Isolation of a Malonyl-CoA-Sensitive CPT/β-Oxidation Enzyme Complex from Heart Mitochondria†

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ABSTRACT: The goal of this study was to establish conditions for solubilization and characterization of CPT_o, the malonyl-CoA sensitive form of mitochondrial carnitine palmitoyltransferase. CPT_o of heart mitochondria is soluble in 1% octyl glucoside with retention of malonyl-CoA sensitivity. The degree of malonyl-CoA sensitivity is dependent on both the concentration of octyl glucoside and the presence of salt (KCl). In mannitol–sucrose, 0.5–1% octyl glucoside solubilizes CPT_o without loss of malonyl-CoA sensitivity; however, either increasing the detergent concentration or addition of KCl promotes loss of malonyl-CoA sensitivity. The immunoglobulin fraction from immune serum obtained from rabbits immunized with the malonyl-CoA-insensitive form of CPT (CPT_i) purified from beef heart mitochondria was used for preparation of an affinity column. The antibody column retained both malonyl-CoA-sensitive and -insensitive CPT activity without apparent selectivity. In addition to CPT, several other major protein bands were detected when the antibody column eluates were subjected to SDS–PAGE; however, native gel electrophoresis gives a large, high molecular weight, diffuse band. After elution of the antibody–CPT column with salt, a 68 000-Da protein is retained by the column. The retained protein contains the CPT activity, but it is not inhibited by malonyl-CoA. Thus, salt elution separates catalysis from inhibition. When the salt eluate is subjected to affinity chromatography using agarose–CoA, two protein peaks are obtained; both bind malonyl-CoA. One of the two fractions contains β-hydroxyacyl-CoA dehydrogenase, β-ketothiolase, and crotonase activity. These data show that octyl glucoside solubilized CPT_o and CPT_i are associated with a complex that contains β-oxidation enzymes.

Although it is well established that the outer form of mitochondrial carnitine palmitoyltransferase (CPT_o), the one in contact with the cytosolic compartment, can be inhibited by malonyl-CoA (McGarry et al., 1978; McGarry & Foster, 1979; Bremer, 1983; Bieber, 1988), considerable ambiguity and controversy exist about the location, as well as the physical and enzymatic properties of this enzyme. The studies of several investigators using primarily rat liver mitochondria indicate that CPT_o is tightly membrane bound (Declercq et al., 1987; Woeltje et al., 1987; Murthy & Pande, 1987a; Lund & Woldegiorgis, 1986; Lund, 1987) and labile to detergent solubilization (Declercq et al., 1987; Woeltje et al., 1987), but other studies (West et al., 1971; Hoppel & Tomec, 1972; Ramsay et al., 1987) indicate it is a loosely bound and easily solubilized enzyme. This latter difference was resolved when it was shown that easily solubilized CPT activity is due to rupture of peroxisomes, which contaminate liver mitochondria isolated by differential centrifugation. Peroxisomes contain a soluble medium-chain carnitine acyltransferase (COT) that uses both medium-chain and long-chain acyl-CoAs as substrate (Healy et al., 1988; Ramsay, 1988). Evidence has also been presented that CPT_o is associated with the outer, rather than the inner, membrane of liver mitochondria (Murthy & Pande, 1987a), and other studies indicate CPT_o of liver mitochondria is a very labile enzyme of approximately 90 000 Da (Declercq et al., 1987). Thus, at least four different enzyme preparations/activities have been attributed to mitochondrial CPT_o (Declercq et al., 1987; Woeltje et al., 1987; Murthy & Pande, 1987a; Hoppel & Tomec, 1972; Clarke & Bieber, 1981; Hoppel et al., 1988), yet most attempts to purify CPT_o and CPT_i (the carnitine palmitoyltransferase in contact with the matrix compartment of mitochondria) have yielded a single protein species (Clarke & Bieber, 1981; Miyazawa et al., 1983; Brady et al., 1987; Bergstrom & Reitz, 1980), with the exception of that of Kopec and Fritz (1973), who obtained two interconvertible forms of CPT. Part of this difficulty is caused by the fact that liver contains at least three carnitine acyltransferases that transfer both long-chain and medium-chain acyl groups from acyl-CoA to carnitine, but only one of these enzymes is mitochondrial CPT [Markwell et al., 1973, 1977; also see Bieber (1988) for discussion of this topic].

The isolation of a single CPT protein from heart mitochondria (Clarke & Bieber, 1981; Bergstrom & Reitz, 1980; Fiol & Bieber, 1984) and rat liver (Miyazawa et al., 1983; Brady et al., 1987; Ozasa et al., 1983) does not prove the existence of a single catalytic species of CPT because CPT_o may be destroyed by detergents (Declercq et al., 1987; Woeltje et al., 1987). Herein, we show that octyl glucoside solubilized CPT_i and CPT_o of heart mitochondria are stable in octyl glucoside and that they can be isolated as a complex that apparently is associated with β-oxidation cycle enzymes.

Materials and Methods

Materials

Most chemicals, including acyl-CoAs, CoASH, and tresyl-activated Sepharose 4B, were purchased from Sigma Chemical Co.; chromatographic supports, Sephacryl S-300, and agarose–CoA type 5 were from Pharmacia, and DE-52 cellulose was from Whatman. L-Carnitine was a gift from Sigma-Tau (Rome, Italy), and Δ^2-*trans*-decenoyl-CoA was kindly provided by Dr. H. Schulz (City College of City University, New York, NY). [1-^{14}C]Decanoyl-CoA was synthesized as described earlier (Fiol et al., 1987). [2-^{14}C]-

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Malonyl-CoA, specific radioactivity 55 mCi/mmol, was purchased from Amersham.

Definitions. CPT is defined herein as the catalytically active form of carnitine palmitoyltransferase that exhibits activity on either or both sides of the inner membrane of mitochondria. Two forms of the enzyme must exist. The one located on the matrix side of the inner membrane of mitochondria is CPT_i, and the other, inhibited by malonyl-CoA in intact mitochondria and in contact with the cytosolic compartment, is CPT_o. Carnitine octanoyltransferase (COT) is the medium-/long-chain carnitine acyltransferase that occurs in peroxisomes, as well as in microsomes.

Methods

Isolation of Mitochondria. Beef heart mitochondria were isolated in 0.25 M sucrose, 5.0 mM HEPES,[1] 0.25 mM EDTA, pH 7.7 (Clarke & Bieber, 1981), and stored frozen at −70 °C. Rat heart mitochondria were isolated from male Sprague-Dawley rats weighing 150–200 g and resuspended either in 0.225 M mannitol, 0.075 M sucrose, and 1 mM EGTA, pH 7.5 (medium A) (Toth et al., 1986), or in 130 mM KCl, 20 mM Tris-HCl, and 1 mM EGTA, pH 7.5 (medium B). Mitochondria were used immediately or stored at −70 °C.

Enzyme and Protein Assays. CPT was measured spectrophotometrically (at room temperature) at 324 nm by using 100 μM decanoyl-CoA (or 80 μM palmitoyl-CoA), 20 mM L-carnitine, and 200 μM dithiopyridine in 25 mM potassium phosphate, pH 7.5, containing 0.1% Triton X-100. E_{324} = 19.6 $cm^2/\mu mol$. Values were corrected for carnitine-independent CoASH release. For determination of the malonyl-CoA sensitivity, the more sensitive radiochemical assay was used (Fiol et al., 1987). The assay mixture contained 120 mM KCl, 20 mM sucrose, 10 mM Hepes, 1 mM EGTA, 1 mM DTT, pH 7.5, 10 mM L-carnitine, 17 μM [1-^{14}C]decanoyl-CoA, and ±50 μM malonyl-CoA in a final volume of 100 μL. The reaction was initiated by adding 2–5 μg of mitochondrial protein in 5 μL; at this protein concentration, less than 25% of the decanoyl-CoA was consumed in 30 s–2 min at 30 °C. When octyl glucoside solubilized mitochondria (18–20 mg/mL) were assayed for CPT, the final detergent concentration in the assay was 0.0017%.

Malonyl-CoA Binding Assay. For assaying the malonyl-CoA binding of the different protein fractions, the method of centrifugal filtration (Penefsky, 1977) was used. Sixty microliters of binding mixture, containing 225 mM mannitol, 75 mM sucrose, 1 mM EGTA, 2 mM DTT, and 4.4 μM [2-^{14}C]malonyl-CoA (specific ratioactivity 55 mCi/mmol), pH 7.5, was added to 60 μL of protein and incubated on ice for 20 min. The reaction was terminated by centrifugal filtration of 100 μL of the incubation mixture on Sephadex G-50, equilibrated with binding mixture devoid of malonyl-CoA. Less than 50 dpm was present in filtrate when protein was replaced by 225 mM mannitol, 75 mM sucrose, and 1 mM EGTA, pH 7.5; 20% glycerol, 300 mM NaCl, and 25 mM potassium phosphate, pH 7.5; or 20% glycerol and 50 mM ammonium acetate, pH 7.1–7.2. No corrections were made for nonspecific binding.

β-Oxidation Enzymes. Acyl-CoA dehydrogenase was assayed as described by Thorpe (1981) using 30 μM decanoyl-CoA as a substrate.

The overall rate of β-oxidation (acetyl-CoA formation) from Δ^2-*trans*-decenoyl-CoA (60 μM) and crotonyl-CoA (60 μM) was determined by following the rate of NADH formation by using the citrate synthase, malate dehydrogenase coupled enzyme assay (Pearson et al., 1969). The same assay was used to determine short-chain 3-hydroxyacyl-CoA dehydrogenase (120 μM D,L-3-hydroxybutyryl-CoA), thiolase (60 μM acetoacetyl-CoA), and malonyl-CoA decarboxylase (250 μM malonyl-CoA) activity.

Protein was determined by the modified Lowry method (Markwell et al., 1981) and by taking readings at two different UV wavelengths as described (Layne, 1957; Whitaker & Granum, 1980).

Slab SDS–PAGE was performed as described by Laemmli (1970), using a 10% separating and 4% stacking gel. The same standards were used for all gels: phosphorylase (97 000 Da), BSA (67 000 Da) ovalbumin (43 000 Da), chymtrypsinogen (25 000 Da), and ribonuclease A (13 700 Da), indicated by the arrows on the figures from top to bottom, respectively.

Rapid Purification of CPT. (*a*) *Solubilization of CPT.* Both frozen and freshly prepared mitochondria were used. Frozen beef or rat heart mitochondria (~20–30 mg/mL) were thawed, diluted 2× with medium A, and centrifuged for 30 min at 144000*g* to remove soluble proteins. The pellet (containing ≥97% CPT) was resuspended in medium A, 20 mg/mL, made 1% in Triton X-100, and stirred on ice for 30 min. After centrifugation for 30 min at 100000*g*, the pellet was reextracted as above. The combined supernatant fluids contained >90% of the CPT activity.

(*b*) *DE-52 Cellulose Column Chromatography.* Washed and degassed DE-52 cellulose in distilled water was poured into a column (2-cm i.d.) to obtain a column volume approximately equal to the volume of the combined supernatant fluids. The column was equilibrated with 5 mM potassium phosphate, pH 7.5. The combined supernatant fluids were adjusted to pH 7.5 with solid Tris base and applied to the column. The column was washed with 3 column volumes of 5 mM potassium phosphate, pH 7.5, containing 0.5% Triton X-100, and the CPT was eluted with a linear gradient of 5–100 mM potassium phosphate, pH 7.5, containing 0.5% Triton X-100. Two column volumes of each buffer was used. Pooled CPT was reduced to 20 mL or less by vacuum filtration.

(*c*) *Sephacryl S-300 Gel Filtration.* Sephacryl S-300 in degassed 5 mM potassium phosphate, pH 7.0, was poured into a column (2.5 cm × 100 cm) and equilibrated with the same buffer containing 0.5% Triton X-100. CPT was loaded and eluted with 5 mM potassium phosphate buffer, pH 7.0, containing 0.5% Triton X-100. Fractions containing CPT were pooled.

(*d*) *Agarose–CoA* (*AG–CoA Type 5*) *Affinity Chromatography.* Twenty milliliters of AG–CoA in 5 mM potassium phosphate, pH 7.0, was poured into a 1.5-cm i.d. column and washed. After CPT was loaded, the column was washed with 5 column volumes of 5 mM potassium phosphate, pH 7.0, containing 0.5% Triton X-100, and CPT was eluted with a linear gradient of 5–100 mM potassium phosphate, pH 7.0, containing 0.5% Triton X-100 formed from 3 column volumes of each buffer. Pooled CPT was reduced to 1–2 mL by vacuum dialysis against 100 mM potassium phosphate, pH 7.5.

This purification procedure gave a nearly homogeneous CPT with approximately 40% yield. The average specific activities for rat heart CPT were 108 and 20.3 μmol min^{-1} (mg of protein)$^{-1}$ with decanoyl-CoA and palmitoyl-CoA, respectively, and 123 and 46 μmol min^{-1} (mg of protein)$^{-1}$ with decanoyl-CoA and palmitoyl-CoA, respectively, for beef heart mitochondria. Neither of these purified enzymes was inhibited by malonyl-CoA.

[1] Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid.

Preparation of Immune Serum. Beef heart mitochondrial CPT (0.6 mg) in 0.6 mL of 150 mM NaCl and 10 mM potassium phosphate, pH 7.5, containing ~1% Triton X-100 was emulsified with Freund's complete adjuvant and injected into rabbits subcutaneously. A booster injection was made 3 weeks later by using 0.4 mg of CPT emulsified with Freund's incomplete adjuvant. Antiserum was prepared from blood taken 8 days after the second antigen injection, and preimmune serum was prepared from blood taken prior to initiation of immunization. Booster injections and antiserum preparations were repeated three times at approximately 6-week intervals. Both preimmune serum and immune serum were stored in 1.0-mL portions at −70 °C.

Immunoglobulin Isolation. The immunoglobulin fraction from rabbit antiserum was isolated by using a 100-mL CM Affi-Gel Blue column as described by the manufacturer. After precipitation by ammonium sulfate (50% saturation), the protein precipitate was dissolved in 150 mM NaCl and 20 mM Tris, pH 7.5, and dialyzed against this buffer for 24 h (2 × 1.4 L). After dialysis, precipitated protein was removed by centrifugation (10 min at 12000*g*), and the protein concentration of the supernatant fluid was adjusted to 5.1 mg/mL. Samples were stored at −70 °C in 1.2-mL aliquots. Gel filtration on HPLC using a Zorbax F250 column gave two peaks. One, 7% of the total protein, eluted with the void volume, and the second eluted with a retention time corresponding to that of IgG (160–170 kD). Forty micrograms of purified immunoglobulins completely immunoprecipitated 150 nmol min^{-1} (mg of protein)$^{-1}$ of purified beef heart mitochondrial CPT. Neither peroxisomal carnitine octanoyltransferase (mouse liver) nor carnitine acetyltransferase (beef heart mitochondria) cross-reacted with the antibodies.

Synthesis of Immunoaffinity Support. Immunoglobulins (130 mg) were passed through a Sephadex G-20 column that had been equilibrated with coupling buffer (0.5 M NaCl, 0.2 M $NaHCO_3$, pH 8.3). The eluate was dialyzed overnight against the buffer and the protein concentration adjusted to 3.0 mg/mL with buffer. After 5.0 g of freeze-dried tresyl-activated Sepharose 4B was swollen in ice-cold 1 mM HCl for 15 min, it was filtered on a sintered G-3 glass filter and washed with 1 L of 1.0 mM cold HCl. The gel was then washed with ice-cold coupling buffer and immediately mixed with 40 mL of immunoglobulins in coupling buffer (30% excess IgG was used). The mixture was left at room temperature for 4 h with a gel to buffer ratio of 1:2. The extent of coupling was determined by measuring the OD at 280 nm. Unreacted active sites were blocked with 1 M ethanolamine, pH 8.0 (2.0 h at room temperature). Then the gel was washed extensively with coupling buffer, followed by 0.5 M NaCl and 0.1 M acetate buffer, pH 4.0, and again with coupling buffer. The immunoglobulin–Sepharose conjugate was stored at 4 °C in 150 mM NaCl, 25 mM potassium phosphate, and 0.02% NaN_3, pH 7.25.

Application to and Elution of CPT from Immunoglobulin Columns. Frozen–thawed rat heart mitochondria in 225 mM mannitol, 75 mM sucrose, and 1 mM EGTA were diluted with the mannitol–sucrose mixture to 18–20 mg of protein/mL and made 1% in octyl glucoside. After standing for 30 min on ice with occasional mixing, the mitochondrial extract was centrifuged for 30 min at 100000*g* and the supernatant fluid was removed. Aliquots of this supernatant fluid were loaded onto the immunoaffinity column at room temperature. The column had been previously equilibrated with 150 mM NaCl and 25 mM potassium phosphate, pH 7.25. Unbound proteins were removed by washing with 225 mM mannitol, 75 mM sucrose, and 1 mM EGTA, pH 7.5, until the OD at 280 nm was <0.01. CPT and other proteins were eluted with ice-cold 20% glycerol and 50 mM NH_3, pH 10.6, as done for Figure 4, peak A. Alternatively, the column was initially eluted with 20% glycerol, 300 mM NaCl, and 25 mM potassium phosphate, pH 7.5, to remove non-CPT proteins (see Figure 6, peak AI). CPT was then eluted with 20% glycerol and 50 mM NH_3, pH 10.6 (see Figure 6, peak AII). The pH 10.6 protein containing fractions were combined, and the pH was adjusted to 7.1–7.2 with 1 M acetic acid (i.e., peak A in Figure 4 and peak AII in Figure 6). After use, the immunoaffinity support is regenerated by extensive washing with 0.5 M NaCl and 0.1 M acetate, pH 4.0, following by washing with coupling buffer containing 1% TritonX-100 and, finally, by washing with 150 mM NaCl, 25 mM potassium phosphate, and 0.02% NaN_3, pH 7.25. It can be stored in the latter mentioned buffer at 4 °C. The performance of this column remained unchanged for several months.

RESULTS

Solubilization and Stabilization of Malonyl-CoA Sensitivity of Rat Heart Mitochondria. Previous attempts to purify mitochondrial CPT_o resulted in loss of malonyl-CoA sensitivity during solubilization with detergents. The cause for the loss of malonyl-CoA sensitivity is not known. It could be due to inactivation of CPT_o by detergents (Declercq et al., 1987; Woeltje et al., 1987; Murthy & Pande, 1987), or it might be due to dissociation of an oligomeric enzyme (Bieber, 1988; Fiol & Bieber, 1984) or dissociation of a regulatory component. The data of Bergseth et al. (1986) strongly suggest that the malonyl-CoA sensitivity of rat liver mitochondria is reversible. Therefore, experiments were performed to determine if the malonyl-CoA sensitivity can be preserved during detergent solubilization of heart mitochondria. Since both KCl and mannitol buffers have been used for isolation of mitochondria, both isolation media were used. The preliminary data showed that total CPT activity and total malonyl-CoA-sensitive CPT_o were different for mitochondria isolated in a KCl-containing media compared to mitochondria isolated in a mannitol–sucrose-containing media. The values for the mannitol–sucrose-isolated mitochondria ($n = 6$) were 112 ± 12.5 nmol of decanoylcarnitine min^{-1} (mg of protein)$^{-1}$ and for KCl-isolated mitochondria ($n = 6$) were 191 ± 21.6 nmol of decanoylcarnitine min^{-1} (mg of protein)$^{-1}$. When mitochondria isolated in mannitol–sucrose were solubilized with 1% octyl glucoside, the average total CPT activity was 405 ± 24 nmol min^{-1} (mg of protein)$^{-1}$, of which 73 ± 8.1 nmol min^{-1} (mg of protein)$^{-1}$ was inhibited by malonyl-CoA. The pilot data showed that malonyl-CoA-sensitive CPT_o could be solubilized in octyl glucoside with retention of malonyl-CoA sensitivity, but addition of KCl to octyl glucoside solubilized mitochondria greatly reduced malonyl-CoA sensitivity without major loss of CPT activity. These studies are summarized in Table I.

The experiments summarized above demonstrated CPT_o activity can be solubilized by using octyl glucoside. Therefore, experiments were done to determine the optimum concentration of octyl glucoside. Frozen heart mitochondria were used for these experiments because freezing in mannitol–sucrose buffer did not alter the amount of malonyl-CoA-sensitive CPT (Table II). The effect of octyl glucoside concentration on total CPT activity and on the amount of carnitine decanoyltransferase inhibited by malonyl-CoA is shown in Figure 1. The numbers above the solid circles show the nanomoles per minute per milligram of protein inhibited by malonyl-CoA. The amount of malonyl-CoA-inhibitable CPT_o for the nonfrozen mitochondria in mannitol–sucrose media is represented

Table I: Effect of Malonyl-CoA on Octyl Glucoside Solubilized CPT Activity of Mannitol–Sucrose- and KCl-Isolated Mitochondria[a]

expt	mannitol–sucrose [nmol min^{-1} (mg of protein)$^{-1}$] (nmoles/min/mg protein)			KCl [nmol min^{-1} (mg of protein)$^{-1}$]		
	−Mal-CoA	+Mal-CoA	Δ	−Mal-CoA	+Mal-CoA	Δ
1% OG	405 ± 24	332 ± 32	73 ± 8.1	384 ± 81.4	324 ± 58.9	60 ± 45.2
1% OG dialysis vs MSE	503 ± 15.2	445 ± 20.4	58 ± 13.6	501 ± 3.3	486 ± 18.6	16 ± 12.5
1% OG dialysis vs KCl media	391 ± 11.7	378 ± 40.6	13 ± 8.5	415 ± 40.0	418 ± 29.3	(−3)3 ± 12.0

[a] The experiments were performed as described under Methods. MSE = 0.225 M mannitol; 0.075 M sucrose; 1 mM EGTA, pH 7.5 KCl = 130 mM KCl; 20 mM Tris, 1 mM EGTA, pH 7.5. The samples were dialyzed for 20 h at 4 °C. OG = octyl glucoside (1%) for 30 min on ice with gentle stirring. The data represent the average values for four experiments ± standard deviation. Malonyl-CoA = 50 μM, and decanoyl-CoA = 17 μM.

Table II: Effect of Freezing and 1% Octyl Glucoside on Malonyl-CoA Sensitivity of CPT[a]

preparation	nmol min^{-1} (mg of protein)$^{-1}$			% inhibition
	control	+malonyl-CoA	Δ	
intact mitochondria	129.0 ± 12.5	31.0 ± 6.9	98.0 ± 12.0	75.6 ± 5.4
freeze–thaw (−70 °C)	243.6 ± 5.3	133.4 ± 6.2	110.2 ± 2.8	45.3 ± 1.6
freeze–thaw, then 1% octyl glucoside	403.6 ± 22.5	288.5 ± 22.3	115.1 ± 6.5	28.8 ± 1.9

[a] Numbers represent mean ± SEM; $n = 4$. Decanoyl-CoA was the substrate. Rat heart mitochondria were isolated and suspended in medium A. The were assayed for CPT activity ± 50 μM malonyl-CoA immediately after isolation, following storage at −70 °C at a protein concentration of 24–34 mg/mL for several weeks, and after solubilization (18–20 mg/mL) in 1% octyl glucoside. Before determination of enzyme activity, each preparation was diluted with medium A to 0.5–1.0 mg of protein/mL.

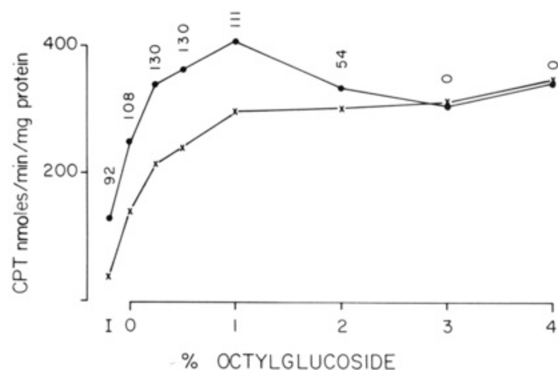

FIGURE 1: Effect of octyl glucoside concentration on malonyl-CoA-sensitive CPT. Frozen–thawed rat heart mitochondria were diluted with medium A to 18–20 mg of protein/mL, and then 20% octyl glucoside was added to make the final detergent concentration indicated on the figure. Mitochondria were kept on ice for 30 min with occasional vortexing and diluted with medium A to 0.5–1.0 mg of protein/mL, and 5-μL aliquots were assayed for CPT activity in the absence (●) and presence (×) of 50 μM malonyl-CoA. The numbers above the solid circles represent the total nmol min^{-1} (mg of protein)$^{-1}$ CPT activity inhibited by malonyl-CoA. I = intact rat heart mitochondria, assayed before freezing, in the absence of octyl glucoside. 0 = rat heart mitochondria stored frozen in medium A at −70 °C for several weeks. $n = 2$.

by I. Approximately 75%, 92 nmol min^{-1} (mg of protein)$^{-1}$, was initially inhibited by malonyl-CoA. When 0.25%, 0.5%, and 1.0% octyl glucoside was added to frozen–thawed preparations, all of the malonyl-CoA-inhibitable CPT_o activity was preserved; compare 108 nmol min^{-1} (mg of protein)$^{-1}$ in the absence of detergent to 130, 130, and 111 nmol min^{-1} (mg of protein)$^{-1}$ in 0.25%, 0.5%, and 1.0% octyl glucoside, respectively. At 2% octyl glucoside, approximately 50% of the malonyl-CoA sensitivity was lost, and at 3% octyl glucoside, all of the sensitivity disappeared. As discussed elsewhere (Fiol et al., 1987), decanoyl-CoA rather than palmitoyl-CoA is used as substrate because saturating amounts of decanoyl-CoA can be used in the absence of both micelles and albumin with a higher V_{max} than that for palmitoyl-CoA; i.e., the V_{max} with decanoyl-CoA is 5.2-fold greater than the V_{max} for palmitoyl-CoA when CPT purified from rat heart mitochondria is used.

Rapid Purification of CPT. The finding that malonyl-CoA-sensitive CPT can be solubilized by octyl glucoside caused us again to attempt to purify CPT_o from CPT_i. The purification procedures used previously to purify CPT are very time-consuming (Clarke & Bieber, 1981; Fiol & Bieber, 1984); therefore, a modified rapid, three-column procedure described under Methods was developed. Some differences in the properties of the enzymes purified from rat and bovine heart mitochondria were found. CPT from rat heart mitochondria has a slightly higher monomeric molecular weight than that from beef heart mitochondria; compare lane R with lane B of Figure 2. The apparent $K_{0.5}$ values for decanoyl-CoA and L-carnitine were similar, 2.0 μM for decanoyl-CoA and 2.0 mM for L-carnitine (beef heart), compared to 6.5 μM for decanoyl-CoA and 3.0 mM for L-carnitine (rat heart), but the Hill coefficients for decanoyl-CoA are quite different. For bovine heart CPT, the Hill coefficient for decanoyl-CoA is slightly greater than 2.0, which is larger than previously found for the enzyme isoalted by different methods (Fiol & Bieber, 1984, 1988; Fiol et al., 1987; Bieber & Fiol, 1986). In contrast, the Hill n for decanoyl-CoA for the rat heart preparation is considerably less, ~1.4. Under identical assay conditions,

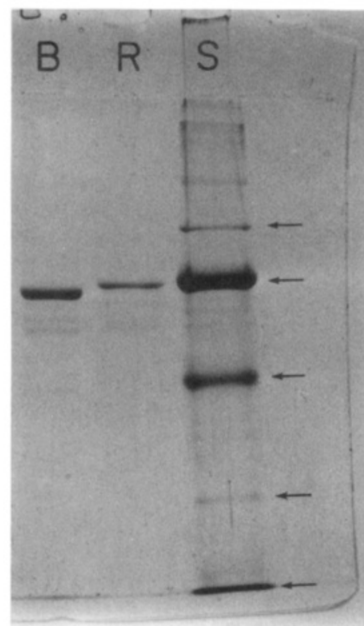

FIGURE 2: SDS–PAGE of purified beef heart (B) and rat heart (R) mitochondrial CPT. S, standards. Separating gel, 10%; stacking gel, 4%. Staining was with Coomassie Brilliant Blue.

Table III: Malonyl-CoA Sensitivity of the CPT Activity of the Proteins Obtained from the Anti-CPT Affinity Column[a]

sample	CPT [nmol min^{-1} (mg of protein)$^{-1}$ × 10^{-3}]			% inhibition
	control	+malonyl-CoA	Δ	
100000*g* extract of octyl glucoside solubilized mitochondria	0.67 ± 0.04	0.53 ± 0.06	0.14 ± 0.05	20.6 ± 7.6
peak A, Figure 4	4.15 ± 0.98	2.37 ± 0.6	1.68 ± 0.4	43.2 ± 1.7
peak AI, Figure 6	<0.005	<0.005	0.0	
peak AII, Figure 6	9.95 ± 0.49	9.03 ± 0.44	0.92 ± 0.65	9.2 ± 6.2

[a] The samples are those obtained by the procedures for Figures 4 and 6. $n = 4$; data are ±SEM. Decanoyl-CoA was the substrate. For these experiments, 20–26 μmol min^{-1} mg^{-1} of CPT was loaded onto a 20-mL antibody column. An average of less than 3% of the total applied CPT activity was not retained.

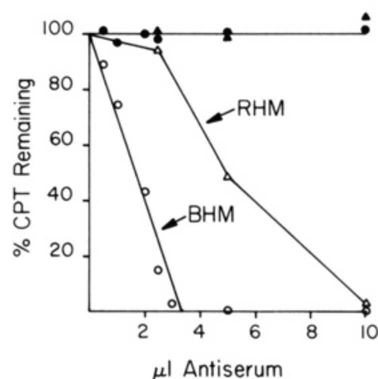

FIGURE 3: Immunoprecipitation of bovine and rat heart mitochondrial CPT. One hundred fifty nmol min^{-1} (mg of protein)$^{-1}$ of purified bovine and rat heart mitochondrial CPT was incubated with increasing amounts of preimmune and immune serum in a final volume of 180 μL, containing 150 mM NaCl and 25 mM potassium phosphate, pH 7.5, at room temperature for 30 min. The samples were centifuged (Eppendorf) for 5 min, and CPT was assayed spectrophotometrically in the supernatant fluid. The solid symbols represent preimmune serum, and the open symbols represent immune serum; triangles represent rat heart (RHM) and circles bovine heart (BHM) mitochondrial CPT. Numbers are averages of two experiments.

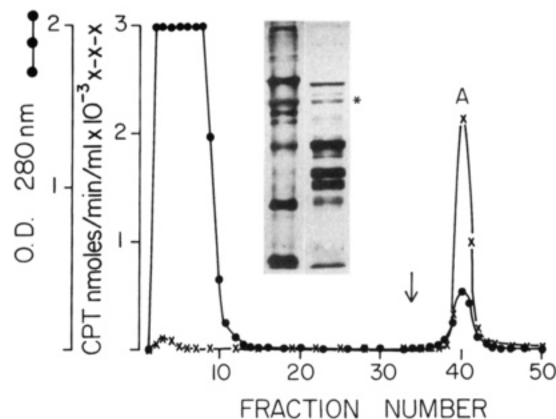

FIGURE 4: Isolation of CPT by immunoaffinity chromatography. Frozen rat heart mitochondria were treated with 1% octyl glucoside as described under Methods. Five milliliters of the 100000*g* supernatant fluid containing 20 μmol min^{-1} mg^{-1} of CPT was applied to a 20-mL immunoaffinity column that had been equilibrated with 150 mM NaCl and 25 mM potassium phosphate, pH 7.25, at room temperature. The flow rate was approximately 0.2 mL/min. Unbound protein was removed by washing with several column volumes of medium A at room temperature; bound proteins were eluted (arrow) with cold 20% glycerol and 50 mM ammonium, pH 10.6 at 4 °C. The ~3.0-mL fractions were assayed for protein (O.D. at 280 nm) and CPT (spectrophotometric assay). Fractions containing CPT (peak A) were combined, and the pH was adjusted to 7.1–7.2 with 1.0 N acetic acid. (Inset) SDS–PAGE of standards (S) and peak A (A); silver stain was used. CPT is indicated by an asterisk.

control, peroxisomal COT, had a Hill $n = 1.0$. The beef heart preparations are stable for months in detergent and buffer, in contrast to the enzyme from rat heart which slowly loses activity with storage. The substrate specificities are different in Triton X-100 compared to those in octyl glucoside; i.e., the ratio of V_{max} in 0.1% Triton X-100/V_{max} in 0.1% octyl glucoside with decanoyl-CoA as substrate is 1.25 for beef heart and 0.67 for the rat heart enzyme. Both purified enzymes are insensitive to malonyl-CoA.

Effect of Anti-CPT Antibody on CPT_o and CPT_i Activity. Even though a single monomeric protein was detected on SDS–PAGE for CPT purified from detergent-solubilized heart mitochondria, antibody was prepared against it to determine if the anti-CPT is selective for either CPT_o or CPT_i. Serum from rabbits immunized against purified CPT from bovine heart mitochondria was used. As shown in Figure 3, the polyclonal antibody essentially precipitated all of the CPT purified from both rat and bovine heart mitochondria, but the rat heart enzyme required considerably more anti-bovine serum antibody. When octyl glucoside solubilized mitochondria were used, no selectivity was observed between inactivation (precipitation) of malonyl-CoA-sensitive versus malonyl-CoA-insensitive CPT activity. For example, in an experiment using rat heart mitochondria extracts, the immune serum precipitated 98% of the total CPT, and it precipitated 94% of the malonyl-CoA-sensitive CPT. The failure to detect selectivity in precipitation of either CPT_i or CPT_o was observed, regardless of whether sufficient antibody was added to completely precipitate, or to partially precipitate, CPT activity.

Since anti-CPT in the IgG fraction of immune serum precipitated both malonyl-CoA-sensitive and -insensitive CPT of octyl glucoside extracts, IgG was covalently attached to an immobile support to make an affinity column, and octyl glucoside extracts of heart mitochondria were passed through the antibody column. As shown in Figure 4, a small amount of CPT activity eluted at the front of the nonretained protein fraction; these fractions have an opaque, milky appearance and appeared to be micellar or vesicular. Most of the CPT, for some preparations >98%, was retained by the antibody column. Washing with 20% glycerol and 50 mM NH_4OH, pH ≅ 10.6, eluted CPT, peak A of Figure 4, but only about half of the total CPT activity was recovered in peak A, an average of 48% for four different preparations. The percent malonyl-CoA inhibition of the CPT activity of peak A was variable. For most preparations, it exceeded the inhibition of the material applied; i.e., the average malonyl-CoA inhibition of the octyl glucoside extract passed over the column was 21%, while the average inhibition of the preparation eluted from the affinity column was 43%. These results are summarized in Table III. Fractions 39–42, peak A of Figure 4, were combined, dialyzed, concentrated, and subjected to SDS–PAGE electrophoresis. Seven major protein bands were present, one corresponding to the subunit molecular weight of CPT (see inset of Figure 4). When the eluate was subjected to nondissociating gel electrophoresis, only one high molecular weight, very diffuse band was obtained (see Figure 5) which had a molecular weight greater than nondissociated phosphorylase. Thus, in addition to CPT, at least six other proteins were retained by the antibody column.

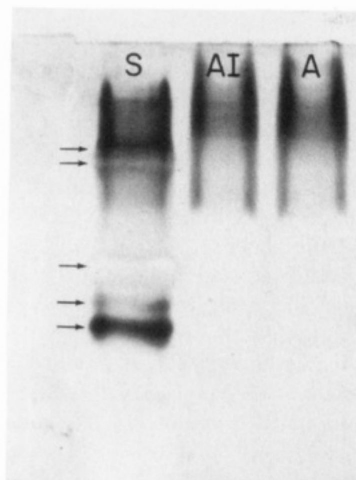

FIGURE 5: Nondenaturing gradient PAGE of immunoaffinity-purified CPT_o and CPT_i. Peak A of Figure 4 and peak AI of Figure 6 were applied to a nondenaturing gradient gel. After running, the gel was silver stained. The stacking gel was 4% and the running gel 4–30%. S, standards; A, peak A of Figure 4; AI, peak AI of Figure 6.

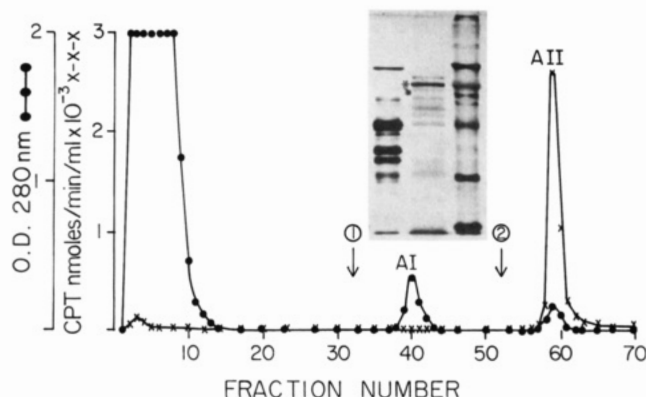

FIGURE 6: Separation of CPT from other proteins retained by the immunoaffinity column. The 100000g octyl glucoside extract was prepared as in Figure 5, and 5.7 mL containing 26 μmol min^{-1} mg^{-1} of CPT was applied to a 20-mL immunoaffinity column. Unbound proteins were removed as for Figure 4. The column was then washed with 20% glycerol, 300 mM NaCl, and 25 mM potassium phosphate, pH 7.5 (arrow 1), until no protein was detected. It was then eluted with 20% glycerol and 50 mM ammonia, pH 10.6 (arrow 2). Peaks AI and AII were combined, and the pH of AII was adjusted to 7.2. (Inset) SDS–PAGE of standards (S), peak AII (AII), and peak AI (AI); silver stain was used. CPT is indicated by an asterisk.

Since the preliminary data showed that addition of salt to octyl glucoside solubilized preparations caused loss of malonyl-CoA sensitivity, the antibody column containing octyl glucoside solubilized CPT was eluted with salt, 300 mM NaCl, 20% glycerol, and 25 mM P_i, pH 7.5. The arrow indicated by 1 in Figure 6 shows the start of the salt elution. Most of the protein, but no CPT activity, was eluted (fraction AI). Elution by high pH was started at the arrow indicated by 2. It eluted CPT, fraction AII. SDS–PAGE profiles of fractions AI and AII are shown in the inset of Figure 6. Fraction AII contained two major proteins; one had a low molecular weight and moved with the tracing dye, and the other had a molecular weight of purified CPT, but it was not inhibited by malonyl-CoA. The presence of two major proteins was confirmed by HPLC (data not shown). With silver staining, other, less prevalent proteins were also detected. Peak AI contained at least six major proteins.

Malonyl-CoA Binding. Experiments were done to determine which fractions of the antibody column eluates bind ^{14}C-labeled malonyl-CoA. These data are summarized in

Table IV: Malonyl-CoA Binding by Antibody Column Eluates[a]

preparation	malonyl-CoA bound (pmol/mg of protein)
100000g octyl glucoside supernatant	43 ± 15
peak A, Figure 4	1419 ± 437
peak AI, Figure 6	1539 ± 107
peak AI, Figure 6 (CPT)	122 ± 32

[a] Malonyl-CoA binding assays were performed as described under Methods. $n = 4$ for all preparations except peak AI, where $n = 3$. Antibody column eluates were prepared as described for Figures 4 and 6.

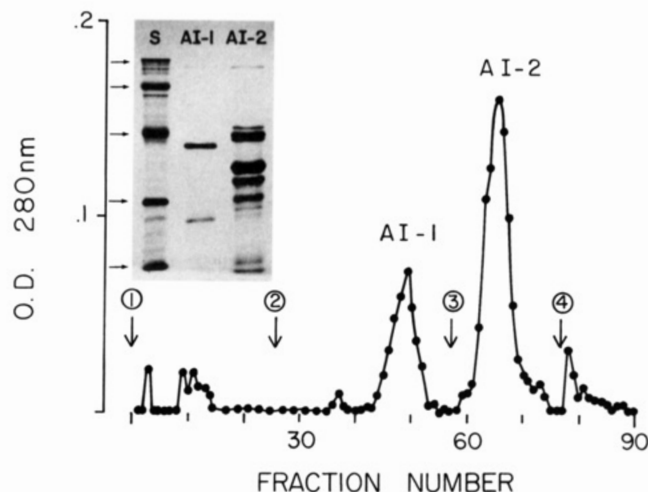

FIGURE 7: Fractionation of peak AI, Figure 6, by agarose–CoA. Peak AI from two separations was combined and concentrated by vacuum dialysis against 5% glycerol and 5 mM potassium phosphate, pH 7.0, to approximately 3 mL. It was applied onto a 5-mL AG–CoA column preequilibrated with the above-mentioned buffer containing 0.2% octyl glucoside. The column was washed with 3 column volumes of the above-mentioned buffer (arrow 1), followed by a linear gradient of 5–100 mM potassium phosphate, pH 7.0, in 5% glycerol and 0.2% octyl glucoside, 10 mL each (arrow 2). Then a linear gradient of 100–500 mM potassium phosphate, pH 7.0, in 5% glycerol and 0.2% octylglucoside was used, 7 mL of each (arrow 3), followed by 500 mM potassium phosphate, pH 7.0, in 5% glycerol and 0.2% octyl glucoside (arrow 4). Protein was assayed at 280 nm. Fractions for peak AI-1 and peak AI-2 were pooled and assayed. (Inset) SDS–PAGE of standards (S), peak AI-1 (AI-1), and peak AI-2 (AI-2); silver strain was used.

Table IV. When octyl glucoside solubilized mitochondria are treated as described for Figure 4, a 35-fold increase in malonyl-CoA binding is obtained. Salt elution of the CPT/β-oxidation complex bound to the antibody column (Figure 6) separates the β-oxidation enzymes (fraction AI) from CPT activity (fraction AII). Most, but not all, of the malonyl-CoA binding is associated with fraction AI, strongly indicating salt dissociates malonyl-CoA binding from catalysis. The low amount of malonyl-CoA binding to peak AII, 122 pmol/mg of protein, is <50% greater than the blank. Although the experiments of Fiol and Bieber (1984) demonstrate that malonyl-CoA is not binding at the catalytic site of CPT (i.e., 600 μM malonyl-CoA does not affect the velocity), those experiments do not indicate whether malonyl-CoA binds to the allosteric site of CPT involved in acyl-CoA cooperativity. Therefore, malonyl-CoA binding to purified CPT was determined. One milligram of purified CPT bind 50 pmol of malonyl-CoA; this represents approximately 0.3% of the CPT subunits (M_r = 69000) binding to malonyl-CoA. Since peak AII also contains a low molecular proteins, it is possible this protein is associated with malonyl-CoA binding. This has not been investigated. None of the fractions that bound to the antibody affinity column contain malonyl-CoA decarboxylase

Table V: Conversion of β-Oxidation Substrates to Acetyl-CoA by the Protein Fractions Eluted from the Antibody Column[a]

preparation	substrate → acetyl-CoA [nmol min^{-1} (mg of protein)$^{-1}$]			
	Δ^2-*trans*-decenoyl-CoA	crotonyl-CoA	D,L-3-hydroxy-butyryl-CoA	aceto-acetyl-CoA
100000*g* octyl glucoside supernatant fluid	120	140	ND[b]	ND[b]
peak AI, Figure 4	1160	1470	1560	1790
peak AI-1, Figure 7	NA[c]	NA[c]	NA[c]	NA[c]
peak AI-2, Figure 7	ND[b]	1428	1680	1975

[a]The numbers represent the average of two experiments. Experimental details are described under Methods. [b]ND, not determined. [c]NA, no activity.

or malonyl-CoA hydrolase activity.

Because peak AI of Figure 6 bound both decanoyl-CoA and malonyl-CoA, it was desalted, concentrated, and passed over an agarose–CoA column. Two major protein-containing peaks, AI-1 and AI-2 of Figure 7, were obtained. Both peaks bind [^{14}C]malonyl-CoA. As shown in the inset, peak AI-1 contains one major protein with a molecular weight of approximately 39 000, and peak AI-2 contains at least four major protein bands and two minor ones.

β-Oxidation Enzymes. Since peak A of Figure 4, peak AI of Figure 6, and peak AI-2 of Figure 7 all had a high affinity for decanoyl-CoA, the presence of one or more of the β-oxidation enzymes was suspected. Large amounts of β-ketothiolase, β-hydroxyacyl-CoA dehydrogenases, and enoyl-CoA hydratase were found in all three peaks, but only traces of acyl-CoA dehydrogenase were found (see Table V). More importantly, this fraction had a large capacity to convert 2,3-unsaturated acyl-CoAs to acetyl-CoA with a lag in the appearance of acetyl-CoA. These results demonstrate the presence of crotonase, the β-hydroxyacyl-CoA dehydrogenase, and thiolase activity in the complex retained by the immunoaffinity column. Under the experimental conditions of Figures 4 and 6, >30% of the acetyl-CoA-forming capacity from Δ^2-*trans*-decenoyl-CoA and crotonyl-CoA present in the octyl glucoside extract was recovered in fractions A (Figure 4) and AI (Figure 6) (data not shown). Furthermore, the proportions of the β-oxidation enzyme activity were approximately 1:1:1 when the overall capacity of the system to convert a specific β-oxidation acyl-CoA intermediate to acetyl-CoA was determined (see Table V). A strong association of these enzymes for each other has been demonstrated in *Escherichia coli* (Binstock & Schulz, 1981). Separation of peak AI of Figure 6 on agarose–CoA resulted in two major protein fractions, peaks AI-1 and AI-2 of Figure 7. The β-oxidation enzymes were found in peak AI-2. Both protein fractions bind malonyl-CoA, and addition of 20 μM decanoyl-CoA inhibits >80% of the malonyl-CoA binding in peak AI-2, but it does not inhibit binding of malonyl-CoA in peak AI-1 (data not shown). To test the possibility that the immunoaffinity column interacts independently with both CPT and the enzymes of β-oxidation, peak AI of Figure 6 was desalted and transferred into medium A. Equal aliquots (equivalent to the amounts used for Figures 4 and 6) were then passed over an immunoaffinity column containing bound CPT and also over a well-washed immunoaffinity column. Both columns bound approximately 7% of the total β-oxidation enzyme activity, demonstrating that the immunoaffinity column has a very low affinity for the β-oxidation portion of the complex in the absence of CPT.

Discussion

The results presented herein show that malonyl-CoA-sensitive CPT, CPT_o of heart mitochondria, is stable in low amounts of octyl glucoside when mannitol–sucrose media are used, but the inhibition of CPT by malonyl-CoA is abolished when octyl glucoside solubilized heart mitochondria are exposed to high concentrations of salt or high concentrations of octyl glucoside. Others have shown that malonyl-CoA-sensitive CPT_o activity can be detected in Tween 20 (McGarry et al., 1987) and in some other detergents (Murthy & Pande, 1987b). Our data do not agree with suggestions that the failure to isolate a catalytically active CPT_o is due to its extreme detergent lability (Declercq et al., 1987; Woeltje et al., 1987), rather the results indicate malonyl-CoA sensitivity can be removed (separated) from catalytic activity. In some experiments (data not shown), more malonyl-CoA-sensitive CPT activity was found after solubilization with octyl glucoside, indicating that some of the malonyl-CoA-sensitive CPT_o is masked or inhibited in mannitol–sucrose-isolated mitochondria. The results do not rule out the possibility that disruption of mitochondria with octyl glucoside promotes interaction of CPT_i with a comonent that confers malonyl-CoA sensitivity to CPT_o.

Separation of CPT activity from malonyl-CoA binding is demonstrated by the experiments of Figures 4, 6, and 7, in which peak A of Figure 4 contains both CPT_o and CPT_i activity, as well as malonyl-CoA binding capacity. Both the malonyl-CoA sensitivity and the malonyl-CoA binding are removed from catalytic activity by washing the antibody column with high salt (see experiments of Figure 6). Limited studies on malonyl-CoA binding show that peak AI contains the malonyl-CoA binding capacity, while peak AII contains the CPT activity. Woldegiorgis et al. (1989), using conventional column chromatographic techniques, also separated CPT activity from malonyl-CoA binding. When their malonyl-CoA binding fraction was separated by using SDS–PAGE, three major proteins with molecular weights of 33 000, 45 000, and 86 000 were found. Fractions A of Figure 4 and AI of Figure 6 both contain proteins with similar molecular weights. Separation of AI into two fractions, peaks AI-1 and AI-2 of Figure 7, produces two fractions that bind malonyl-CoA. Thus, our preparations contain more than one protein that binds malonyl-CoA. Regardless, the data show that salt elution of the antibody–CPT complex separates malonyl-CoA binding from CPT activity and also abolishes the malonyl-CoA sensitivity of CPT. These data indicate salt promotes loss of malonyl-CoA sensitivity due to removal of one or more of the proteins eluted in fraction AI of Figure 6.

The reason for the differences between CPT_o activity of KCl- versus mannitol–sucrose-isolated mitochondria was not investigated. It has been shown that the rate of β-oxidation of acylcarnitines can be altered by the osmotic state of mitochondria (Halestrap, 1987) and that the ionic strength can alter CPT_o activity (McMillin-Wood, 1973), consistent with the findings that ionic strength affects palmitoyl-CoA plus carnitine, but not palmitoylcarnitine oxidation by heart mitochondria (Brosnan & Fritz, 1971). The data of Saggerson (1982) indicate that some salt effects, such as the lags in CPT activity, may be more complex than osmotic effects. Regardless, our data show that the combination of salt plus high detergent concentrations promotes loss of malonyl-CoA sensitivity and indicate both ionic interactions and hydrophobic interactions are involved in maintaining the malonyl-CoA sensitivity of CPT_o. This conclusion is supported by the antibody affinity column data, which show both CPT_o and CPT_i are retained in the presence of 1% octyl glucoside when a

mannitol–sucrose media is used. Washing with salt removes several proteins, but not CPT, with subsequent loss of sensitivity to malonyl-CoA. Such data indicate KCl dissociates malonyl-CoA sensitivity from catalytic activity, possibly by dissociating a regulator subunit. The existence of a regulator subunit (a malonyl-CoA binding component) has been previously proposed (Fiol & Bieber, 1984; Bergseth et al., 1986; Bieber & Fiol, 1986); however, these results do not preclude other interpretations such as lipid involvement.

Liver mitochondria contain a malonyl-CoA binding protein with a molecular weight >90 000 (Declercq et al., 1987; Kiorpes et al., 1984; Zammit et al., 1988), which is thought by some to be CPT_o because it forms a tetradecylglycidyl adduct in the presence of tetradecylglycidyl-CoA. Tetradecylglycidyl-CoA potently inhibits CPT_o in intact mitochondria. Fractions A, AI, and AI-2 of Figures 4, 6, and 7, respectively, all contain a protein with a molecular weight between 80 000 and 90 000. Its malonyl-CoA binding capacity has not, as yet, been determined, but the data of Figure 6 show that elution of the antibody–CPT complex with salt separates the 80–90-kDa protein from CPT. Our data are compatible with CPT_o and CPT_i being associated with the inner membrane of mitochondria, but do not preclude suggestions that CPT_o is associated with the outer mitochondrial membrane (Murthy & Pande, 1987a; Murthy & Pande, 1988; Grantham & Zammit, 1986). However, our preparations of CPT_o would have to have undergone reassociation during octyl glucoside solubilization, since it apparently is associated with the β-oxidation enzymes on the antibody column. Native gel electrophoresis (Figure 5) shows the presence of a large, high molecular weight complex with a molecular weight greater than that of phosphorylase. Lower molecular weight proteins were not detected. It is well established that the β-oxidation system of heart mitochondria occurs in the matrix compartment of mitochondria. The fact that the antibody prepared against purified CPT, which must contain CPT_i, complexes both CPT_i and CPT_o without any apparent selectivity indicates CPT_i and CPT_o have common antigenic determinants. This finding is in agreement with other studies that showed antibody prepared against CPT purified from rat liver mitochondria can precipitate all of the CPT activity (Brady & Brady, 1987), but those data and our results are not in agreement with reports that the inner (CPT_B) and outer (CPT_A) forms of CPT of liver mitochondria are antigenically different, with very little cross-reactivity (Hoppel et al., 1988; Murthy & Pande, 1988). The CPT_A of Murthy and Pande (1988) has a molecular weight of 77 000, while that of Hoppel et al. (1988) has a molecular weight of 66 300. Both molecular weights are very different from the >90 000 molecular weight of the TDGA-binding protein of rat liver mitochondria, also thought to be CPT-1. These differences may be due, in part, to different sources of rat liver enzymes. The organelle origin of CPT_A (Hoppel et al., 1988) is ambiguous, and, in most respects, it has the properties of "easily soluble" CPT, which was shown to be entirely of nonmitochondrial origin (Ramsay et al., 1987; Healy et al., 1988; Ramsay, 1988). It is plausible, but not proven, that the malonyl-CoA-sensitive CPT activity that occurs in preparations enriched with outer mitochondrial membrane of liver mitochondria (Murthy & Pande, 1987a,b; Cook et al., 1988) are due, at least in part, to the malonyl-CoA-sensitive microsomal carnitine octanoyltransferase (Lilly et al., 1988). This enzyme is sensitive to malonyl-CoA, is very labile, and is potently inhibited by micellar concentrations of palmitoyl-CoA (data submitted elsewhere), similar to the inhibition pattern shown for the preparation of Murthy and Pande (1987b). Our data indicate CPT_o and CPT_i are different catalytic expressions of the same protein, since preparations which contain all of the CPT_o activity show the same subunit molecular weight as that of the purified enzyme. Clearly, additional studies are required to definitely resolve the location issue.

The oligomeric nature of CPT purified from different sources has been documented (Miyazawa et al., 1983; Ozasa et al., 1983; Fiol & Bieber, 1984) and discussed elsewhere (Bieber, 1988). Our data indicate that the malonyl-CoA-sensitive CPT, peak A of Figure 4, is complexed to several other proteins. When this preparation was subjected to native gel electrophoresis, using nondissociating conditions, proteins with molecular weight less than 300 000 were not detected; rather a large, diffuse complex of undefined molecular weight was found. This indicates, but does not prove, that the proteins present in the eluate of the antibody column occur as a complex. In addition to CPT, the complex contains at least one malonyl-CoA binding component and high activities of three of the β-oxidation enzymes, the β-ketothiolase, the β-hydroxyacyl-CoA dehydrogenase, and the enoyl-CoA hydratase. It has a high capcity to convert 2,3-unsaturated acyl-CoAs to acetyl-CoA. Only low amounts of the flavin-linked acyl-CoA dehydrogenase were found, but studies of others have shown that this enzyme is easily solubilized. The monomeric molecular weights shown in Figures 4, 6, and 7, and especially for those in peak AI-2 of Figure 7, correspond to the subunit molecular weights of the acyl-CoA dehydrogenase (Thorpe, 1981; Hall, 1981), enoyl-CoA hydratases (Fong & Schulz, 1981), β-hydroxyacyl-CoA dehydrogenases (Bitar et al., 1980; Noyes & Bradshaw, 1973), and β-ketothiolases (Schulz & Staack, 1981). The immunoaffinity column retains the overall capacity for conversion of Δ^2-*trans*-decenoyl-CoA and crotonyl-CoA to acetyl-CoA from the octyl glucoside extract (30%). When CPT is separated from the β-oxidation enzymes (fraction AI, Figure 6) and this fraction is repassed over the antibody column, <10% of the β-oxidation activity is retained, indicating that CPT is required for the binding (data not shown).

If, indeed, CPT_o and CPT_i are associated with the enzymes of β-oxidation, communication between β-oxidation enzymes and CPT_o might occur. This could provide a partial explanation for the apparent different kinetic properties of CPT_o and CPT_i. If these enzymes are catalytic expressions of the same oligomeric protein, then it seems likely that association of CPT with a regulator component on the cytosolic face of the inner membrane conveys a set of kinetic parameters that are different from the kinetic parameters for the enzyme exposed to the matrix surface of the inner membrane which may be associated with β-oxidation enzymes.

The average recovery of total CPT from the immunoaffinity column (peak A of Figure 4) was 46%. One possibility for the low recovery is that the high elution pH, $\cong$10.6, causes hydrolysis of bound cardiolipin, thereby altering catalytic capacity. Fiol and Bieber (1984) demonstrated purified beef heart CPT contains approximately 17 mol of lipid phosphorus/69 000, the subunit molecular weight. Preliminary, unpublished data indicate addition of mitochondrial lipids to neutralized peak A of Figure 4 approximately doubles the CPT activity. Alternatively, removal of octyl glucoside by the antibody column could significantly reduce the V_{max} of CPT, since octyl glucoside stimulates CPT activity of rat heart mitochondria about 1.6-fold. Regardless, the antibody column eluates contain both malonyl-CoA-sensitive and malonyl-CoA-insensitive CPT activities without any detectable dif-

ferences in molecular weight using SDS-PAGE.

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