Kinetic Studies on the Chain Length Specificity of Long Chain Acyl Coenzyme A Synthetase from Rat Liver Microsomes*

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SUMMARY

The kinetics of activation of saturated fatty acids by long chain acyl-CoA synthetase from rat liver microsomes has been studied with a method of selective extraction of free fatty acids based on the insolubility of acyl-CoA in diethyl ether. Saturated fatty acids with a chain length ranging from C_{16} to C_{14} were assayed at concentrations varying from 0.5 to 10 \mu M. Under these conditions, \( V_{\text{max}} \) is maximum for palmitic, myristic, and lauric acids, and decreased drastically with octanoic and hexanoic acids. As judged by the values of \( K_{m} \), the affinity of long chain acyl-CoA synthetase is greatest with palmitic acid and decreases very progressively as the chain length of the fatty acids decreases. Finally, palmitic acid is a competitive inhibitor of the activation of octanoic acid.

From these results, it has been concluded that there is evidence for only one long chain acyl-CoA synthetase in rat liver microsomes, and that this enzyme has a rather broad chain length specificity.

The influence of chain length of saturated fatty acids on the specificity of long chain acyl-CoA synthetase (acid:CoA ligase (AMP), EC 6.2.1.3) of microsomal fractions has been the subject of several studies. In the microsomes of guinea pig liver, the optimum chain length was found to be that of lauric acid (1). In the microsomes of the intestinal mucosa of guinea pig and cat, the optimum rates of reaction were obtained with myristic and palmitic acids, respectively (2). Contradictorily, in rat liver microsomes, the optimum chain length was found to be that of lauric acid (1). In the microsomes of guinea pig liver, the optimum chain length was found to be that of lauric acid (1).

In the present report, the kinetics of activation of saturated fatty acids by microsomal preparations of rat liver has been studied by a method which permits us to assay the substrates in the micromolar range of concentration (6, 7). When it was found that \( V_{\text{max}} \) of long chain acyl-CoA synthetase was similar for palmitic, myristic, and lauric acids and declined sharply with octanoic and hexanoic acids, the kinetics of inhibition of octanoate activation by palmitic acid was also studied.

EXPERIMENTAL PROCEDURE

Materials—1-\(^{14}\)C-labeled saturated fatty acids with chain lengths of C_{18}, C_{16}, C_{14}, C_{12}, C_{10}, C_{8}, and C_{6} were purchased from Amersham-Searle (Arlington-Heights, Ill.). Other nonradioactive fatty acids were products of the Hormel Institute (Austin, Minn.), and of Nu-Chek-Prep (Elysean, Minn.). The source of other chemicals has been described previously (6).

Assay for Long Chain Acyl CoA Synthetase—The microsomal fraction was prepared (6) and was stored (7) as described before. Stock solutions of fatty acid (ammonium salts) were prepared with a solution of Triton WR-1339 (1%) and were stored for short periods of time (7). The method of assay for long chain acyl-CoA synthetase was the same as described previously (6, 7). However when activation of stearic acid was studied, the following modification had to be introduced in order to decrease the radioactivity in the control experiments. The reaction was stopped by addition of 0.2 ml of methanol, and 0.6 ml of water was added. After extraction of the remaining free fatty acid with diethyl ether (four times, 5 ml), the water phase was analyzed as previously described (7).

RESULTS AND DISCUSSION

The kinetics of activation of the different saturated fatty acids was first studied. Each substrate was assayed at concentrations (0.5 to 10 \mu M) which were below known or estimated critical micelle concentrations (8, 9). The reaction time and the enzyme concentration were varied in order to also maintain the concentration of the acyl-CoA formed below its known or estimated critical micelle concentration (9). The results of the kinetics studies are summarized in Figs. 1 and 2 and in Table I. As judged by the values of \( V_{\text{max}} \), long chain acyl-CoA synthetase shows the same activity with palmitic, myristic, and lauric acids as substrates but its activity is more than 30 times lower with fatty acids with a chain length smaller than decanoic acid.

When we consider the Michaelis constants obtained for each substrate, the affinity of the enzyme is greatest for palmitic acid and decreases progressively with myristic and lauric acids. It should be noted that the \( K_{m} \) value reported here for palmitate activation (1.25 \mu M) is about half of that reported previously (7). This discrepancy is probably attributable to differences in mi-
Figs. 1. Kinetics of activation of stearate (Δ—Δ), palmitate (O—O), myristate (X—X), laurate (●—●), and decanoate (Δ—Δ). The assay was as described under “Experimental Procedure.” Protein and substrate concentrations were varied together in order to maintain the linearity of the reaction up to at least 2 min. V is expressed as micromoles of acyl-CoA formed per hour per mg of protein, and S is expressed as μM.

Fig. 2. Kinetics of activation of octanoate (●—●), and those of inhibition of octanoate activation by palmitate (O—O). The inhibition study was carried out at 5 μM palmitate and under standard incubation conditions. The units are as in Fig. 1.

Table I
Michaelis constants and Vₘₐₓ values calculated from intercepts at both axes of Figs. 1 and 2

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Michaelis constants</th>
<th>Vₘₐₓ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td>umoles/hr/mg proteina</td>
</tr>
<tr>
<td>Stearate</td>
<td>3.57</td>
<td>7.30</td>
</tr>
<tr>
<td>Palmitate</td>
<td>1.25</td>
<td>13.33</td>
</tr>
<tr>
<td>Myristate</td>
<td>2.33</td>
<td>14.20</td>
</tr>
<tr>
<td>Laurate</td>
<td>4.55</td>
<td>14.71</td>
</tr>
<tr>
<td>Decanoate</td>
<td>7.69</td>
<td>5.88</td>
</tr>
<tr>
<td>Octanoate</td>
<td>11.10</td>
<td>1.00</td>
</tr>
<tr>
<td>Hexanoate</td>
<td>20.80</td>
<td>0.045</td>
</tr>
</tbody>
</table>

a The kinetics of hexanoate activation has not been graphically elaborated.

In order to evaluate further the hypothesis of two different activating enzymes, the inhibition of the activation of a medium chain fatty acid by a long chain fatty acid was studied. Because of the possible overlapping in the specificities of the hypothetical enzymes, we selected to study the inhibition of octanoate activation by palmitate. The results (Fig. 2) show a clear example of competitive inhibition with a Kᵢ of 5.71 μM. This is compatible with the results of Aas (5), who could not obtain inhibition effects to support the hypothesis of two activating enzymes (4).

The existence of four different ATP-dependent acyl-CoA synthetases is generally admitted (4): first, acetyl-CoA synthetase (10) which reacts mainly on acetate; second, butyryl-CoA synthetase which catalyzes the activation of propionate to heptanoate (11); third, heptanoyl-CoA synthetase which has a substrate spectrum ranging from butyrate to dodecanoate (12); fourth, long chain acyl-CoA synthetase which reacts with higher fatty acids with a chain length up to C₁₈ (1). Because several authors have reported a peak of activity of the latter enzyme for fatty acids with chain length of C₁₀ to C₂₀ (1, 5, 13, 14), the existence of another long chain acyl-CoA synthetase specific for dodecanoate has been proposed (4, 5). However the results of other works are not in agreement with this hypothesis (2, 3). Also the values of Kᵢ and Vₘₐₓ as well as the competitive inhibition exerted by palmitate on octanoate activation obtained in the present study do not support the hypothesis of a second long chain acyl-CoA synthetase specific for dodecanoate in rat liver microsomes. The most likely explanation for this contradiction resides in the difference in the methods used. The former studies which had been done used various methods—hydroxylamine trapping, measure of CoA-SH disappearance, or enzymatic conversion to acyl-carnitines—which all have a common denominator; the substrates were assayed in the millimolar range, whereas in the present study the substrates were assayed in the micromolar range. It has been shown previously (15) that both fatty acids and acyl-CoA are inhibitors of long chain acyl-CoA synthetase when these compounds are present in the 1 mM range and in the 0.1 mM range, respectively. Such inhibitory effects have been attributed to the nonspecific detergent properties of these compounds. In addition, when fatty acids are used in the millimolar range of concentration for acyl-CoA synthetase assay, substrates (8) and most likely products (9) of the reaction are present above their critical micelle concentrations. Because the accumulation of free fatty acids inside the liver cell has never been demonstrated, it is probable that the micellar form is not the natural or preferred form of substrates for acyl-CoA synthetase. For these reasons, we think that the results obtained in the present report with very low concentrations of substrates are the most reliable and the closest to in vivo conditions.

In conclusion, we have presented evidence that there is only one long chain acyl-CoA synthetase in rat liver microsomes and that this enzyme has an optimum affinity for saturated acids with a chain length ranging from C₆ to C₁₈. Therefore, long chain acyl-CoA synthetase in microsomes activates both saturated and unsaturated fatty acids (7); it has a broad chain length specificity and a definite specificity for the position of double bonds in fatty acids (6, 7).

REFERENCES
Kinetic Studies on the Chain Length Specificity of Long Chain Acyl Coenzyme A Synthetase from Rat Liver Microsomes
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