Enzymes of carnitine acylation

Is overt carnitine palmitoyltransferase of liver peroxisomal carnitine octanoyltransferase?

Michael J. HEALY,* Janos KERNER and Loran L. BIEBER

Department of Biochemistry, Michigan State University, East Lansing, MI 48824, U.S.A.

Liver mitochondria prepared by differential centrifugation are contaminated by significant quantities of peroxisomes and microsomal fractions. 'Easily solubilized carnitine palmitoyltransferase' prepared from liver mitochondria is thought to originate from the outer surface of the mitochondrial inner membrane. We have characterized the carnitine palmitoyltransferase activities of freeze-thaw extracts of rat liver mitochondrial preparations. Chromatography on Sephadex G-100 yields two broad peaks of carnitine decanoyltransferase activity: one eluted at the end of the void volume, which can be removed (precipitated) by ultracentrifugation; the second peak represents the soluble activity and is eluted at an M_r near 70000. The activity in the soluble peak is precipitated by an antibody raised against carnitine octanoyltransferase purified from mouse liver peroxisomes. In contrast, antibody raised against carnitine palmitoyltransferase purified from liver mitochondrial membranes had no effect (P. Brady & L. Brady, personal communication). The carnitine acyltransferase activities of the M_r -70000 peak in the presence or absence of Tween 20 showed maximum activity with decanoyl-CoA and about one-third of this activity with palmitoyl-CoA, similar to peroxisomal carnitine octanoyltransferase. These data show that 7500 g preparations of liver mitochondria isolated by differential centrifugation are enriched by peroxisomal carnitine octanoyltransferase (approx. 20 % of the protein of the fraction is peroxisomal) and indicate that this enzyme may be the one reported as 'overt' or 'easily solubilized' mitochondrial carnitine palmitoyltransferase.

INTRODUCTION

Carnitine (γ -trimethylamino- β -hydroxybutyrate) possesses the function of transporting acyl moieties that are straight-chain, branched-chain or unsaturated hydrocarbons with chain lengths between C_2 and C_{18} from the cytosol across the mitochondrial inner membrane into the matrix. The acylated carnitine is subsequently transformed into free carnitine and an acyl derivative of CoA, which is subjected to mitochondrial oxidation.

Liver mitochondria contain at least two carnitine-dependent acyltransferases (Bieber & Farrell, 1983). One is specific for short-chain-length acyl groups, particularly C_2 -CoA and C_3 -CoA (Miyazawa *et al.*, 1983a), and the other enzyme exhibits broad chain-length specificity, from C_8 -CoA up to at least C_{18} -CoA; it is referred to as CPT (Bergstrom & Reitz, 1980; Miyazawa *et al.*, 1983b).

CPT is a membrane-bound enzyme, and is proposed by many to exist in two functionally distinct forms in the mitochondrial inner membrane. The model advocated since about 1967 is that one form (an overt form) exists as an enzyme that is loosely attached to the inner membrane, partly projects into the intermembrane space, and is easily solubilized, owing to its exposed environment, by techniques such as freeze-thawing, ultrasonication or digitonin treatment. The other form (a latent form) is more firmly embedded within the mitochondrial inner membrane (Hoppel, 1976) and requires detergents such as Triton, octyl glucoside or bile salts for solubilization. More recently, evidence has

been presented indicating that CPT-I (CPT_o) may be associated with the mitochondrial outer membrane (Murthy & Pande, 1987). The overt and latent forms of CPT were considered, on the basis of immunological and M_r studies of the liver enzymes by Bergstrom & Reitz (1980) and of the bovine heart enzymes by Clarke & Bieber (1981), to be identical polypeptides.

More recent results have raised some questions about the identity in structural terms of the two forms of CPT. Enzymes, isolated apparently from the mitochondrion by two groups, were thought to be the easily solubilized enzyme. This enzyme was distinguished from the latent enzyme on the basis of differences in activity by using substrate analogues as inhibitors (Derrick *et al.*, 1985; Hoppel *et al.*, 1986).

Many of the characteristic properties of the easily solubilized transferase resemble those of peroxisomal COT. Both transferases show activity with palmitoyl-CoA, are easily solubilized from liver, are monomers with apparent M_r values of 60000–70000, and are inactivated by 2-bromopalmitoyl-CoA. But liver carnitine-dependent transferases exist in at least three sub-cellular locations: mitochondria, peroxisomes and endoplasmic reticulum (Markwell *et al.*, 1973). Furthermore, these enzymes exhibit a broad range of specificities with regard to C_n -CoA, and their kinetic properties, particularly those of the mitochondrial transferase, are exquisitely sensitive to particular assay conditions.

Liver mitochondrial preparations, unless prepared by continuous sucrose-density-gradient centrifugation, are contaminated by significant quantities of peroxisomes

Abbreviations used: C_n -CoA, acyl-CoA, where n = acyl chain length; CPT, carnitine palmitoyltransferase; COT, carnitine octanoyltransferase; DTNB, 5,5'-dithiobis-(2-nitrobenzoate).

* On leave of absence during 1986 from the Commonwealth Department of Science, P.O. Box 65, Belconnen, A.C.T. 2616, Australia.

and microsomal fractions (de Duve & Baudhuin, 1966; Schnaitman & Greenawalt, 1968; Tolbert, 1981). The findings reported herein reconfirm that liver mitochondrial preparations isolated by differential centrifugation are enriched in peroxisomal COT and strongly indicate that this enzyme may be the one reported as overt or easily solubilized CPT from mitochondria.

MATERIALS AND METHODS

Preparation of mitochondria

Mitochondria were prepared by differential centrifugation as follows: Sprague-Dawley rats were fed *ad libitum* and killed by exposure to CO_2 , followed by decapitation. Livers were rinsed and cooled in ice-cold 0.25 M-sucrose/1 mM-EDTA/phenylmethanesulphonyl fluoride (25 $\mu\text{g}/\text{ml}$). Then 3 vol. of the sucrose solution was added to 1 vol. of liver, and the chopped liver was homogenized with a loose-fitting Teflon pestle until the suspension appeared homogeneous. The suspension was centrifuged for 20 min at 600 g . The supernatant fluid was decanted through cheesecloth and centrifuged for 20 min at 7400 g . The pellet was gently resuspended in 3 vol. of sucrose solution and re-centrifuged at 6400 g for 20 min. The supernatant was discarded, and the pellet containing the mitochondria-enriched 600 g /7400 g fraction of rat liver extract was immediately subjected to freeze-thawing as described below.

Solubilization of transferases

The pellet from the previous step was suspended in 10 vol. of ice-cold 10 mM-potassium phosphate/1 mM-EDTA/phenylmethanesulphonyl fluoride (25 $\mu\text{g}/\text{ml}$) (pH 7.5) and freeze (-60°C)-thawed (10°C) three times over 24 h. The suspension was centrifuged for 20 min at 40000 g , and the supernatant was collected and re-centrifuged at 105000 g for 2 h. The supernatant was decanted, and appeared as a dark-brown solution visibly clear of particulate matter.

Anti-(mouse liver peroxisomal COT) antibody

Serum containing this antibody was that described by Farrell *et al.* (1984).

Assays

Carnitine acyltransferases were assayed in the forward direction (formation of acylcarnitine) with 50 μM -acyl-CoA (Bieber *et al.*, 1972) by continuously monitoring release of CoA with DTNB at 412 nm. The 2.0 ml reaction mixture contained 115 mM-Tris buffer, 1 mM-EDTA, 0.1 mM-DTNB, 6 mM-L-(–)-carnitine, and acyl-CoA, pH 7.5, at 25°C .

Kinetic data were obtained with a semi-automated system described by LeBlond *et al.* (1980a,b). Raw absorbance/time data were obtained by continuously increasing the acyl-CoA concentration of a stirred enzyme-assay mixture with the use of a precision syringe drive during a reaction time of 3.6 min, and collected with a Gilford model 2600 spectrophotometer. The total volume change in the cuvette during the reaction was always less than 5%, and the final varied substrate concentration was optimized to give a value of $3\text{--}5 \times K_m$. The raw data were transformed into velocity/substrate data by a tangent slope procedure and then analysed as linear plots by using the TANKIN program with a Hewlett-Packard calculator. All assays were corrected

Fig. 1. Kinetic profile of freeze-thawed extracts of rat liver mitochondria: v versus $[\text{C}_{10}\text{-CoA}]$

The assay was conducted with 0.1% Triton X-100 present. Control assays were made either without the addition of carnitine or with omission of enzymic extract. The insert shows the same data in the form $1/v$ versus $1/[\text{C}_{10}\text{-CoA}]^{1.2}$.

for acyl-CoA hydrolase activity as described previously by Fiol & Bieber (1984). Citrate synthase was assayed by the method of Srere (1969), urate oxidase as described in Bergmeyer (1974) and catalase as described by Tolbert (1974). Protein was determined by the modified Lowry method (Markwell *et al.*, 1981).

Gel filtration

Sephadex G-100 (bead size 40–120 μm), with a protein exclusion limit for average M_r above 150000, was equilibrated in 10 mM-potassium phosphate/1 mM-EDTA and loaded into a Pharmacia column to give a bed volume of 1.4 litres (5 cm $\times$ 71 cm). Sample volumes of 36 ml were loaded with a Pharmacia SA-50 Sample Applicator. Fractions of volume 7.5 ml were collected at a flow rate of 1 ml/min, by using a Gilson micro fractionator. All operations were conducted at $5\text{--}7^\circ\text{C}$. The column was calibrated twice with a mixture of Blue Dextran and bovine serum albumin (M_r 68000; Sigma product).

RESULTS AND DISCUSSION

Kinetic profile of freeze-thawed extracts of rat liver mitochondria

A mitochondria-enriched fraction from rat liver was prepared by differential centrifugation, then freeze-thawed, centrifuged at 40000 g (see the Materials and methods section for details), and the supernatant fluid was analysed. 'Soluble' detergent-free extracts prepared by freeze-thawing showed about 4 times more activity with $\text{C}_{10}\text{-CoA}$ than with $\text{C}_{16}\text{-CoA}$. Such a ratio is expected for COT on the basis of published substrate-activity ratios of enzymes purified from rat liver: COT is about 3 and CPT is 1 (Miyazawa *et al.*, 1983b).

Soluble extracts were also examined kinetically with $\text{C}_{10}\text{-CoA}$ as variable substrate (Fig. 1). The profiles obtained from a number of preparations showed mixed, albeit predominantly hyperbolic, enzyme kinetics, with Hill coefficients (h) in the range 1–1.2 and $K_{0.5}$ values for

Fig. 2. Sephadex G-100 gel filtration of the freeze-thawed extract before (a) and after (b) ultracentrifugation

Soluble freeze-thawed extracts of a mitochondria-enriched fraction from rat liver (starting livers weighed 132 g; details in the Materials and methods section) were centrifuged (a) or ultracentrifuged (b) and filtered on a column of Sephadex G-100. After elution of most (400 ml) of the void volume, fractions were collected and assayed for C_{10} -CoA/carnitine acyltransferase activity. (Profile a was obtained from proportionally smaller sample volumes and columns than was profile b; variables were normalized to those of profile b to enable more direct comparison.)

C_{10} -CoA between 3 and 10 μ M. Hyperbolic kinetics with a Hill coefficient of 1 have been reported for purified COT (Farrell *et al.*, 1984), whereas sigmoidal kinetics with a Hill coefficient of 2 or greater have been reported for CPT I (the CPT associated with the cytosolic face of the mitochondrial inner membrane) (Saggerson & Carpenter, 1981; C. J. Fiol, unpublished work). Extracts were also examined with C_{16} -CoA as the variable substrate up to concentrations as high as 1 mM, but the profiles obtained were not readily interpreted, possibly because of substrate detergent and micelle effects (Bartlett *et al.*, 1985). Although the profile shown in Fig. 1 suggests that peroxisomal COT is dominant in these crude extracts, the presence of mitochondrial CPT is not ruled out.

Sephadex G-100 filtration of freeze-thawed extracts of rat liver mitochondria

Ultracentrifuged soluble extracts were filtered on Sephadex G-100, and fractions were examined for catalytic activity by using C_{10} -CoA (Fig. 2). Two separate peaks were observed, the first being eluted at the end of the void volume and the second at the elution position of bovine serum albumin (a standard). Both peaks exhibited CPT activity. The total decanoyltransferase activity eluted from the column was distributed in the ratio of 8% to 92% between the two peaks respectively. When the 40 000 g-supernatant fluid was used without ultracentrifugation, the first peak increased in size and the distribution of relative activity became approx. 35% to 65%.

The substantial absence of the first peak when the extract was ultracentrifuged, as well as its position within the void volume, suggested that its transferase activity arose from insoluble membrane-bound material. The

Fig. 3. C_n -CoA substrate-specificity profile of '70000- M_r ' pool

Assays were conducted as described in the Materials and methods section, except for the absence (○) or presence (●) of 0.2% Tween 20. The acyl-CoA concentration was 100 μ M.

two most likely sources of particulate material were microsomal fractions, which are known to contain a membrane-bound COT, and/or mitochondrial membrane fragments containing CPT. The second peak contained one or more soluble carnitine acyltransferases with M_r value(s) near 70000. Fractions containing the '70000- M_r ' peak (fractions 24-44) were pooled and subjected to further characterization as described below.

C_n -CoA substrate specificity of '70000- M_r ' pool from gel-filtration column

The substrate-specificity profile for the '70000- M_r ' pool is illustrated in Fig. 3. Activities of the pool with acyl-CoAs of chain lengths C_4 to C_{18} in the presence or absence of Tween 20 showed maximum activity near C_{10} , and about one-third of this activity with C_{16} . This profile is very similar to that shown for COT by Miyazawa *et al.* (1983b). Both profiles were obtained with nearly identical assay conditions. [Studies on CPT substrate specificity indicate that CPT (rat heart) is very sensitive to the detergent used. Profiles of C_n -CoA activities are completely different in assays with 0.1% Triton X-100 from those with 12 mM-octyl glucoside (Fiol, 1986).]

The substrate-specificity profile for rat liver CPT (Miyazawa *et al.*, 1983b) shows strong stimulation of activities with C_{10} -CoA to C_{16} -CoA when Tween 20 is included in the assays. The substrate-specificity profile for COT (Miyazawa *et al.*, 1983b) shows comparatively

Fig. 4. Immunotitration of transferase activity in the '70000- M_r ' pool with rabbit serum containing anti-(mouse liver peroxisomal COT) antibody

Assays were conducted as described in the Materials and methods section, except for addition of portions of diluted serum. Controls employed serum from a non-immunized rabbit.

little stimulation of activities with C_{10} -CoA to C_{16} -CoA when Tween 20 is included in the assays. Since the '70000- M_r ' pool also exhibited little stimulation of activities by Tween 20, it is apparent that the transferase(s) activity primarily includes COT and not CPT in the form isolated by Miyazawa *et al.* (1983b).

The skewed form of the profile in Fig. 3 may be caused by the presence of soluble peroxisomal carnitine acetyltransferase. This transferase is slightly active with C_{10} -CoA and is readily released by lysis of the fragile peroxisomal membrane (Bieber & Farrell, 1983). It has an M_r near 60000.

Immunoreaction of '70000- M_r ' transferase pool from gel-filtration column and easily soluble bound CPT

Fig. 4 represents a characteristic immunotitration of the transferase activity in the '70000- M_r ' pool with rabbit antiserum raised against pure mouse liver COT; approx. 95% of the transferase activity was precipitated. The antibody partially immunoprecipitates crude rat liver preparations of COT (Farrell *et al.*, 1984) that may contain enzymes from both peroxisomal and microsomal sources. Western blots of the '70000- M_r ' pool did not produce precipitation bands in the 70000- M_r region. However, purified COT also does not produce precipitation bands in Western blots, presumably owing to denaturation of COT by SDS in the gel. The anti-COT serum appears to be an active-site-directed antibody; thus treatments which destroy catalytic activity can also destroy antibody binding. Mouse peroxisomal COT antibodies appear to be quite specific for COT, whether the assays are based on immunoprecipitation or activity; they do not exhibit specificity towards mitochondrial CPT (Miyazawa *et al.*, 1983b; Farrell *et al.*, 1984).

Similarly, rabbit antiserum raised against pure rat liver CPT does not titrate or cross-react with COT (Miyazawa *et al.*, 1983b). A similar antibody to inner CPT from ox liver mitochondria did not react with the '70000- M_r ' pool, nor form a line on an Ouchterlony plate (results not shown; R. R. Ramsay, personal communication). In

Fig. 5. Immunotitration of the 'easily soluble' carnitine acyltransferase from bovine liver and mouse liver peroxisomal COT

Purified 'easily soluble' CPT was kindly provided by Dr. Rona Ramsay, Veterans Administration Medical Center, University of California, San Francisco, CA, U.S.A., and purified COT from mouse liver peroxisomes was that purified by Farrell (see Farrell *et al.*, 1984). Assays were performed as for Fig. 4.

the enzyme-linked immunosorbent assay test, purified 'soluble CPT' from ox liver also did not react with the antibody to purified inner CPT, whereas anti-COT antibody showed strong cross-reactivity. In addition, the activity of the soluble CPT isolated from bovine liver mitochondrial fractions by Dr. R. R. Ramsay was inhibited by anti-COT antibody (Fig. 5).

Properties of the first peak from the Sephadex G-100 column

Fig. 2 illustrates gel-filtration results with a conventional, but crude, preparation of rat liver mitochondria. Mitochondria were freeze-thawed, ultracentrifuged, and the supernatant was filtered. However, replacing ultracentrifugation by a centrifugation at 40000 g altered the profile shown in Fig. 2(b) to one when the first peak is substantially increased. This peak was pooled, and some of its properties were examined.

The carnitine decanoyltransferase activity in this first peak appeared to be particulate (insoluble), since it was eluted in the void volume and could be removed by ultracentrifugation before filtration.

Neither the first nor the second peaks from the gel filtration was inhibited by 20 μM -malonyl-CoA. No inhibition is expected for the second peak if it consists of peroxisomal COT. The cause for the lack of inhibition of the first peak is more difficult to explain. The difficulty probably lies in the assay conditions. Although intact mitochondria exhibit sensitivity of CPT to malonyl-CoA when the DTNB assay is used, this may not be the case once the membrane environment is disrupted by freeze-thawing (McGarry *et al.*, 1983). Our preliminary conclusion is that this first peak comprises mitochondrial membrane fragments.

Table 1. Peroxisomal contribution to CPT activity in rat liver mitochondria isolated by differential centrifugation

The '%' values refer to the distribution of activities in the three fractions. Liver mitochondria from three fed animals were isolated by differential centrifugation at 8000 *g* and washed once. For 20 h-starved animals, the mitochondria were isolated by differential centrifugation at 8000 *g* and washed twice. The isolation medium is described in the Materials and methods section. The sucrose gradient for mitochondria from starved animals was, from top to bottom, 36–56% (w/w) sucrose in 2% increments (each increment 5 ml). A 62%-sucrose cushion was at the bottom. For each gradient 2.0 ml of mitochondria in 25% sucrose was placed on top of the gradient, and the samples were spun for 3 h at 77000 *g*_{av}. The sucrose gradient for mitochondria from fed animals ranged from 25 to 50% (w/w), with 5% increments (each increment was 10 ml). Abbreviation: AB, anti-(peroxisomal COT) antibody. Palmitoyl-CoA was used as substrate for preparations from fed, and decanoyl-CoA as the substrate for preparations from starved, animals. —, Not determined.

Fraction	Animals	Protein (%)	Uricase			Citrate synthase			CPT			Catalase (%)
			(munits/mg)	(munits)	(%)	(munits/mg)	(munits)	(%)	(munits)	(%)	(% inhibition by AB)	
Supernatant	Fed	21.1	Not detected	—	4	49	1544	6.1	760	25	95	—
	Starved	17	—	—	—	—	437	5.7	285	22	95	33
Mitochondria	Fed	47.5	24.5	1749	23.6	251.3	18421	73	1635	54.1	16.7	—
	Starved	59	—	—	28	—	6421	84	702	56	14.3	8.5
Peroxisomes	Fed	15	126.5	3000	40.5	97	1843	7.3	319	10.6	92.0	—
	Starved	24	—	—	68	—	746	9	278	23	86	59

Table 2. Comparison of the properties of the two enzymes isolated by Derrick *et al.* (1985) and Hoppel *et al.* (1986) with the enzymes isolated by several other groups

Enzyme characteristic	Reference ...	The present paper	Hoppel <i>et al.</i> (1986)	Derrick <i>et al.</i> (1985)	Farrell <i>et al.</i> (1984)	Miyazawa <i>et al.</i> (1983b)	Miyazawa <i>et al.</i> (1983b)
Hepatic source	Crude rat liver mitochondria, freeze-thawed	Crude rat liver mitochondria, freeze-thawed	Ox liver mitochondria, sonicated	Ox liver mitochondria, thawed and homogenized	Mouse liver peroxisomes, freeze-thawed	Rat liver, freeze-thawed	Rat liver crude mitochondrial membranes, freeze-thawed
Stage of purification	Ultracentrifugation, gel filtration	Ultracentrifugation, gel filtration	Homogeneity	Homogeneity	Homogeneity	Homogeneity	Homogeneity
<i>M_r</i>	60000–70000	60000	66000	62500	60000	65000	4 × 69200
Effect of 2-bromo- <i>C</i> ₁₆ -CoA	—	Inactivated	Inactivated	Inactivated	—	Inactivated	Fully active
Effect of malonyl-CoA	—	Inhibited	Inhibited	Fully active	Fully active	Fully active	Inhibited
Effect of anti-COT serum	Fully active	—	—	—	Inhibited	Inhibited	Fully active
Effect of anti-CPT serum	Fully active	—	—	—	—	Fully active	Inhibited
Activity ratio <i>C</i> ₁₆ -CoA/ <i>C</i> ₈ -CoA	0.5	—	—	2	0.1	0.3	2.5
Identification	Peroxisomal COT	Mitochondrial overt CPT	Mitochondrial overt CPT	Mitochondrial overt CPT	Peroxisomal COT	Peroxisomal COT	Mitochondrial CPT

Sucrose-gradient distribution of COT and the protein of a 7500 g rat liver mitochondria preparation

Since the results show that rat liver mitochondria isolated by differential centrifugation are highly contaminated by peroxisomal COT, rat liver mitochondria from both fed and starved animals were isolated by conventional 8000 g differential centrifugation and washed, once for those from fed and twice for those from starved animals. The mitochondria-enriched fractions were subjected to sucrose-density-gradient fractionation, which separates peroxisomes from mitochondria. Urate oxidase was used as a peroxisomal marker and citrate synthase as a mitochondrial marker. The percentage distribution of citrate synthase, protein, urate oxidase and soluble decanoyltransferase activity (starved rats) and palmitoyltransferase activity (fed rats) as well as the percentage of CPT inactivated by peroxisomal anti-COT serum are shown in Table 1. The data show that most of the soluble carnitine acyltransferase (easily soluble CPT) is associated with the peroxisomal fraction and the soluble fraction. The soluble fraction should contain the carnitine acyltransferase from broken peroxisomes (see Markwell *et al.*, 1973). Approx. 20% of the protein of the 8000 g fraction was found in the peroxisome band, with another approx. 20% in the soluble fraction, much of which must be derived from peroxisomes, owing to their fragility and owing to the occurrence of high amounts of catalase and low quantities of citrate synthase. Only about 60% of the protein is associated with the mitochondria band. It is interesting that Ishii *et al.* (1985) have shown that approx. 50% of the total CPT activity of chick liver is due to a soluble peroxisomal enzyme with apparent K_m values for acyl-CoAs that are as low as, or lower than, those for mitochondrial CPT. These data re-affirm the necessity for caution about claims for the cellular location of soluble CPT in fractions isolated by differential centrifugation.

Conclusion

Table 2 is a summary and comparison of our investigation with the conclusions of Hoppel *et al.* (1986), Derrick *et al.* (1985), Farrell *et al.* (1984) and Miyazawa *et al.* (1983b) on liver carnitine medium-/long-chain-length acyltransferases. All of these workers purified an enzyme from mouse, rat or bovine liver to homogeneity.

Of particular interest are the conclusions of Hoppel *et al.* (1986) and Derrick *et al.* (1985) that their transferases originated from the outer surface of the mitochondrial inner membrane. For each case, mitochondria isolated by differential centrifugation are almost certainly contaminated by slightly heavier peroxisomes. Lysis of mitochondria is accompanied by lysis of the more fragile peroxisomes, whether the method is freeze-thaw, sonication, or detergent disruption. Assignments of a subcellular location for an enzyme are not justified on the basis of starting preparations that employ mixed populations of subcellular organelles. The data in Table 1 show that preparation of mitochondria by using density-gradient centrifugation to separate peroxisomes, microsomal fractions and other organelles from mitochondria is highly desirable for liver.

Both COT and CPT show activity towards the same substrates, i.e. C_8 -CoA, C_{10} -CoA or C_{16} -CoA. The enzymes change their K_m and V_{max} according to the

assay conditions. CPT is particularly tractable. Depending on assay conditions, the activity ratio of C_{16} -CoA to C_{10} -CoA can be inverted (Fiol, 1986). It appears that CPT, either in pure form or in mitochondria, shows complex kinetics. The use of C_{16} -CoA alone to assess CPT activity can be misleading, owing to micelle formation etc. C_{10} -CoA has some advantages over C_{16} -CoA, because assays can be done in the absence of micelles and albumin; however, the $K_{0.5}$ for L-carnitine is higher for decanoyl-CoA than for palmitoyl-CoA (Fiol & Bieber, 1984).

Most CPT remains tightly membrane-bound after freeze-thawing of mitochondria (Woldegiorgis *et al.*, 1985). However, COT from contaminating peroxisomes is readily solubilized by freeze-thawing. Our preparations have reconfirmed that liver mitochondrial preparations isolated by differential centrifugation are enriched by peroxisomal COT, and indicate that this enzyme may be the one reported as overt or easily solubilized CPT.

Similar findings have been reported by Ramsay (1988).

Portions of this work were presented at the 71st Annual Meeting of the Federation of American Societies for Experimental Biology, March 29 to April 3, 1987, in Washington, D.C. (Bieber & Healy, 1987). This work was supported by U.S. National Institutes of Health Research Grant AM-18427. L-Carnitine was generously given by Sigma Tau Pharmaceutical. We thank Dr. Rona Ramsay for her generous gift of easily soluble bovine liver CPT. This is Paper No. 12301 from the Michigan State Agricultural Experiment Station.

REFERENCES

- Bartlett, K., Bartlett, P., Bartlett, N. & Sherratt, H. S. A. (1985) *Biochem. J.* **229**, 559–560
- Bergstrom, J. D. & Reitz, R. C. (1980) *Arch. Biochem. Biophys.* **204**, 71–79
- Bergmeyer, H. U. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), vol. 1, p. 518, Verlag Chemie, Weinheim, and Academic Press, New York
- Bieber, L. L. & Farrell, S. O. (1983) *Enzymes* 3rd Ed. **16**, 627–644
- Bieber, L. L. & Healy, M. J. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46**, paper no. 4058
- Bieber, L. L., Abraham, T. & Helmrath, T. (1972) *Anal. Biochem.* **50**, 509–518
- Clarke, P. R. & Bieber, L. L. (1981) *J. Biol. Chem.* **256**, 9861–9868
- de Duve, C. & Baudhuin, P. (1966) *Physiol. Rev.* **46**, 323–357
- Derrick, J. P., Tubbs, P. K. & Ramsay, R. R. (1985) *Biochem. Soc. Trans.* **14**, 698
- Farrell, S. O., Fiol, C. J., Reddy, J. K. & Bieber, L. L. (1984) *J. Biol. Chem.* **259**, 13089–13095
- Fiol, C. J. (1986) Ph.D. Thesis, Michigan State University
- Fiol, C. J. & Bieber, L. L. (1984) *J. Biol. Chem.* **259**, 13084–13088
- Hoppel, C. L. (1976) in *The Enzymes of Biological Membranes* (Martonosi, A., ed.), vol. 2, pp. 119–143, Plenum Publishing Corp., New York
- Hoppel, C., Turkaly, J., Albers, L. & Wilson, A. (1986) *Abstr. Annu. Meet. Am. Soc. Biol. Chem.* 76th abstr. no. 676
- Ishii, S., Ishii, H. & Suga, T. (1985) *J. Biochem. (Tokyo)* **98**, 747–755
- LeBlond, D. J., Ashendel, C. L. & Wood, W. A. (1980a) *Anal. Biochem.* **104**, 355–369

- LeBlond, D. J., Ashendel, C. L. & Wood, W. A. (1980*b*) *Anal. Biochem.* **104**, 370–385
- Markwell, M. A. K., McGroarty, E. J., Bieber, L. L. & Tolbert, N. E. (1973) *J. Biol. Chem.* **248**, 3426–3432
- Markwell, M. A. K., Haas, S. M., Tolbert, N. E. & Bieber, L. L. (1981) *Methods Enzymol.* **72**, 296–303
- McGarry, J. D., Mills, S. E., Long, C. S. & Foster, D. W. (1983) *Biochem. J.* **214**, 21–28
- Miyazawa, S., Ozasa, H., Furuta, S., Osumi, T. & Hashimoto, T. (1983*a*) *J. Biochem. (Tokyo)* **93**, 439–451
- Miyazawa, S., Ozasa, H., Osumi, T. & Hashimoto, T. (1983*b*) *J. Biochem. (Tokyo)* **94**, 529–542
- Murthy, S. R. & Pande, S. V. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 378–382
- Ramsay, R. R. (1988) *Biochem. J.* **249**, 239–245
- Saggerson, E. D. & Carpenter, C. A. (1981) *FEBS Lett.* **132**, 166–168
- Schnaitman, C. & Greenawalt, J. W. (1968) *J. Cell Biol.* **38**, 158–175
- Srere, P. A. (1969) *Methods Enzymol.* **13**, 3–11
- Tolbert, N. E. (1974) *Methods Enzymol.* **31**, part A, 734–746
- Tolbert, N. E. (1981) *Annu. Rev. Biochem.* **50**, 133–157
- Woldegiorgis, G., Bremer, J. & Shrago, E. (1985) *Biochim. Biophys. Acta* **837**, 135–145

Received 5 January 1987/5 May 1987; accepted 11 September 1987