Preliminary Communications

Enzymic Hydrolysis and Synthesis of Ceramides*

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Ceramides (N-acyl derivatives of sphingosine or dihydro-sphingosine) are the simplest sphingolipids isolated from animal tissue. As in all other sphingolipids, a long chain fatty acid is bound to the sphingosine base in amide linkage. Ceramides have been implicated as precursors in the biosynthesis of sphingomyelin (1), and their structure would imply that they might also be intermediates in the catabolism of the various sphingosine derivatives. The biosynthesis of these compounds by tissue preparations has been reported (2, 3), and the incorporation of several precursors into the ceramides of rat and human brain has been described (4, 5). The present communication reports the isolation of a soluble enzyme preparation from rat brain which hydrolyzes the amide linkage of ceramides yielding sphingosine and free fatty acid; the same preparation also catalyzes the reverse reaction.

Ceramides labeled with radioactive tracer were synthesized as follows. (a) Palmitoyl-1-14C chloride was allowed to react with oleyl chloride (8) to yield N-oleyl-dipalmitoylsphingosine. The mixture was subjected to partitioning according to Dole (8). The radioactive fatty acid or sphingosine was then isolated from the heptane or the aqueous isopropyl alcohol phase, respectively, and counted in a Packard Tri-Carb scintillation counter. When nonradioactive ceramides were used, the sphingosine formed by the hydrolytic reaction was measured by the method of Lowry et al. (10). The protein content of the soluble enzymes was determined by absorption at 280 mp, corrected for the nucleic acid content.

The reaction mixture, in a volume of 0.25 ml, contained 30 pmol of acetate buffer, pH 5.0; 0.05 pmole of oleyldi-H3-sphingo-sine (2 × 106 c.p.m. per pmole) suspended in 0.1 mg of Tween 20; 0.25 mg of Triton X-100; and 0.4 mg of sodium cholate. This mixture was incubated for 2 hours at 37°C. The protein content of the homogenate and particles was determined by the method of Lowry et al. (10). The protein content of the soluble enzymes was determined by absorption at 280 mp, corrected for the nucleic acid content.

### Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Protein</th>
<th>Activity</th>
<th>Specific activity</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>mg</td>
<td>%</td>
<td>c.p.m.</td>
<td>%</td>
</tr>
<tr>
<td>Homogenate</td>
<td>280</td>
<td>3,650</td>
<td>40</td>
<td>2.48 × 10⁴</td>
<td>680</td>
</tr>
<tr>
<td>Particles</td>
<td>120</td>
<td>1,440</td>
<td>40</td>
<td>2.6 × 10⁴</td>
<td>1,800</td>
</tr>
<tr>
<td>Cholate extract of sonically treated particles (15,000 × g supernatant)</td>
<td>16</td>
<td>48</td>
<td>1.3</td>
<td>8.6 × 10⁴</td>
<td>3.5</td>
</tr>
<tr>
<td>Cholate extract of sonically treated particles (100,000 × g supernatant)</td>
<td>12</td>
<td>18</td>
<td>0.5</td>
<td>5.7 × 10³</td>
<td>23</td>
</tr>
<tr>
<td>Extract after Sephadex treatment</td>
<td>22</td>
<td>12</td>
<td>0.33</td>
<td>3 × 10²</td>
<td>12</td>
</tr>
<tr>
<td>Ammonium sulfate fractionation:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 40% saturation</td>
<td>0.5</td>
<td>4.1</td>
<td>0.12</td>
<td>8.3 × 10⁴</td>
<td>3.35</td>
</tr>
<tr>
<td>40 to 50% saturation</td>
<td>5.0</td>
<td>1.05</td>
<td>0.029</td>
<td>5.3 × 10⁴</td>
<td>2.15</td>
</tr>
<tr>
<td>50 to 60% saturation</td>
<td>4.5</td>
<td>1.35</td>
<td>0.037</td>
<td>10⁴</td>
<td>4.0</td>
</tr>
<tr>
<td>60 to 80% saturation</td>
<td>5.5</td>
<td>1.93</td>
<td>0.053</td>
<td>5.5 × 10⁴</td>
<td>2.2</td>
</tr>
</tbody>
</table>

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Enzymic Hydrolysis and Synthesis of Ceramides

Vol. 238, No. 9

Sphingosine; extraction of the fragments. These two steps resulted in a 100-fold purification of the enzymic activity. The enzyme had an optimal pH of about 5, which was not activated by magnesium, manganese, or calcium salts.

An isotope exchange reaction between tritium-labeled sphingosine and palmitoyl-CoA was observed in the presence of sodium cholate or potassium cholate. The reaction mixture was partitioned according to Dole (8), and the radioactive free fatty acid was removed from the heptane phase after incubation with alkaline ethanol. The ceramide was counted. The enzyme also catalyzed the reverse reaction, namely, the synthesis of ceramide from sphingosine and fatty acid. The synthetic reaction was readily assayed with 1-C^14-palmitate and nonradioactive sphingosine or with tritium-labeled sphingosine and nonradioactive fatty acid as substrates. After incubation, the reaction mixture was partitioned according to Dole (8), and the radioactive free fatty acid was removed from the heptane phase with alkaline ethanol. The ceramide was counted.

In summary, a soluble enzyme preparation has been isolated from rat brain which catalyzes both the hydrolysis of ceramides and the synthesis of sphingosine from these components. This enzyme differs from the two other preparations reported (2, 3) in that it catalyzes the synthesis of ceramides from sphingosine and fatty acid and its synthesis from these components.
from sphingosine and free fatty acid rather than the coenzyme A derivative of the acid.

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REFERENCES

On the Mechanism of the Enzymatic Synthesis of Unsaturated Fatty Acids in Escherichia coli*

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The fatty acid synthetase from Escherichia coli B is unique in that it catalyzes the formation of both saturated and unsaturated fatty acids from acetyl coenzyme A and malonyl-CoA (1-3), forming cis-vaccenic acid as the major product (4). The crude enzyme (ammonium sulfate fraction) also catalyzes a dehydration of β-hydroxydecanoyl-CoA to a mixture of α,β-trans- and β,γ-(cis?)-decanoates (1). This reaction is of particular interest since the formation of cis-vaccenic acid has been attributed to a β,γ-dehydration step at the C10 level and subsequent chain elongation of cis-3-decanoate (4). We have now succeeded in separating the dehydrase from the synthetase complex, and have partially purified this novel enzyme.

When either crude or partially purified synthetase is heated at 50° in 0.01 M potassium phosphate buffer at pH 7 for 2 minutes, enzyme activity for long chain fatty acid synthesis from malonyl-CoA and acetyl-CoA is completely destroyed. However, the ability to dehydrate β-hydroxydecanoyl thioester is fully retained (95%) even after 10 minutes of heating.

Crude or purified synthetase preparations obtained from different batches of cells produce fatty acid mixtures of variable composition, the proportion of unsaturated acids ranging from 20 to 90% of the total. Dehydrases prepared from these different synthetases by heat treatment produce correspondingly higher or lower percentages of the β,γ isomer of deconate from β-hydroxycanoyl-CoA and free fatty acid rather than the coenzyme A derivative of the acid.

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