Purification of Rabbit Myocardial Cytosolic Acyl-CoA Hydrolase, Identity with Lysophospholipase, and Modulation of Enzymic Activity by Endogenous Cardiac Amphiphiles†

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ABSTRACT: Rabbit myocardial cytosolic acyl coenzyme A (acyl-CoA) hydrolase activity was purified to near-homogeneity by ammonium sulfate precipitation and ion-exchange, gel filtration, chromatofocusing, and hydroxylapatite chromatographies. Kinetic analysis of the purified protein demonstrated a maximum velocity of 24 μmol/(mg·min) and an apparent Michaelis constant of 50 μM. Cytosolic acyl-CoA hydrolase and lysophospholipase activities cochromatographed in every fraction of every step. The purified protein was a single band (M_r 23 000) after sodium dodecyl sulfate–polyacrylamide gel electrophoresis and silver staining. These results suggest that cytosolic lysophospholipase and palmitoyl-CoA hydrolase activities are catalyzed by a single polypeptide with dual activities. Palmitoyl-CoA competitively inhibited lysophospholipase activity (K_i = 4 μM). Low concentrations (20 μM) of lysophosphatidylcholine or L-palmitoylcarnitine increased palmitoyl-CoA hydrolase activity at low palmitoyl-CoA concentrations but had little effect at high concentrations of palmitoyl-CoA. In contrast, high concentrations (100 μM) of lysophosphatidylcholine or L-palmitoylcarnitine inhibited palmitoyl-CoA hydrolase activity. The results suggest that interactions between endogenous cardiac amphiphiles and palmitoyl-CoA hydrolase contribute to the regulation of intracellular long-chain acyl-CoA concentrations and therefore potentially modulate fluxes of fatty acid through several biochemical pathways.

Thioesterification of long-chain fatty acids is the first step of fatty acid utilization. The subsequent metabolic fate of activated fatty acids in myocardial cytosol can be grouped into three main pathways: (1) transfer to carnitine by palmitoylcarnitine acyltransferase; (2) transfer to glycerol phosphate as the first committed step toward the synthesis of lipid metabolites; and (3) hydrolysis by acyl coenzyme A (acyl-CoA)[1] hydrolase to regenerate free fatty acid. Little is known of the physiologic significance of the role of the net futile cycle of thioesterification and hydrolysis, but cytosolic acyl-CoA occupies an ideal position to provide control over several metabolic pathways (Holroyde et al., 1976; Bloch & Vance, 1977; Weinhouse, 1976). Recently acyl-CoA was demonstrated to be an allosteric modulator of glucokinase (Tippett & Neet, 1982), and thus cytosolic acyl-CoA levels are likely to modify rates of glycolysis, β-oxidation, and lipid synthetic pathways. Thus, enzymes which modulate cystolic acyl-CoA concentration, such as acyl-CoA hydrolase, could potentially provide significant metabolic control.

Acyl-CoA hydrolase activity has previously been identified in rabbit myocardial cytosol, but its low reported specific activity (Kako & Patterson, 1975) had placed the physiological significance of hydrolysis of cytosolic thio esters in myocardium in doubt. This study demonstrates the presence of palmitoyl-CoA hydrolase activity in rabbit myocardial cytosol 2 orders of magnitude higher than previously reported, the purification of cytosolic palmitoyl-CoA hydrolase to near-homogeneity and its identity with cytosolic lysophospholipase, and also presents kinetic evidence which demonstrates that endogenous cardiac amphiphiles can be either positive or negative modulators of acyl-CoA hydrolase activity.

Materials and Methods

Preparation of Subcellular Fractions. New Zealand White rabbits were sacrificed by cervical dislocation, their hearts promptly removed after a left thoracotomy, and subcellular fractions prepared after Polytron homogenization by differential centrifugation as previously described (Gross & Sobel, 1982). The $10000g_{max}$ pellet (mitochondria) was washed with homogenization buffer and resuspended by utilizing a Duall apparatus (5.5 mg of protein/mL). The $105000g_{max}$ pellet (microsomes) was resuspended in homogenization buffer (2 mg/mL). The supernatant after the $105000g_{max}$ centrifugation, cytosol (7 mg/mL), was utilized for the subsequent protein purification.

Purification of Cytosolic Acyl-CoA Hydrolase Activity. Palmitoyl-CoA hydrolase activity from rabbit myocardial cytosol was precipitated by slowly adding solid ammonium sulfate (to 45% saturation), stirred for 10 min, and centrifuged at $14000g_{max}$ for 5 min. The pellet was resuspended in 20 mM phosphate, 10 mM HSEtOH, 10% glycerol, and 10 μM lyso-PC, pH 7.6, and dialyzed for 14 h against 100 volumes of the same buffer. The purification scheme was nearly identical with that previously reported for rabbit myocardial cytosolic lysophospholipase (Gross & Sobel, 1983) with the following modifications: (a) AcA-54 resin was utilized for both gel filtration columns, resulting in improved resolution of the low molecular weight polypeptide; (b) active fractions from hydroxylapatite chromatography were dialyzed against pH 6.7 imidazole buffer (20 mM) containing 10 μM lyso-PC, 10 mM HSEtOH, and 10% glycerol, pH 6.7 (adjusted with 6 N HCl), and loaded onto a chromatofocusing column packed with Polybuffer exchanger 94 (0.9 × 18 cm). The retained proteins were washed with 1 column volume of starting buffer, and the column was subsequently developed with Polybuffer 74 (1:8

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[1] Abbreviations: CK, creatine kinase; CoA, coenzyme A; HSEtOH, β-mercaptoethanol; LDH, lactate dehydrogenase; lyso-PC, 1-palmitoyl-*sn*-glycero-3-phosphocholine; SDS, sodium dodecyl sulfate; TLC, thin-layer chromatography; DEAE, diethylaminoethyl.

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dilution) containing 10% glycerol, 10 mM HSEtOH, and 10 μM lyso-PC, at pH 5.3 (adjusted with 6 N HCl).

Assay of Palmitoyl-CoA Hydrolase and Lysophospholipase Activities. Palmitoyl-CoA hydrolase activity from column eluates was quantitated by incubating 50 μL of column fractions in 700 μL of 0.1 M phosphate buffer (pH 7.0) containing 140 μM [1-^{14}C]palmitoyl-CoA (specific activity approximately 2000 dpm/nmol) at 37 °C for 15 min. Reaction products were extracted into 500 μL of butanol by vortexing twice for 30 s and centrifuged at $2000g_{max}$ for 2 min to separate layers. Thirty microliters of the butanol layer (upper phase) was applied to a silica 0F TLC plate (Analabs). Reaction products were separated by utilizing a 3:4:1:1:0.5 $CHCl_3$:acetone:MeOH:HOAc:H_2O solvent system. Regions corresponding to fatty acid (R_f = 1) were scraped into scintillation vials, 10 mL of Aquasol II was added, and radioactivity was quantitated by scintillation spectrometry. Kinetic assays were performed similarly except that a 10-min incubation in 1 mL of buffer was used. Palmitoyl-CoA hydrolase activity assayed with this procedure was linear with respect to incubation time and mass of protein under the conditions utilized in this study (<15% substrate hydrolyzed). Lysophospholipase activity from column eluates was assayed by quantitating the release of labeled palmitic acid from [^{14}C]-lyso-PC by utilizing a similar procedure described previously (Gross & Sobel, 1983).

Thermal Denaturation. The purified protein was incubated at 37 °C in 0.1 M PO_4 buffer (pH 7.0) alone or in the presence of unlabeled substrate or amphiphile for 0–20 min at which time radiolabeled substrate was added. This mixture was incubated for an additional 10 min, extracted into butanol, and separated by TLC, and the products were quantified as described above.

Liberation of Latent Enzymic Activity by Sonication. The mitochondrial and microsomal fractions were sonicated with a Branson sonicator equipped with a microtip at a setting of 3 for 60 s followed by a 2-min period of cooling in ice. This process was repeated 3 times. The sonicated suspensions were centrifuged at $105000g_{max}$ for 60 min, the supernatant was decanted, and the pellets were washed and resuspended in homogenization buffer.

Effects of Dilution and Dialysis on Amphiphile Modulation of Acyl-CoA Hydrolase Activity. Palmitoyl-CoA hydrolase was incubated with 100 μM lyso-PC or L-palmitoylcarnitine in 100 μL of buffer for 5 min. After this preincubation, 600 μL of buffer (0.1 M PO_4, pH 7.0) was added, followed by addition of [^{14}C]palmitoyl-CoA (10 μM final concentration). This mixture was incubated for 10 min at 37 °C and extracted in butanol and released fatty acids quantified as described above. In experiments examining the reversibility of inhibition by dialysis, enzyme was incubated with 100 μM amphiphile for 10 min and dialyzed against 100 volumes of 20 mM PO_4 containing 10% glycerol–10 mM HSEtOH, pH 7.0, for 8 h.

Marker Enzyme Assays. Cytosolic markers, CK and LDH, were assayed by methods previously described (Rosalki, 1967; Reeves & Fimognari, 1966). Patent and latent citrate synthetase activity, a marker for mitochondrial matrix proteins and mitochondrial damage, was assayed by the method of Shepherd (Shepherd & Garland, 1969) before and after sonication. Sodium azide inhibitable Mg-ATPase was quantitated by measuring the difference in inorganic phosphate released from ATP in the presence and absence of 5 mM sodium azide as described previously (Robertson & Boyer, 1955). Oligomycin-inhibitable Mg-ATPase activity, a mitochondrial marker, was assayed by the method of Reeves (Reeves & Sutko, 1979). The sarcolemmal marker Na–K-ATPase was assayed as described previously (Beller et al., 1976). Acylcoenzyme A:lyso-PC acyltransferase was assayed by the method of Gross (Gross & Sobel, 1982).

Table I: Subcellular Localization of Acyl-CoA Hydrolase[a]

	mitochondria[b]	microsomes	cytosol
palmitoyl-CoA hydrolase (nmol mg^{-1} min^{-1})	3.6 (12)[c]	7.9 (13)[c]	8.6 (75)[c]
LDH (μmol mg^{-1} min^{-1})	0.7	0.8	12.1
CK (μmol mg^{-1} min^{-1})	8.9	7.9	58.0
Na-K-ATPase (μmol mg^{-1} h^{-1})	4.6	4.5	0
NaN_3-inhibitable Mg-ATPase (μmol mg^{-1} h^{-1})	23.6	6.5	1.4
oligomycin-inhibitable Mg-ATPase (μmol mg^{-1} h^{-1})	20.0	4.4	0.4
citrate synthetase (μmol mg^{-1} min^{-1})	1.8	0.3	0.3
CoA:lyso-PC acyltransferase (nmol mg^{-1} min^{-1})	0.7	4.3	0.9

[a] The data represent the mean of at least three determinations. [b] Specific activities of enzymes in the mitochondrial fraction were determined after sonication as described under Materials and Methods. No significant differences of enzymic activities were found in the microsomal or cytosolic fractions after sonication. [c] Numbers in parentheses are the percent of total palmitoyl-CoA hydrolase activity.

SDS Electrophoresis. SDS–polyacrylamide gel electrophoresis was used to determine the purity and molecular weight of acyl-CoA hydrolase. Ten percent polyacrylamide gels were prepared by the method of Laemmli (1970). Five hundred nanograms of the purified protein was heated with 50% glycerol, 100 mM HSEtOH, and 20% SDS for 5 min at 90 °C. Electrophoresis was carried out at 110 V for 8 h. Protein was visualized by silver staining (Merril et al., 1981).

Protein Determinations. Throughout the purification procedure, protein was determined by utilizing a Bio-Rad protein assay kit with bovine serum albumin as the standard. The amount of protein present after the chromatofocusing column was not determined due to interference from the eluting buffer, and therefore, specific activities from that purification step were not determined.

Results

Subcellular Distribution of Rabbit Myocardial Palmitoyl-CoA Hydrolase Activity. Rabbit myocardial homogenates contained palmitoyl-CoA hydrolase with a specific activity of 1.2 nmol/(mg·min). The highest specific activities of palmitoyl-CoA hydrolase were present in the microsomal and cytosolic fractions in each of five preparations (Table I). The cytosolic fraction contained the largest amount of palmitoyl-CoA hydrolase activity per gram wet weight (Table I). Several membrane markers including Na–K-ATPase, CoA: lyso-PC acyltransferase, sodium azide inhibitable Mg-ATPase, and oligomycin-inhibitable Mg-ATPase had low specific activities in the cytosolic fraction and thus could not account for the relatively high specific activity as well as total activity present in the cytosolic fraction (Table I).

The mitochondrial fraction contained latent palmitoyl-CoA hydrolase activity which was expressed after brief sonication. The large majority of activity was in the supernatant after centrifugation (Table II). The increase in the specific activity of palmitoyl-CoA hydrolase in the supernatant paralleled the increase of specific activity of the mitochondrial matrix marker

Table II: Liberation of Latent Enzymic Activity by Mitochondrial Sonication[a]

	palmitoyl-CoA hydrolase (nmol mg^{-1} min^{-1})	lysophospholipase (nmol mg^{-1} min^{-1})	citrate synthetase (μmol mg^{-1} min^{-1})	LDH (μmol mg^{-1} min^{-1})
mitochondria	2.74	0.49	0.3	0.59
mitochondria[b]	3.56	0.34	1.8	0.69
mitochondrial pellet[c]	0.93 (17)[e]	0.15	0.7	0.18
mitochondrial supernatant[d]	9.09 (83)[e]	1.16	4.4	2.82

[a] The data represent the mean of at least three determinations. [b] Mitochondria were sonicated as described under Materials and Methods prior to assay. [c] Mitochondria were sonicated and centrifuged, and the pellet was resuspended as described under Materials and Methods prior to assay. [d] Mitochondria were sonicated and centrifuged as described under Materials and Methods, and the supernatant was utilized for assay. [e] Numbers in parentheses refer to the percent of total activity recovered in that fraction after centrifugation.

Table III: Purification of Rabbit Myocardial Palmitoyl-CoA Hydrolase and Lysophospholipase

		sp act.[c]		% yield[d]		purification	
step	protein (mg)	LPL[a]	PCH[b]	LPL[a]	PCH[b]	LPL[a]	PCH[b]
homogenate	11480	0.20	1.3	100	100	1	1
cytosol	1404	1.5	8.5	91	78	7.4	6.5
ammonium sulfate	381	2.8	14	46	35	14	11
DEAE-Sephacel	41	11	99	20	27	57	76
Amicon concn	38	7	43	12	11	36	33
AcA-54	3.9	84	531	14	14	420	408
hydroxylapatite	0.23	957	4065	10	6	4785	3127
chromatofocusing				4	2		
AcA-54	0.010	5900	23000	3	2	29500	17692

[a] Lysophospholipase. [b] Palmitoyl-CoA hydrolase. [c] One unit of activity is defined as that necessary to hydrolyze 1 nmol of fatty acid from either lyso-PC or palmitoyl-CoA per min at 37 °C. [d] No corrections for loss of activity due to the presence of other enzymes with lysophospholipase or palmitoyl-CoA hydrolase activity have been made.

citrate synthetase, suggesting the presence of palmitoyl-CoA hydrolase in the mitochondrial matrix (Table II). In contrast, the microsomal fraction did not contain significant latent palmitoyl-CoA hydrolase activity, and the large majority of activity (>80%) remained membrane bound even after sonication. Palmitoyl-CoA hydrolase activity in the microsomal or mitochondrial fractions could not be accounted for by cytosolic contamination (Tables I and II).

To exclude the possibility that cytosolic palmitoyl-CoA hydrolase activity resulted from mitochondrial breakage during homogenization, patent and latent citrate synthetase activities were quantitated. The specific activity of citrate synthetase in the cytosol was 17% of that in the mitochondrial fraction and was not increased after sonication. Furthermore, over 80% of the citrate synthetase activity in the homogenate was latent, demonstrating only modest release of mitochondrial matrix proteins during homogenization. Since both the total activity and specific activity of palmitoyl-CoA hydrolase in the mitochondrial fraction could not account for the activity in the cytosolic fraction and since mitochondrial palmitoyl-CoA hydrolase activity was present in the supernatant after sonication and centrifugation, these results demonstrate a dual localization of soluble palmitoyl-CoA hydrolase activity in both the mitochondrial matrix and the cytosolic fractions.

Differentiation of cytosolic and mitochondrial matrix acyl-CoA hydrolase activities was accomplished by comparisons of their kinetic and chromatographic characteristics. The large majority (>85%) of mitochondrial acyl-CoA hydrolase activity in the supernatant after sonication did not bind to chromatofocusing resin under conditions utilized for the purification of cytosolic acyl-CoA hydrolase. The small amount of mitochondrial acyl-CoA hydrolase activity which bound to chromatofocusing resin eluted with an apparent isoelectric point of 5.6 and can be accounted for by cytosolic contamination of the mitochondrial supernatant (Table II). In contrast to cytosolic acyl-CoA hydrolase activity which could not be separated from lysophospholipase activity (see below), mitochondrial matrix acyl-CoA hydrolase activity in the void volume after chromatofocusing was not accompanied by lysophospholipase activity. Furthermore, although cytosolic acyl-CoA hydrolase was not bound by hydroxylapatite resin, mitochondrial matrix cytosolic acyl-CoA hydrolase activity was avidly bound. Microsomal acyl-CoA hydrolase activity had an apparent Michaelis constant of 25 μM. Mitochondrial acyl-CoA hydrolase activity (obtained from the void volume after chromatofocusing) was inhibited by palmitoyl-CoA at concentrations greater than 50 μM. Taken together, these results demonstrate the presence of at least three separate and distinct proteins in rabbit myocardium catalyzing the hydrolysis of palmitoyl-CoA. Since the cytosol contained the overwhelming majority of palmitoyl-CoA hydrolase activity, the protein catalyzing this activity was purified and characterized.

Purification of Myocardial Cytosolic Palmitoyl-CoA Hydrolase Activity. Cytosolic palmitoyl-CoA hydrolase activity was purified by ammonium sulfate precipitation and sequential DEAE, gel, hydroxylapatite, chromatofocusing, and repeat gel chromatographies. Cytosolic acyl-CoA hydrolase activity was purified 17 000-fold from the homogenate to a final specific activity of 23 μmol/(mg·min) (Table III). Rabbit myocardial cytosolic lysophospholipase and cytosolic palmitoyl-CoA hydrolase activities cochromatographed in every fraction of every step (Figure 1). The purified preparation containing both lysophospholipase and palmitoyl-CoA hydrolase activities was a single band after SDS–polyacrylamide gel electrophoresis with an apparent molecular weight of 23 000 in comparisons with standards (Figure 2).

Kinetic Analysis of Purified Cytosolic Acyl-CoA Hydrolase. Purified palmitoyl-CoA hydrolase had a maximum velocity of 24 μmol/(mg·min) and an apparent K_m = 50 μM (Figures 3 and 4). The enzyme had a broad pH optimum centered at pH 7.5. Incubation of the purified protein with 20 μM

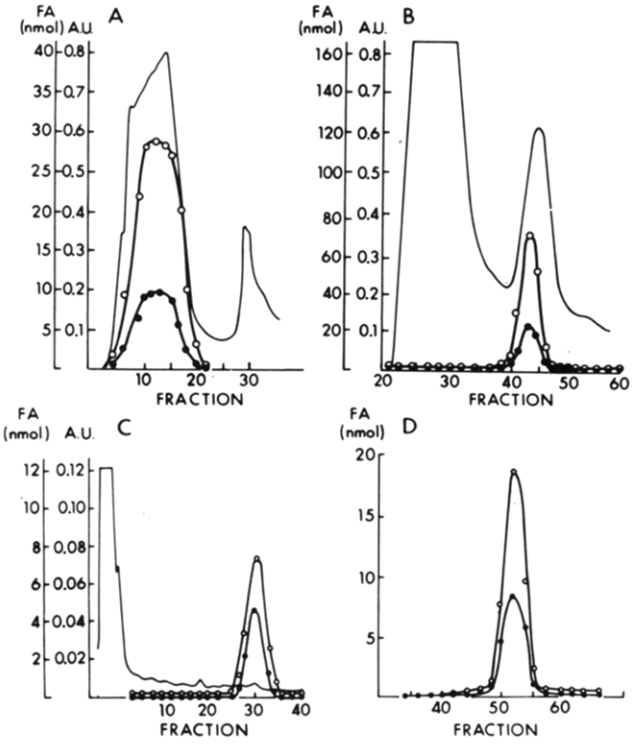

FIGURE 1: Copurification of myocardial cytosolic palmitoyl-CoA hydrolase and lysophospholipase. The ammonium sulfate precipitate was resuspended, dialyzed, and chromatographed on (A) DEAE-Sephacel, (B) AcA-54 and hydroxylapatite (not shown), (C) chromatofocusing resin, and (D) AcA-54 (no UV absorbance was detectable). Aliquots of column eluates (50 μL for determinations of palmitoyl-CoA hydrolase activity and 100 μL for determinations of lysophospholipase activity) were incubated with radiolabeled substrate (palmitoyl-CoA or lysophosphatidylcholine) for 15 min at 37 °C. Reaction products were extracted in butanol, separated by TLC, and quantitated by scintillation spectrometry. (○) Nanomoles of fatty acid released from palmitoyl-CoA; (●) nanomoles of fatty acid released from lysophosphatidylcholine; (—) absorbance at 280 nm.

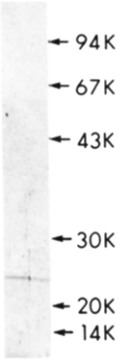

FIGURE 2: SDS–polyacrylamide gel electrophoresis of myocardial cytosolic palmitoyl-CoA hydrolase. 500 ng of protein from the final gel filtration column was heated with 100 mM HSEtOH and 20% SDS for 5 min at 90 °C and loaded onto a 10% polyacrylamide gel. Electrophoresis was carried out at 110 V for 10 h; proteins were fixed in 50% methanol and visualized by silver staining.

lyso-PC significantly increased the initial reaction velocity at low palmitoyl-CoA concentrations although the velocity at saturating concentrations of palmitoyl-CoA was only marginally exceeded (Figure 3). Incubations of the purified enzyme with 50 or 100 μM lyso-PC resulted in potent inhibition at low palmitoyl-CoA concentrations but had only a modest effect at saturating concentrations of palmitoyl-CoA. Similarly, L-palmitoylcarnitine was both a positive and a negative modifier of cytosolic palmitoyl-CoA hydrolase (Figure 4). L-Palmitoylcarnitine (20 μM) significantly increased the initial

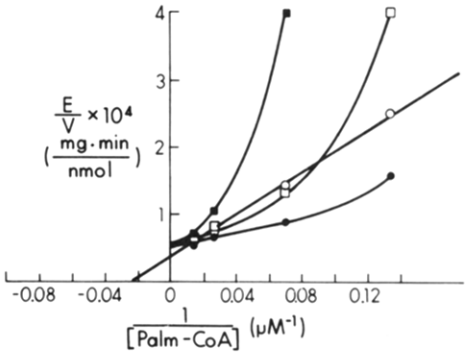

FIGURE 3: Modulation of palmitoyl-CoA hydrolase activity by lyso-PC. Purified protein was incubated with 8, 16, 40, or 80 μM radiolabeled palmitoyl-CoA in the presence of lyso-PC [(○) 0, (●) 20, (□) 50, or (■) 100 μM] for 10 min at 37 °C. Fatty acid release from palmitoyl-CoA was quantitated by fatty acid extraction into butanol, separation by TLC, and scintillation spectrometry. Points are the mean of duplicate determinations.

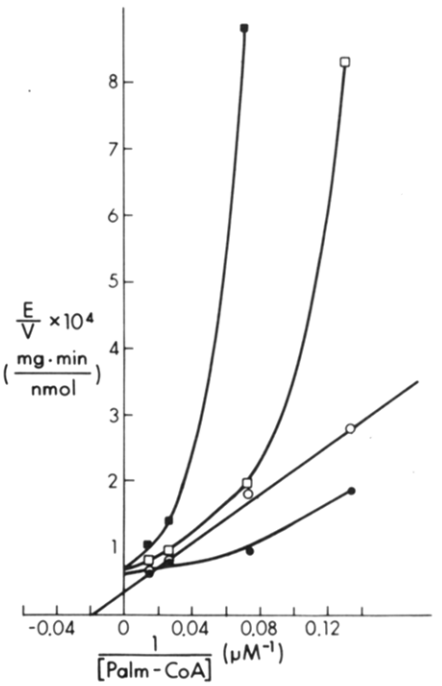

FIGURE 4: Modulation of palmitoyl-CoA hydrolase activity by L-palmitoylcarnitine. Purified protein was incubated with 8, 16, 40, or 80 μM radiolabeled palmitoyl-CoA in the presence of L-palmitoylcarnitine [(○) 0, (●) 20, (□) 50, or (■) 100 μM] for 10 min at 37 °C. Fatty acid release from palmitoyl-CoA was quantitated by extraction of fatty acids into butanol, separation by TLC, and scintillation spectrometry. Points are the mean of duplicate determinations.

velocity at low palmitoyl-CoA concentrations, but high concentrations of L-palmitoylcarnitine (50 or 100 μM) attentuated the reaction velocity especially at low palmitoyl-CoA concentrations.

Effects of Palmitoyl-CoA on Lysophospholipase Activity. Lysophospholipase activity of the purified preparation had a maximum velocity of 6 μmol/(mg·min) and an apparent K_m of 10 μM. Incubation of the purified protein with selected concentrations of lyso-PC in the presence of 25 or 50 μM palmitoyl-CoA resulted in potent competitive inhibition. A slope replot demonstrated an apparent $K_i = 4$ μM. Incubation of the purified protein with 100 μM palmitoyl-CoA resulted in only trace amounts of detectable activity at a lyso-PC concentration of 50 μM ($5K_m$).

Reversibility of Inhibition by Dilution or Dialysis. To further characterize the interactions of the enzyme with en-

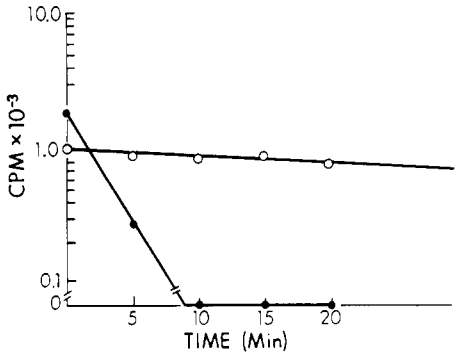

FIGURE 5: Thermal denaturation of palmitoyl-CoA hydrolase and lysophospholipase. Purified enzyme obtained from the final gel filtration column was incubated at 37 °C for the period indicated on the abscissa at which time radiolabeled substrate [(●) palmitoyl-CoA or (○) lyso-PC] was added and the incubation continued for 10 min. Liberated fatty acid was extracted into butanol and separated by TLC, and reaction products were quantitated by scintillation spectrometry. Points are the mean of duplicate determinations.

dogenous cardiac amphiphiles, incubations were performed with high concentrations of amphiphile (100 μM) in the absence of substrate, and the effects of dilution or dialysis were quantitated. Dilution of either palmitoylcarnitine or lyso-PC from 100 to 15 μM resulted in an increase of palmitoyl-CoA hydrolase activity of 180% compared to enzyme not incubated with lyso-PC or L-palmitoylcarnitine. Furthermore, incubation of palmitoyl-CoA hydrolase and 100 μM amphiphile (lyso-PC or palmitoylcarnitine) and subsequent dialysis resulted in similar palmitoyl-CoA hydrolase activities in comparison to enzyme that was not exposed to 100 μM amphiphile. These results demonstrate that the effects of these modifiers are rapidly reversible and are concentration dependent.

Similarly, although palmitoyl-CoA was a potent competitive inhibitor of lysophospholipase activity, dilution from 100 to 10 μM palmitoyl-CoA restored over 50% of lysophospholipase activity. Furthermore, incubation of enzyme with 100 μM palmitoyl-CoA and subsequent dialysis did not result in a significant difference of lysophospholipase activity in comparison to enzyme not exposed to 100 μM palmitoyl-CoA.

Thermal Denaturation Studies. Incubation of the purified protein at 37 °C in 0.1 M phosphate buffer (pH 7.0) resulted in the rapid denaturation of palmitoyl-CoA hydrolase activity with a $t_{1/2}$ = 2.5 min (Figure 5). In contrast, lysophospholipase activity was not rapidly denatured under identical conditions (Figure 5). Loss of palmitoyl-CoA hydrolase activity at 37 °C could be prevented by incubating the purified enzyme with low concentrations of palmitoyl-CoA prior to the addition of radiolabeled palmitoyl-CoA. Dialysis of the purified protein (to remove lyso-PC from the buffer) and subsequent thermal denaturation analysis demonstrated that palmitoyl-CoA hydrolase and lysophospholipase had similar half-lives at 37 °C (2.5 vs. 3 min) in the absence of lyso-PC.

Discussion

This study demonstrates the presence of a high specific activity palmitoyl-CoA hydrolase in rabbit myocardial cytosol and its modulation by endogenous cardiac amphiphiles. Marker enzyme analysis demonstrated that palmitoyl-CoA hydrolase activity present in the cytosolic fraction could not be accounted for by contamination from mitochondrial or microsomal proteins. Sequential column chromatographies resulted in a 17 000-fold purification of palmitoyl-CoA hydrolase activity to near-homogeneity. Rabbit myocardial cytosolic palmitoyl-CoA hydrolase has a higher specific activity than either mitochondrial or microsomal palmitoyl-CoA hydrolase previously isolated from rat liver (Berge & Farstad, 1979; Berge, 1979) or cytosolic acyl-CoA hydrolase isolated from lactating rabbit mammary glands (Knudsen et al., 1981). Furthermore, the molecular mass of cytosolic myocardial palmitoyl-CoA hydrolase activity is different than that of rat liver mitochondrial, rat liver microsomal, porcine myocardial mitochondrial, and rabbit mammary gland palmitoyl-CoA hydrolase (Berge & Farstad, 1979; Berge, 1979; Lee & Schulz, 1979; Knudsen et al., 1981).

Lysophospholipase and palmitoyl-CoA hydrolase activities cochromatographed in every fraction of every step, and the purified protein was a single band after visualization by the highly sensitive method of silver staining. These results suggest that lysophospholipase and acyl-CoA hydrolase activities in myocardial cytosol are catalyzed by a single polypeptide with dual activities. This conclusion is supported by the similar pH optima for palmitoyl-CoA hydrolase and lysophospholipase activities, competitive inhibition of lysophospholipase activity by palmitoyl-CoA, modulation of palmitoyl-CoA hydrolase activity by lyso-PC and palmitoylcarnitine, and similar rates of thermal denaturation of both activities in the absence of amphiphile. The relative functional specificities of the protein, as ascertained by the ratio of the maximum velocity to the apparent Michaelis constant, were similar (0.48 and 0.60 for palmitoyl-CoA hydrolase and lysophospholipase, respectively). Lysophospholipases purified from beef pancreas and liver possess general carboxylesterase activity (DeJong et al., 1973, 1974). The present study demonstrates that myocardial lysophospholipase can hydrolyze both oxy and thio ester linkages present in structurally dissimilar substrates. The possibility that lysophospholipase and palmitoyl-CoA hydrolase activities are catalyzed by highly homologous yet different polypeptides of nearly identical molecular mass that are not separated after a 20 000-fold purification cannot be definitively excluded but seems unlikely.

Kinetic analysis demonstrated several unusual features including a modulation of enzymic activity by amphiphilic compounds, a discordance between the rate of thermal denaturation of lysophospholipase and palmitoyl-CoA hydrolase activities with low concentrations of lyso-PC, and a marked difference in the apparent Michaelis constant for palmitoyl-CoA hydrolase activity compared to the apparent inhibitory constant of palmitoyl-CoA for lysophospholipase activity. The structures of palmitoyl-CoA and lysophosphatidylcholine are markedly different from either a stereochemical or an electrostatic perspective, yet both are effectively hydrolyzed. In contrast, L-palmitoylcarnitine, which bears a close structural resemblance to lysophosphatidylcholine and is a competitive inhibitor of lysophospholipase activity, is not hydrolyzed by this protein under identical conditions (Gross & Sobel, 1983). Thus, there must exist other factors, such as the orientation of the carbonyl in the active site, the ability of the enzyme to induce conformational changes in the substrate, or the affinity of the transition state for the enzyme, which are required for catalysis.

Thermal denaturation profiles demonstrated that palmitoyl-CoA hydrolase activity was more labile than lysophospholipase activity. This conclusion is supported by the higher lability of palmitoyl-CoA hydrolase activity in comparison with lysophospholipase activity observed during the purification especially with respect to the loss of activity noted during concentration (Table III). Since the results suggest that a single polypeptide catalyzes both lysophospholipase and palmitoyl-CoA hydrolase activities, it is necessary to postulate the presence of a labile portion of the active site which is

required for hydrolysis of CoA thio esters but not lyso-PC. The possibility that catalysis of palmitoyl-CoA and lysophosphatidylcholine occurs at separate and distinct active sites on this low molecular weight polypeptide cannot be excluded.

Nonproductive binding of substrate to enzyme is an essential kinetic feature of several hydrolytic enzymes [e.g., see Ingles & Knowles (1967) and Dunn & Brucie (1973)]. The presence of nonproductive binding modes of substrate alters the observed dissociation constants and reflects contributions from multiple equilibria. If lyso-PC or L-palmitoylcarnitine altered the fraction of palmitoyl-CoA or lyso-PC productively bound or alternatively if two or more conformations of the polypeptide were preferentially stabilized by amphiphile, a complicated kinetic picture would emerge. Furthermore, possible contributions from ternary complexes of enzyme, substrate, and amphiphile such as those proposed for a general modifier mechanism (Botts & Morales, 1953) and possible allosteric effects remain to be determined. The present study does not permit conclusions regarding the mechanism through which this biphasic modulation is mediated.

Several kinetic features of myocardial acyl-CoA hydrolase uncovered in this investigation have potential physiological significance and merit consideration. First, although the relatively high apparent Michaelis constant suggests that only modest palmitoyl-CoA hydrolase activity is present at physiologic concentrations of acyl-CoA, interaction with palmitoylcarnitine could increase hydrolytic rates appreciably. Since palmitoylcarnitine is present in the cytosolic compartment in concentrations similar to those utilized in this study (Oram et al., 1975; Idell Wenger et al., 1978), it seems likely that protein–amphiphile interactions modulate cytosolic palmitoyl-CoA hydrolase activity in vivo. Second, the amount of acyl-CoA hydrolase activity in myocardial cytosol is approximately 20% of myocardial acyl-CoA synthetase activity (Oram et al., 1973), and thus acyl-CoA hydrolase could exert significant control over cytosolic acyl-CoA concentrations and thereby potentially modulate fluxes through several biochemical pathways. Third, exogenously administered lyso-PC has been demonstrated to partition into both myocardial membranes as well as cytosol by autoradiography (Gross et al., 1982), and long-chain acylcarnitines accumulate in the cytosolic compartment of ischemic myocardium to concentrations approaching 1 mM (Idell Wenger et al., 1978). Thus, palmitoyl-CoA, palmitoylcarnitine, and lyso-PC which accumulate during myocardial ischemia (Oram et al., 1973; Corr et al., 1982) have access to this cytosolic protein, and the interaction of these moieties with a metabolically interwoven catabolic protein would be expected to amplify the amphiphilic burden presented to ischemic myocardium potentially resulting in damage to cellular membranes.

This investigation demonstrates the presence of three enzymes in rabbit myocardium catalyzing the hydrolysis of palmitoyl-CoA. The cytosolic enzyme quantitatively accounts for the majority of acyl-CoA hydrolase activity and is also a lysophospholipase. The results suggest that metabolism of cardiac amphiphiles is modulated by complex protein–lipid interactions.

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Registry No. Lyso-PC, 17364-16-8; palmitoyl-CoA hydrolase, 9025-87-0; lysophospholipase, 9001-85-8; palmitoyl-CoA, 1763-10-6; L-palmitoylcarnitine, 2364-67-2.

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