Properties of Partially Purified Carnitine Acetyltransferase*

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Friedman and Fraenkel (1) demonstrated that extracts of pigeon and sheep livers contain an enzyme, carnitine acetyltransferase (EC 2.3.1.7), which catalyzes the reaction

\[ \text{Acetylcarnitine} + \text{CoA} \rightarrow \text{acetyl-CoA} + \text{carnitine} \]

Subsequent reports from our laboratory showed that both pigeon and sheep livers contain an enzyme, carnitine acetyltransferase (EC 2.3.1.7), which catalyzes the reaction

\[ \text{Acetylcarnitine} + \text{CoA} \rightarrow \text{acetyl-CoA} + \text{carnitine} \]

Acetylcarnitine formation in the absence of added carnitine had no such effect (3). Further, acetylcarnitine transferases might be directly involved in mediating the metabolic effects of carnitine on fatty acid metabolism has been discussed in previous publications (3-5). In the present investigation we shall describe the properties of carnitine acetyltransferase and will give results demonstrating that the O-acyl structure of acetylcarnitine derivatives has a high group potential.

**EXPERIMENTAL PROCEDURE**

**Assay Procedures**

**Hydroxamate Formation at pH 7.4**—In crude preparations, which were unsuitable for spectrophotometric assays to be described, the hydroxamate procedure suggested by Friedman and Fraenkel (1) was employed. The assay takes advantage of the quantitative conversion of acetyl-CoA to hydroxymalonate at pH 7.4, whereas acetylcarnitine reacts with hydroxylamine to only a slight extent at this pH. The amount of hydroxamate formed nonenzymatically from acetylcarnitine was variable, depending upon pH, initial acetylcarnitine concentration, and time of incubation at 35°. When this assay was used, enzyme units were calculated on the basis of micromoles of hydroxamate formed in 30 minutes at 35° in tubes containing 10.0 μmoles of [U-14C]-acetylcarnitine, 400 μmoles of hydroxylamine at pH 7.4, variable amounts of enzyme preparation, and 0.34 mg of CoA (Pabst, 55% pure) in a total volume of 1.5 ml. Control tubes contained no CoA.

**NADH Production in a Coupled Enzyme System**—After partial purification of the enzyme preparation, it was possible to use a spectrophotometric method dependent upon the rate of generation of acetyl-CoA from acetylcarnitine, in which the citrate-condensing enzyme assay system of Ochoa (6) was employed. Cuvettes contained an excess of malate, malate dehydrogenase (California Corporation for Biochemical Research, porcine heart), NAD (Pabst), crystalline pig heart citrate-condensing enzyme (7), and CoA buffered at pH 8.0 with Tris-HCl. Changes in absorbancy at 340 μm were followed after addition of [O-14C]-acetylcarnitine and the enzyme preparation. It was necessary to add cyanide to inhibit NADH oxidase, which remained in enzyme preparations until final purification steps. Concentrations of all constituents were listed in legends to Table II and Fig. 1. The reaction could be initiated by addition of either CoA, acetylcarnitine, or carnitine acetyltransferase as the final component. Concentrations of acetyl-CoA synthesized from acetate anhydride and CoA (8) were estimated as equivalents of NADH formed when all other components of the coupled citrate-condensing enzyme-malate dehydrogenase assay were in excess.

**Formation of CoA from Acyl-CoA Derivatives**—With purified enzyme preparations, the reaction was followed by determining the rate of appearance of sulphydryl groups from fatty acyl-CoA derivatives according to the procedure of Elman (9). Constituents added to cuvettes are listed in the legend to Fig. 4. The reagent, 5,5'-dithiobis-(2-dinitrobenzoic acid), did not inhibit the initial velocity of the transfer reaction. When carnitine acetyltransferase was preincubated with the above reagent for 15 minutes under conditions cited, enzymatic activity was abolished.

**Changes in Absorbancy at 232 μm**—With purified preparations, the cleavage of the thioester bond of acetyl-CoA or other acyl-CoA derivatives catalyzed by carnitine acetyltransferase in the presence of carnitine was assayed by recording the decrease in absorbancy at 232 μm (10). Alternatively, the formation of thioester bonds was followed by recording the increase in absorbance at 232 μm when acetylcarnitine and CoA were added initially. Spectrophotometric determinations were performed manually with the aid of a Beckman DU spectrophotometer in early experiments, but data reported were obtained primarily
with a Gilford automatic recording attachment to the spectrophotometer.

**Enzyme Extraction and Purification Procedures**

Pig hearts were obtained immediately after slaughter from Peters Sausage Company, Ann Arbor. They were either extracted directly or frozen for subsequent use, since it was found that storage of frozen hearts for several months did not lower enzyme yields.

Approximately 200 g of heart ventricle were trimmed free of visible fat and were cut into thin strips. The weighed muscle was rinsed several times in an excess of ice-cold 0.25 M sucrose containing 0.005 M Versene (sodium EDTA) at pH 7.4, and 50-g portions were homogenized in an ice-jacketed semimicro Monel container. The Waring Blendor motor was run at low speeds by employing a Variac at 60 to 70 volts. The mixture was kept cold by interrupting homogenization at 30-second intervals for 60-second cooling periods. Total homogenization time exclusive of cooling was 5 minutes per 50-g portion. The pooled homogenate in approximately 400 ml of the sucrose-Versene solution was filtered through one layer of cheesecloth at 0 to 5°C and centrifuged at 10,000 × g for 10 minutes. All subsequent steps were performed with reagents maintained at 0-2°C. The residue was extracted by homogenization twice in the Monel blender as described above, with 150 ml of 0.1 M K2HPO4 containing 5 × 10-4 M sodium EDTA for each extraction of the residue. The supernatant fractions were pooled, protein was determined spectrophotometrically (11), and Ca3(PO4)2 gel (12) was added to give a final ratio of 1 mg of gel per mg of protein. After the gel had been left in contact with the protein solution for 30 to 60 minutes with occasional stirring, it was collected by centrifugation, washed once with 150 ml of 0.05 M K2HPO4, and extracted three times by successive addition of 50-, 30-, and 20-ml portions of 0.4 M K2HPO4 buffer at pH 6.5 containing 0.005 M KH2PO4, and then 20 g of solid (NH4)2SO4 were gradually added per 100 ml of solution to give a 35% saturated solution. After about 15 minutes, the precipitate was collected by centrifugation at 10,000 × g for 10 minutes. The supernatant solution was brought to pH 8.0 by the addition of 1 N NaOH, and approximately 40 g more of (NH4)2SO4 per 100 ml of solution were added over a period of hours at 0°C to -5°C with stirring until the solution was saturated. The suspension was allowed to stand for approximately 1 hour at -5°C, and then centrifuged at 18,000 × g for 1 hour. In other preparations, the saturated solution had been allowed to stand overnight, with no perceptible difference in results. The residue was taken up in 25 to 50 ml of 0.01 M KH2PO4 buffer at pH 6.5 containing 0.005 M sodium EDTA. To 30 ml of solution containing approximately 2.5 mg of protein per ml, 7.5 ml of absolute ethanol at -15°C were added very slowly with stirring. The final temperature of the alcoholic solution was -5°C. One hour later, the suspension was centrifuged at 10,000 × g for 20 minutes at -5°C, and the pellet was discarded. Precipitates subsequently formed in the supernatant solutions stored at 0°C to -5°C were discarded without loss of enzymatic activity. Carnitine acetyltransferase in the supernatant solution proved stable for at least 3 months when stored at this temperature.

Enzyme purification up to the ethanol step has been performed for five different preparations, and fairly uniform yields have been obtained with specific activities varying from 0.18 to 0.40 unit per mg of protein; a carnitine acetyltransferase unit is defined as the amount of enzyme required to catalyze the formation of 1 μmol of acetyl-CoA per minute. This represents an average purification of 10- to 25-fold. Ethanol precipitation has given more variable results. In our best of three preparations with this step, the supernatant of the ethanol fraction had a specific activity of 4.6 units per mg, representing a 250-fold purification. In other preparations, however, the comparable specific activity figures were only 0.58 and 0.77. If the alcoholic supernatant fraction contained acetyl-CoA hydrolase, we were able to free it from carnitine acetyltransferase by first removing alcohol by dialysis, reprecipitating the enzyme with 80% saturated (NH4)2SO4, and then repeating the Ca3(PO4)2 gel adsorption and elution procedure described above. All experiments reported with "purified preparations" were performed with enzyme solutions having a specific activity of 0.58 to 4.6 units per milligram.

**Materials and Analytical Procedures**

Acetyl-, propionyl-, butyryl-, pentanoyl-, hexanoyl-, octanoyl-, and decanoyl-CoA were synthesized from their respective anhydrides and CoA. For the synthesis of octanoyl and decanoyl derivatives, equal volumes of tetrahydrofuran and water were used to solubilize the anhydride. After anhydride addition and when no free sulfhydryl groups remained, excess anhydride and free acid were extracted with ether, and tetrahydrofuran was removed under vacuum. Acyl CoA concentration was approximated by determining absorbancy at 232 and 260 μm (13). A more accurate analysis was performed by measuring CoA released in the coupled carnitine acetyltransferase reaction described above. Protein was estimated spectrophotometrically as described by Layne (11). Carnitine was generously supplied by International Minerals and Chemical Corporation, Skokie, Illinois, and carnitine derivatives were obtained from sources previously reported (3). Norcarnitine and its derivatives were gifts from Riker Laboratories, Inc., Northridge, California.

**RESULTS**

**Purification of Enzyme and Properties of Assay System**

Table I shows the results obtained during a typical purification procedure. It is apparent that carnitine acetyltransferase was not soluble in the sucrose fraction under conditions of homogenization described in "Experimental Procedure," but that the transferase could readily be extracted with K2HPO4. The final enzyme preparation was free of acetyl-CoA hydrolase and acetyl-carnitine hydrolase. Both these substrates could be recovered quantitatively when incubated with carnitine acetyltransferase for 30 minutes at 35°C in the absence of carnitine or CoA.

An outline of the reactions of the coupled enzyme system employed is presented in Table II, together with data demonstrating the dependency of NADH production on components listed. Since NADH formation did not occur in the citrate-condensing enzyme assay in the absence of CoA, it appears that acetylcarnitine was unable to condense directly with oxaloacetate. Acetyl-CoA formation from acetylcarnitine was necessary to permit the reaction sequence to proceed. Purified preparations were nearly free of NADH oxidase, but still retained citrate-condensing enzyme and malate dehydrogenase.

When all components except acetylcarnitine were present in excess, the assay could be used to determine acetylcarnitine...
TABLE I
Partial purification of carnitine acetyltransferase from pig heart ventricle

Pig heart ventricle was extracted and purified as described in "Experimental Procedure." Acetyl-CoA generation in all assays except in the initial homogenate was estimated by assuming that 1.0 μmole of NADH was formed for every micromole of acetyl-CoA presented to the malate dehydrogenase-citrate-condensing enzyme assay system (6). NADH was measured according to the method of Friedman and Fraenkel (1). For details, see the text.

<table>
<thead>
<tr>
<th>Fraction and procedure</th>
<th>Protein</th>
<th>Total yield</th>
<th>Approx. activity</th>
<th>Specific activity</th>
<th>Approx. purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole homogenate (300 g/400 ml)</td>
<td>14,350</td>
<td>(217) 100 (0.014)</td>
<td>1.30</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Succrose supernatant</td>
<td>2,750</td>
<td>0 0 0</td>
<td>0</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>K2HPO4 supernatant</td>
<td>2,100</td>
<td>157 72 0.075</td>
<td>5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elution from Ca3(PO4)2 gel with 0.4 M K2HPO4</td>
<td>875</td>
<td>150 69 0.18</td>
<td>13.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35% saturation with (NH4)2SO4; precipitate discarded</td>
<td>250</td>
<td>98 45 0.40</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NH4)2SO4; precipitate taken up in 0.01 M KH2PO4 at pH 6.5</td>
<td>250</td>
<td>98 45 0.40</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant after treatment with 20% ethanol</td>
<td>99 76 35 0.77</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After dialysis; 90% saturation with (NH4)2SO4; precipitate taken up in 0.01 M KH2PO4 at pH 6.5</td>
<td>38 52 24 1.40</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

concentrations (Fig. 1). Approximately 0.8 μmole of NADH was formed per 2.0 μmole of dl-acetylcarnitine added, suggesting that only one-half of the added substrate was acceptable to the enzyme. It is likely that (+)-acetylcarnitine is an unsuitable substrate, since (-)-carnitine is the natural isomer in muscle (14). Further, the acetyl group of (-)-acetylcarnitine was completely oxidized by mitochondrial preparations investigated by Bremer (5), whereas only one-half of the dl-derivative was utilized.

Initial velocities in this coupled reaction sequence have been plotted against acetylcarnitine concentrations in a Lineweaver and Burk graph (15). Under these conditions, the apparent \( K_m \) is 6.2 \( \times 10^{-4} \) M for dl-acetylcarnitine, and the \( K_m \) for (-)-acetylcarnitine may be estimated at 3.1 \( \times 10^{-4} \) M if the (++)-isomer proves to be enzymatically active (Fig. 2).

Estimation of Equilibrium Constant

The equilibrium constant has been estimated by following the disappearance or appearance of thioester bonds spectrophotometrically at 232 μm (10), with a \( \epsilon_m \) of 4.5 \( \times 10^{4} \) M⁻¹ cm⁻¹ for acetyl-CoA at this wave length (13). Data shown from a single experiment in Fig. 3 and results from eight experiments summarized in Table III indicate that the apparent equilibrium constant at pH 7.0 was approximately 0.6 toward acetyl-CoA formation when the initial reactants were either DL-acetylcarnitine and CoA or acetyl-CoA and DL-carnitine. For these calculations, it was assumed that only the (-)-isomers were enzymatically active and that the reactants and products were exclusively those cited in Table II for the carnitine acetyltransferase reaction. In the experiment shown in Fig. 3, acetyl-CoA and carnitine were initially incubated with carnitine acetyltransferase. After no further change in absorbancy occurred, acetylcarnitine and CoA were added at separate times. Calculations to determine the apparent equilibrium constant were made by assuming that acetylcarnitine formed initially was equal to acetyl-CoA which disappeared. By substitution into the equation,

\[
K_{eq} = \frac{(\text{acetyl-CoA})(\text{carnitine})}{(\text{CoA})(\text{acetylcarnitine})}
\]

the values obtained in the experiment cited in Fig. 3 were 0.55 in the first phase, in which there was a decrease in absorbancy of 0.129, corresponding to a disappearance of 0.0287 μmole of acetyl-CoA; 0.50 after acetylcarnitine addition, in which there was an increased absorbancy of 0.031, corresponding to an increase of 0.007 μmole of acetyl-CoA; and 0.44 after CoA addition, in which the absorbancy increased by 0.054, corresponding to an increase of 0.012 μmole of acetyl-CoA. This experiment is the same as Experiment 3, Series B, of Table III.

<table>
<thead>
<tr>
<th>Components added</th>
<th>NADH formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>All minus carnitine acetyltransferase</td>
<td>0</td>
</tr>
<tr>
<td>All (0.005 mg of carnitine acetyltransferase)</td>
<td>16</td>
</tr>
<tr>
<td>All (0.010 mg of carnitine acetyltransferase)</td>
<td>32</td>
</tr>
<tr>
<td>Minus acetylcarnitine</td>
<td>0</td>
</tr>
<tr>
<td>Minus CoA</td>
<td>0</td>
</tr>
<tr>
<td>Minus malate</td>
<td>0</td>
</tr>
<tr>
<td>Minus citrate-condensing enzyme</td>
<td>9</td>
</tr>
<tr>
<td>Minus malate dehydrogenase</td>
<td>15</td>
</tr>
</tbody>
</table>
Fig. 1. Total NADH formed in the coupled carnitine acetyltransferase-malate dehydrogenase-citrate-condensing enzyme system at various acetylcarnitine concentrations. Cuvettes contained all components listed in the legend to Table II at identical concentrations, except that amounts of D,L-acetylcarnitine added were varied as indicated, and 0.04 mg of carnitine acetyltransferase of specific activity 4.6 was present in each cuvette. The reaction was initiated by acetylcarnitine addition, and absorbancy at 340 nm was recorded until no further changes occurred. For samples containing more than 0.25 µmole of D,L-acetylcarnitine per ml, absorbancy at 340 nm was determined in aliquots which had been diluted 1:10 after incubation for 30 minutes at 35°C, by which time the reaction had gone to completion.

Final concentrations of acetylcarnitine and acetyl-CoA in these cuvettes were determined by an independent method. Acetyl-CoA and carnitine were incubated at higher concentrations with carnitine acetyltransferase as indicated above. Cuvettes were then heated at 70°C for 1 minute to inactivate carnitine acetyltransferase, after which acetyl-CoA was measured with the citrate-condensing enzyme (6). After the analysis was completed, carnitine acetyltransferase and CoA were added to the same cuvette for determination of acetylcarnitine by methods shown in Fig. 1. Equilibrium constant values calculated were in fair agreement with other determinations (see Experiments 8 of Series A and B, Table III).

Substrate Specificity

Fatty Acyl-CoA Derivatives—Under conditions shown in Table IV, the acyl groups of acetyl-CoA, propionyl-CoA, and butyryl-CoA were transferred to carnitine at approximately the same rates, but the transfer of longer chain acyl groups proceeded at reduced velocities. We have no evidence concerning the possibility that more than one short chain carnitine acyltransferase exists. The rate of release of CoA from acyl-CoA derivatives when a different amount of enzyme was used is plotted in Fig. 4. The acyl group of palmitoyl-CoA was not released by carnitine acetyltransferase, nor did the preparation contain a palmitoyl-CoA hydrolase. After reactions shown in Fig. 4 and Table IV had apparently stopped, addition of more enzyme resulted in further CoA release from hexanoyl-, octanoyl-, and decanoyl-CoA, but in no further CoA release when acetyl-, propionyl-, or butyryl-CoA was substrate. This probably occurred because carnitine acetyltransferase was slowly inactivated by interaction with the reagent, 5,5′-dithiobis(2-nitrobenzoic acid), used in the sulfhydryl group analysis (9). It is possible, however, to determine concentrations of specific acyl-CoA derivatives by measurement of sulfhydryl released when sufficient amounts of enzyme are added.

Reversible transfer of acyl groups from other acyl-CoA derivatives to carnitine was also followed by the 232 nm assay, and similar results were obtained in that the rate of transfer was slower for longer chain acyl groups. At each enzyme concentration tried, the initial velocity for short chain length acyl transfer, i.e. acetyl, propionyl, and butyryl, was greater than that observed for transfer of longer chain length acyl groups. Acyl-CoA derivatives incubated in the presence of carnitine acetyltransferase did not release CoA unless carnitine was added to the system, demonstrating that the enzyme preparation did not contain acyl-CoA hydrolase.

Carnitine and Derivatives—CoA liberation from acetyl-CoA incubated with carnitine acetyltransferase was proportional to carnitine added to the system (Fig. 5). The only carnitine

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**Fig. 2.** Determination of the apparent $K_a$ for acetylcarnitine in the coupled carnitine acetyltransferase-malate dehydrogenase-citrate-condensing enzyme system. Cuvettes contained all components listed in the legend to Table II at identical concentrations, except that concentrations of D,L-acetylcarnitine were varied as indicated, and 0.04 mg of carnitine acetyltransferase of specific activity 4.6 was present in each cuvette. The reaction was initiated by acetylcarnitine addition, and absorbancy change at 340 nm was recorded. The rate of NADH formation was linear for at least 1 minute after addition of 0.2 or more µmole of D,L-acetylcarnitine.
Fig. 3. Transfer of acetyl group from acetyl-CoA to carnitine. Cuvettes initially contained 0.05 μmole of acetyl-CoA, 100 μmoles of Tris-HCl buffered at pH 7.0, 0.17 mg of carnitine acetyltransferase of specific activity 0.58, and water to make a total volume of 0.95 ml. At zero time (arrow labeled C), 0.05 ml of water was added to the blank and to Cuvette 1 (top curve), and 0.1 μmole of dl-carnitine in 0.05 ml of water at pH 7.0 was added to Cuvette 2 (bottom curve). When no further change in absorbancy was observed (18 minutes, arrow labeled AC), 0.1 μmole of dl-acetyl-carnitine at pH 7.0 (0.05 ml) was added to Cuvettes 1 and 2 and 0.05 ml of water alone was added to the blank. At 28 minutes no further absorbancy changes were seen, and 0.05 pmole of CoA was added to all cuvettes (arrow labeled CoA). Absorbancy changes in each sample were recorded consecutively for 1-second intervals at 232 mm. Curves are corrected for changes in the absorbancy in the blank and for those due to volume changes. The temperature of the contents of the cuvettes was held constant at 35°.

Derivative tried which was also able to accept transfer of the acetyl group from acetyl-CoA was "norcarnitine," (p-hydroxy-y-dimethylaminobutyrate). As measured by the 232 mm assay system at pH 7, the equilibrium of the reaction,

\[ \text{Acetyl-CoA} + \text{norcarnitine} \rightleftharpoons \text{acetylnorcarnitine} + \text{CoA} \]

was the same as that reported in Table III for acetyl-CoA and carnitine, but the velocity was reduced.

Compounds tested which were unable to substitute for carnitine in accepting transfer of the acetyl group from acetyl-CoA included deoxycarnitine (γ-trimethylammonium butyrate), dl-carnitine nitrile (l-cyano-2-hydroxy-3-trimethylammonium propane), dl-β-hydroxy-γ-aminobutyrate, the ethyl ester of dl-norcarnitine, dl-norcaritinamide (β-hydroxy-γ-dimethylaminobutyramide), dl-norcarnitol (β-hydroxy-γ-dimethylaminobutanol), and choline. Concentrations of compounds examined were 0.5 pmole per ml for choline and deoxycarnitine, 1.0 μmole per ml for carnitine nitrile, and 0.2 pmole per ml for the remaining derivatives (Fig. 5).

Acetyl-CoA was formed from CoA in the presence of carnitine acetyltransferase and acetylcarnitine or acetylnorcarnitine, as estimated by following the increase in absorbancy in the 232 mm assay system. Acetylcholine was unable to transfer its acetyl group to CoA under these conditions. Neither glutathione, cysteine, nor mercaptoethanol could substitute for CoA.

In experiments shown in Fig. 5, the amount of sulfhydryl released from acetyl-CoA in the presence of carnitine acetyltransferase was proportional to carnitine concentration, thereby providing an enzymological assay for carnitine analysis. Deoxycarnitine at concentrations of 0.5 μmole per ml did not alter the rate or amount of CoA released under conditions described in Fig. 5, but 5 and 50 pmole of deoxycarnitine per ml inhibited the transfer of acetyl group from acetyl-CoA.

Table III

<table>
<thead>
<tr>
<th>Initial reactants</th>
<th>Experiment</th>
<th>( K'_{eq} )</th>
<th>Initial reactants</th>
<th>Experiment</th>
<th>( K'_{eq} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series A</td>
<td></td>
<td></td>
<td>Series B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoA (0.05 μmole) + dl-acetylcarnitine (0.1 μmole)</td>
<td>1</td>
<td>0.92</td>
<td>Acetyl-CoA (0.05 μmole) + dl-carnitine (0.1 μmole)</td>
<td>1</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.48</td>
<td></td>
<td>2</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.66</td>
<td></td>
<td>3</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.47</td>
<td></td>
<td>4</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.98</td>
<td></td>
<td>5</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.46</td>
<td></td>
<td>6</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.49</td>
<td></td>
<td>7</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>8*</td>
<td>0.49 (0.40)</td>
<td></td>
<td>8*</td>
<td>0.67 (0.42)</td>
</tr>
<tr>
<td>Average</td>
<td>0.69 ± 0.077†</td>
<td></td>
<td>Average</td>
<td>0.61 ± 0.060†</td>
<td></td>
</tr>
</tbody>
</table>

* In these experiments, 0.2 μmole of dl-acetylcarnitine and 0.1 μmole of CoA (Series A) or 0.2 μmole of dl-carnitine and 0.1 μmole of acetyl-CoA (Series B) were present at zero time. The numbers in parentheses are explained in the legend above.
† Mean ± standard error of the mean.

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The long chain carnitine acyltransferase is not yet sufficiently pure to permit characterization of its properties. It will be of interest to determine whether the substrate specificity toward carnitine and its derivatives is the same as that reported here for carnitine acetyltransferase, since an identical specificity is required to obtain stimulation of long chain fatty acid oxidation by heart muscle preparations (8). If this is the case, it will offer additional support to recent suggestions that acylcarnitine derivatives are involved in processes by which carnitine catalytically enhances oxidation of fatty acids by several tissues (2-4). Evidence indicating that carnitine may serve as a shuttle for the transport of long chain acyl groups to the fatty acid oxidase site from extramitochondrial portions of the cell has been presented both by Bremer (19) and by us (17, 18), and has recently been reviewed (20).

A function similar to that formulated for long chain carnitine acyltransferase (18) may be postulated for carnitine acetyltransferase, in that the enzyme whose properties have been reported here could mediate the transfer of acetyl groups from mitochondria to sites of fatty acid synthesis in the cytoplasm. Although this hypothesis remains to be tested, there is ample evidence

<table>
<thead>
<tr>
<th>Substrate</th>
<th>CoA released in initial 30 seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-CoA</td>
<td>30</td>
</tr>
<tr>
<td>Propionyl-CoA</td>
<td>37</td>
</tr>
<tr>
<td>Butyryl-CoA</td>
<td>30</td>
</tr>
<tr>
<td>Pentanoyl-CoA</td>
<td>11</td>
</tr>
<tr>
<td>Hexanoyl-CoA</td>
<td>7</td>
</tr>
<tr>
<td>Octanoyl-CoA</td>
<td>4</td>
</tr>
<tr>
<td>Decanoyl-CoA</td>
<td>1</td>
</tr>
<tr>
<td>Palmitoyl-CoA*</td>
<td>0</td>
</tr>
</tbody>
</table>

* Palmitoyl-CoA was synthesized by the method of Seubert (16).

The decrease in absorbancy at 232 μm at various hydrogen ion concentrations during the first minute after addition of carnitine acetyltransferase to cuvettes containing 0.05 μmole of both acetyl-CoA and carnitine is plotted in the top part of Fig. 6. Enzyme activity decreased at pH values below 7.1, and maximal activity was unchanged from pH 7.1 to 8.2. The rate decreased to less than half maximum at pH 8.9. Total absorbancy change in a 15-minute period was constant at all hydrogen ion concentrations examined up to pH 8.6, but at higher pH values, the enzyme was inactivated (Fig. 6, bottom).

We have obtained carnitine acetyltransferase activity in extracts of rat liver and brain, but levels were lower than those found in pig or rat heart preparations. A detailed study of enzyme distribution in these and other organs has not yet been attempted, but we have observed that extracts of heart mitochondria gave highest yields of carnitine acetyltransferase per mg of initial protein. The enzyme was also present, however, in 0.25 g supernatant fractions of fresh rat hearts homogenized in 0.25 M sucrose containing 1 mM sodium EDTA when an all glass conical tissue homogenizer was employed.

**DISCUSSION**

Carnitine acetyltransferase catalyzes the reversible transfer of short chain acyl groups to carnitine from acyl-CoA derivatives. Although this enzyme lacks the ability to transfer long chain groups, a separate transferase has been found in both mitochondria and soluble cytoplasmic fractions which catalyzes the reaction (17, 18).

Palmitoylcarnitine + CoA ⇌ palmitoyl-CoA + carnitine
that acetylcarnitine can penetrate mitochondrial barriers. Addition of acetylcarnitine stimulated oxygen uptake by heart muscle preparations (3) and by mitochondria from several tissues (5) in the absence of added CoA. The increase in respiration most probably resulted from oxidation of acetyl groups in the tricarboxylic acid cycle after initial conversion of acetylcarnitine to acetyl-CoA within mitochondria, catalyzed by mitochondrial carnitine acetyltransferase. As Brenner indicated, however, the possibility remained that the increased respiration that followed acetylcarnitine addition could have resulted from transacetylase independent of acetyl-CoA formation, possibly by direct condensation with oxaloacetate to form citrate (5). This possibility can now be ruled out on the basis of data shown in Table II. Since NADH was not formed in the coupled citrate-condensing enzyme system unless both CoA and carnitine acetyltransferase were added to cuvettes containing acetylcarnitine, it may be concluded that the citrate-condensing enzyme cannot accept the acetyl group directly from acetylcarnitine. Available evidence is consonant with the concept that acetylcarnitine must first be converted to acetyl-CoA before being metabolized further.

The partial purification of carnitine acetyltransferase has permitted us to estimate the equilibrium constant of that reaction by employing procedures described in Fig. 3. When Friedman and Fraenkel (1) initially demonstrated the carnitine acetyltransferase reaction, they postulated that the O-acetyl ester of acetylcarnitine might have a high group potential. The impure nature of their enzyme preparation did not allow Fraenkel and Friedman to substantiate their hypothesis. The present data, which demonstrate an equilibrium constant of approximately 0.6 toward acetyl-CoA formation under conditions described, show that the group potential of the O-acyl structure is indeed of a higher order than would be expected of an ordinary secondary ester. The \( \Delta F' \) for hydrolysis of acetylcarnitine to carnitine and acetate may be estimated by summing the following reactions.

\[
\text{Acetylcarnitine} + \text{CoA} \rightleftharpoons \text{acetyl-CoA} + \text{carnitine} \quad (\Delta F' = +0.5 \text{ kcal}) \quad (A)
\]

\[
\text{Acetyl-CoA} + \text{H}_2\text{O} \rightleftharpoons \text{acetate} + \text{H}^+ + \text{CoA} \quad (\Delta F' = -8.2 \text{ kcal at pH 7.0}) \quad (B)
\]

Sum: \[
\text{Acetylcarnitine} + \text{H}_2\text{O} \rightleftharpoons \text{acetate} + \text{H}^+ + \text{carnitine} \quad (\Delta F' = -7.9 \text{ kcal at pH 7.0}) \quad (C)
\]

As Jaenicke and Lynen (21) have pointed out, a high group potential of the O-acyl ester of acetylcarnitine is contrary to usual expectations. It is not found, for example, in the O-acyl group of acetylcholine (21). Since norcarnitine was able to substitute for carnitine in the acetyltransferase reaction at pH 7.0 without changing the equilibrium position, it appears that the energy of the O-acyl group was not altered by removal of a methyl group from the nitrogen of acetylcarnitine.

In an examination of substrate specificity of carnitine acetyltransferase, we have demonstrated that removal of a methyl group to form norcarnitine decreased the velocity of the reaction, whereas substitution of a cyano, a hydroxyl, an amide, or an ester grouping for the original carboxyl group abolished activity of the derivative molecule in the enzymatic assay at concentrations employed. Since a free carboxyl group appears to be required, it is possible that the sequence shown in Diagram 1 occurs during the carnitine acetyltransferase reaction.

---

**Figure 5. Effects of carnitine and derivatives on the release of CoA from acetyl-CoA in the presence of carnitine acetyltransferase.** Results were obtained by following absorbancy at 412 m\( \mu \). Blank cuvettes, contained, in 1.0 ml, the following components: 0.2 \( \mu \)mole of 5,5′-dithiobis-(2-nitrobenzoic acid), 100 \( \mu \)moles of Tris-HCl buffered at pH 8.0 at 35°C; 0.05 \( \mu \)mole of acetyl-CoA, and 0.02 mg of carnitine acetyltransferase of specific activity 4.6. Other cuvettes, appropriately labeled on the graph, contained the same components plus indicated amounts of \( \Delta_l \)-carnitine or \( \Delta_l \)-norcarnitine. In experiments labeled R on the graph, cuvettes contained one of the following derivatives: choline, deoxycarnitine, \( \Delta_l \)-hydroxy-\( \gamma \)-aminobutyrate, \( \Delta_l \)-norcarnitol, \( \Delta_l \)-carnitine nitrile, \( \Delta_l \)-norcarnitinamide, or the ethyl ester of \( \Delta_l \)-norcarnitine. Maximal concentrations examined of these latter derivatives were varied between 0.2 and 1.0 \( \mu \)mole per ml, as stated in the text. CoA was measured by the procedure of Ellman (9). \( \Delta_l \)-Norcarnitine at 0.2 \( \mu \)mole per ml resulted in a release of CoA corresponding to an absorbancy of 0.289, giving a continuation of the straight line labeled norcarnitine. Reactions were started by addition of enzyme. The blank absorbancy, subtracted from all points on the above graph, was 0.114 and was contributed chiefly by interaction of the dye with protein. Solid points represent analyses performed in the presence of 0.5 \( \mu \)mole of deoxycarnitine per ml.

Intermediate I might be stabilized by interaction of the O− with positively charged groups on the enzyme. Similar reaction sequences have been postulated to account for unexpectedly high rates of hydrolyses of certain esters (22). Alternatively, the positively charged amino group may be involved in the transition states (23). It will be of interest to examine acetyl-CoA formation from acetyl-norcarnitine and CoA at pH values above the pK of the amino group to see whether a positively charged nitrogen is in fact required in the carnitine acetyltransferase reaction.
The effects of pH on carnitine acetyltransferase activity were studied. Acetyl-CoA (0.05 μmole per ml) and 0.1 μmole of d,l-carnitine per ml were present in all cuvettes together with buffer at pH values indicated. The reaction was initiated by addition of 0.035 mg of carnitine acetyltransferase of specific activity 1.4. Absorbancy change at 232 mp during the first minute is plotted against pH in the top graph; the bottom graph shows total absorbancy change obtained with a single addition of enzyme. Buffers used were 0.05 M KH₂PO₄ (circles), 0.1 M Tris-HCl (squares), and 0.05 M glycine with NaOH added to give designated pH values (triangles). Immediately after the end of incubation at 35°, pH values of these solutions were measured with glass electrodes (Beckman model G) at room temperature, and suitable correction factors were introduced to calculate pH values at 35°.

**SUMMARY**

Carnitine acetyltransferase was extracted from pig heart and was partially purified. It catalyzed the reaction

\[
\text{Acetylcarnitine} + \text{CoA} \rightleftharpoons \text{acetyl-CoA} + \text{carnitine}
\]

Since enzymatic activity was abolished when carnitine acetyltransferase was incubated with 5,5'-dithiobis-(2-dinitrobenzoic acid), it is possible that sulfhydryl groups of the enzyme may be involved in the formation of an acylthio-enzyme intermediate in the reaction sequence.

**Diagram 1**

Since enzymatic activity was abolished when carnitine acetyltransferase was incubated with 5,5'-dithiobis-(2-dinitrobenzoic acid), it is possible that sulfhydryl groups of the enzyme may be involved in the formation of an acylthio-enzyme intermediate in the reaction sequence.

**Diagram 1**

\[
\begin{align*}
\text{Acetylcarnitine} & \rightleftharpoons \text{Acetyl-CoA} + \text{carnitine} \\
\text{CH₃} & \text{O} \\
\text{O} & \text{C} \\
\text{O} & \text{CH₃CH₂CH₂CO} \\
\text{CH₃NCH₂CHCH₂COO} & \rightleftharpoons \text{[(CH₃)₂NCH₂CHCH₂COO]⁻} \\
\end{align*}
\]

Carnitine acetyltransferase was extracted from pig heart and was partially purified. It catalyzed the reaction

Acetylcarnitine + CoA ⇌ acetyl-CoA + carnitine

The apparent equilibrium constant was 0.0 at pH 7.0 and at 35°, on the assumption that only the (-)-isomers were enzymatically active. It was concluded that the O-acyl ester of acetylcarnitine derivatives had a high group potential, and that the ΔGro for acetylcarnitine hydrolysis was approximately -7.9 kcal.

Substrate specificity of carnitine acetyltransferase toward carnitine or its derivatives was investigated by examining conversion of enzyme A (CoA) release from acetyl-CoA, and it was found that the analogous compounds containing an amide, cyano, hydroxyl, or ester group instead of the carboxyl were inactive at concentrations examined. The removal of one methyl group from carnitine to form norcarnitine did not abolish activity in the enzymatic assay, although the velocity of the transfer was less than that observed with equimolar carnitine concentrations. \(\beta\)-Hydroxy-\(\gamma\)-aminobutyric acid was unable to accept acetyl transfer. Acetyl-CoA, propionyl-CoA, and butyryl-CoA reacted at approximately the same rates, but carnitine acetyltransferase catalyzed the transfer of acyl groups of longer chain lengths at slower velocities under the conditions described. Decanoyl-CoA reacted slowly, and palmitoyl-CoA was inactive. The enzyme had slight or no fatty acyl-CoA hydrolase or acetylcarnitine esterase activity. Carnitine acetyltransferase did not react with choline or its derivatives. Of various assay procedures used in the purification and characterization of carnitine acetyl-
transferase, the system employed to measure CoA release from acetyl-CoA seemed most appropriate as an enzymological assay for carnitine in biological material, since a linear relationship was established between carnitine concentration and sulfhydryl groups released. The possible functional significance of carnitine acetyltransferase was discussed, and efforts were made to relate data presented to previous findings that carnitine catalytically enhances fatty acid oxidation. On the basis of the observed substrate specificity, a reaction sequence was postulated.

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REFERENCES

Properties of Partially Purified Carnitine Acetyltransferase
Irving B. Fritz, Suzzanne K. Schultz and Paul A. Srere


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