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Properites of the Medium-Chain/Long-Chain Carnitine Acyltransferase Purified from Rat Liver Microsomes*

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쥐 간의 마이크로소옴에서 분리한 Medium-Chain/Long-Chain Carnitine Acyltransferase의 특성에 관한 연구*

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Introduction

We previously reported the purification of a carnitine acyltransferase that exhibits both medium-chain and long-chain carnitine acyltransferase activity (COT) in rat liver microsomes (Murthy and Bieber, 1992). This enzyme is one of a group of enzymes that catalyze the reversible transfer of the acyl groups of medium-chain and long-chain acyl-CoAs to L-carnitine (McGarry et al., 1978, 1979). The existence of such enzyme activity in mitochondria was reported in the early 1960s, and the existence of extramitochondrial activity associated with microsomes and peroxisomes was documented about a decade later (Markwell et al., 1973, 1977). Although an extensive literature exists about the properties, characteristics, and regulation of the medium-chain/long-chain carnitine acyltransferase (s) associated with β-oxidation of fatty acids in mitochondria (Bieber, 1988) and considerable information exists about the properties of the enzyme associated with peroxisomes (Farrell et al., 1984, Miyazawa et al., 1983, Chatterjee et al., 1988), characterization of the enzyme associated with microsomes is limited due to its instability. Microsomal COT in inhibited by malonyl-CoA (Lilly et al. 1990, Lilly and Bieber, 1990); thus, the regulation of this activity by malonyl-CoA may be an important parameter in the control of the β-oxidation of acyl-CoAs formed in the cytosolic compartment of cells. Herein, some of the kinetic properties, immunological properties, and inhibitory characteristics of the enzyme purified from rat liver microsomes are reported.

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Methods

1. Enzyme Purification

Microsomal COT was purified from rat liver microsomes essentially as described (Murthy and Bieber, 1992), except that Cibacron Blue Sepharose 6B from Pharmacia was used for the first chromatographic step; the pH of the phosphate buffer for DE-52 chromatography was 7.4 rather than 7.2; and the polyethylene glycol 35,000 was from Fluka rather than Sigma. Several preparations of enzyme were made; the specific activity varied between 3 and 103 units/mg protein. SDS-PAGE analyses of the enzyme isolated from the Matrix gel Orange -A columns gives a major band with a molecular weight near 53,000 daltons and two other proteins, as shown in lane 3 of Figure 1A. The enzyme was stored in 25 mM KPi, pH 7.2; 20% glycerol, 5 mM L-carnitine, 25μg bestatin, pepstatin, and leupeptin per ml plus 0.1 mM phenylmethylsulfonyl fluoride in 1% octylglucoside at 4℃. These preparations were used for the experiments described herein, except for the protein used for antibody preparation. For antibody preparation, the Matrix gel Orange-A COT was further purified using SDS-PAGE and the 53,000 M.W. material was electroeluted and used for immunization. The SDS-PAGE profile of the electroeluted fractions is shown in Figure 1B.

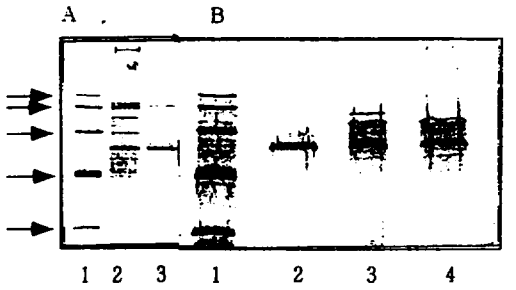

Fig. 1. SDS-PAGE of microsomal COT. For both panels, lane 1 shows the molecular weight markers. The molecular weights of the standards from top to bottom are 116, 97, 66, 45, and 29 kD. In panel A, lane 2 represents the DE-52 extract and lane 3 is the Matrix gel Orange-A eluate used for most of the assays. In panel B, lane 2 is the protein after electro-elution that was used for the primary immunization; lane 3 shows the protein profile before electroelution, and lane 4 shows the protein profile used for the booster injection for antibody production. The gels were silver-stained.

2. Assays

Rate forward. For most assays, COT activity was assayed spectrally in the forward direction (acyl-CoA→acylcarnitine) at 324 nm in the presence of 4,4′-dipyridyldisulfide (DTBP) as the CoA trapping agent as described (McMillin et al., 1992). For this assay, the media contained: L-carnitine, 3.1 mM; acyl-CoA, 12.5 μM; phosphate, 39 mM; pH 7.5; DTBP, 20 μM; the assays were run at ambient temperature.

Reverse assay and preparation of tritiated decanoylcarnitine. Tritiated decanoylcarnitine was prepared from decanoyl-CoA and [^{3}H$_3$C] carnitine using the conditions for the rate forward assay, except 0.4 mM decanoyl-CoA and 0.1 mM methyl[^{3}H]carnitine were used with 30 units of purified mitochondrial carnitine palmitoyltransferase. The mixture was incubated for 4 hrs at 18℃ in a final volume of 805 μl. The reaction was stopped by the addition of 2.8 ml of methanol, and tritiated carnitine was separated from tritiated decanoylcarnitine using the HPLC procedure

described elsewhere (Lysiak et al., 1986): the peak containing decanoylcarnitine (55% conversion of carnitine to decanoylcarnitine) was collected and concentrated. The specific radioactivity of decanoylcarnitine was 1.7×10^{8} DPM per nmol. Figure 2B shows an HPLC radiochromatogram tracing of the purified decanoylcarnitine and Figure 2A shows a typical profile of the substrate decanoylcarnitine and the product L-carnitine after incubating decanoylcarnitine with COT and CoASH. The nmoles of tritiated carnitine formed represent COT activity. This assay was used for determination of the $K_{i.s}$s for CoA and for decanoylcarnitine, as well as for determination

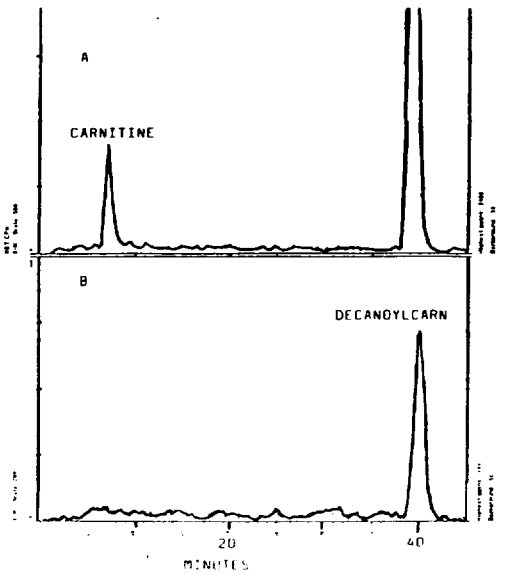

Fig. 2. Radiochromatogram of [^{3}H] methyldecanoylcarnitine and its reaction products. [^{3}H] Methyldecanoylcarnitine was prepared enzymatically from [^{3}H]methyl-L-carnitine and decanoyl-CoA as described in the Methods. Panel A shows a typical separation of ^{3}H-carnitine from [^{3}H]decanoylcarnitine after incubation with purified COT. Panel B is a radiochromatogram of [^{3}H]decanoylcarnitine.

of the effect of valproylcarnitine, 3-methylglutarylcarnitine, and pivaloylcarnitine on the reverse reaction. For these inhibitor studies, equal amounts of decanoylcarnitine and other acylcarnitines were used. Other details are included in the figure and table legends.

3. Antibody Preparation and Western Blotting

Preparation of antigen. COT from the Matrix gel Orange-A columns was concentrated and subjected to Sephacryl S-300 gel filtration because the Matrix gel Orange-A eluates contain an impurity with a molecular weight protein near 100,000 daltons. Although Sephacryl S-300 gel filtration gave a single peak of COT activity, this chromatography did not remove appreciable quantities of the 100,000 M.W. material; therefore, the Matrix gel Orange-A eluates were concentrated and subjected to preparative SDS-PAGE essentially as described (Hunkapellar et al., 1983). Protein with a molecular weight between, 50,000 and 55,000 daltons was electroeluted, concentrated, and used for immunization. The diced gel containing COT was soaked 3-5 hrs in buffer prior to the 30 hr electroelution. The eluted protein, after dialysis, was concentrated to dryness using a speed-vac concentrator and the sample treated with 90% ethanol for 18 hrs at -20°. After centrifugation, the protein was menbrane-filtered and 0.2mg COT in 330μl in distilled water was added to 240μl of Titer Max™ and the solution was agitated in a spin mixer for 15 min until a thick emulsion formed. The total solution (570μl containing 0.2mg of protein) was injected subcutaneously into a mature rabbit. After three weeks, 0.2mg of COT in Titer Max™, prepared as described above, was used for a booster injection. Seven days

after the booster injection, 30ml of blood was withdrawn and immune serum prepared and stored as described (Kerner and Bieber, 1990).

Western blotting. Proteins were subjected to SDS-PAGE and then electroblotted onto an Immobilon (polyvinylidene difluoride) membrane in 0.25 M Tris, 1.92 M glycine, 20% methanol, pH 8.3 buffer at 100 V for 30 min. Following transfer, the molecular weight marker region was removed and stained with Coomassie blue. The remaining membrane was washed once with 20 mM Tris, 500 mM NaCl, pH 7.5 (TBS) for 5 min. The membrane was then incubated in a blocking solution consisting of 3% chicken egg albumin in TBS at room temperature for 1 hr and air-dried overnight. The next morning, the membrane was reconstituted by washing in a solution of TBS containing. 5% Tween 20 for 15 min and washed twice with a solution of TBS containng .1% Tween (TTBS) for 5 min. The antibody was diluted in a solution of .1% chicken egg albumin in TTBS and the membrane was incubated in this solution at room temperature for 1 hr. After incubation, the membrane was washed three times with TTBS for 5 min. The second antibody (goat anti-rabbit IgG alkaline phosphatase conjugate, 1 : 3000 dilution) was diluted in .1% chicken egg albumin in TTBS and the membrane was incubated in this solution at room temperature for 1 hr and washed three times in TTBS for 5 min, followed by one wash in TBS for 5 min. The membrane was then placed in a container for detection of alkaline phosphatase activity; the incubation solution contained a 100 : 1 : 1 mixture of 100 mM sodium bicarbonate, 1 mM magnesium chloride, pH 9.8; 1.5% 5-bromo-4-chloro indoxyl phosphate in N, N-dimethylformamide 3% nitroblue tetrazolium, in 80% N, N-dimethylformamide. After color development, the membrane was washed twice with water and air-dried.

Results

1. Determination of $K_{0.5}$ and V_{max} for the Forward Reaction

Since previous studies had shown that COT associated with intact microsomes exhibits catalytic activity with both medium-chain and long-chain acyl-CoAs, the substrate concentration required for half-maximal V_{max} ($K_{0.5}$) was determined for straight-chain aliphatic acyl-CoA derivatives. These data are given in Table I. The enzyme uses substrates from 6-carbon to 16-carbon in chain length, with very little difference in $K_{0.5}$ or V_{max}. No activity was found with butyryl-CoA, and acyl-CoAs of chain -length greater than 16-carbon were not tested.

Table 1. $K_{0.5}$ and V_{max} values for even chain length saturated acyl-CoAs

Acyl-CoA	$K_{0.5}$ (μM)	V_{max} (μmol/min/mg protein)
C- 6	1.0	5
C- 8	2.0	5
C-10	4.0	23
C-12	1.0	8
C-14	1.0	5
C-16	1.0	5

The experimental conditions were the same as for the controls described in the legend of Table Ⅱ, except the acyl-CoA concentrations were 0.3, 0.5, 0.7, 1.0, 3.0, 5.0, 7.0, 9.0, 12.5, 25 and 50 μM; $K_{0.5}$ (K_ms) and v_{max} values were calculated using Lineweaver-Burk plots. The data represent the average from two experiments for each acyl-CoA. Each assay contained o.25 μg protein.

The $K_{0.5}$s varied between 1.0 and 4.0 μM, and the V_{max}s varied between 5 and 23 μmoles/min/mg of protein for the enzyme preparation used. Since the highest V_{max} obtained was with decanoyl-CoA, all subsequent studies were performed using decanoyl-CoA and/or decanoylcarnitine as substrate.

2. Effect of Inhibitors on the Forward Reaction

The effects of some potential inhibitors of COT activity were examined. As shown in Table Ⅱ, D-carnitine is a very weak inhibitor of COT, while malonyl-CoA, a potent inhibitor of the microsome-associated COT, essentially showed no inhibition, even at 500 μM, while etomoxiryl-CoA, also an inhibitor of the membrane-associated enzyme, gave weak inhibition. In contrast, purified COT was inhibited by aminocarnitine to the same degree as reported for the membrane-associated enzyme (Lilly et al., 1992). Since some carnitine acyltransferases are inhibited by CoA trapping agents, the effect of incubation of the enzyme with DTBP in the absence of substrate was determined. Preincubation with 20 μM DTBP, the concentration used in the assays, inhibited the enzyme > 70%. However, no inhibition was observed when the substrates decanoyl-CoA and

Table 2. Effect of inhibitors on the conversion of decanoyl-CoA to decanoylcarnitine

Inhibitor Added	mM	% of Control	Exp.#
None	—	100 ± 3.8	—
D-carnitine	1 10 100	75.2 ± 2.7 68.0 ± 2.5 43.1 ± 0.7	A
Malonyl-CoA	.050 .250 .50	100.7 ± 1.6 98. 4± 3.9 95.5 ± 3.5	B
Etomoxiry-CoA	.001 .005 .01	93.9 ± 0.78 79.7 ± 5.5 80.0 ± 1.5	C
DL-aminocarnitine	0.10 0.50	63.2 ± 1.9 46.3 ± 1.5	D
Preincubation of COT with DTBP for 10 min	0.2	28.5 ± 0.71	E

Each assay contained 12.5 μM decanoyl-CoA, 3.1 mM L-carnitine, 20 μM DTBP, and 41.3 mM potassium phosphate, pH 7.4. The reactions were started by addition of 10 μl of COT. The final volume was 200 μl. For experiments A to D, the inhibitor was added to the assay mixture before the enzyme. For experiment E, COT was incubated with DTBP for 10 min and then the substrates were added. The specific activity of COT was 10.0 μmoles/min/mg protein and 0.25 μg protein were used per assay. n=3 for each experiment: ± represents the standard deviation. The reaction was monitored at 324 nm (E_{324} = 19,600 M^{-1} cm^{-1}).

carnitine were present in the assay media; the activities for the spectral assay were linear (data not shown).

3. Conversion of Acylcarnitine→ Acyl-CoA

The function of microsomal COT is not known. Our working hypotheses has been that this enzyme functions in the reverse direction, namely the conversion of medium-chain/long-chain acylcarnitines to acyl-CoAs in the cytosolic compartment, or, alternatively, it may be involved in the formation of unusual acylcarnitines which arise in certain physiological situations. Some experiments were done to determine kinetic parameters in the reverse direction and to determine the effect of specific, unusual acylcarnitines on COT activity. Although the reaction in the forward direction using a rate spectral assay is linear with time, the data in Figure 3 show the enzyme exhibits a concave velocity versus time response for the reverse direction; thus, the enzyme appears to

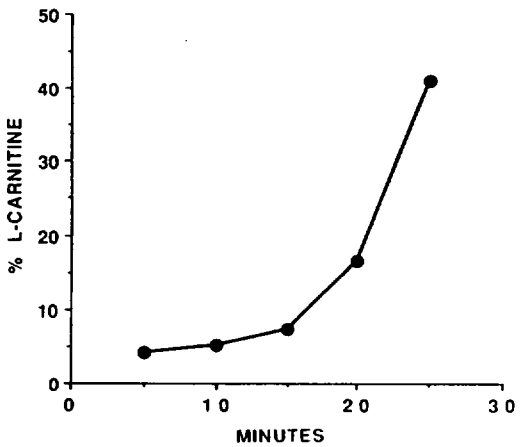

Fig. 3. Time course for conversion of decanoylcarnitine to carnitine by microsomal COT. The reaction and assay conditions were those described for the controls in Table Ⅲ, except the incubation time was varied.

be activated during the reaction. This was confirmed using a different assay, in which the appearance of decanoyl-CoA from decanoylcarnitine was monitored using a rate spectral assay at 232 nm. As with the isotope forward assay, the enzyme exhibited increased velocity with time. However, the increase in rate was not as great as shown in Figure 3 (data not shown). Since we did not establish conditions for maximum activation of the enzyme, all experiments for the reverse reaction (acylcarnitine→acyl-CoA) were done using standardized conditions with attention to time and incuation conditions. The enzyme preparation used for these experiments had approximately 100 units/mg protein activity in the forward direction using the rate spectral assay, and 12.7 units/mg protein initial activity in the reverse direction using the 1.8 mM decanoylcarnitine and 2.5 mM CoASH at pH 7.4. The spectral assay for the reverse reaction, namely monitoring the appearance of thioester at 232 nm, requires considerable amounts of enzyme and is much less sensitive than the isotope assay; thus, a radiochemical HPLC assay was developed and used to monitor the reaction in the reverse direction, as described in the Methods. This assay is very similar to the radiochromatographic HPLC procedure developed for measurement of the forward reaction, except the appearance of [^{3}H]carnitine rather than appearance of radioactive acylcarnitines is monitored.

4. Effects of Inhibitors on the Reverse Reaction

Since one of our working hypotheses has been that the microsomal enzyme may be responsible for formation of unusual acylcarnitines, such as the 3-methylglu-

tarylcarnitine, that occur in certain human disease states, the effect of unusual acylcarnitines on COT was determined. As shown in Table Ⅲ, 3-methylglutarylcarnitine, pivaloylcarnitine, and valproylcarnitine all had little effect on COT. The concentration of inhibitor was identical to the concentration of the substrate decanoylcarnitine; thus, each should have had equal opportunity to interact at the acylcarnitine binding site. No radioactivity was detected in regions where pivaloylcarnitine, valproylcarnitine, or methylglutarylcarnitine should appear on the HPLC chromatogram. Consequently, the enzyme did not exhibit ability to exchange carnitine into these acylcarnitine derivatives. It was shown previously that COT is monomeric and shows Michaelis-Menten kinetics with Hill n's = 1 for decanoyl-CoA and L-carnitine (Murthy and Bieber, 1992)

Table 3. Effect of unusual acylcarnitines on the conversion of decanoylcarnitine to L-carnitine

Inhibitor	% Decanoyl-Carnitine Converted to L-Carnitine
No Inhibitor (Control)	27.6 ± 3.1
1.8 mM 3-Methylglutaryl-L-Carnitine	25.5 ± 1.4
1.8 mM Pivaloyl-L-Carnitine	32.9 ± 0.4
1.8 mM Valproyl-L-Carnitine	32.1 ± 0.83

Each assay contained 2.5 mM CoASH, 1 mM dithiothreitol, 34 mM potassium phosphate, pH 7.4, and 1.8 mM ^{3}H-decanoyl-L-carnitine, 50,000 DPM per assay. The samples (40 μl) were incubated for 3 min at 30℃, and the reaction was stopped by the addition of 160 μl methanol and the methanol extract analyzed by radio-HPLC as described in the Methods. The specific activity of the COT was 12.7 U/mg in the reverse direction and 67 U/mg in the forward direction. n=3, ± represents the standard deviation.

5. Estimation of $K_{0.5}$ for CoASH and Decanoylcarnitine

Figure 4 shows the velocity versus CoASH concentration curve for microsomal COT using 1.8mM decanoylcarnitine. The data represent a composite of three separate experiments. Although a precise measurement of the $K_{0.5}$ was not made, it was between 0.1 and 0.4 mM for each of the three determinations; the $K_{0.5}$ is estimated to be 0.3 mM. Similar experiments were done in which the concentration of CoASH was 2.5 mM, and the concentration of decanoyl-carnitine varied. These data were more variable than those shown for CoASH, and some apparent substrate inhibition was observed at higher decanoylcarnitine concentrations. However, all studies showed that the $K_{0.5}$ for

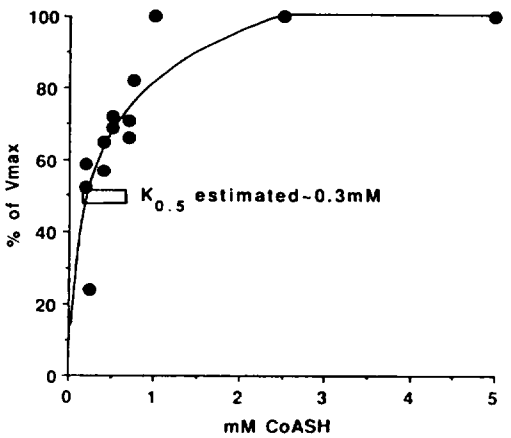

Fig. 4. The effect of the concentration of CoASH on COT activity. The assay and reaction conditions are identical to those described in the legend of Table Ⅲ, except the amount of CoASH was varied. The data represent a composite of three experiments; the estimated $K_{0.5}$ represents the [CoASH] required for 1/2 V_{max}.

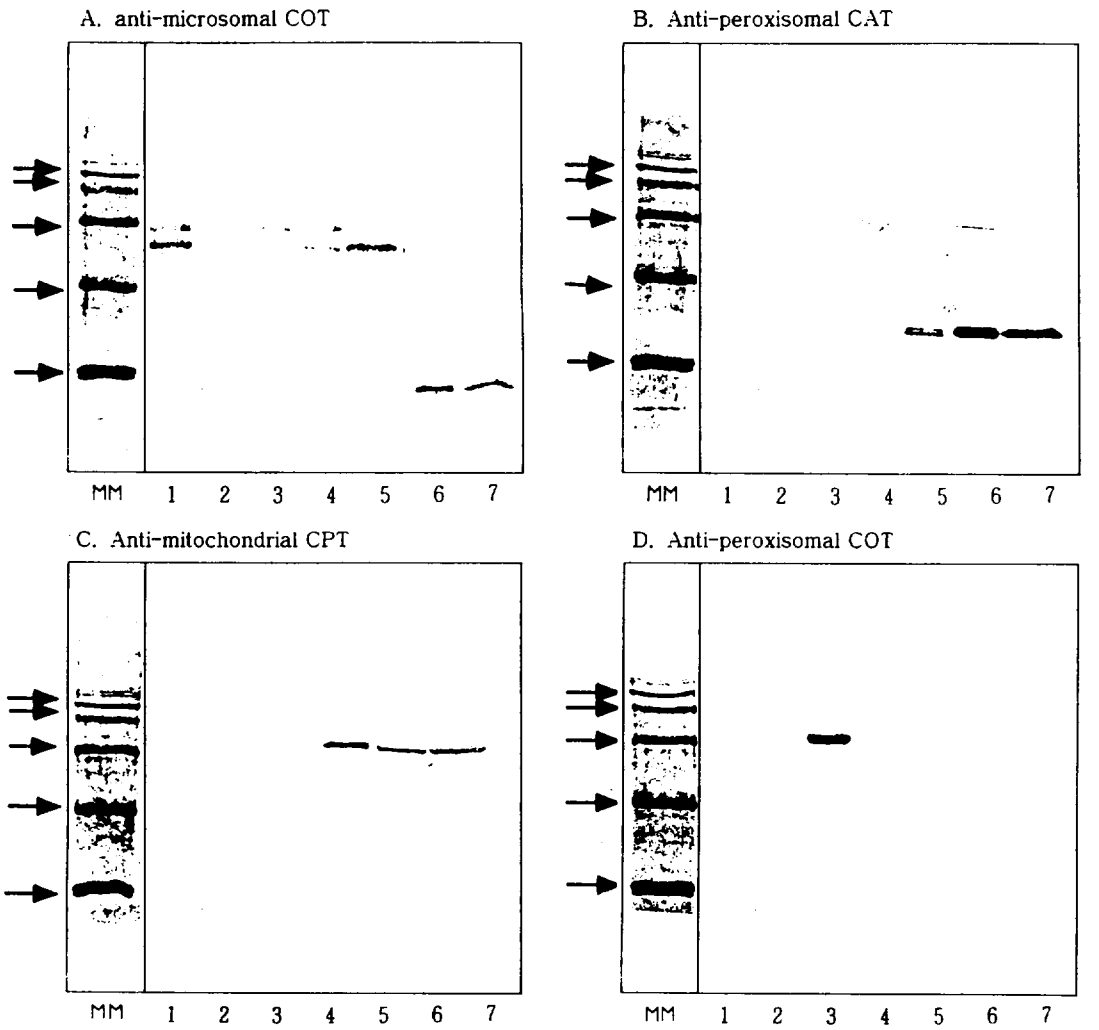

Fig. 5. Western blots of microsomal COT. For each panel, MM represents the molecular weight markers, from top to bottom : 116,000; 97,000; 66,000; 45,000; and 29,000 daltons. CAT represents carnitine acetyltransferase and CPT represents carnitine palmitoyltransferase. Four and three-tenths μg microsomal COT was used in lane 1 of Figures A and B and in lane 2 of Figures C and D. Eight-tenths μg beef liver of peroxisomal COT was used in lane 2 of Figures A and B and lane 3 of Figures C and D. Five-tenths μg of rat heart mitochondrial CPT was used in lane 3 of Figures A and B and lane 4 of Figures C and D. Lane 4 of Figures A and B contained 9.9 μg of pigeon breast muscle CAT. Lane 5 of Figures A and B contained 204 μg of solubilized rat liver microsomes. Sixty-four μg of solubilized rat heart mitochondria was used in lane 6 of Figures A and B and lane 5 of Figures C and D. For panels A, B, and D, the IgG fractions of serum obtained from rabbits immunized against microsomal COT, rat liver peroxisomal CAT, and mouse liver peroxisomal COT, respectively, were used. For panel C, serum from a rabbit immunized against purified CPT was used. The blots were incubated with the primary antibody for 1 hr at room temperature as described in the Methods.

decanoylcarnitine was > 1 mM. Four separate determinations were made and, from these data, it is estimated that the $K_{0.5}$ for decanoylcarnitine is between 1.0 and 4.0 mM. Because of the apparent substrate inhibition at higher concentrations of decanoylcarnitine, the studies with the inhibitors shown in Table Ⅲ were done using 1.8 mM decanoylcarnitine rather than with saturating quantities of decanoylcarnitine.

6. Polyclonal Antibody Production and Western Blotting

Sine the COT preparations used for these studies contained small amounts of other proteins, an enzyme preparation was subjected to SDS-PAGE preparative electrophoresis, and the protein with a molecular weight 50,000-55,000 was electroeluted as described in the Methods. Figure 1B shows the SDS-PAGE of COT before electroelution (lane 3), and after electroelution (lane 2). Lane 4 shows the protein used for the booster injection; it was obtained from a second SDS-PAGE electroelution preparation. An IgG fraction from serum was prepared, and this fraction was used for western blot analysis of the following purified enzymes : microsomal COT, heart mitochondrial carnitine palmitoyltransferase, peroxisomal COT, and carnitine acetyltransferase, as well as analysis of solubilized extracts of mitochondria and mocrosomes. As shown in Figure 5A, the antibody cross-reacted with purified COT and with a protein in rat liver microsomes that has a molecular weight equal to purified COT, but it did not exhibit cross-reactivity against carnitine acetyltransferase, peroxisomal COT, or CPT when these enzymes were used at concentrations similar to those exhibiting cross-reactivity with COT, but the antibody gave weak cross-reactivity with both purified peroxisomal COT and purified carnitine palmitoyltransferase when the enzyme concentration was increased. As shown in lane 6 of Figure 5A, the antibody cross-reacted strongly with a low molecular weight protein (22,000 daltons) in rat heart mitochondria. The identity of this protein is not known. Polyclonal antibody prepared against rat liver peroxisomal carnitine acetyltransferase, panel B (see ref. 18 for a description of this antibody), and polyclonal antibody prepared against mitochondrial carnitine palmitoyltransferase, panel C (see ref. 15 for a description of this antibody) did not cross-react with purified COT. Although not visible in panel D, purified microsomal COT showed a very weak interaction with antibody prepared against mouse liver peroxisomal COT.

Discussion

Although the different preparations of COT isolated from the Matrix-gel Orange-A columns had very similar protein profiles on SDS-PAGE, the specific activities ranged from 3 to 103 U/mg protein. The basis for the variability is not known. The data in Figure 3 imply that purified COT is only partially active because it exhibits increases in rate with time for conversion of acylcarnitine to acyl-CoA However, this behavior was not observed in the forward direction, indicating that the less active conformer is either rapidly converted to a more active conformer in the presence of acyl-CoA and carnitine or that such a conversion does not occur during the forward reaction. Regardless, it seems likely that the variable specific activity is due to the isolation of conformers that are not fully active. The 103 U/mg protein for the preparation with the highest activity is similar

to the 108 and 123 U/mg protein of carnitine palmitoyltransferase from rat and beef heart mitochondria using decanoyl-CoA as substrate (Kerner and Bieber, 1990). Because of the non-linear time course responses shown for the reverse reaction, all assays for the forward direction were performed using a rate spectral assay rather than the end-point radiochemical assay. The spectral assay allows continuous monitoring of the rate of reaction. The rates were linear for the forward reaction !

Although the lack of linearity with time for the reverse direction presented experimental difficulties, two conclusions can be made from the data. The $K_{0.5}$ for decanoylcarnitine is in the mM rather than μM range. This indicates that microsomal COT does not normally function in the reverse direction. The data seem to preclude it functioning for formation of medium-chain acyl-CoAs from medium-chain acylcarnitines under physiological conditions. The low $K_{0.5}$ for 6-carbon to 16-carbon acyl-CoAs strongly indicate COT functions physiologically in the forward direction. Howeveer, COT could function in the reverse direction if the concentrations of medium-chain acylcarnitines became high in the cytosol. There is little physiological evidence for this. We previously showed (Murthy and Bieber, 1992) the $K_{0.5}$ for L-carnitine is in the physiological range for carnitine with decanoyl-CoA as substrate; the $K_{0.5}$ for L-carnitine with the other substrate was not determined. The $K_{0.5}$ for decanoyl-CoA and palmitoyl-CoA shown in Table I are higher than the 0.6 μM values found previously (Murthy and Bieber, 1992), but different assay conditions and different preparations of COT were used. It is not konwn if the thiol reducing conditions used for the end-point isotope assay used previously (Murthy and Bieber, 1992) (presence of DTT) affects the $K_{0.5}$; the rate forward assay used for these studies is performed in the presence of thiol oxidizing conditions. The lack of inhibition of the reverse reaction by acylcarnitines such as methylglutarylcarnitine, pivaloylcarnitine, and valproylcarnitine, when concentrations identical to the decanoylcarnitine concentration were used, indicates that these acylcarnitines did not bind to the acylcarnitine binding site. This conclusion agrees with previous data using intact microsomes (Bieber et al., 1992).

The inhibitor data shown in Table II agrees with data for other carnitine acyltransferases and for the membrane-bound COT. D-carnitine is a weak inhibitor of most of the carnitine acyltransferases (Bieber, 1988) and aminocarnitine was previously shown to be a modest inhibitor of membrane-associated microsomal COT (Lilly at al., 1992). Although the inhibition by malonyl-CoA and by etomoxiryl-CoA is different for purified COT compared to the membrane-associated enzyme, the inhibition pattern is very similar to that of mitochondrial carnitine palmitoyltransferase which is inhibited by etomoxiryl-CoA and malonyl-CoA in intact mitochondria. The malonyl-CoA inhibition of mitochondrial carnitine palmitoyltransferse is also lost as mitochondria are disrupted with some detergents (McGarry et al., 1992, 1979). Although several interpretations for such data have been presented by different investigators, our interpretation is the data indicate that the malonyl-CoA binding site, as well as the primary etomoxiryl-CoA binding protein are lost during purification. We previously demonstrated that microsomes, like mitochondria, contain an etomoxiryl-CoA binding protein with molecular weight near 90,000 daltons (Murthy and Bieber, 1992, Lilly et al., 1992). Most investigators agree that a protein with a molecular weight between 86,000 and 90,000 daltons is

responsible for both etomoxiryl-CoA and maloyl-CoA inhibition of mitochondrial carnitine palmitoyltransferase, but there are different opinions about the function of the function of this protein in intact systems. Attempts are currently underway to determine if a malonyl-CoA-binding/etomoxiryl-CoA-binding protein is involved in conferral of malonyl-CoA sensitivity to COT associated with microsomes.

We previously purified mitochondrial carnitine acetyltransferase and canitine palmitoyltransferase and peroxisomal COT, and prepared antibodies against each. The antibody prepared against microsomal COT does not cross-react with carnitine acetyltransferase, but it exhibits weak interaction with the medium-chain/long-chain carnitine acyltransferases purified from peroxisomes and from mitochondria. The antibodies prepared against peroxisomal carnitine acetyltransferase and against mitochondrial carnitine palmitoyltransferase did not cross-react with the COT from microsomes, but anti-peroxisomal COT antibody showed a weak interaction with microsomal COT. The primary sequence of peroxisomal COT (Chatterjee et al., 1988) is different than that of liver mitochondrial carnitine palmitoyltransferase (Woeltje et al., 1990, Finocchiaro et al., 1991): although the amino acid sequence of microsomal COT is not known, the different antigenic properties and molecular weight indicate its sequence must be different than the above-mentioned carnitine acyltransfer-ases.

Thus, the solubility, the lability, the different molecular weights, and the immunological properties, as well as the kinetic profiles all support the conclusion that microsomal COT is different than the enzymes associated with peroxisomes or mitochondria, and the kinetic data strongly indicate that microsomal COT functions in the forward direction, namely conversion of acyl-CoA to acylcarnitines.

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Abbreviations

COT represents carnitine octanoyltransferase.
SDS-PAGE represents polyacrylamide gel electrophoresis.
DTBP represents (4, 4'-dipyridyl disulfide), also referred to as dithiodipyridine (DTP).

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〈국문초록〉

쥐 간의 마이크로소옴에서 분리한 Medium-Chain/Long-Chain Carnitine Acyltransferase의 특성에 관한 연구

쥐 간의 마이크로소옴에서 분리한 medium-chain/long-chain carnitine acyltransferase의 몇가지 특성과 kinetic parameter들을 결정하였다. 이 효소는 carnitine octanoyltransferase라고 하며 탄소수가 6개에서 16개의 탄소사슬을 가지는 포화 지방산의 acyl-CoA류에 대한 반응에서 $K_{o.5}$는 1.0에서 4.0μM의 값을 가졌으며, Vmax값은 이들에 대하여 조금 씩의 차이를 보였다. 이 효소는 정반응에서 반응 시간에 따라 일정비의 증가를 보였으나 역반응에서는 반응시간이 경과하면 증가비율의 더 커지는 경향을 나타내었다. 이러한 양상이 역반응을 조사하는데 대하여 어려움을 주었으나 $K_{o.5}$는 decanoylcarnitine과 CoASH의 반응에서 보면, CoASH에 대하여는 0.3mM, decanoylcarnitine에 대하여는 1.0과 4.0mM사이에 있었다. 이 효소에 대한 kinetic data는 acylcarnitine을 합성하는 반응에 대한 효소의 기능을 말해준다.

그리고 이 효소는 aminocarnitine에 의하여 어느 정도 저해가 되며 D-carnitine의 저해작용은 약했다. 이 효소를 기질의 없는 상태에서 4,4'dipyridildisulfide(-SH modifier의 일종)과 항온처리반응을 시킨 후에는 활성이 저해되었다. 이러한 억제 작용은 여러가지 acyl-CoA들과 carnitine이 존재하에서는 일어나지 않았다. 다른 실험 결과들이 보여주는 것처럼 valproylcarnitine, 3-methylglutarylcanrnitine 또는 pivaloylcarnitine을 기질로 한 경우에는 조금 사용하거나 사용하지 못하였다. 막에 존재하는 다른 효소들의 연구결과와 비교하면 이 효소는 malonyl-CoA에 의해서 저해 받지 않았으나 etomoxiryl-CoA는 약간 저해시키는 효과를 나타내었다.

COT에 대한 polyclonal antibody를 얻어서 순수 분리된 효소에 대한 Western blot을 한 결과 마이크로소옴의 단백질에 대한 COT의 M. W. 는 53,000dalton이었다. anti-microsomal COT antibody는 순수 분리된 쥐 심장의 미토콘드리아의 carnitine palmitoyltransferase와 peroxisome의 COT에 대하여 약한 cross 반응을 보였으나 carnitine acetyltransferase와는 cross 반응을 하지 않았다. anti-mitochondrial CPT와 anti-peroxsomal CAT에 대한 microsomal COT는 cross반응을 보이지 않았다. 억제제에 대한 실험 data와 분자량 Western blot분석결과는 쥐 간의 마이크로소옴에서 정제된 효소에 대한 결과이나 다른 세포 소기관에서 분리한 carnitine acyltransferanse는 이전에 정제된 효소를 사용하였다.