which includes both phosphorylated enzyme and enzyme-CoA intermediates (7).

REFERENCES

Synthesis of Oleic Acid by Euglena gracilis*

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(Received for publication, June 10, 1965)

While mechanisms for the enzymatic synthesis of oleic acid and related monounsaturated fatty acids have been established in a variety of biological systems, the origin of oleic acid in plants has remained unknown. As Mudd and Stumpf have shown, the synthesis of oleic acid in extracts of avocado mesocarp requires oxygen as it does in yeast, animal tissues, and in certain bacteria (1). However, unlike other oxygen-dependent systems, plant extracts apparently cannot convert stearate or palmitate into the corresponding olefins (2). Confirming Stumpf's findings on plant systems, we have previously reported that intact cells of Euglena gracilis and various other algae fail to desaturate stearate or palmitate (3). Further studies with Euglena have, however, led to results which require a revision of the earlier conclusion that stearate is not converted to oleate in photosynthetic systems. We have now shown that in cell-free extracts of Euglena, thioesters of long chain saturated fatty acids are desaturated, and we have further found that the enzyme systems from photosynthetic and from etiolated cells have markedly different properties. As shown in Fig. 1, an enzyme system prepared from colorless cells, grown on sucrose in the dark, readily forms unsaturated acids from the coenzyme A esters of stearic and palmitic acids. For these transformations both particulate and soluble fractions are required. Extracts from photosynthetic Euglena cells do not act on the CoA esters but they desaturate stearyl-ACP to oleate (Fig. 1). Conversely stearyl-ACP (or palmityl-ACP) is a poor substrate for the enzyme system from etiolated cells. The enzyme from green cells is soluble. It is obtained by centrifugation of broken cells at 105,000 X g for 45 minutes, precipitation of protein from the supernatant by 0 to 75% ammonium sulfate, and passage of the dissolved precipitate through Sephadex G-25. Two fractions, one precipitated between 20 and 45% and the other between 45 and 70% ammonium sulfate, show desaturating activity when combined, but they are inactive when tested singly (Table I). The soluble desaturase is specific for stearyl-ACP; the ACP derivatives of palmitate or myristate are not metabolized. By requiring O2 and TPNH for activity (Table I), the enzyme resembles the known stearyl-CoA desaturases. Ascorbate (9), FAD, FMN (10-12), or 2-amino-4-hydroxy-6-methyltetrahydropteridine (13, 14), the cofactors required by some oxygenase systems, do not stimulate the soluble Euglena enzyme.

The ACP used in these experiments was the heat-stable protein isolated from Escherichia coli (15, 16). It appears from the present and other findings (17, 18), that the bacterial protein can serve as acyl carrier in plants as well.

* Supported by grants-in-aid from the United States Public Health Service, the National Science Foundation, the Life Insurance Medical Research Fund, and the Eugene P. Higgins Trust Fund of Harvard University.

1 The abbreviation used is: ACP, acyl carrier protein.

2 Private communication from Dr. P. R. Vagelos.
tions were the same as described in the legend for Fig. 1A.

The composition of the reaction mixture and the experimental conditions were the same as described in the legend for Fig. 1A.


Table 1

<table>
<thead>
<tr>
<th>Enzyme and additions</th>
<th>Desaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 75% (NH₄)₂SO₄ fraction, 0.9 mg of protein</td>
<td>15.2</td>
</tr>
<tr>
<td>TPNH</td>
<td>0.4</td>
</tr>
<tr>
<td>DPNH</td>
<td>5.2</td>
</tr>
<tr>
<td>TPNH, anaerobic</td>
<td>0.5</td>
</tr>
<tr>
<td>TPNH, air</td>
<td>0.4</td>
</tr>
<tr>
<td>20 to 45% (NH₄)₂SO₄, 0.45 mg of protein (A)</td>
<td>11.5</td>
</tr>
<tr>
<td>TPNH</td>
<td>2.1</td>
</tr>
<tr>
<td>45 to 75% (NH₄)₂SO₄, 0.26 mg of protein (B)</td>
<td>1.6</td>
</tr>
<tr>
<td>A + B, 0.71 mg of protein</td>
<td>19.7</td>
</tr>
</tbody>
</table>

* Incubations were carried out in Thunberg tubes. Air was replaced by flushing three times with helium followed by evacuation.
† This experiment served as a control. After flushing with He and evacuation, air was admitted and the tube was incubated for 10 min.

Δ⁴-C₁₀ → Δ⁶-C₁₂ → Δ⁸-C₁₄ → Δ₁₀-C₁₆ → Δ₁₂-C₁₈
Δ⁴-C₁₀ → Δ⁴-C₁₂ → Δ⁶-C₁₄ → Δ₁₀-C₁₆ → Δ₁₂-C₁₈

Fig. 2. Postulated pathways to unsaturated fatty acids in photoautotrophic Euglena. The ACP derivatives are assumed to be the substrates in all of the indicated transformations.

The activity of the stearyl-ACP specific enzyme from photoautotrophic Euglena accounts for the formation of oleic acid, but it fails to explain the presence of Δ¹-octadecenoic acid, and of the Δ¹- and Δ³-isomers of hexadecenoic acid in photoautotrophic Euglena (19, 20). Double bond isomers of this type are also found in Clostridium butyricum and in other bacteria which synthesize long chain unsaturated fatty acids anaerobically by chain-elongation of unsaturated acids, e.g. Δ⁸-decenoate or Δ⁶-dodecenoate (21, 6, 22). On structural grounds, it therefore seems likely that Euglena and perhaps other photosynthetic organisms employ a mechanism of the bacterial type, in addition to stearyl-ACP desaturation. Evidence in favor of this assumption is the ability of cell-free extracts of photoautotrophic Euglena to elongate the ACP-derivatives of octanoate, decanoate, and dodecanoate to long chain saturated and unsaturated acids.² Analogous transformations occur in extracts of E. coli with octanyl-ACP.² However, unlike the bacterial system, the Euglena-elongating system produces long chain unsaturated acids only in the presence of oxygen.² These findings are consistent with the sequence of reactions shown in Fig. 2, a scheme already proposed on the basis of other evidence (23). The principal and so far specula-
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