BACTERIAL OXIDATION OF p-AMINOBENZOIC ACID BY
PSEUDOMONAS FLUORESCENS

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The importance of p-aminobenzoic acid as a growth factor has been recognized for some time. However, little is known concerning its oxidative metabolism. Sloan (1951) reported that washed cells of Mycobacterium smegmatis were capable of forming p-aminophenol from p-aminobenzoic acid. Davis (1951) reported that p-aminobenzoic acid affected the metabolism of Escherichia coli in three ways: at low concentrations (0.005 μg/ml) it acted as a normal vitamin, in moderate concentrations (0.1 to 1.0 μg/ml) it served as a source of another vitamin, p-hydroxybenzoic acid, and in high concentrations (150 to 1600 μg/ml) it functioned as a growth inhibitor. This paper is concerned with the utilization of p-aminobenzoic acid by Pseudomonas fluorescens and the possible intermediates in the oxidative scheme.

MATERIALS AND METHODS

A strain of P. fluorescens was selected that was capable of oxidizing p-aminobenzoic acid as the sole source of energy for aerobic growth. Stock cultures were maintained on synthetic medium containing p-aminobenzoic acid. Simultaneous adaptation (Stanier, 1947) was the principal method of investigation. In order to produce cell suspensions with the desired enzymatic patterns for manometric work, the organism was grown on synthetic medium containing a single compound as the source of carbon and energy. The synthetic medium had the following basal composition: NaCl, 0.2 g; KH₂PO₄, 0.32 g; K₂HPO₄, 0.42 g; NH₄Cl, 0.2 g; agar, 2.0 g; and distilled water, 100 ml. To this was added 0.1 ml of a mineral salts solution consisting of MgSO₄·7H₂O, 5.0 g; MnSO₄, 0.1 g; FeCl₃, 1.0 g; CaCl₂, 0.5 g;

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Cells grown on p-aminobenzoic acid show simultaneous adaptation to p-hydroxybenzoic acid as a source of carbon and energy. The specific compound that served as a source of carbon and energy was added at a final concentration of 0.1 to 0.2 per cent, and the pH was adjusted to 7.2. The "unadapted" cells were grown using asparagine as the carbon source.

All respirometer experiments were performed in the Warburg apparatus (Umbreit et al., 1945) at a temperature of 30°C with air as the gas phase. The enzymatically adapted cell suspensions were prepared by harvesting the growth from plate cultures 20 hr old, washing twice, and resuspending in M/50 phosphate buffer of pH 7.0. The substrate concentration was two micromoles per flask.

Dried cell preparations were prepared by harvesting the growth from plate cultures, washing twice with phosphate buffer (M/50, pH 7.0) and a third time with distilled water. The cell paste was then spread over the bottom of a beaker and slowly dried in vacuo at 25°C. The dried residue was ground to a fine powder and stored at 0°C until used. The dried cells were resuspended in phosphate buffer (M/50, pH 7.0) for manometric experimentation. The oxidation of protocatechuic acid results in the liberation of a large amount of acid: the phosphate buffer concentration was therefore increased to M/10 for both living and dried cells.

RESULTS

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acid and protocatechuic acid (figure 2) which suggests that these substances are possible intermediates.

The p-hydroxybenzoic acid-grown cells exhibit simultaneous adaptation to protocatechuic acid (figure 3) which is in agreement with the report of Sleeper and Stanier (1950). In addition,

**Figure 1.** The oxidation of protocatechuic acid, p-hydroxybenzoic acid, and p-aminobenzoic acid by *Pseudomonas fluorescens* grown on asparagine.

**Figure 2.** The oxidation of protocatechuic acid, p-hydroxybenzoic acid, and p-aminobenzoic acid by *Pseudomonas fluorescens* grown on p-hydroxybenzoic acid.

**Figure 3.** The oxidation of protocatechuic acid, p-hydroxybenzoic acid, and p-aminobenzoic acid by *Pseudomonas fluorescens* grown on protocatechuic acid.

these cells required a period of adaptation for p-aminobenzoic acid oxidation, thus indicating that p-aminobenzoic acid is not an intermediate in the oxidation of p-hydroxybenzoic acid while
substrates employed.

Enzymatic activity of dried cell preparations. In order to substantiate the results obtained with living cells, the enzymatic activity, as measured by oxygen uptake, of dried cells with preestablished adaptive patterns was studied. Sleeper et al. (1950) reported that high levels of enzymatic activity against substrates could be found in dried cells, but only when the substrate previously employed for growth was one known to cause complete adaptation to the compounds in question. Tables 1 and 2 compare the patterns of adaptation in living cells with the enzymatic activity of dried cells after growth on the various substrates employed.

Dried cells that were grown on p-aminobenzoic acid demonstrated a high level of enzymatic activity for p-hydroxybenzoic acid and protocatechuic acid, thus offering further evidence that the latter two compounds are intermediates in the oxidative pathway. Dried p-hydroxybenzoic acid-grown cells showed activity for protocatechuic acid but not for p-aminobenzoic acid, while dried cells that were grown on protocatechuic acid showed no activity for either p-aminobenzoic acid or p-hydroxybenzoic acid.

Since the establishment of adaptive enzyme systems in vivo is essential in order for dried cells to show enzymatic activity against the compounds in question, these results strongly support the data obtained with living cells which suggest that p-hydroxybenzoic acid and protocatechuic acid are intermediates in the oxidative metabolism of p-aminobenzoic acid.

Stanier et al. (1950) reported that protocatechuic acid was oxidized quantitatively to β-ketoadipic acid. In studying this step of the oxidative pathway, the Rothera reaction was used, which is specific for β-ketoadipic acid (Kilby, 1948). The flasks containing protocatechuic acid as the substrate were tested when oxygen uptake ceased and showed the formation of a compound which gave an intense violet Rothera reaction. Stanier et al. (1950) reported similar results and stated that the compound responsible for this reaction was β-ketoadipic acid.

The rate of oxidation of a substrate by the dried cells remained constant; however, the oxygen uptake per mole of substrate was approximately one-fourth the uptake observed with living cells. Methylene blue was added, at first, to provide an extraneous hydrogen carrier but was omitted when the oxidations by the dried cells proceeded with equal rapidity in its absence.

Oxidation of additional aromatic compounds. In addition to the substrates already tested, the following compounds were tested as possible intermediates: p-nitrophenol, p-aminophenol, p-aminosalicylic acid, aniline, catechol, and benzoic acid. The p-aminobenzoic acid-grown cells oxidized benzoic acid and catechol after a period of adaptation, but oxygen uptake in excess of the endogenous respiration was not observed with aniline, p-aminophenol, p-aminosalicylic acid, or p-nitrophenol. Thus, none of the compounds listed can be a principal intermediate in the dissimilation of p-aminobenzoic acid.

### Table 1

<table>
<thead>
<tr>
<th>Cells Grown On</th>
<th>p-Aminobenzoate</th>
<th>p-Hydroxybenzoate</th>
<th>Protocatechuate</th>
<th>Benzoate</th>
<th>Catechol</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Aminobenzoate</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td></td>
</tr>
<tr>
<td>p-Hydroxybenzoate</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td></td>
</tr>
<tr>
<td>Protocatechuate</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Cells Grown On</th>
<th>Dried Cell Activity (μL Oxygen Uptake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Aminobenzoate</td>
<td>55 63 50 0 0</td>
</tr>
<tr>
<td>p-Hydroxybenzoate</td>
<td>0 59 51 0 0</td>
</tr>
<tr>
<td>Protocatechuate</td>
<td>0 0 51 0 0</td>
</tr>
</tbody>
</table>

Substrate concentration was 2 micromoles.
DISCUSSION

Para-aminobenzoic acid fulfills the role of an oxidizable substrate in addition to functioning as a growth factor. Cells adapted to p-aminobenzoic acid are simultaneously adapted to p-hydroxybenzoic acid and protocatechuic acid, thus indicating that the latter two compounds are intermediates in the oxidative scheme. Additional data indicate that protocatechuic acid may be further oxidized to \(\beta\)-ketoacidic acid. The conclusions may be formulated in the following sequence:

\[
\begin{align*}
\text{COOH} & \xrightarrow{-\text{NH}_2} \text{COOH} \\
\text{NH}_2 & \xrightarrow{+\text{H}_2\text{O}} \text{OH} \\
\text{COOH} & \xrightarrow{+\text{O}_2} \text{CH}_3 \\
\text{OH} & \xrightarrow{-\text{CO}_2} \text{C=O} \\
& \xrightarrow{+\text{H}_2\text{O}} \text{COOH}
\end{align*}
\]

Cells of *Pseudomonas fluorescens* grown on p-aminobenzoic acid show complete simultaneous adaptation to p-hydroxybenzoic acid and protocatechuic acid. In addition, protocatechuic acid may be further oxidized to \(\beta\)-ketoacidic acid. Cells grown on p-hydroxybenzoic acid are adapted to protocatechuic acid but not to p-aminobenzoic acid, while protocatechuic acid-grown cells are not adapted to either p-aminobenzoic acid or p-hydroxybenzoic acid. These facts suggest that p-hydroxybenzoic acid, protocatechuic acid, and \(\beta\)-ketoacidic acid may serve as intermediates in the oxidation of p-aminobenzoic acid and the sequence of appearance is in this order.

Dried cell preparations have been obtained that were capable of oxidizing p-aminobenzoic acid, p-hydroxybenzoic acid, and protocatechuic acid.

REFERENCES


