streptomyces griseus* the tricarboxylic acid cycle plays an important role in biosynthetic as well as in energy yielding processes (Gilmour et al., 1955). Inasmuch as the biosynthesis of carbon skeletons of amino acids and other cellular constituents requires the net synthesis of the all-important four carbon acids which cannot be obtained by the tricarboxylic acid operation, the present study was carried out to investigate the possible occurrence of a $\text{C}_4 + \text{C}_3$ type condensation.

EXPERIMENTAL METHODS

*Streptomyces griseus* strain 3475 was cultivated and cells harvested as outlined in the previous study. Aliquots of the resulting cell mass were resuspended in the described synthetic growth medium (Gilmour et al., 1955).

The apparatus consisted of a three-neck flask with attachments as follows. One neck was equipped with a vial seal through which the carbon dioxide, oxygen, and pyruvate could be administered with a hypodermic needle. An outlet was provided on another neck of the flask for attachment to a water aspirator. The third neck was connected to a mercury manometer. Prepared cells of *S. griseus* were added to the reaction flask which contained 100 ml of the growth medium and two mm of nonisotopic sodium pyruvate. C\textsuperscript{14}O\textsubscript{2} liberated from barium carbonate with a total activity of $5.8 \times 10^8$ counts per minute was admitted into the partially evacuated system. Oxygen was then introduced to bring the system back to atmospheric pressure. The culture flask was agitated mechanically and maintained at a temperature of 28 C for a period of 8 hours. At the end of this period 10 ml of 6 N HCl were added, and the system was swept with carbon dioxide-free air to remove the residual C\textsuperscript{14}O\textsubscript{2}.

The cells were then harvested by centrifugation and washed thoroughly with water, alcohol, and ether in succession. The cells were hydrolyzed under reflux with 6 N HCl for 20 hours.

The incorporation of C\textsuperscript{14}O\textsubscript{2} into various amino acids and their relative labeling level were examined by subjecting the HCl-free hydrolyzate to paper chromatography in combination with radioautography (Benson et al., 1950).

Glutamic acid and aspartic acid were isolated from the cell hydrolyzate following the addition of nonisotopic carrier by the operation of a "Dowex 3" ion exchange resin column. Aspartic acid was isolated as its copper salt from the HCl eluate of the column and glutamic acid as the hydrochloride (Block and Bolling, 1951). Controls were maintained throughout the isolation operations by means of ninhydrin spot tests. Dilution factors were obtained on the basis of the content of glutamic acid and aspartic acid as determined by microbiological assays (Horn et al., 1950).

The aspartic acid was subjected to the following degradation procedures: (1) combustion to carbon dioxide and subsequent radioassay provided a measure of the activity of the whole molecule, (2) ninhydrin decarboxylation (Van Slyke et al., 1943) and counting of the carbon dioxide gave the activity of the two carboxyl groups, (3) deamination with nitrous acid (Davis, 1954) produced maleic acid which was subjected to decarboxylation with concentrated sulfuric acid (Uter, 1951). The carbon monoxide obtained was oxidized to carbon dioxide which was equivalent to the alpha carboxyl carbon atom (Davis, 1954).

The glutamic acid hydrochloride was partially degraded by total combustion and by ninhydrin decarboxylation (alpha carboxyl carbon atom). The gamma carboxyl activity was obtained by difference, assuming that no activity was located at the middle carbon atoms.

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All samples were counted as barium carbonate with appropriate correction for background and self-absorption.

RESULTS

From Table 1 it can be seen that about 7 per cent of the initial activity of the C4O2 was fixed by growing cells of S. griseus in spite of the rapid dilution of the initial C4O2 by metabolic CO2 from the combustion of nonlabeled pyruvate. Since the radioactivity of the isolated glutamic acid and aspartic acid accounted for 15 per cent of the total activity of the cell hydrolyzate, it is apparent that the incorporation of carbon dioxide into other amino acids was fairly extensive. In fact, radioautography revealed that the following amino acids, arranged in order of decreasing activity, were found to be extensively labeled: aspartic acid, glycine, glutamic acid, threonine, and serine.

That a C4-C3 condensation was the principal carbon dioxide fixation process was indicated by the preferential labeling on the beta carboxyl carbon atom of aspartic acid (Table 2). The little activity found in the alpha carboxyl carbon atom may have been derived through the partial equilibration of oxalacetic acid with symmetrical four-carbon acids (Wood, 1948).

The appreciable fixation of C4O2 into various amino acids in this organism together with the isotopic distribution pattern of aspartic acid thus suggests that the C4-C3 type condensation could have served as one of the major pathways of four-carbon acid synthesis in the terminal respiration of S. griseus. The finding of practi-

TABLE 1
Incorporation of C4O2 by Streptomyces griseus in the presence of nonisotopic pyruvate

<table>
<thead>
<tr>
<th>Radioactivity</th>
<th>cpm</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4O2 (initial)</td>
<td>5.8 x 10^4</td>
<td>100.0</td>
</tr>
<tr>
<td>C4O2 (final)</td>
<td>5.4 x 10^4</td>
<td>93.0</td>
</tr>
<tr>
<td>Cell hydrolyzate</td>
<td>4.3 x 10^4</td>
<td>7.0</td>
</tr>
<tr>
<td>Humin</td>
<td>0.3 x 10^4</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Activity in Hydrolyzate Amount

| Glutamic acid | 12.7 | 2.4 x 10^4 | 5.5 |
| Aspartic acid | 9.0 | 4.1 x 10^4 | 9.5 |

DISCUSSION

The demonstration of the fixation of C4O2 into the protein fraction of S. griseus leaves little doubt that this organism makes active use of this reaction during cell growth. Aspartic acid, glutamic acid, serine, glycine, and threonine were radioactive. As would be expected, alanine was not radioactive. The intramolecular distribution of C4 in aspartic acid and in glutamic acid was in accord with a C4-C3 condensation. The total activity of aspartic acid was found in the carboxyl carbons. The higher activity in the beta carboxyl carbon is in agreement with the oc-
currence of a C4-C1 condensation. A partial equilibrium with a symmetrical four carbon acid such as fumaric would account for the activity on the alpha carboxyl of aspartic acid (Wood, 1948).

The activity of glutamic acid was located almost exclusively in the alpha carboxyl carbon. This labeling is in agreement with the existence of an extensive cyclic pathway through an asymmetrical C4 compound (Wang et al., 1953). These data would indicate that carbon dioxide enters the carbon skeletons of glutamic acid and aspartic acid via carbon dioxide fixation and the tricarboxylic acid cycle. Thus carboxylation of pyruvate leading to malate or oxalacetate (Ochoa et al., 1948) appears to represent a major pathway of carbon dioxide utilization in S. griseus.

SUMMARY

It has been shown that a C4-C1 condensation coupled with tricarboxylic acid cycle activity is a major pathway of carbon dioxide fixation by Streptomyces griseus.

The intramolecular distribution of C14 in glutamic acid rising from the incorporation of labeled carbon dioxide further confirmed that the tricarboxylic acid cycle operates extensively in the terminal respiration of S. griseus.

REFERENCES


