Purification and Properties of a Pig Heart Thiolase with Broad Chain Length Specificity and Comparison of Thiolases from Pig Heart and Escherichia coli*

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HAROLD STAACK, JUDITH F. BINSTOCK, AND HORST SCHULZ†
From the Department of Chemistry, City College of the City University of New York, New York, New York 10031

A thiolase (acetyl CoA acyltransferase, EC 2.3.1.16) which acts on substrates of various chain lengths (thiolase I) has been purified from pig heart muscle 366-fold to near homogeneity as judged by gel electrophoresis. Its molecular weight was estimated to be 200,000 in the absence and 46,000 in the presence of sodium dodecyl sulfate. Kinetic measurements with acetocacetyl coenzyme A, 3-ketohexanoyl-CoA, 3-ketoctanoyl-CoA, and 3-ketodecanoyl-CoA yielded apparent $K_m$ values of 16, 8.3, 2.4, and 1.8 $\mu$M, respectively, whereas apparent $V_{max}$ values of 65 to 69 $\mu$mol/min/mg were obtained with all substrates except for acetacetyl-CoA, with which a value of 28.5 $\mu$mol/min/mg was observed. Antibodies prepared against this thiolase were used to demonstrate that thiolase I and acetacetyl-CoA thiolase (thiolase II) from pig heart mitochondria are immunologically unrelated. The antibodies cross-reacted, however, with thiolase I from beef heart.

Kinetic constants ($K_m$, $V_{max}$) were also determined for thiolases I and II from Escherichia coli, as were the native and subunit molecular weights of $E. coli$ thiolase II. Although the $E. coli$ thiolases were found to be immunologically distinct from the pig heart enzymes, their physical and kinetic properties are strikingly similar to those of the heart thiolases. In view of this finding and in view of the known physiological functions of the $E. coli$ thiolases, it is proposed that thiolase I from pig heart is only involved in fatty acid metabolism, whereas thiolase II functions solely in ketone body degradation.

Thiolases (acetyl-CoA acyltransferase, EC 2.3.1.9 and acetyl-CoA acyltransferase, EC 2.3.1.16) are ubiquitous enzymes which occur in multiple forms within one tissue. Mammalian liver, for example, contains three types of thiolases which differ in intracellular location or substrate specificity (or both) (1). In the cytoplasm of liver, an acetacetyl coenzyme A-specific thiolase is present which is believed to function in cholesterol biosynthesis (1-3), whereas in heart, which ordinarily does not synthesize cholesterol, this enzyme was not observed (1). In mitochondria, two types of thiolases have been identified, one (thiolase I) which acts on substrates of various chain lengths and which therefore must be involved in $\beta$ oxidation, and an acetacetyl-CoA-specific thiolase (thiolase II) which may function in ketone body metabolism (1).

As part of our study aimed at elucidating the regulation of fatty acid metabolism in heart, we have purified and studied the physical as well as kinetic properties of thiolase I from pig heart. Antibodies raised against this thiolase were used to evaluate the immunological relationships of various thiolases. Additionally, the two functionally well-defined thiolases from $E. coli$ were studied for the purpose of comparing their properties with those of the pig heart thiolases and with the aim of thereby obtaining further evidence concerning the physiological roles of the latter two enzymes.

**EXPERIMENTAL PROCEDURES**

**Materials**—Fresh pig hearts were purchased from Max Insel Cohen Co., N. J. New Zealand white rabbits were bought from Marland Breeding Farms, N. J. Immunodiffusion plates and Freund's complete adjuvant were obtained from Cappel Laboratories. Crotonase was prepared from beef liver by following the procedure of Steinman and Hill (4) and thiolases I and II were purified from $E. coli$ B cells as previously described (5). All other enzymes, except for 3-hydroxyacyl-CoA dehydrogenase, which was purchased from Boehringer Mannheim, were obtained from Sigma Chemical Co. The sources, respectively, of the syntheses of trans-$\Delta^2$-enoic acids have been previously described (6). The CoA derivatives of the trans-$\Delta^2$-enoic acids were prepared by the mixed anhydride method of Goldman and Vagelos (7) as previously described (6). $E. coli$ was synthesized according to the method of Seubert (8), while all other 3-ketocacyl-CoA compounds were prepared enzymatically as described by Seubert et al. (9).

**Protein and Enzyme Determinations**—Protein concentrations were determined by the method of Lowry et al. (10). Thiolase activities were routinely measured as described by Lynen and Ochoa (11). Since the extinction coefficients of the $Mg^2+$-3 ketocacyl-CoA complexes are dependent on pH, the 3-ketocacyl-CoA, and $Mg^2+$ concentrations, all routine assays were performed in Tris/HCl (pH 8.3) in the presence of 25 mM MgCl$_2$ and 10 $\mu$M 3-ketocacyl-CoA (32 $\mu$M in the case of acetocacetyl-CoA). The extinction coefficients under these conditions were determined by measuring the total change of absorbance at 308 nm in the presence of 70 $\mu$M CoASH and thiolase (5 milliunits/0.6 ml) and by measuring the concentration of the 3-ketocacyl-CoA substrates with 3-hydroxyacyl-CoA dehydrogenase (12 milliunits/0.6 ml) as previously described (5). The molar extinction coefficients ($\varepsilon$) thus obtained were: acetocacetyl-CoA, $21,400 \text{ M}^{-1}$,

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† To whom correspondence should be addressed.
Purification of Thiolase with Broad Chain Length Specificity (Thiolase I) and Acetoacetyl-CoA Thiolase (Thiolase II) from Pig Heart—All operations were performed at 4°C. Fresh pig heart (579 g) was minced with a meat grinder and homogenized together with 2.5 liters of 0.05 M potassium phosphate (pH 6.6) containing 5% glycerol and 10 mM mercaptoethanol. The homogenate was centrifuged at 11,900 × g for 15 min. The supernatant was then applied to a phosphocellulose column (5 × 50 cm) which had been extensively dialyzed against saline solution containing 20 mM sodium phosphate (pH 7.2) and had been emulsified with sodium dodecyl sulfate (13) to be 50%. No significant loss of activity was observed.

Table I

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Purification</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>20.24</td>
<td>3300</td>
<td>0.163</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Phosphocellulose (batch)</td>
<td>1.468</td>
<td>2935</td>
<td>2.0</td>
<td>12</td>
<td>89</td>
</tr>
<tr>
<td>Phosphocellulose (gradient)</td>
<td>0.110</td>
<td>1494</td>
<td>12.8</td>
<td>79</td>
<td>45</td>
</tr>
<tr>
<td>CM-cellulose</td>
<td>0.018</td>
<td>1056</td>
<td>59.6</td>
<td>366</td>
<td>32</td>
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</tbody>
</table>
yielded subunit molecular weights for these two thiolases of *Escherichia coli*—Pig Heart and *Escherichia coli*—units as has been established for pig heart thiolase II (16), for these thiolases are composed of four possibly identical subunits with molecular weights of 46,000 and 41,500, respectively. Hence, it is concluded that thiolase I and thiolase II from pig heart and thiolase II, respectively. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (13) yielded subunit molecular weights for these two thiolases of 46,000 and 41,500, respectively. Hence, it is concluded that these thiolases are composed of four possibly identical subunits as has been established for pig heart thiolase II (16), for cytoplasmic thiolases (2, 3), and recently for *E. coli* thiolase II (21).

The chain length specificity of pig heart thiolase I was determined by measuring the rates of the thiolytic cleavage of a number of CoA derivatives of even-numbered 3-keto acids having 4 to 14 carbons. As shown in Fig. 2, the rate observed with acetoacetyl-CoA at 10 μM concentration was approximately one-fifth of those measured with longer chain substrates for all of which nearly equal rates were determined. The decrease in rate seen with 3-ketotetradecanoyl-CoA is most likely due to its nonspecific inhibition of thiolase I because this inhibition was found to be dependent on the bovine serum albumin concentration in the assay mixture. In order to further analyze the rate difference between the cleavage of acetoacetyl-CoA and those of longer chain substrates, the apparent $K_m$ and $V_{max}$ values for several substrates were determined. Since the thiolase assay is based on the disappearance of the Mg$^{2+}$-acetoacetyl-CoA complex, whose extinction coefficient ($ε$) is a function of the 3-ketoacyl-CoA concentration, values of $ε$ were determined at various substrate concentrations as described under "Experimental Procedures." The results obtained with 3-ketohexanoyl-CoA are shown in the inset of Fig. 3. An increase in the substrate concentration from approximately 4 to 40 μM resulted in a decrease of $ε$ from 18 to 12 mmol$^{-1}$ cm$^{-1}$. Only in the case of the Mg$^{2+}$-acetoacetyl-CoA complex did the extinction coefficient not change dramatically. When rates, measured in the presence of various concentrations of 3-ketohexanoyl-CoA, were corrected for the concentration dependence of the extinction coefficient and were plotted according to Lineweaver and Burk, they fell on a straight line (see Fig. 3). However, when the values were calculated using a constant extinction coefficient, a complex result was obtained which could be taken as evidence for substrate inhibition (see Fig. 3). The $K_m$ and $V_{max}$...
values obtained by the above outlined approach and in the presence of a saturating level of CoA (70 μM) are listed in Table II. Kinetic parameters for longer chain substrates then listed were not determined because these compounds severely inhibited the enzyme. For comparative purposes, several kinetic constants were determined with pig heart thiolase II and with thiolases I and II from E. coli (see Table III).

Immunological Studies – Antibodies prepared against purified pig heart thiolase I were used to study the structural relationships between the two thiolases of pig heart and between E. coli and pig heart thiolases. Since antibodies to pig heart thiolase I inhibited this enzyme maximally only 60%, it was necessary to remove the thiolase-antibody complex by either ultrafiltration or centrifugation before determining the remaining activity. The results of such an experiment with purified thiolase I are shown in Fig. 4. The smooth immunotitration curve obtained is indicative of the specific and complete precipitation of thiolase I. This experiment also demonstrates that the antibodies to thiolase I did not cross-react with pig heart thiolase II even at 2500 times higher concentration than required to achieve 50% inhibition of thiolase I. This finding leads to the conclusion that the two mitochondrial thiolases differ greatly in their structures. Not surprising is the observation that antibodies prepared against E. coli thiolase II did not react with either of the two pig heart thiolases. The above mentioned conclusions were confirmed by double immunodiffusion experiments (data not shown) according to Ouchterlony (23). Antibodies prepared against pig heart thiolase I were found to cross-react with thiolase I from beef heart (data not shown), a finding which conforms to

TABLE II

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Apparent $K_a$, μM</th>
<th>Apparent $V_{max}$, units/mg</th>
<th>Relative $V_{max}$</th>
</tr>
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<tbody>
<tr>
<td>Acetoacetyl-CoA</td>
<td>17</td>
<td>29</td>
<td>1.0</td>
</tr>
<tr>
<td>3-Ketohexanoyl-CoA</td>
<td>8.3</td>
<td>69</td>
<td>2.4</td>
</tr>
<tr>
<td>3-Ketoctanoyl-CoA</td>
<td>2.4</td>
<td>65</td>
<td>2.2</td>
</tr>
<tr>
<td>3-Ketodecanoyl-CoA</td>
<td>1.8</td>
<td>67</td>
<td>2.3</td>
</tr>
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</table>

TABLE III

<table>
<thead>
<tr>
<th>Chain length specificity</th>
<th>Activity ratio</th>
<th>$K_a$, μM</th>
<th>Molecular weight</th>
<th>Physiological function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig heart thiolase I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁₂-C₁₅</td>
<td>1:6</td>
<td>C₁₂-CoA 17.3</td>
<td>7.6 (4 x 46,000)</td>
<td>Fatty acid oxidation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CoASH 8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pig heart thiolase II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁₀</td>
<td></td>
<td>C₁₀-CoA 13.0</td>
<td>7.7</td>
<td>Ketone body degradation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CoASH 8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli thiolase I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁₂-C₁₅</td>
<td>1:20³</td>
<td>C₁₂-CoA 31</td>
<td>4.1 (4 x 41,000)</td>
<td>Fatty acid oxidation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C₁₂-CoA -2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CoASH 7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli thiolase II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁₀</td>
<td></td>
<td>C₁₀-CoA 7.5</td>
<td>7.7</td>
<td>Acetoacetyl degradation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CoASH 7.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

³ See Ref. 16.
³ See Ref. 5.
³ See Ref. 22.

The pig heart thiolase which acts on substrates of various chain lengths (thiolase I) is the second mammalian thiolase of its type to be purified to near homogeneity and it is the first of its type to be studied with respect to its native and subunit molecular weights and with respect to its kinetic parameters with longer chain substrates. Since the acetoacetyl-CoA-specific thiolase (thiolase II) from pig heart has previously been purified and studied (15-17), this report completes the in depth investigation of the mitochondrial thiolases present in one mammalian organ. Of great importance to a full understanding of the regulation of β oxidation is the possible cooperation of thiolases I and II which, if effective, would assure a high rate of degradation of all 3-ketodecanoyl-CoA compounds formed during fatty acid oxidation. Although no definite evidence for or against this cooperation is available, a comparison of the Escherichia coli fatty acid oxidation enzymes with those found in animals might provide further insight into this problem. Most important in this context is the recent demonstration

Fig. 4. Immunotitrations of thiolases I and II from pig heart. Antibody and thiolase solutions were combined and kept for 1 min at 25°. After adding 0.25 ml of 0.3 M Tris/HCl (pH 8.3) containing 75 mM MgCl₂ and 0.67 ml of 0.1 M KCl, the solutions were filtered through a Millipore filter (0.45 μ) and assayed for remaining thiolase activity. The immunotitration curves shown are; A, pig heart thiolase I (0.26 μg) with antibodies to pig heart thiolase I; B, pig heart thiolase I (0.26 μg) with antibodies to Escherichia coli thiolase II (no inhibition was observed in the presence of up to 685 μg of antibodies (data not shown)); C, pig heart thiolase II (75 ng) with antibodies to pig heart thiolase I (no inhibition was observed in the presence of up to 376 μg of antibodies (data not shown)); D, pig heart thiolase II (75 ng) with antibodies to E. coli thiolase II (no inhibition was observed in the presence of up to 1.37 mg of antibodies (data not shown)).

DISCUSSION

The pig heart thiolase which acts on substrates of various chain lengths (thiolase I) is the second mammalian thiolase of its type to be purified to near homogeneity and it is the first of its type to be studied with respect to its native and subunit molecular weights and with respect to its kinetic parameters with longer chain substrates. Since the acetoacetyl-CoA-specific thiolase (thiolase II) from pig heart has previously been purified and studied (15-17), this report completes the in depth investigation of the mitochondrial thiolases present in one mammalian organ. Of great importance to a full understanding of the regulation of β oxidation is the possible cooperation of thiolases I and II which, if effective, would assure a high rate of degradation of all 3-ketodecanoyl-CoA compounds formed during fatty acid oxidation. Although no definite evidence for or against this cooperation is available, a comparison of the Escherichia coli fatty acid oxidation enzymes with those found in animals might provide further insight into this problem. Most important in this context is the recent demonstration

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that the *E. coli* fatty acid oxidation system exists as a multienzyme complex which includes thiolase, 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase (22). Although it has been suggested that the enzymes of fatty acid oxidation exist in mitochondria in a highly organized arrangement (24), *E. coli* is the first organism in which this was shown to be the case. This finding adds weight to the contention that a similar situation may exist in mitochondria except that the enzymes would be more loosely bound to one another. Since thiolase II of *E. coli* was shown to be a separate enzyme (51, it is suggested that also in mitochondria thiolase II is not part of the putative multienzyme arrangement of \( \beta \) oxidation. Further suggestive evidence concerning the physiological functions of thiolases I and II of heart is derived from a comparison of *E. coli* and pig heart thiolases. A compilation of properties of both thiolases from *E. coli* and pig heart is presented in Table III. Clearly, the two thiolases I are strikingly similar in that they both act on substrates of various chain lengths and both act more efficiently on longer chain substrates than on acetoacetyl-CoA. Additionally, their kinetic parameters are very similar, specifically the low \( K_m \) values for the longer chain substrates. Since the *E. coli* thiolase I is part of a multienzyme complex, its true molecular weight and subunit structure are still unknown. Also, the two thiolases II are nearly identical with respect to the listed properties except for their \( K_m \) values for CoASH which differ by a factor of 7. It is thus concluded that the two types of thiolases present in heart mitochondria and *E. coli*, which are strikingly similar, may have identical functions. Since thiolase I of pig heart mitochondria is the sole thiolase required for \( \beta \) oxidation of fatty acids, whereas thiolase II functions only in ketone body degradation.

**REFERENCES**

Purification and properties of a pig heart thiolase with broad chain length specificity and comparison of thiolases from pig heart and Escherichia coli.
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