Comparative Effect of Oxygen and Nitrate on Protoporphyrin and Heme Synthesis from Δ-Amino Levulinic Acid in Bacterial Cultures

N. J. JACOBS, J. M. JACOBS, AND H. E. MORGAN, JR.

Department of Microbiology, Dartmouth Medical School, Hanover, New Hampshire 03755

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Pseudomonas aeruginosa and P. denitrificans accumulate more protophe and considerably more protoporphyrin during anaerobic growth under denitrifying conditions than during aerobic growth. In Escherichia coli, the small accumulation of protoporphyrin and protoheme which occurs during anaerobic growth is slightly stimulated by nitrate and markedly stimulated by oxygen.

The role of oxygen in the penultimate step of bacterial heme synthesis, involving the oxidative conversion of coproporphyrinogen (COPRO) to protoporphyrin (PROTO), remains unclear. In mammalian tissue, molecular oxygen is absolutely essential for this conversion, but an anaerobic mechanism must exist in certain facultative and anaerobic bacteria which contain cytochromes (see 6 for references). Previous studies with cell-free extracts of several species of anaerobic and facultative bacteria have either failed to demonstrate or not clarified the anaerobic mechanism for this conversion (2, 3, 5, 8, 9). Therefore, it seemed important to confirm its existence by studies of porphyrin production (1, 4, 7) in growing cultures of various heterotrophic, facultative bacteria such as denitrifying pseudomonads and Escherichia coli, which form cytochromes during anaerobic growth.

Pseudomonas denitrificans was grown anaerobically at 30 C in 250 ml of nitrate-containing media as previously described (6). After 17 hr, a vigorous evolution of nitrogen gas aided in maintaining anaerobiosis, and 13 mg of Δ-amino levulinic acid (ALA) was added, where indicated. The flasks were then incubated for a further 4 hr either anaerobically or aerobically by transferring the contents to a 1-liter Erlenmeyer flask on a vigorously rotating shaker. P. aeruginosa was grown (37 C) anaerobically and incubated anaerobically with ALA as described above. For aerobic conditions, ALA was incubated for 4 hr with young aerobic cultures of P. aeruginosa grown aerobically in the same medium without added nitrate. E. coli was grown anaerobically (4) for 14 hr in 250 ml of the above medium modified by addition of 0.2% glucose (sterilized separately), 0.08 M potassium phosphate (pH 7.6), 0.4% KNO₃, and ALA (13 mg) were indicated. For aerobic conditions, a culture was first grown anaerobically (without nitrate) for 10 hr and then aerated for 4 hr after addition of ALA.

After incubation, cultures were chilled and centrifuged (4 C) without aeration. Porphyrins were extracted from both the cells and supernatant fluid with ethyl acetate and acetic acid, and analyzed as previously described (6). Porphyrin was characterized spectrophotometrically and chromatographically (5). The heme which was extracted from the cells and supernatant fluid by ethyl acetate and acetic acid was extracted from this solvent with 1 N NaOH and analyzed spectrophotometrically (4). Any heme covalently linked to protein, such as the heme of cytochrome c, would not be extracted by this procedure.

In P. aeruginosa, aerobic growth led to a large accumulation of COPRO from ALA and a smaller accumulation of PROTO, while anaerobic growth with nitrate under denitrifying conditions led to a marked increase in PROTO accumulation (Table 1). Heme accumulation was slightly stimulated by anaerobic growth with nitrate. In P. denitrificans, significant levels of PROTO accumulated only during anaerobic growth with nitrate, and heme formation was also maximal under these conditions (Table 1). These results establish that, in these two denitrifying pseudomonads, the conversion of COPRO to PROTO can occur readily under anaerobic conditions when nitrate is the electron acceptor for growth. Anaerobic conditions appear to specifically favor PROTO accumulation. However, these experiments do not distin-
with Aerobic, with Aerobic, with Anaerobic, with Anaerobic,

with Anaerobic, with Aerobic, with Anaerobic, with Anaerobic,

Aerobic, no ALA Aerobic, with ALA Anaerobic, no ALA Anaerobic, with ALA

Aerobic, no ALA Aerobic, with ALA Anaerobic, no ALA Anaerobic, with ALA

TABLE 2. Effect of oxygen and nitrate on porphyrin and heme accumulation in cultures of E. coli

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<table>
<thead>
<tr>
<th>Conditions of incubation</th>
<th>Picosomes per milligram(^{a})</th>
<th>Amt of growth (mg)(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coproporphyrin</td>
<td>Protoporphyrin</td>
</tr>
<tr>
<td>Anaerobic, with ALA</td>
<td>1,370</td>
<td>6</td>
</tr>
<tr>
<td>Anaerobic, with ALA</td>
<td>912</td>
<td>68</td>
</tr>
<tr>
<td>Anaerobic with nitrate</td>
<td>235</td>
<td>26(^{b})</td>
</tr>
<tr>
<td>Anaerobic with nitrate,</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^{a}\) Expressed as picomoles per milligram (dry weight) of cells in the culture.

\(^{b}\) Expressed as milligrams (dry weight) of cells per 250 ml of growth medium.

\(^{p}\) Unidentified porphyrin(s) with absorption peaks at 422, 563, and 612 nm was also in this fraction.

\(^{p}\) The pyridine hemochromogen of this heme exhibited a visible peak at 553 rather than 556 nm.

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LITERATURE CITED


In E. coli, anaerobic growth without added electron acceptors leads largely to COPRO accumulation with a very small accumulation of PROTO and heme (Table 2). Growth with oxygen causes a marked increase in PROTO and heme formation, but the addition of nitrate causes a considerably smaller increase. These findings suggest that the facultative anaerobe E. coli requires oxygen for maximal conversion of COPRO to PROTO. However, the presence of the alternative electron acceptor nitrate in the anaerobic growth medium, although stimulating the conversion, is considerably less effective than oxygen. Again, these experiments do not distinguish between a direct or an indirect effect of nitrate. In previous studies with cell-free extracts of P. denitrificans and E. coli, we were unable to show that nitrate or other electron acceptors could replace oxygen in this conversion (5, 6).