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SUBSTRATE SPECIFICITY OF SUCCINYL-CoA TRANSFERASE FROM RAT KIDNEY MITOCHONDRIA*

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1. Succinyl-CoA: 3-oxoacid transferase (EC 2.8.3.5) from rat kidney mitochondria, purified about 200-fold, catalyses the CoA transfer from acetoacetyl-CoA to succinate, acetoacetate, maleate, glutarate and malonate; maleate proved to be a true substrate of the enzyme.

2. Double-reciprocal plots of the initial reaction rates against substrates concentrations are best fitted by parallel lines. Inhibition by each acid product of the reaction is competitive with respect to the acid acceptor of CoA.

3. CoA-transferase from rat kidney shows similar kinetics as, but different substrate specificity than, the enzyme from other sources.

Succinyl-CoA: 3-oxoacid coenzyme A transferase (EC 2.8.3.5), further called CoA-transferase, catalyses CoA transfer from succinyl-CoA to 3-oxoacids according to the reaction:

\[
\text{Succinyl-CoA} + 3\text{-oxoacid} \rightleftharpoons 3\text{-oxoacetyl-CoA} + \text{succinate}.
\]

The enzyme isolated and purified from pig heart by Stern et al. (1956) was reported to be specific for succinyl-CoA, while various C_4 to C_6 3-oxoacids might serve as CoA acceptors. However, in 1960 Menon & Stern (1960) demonstrated that dog muscle and pig heart extracts catalyse the following other CoA transfer reactions: \(a\), acetoacetyl-CoA + malonate \(\rightleftharpoons\) malonyl-CoA + acetoacetate; \(b\), acetoacetyl-CoA + glutarate \(\rightleftharpoons\) glutaryl-CoA + acetoacetate; and \(c\), succinyl-CoA + malonate \(\rightleftharpoons\) malonyl-CoA + succinate, as well as \(d\), acetoacetyl-CoA + succinate \(\rightleftharpoons\) succinyl-CoA + acetoacetate. In spite of these and other findings, CoA-transferase is still generally considered as strictly specific for succinyl moiety (Jencks, 1973).

In our studies on the inhibitory effect of maleate on oxidation of 2-oxoacids in rat kidney mitochondria, indirect evidence was obtained for participation of

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* This work was supported by the Polish Academy of Sciences, Grant no. II.1.1.5.
maleate as a substrate in the reaction catalysed by CoA-transferase (Pacanis et al., 1975). When these studies were in progress, Fenseleau & Wallis (1974) reported that maleate might be a substrate for the enzyme isolated from rat heart. Data reported in the present paper and the results of the afore-mentioned authors prove that maleate serves as CoA acceptor in CoA-transferase reaction. This, in turn, supports our hypothesis that maleate primarily affects succinyl-CoA metabolism (Pacanis et al., 1975).

MATERIALS AND METHODS

Animals. Rats of Wistar strain weighing 200 - 250 g were killed by cervical dislocation. Kidney, heart, brain, liver and skeletal muscle were rapidly removed and placed in a chilled solution containing 250 mm-sucrose buffered with 10 mm-Tris-HCl to pH 7.4.

Pig kidneys were obtained from the slaughterhouse immediately after killing of the animals, and transported to the laboratory in ice.

Reagents. Sodium acetoacetate was prepared by the method of Seeley (1955), acetoacetyl-CoA according to Lynen et al. (1958) and succinyl-CoA by the method of Simon & Shemin (1953). CoA-SH (85 - 90% pure) was purchased from Sigma Chem.Co. (St.Louis, Mo., U.S.A.).

Methods. Acetoacetyl-CoA concentration was determined by the method of Stern et al. (1956), and succinyl-CoA concentration by the hydroxamate method of Lipmann & Tuttle (1945).

CoA-transferase activity was assayed by measuring changes in absorbance of the enolate form of acetoacetyl-CoA at 310 nm in the presence of magnesium chloride at pH 8.1 (Hersh & J encks, 1967). The assay medium containing 67 mm-Tris- HCl, pH 8.1, 5 mm-MgCl₂ and 0.06 mm-acetoacetyl-CoA in a total volume of 0.45 ml, was incubated at 30°C with the enzyme (60 - 300 ng; spec. act. 80 μmoles/ min/mg protein). Disappearance of acetoacetyl-CoA absorbance was recorded continuously for 2 to 3 min. CoA acceptor was then added to a final concentration of 67 mm and consumption of acetoacetyl-CoA was measured for an additional 3 to 5 min. The reaction was linear within the first 90 sec, and this rate was used to calculate the enzyme activity. Molar extinction coefficient for acetoacetyl-CoA under the applied conditions is 1.19 x 10⁴.

Kinetics of the reaction was studied in the same medium except for varying substrate concentration and, where noted, elimination of MgCl₂. Ionic strength of the medium in these experiments was maintained at 1.0 x by addition of sodium sulphate (Hersh & J encks, 1967).

Protein concentration was measured by the biuret method (Gornall et al., 1949) or according to Warburg & Christian (1941). The enzyme activity is expressed in units (1 μmole of acetoacetyl-CoA used per minute at 30°C).

Purification of CoA-transferase from rat kidney. The procedure described below is based on the principles reported by Tildon & Sevdalian (1972) and Benson & Boyer (1969). All steps were carried out at 4°C. About 60 g of rat kidneys was
washed with 0.9% NaCl solution, minced and homogenized (2 x 1 min at 1900 rev./min) in 5 vol. of the solution containing 150 mm-KCl, 1 mm-EDTA and 5 mm-Tris-HCl buffer, pH 7.0. The gross particulate fraction was removed by centrifugation at 800 g for 3 min. Mitochondria were then sedimented at 8000 g for 10 min, suspended in 1 vol. of water and frozen at -20°C for 12 h. After thawing, the mitochondrial suspension was frozen twice in liquid nitrogen, thawed, and centrifuged at 20 000 g for 30 min. The volume of the soluble fraction was adjusted to 60 ml, ammonium sulphate (3.13 g/10 ml) was added, the mixture stirred for 60 min and centrifuged at 20 000 g for 30 min. Ammonium sulphate (1.37 g/10 ml) was added to the supernatant and stirred for 2 h. The precipitate separated on centrifugation as above was dissolved in a minimum volume of 2 mM-potassium phosphate buffer, pH 7.0, and dialysed overnight against the same buffer. The dialysed preparation (50 - 100 mg of protein) was applied on a DEAE-Sephadex column (20 x 1.5 cm) equilibrated with 50 mM-potassium phosphate, pH 7.0. The column was washed with 300 - 350 ml of the same buffer at a flow rate of 9 ml/h until complete disappearance of absorbance at 280 nm as monitored by Uvicord II (LKB). The CoA-transferase activity was then eluted with 50 mM-potassium phosphate buffer containing 500 mM-KCl, pH 7.0. Fractions of 5 ml were collected. The two 5-ml fractions showing the highest activity, and containing more than 90% of the total activity placed on the column, were used as the final enzyme preparation.

The enzyme was purified about 900-fold as compared with crude tissue extract, or about 200-fold as compared with the mitochondrial extract (Table 1). The applied method is simple, fairly reproducible and not time-consuming. The enzyme showing specific activity of 83 μmoles/min/mg protein was essentially free of detectable thiolase or deacylase activities. It can be stored at -20°C for at least 6 months without loss of activity.

Table 1

<table>
<thead>
<tr>
<th>Step</th>
<th>Protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg protein)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>6900</td>
<td>650</td>
<td>0.094</td>
<td>100</td>
</tr>
<tr>
<td>Mitochondrial extract</td>
<td>480</td>
<td>192</td>
<td>0.40</td>
<td>30</td>
</tr>
<tr>
<td>Ppt. at 0.5 - 0.7 (NH₄)₂SO₄ sat.</td>
<td>80</td>
<td>109</td>
<td>1.36</td>
<td>17</td>
</tr>
<tr>
<td>DEAE-Sephadex eluate</td>
<td>0.82</td>
<td>68</td>
<td>83.0</td>
<td>10</td>
</tr>
</tbody>
</table>

Purification of CoA-transferase from pig kidney. Exactly the same procedure as described above was used for isolation and purification of CoA-transferase from pig kidney mitochondria. The final preparation was purified about 400-fold and its specific activity was about 17 μmoles/min/mg protein.
Preparation of mitochondrial extracts from rat tissues. Kidneys were bisected along the longitudinal axis and the cortex was separated from the medulla by dissection through the corticomedullary junction. The homogenates of all tissues studied were prepared with 9 vol. of 250 mM-sucrose solution buffered with 10 mM-Tris-HCl to pH 7.4, per gram of tissue, using a glass homogenizer with Teflon pestle. The gross particulate fraction was removed by centrifugation at 800 g for 7 min. Mitochondria were sedimented at 10 000 g for 10 min, washed with sucrose and centrifuged again at 20 000 g for 1 h. Pellets were suspended in 1 vol. of water and frozen in liquid nitrogen. Freezing and thawing were repeated four times, then the suspensions were centrifuged at 20 000 g for 30 min and the soluble fractions used for enzymatic measurements.

RESULTS

As illustrated in Fig. 1, the addition of maleate as well as succinate, malonate or glutarate accelerated the rate of acetoacetyl-CoA disappearance in the presence of the 200-fold purified preparation of CoA-transferase from rat kidney mitochondria. The rate of CoA transfer from acetoacetyl-CoA to maleate was about 5 times slower than the rate of CoA transfer to succinate. It was comparable to the rate of CoA transfer to glutarate and was considerably faster than CoA transfer to malonate. It is evident from the results presented that decomposition of acetoacetyl-CoA due to contaminating deacetylase and thiolase, or to spontaneous hydrolysis at pH 8.1 was negligible. Also, there was no chemical reaction of acetoacetyl-CoA with maleate nor the reaction did proceed with the boiled enzyme. The rate of the enzymatic reaction was proportional to the amount of enzyme added.

Double-reciprocal plots of the effect of succinate on the rate of the acetoacetyl-CoA + succinate ⇌ succinyl-CoA + acetoacetate reaction at two different concentrations of acetoacetyl-CoA are shown in Fig. 2A, and for the reaction with
maleate as a substrate, in Fig. 2B. In both cases the data are best fitted by parallel lines, and the results obtained suggest that the reaction of acetoacetyl-CoA with maleate exhibits similar kinetics to that with succinate. $K_m$ for maleate, calculated

![Fig. 2. Double-reciprocal plots for the effect of succinate (A) or maleate (B) on the initial velocity of the reaction catalysed by CoA-transferase at a series of acetoacetyl-CoA concentrations. The reaction mixture contained 55 - 304 ng of enzyme protein, 67 mM-Tris-sulphate, pH 8.1, 5 mM-MgCl₂, and succinate, maleate and acetoacetyl-CoA as indicated. Sodium sulphate was added to give a ionic strength of 1.0 M. The rate is expressed as μmoles/min/mg protein.](image)

![Fig. 3. Product inhibition by acetoacetate of the acetoacetyl-CoA+succinate (A) or the acetoacetyl-CoA+maleate (B) reaction. The reaction mixture contained 0.058 mM-acetoacyl-CoA, 67 mM-Tris-sulphate, pH 8.1, 58 - 304 ng of enzyme protein, sodium sulphate to obtain 1.0 M ionic strength, and acetoacetate and succinate or maleate at the indicated concentrations. The rate is expressed as μmoles/min/mg protein.](image)

according to Hersh & Jencks (1967) was similar to that for succinate, i.e., respectively, 25 and 14 mM. The effect of acetoacetate on the reaction of acetoacetyl-CoA with maleate or succinate (Fig. 3A, B) shows the pattern of competitive product inhibition. The reaction of succinyl-CoA with acetoacetate was also inhibited competitively by succinate and maleate (Fig. 4A, B), which indicates that maleate is a substrate for CoA-transferase, the $K_i$ values being, respectively, 0.32 and 2.9 mM.
Fig. 4. Product inhibition of the succinyl-CoA-acetoacetate reaction by succinate (A) or maleate (B). The reaction mixture was as in Fig. 3 except for the presence of 0.12 mM-succinyl-CoA and the greater amount of enzyme protein (2.7 μg). Acetoacetate and succinate or maleate were at the indicated concentrations. The rate is expressed as μmoles/min/mg protein.

To determine whether one or more enzymes are involved in the transfer reaction, the relative rates of CoA transfer from acetoacetyl-CoA to maleate and succinate and to other acid substrates were measured in rat and pig kidney preparations. In rat kidney, the rate of CoA transfer from acetoacetyl-CoA to maleate equalled that of CoA transfer to glutarate, and was about three times faster than the rate of CoA transfer to malonate in crude extracts and at consecutive steps of purification. On the other hand, the ratio of activities with succinate and maleate or glutarate rose from about 3.5 in crude tissue extracts to about 5.0 in final preparations (Table 2). The succinate to malonate ratio of the rate of CoA transfer

| Table 2 |
|---|---|---|---|---|
| Fraction | Succinate | Maleate | Glutarate | Malonate |
| Rat kidney | | | | |
| Crude extract | 0.094 | 0.025 | 0.027 | 0.008 |
| Mitochondrial extract | 0.400 | 0.090 | 0.090 | 0.033 |
| Ppt. at 0.5-0.7 (NH₄)₂SO₄ sat. | 1.360 | 0.250 | 0.270 | 0.080 |
| DEAE-Sephadex eluate | 83.000 | 15.200 | 16.200 | 5.350 |
| Pig kidney | | | | |
| Crude extract | 0.040 | 0.012 | 0.010 | 0.0008 |
| Mitochondrial extract | 0.146 | 0.030 | 0.023 | 0.0025 |
| Ppt. at 0.5-0.7 (NH₄)₂SO₄ sat. | 0.506 | 0.083 | 0.080 | 0.0120 |
| DEAE-Sephadex eluate | 17.150 | 1.870 | 2.170 | 0.3400 |
showed a similar increase. The pig kidney CoA-transferase was 50 times more active with succinate than with malonate and this ratio did not change over all purification steps, which is in agreement with the data of Menon & Stern (1960). The ratio of activities with succinate and maleate or glutarate rose about 2 to 3 times, while the ratio of activities with maleate or glutarate as compared with malonate decreased by the same factor.

Table 3

The activity of mitochondrial CoA transferase in rat tissues

The measurements were made on mitochondrial extracts, obtained as described in Methods.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific activity (units/mg protein)</th>
<th>Succinate</th>
<th>Maleate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Succinate</td>
<td>Maleate</td>
</tr>
<tr>
<td>Kidney (whole)</td>
<td>500</td>
<td>80</td>
<td>6.25</td>
</tr>
<tr>
<td>Kidney cortex</td>
<td>525</td>
<td>85</td>
<td>6.20</td>
</tr>
<tr>
<td>Kidney medulla</td>
<td>318</td>
<td>42</td>
<td>7.55</td>
</tr>
<tr>
<td>Heart</td>
<td>164</td>
<td>24</td>
<td>6.85</td>
</tr>
<tr>
<td>Brain</td>
<td>91</td>
<td>15</td>
<td>6.05</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>27</td>
<td>4</td>
<td>6.75</td>
</tr>
<tr>
<td>Liver</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The activities of mitochondrial CoA-transferase in various rat tissues given in Table 3 are consistent with the data reported by Tildon & Sevdalian (1972) and by Williamson et al. (1971) for the whole tissue extracts. The highest activity was found in kidney cortex and no activity was detected in liver. Although the enzyme activity varied in the tissues examined, the activity ratio with succinate and maleate was practically the same.

DISCUSSION

Data reported on specificity of CoA-transferase show species- and tissue-dependent differences. Malonate was shown to be a substrate for the pig heart, dog heart and dog skeletal muscle enzyme (Menon & Stern, 1960), and for cow skeletal muscle (Blair, 1969) and rat heart (Fenselau & Wallis, 1974) CoA-transferase. On the other hand, glutarate has been shown to participate in CoA transfer reaction in dog heart and skeletal muscle, but not in pig heart (Menon & Stern, 1960) and rat heart (Fenselau & Wallis, 1974). The data presented in this report show that purified CoA-transferase from rat and pig kidney catalyses CoA transfer to succinate, acetoacetate, maleate, glutarate and malonate. Fenselau & Wallis (1974) reported that maleate might be a substrate for rat heart CoA-transferase. The relative rates of the exchange of CoA between various acid substrates differ largely, depending on the tissue and animal species. The data for rat kidney may be interpreted as indicating the existence of two similar but distinct enzymes, differing in substrate specificity or the substrate affinity. On the other hand, it might be
assumed that in pig kidney one enzyme acts with succinate and malonate, while the other one with maleate and glutarate. It was, however, demonstrated repeatedly that CoA-transferase from different sources has essentially the same kinetic properties, despite different substrate specificities (Tildon & Sevdalian, 1972).

The data presented in this paper point to close similarities between maleate and succinate as the substrates for CoA-transferase: $K_m$ values are similar and the rate of CoA transfer to maleate appeared to be at least 20% that to succinate. Moreover, maleate, succinate and acetoacetate show a pattern of competitive product inhibition with respect to each other. This means that maleate is not a dead-end inhibitor. This type of kinetic behaviour is consistent with "ping-pong" kinetics described for CoA-transferase from different animal sources (Hersh & Jencks, 1967; Blair, 1969; Tildon & Sevdalian, 1972; Fenselau & Wallis, 1974). In the CoA-transferase reaction this type of kinetics implies that the reaction proceeds through two half-reactions in which the product from the first substrate is formed prior to the reaction with the second substrate, with concomitant conversion of the enzyme to the intermediate enzyme-CoA derivative (Hersh & Jencks, 1967).

\[
\text{Acetoacetyl CoA} + E \rightleftharpoons E\text...\text{CoA} + \text{acetoacetate}
\]

\[
\text{Succinate} + E\text...\text{CoA} \rightleftharpoons E + \text{succinyl-CoA}.
\]

The data presented suggest that CoA-transferase from rat and pig kidney mitochondria can catalyse the exchange of CoA between maleate and acetoacetate or succinate. CoA transfer to maleate should result in formation of maleyl-CoA, according to the reactions:

\[
\text{Maleate} + \text{acetoacetyl-CoA} \rightleftharpoons \text{acetoacetate} + \text{maleyl-CoA}
\]

\[
\text{Maleate} + \text{succinyl-CoA} \rightleftharpoons \text{succinate} + \text{maleyl-CoA}.
\]

This product, however, has not so far been isolated because of its reactivity and instability.

The authors wish to express their gratitude to Prof. Dr. S. Angielski for his interest, advice and helpful discussion.

REFERENCES

SPECIFICITY OF SUCCINYL-COA TRANSFERASE


SPECYFIKNOŚĆ SUBSTRATOWA TRANSFERAZY KOENZYMU A Z MITOCHONDRII NEREK SZCZURY

Streszczenie

1. Transferazę CoA z mitochondrii nerek szczury oczyszczone ok. 200-krotnie. Enzym katalizuje reakcję przeniesienia CoA z acetocetylo-CoA na bursztynian, acetooctan, maleinian, glu- taran i malonian.

2. Maleinian, podobnie jak bursztynian i acetooctan, jest kompetencyjnym inhibitorym w stosunku do innych akceptorów CoA.

3. Transferaza z nerki szczury wykazuje podobne właściwości kinetyczne do enzymu izolowanego z innych tkanki lub innych zwierząt, różni się natomiast specyficznością substratową.

Received 18 March, 1976.

Note added in proof

While this paper was in press, maleate was reported to be a substrate for bovine heart CoA-transferase (White, H. & Jencks, W. P., 1976, J. Biol. Chem., 251, 1688 - 1699).