Palmitoyl-coenzyme A hydrolyzing activity in rat kidney and its relationship to carboxylesterase

Takahiro Tsujita[1] **and Hiromichi Okuda**

Department of Medical Biochemistry, School of Medicine, Ehime University, Shigenobu, Onsen-gun, Ehime 791-02, Japan

Abstract Palmitoyl-coenzyme A (palmitoyl-CoA) hydrolase was obtained from rat kidney in an electrophoretically homogeneous form. The enzyme associated with carboxylesterase activity was purified by acetone precipitation of microsomes, followed by successive chromatographies on DEAE-cellulose, phenyl-Sepharose, and Sephadex G-100 gel. The two activities in rat kidney were associated as judged by their co-elution profiles, co-purification at different steps, co-precipitation by an antibody raised against the purified enzyme, and identical profiles of inhibition by diisopropylfluorophosphate. The enzyme catalyzed the hydrolysis of long- and medium-chain acyl-CoA, but not short-chain acyl-CoA. The N-terminal amino acid sequence of the first 27 residues of the purified enzyme was 80% identical with that of the carboxylesterase from rat adipose tissue. Using a polyclonal rabbit antibody against the rat kidney palmitoyl-CoA hydrolase, the enzyme was demonstrated in liver but not in adipose tissue. The antibody reacted with the carboxylesterase(s) (pI 6.3 and pI 6.6) in rat liver microsomes. The antibody removed the palmitoyl-CoA hydrolase in kidney (75%) and liver (68%). The antibody also removed the monoolein hydrolase in kidney (77%) and liver (61%). These results suggest that carboxylesterase contributes to the hydrolysis of long-chain acyl-CoA and monoglyceride in kidney and liver.—**Tsujita, T., and H. Okuda.** Palmitoyl-coenzyme A hydrolyzing activity in rat kidney and its relationship to carboxylesterase. *J. Lipid Res.* 1993. **34:** 1773–1781.

Supplementary key words palmitoyl-CoA hydrolase • rat liver microsomes

Most cells are able to metabolize fatty acids. Fatty acid molecules are chemically unreactive toward nucleophilic substitution. Therefore, for there to be greater reactivity, the carboxyl group should be changed to an ester, thioester, or acid anhydride. The most important activation of fatty acids is esterification by coenzyme A. Long-chain fatty acyl-CoA is utilized in energy production by β-oxidation and in the synthesis of triglyceride, phospholipids, and cholesteryl esters (1). Recently, fatty acyl-CoAs were suggested to have other biological functions: modulation of binding of the thyroid hormone to its nuclear receptor (2), activation of protein kinase C (3), and vesicle budding from multiple Golgi cisternae (4).

Fatty acyl-CoA hydrolase (E.C. 3.1.2.2) hydrolyzes fatty acyl-CoA thioesters to CoA and fatty acids. It is important in lipid metabolism: it could control the chain length of synthesized fatty acids and modify the product specificity of fatty acid synthetase (5, 6). Fatty acyl-CoA hydrolase has been found in various mammalian tissues, and its various subcellular localizations have been described (7, 8). High activities are in the kidney, liver, spleen, testes, and heart (7). The enzyme has not previously been purified from rat kidney, which is one of its main sources. In the present work, we describe in detail the purification procedure and some characteristics of the purified enzyme from rat kidney, and show that it is associated with carboxylesterase.

Carboxylesterases are widely distributed in animals and plants (9, 10). Despite the wide distribution of this enzyme, most of its known substrates are foreign compounds that are not normally involved in intermediary metabolism. Thus the physiological function of carboxylesterase is not known. In this paper, we demonstrate that palmitoyl-CoA hydrolase in rat kidney is the same enzyme as rat kidney carboxylesterase and it contributes to hydrolysis of long-chain fatty acyl-CoA and monoglyceride in kidney and liver.

MATERIALS AND METHODS

Materials

Compounds were obtained from the following sources: [1-^{14}C]palmitoyl-coenzyme A (2.11 GBq/mmol) from New England Nuclear (Boston, MA); phenyl-Sepharose and Sephadex G-100 from Pharmacia LKB Biotechnology (Uppsala, Sweden); DEAE-cellulose (DE-52) from Whatman BioSystems (Maidstone, UK); *p*-nitrophenyl

Abbreviations: CoA, coenzyme A; PNPB, *p*-nitrophenyl butyrate; DFP, diisopropylfluorophosphate; DTNB, 5',5'-dithiobis(2-nitrobenzoic acid).

[1]To whom correspondence should be addressed.

butyrate (PNPB), acyl-CoAs, and diisopropylfluorophosphate (DFP) from Sigma (St. Louis, MO); acylglycerols from Serdary Research Laboratories (London, Canada); and peroxidase anti-rabbit IgG (H + L) from Daiichi Kayaku (Tokyo, Japan). Bovine serum albumin from Wako Pure Chemical Industries (Osaka, Japan) was extracted by the method of Chen to remove free fatty acid (11).

Methods

Enzyme purification. All purification procedures were carried out at 0–4°C. Wistar strain rats, weighing 300–400 g, were killed and their kidneys were excised, chilled, minced, and homogenized in 3 volumes of 10 mM Tris-HCl (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA in a pestle-type homogenizer. The homogenate from 150 g of kidney was centrifuged for 10 min at 1,000 *g* and the supernatant was centrifuged for 10 min at 8,000 *g*. The 8,000 *g* supernatant was brought to pH 5.0 by dropwise addition of acetic acid (0.2 M) with constant stirring; after 10–15 min, the mixture was centrifuged at 8,000 *g* for 10 min. The precipitate was resuspended in 100 mM Tris-HCl at half the original volume and stored overnight at −20°C. Then 0.11 volume of Triton X-100 (35 mg per ml in the buffer) was added slowly with stirring; after 10–15 min, the pH was adjusted to 5.0 by dropwise addition of 0.2 M acetic acid. After 15 min, the mixture was centrifuged at 8,000 *g* for 15 min and the clear supernatant was mixed with an equal volume of cold acetone (−20°C). The mixture was centrifuged at 8,000 *g* for 10 min and the precipitate was washed with 300 ml cold acetone and cold ethyl ether. The precipitate was dried under reduced pressure (in a desiccator) for 1 h. Then it was extracted with 10 mM NH_4OH (one-quarter of the original homogenate volume) at 0°C for 1 h and centrifuged at 100,000 *g* for 1 h. The supernatant fraction was dialyzed against 10 mM potassium phosphate buffer (pH 8.0) and applied to a DEAE-cellulose column (2.7 × 35 cm) equilibrated with the same buffer. The column was washed with the starting buffer, and then material was eluted with a linear gradient formed with one-liter volumes of 0 and 300 mM NaCl. Active fractions were pooled and solid NaCl was added to a final concentration of 1 M. The mixture was applied to a phenyl-Sepharose column (2.1 × 11 cm) equilibrated with 10 mM potassium phosphate buffer (pH 7.0) containing 1 M NaCl. Material was eluted with a linear gradient formed with 400-ml volumes of the same buffer and of 10 mM potassium phosphate buffer (pH 7.0), containing 0.2% Brij-35. Active fractions were pooled, dialyzed against 5 mM potassium phosphate buffer (pH 7.0), and concentrated. The enzyme was applied to a Sephadex G-100 column (2.7 × 120 cm) equilibrated with 10 mM potassium phosphate buffer (pH 7.0) containing 200 mM NaCl. Active fractions were pooled and dialyzed against 10 mM potassium phosphate buffer (pH 7.0).

Enzyme assay. Acyl-CoA-hydrolyzing activity was measured by two methods. Unless otherwise stated, the activity was determined by measuring the rate of release of free thiol groups using 5',5'-dithiobis (2-nitrobenzoic acid) (DTNB) at 25°C by a modification of the procedure of Alexson and Nedergaard (12). The assay mixture consisted of 0.8 ml of 10 mM HEPES buffer (pH 7.4) containing 100 μM acyl-CoA, 0.3 mM DTNB, and 0.025% Triton X-100. Released free thiol groups were determined continuously by monitoring the absorbance at 412 nm using incubation mixture without enzyme as a blank. The molar extinction coefficient of reduced glutathione was taken as 13,300 M^{-1} cm^{-1}. The hydrolase activity was also determined by a radiochemical method where the release of [1-^{14}C]palmitic acid from [1-^{14}C]palmitoyl-CoA was measured. The assay mixture consisted of 0.25 ml of 10 mM HEPES buffer (pH 7.4) containing 100 μM [1-^{14}C]palmitoyl-CoA (29.6 kBq/μmol). Incubation was carried out at 25°C for 2 min. The reaction was stopped by adding 1.0 ml Dole's reagent and unesterified [1-^{14}C]palmitic acid was extracted by the method of Bar-Tana, Rose, and Shapiro (13). Equivalent results were obtained by the two methods (specific activity of purified enzyme was 13.5 and 15.1 μmol/mg per min by using spectrophotometric and radiochemical methods, respectively). The enzyme activity determined by the radiochemical method was not affected by the addition of 1 mM DTNB.

PNPB-hydrolyzing activity was determined by measuring the release of *p*-nitrophenol (absorbance at 400 nm at 25°C) by the procedure described previously (14).

Monoglyceride-hydrolyzing activity was assayed with synthetic monoolein emulsified with gum arabic. The assay system contained the following components in a total volume of 0.2 ml: 2 μmol of 1-monoolein, 2.5 mg of gum arabic, 5 mg of bovine serum albumin, 2.5 μmol of potassium phosphate, and 30 μmol enzyme solution. Incubation was carried out at pH 7.0 for 1 h. The oleic acid produced was extracted and determined as described previously (15).

Purification of antibody. Antiserum against purified rat kidney palmitoyl-CoA hydrolase was raised in rabbits. The rabbit serum was treated with ammonium sulfate at 33% saturation, and the precipitate was collected and dissolved in saline. The resulting solution was treated with ammonium sulfate at 33% saturation. The precipitate was dissolved in and dialyzed against 20 mM potassium phosphate buffer (pH 8.0) at 4°C. The dialysate was then applied to a DEAE-cellulose column (1.5 × 20 cm). The unadsorbed fractions were pooled, dialyzed against 5 mM potassium phosphate buffer (pH 7.0), and lyophilized. Anti-IgG against rat adipose tissue carboxylesterase was prepared as described previously (16).

Immunochemical methods. Immunodiffusion was performed by the method for the double diffusion test. Agar plates were prepared with 1.5% agarose in 50 mM potas-

sium phosphate buffer (pH 7.0) containing 0.1 M NaCl and 0.01% NaN_3. SDS-gel electrophoresis was performed in an 8% gel under reducing conditions as described by Laemmli (17). Proteins were transferred electrophoretically to nitrocellulose as described previously (16). The membranes were blocked by treatment with 3% bovine serum albumin in TBS (20 mM Tris, pH 7.5, containing 0.15 M NaCl) for 2 h and then incubated with primary antibody (rabbit anti-rat kidney palmitoyl-CoA hydrolase, 2 μg/ml) in TBS containing 1% bovine serum albumin for 1 h. The membranes were then washed three times with T-TBS (TBS containing 0.1% Tween-20), and incubated with the second antibody (goat anti-rabbit IgG conjugated to peroxidase at 2000-fold dilution) in TBS containing 1% bovine serum albumin for 1 h. After three washes with T-TBS, the membranes were incubated with TBS containing 0.5 mg/ml 3,3-diaminobenzdine and 0.003% H_2O_2, rinsed with water to stop the reaction, and air-dried.

Isoelectric focusing of rat liver microsomal esterases. Rat livers were homogenized in 4 volumes of 25 mM Tris-HCl (pH 7.4) containing 0.25 M sucrose and 1 mM EDTA. The homogenate from 60 g of liver was centrifuged for 10 min at 1,000 *g*, for 10 min at 10,000 *g* and then for 1 h at 100,000 *g*. The 100,000 *g* precipitate was suspended in 5 mM potassium phosphate buffer (pH 7.2) containing 0.2% Triton X-100 with constant stirring at 0°C. After 30 min, the mixture was centrifuged at 100,000 *g* for 1 h and the clear supernatant was mixed with 2 volumes of cold acetone (−20°C). The mixture was centrifuged at 8,000 *g* for 15 min and the precipitate was washed with cold acetone and cold ethyl ether. The precipitate was dried under reduced pressure (in a desiccator) for 1 h. Then it was extracted with 5 mM potassium phosphate buffer (pH 7.0) at 4°C for 12 h and centrifuged at 100,000 *g* for 1 h. The supernatant fraction was dialyzed against 5 mM potassium phosphate buffer (pH 7.0). After addition of carrier ampholyte of pH 4 to 8 (1.2% final concentration), the mixture was transferred to a 110-ml electrofocusing column. Electrofocusing was carried out for 60 h with a gradient of 0 to 50% sucrose at 600 volts.

Other procedures. SDS-gel electrophoresis was performed in 8% gels under reduced conditions as described by Laemmli (17). The gel was stained with Coomassie Brilliant Blue R-250. Relative protein concentration was determined with a Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin as a standard.

RESULTS

The palmitoyl-CoA- and PNPB-hydrolyzing activities of the acetone powder extract were adsorbed to a column of DEAE-cellulose. The palmitoyl-CoA-hydrolyzing activity was eluted in two peaks, both coinciding with PNPB-hydrolyzing activity (**Fig. 1**). The enzyme activities of both peaks were completely removed by addition of anti-IgG against purified rat kidney enzyme (data not shown). The first major peak (fractions 34–41) was applied to a phenyl-Sepharose column. Much of the protein was not adsorbed to the column, but the palmitoyl-CoA- and PNPB-hydrolyzing activities were both completely bound and showed similar elution profiles (data not shown). Active fractions were pooled, concentrated, and subjected to Sephadex G-100 gel filtration (**Fig. 2**). The palmitoyl-CoA- and PNPB-hydrolyzing activities were recovered together in a single peak. The steps of enzyme purification and the yields of enzyme at each step are summarized in **Table 1.** At the final step of purification the specific activities for palmitoyl-CoA and PNPB hydrolysis were 13.5 μmol/mg protein per min and 136 μmol/mg protein per min, respectively. In the steps of purification, the ratios of specific activities to percentage recoveries

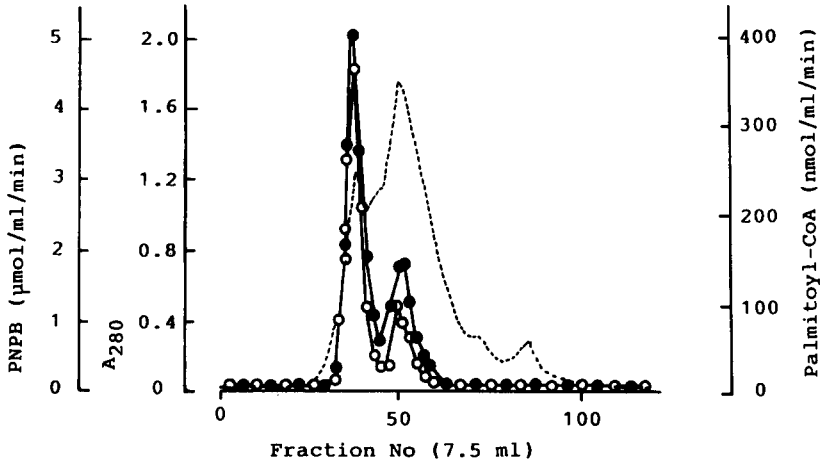

Fig. 1. Ion-exchange column chromatography of rat kidney palmitoyl-CoA hydrolase on DEAE-cellulose. An acetone powder extract from rat kidney microsomes was applied to the column. The column was washed with buffer and developed with a linear gradient of 0–0.3 M NaCl. Fractions of 7.5 ml were collected. A_{280} (----) and palmitoyl-CoA- (●) and PNPB- (○) hydrolyzing activities were measured.

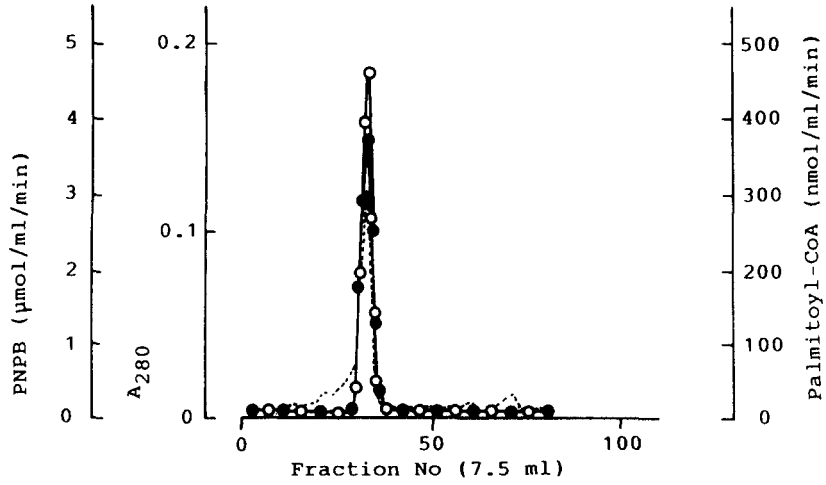

Fig. 2. Gel filtration of rat kidney palmitoyl-CoA hydrolase on Sephadex G-100. The eluent was 10 mM potassium phosphate buffer, pH 7.0, containing 200 mM NaCl. Fractions of 10 ml were collected. A_{280} (----) and palmitoyl-CoA- (●) and PNPB- (○) hydrolyzing activities were measured.

were the same. The purified enzyme gave a single band on SDS-polyacrylamide gel electrophoresis from which its molecular weight was estimated to be 60,000 (**Fig. 3**).

Fig. 4 represents the enzymatic activities as functions of palmitoyl-CoA concentration in the presence and absence of 0.025% Triton X-100. The enzyme activities were about 2- to 3-fold higher in the presence of 0.025% Triton X-100. With Triton X-100, the enzyme activity increased with the concentration of palmitoyl-CoA to 200 mM and then decreased. Without Triton X-100, the activities increased with the concentration of substrate to 70 mM and then slightly decreased. The enzyme catalyzed the hydrolysis of long- and medium-chain acyl-CoA, but not short-chain acyl-CoA. The activities for lauroyl-CoA and decanoyl-CoA were about 150% and 44%, respectively, of that for palmitoyl-CoA. The enzyme did not hydrolyze hexanoyl-CoA or acetyl-CoA (**Fig. 5**). The susceptibility of fatty acid chain length of acyl-CoA was not changed in the presence and absence of Triton X-100. **Fig. 6** represents the effect of PNPB concentration on palmitoyl-CoA hydrolysis. PNPB inhibited palmitoyl-CoA hydrolysis in a concentration-dependent manner (50% inhibition at about 1 mM). On the other hand, PNPB-hydrolyzing activity was inhibited by addition of palmitoyl-CoA (50% inhibition at about 0.5 mM) (data not shown). Bovine serum albumin influenced the palmitoyl-CoA-hydrolyzing activity to a maximal level (125 nmol palmitoyl-CoA/mg albumin), and further increase in the ratio of palmitoyl-CoA to albumin inhibited the activity (**Fig. 7**). Fig. 7 also shows that Triton X-100 influenced the palmitoyl-CoA-hydrolyzing activity. At 100 μM palmitoyl-CoA concentration, the activity was maximal at ca. 2 μmol palmitoyl-CoA/% Triton X-100 and further increase in the ratio inhibited the activity.

The purified enzyme was mixed with various amounts of anti-IgG prepared with rat kidney palmitoyl-CoA hydrolase, left to stand overnight at 4°C, and then centrifuged to remove insoluble material. Residual enzyme activities in the supernatant were measured with each substrate (**Fig. 8**). The palmitoyl-CoA- and PNPB-hydrolyzing activities of the supernatant decreased in parallel with increase in the amount of anti-IgG. Neither activity was affected by nonimmune IgG, which was used as a control (data not shown).

TABLE 1. Purification of palmitoyl-CoA hydrolase from rat kidney

Purification Step	Total Protein	Palmitoyl-CoA Hydrolysis		PNPB Hydrolysis	
		Total Activity	Specific Activity	Total Activity	Specific Activity
	mg	*μmol/min*	*μmol/mg/min*	*μmol/min*	*μmol/mg/min*
Extraction of acetone powder	253	47.6	0.188	510	2.02
DEAE-cellulose	30.5	23.7	0.776	221	7.25
Phenyl-Sepharose	2.7	20.9	7.76	199	73.5
Sephadex G-100	0.83	11.2	13.5	113	136

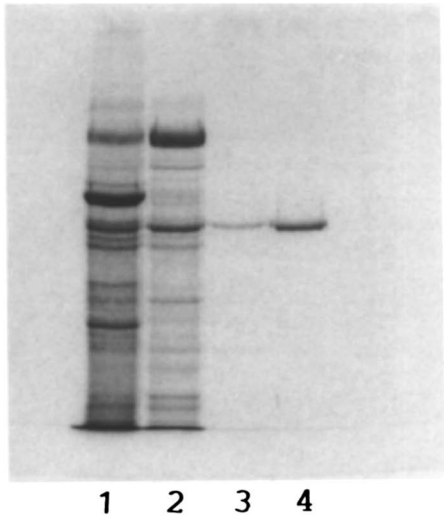

Fig. 3. SDS-polyacrylamide gel electrophoresis of rat kidney palmitoyl-CoA hydrolase. (1) Acetone powder extract (approximately 42 μg of protein); (2) DEAE-cellulose (approximately 10 μg of protein); (3) phenyl-Sepharose (approximately 2 μg of protein); (4) Sephadex G-100 (approximately 3 μg of protein).

DFP inhibited the palmitoyl-CoA- and PNPB-hydrolyzing activities of the purified enzyme, the profiles of inhibition of the two activities being essentially identical (**Fig. 9**). DFP at 5×10^{-7} M inhibited the activities about 50% after 10 min preincubation at 25°C.

The amino acid sequence of the first 27 N-terminal residues of the purified enzyme are Asn-Pro-Ser-Ser-Pro-Pro-Val-Val-Asp-Thr-Thr-Lys-Gly-Lys-Val-Leu-Gly-Lys-Tyr-Val-Ser-Leu-Glu-Gly-Val-Thr-Gln-. This sequence is about 80% identical with that of the carboxylesterase from rat adipose tissue. Anti-IgG against purified rat kidney enzyme gave a single precipitin line against acetone powder extracts of kidney and liver, but not of adipose tissue (**Fig. 10 A**). Western blot analysis showed the immunoreactive protein in kidney and liver, but not adipose tissue (Fig. 10 B).

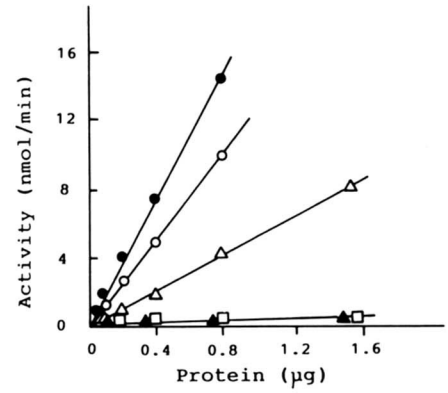

Fig. 5. Acyl specificity of the hydrolase on acyl-CoA. The acyl-CoA concentration was 100 μM; palmitoyl-CoA (○), lauroyl-CoA (●), decanoyl-CoA (△), hexanoyl-CoA (▲), and acetyl-CoA (□).

The rat liver microsomal esterases acting on PNPB with isoelectric points 5.1, 5.5, 6.0, 6.3, and 6.6 were resolved by isoelectric focusing (**Fig. 11 A**). The palmitoyl-CoA-hydrolyzing activity was separated in two peaks, pI 6.3 and 6.6, the elution profiles of the two peaks coinciding with those of PNPB-hydrolyzing activity. On addition of anti-IgG against purified rat kidney enzyme to each fraction, the peaks of pI 6.3 and 6.6 disappeared. No palmitoyl-CoA-hydrolyzing activity was detected (Fig. 11 B). The enzyme peak of pI 6.0 disappeared on addition of anti-IgG against rat adipose tissue carboxylesterase (Fig. 11 C).

Table 2 shows data on the palmitoyl-CoA-, PNPB-, and monoolein-hydrolyzing activities of acetone powder extracts of rat kidney and liver. Anti-IgG against the rat kidney enzyme removed the palmitoyl-CoA-hydrolyzing activity in kidney (75%) and that in liver (68%). The

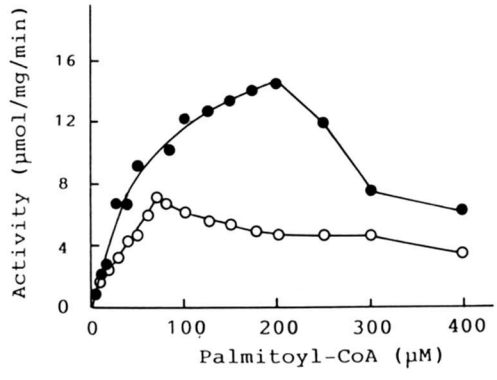

Fig. 4. Effect of substrate concentration on palmitoyl-CoA-hydrolyzing activity. The activity was assayed with 0.25 μg of purified enzyme with (●) and without (○) 0.025% Triton X-100.

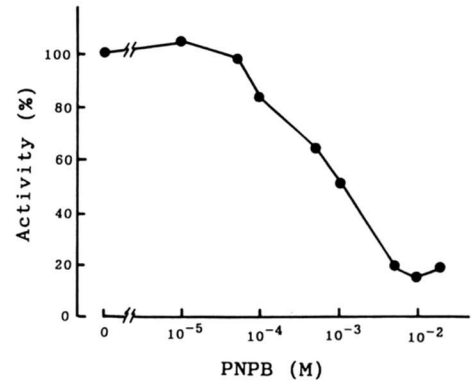

Fig. 6. Inhibition by PNPB of palmitoyl-CoA-hydrolyzing activities of purified rat kidney palmitoyl-CoA hydrolase. Activity was assayed with 100 μM [1-^{14}C]palmitoyl-CoA and 0.2 μg of enzyme.

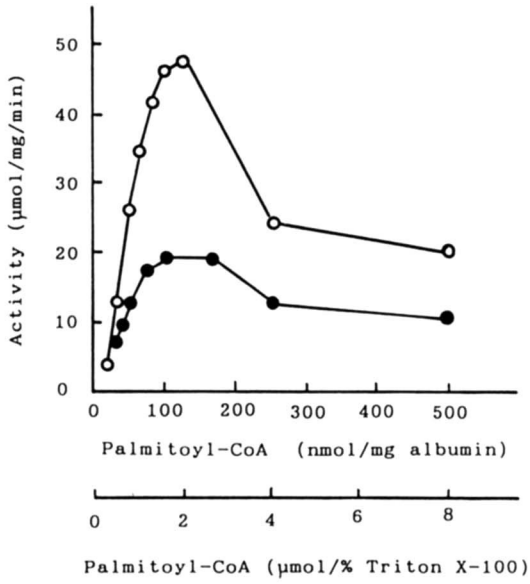

Fig. 7. Effect of the ratio of palmitoyl-CoA to bovine serum albumin and Triton X-100 as a result of various amounts of bovine serum albumin, and Triton X-100 in the presence of a constant amount of palmitoyl-CoA and purified enzyme. The reaction mixture contained 10 mM HEPES buffer (pH 7.4), 2.5 μg of purified enzyme, and decreasing amounts of bovine serum albumin from 5.0 to 0.2 mg/ml or Triton X-100 from 0.2 to 0.01, and 100 μM of palmitoyl-CoA. Bovine serum albumin (○); Triton X-100 (●).

antibody also removed the monoolein-hydrolyzing activities in kidney (77%) and liver (61%). It also removed 81% of the PNPB-hydrolyzing activity in kidney.

DISCUSSION

Long-chain fatty acyl-CoA hydrolase is present in various mammalian tissues and seems to be important in lipid and carbohydrate metabolism. In this study, we purified

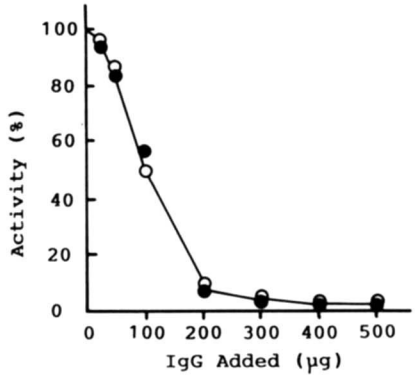

Fig. 8. Effect of palmitoyl-CoA hydrolase antibody on palmitoyl-CoA- and PNPB-hydrolyzing activities. The purified enzyme (2.5 μg) was mixed with various amounts of anti-IgG. After overnight incubation at 4°C, the mixtures were centrifuged to remove insoluble materials. Residual palmitoyl-CoA- (●) and PNPB- (●) hydrolyzing activities were then measured.

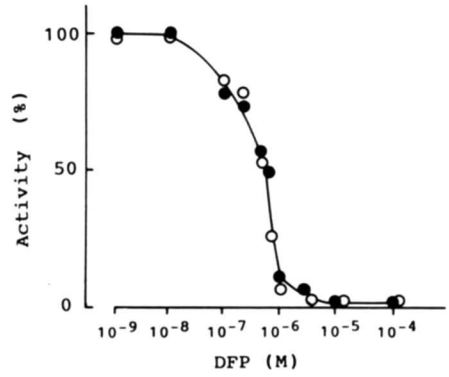

Fig. 9. Inhibition by DFP of palmitoyl-CoA- and PNPB-hydrolyzing activities of purified rat kidney palmitoyl-CoA hydrolase. The enzyme was incubated with various amounts of DFP for 10 min at 37°C. Remaining palmitoyl-CoA- (●) and PNPB- (○) hydrolyzing activities were then measured.

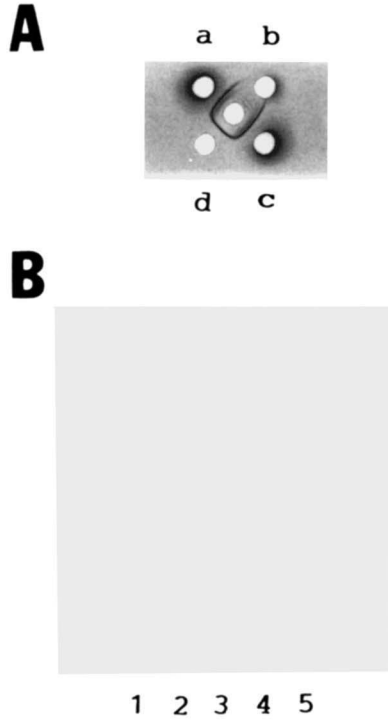

Fig. 10. A: Immunodiffusion study of palmitoyl-CoA hydrolase. The wells contain 20 μl of acetone powder extract from rat kidney (120 μg, a), purified rat adipose tissue carboxylesterase (0.8 μg, b), acetone powder extract from rat liver (103 μg, c), purified rat kidney palmitoyl-CoA hydrolase (0.5 μg, d), and anti-IgG prepared against rat kidney palmitoyl-CoA hydrolase (200 μg, center well). The gel was stained with Coomassie Brilliant Blue R-250. B: Immunoblotting analysis with antibody against rat kidney palmitoyl-CoA hydrolase. Proteins (10 μl) were separated by SDS-polyacrylamide gel (8%) electrophoresis and transferred to a nitrocellulose membrane. Protein was detected with anti-palmitoyl-CoA hydrolase IgG. Lane 1, 2 μg of purified rat kidney palmitoyl-CoA hydrolase; lane 2, 71 μg of acetone powder extract from rat kidney; lane 3, 43 μg of acetone powder extract from rat liver; lane 4, 50 μg of acetone powder extract from rat adipose tissue; lane 5, 4 μg of purified rat adipose tissue carboxylesterase.

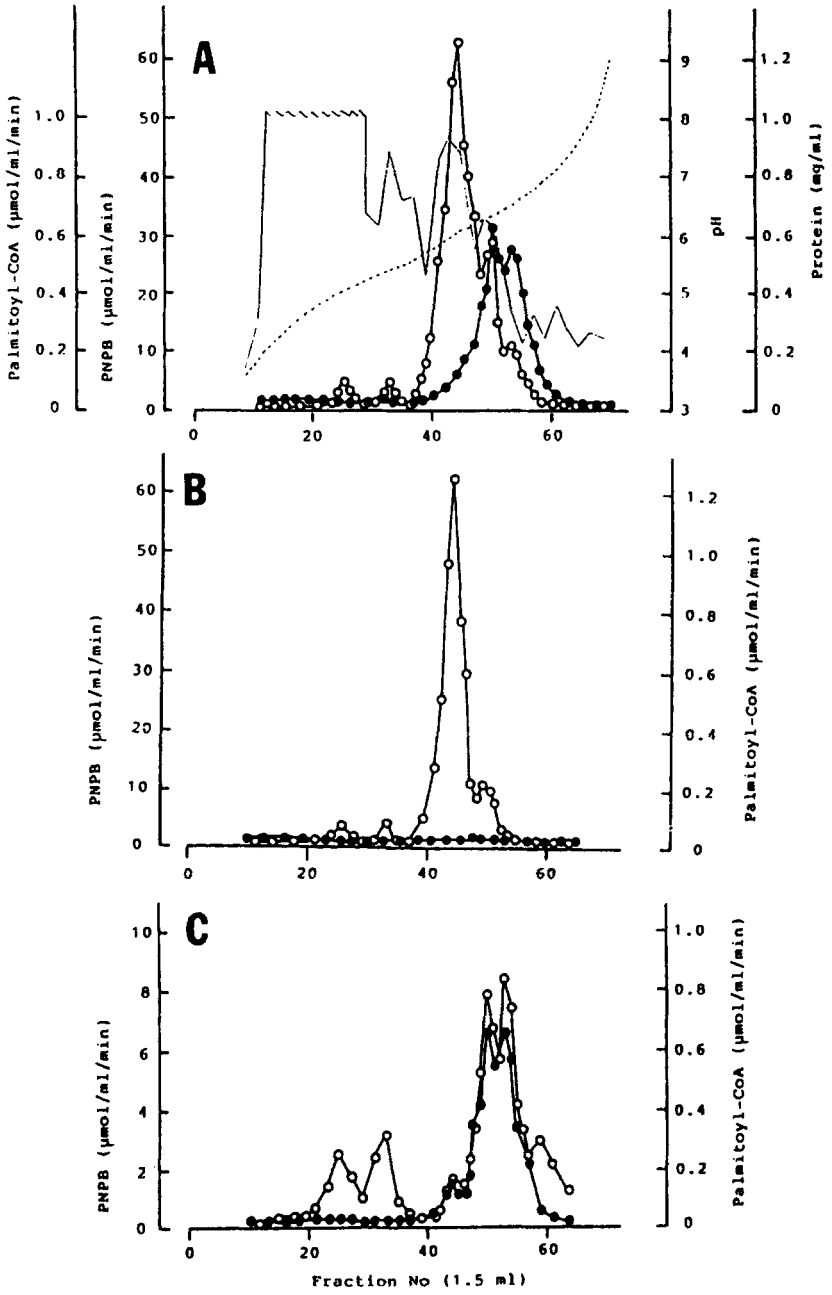

Fig. 11. A: Isoelectric focusing of esterases from rat liver microsomes. Esterases were extracted by Triton X-100 and resolved by isoelectric focusing in a pH 4–8 gradient as described in Materials and Methods. Fractions were 1.5 ml each. Palmitoyl-CoA- (●) and PNPB- (○) hydrolyzing activities were measured; A_{280} (——) and the pH at 4°C (----). B: Effect of anti-IgG against rat kidney palmitoyl-CoA hydrolase. Each fraction (100 μl) was mixed with anti-IgG (3 mg) against rat kidney palmitoyl-CoA hydrolase. After overnight incubation at 4°C, the mixtures were centrifuged to remove insoluble materials. Residual palmitoyl-CoA- (●) and PNPB- (○) hydrolyzing activities were then measured. C: Effect of anti-IgG against rat adipose tissue carboxylesterase. Each fraction (100 μl) was mixed with anti-IgG (3 mg) against rat adipose tissue carboxylesterase. Palmitoyl-CoA- (●) and PNPB- (○) hydrolyzing activities were measured as described in the legend of Fig. 11 B.

acyl-CoA hydrolase from rat kidney and showed that it was associated with carboxylesterase. The association of the two enzyme activities was concluded from the following observations. *a)* The elution pattern of the palmitoyl-CoA-hydrolyzing activity coincided with that of PNPB-hydrolyzing activity during the purification steps (Figs. 1 and 2). *b)* During the purification procedure, the ratio of palmitoyl-CoA- and PNPB-hydrolyzing activities was almost constant (Table 1). *c)* The purified preparation had both palmitoyl-CoA- and PNPB-hydrolyzing activities. *d)*

TABLE 2. Effect of anti-palmitoyl-CoA hydrolase antibody on palmitoyl-CoA-, PNPB-, and monoolein-hydrolyzing activities

Organ	Palmitoyl-CoA Hydrolysis		PNPB Hydrolysis		Monoolein Hydrolysis	
	Control IgG	Anti-IgG	Control IgG	Anti-IgG	Control IgG	Anti-IgG
	nmol/mg/min		*μmol/mg/min*		μmol/mg/h	
Kidney	22.9 ± 1.30	5.65 ± 0.59	0.350 ± 0.02	0.066 ± 0.01	4.47 ± 0.27	1.02 ± 0.11
Liver	15.4 ± 1.05	4.98 ± 0.28	0.740 ± 0.03	0.481 ± 0.03	2.59 ± 0.10	1.02 ± 0.06

Five male Wistar rats (body weight 202 ± 2.3 g) were used. Acetone powders of rat kidney and liver were extracted with 50 mM NH_4Cl buffer, pH 8.5, overnight at 4°C and centrifuged at 100,000 *g* for 1 h. Samples of supernatants (100 μl) were mixed with IgG (1 mg), kept at 4°C overnight, and then centrifuged to remove insoluble material. Residual palmitoyl-CoA-, PNPB-, and monoolein-hydrolyzing activities were measured. Values are mean ± SE.

Palmitoyl-CoA-hydrolyzing activity of the purified enzyme was reduced by addition of PNPB (Fig. 6) and, conversely, PNPB-hydrolyzing activity was reduced by addition of palmitoyl-CoA. *e)* Antibody against the purified enzyme affected both the palmitoyl-CoA- and PNPB-hydrolyzing activities, and the profiles of their precipitations were essentially identical (Fig. 8). *f)* The DFP inhibition profiles of the two enzymes were identical (Fig. 9).

The mobility of this enzyme and standard proteins on SDS-gels and Sephadex G-100 indicated that the purified enzyme is composed of one polypeptide with a molecular weight of ca. 60,000. This value was essentially the same as those of subunits of the carboxylesterase from rat liver (18), pig liver (19), and human liver (20). A similar molecular weight (59,000) was estimated for a long-chain acyl-CoA hydrolase from rat liver microsomes (8). However, proteins of various molecular weights with long-chain acyl-CoA hydrolase activity have been isolated from several tissues. Anderson and Erwin (21) reported that bovine brain acyl-CoA hydrolase seemed to be a dimer with a molecular weight of 96,000. Berge and Farstad (22) reported an enzyme from rat liver mitochondria with a molecular weight of 19,000. The molecular weight of acyl-CoA hydrolase from rat lactating mammary gland was found to be 29,000 (23).

The enzyme catalyzed the hydrolysis of both tri- and monoacylglycerols (data not shown). The susceptibility of substrates decreased with increase in length of the acyl chain of the fatty acid moiety in glyceride. The enzyme had little or no activity on long-chain triacylglycerols such as triolein and tripalmitin. This enzyme hydrolyzed water-soluble carboxyl esters such as tributyrin and methyl butyrate. Thus the enzyme is an esterase. Although the susceptibility of glycerides decreased with an increase in length of the acyl chain of the fatty acid moiety, the enzyme showed preference for long-chain acyl-CoA.

The palmitoyl-CoA-hydrolyzing activity was strongly influenced by albumin. The activity of the hydrolase was maximal when the substrate to protein ratio was ca. 125 nmol palmitoyl-CoA/mg albumin, and a higher as well as a lower ratio resulted in a lower activity (Fig. 7). Similar results were observed with rat liver mitochondria and microsomal acyl-CoA hydrolase, for which maximal ratios were 115 nmol/mg albumin and 120 nmol/mg albumin, respectively (8, 22). Berge (8) suggested that bovine serum albumin could bind to palmitoyl-CoA and it could have prevented enzyme inhibition by substrate in the micellar form at far above the critical micellar concentration.

Mentlein et al. (24, 25) reported the isolation of five carboxylesterases from rat liver microsomes. All these enzymes were capable of cleaving monoglycerides and small synthetic esters, and all had a subunit molecular weight of about 60,000, but differed in their isoelectric points (pIs 5.2, 5.6, 6.0, 6.2, and 6.4). These enzymes also differed in the substrate specificity for neutral lipids (26). The pI 6.0 enzyme had the highest activity toward short aliphatic esters. Long-chain acyl-CoA was hydrolyzed by the enzymes of pIs 6.2 and 6.4. We also resolved five microsomal esterases (pIs 5.1, 5.5, 6.0, 6.3, and 6.6) by isoelectric focusing (Fig. 11 A). The palmitoyl-CoA-hydrolyzing activities of rat liver microsomes were completely removed by antibody against the rat kidney enzyme. The antibody against the rat adipose tissue enzyme reacted with the enzyme of pI 6.0 (Fig. 10 C). We have reported that antibody against rat adipose tissue enzyme reacted with the enzymes of liver, lung, and testis, but not kidney (16), and that the sequence of the first 19 N-terminal amino acid residues of the adipose tissue enzyme is identical to those of the enzymes of liver (pI 6.0) and lung (16). The N-terminal amino acid sequence of the rat kidney enzyme is not identical to that of the adipose tissue enzyme. From these results, we conclude that rat liver microsomes contain both the adipose tissue type enzyme (pI 6.0) and the kidney type enzyme (pIs 6.3 and 6.6), and the kidney type enzyme has long-chain acyl-CoA-hydrolyzing activity.

Most of the palmitoyl-CoA- and monoolein-hydrolyzing activities in kidney and liver were removed by antibody

against the rat kidney enzyme (Table 2). We conclude from this study that long-chain acyl-CoA hydrolase in rat kidney is the same enzyme as rat kidney carboxylesterase and that it is also present in liver (pIs 6.2 and 6.4 carboxylesterase (26)). Therefore, this enzyme in kidney and liver contributes to hydrolysis of long-chain acyl-CoAs and long-chain monoacylglycerols.

We thank Mr. Takeshi Takaku (Central Research Laboratory, University of Ehime) for N-terminal amino acid sequencing.

Manuscript received 15 February 1993 and in revised form 7 May 1993.

REFERENCES

1. Waku, K. 1992. Origins and fates of fatty acyl-CoA esters. *Biochim. Biophys. Acta.* **1124:** 101–111.
2. Li, Q., N. Yamamoto, A. Inoue, and S. Morisawa. 1990. Fatty acyl-CoAs are potent inhibitors of the nuclear thyroid hormone receptor in vitro. *J. Biochem.* **107:** 699–702.
3. Bronfman, M., A. Orellana, M. N. Morales, F. Bieri, F. Waechter, W. Staubli, and P. Bentley. 1989. Potentiation of diacylglycerol-activated protein kinase C by acyl-coenzyme A thioesters of hypolipidaemic drugs. *Biochem. Biophys. Res. Commun.* **159:** 1026–1031.
4. Pfanner, N., L. Orci, B. S. Glick, M. Amherdt, S. R. Arden, V. Malhotra, and J. E. Rothman. 1989. Fatty acyl-coenzyme A is required for budding of transport vesicles from Golgi cisternae. *Cell.* **59:** 95–102.
5. Libertini, L. J., and S. Smith. 1978. Purification and properties of a thioesterase from lactating rat mammary gland which modifies the product specificity of fatty acid synthetase. *J. Biol. Chem.* **253:** 1393–1401.
6. Knudsen, J., S. Clark, and R. Dils. 1975. Acyl-CoA hydrolase(s) in rabbit mammary gland which control the chain length of fatty acids synthesized. *Biochem. Biophys. Res. Commun.* **65:** 921–926.
7. Kurooka, S., K. Hosoki, and Y. Yoshimura. 1972. Some properties of long fatty acyl-coenzyme A thioesterase in rat organs. *J. Biochem.* **71:** 625–634.
8. Berge, R. K. 1979. Purification and characterization of a long-chain acyl-CoA hydrolase from rat liver microsomes. *Biochim. Biophys. Acta.* **574:** 321–333.
9. Krish, K. 1971. Carboxylic ester hydrolases. *In* The Enzymes. Vol. 5. P. D. Boyer, editor. Academic Press, New York. 43–69.
10. Tsujita, T., T. Miyada, and H. Okuda. 1988. Purification of rat kidney carboxylesterase and its comparison with other tissue esterases. *J. Biochem.* **103:** 327–331.
11. Chen, R. F. 1967. Removal of fatty acid from serum albumin by charcoal treatment. *J. Biol. Chem.* **242:** 173–181.
12. Alexson, S. E. H., and J. Nedergaard. 1988. A novel type of short- and medium-chain acyl-CoA hydrolase in brown adipose tissue mitochondria. *J. Biol. Chem.* **23:** 13564–13571.
13. Bar-Tana, J., G. Rose, and B. Shapiro. 1971. The purification and properties of microsomal palmitoyl-coenzyme A synthetase. *Biochem. J.* **122:** 353–362.
14. Tsujita, T., and H. L. Brockman. 1987. Regulation of carboxylester lipase adsorption to surface. 1. Chemical specificity. *Biochemistry.* **26:** 8423–8429.
15. Tsujita, T., and H. Okuda. 1983. Carboxylesterases in rat and human sera and their relationship to serum aryl acylamidases and cholinesterases. *Eur. J. Biochem.* **133:** 215–220.
16. Tsujita, T., and H. Okuda. 1992. Fatty acid ethyl ester synthase in rat adipose tissue and its relationship to carboxylesterase. *J. Biol. Chem.* **267:** 23489–23494.
17. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **224:** 1253–1258.
18. Mentlein, R., A. Ronai, M. Robbi, E. Heymann, and O. V. Deimling. 1987. Genetic identification of rat liver carboxylesterases in different laboratories. *Biochim. Biophys. Acta.* **913:** 27–38.
19. Junge, W., E. Heymann, and H. Hollandt. 1974. Further investigations on the subunit structure of microsomal carboxylesterase from pig and ox livers. *Eur. J. Biochem.* **43:** 379–389.
20. Tsujita, T., and H. Okuda. 1983. Human liver carboxylesterase. Properties and comparison with human serum carboxylesterase. *J. Biochem.* **94:** 793–797.
21. Anderson, A. E., and V. G. Erwin. 1971. Brain acyl-coenzyme A hydrolase: distribution, purification and properties. *J. Neurochem.* **18:** 1179–1186.
22. Berge, R. K., and M. Farstad. 1979. Purification and characterization of long-chain acyl-CoA hydrolase from rat liver mitochondria. *Eur. J. Biochem.* **96:** 393–401.
23. Knudsen, J., S. Clark, and R. Dils. 1976. Purification and some properties of a medium-chain acyl-thioester hydrolase from lactating-rabbit mammary gland which terminates chain elongation in fatty acid synthesis. *Biochem. J.* **160:** 683–691.
24. Mentlein, R., S. Heiland, and E. Heymann. 1980. Simultaneous purification and comparative characterization of six serine hydrolases from rat liver microsomes. *Arch. Biochem. Biophys.* **200:** 547–559.
25. Mentlein, R., M. Schumann, and E. Heymann. 1984. Comparative chemical and immunological characterization of five lipolytic enzymes (carboxylesterases) from rat liver microsomes. *Arch. Biochem. Biophys.* **234:** 612–621.
26. Heymann, E., and R. Mentlein. 1981. Carboxylesterases-amidases. *Methods Enzymol.* **77:** 333–345.