PALMITYL COENZYME A DEACYLASE*

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SUMMARY

Enzymes have been found in various tissues which deacylate long chain fatty acyl CoA compounds. Methods of assay and the partial purification of palmityl CoA deacylase from pig brain are described.

INTRODUCTION

Several different enzymes have been described which catalyze hydrolysis of acyl CoA derivatives. Acetyl CoA deacylase[2], and succinyl CoA deacylase[2-4], hydroxyisobutyryl CoA deacylase[5] and a hydroxymethyl glutaryl CoA deacylase[6] have been described. Enzyme preparations that deacylate acetoacetyl CoA have been described[7,8] but recent evidence points to a more involved mechanism than one of simple hydrolysis[9,10]. In addition, cleavage of long chain fatty acyl glutathione compounds have been reported[11,12]. PORTER AND TIETZ and PORTER AND LONG have reported the presence of palmityl CoA deacylase in pigeon liver[13,14]. The purpose of this paper is to present an isolation procedure and several assay methods for a long chain fatty acyl CoA deacylase found in pig brain.

METHODS

Fatty acyl CoA

Preparations of fatty acyl CoA compounds were made by adding the appropriate fatty acid chlorides to CoASH (glutathione-free) according to the method of SEUBERT[15]. As a standard substrate, palmityl CoA was employed. The enzymic purity of this compound was assayed as 80 %.

Enzyme assay methods

Enzyme assays were performed in one of the four following ways:

1. By measurement of the free CoASH formed during the reaction. After the hydrolysis had taken place the CoASH was converted to acetoacetyl CoA by the addition of diketene[16]. The acetoacetyl CoA thus formed was measured spectrophoto-

The following abbreviations are used: CoA and CoASH = bound and free Coenzyme A. DPN and DPNH = oxidized and reduced diphosphopyridinonucleotide.

* Some of this work has appeared in a preliminary form[1].

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metrically by its stoichiometric reaction with DPNH in the presence of β-hydroxyacyl dehydrogenase. The oxidation of DPNH to DPN^+ was followed at 366 mμ.

2. Fatty acyl CoA derivatives have a strong absorption band at 233 mμ which is due to the thiolester linkage[17,18]. The reaction which involves a hydrolysis of such a linkage can be followed spectrophotometrically by measuring the decrease in absorption at 233 mμ.

3. Free sulfhydryl compounds are known to reduce dichlorophenol indophenol[19]. This reduction is accompanied by a decrease in absorption of this dye at 578 mμ[11,19]. The liberated CoASH can be measured by determining the rate of dye reduction at 578 mμ.

4. Measurement of release of sulfhydryl groups by the method of GRUNERT AND PHILLIPS[20].

All four of these methods were used to establish the nature of the reaction involved. Because of the simplicity of assay method 2 it was chosen as the routine assay for the purification procedure. This method gave non-linear kinetics owing to the precipitation of the released palmitic acid, and subsequent increase in optical density due to this precipitation. Satisfactory results could be obtained by comparing only closely equivalent rates of reaction.

Enzyme purification

All procedures were carried out at 3° unless otherwise specified. Pig brain obtained at the slaughter house was placed in ice for transport to the laboratory. The brain tissue was placed in a Star Mix blendor with four volumes of 2 *M* ammonium sulfate (pH 7.4) containing 0.001 *M* ethylenediaminetetraacetate. This mixture was homogenized for 1.5 min. Celite (60 % of the weight of the fresh brains) was added to the homogenate and the entire mixture was filtered through a Buchner funnel. The Celite cake was resuspended in one-fifth the original volume of 2 *M* ammonium sulfate solution and then refiltered.

Solid ammonium sulfate was added to the combined filtrates to bring the final ammonium sulfate concentration to 0.7 saturation. The precipitate was then collected by centrifugation at 20,000 × *g* and dissolved in a small volume of potassium phosphate buffer (pH 7.0, *M*/15).

The solution was cooled to 0°, 0.1 *M* zinc acetate solution added to a concentration of 0.01 *M* Zn^{++}, and 95 % ethanol then added slowly to bring the final ethanol concentration to 33 %. The precipitate was collected by centrifugation and dissolved

TABLE I

PURIFICATION OF PALMITYL CoA DEACYLASE

Fraction	*Specific activity**	*Total activity* 10^6 *units*	*Purification*
Ammonium sulfate extract	4,000	6.3	1
70 % ammonium sulfate fraction	4,900	5.0	1.2
0–33 % zinc-alcohol ppt.	39,000	0.1	9.7

* Specific activity has been defined as the decrease in optical density units (0.001 O.D. = 1 unit) per 5 min per mg protein at 233 mμ in the Zeiss spectrophotometer (length = 1 cm). The test cuvette contained 1.6 ml of *M*/15 KPO_4, pH 7.4 and enzyme. The reaction was initiated by the addition of palmityl CoA (10–12 μ*M*).

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in $M/15$ potassium phosphate buffer (pH 7.0) containing 0.1 M ethylenediaminetetraacetate. The data for one purification run is shown in Table I.

RESULTS

Fig. 1 shows the spectrum of palmityl CoA as well as the spectra of the alkaline (NaOH) and enzymic hydrolysates. Table II presents results showing evidence for the release of CoASH as measured by the highly specific method 1 (above). The reaction produced is clearly a simple hydrolysis of palmityl CoA.

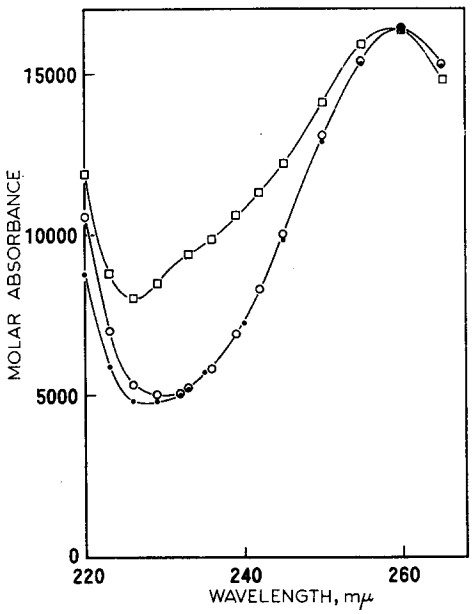

Fig. 1. Spectra of palmityl CoA before (□) and after enzymic hydrolysis (·) and after sodium hydroxide hydrolysis (○).

TABLE II

ENZYMIC RELEASE OF CoASH FROM PALMITYL CoA

Tubes were incubated 30 min at 37°. Each tube contained 70 μM potassium phosphate buffer pH 7.0.

Rat brain homogenate Expt. No.	*Palmityl CoA added μM*	*CoASH formed* μM*
1	0.06	0.034
2	0.06	0.020
3	1.0	0.42

* CoASH was converted to acetoacetyl CoA with diketene. The acetoacetyl CoA was assayed with β-hydroxybutyryl CoA dehydrogenase and DPNH. Palmityl CoA was omitted from control tubes.

Enzyme distribution

Table III shows a comparison of various tissues assayed for this fatty acyl CoA deacylase. Brain tissue is clearly the best source of this enzyme. In addition to showing,

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that pig brain is an excellent source of this enzyme, Table V shows that the enzyme is soluble in 0.5 saturated ammonium sulfate.

TABLE III

DISTRIBUTION OF PALMITYL CoA DEACYLASE

Tissue	*Homogenizing medium*	*Specific activity**
Rat brain (2 day old rats)	Sucrose	740
Rat liver (2 day old rats)	Sucrose	90
Rat kidney (2 day old rats)	Sucrose	210
Pig brain	Sucrose	890
Pig brain	2 *M* ammonium sulfate	4000
Yeast	*M*/15 KPO_4	0

* See definition of specific activity in footnote of Table I. Total activity refers to the specific activity of a fraction times its total protein content.

Stability of enzyme

Most preparations lost 50 % of their activity on storage at —20° for 3 days. Ammonium sulfate fractions could be dialyzed against water at 3° for 15 h with no appreciable loss of activity. The enzyme was most stable at pH 7.0, losing half of its activity after 15 min incubation at pH 5.0 or pH 8.0 at 0°. The enzyme exhibited full activity in a 20 % alcohol solution.

Enzyme kinetics

Though method 2 gave non-linear kinetics, linearity could be shown using the dye reduction method (Table IV). With palmityl CoA as a substrate in the dye reduction method a Michaelis constant of $2.2 \cdot 10^{-5}$ *M* was obtained.

TABLE IV

EFFECT OF ENZYME CONCENTRATION ON DYE REDUCTION

Enzyme μg	*Δ O.D. 578 mμ/5 min*
5.4	0.022
13.5	0.044
27.0	0.088
54.0	0.160

Each cuvette contained 0.16 μM palmityl CoA, dichlorophenolindophenol to give an initial optical density of 0.700, 60 μM KPO_4 pH 7.0 in a total volume of 1 ml.

Specificity

Table V shows the activity of various fractions toward fatty acyl CoA derivatives of different chain length. In order to explain these results one must assume either that there are two enzymes, each of which exhibits a variable specificity against different thioesters, or that there are separate enzymes for each substrate. Table VI shows the activity of two enzyme fractions against short chain thioesters.

The enzyme is not inhibited in the dye assay by diisopropylfluorophosphate, in contrast to a number of simple esterases.

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Table VII shows that the initial extract contains the thioesterase described by STRECKER *et al.*[21] since it has activity against acetyl glutathione. This activity can be removed by heating the extract to 65° for three minutes without significantly effecting the palmityl CoA deacylase activity.

TABLE V

ACTIVITY OF THE ACYL CoA DEACYLASE AGAINST DERIVATIVES OF DIFFERENT CHAIN LENGTHS

CoA Derivative	*Relative activity**					
	Enzyme prep. I		*Enzyme prep. II*			
	Am. sulfate ext.	*70% Am. sulfate*	*Am. sulfate ext.*	*70% Am. sulfate*	*Dialyzed*	*Heated to 55°*
Hexanoyl			50	41		
Octanoyl	138	318	88	54	56	43
Decanoyl	134	234	94	70		
Myristyl	88	148				
Palmityl	100	100	100	100	100	100
Stearyl	112	104				

* The decrease in absorption at 233 mμ under conditions listed in footnote of Table I after five min with palmityl CoA as substrate was arbitrarily set equal to 100. All other assays were run under as closely equal conditions to this as possible (*i.e.* substrate concentrations equal, enzyme concentration equal etc.). And the decrease in absorption at 233 mμ after five min related to that of palmityl CoA.

TABLE VI

ENZYMIC RELEASE OF SULFHYDRYL GROUPS FROM THIOESTERS

Substrate	*μM SH released*	
	Enzyme 1	*Enzyme 2*
Hydroxymethylglutaryl CoA	0.17	0.0
α-Hydroxyisobutyryl CoA	0.0	0.0
Succinyl CoA	0.0	0.0
Acetyl CoA	0.09	0.06
Methyacrylyl CoA	0.01	0.09
Palmityl CoA	0.2	0.15

All experiments were run at 37° for 30 min with 200 μ*M* of tris(hydroxymethyl)-aminomethane pH 7.4, 0.5 μ*M* substrate and enzyme in a total volume of 1 ml. Enzyme 1 was a 50–70% ammonium sulfate fraction and enzyme 2 was this fraction after heating to 65° for 3 min.

TABLE VII

SEPARABILITY OF DEACYLASE ACTIVITY TOWARD ACETYL CoA, ACETYL GLUTATHIONE, AND PALMITYL CoA

All cuvettes contained 300 μ*M* (or 30 μ*M* when marked with an asterisk) of tris (hydroxymethyl)-aminomethane pH 7.4. Total volumes were 3 ml.

Substrate	*Extract μg*	*Heated extract μg*	*—O.D. 235 mμ/5 min*
Acetyl CoA	—	100	0.00
Palmityl CoA	40	—	0.044
Palmityl CoA	—	20	0.037
Palmityl CoA*	—	20	0.038
Acetyl glutathione	200	—	0.056
Acetyl glutathione*	—	100	0.005
Acetyl glutathione*	—	100	0.006

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DISCUSSION

The role of hydrolytic enzymes in metabolism has been the subject of considerable discussion. Four roles can be considered for them: 1. To act synthetically by reversing hydrolysis, 2. to transfer one of the products, a reaction which can be detected only when the propert acceptor (other than water) is used *in vitro*, 3. to regulate the concentration of the materials involved in the reaction, or 4. to form an intermediate lying directly in a metabolic path.

Evidence for each of these roles has been obtained, using various hydrolases.

It has been shown that cholesterol ester synthesis is probably catalyzed by the same enzyme which catalyzes its hydrolysis[22]. This is true also for certain hydrolases acting upon triglycerides. It has been impossible to demonstrate any synthesis of this type with palmityl CoA deacylase.

Certain proteolytic enzymes have been shown to exhibit transfer activity and synthetic activity under special conditions[23]. Several preliminary experiments were made with the addition of cholesterol, glycerol and β-glycerophosphate to the system but no stimulation of CoASH release could be found. It seems unlikely that palmityl CoA deacylase could serve for internal maintenance of a given CoASH concentration for one might expect to find free fatty acids in brain tissue and this is contrary to what is observed. PORTER AND LONG[14] show, however, that palmityl CoA deacylase can play such a regulatory role in their reconstructed fatty acid synthesis system where free fatty acid formation is observed.

RENDINA AND COON[5] have suggested that the enzyme β-hydroxypropionyl CoA deacylase plays an important part in propionate metabolism. Aside from their role in the transport of fat, unesterified fatty acids do not enter into any well known metabolic role, so a like function for the deacylase does not seem probable.

NOTE ADDED IN PROOF

Since submission of this paper for publication, VIGNAIS AND ZABIN[24] have published a paper concerning the synthesis of palmityl CoA, palmityl glutathione and palmityl adenylate. In addition, enzymes hydrolyzing these substrates as well as enzymes having transferase activity toward these substrates were described.

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THE STRUCTURE OF PLASMALOGENS

IV. LIPIDS IN NORMAL AND NEOPLASTIC TISSUES OF MAN AND IN NORMAL TISSUES OF RABBIT AND RAT

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SUMMARY

1. Total lipid extracts of rabbit tissues (9 normal), rat tissues (9 normal, 1 cancerous), and human tissues (7 normal, 10 neoplastic) have been analyzed for their plasmalogen content by 2 independent methods: formation of the aldehyde *p*-nitrophenylhydrazone and addition of iodine under conditions specific for α,β-unsaturated ethers.

2. The results agree with an average variation of less than 10 %, indicating that almost all plasmalogens in mammalian organs have, as a common structural feature, an α,β-unsaturated ether linkage in a relatively long fatty chain.

3. It is suggested that as the plasmalogen–lipid phosphorus ratio for a given organ is relatively constant, this ratio may be a more significant criterion than plasmalogen content for relating plasmalogen changes to organ function.

INTRODUCTION

In earlier papers in this series it was established that the aldehydogenic linkage in plasmalogens is an α,β-unsaturated ether. This was first shown with lysophosphatidal ethanolamine (formerly believed to be "acetal phosphatide") isolated from bovine muscle (RAPPORT, LERNER, ALONZO AND FRANZL[1]). Conclusive evidence was then

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