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Differences between Microsomal and Mitochondrial-Matrix Palmitoyl-Coenzyme A Hydrolase, and Palmitoyl-L-carnitine Hydrolase from Rat Liver

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Palmitoyl-CoA hydrolase (EC 3.1.2.2) and palmitoyl-L-carnitine hydrolase (EC 3.1.1.28) activities from rat liver were investigated. 1. Microsomal and mitochondrial-matrix palmitoyl-CoA hydrolase activities had similar pH and temperature optima, although the activities showed different temperature stability. They were inhibited by Pb^{2+} and Zn^{2+}. The palmitoyl-CoA hydrolase activities in microsomal fraction and mitochondrial matrix were differently affected by the addition of Mg^{2+}, Ca^{2+}, Co^{2+}, K^+ and Na^+ to the reaction mixture. ATP, ADP and NAD^+ stimulated the microsomal activity and inhibited the mitochondrial-matrix enzyme. The activity of both the microsomal and mitochondrial-matrix hydrolase enzymes was specific for long-chain fatty acyl-CoA esters (C_{12}–C_{18}), with the highest activity for palmitoyl-CoA. The apparent K_m for palmitoyl-CoA was 47μM for the microsomal enzyme and 17μM for the mitochondrial-matrix enzyme. 2. The palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase activities of microsomal fraction had similar pH optima and were stimulated by dithiothreitol, but were affected differently by the addition of Pb^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+} and cysteine. The two enzymes had different temperature-sensitivities. 3. The data strongly suggest that palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase are separate microsomal enzymes, and that the hydrolysis of palmitoyl-CoA in the microsomal fraction and mitochondrial matrix was catalysed by two different enzymes.

Long-chain fatty acyl-CoA thioesterase (palmitoyl-CoA hydrolase, EC 3.1.2.2) is present in various mammalian tissues and seems to be involved in both lipid and carbohydrate metabolism (Kurooka *et al.*, 1972; Porter & Long, 1956). However, no definite metabolic significance has been assigned to palmitoyl-CoA hydrolase (McMurray & Magee, 1972), although its properties have been described in some detail (Barden & Cleland, 1969; Jezyk & Hughes, 1971; Barber & Lands, 1971; Jansen & Hülsmann, 1973).

In a study from this laboratory, the enzymic hydrolysis of palmitoyl-CoA in the matrix fraction of rat liver mitochondria has been described, in addition to the well-established palmitoyl-CoA hydrolase activity found in microsomal fraction (Berge & Farstad, 1977, 1979). As the two palmitoyl-CoA hydrolase activities had different subcellular localizations, it was suggested that the hydrolysis of palmitoyl-CoA in the microsomal fraction and in the mitochondrial matrix was mediated by different enzymes. The present results support this suggestion.

In addition, as the palmitoyl-L-carnitine hydrolase activity was confined to the microsomal fraction of rat liver cells (Mahadevan & Sauer, 1969; Berge & Farstad, 1979), the possibility that microsomal palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase might be due to dual activities of a single microsomal enzyme (Berge & Farstad, 1977, 1979) was tested. Evidence in the present paper indicates that microsomal palmitoyl-CoA hydrolase activity differs from the microsomal palmitoyl-L-carnitine hydrolase activity.

Materials and Methods

Materials

All radioactive compounds were purchased from New England Nuclear Corp., Boston, MA, U.S.A. Palmitoyl-L-carnitine and L-carnitine were from Supelco, Bellefonte, PA, U.S.A. ADP, ATP, FAD, NAD^+, riboflavin, dithiothreitol, cysteine, palmitoyl-CoA, myristoyl-CoA, stearoyl-CoA, lauroyl-CoA, oleoyl-CoA, acetyl-CoA, 5,5′-dithiobis-(2-nitrobenzoic acid), *sn*-glycerol 3-phosphate and different tricarboxylic acid-cycle intermediates were from Sigma Chemical Co., St. Louis, MO, U.S.A. Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], Pipes (1,4-piperazinediethanesulphonic acid), Hepps [4-(2-hydroxyethyl)-1-piperazinepropanesulphonic acid] and Taps (3-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}-1-propanesulphonic acid) were from Calbiochem, Lucerne, Switzerland. Silica-gel plates were obtained from Merck, Darmstadt, Germany. Other chemicals

were of the highest purity commercially available. Deionized water which had been subsequently double-quartz-distilled was used throughout.

Preparation of microsomal fraction, mitochondria and mitochondrial matrix

Rat liver microsomal fraction, mitochondria and mitochondrial-matrix protein were isolated as previously described (Berge & Farstad, 1979) in 0.25mM-sucrose/10mM-Hepes buffer, adjusted to pH7.40 by 2M-KOH, at a concentration of approx. 30mg of protein/ml, 30mg of protein/ml and 4.8mg of protein/ml, respectively. The purity of the subcellular fractions and mitochondrial matrix was tested by measuring the activity of marker enzymes (Table 1).

Protein determination

Protein was determined by Folin–Ciocalteau reagent (Lowry *et al.*, 1951), with bovine serum albumin as a standard.

Enzymic assays

Rotenone-insensitive NADPH–cytochrome *c* oxidoreductase (EC 1.6.2.4), succinate–phenazine methosulphate oxidoreductase (EC 1.3.99.1) and malate dehydrogenase (EC 1.1.1.37) were assayed by standard techniques [Sottocasa *et al.* (1967), Arrigoni & Singer (1962) and Bergmeyer & Bernt (1974) respectively]. Spectrophotometric measurements were performed in a Shimadzu recording spectrophotometer (model MPS-5000). The hydrolysis of tripalmitoylglycerol was assayed as described by Jansen *et al.* (1977), that of cholesteryl oleate as described by Coleman & Bell (1976) and that of dipalmitoylglycero-3-phosphocholine as described by Khoo & Steinberg (1975).

The identities of the [1-^{14}C]palmitate and [1-^{14}C]-oleate were checked by t.l.c. on a silica-gel plate together with appropriate standards run in a system of light petroleum (b.p. 40–60°C)/diethyl ether/acetic acid (80:20:1, by vol.).

Assay of the hydrolysis of long-chain acyl-CoA esters

Spectrophotometric assay. Hydrolysing activity was assayed by measuring the release of thiol groups from the different acyl esters, all added to concentrations from 0 to 200μM. The assay mixture contained 0.25M-sucrose, 10mM-Hepes buffer, pH7.5, 1mM-EDTA, 0.3mM-5,5′-dithiobis-(2-nitrobenzoic acid), acyl-CoA esters, and enzymes in isolated microsomal fraction or mitochondrial matrix. The reaction was started by adding enzyme and was followed spectrophotometrically at 412nm. The amount of thiol released was calculated from the molar absorption coefficient, 1.36×10^4 litre$\cdot$mol$^{-1}\cdot$cm^{-1} (Means & Feeney, 1971). Since 5,5′-dithiobis-(2-nitrobenzoic acid) also reacts slowly with the thiol groups of proteins, the initial rates were corrected by controls run in the absence of acyl-CoA.

Radiochemical assay. The chain-length specificities for acyl-CoA esters of the mitochondrial-matrix enzyme and microsomal enzyme were determined by release of [1-^{14}C]fatty acids from [1-^{14}C]acyl-CoA esters as previously described (Berge & Farstad, 1977). The assay mixture contained: 0.25M-sucrose, 10mM-Hepes buffer, pH7.5, and the concentrations of [1-^{14}C]acyl-CoA esters (0.8–1.6nCi/nmol) given in the Tables or legends to Figures. The reaction mixtures were incubated at 35°C for 2–5min, and stopped by Dole (1956) extraction mixture. The microsomal palmitoyl-L-carnitine hydrolase activity was measured in the same assay mixture as mentioned above, but with [1-^{14}C]palmitoyl-L-carnitine as substrate.

Results

A high purity of the mitochondrial matrix and microsomal fraction was found, as judged by the distribution of marker enzymes (Table 1). When mitochondrial matrix was used as source of enzyme, equimolar quantities of unesterified fatty acid and CoA (measured as thiol groups) were released by the enzyme, showing that the reaction catalysed by the enzyme is a simple hydrolytic one involving no intermediates (results not shown).

Table 1. *Relative specific activity of marker enzymes in microsomal fraction, mitochondria and mitochondrial matrix*
The subcellular fractions were obtained by differential centrifugation and the mitochondrial matrix was obtained from digitonin-fractionated mitochondria (see the Materials and Methods section). The relative specific activity of the marker enzymes of the various fractions was calculated relative to the specific activity of the marker enzymes of the crude homogenate = 1.00.

	Rotenone-insensitive NADPH–cytochrome *c* oxidoreductase	Succinate–phenazine methosulphate oxidoreductase	Malate dehydrogenase
Microsomal fraction	3.72	0.01	0.21
Mitochondria	0.21	3.79	2.4
Mitochondrial matrix	0.02	0.28	6.8

Microsomal palmitoyl-CoA hydrolase activity

The palmitoyl-CoA hydrolase activity of rat liver microsomal fraction was proportional to the amount of protein up to 0.5mg/ml, and to time up to 2min when a protein concentration of 0.5mg/ml was used (results not shown). The activity had a broad pH optimum from 7.0 to 8.0 (results not shown).

The effects of several compounds on the microsomal palmitoyl-CoA hydrolase activity are shown in Fig. 1 and Table 2. EDTA at a concentration of 2mM moderately increased the activity (Fig. 1*d*), whereas addition of Mg^{2+} and Ca^{2+} (chloride salts) inhibited the microsomal palmitoyl-CoA hydrolase activity at all concentrations tested (Figs. 1*a* and 1*b*). Cations such as Mn^{2+} and Cu^{2+} (chloride salts) strongly inhibited at concentrations of 1.2mM, whereas Zn^{2+} and Pb^{2+} inhibited the activity by 20–50% at these concentrations (Fig. 1*f* and Table 2). No changes in activity occurred after the addition of dithiothreitol, whereas cysteine had a stimulating effect (Table 2). K^+ stimulated the activity up to a concentration of approx. 20mM, above which inhibition occurred (Fig. 1*e*). Sulphate and nitrate anions slightly inhibited the activity, whereas fluoride inhibited the activity by approx. 60%. Cyanide increased the activity by about 25% (Table 2). Freezing and thawing the microsomal preparation five times inhibited the activity by approx. 60%.

Mitochondrial-matrix palmitoyl-CoA hydrolase activity

The palmitoyl-CoA hydrolase activity from matrix of rat liver mitochondria was proportional to the amount of protein added up to 0.5mg/ml, and to time up to 5min when 0.5mg of protein/ml was used (results not shown). The activity had a pH optimum between 8.0 and 8.2 (results not shown). However, assays are not routinely run at this pH optimum, owing to the slow, non-enzymic hydrolysis of palmitoyl-CoA under these conditions. The effects of different compounds on mitochondrial-matrix palmitoyl-CoA hydrolase activity are summarized in Fig. 1 and Table 2. EDTA above 2mM stimulated the activity, but at a concentration of approx. 0.5mM inhibited the activity. The mitochondrial-matrix palmitoyl-CoA hydrolase activity was stimulated by 0.5mM-Mg^{2+}, and inhibited at high concentrations. A similar effect was found with Ca^{2+} (Figs. 1*a* and 1*b*). Co^{2+} and Pb^{2+} (chloride salts) slightly inhibited the activity, whereas Mn^{2+} and Zn^{2+} (chloride salts) inhibited the activity more strongly. Cu^{2+} inhibited the activity at all concentrations tested (Fig. 1*f*). Dithiothreitol at a concentration of 7.5mM stimulated the activity (30%), but essentially no change in activity was seen after addition of cysteine (Table 2). KCl stimulated ($P<0.05$) the hydrolase activity up to a concentration of approx. 125mM, above which

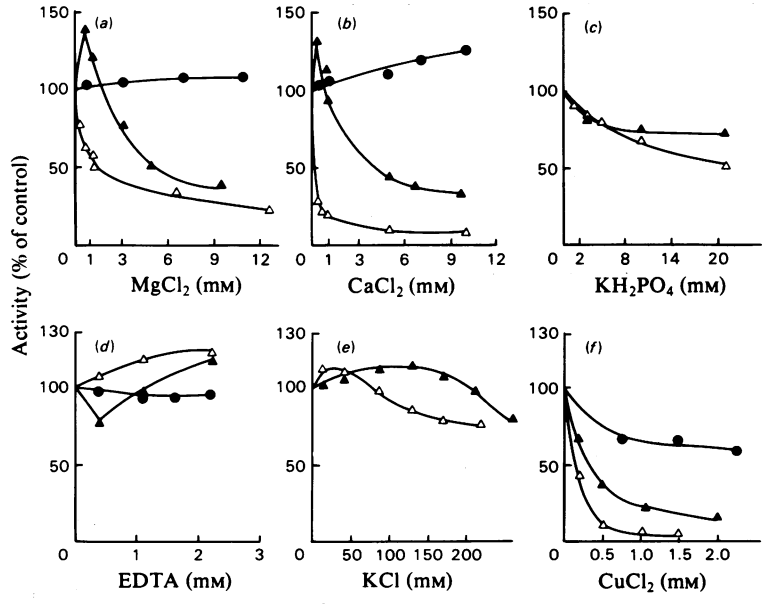

Fig. 1. *Effect of varying the concentration of (a) Mg^{2+}, (b) Ca^{2+}, (c) P_i, (d) EDTA, (e) K^+ and (f) Cu^{2+} on the rates of hydrolysis of palmitoyl-CoA and palmitoyl-L-carnitine*

Mitochondrial matrix (▲), and microsomal fraction (△) (approx. 0.5mg of protein/ml) were incubated for 3min with 50- and 125μM-palmitoyl-CoA respectively, or microsomal fraction was incubated with 100μM-palmitoyl-L-carnitine (●).

inhibition occurred (Fig. 1*e*). Sulphate and nitrate (1.25mM) inhibited the activity by approx. 20 and 30% respectively (results not shown), but fluoride had no effect (Table 2). Cyanide stimulated the activity. Freezing and thawing the mitochondrial matrix preparation five times caused a loss of approx. 55% of the activity.

Effect of different nucleotides

The activity of both microsomal and mitochondrial-matrix palmitoyl-CoA hydrolase was influenced by the presence of different nucleotides (Fig. 2). With microsomal fraction a moderate stimulation ($P<0.05$) was seen with ATP and ADP

Table 2. *Effect of ions and thiol reagents on the hydrolysis of palmitoyl-CoA and palmitoyl-L-carnitine by microsomal fraction and mitochondrial matrix isolated from rat liver*

The reaction mixture contained, in a total volume of 0.25ml, 0.25M-sucrose, 10mM-Hepes buffer, pH 7.5, and 0.5mg of both microsomal and mitochondrial-matrix protein/ml. The concentration of palmitoyl-L-carnitine was 100μM, of palmitoyl-CoA with the mitochondrial matrix 50μM and with microsomal fraction 125μM. Further additions were 1.2mM-cations and anions, 7.5mM-dithiothreitol and 10.0mM-cysteine. The results are means ± S.D., calculated from six determinations. Significance of differences of the activity in the absence and presence of stated additions was calculated by Student's *t* test: $^{**}P<0.005$; $^{*}P<0.05$.

Addition	Palmitoyl-CoA hydrolase activity (nmol/min per mg of protein)		Palmitoyl-L-carnitine hydrolase activity (nmol/min per mg of protein)
	Mitochondrial matrix	Microsomal fraction	Microsomal fraction
No addition	14.2±0.6	46.8±0.6	2.7±0.3
+Mn^{2+}	3.7±0.5**	1.9±0.6**	3.2±0.4*
+Co^{2+}	10.8±0.6**	2.3±0.7**	2.5±0.3
+Zn^{2+}	5.7±0.8**	22.5±0.5**	0.2±0.3**
+Pb^{2+}	11.8±0.5**	38.8±0.4**	3.1±0.4*
+Cyanide (K^+)	24.2±1.0**	59.0±1.0**	3.1±0.4*
+Fluoride (K^+)	13.6±0.4*	17.8±0.8**	1.3±0.3**
+Dithiothreitol	18.5±0.5**	48.2±0.5*	2.7±0.3
+Cysteine	15.4±0.4*	61.3±1.0**	2.3±0.3*

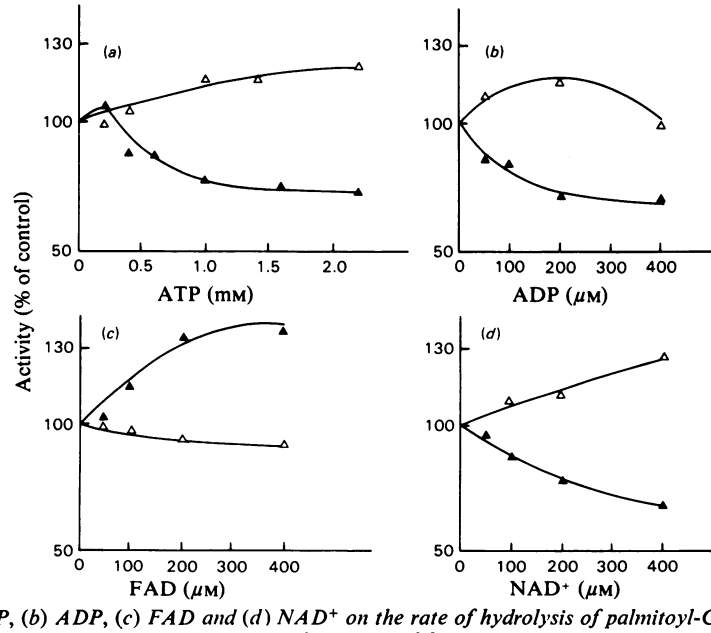

Fig. 2. *Effect of (a) ATP, (b) ADP, (c) FAD and (d) NAD^+ on the rate of hydrolysis of palmitoyl-CoA by mitochondrial matrix and microsomal fraction*

Matrix (▲) and microsomal fraction (△) (about 0.5mg of protein/ml) were incubated as described in the Materials and Methods section in the presence of 50 and 125μM-palmitoyl-CoA, respectively.

added in concentrations up to 2mM and 200μM, respectively. With mitochondrial matrix both ATP and ADP were, however, inhibitory. Addition of FAD up to 400μM stimulated the activity of the mitochondrial-matrix enzyme and slightly inhibited ($P<0.05$) the microsomal palmitoyl-CoA hydrolase activity. The microsomal palmitoyl-CoA hydrolase activity was stimulated by NAD^+, at least up to 400μM, but NAD^+ inhibited the mitochondrial-matrix palmitoyl-CoA hydrolase activity. FMN had a similar effect to NAD^+ on the hydrolase activities, and addition of riboflavin stimulated the activity of both enzymes (results not shown).

Effect of different metabolites and acids on the microsomal and mitochondrial-matrix palmitoyl-CoA hydrolase activities

No changes in microsomal and mitochondrial-matrix palmitoyl-CoA hydrolase activities were seen after addition of oxaloacetate, α-oxoglutarate, glutamate, pyruvate, α-glycerophosphate, aspartate and ascorbate (results not shown). Nicotinic acid and fumarate inhibited the mitochondrial-matrix palmitoyl-CoA hydrolase activity, whereas microsomal activity was stimulated (Table 3). Malate, succinate and β-hydroxybutyrate stimulated the mitochondrial-matrix activity. No change in microsomal activity was seen after the addition of succinate, whereas malate and β-hydroxybutyrate moderately inhibited the activity. Citrate increased the activity of both enzymes, especially that from the matrix.

Chain-length specificity

The microsomal and mitochondrial-matrix enzymes showed different specificities for thioesters of long-chain fatty acids (Fig. 3). At saturating concentrations of substrates the maximal rate of hydrolysis was found with palmitoyl-CoA, and activity decreased with either increasing and decreasing chain length of the substrates (Fig. 3*a*).

In the mitochondrial-matrix fraction the rates of hydrolysis of myristoyl-CoA and palmitoyl-CoA were approximately the same (Fig. 3*b*). At saturating concentration the rates of hydrolysis of stearoyl-CoA and oleoyl-CoA were about 55–60% of that of palmitoyl-CoA (Fig. 3*b*). Lower rates were observed with lauroyl-CoA (46%) and minimal hydrolysis was

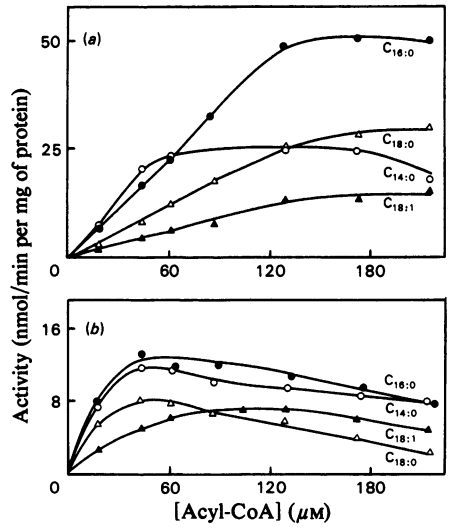

Fig. 3. *Comparison of rates of hydrolysis of fatty acyl-CoA derivatives as a function of acyl-CoA concentration*

Microsomal fraction (*a*) and mitochondrial matrix (*b*) (approx. 0.5mg of protein/ml) in the presence of the added acyl-CoA concentration as indicated were incubated for 2min. The rates of hydrolysis were determined radiochemically as described in the Materials and Methods section.

Table 3. *Effect of metabolites and acids on the microsomal and mitochondrial-matrix palmitoyl-CoA hydrolase activity*

Matrix and microsomal fraction (about 0.5mg of protein/ml) were incubated for 3min in the presence of 50 and 125μM-palmitoyl-CoA, respectively. Further additions were 4mM of the indicated acids. The results represent the means ± S.D., calculated from six determinations. The significance of differences between the activity in the absence and presence of stated additions was calculated by Student's *t* test: $^{**}P<0.005$; $^{*}P<0.05$.

Conditions	Relative activities of palmitoyl-CoA hydrolase (%) from Mitochondrial matrix	Microsomal fraction
Complete system	100	100
+Citrate	149 ± 10**	110 ± 8*
+Succinate	140 ± 8**	100 ± 5
+Fumarate	87 ± 8*	119 ± 7*
+Malate	120 ± 5**	84 ± 8**
+β-Hydroxybutyrate	111 ± 8*	79 ± 7**
+Nicotinic acid	68 ± 6**	147 ± 10**

detectable with acetyl-CoA (4.3%) (results not shown). The apparent K_m for palmitoyl-CoA was 47 μM for the microsomal enzyme and 17 μM for the mitochondrial-matrix enzyme.

Hydrolysis of tripalmitoylglycerol, cholesteryl oleate and dipalmitoylglycero-3-phosphocholine compared with palmitoyl-CoA hydrolysis

When tested under optimal conditions by the methods mentioned in the Materials and Methods section, the rates (Jansen *et al.*, 1977; Coleman & Bell, 1976; Khoo & Steinberg, 1975) of hydrolysis of tripalmitoylglycerol, cholesteryl oleate and dipalmitoylglycero-3-phosphocholine by microsomal fraction and mitochondrial matrix were only 1–5% of that obtained with palmitoyl-CoA as the substrate (results not shown). Addition of Ca^{2+} (5 mM) strongly inhibited the hydrolysis of palmitoyl-CoA (approx. 90%) and tripalmitoylglycerol (approx. 75%), but stimulated the hydrolysis of dipalmitoylglycero-3-phosphocholine (approx. 70%). Maximal hydrolysis of cholesteryl oleate and tripalmitoylglycerol was obtained in the presence of the detergent Triton X-100 (0.04%, v/v), which inhibited the hydrolysis of palmitoyl-CoA (approx. 20%) (results not shown).

Microsomal palmitoyl-L-carnitine hydrolase activity

The palmitoyl-L-carnitine hydrolase activity of the microsomal fraction of rat liver was proportional to the amount of protein added up to a concentration of 0.5 mg/ml, and to incubation time up to 6–7 min with 0.5 mg of protein/ml (results not shown). The activity had a broad pH optimum from 7.0 to 8.0 (results not shown). The effects of various compounds on the microsomal palmitoyl-L-carnitine hydrolase activity are shown in Fig. 1 and Table 2. No significant change in activity was seen in the presence of EDTA (Fig. 1*d*) or Mg^{2+} (Fig. 1*a*). Ca^{2+} at concentrations of about 10 mM moderately stimulated the activity (Fig. 1*b*). Mn^{2+} and Pb^{2+} (chloride salts) moderately stimulated the activity, whereas the chloride salt of Zn^{2+} was inhibitory at concentrations of about 1.2 mM (Table 2). Cu^{2+} inhibited activity at all concentrations tested (Fig. 1*f*). No change in activity was seen with the addition of 7.5 mM-dithiothreitol, whereas cysteine at about 10 mM slightly inhibited the activity (Table 2). Sulphate and nitrate (1.2 mM) inhibited the activity by approx. 15% (results not shown), and fluoride inhibited the activity by approx. 50% (Table 2).

Effect of incubation temperature on the hydrolysis of palmitoyl-CoA and palmitoyl-L-carnitine

The hydrolysis of palmitoyl-CoA and palmitoyl-L-carnitine was strongly influenced by change in the temperature. Maximal microsomal and mitochondrial-matrix palmitoyl-CoA hydrolase activities were both at approx. 50°C (results not shown). The microsomal palmitoyl-L-carnitine hydrolase activity revealed a maximum at around 42°C with an energy of activation of approx. 60 kJ/mol (results not shown).

The energy of activation for the microsomal and mitochondrial-matrix palmitoyl-CoA hydrolase was approx. 52 kJ/mol and 28 kJ/mol respectively (results not shown). Boiled microsomal fraction and mitochondrial matrix showed no hydrolase activity.

Thermal inactivation

Microsomal fraction and mitochondrial matrix were heated and samples were taken at various times for assay of palmitoyl-CoA and palmitoyl-L-carnitine hydrolase activities at 32°C. Both activities were stable with preincubation at 35°C for 5 min. At 45°C, 30% of the mitochondrial-matrix palmitoyl-CoA hydrolase activity was lost after 15 min of heating (results not shown). The microsomal palmitoyl-CoA hydrolase activity was more heat-stable, with none of its activity lost during 15 min of heating at 45°C. The microsomal palmitoyl-L-carnitine hydrolase activity was moderately thermolabile, with 25% of its activity lost during 15 min of heating at 45°C (results not shown).

Discussion

A high purity of the isolated mitochondrial matrix and microsomal fraction was obtained.

Triacylglycerol hydrolase has been reported to have some activity also with palmitoyl-CoA as the substrate (Jansen *et al.*, 1973; Jansen & Hülsmann, 1974). The low activities with tripalmitoylglycerol, cholesteryl oleate and dipalmitoylglycero-3-phosphocholine as substrates excluded the possibility that the hydrolysis of palmitoyl-CoA in the present study is catalysed by an unspecific lipase activity.

In view of the similarities of the reactions, products and the reactions catalysed by the microsomal and mitochondrial-matrix palmitoyl-CoA hydrolase activity, it was not surprising that the activity was similar with respect to their pH and temperature optima. The energy of activation for the microsomal and mitochondrial-matrix palmitoyl-CoA hydrolase is, however, different ($P<0.005$). Our results suggest that the hydrolysis of palmitoyl-CoA in microsomal fraction and mitochondrial matrix is mediated by different enzymes; the two palmitoyl-CoA hydrolase activities had different subcellular localizations, the activities had optima for different lengths of chains of acyl-CoA, the $V_{max.}$ of palmitoyl-CoA for the microsomal enzyme was 3.5 times that for the mitochondrial matrix enzyme, and they had different apparent K_m

values. Estimated kinetic parameters must, however, be considered tentative, as the maximal enzymic activity was seen at concentrations considerably above the critical micelle concentration for palmitoyl-CoA (Zahler *et al.*, 1967). Furthermore, they were differently inactivated by heating, differently affected by the addition of cations, nucleotides and metabolites, and differently inactivated by thiol reagents and EDTA.

A physiological role of palmitoyl-CoA as a regulator of mitochondrial metabolism has been postulated, which involves nicotinamide nucleotide transhydrogenase, the di- and tri-carboxylic acid carrier, pyruvate carboxylation, phosphate transport, adenine nucleotide translocase, changes in the permeability to cations and effect of energy coupling (Wojtczak, 1976). β-Oxidation in the mitochondrial matrix has three substrates, namely acyl-CoA, hydrogen acceptor (NAD^+ and flavin) and free CoA (for thiolase action). The first and second dehydrogenation steps in fatty acid oxidation involve flavin and NAD^+, respectively, as the specific electron acceptors. As the microsomal and mitochondrial-matrix palmitoyl-CoA hydrolase activities behaved differently on addition of metabolites and nucleotides, the availability of these electron acceptors, especially NAD^+, may influence the intramitochondrial acyl-CoA for fatty acid oxidation in tissues (Tubbs, 1977).

A comparative study of microsomal palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase is also presented. Several lines of evidence strongly suggest that palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase are separate enzymes: microsomal palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase were affected differently by the addition of cations, had different ($P<0.05$) temperature optima and were differently inactivated by heating.

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