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FORMATION OF *S*-ACYL PANTETHEINE FROM ACYL-COENZYME A BY PLASMA MEMBRANES

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SUMMARY

1. During incubation of a variety of plasma membrane preparations with *S*-acyl-CoA esters substantial amounts of a novel lipid were produced. This material was identified as *S*-acyl pantetheine.

2. Characterization of palmityl pantetheine was achieved by infrared and mass spectrometry, by analysis for its constituent groups and by chemical synthesis.

3. Formation of acyl pantetheines was studied in a variety of liver subcellular fractions. Plasma membrane preparations gave by far the highest yield, as did membrane preparations from adipose and electric tissue.

4. Formation of acyl pantetheine from acyl-CoA was accompanied by P_i liberation and the production of free fatty acid. The reactions leading to the formation of *S*-acyl pantetheines depend on a membrane nucleotide pyrophosphatase and a phosphomonoesterase activity.

INTRODUCTION

In the preceding communication we have shown that long chain fatty acids from acyl-CoA esters were incorporated into the lipids of isolated liver plasma membrane preparations. During the incubation of liver plasma membranes with acyl-CoA, substantial amounts of the acyl moiety were converted, either to free fatty acids or to a more complex, and more polar material. The formation of the latter took place only if reasonably pure membrane preparations were employed. It will be shown in this communication that the lipid in question was *S*-acyl pantetheine. Although the *S*-acyl derivatives of pantetheine had been used experimentally, substituting for the CoA derivatives[2,3], the enzymatic formation of long chain acyl pantetheine thioesters has not been reported previously.

The present communication deals with the formation *in vitro*, isolation and characterization of *S*-acyl pantetheine.

Abbreviation: BAL, 2,3-dimercaptopropanol.

MATERIALS AND METHODS

Liver cell membranes were prepared from male Sprague-Dawley rats weighing 120–180 g according to the improved method of NEVILLE[4] as described in the preceding paper[1]. Liver cell nuclei, mitochondria and microsomes were prepared according to SCHNEIDER AND HOGEBOOM[5] or alternatively, as described in the text. Adipose tissue homogenates were prepared in 1 mM $NaHCO_3$. Isolated adipose tissue cells and adipose tissue cell ghosts were prepared by the methods of RODBELL[6,7]. The cells were suspended in 0.9 % NaCl, and the ghosts washed and suspended in 1 mM $KHCO_3$ before assay. Vesicular membrane fractions from the electroplax of *Electrophorus electricus* were prepared according to ALBERS, FAHN AND KOVAL[8]. Erythrocyte ghosts were prepared by lysis according to standard techniques. Long chain acyl-CoA derivatives were synthesized enzymatically as previously described[1] and their relative purity was estimated by determining the nucleotide:ester ratio and by thin-layer chromatography. Calculation of the specific activity of the acyl-CoA compounds was based on the ratio of the disint./min per nucleotide (or ester). Protein determinations were based on the method of LOWRY *et al.*[9]. Succinic-dehydrogenase activity was measured essentially according to DAVENPORT[10]. Glucose-6-phosphatase activity was measured by the liberation of P_i according to the procedure of LOWRY AND LOPEZ[11]. Ester determinations were made according to RAPPORT AND ALONZO[12] and identification and separation of hydroxamic acids by thin-layer chromatography as described elsewhere[13]. The incubation and thin-layer chromatography procedures utilized have been described in the previous paper[1]. Measurement of the liberation of inorganic phosphate (P_i) in incubation mixtures was carried out on trichloroacetic acid supernatants by the method of AMES AND DUBIN[14].

Synthesis of S-palmityl pantetheine

1.0 mmole of D-pantetheine (Sigma) was dissolved in 1 ml of water and 5 ml of tetrahydrofuran were added. After the addition of 0.4 ml of 2-mercaptoethanol, the solution was stirred in a closed flask by bubbling dry N_2 through it. 0.5 ml of 0.5 M Tris buffer (pH 8.1) was then added and the entire reaction mixture was maintained at pH 8 by occasional addition of 20 % KOH. 2.0 mmoles of palmitylchloride in 5 ml of tetrahydrofuran were added over a period of 30 min at room temperature and the entire mixture stirred for another 30 min. 0.5 ml of 4 M HCl was added and about 50 ml of crushed ice. The mixture was filtered in the cold by suction and the precipitate washed with water. The solid material was dissolved in 5 ml of tetrahydrofuran and after the addition of 35 ml of ethyl ether, the suspension was centrifuged. The clear supernatant was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. After drying the crude palmityl pantetheine in a vacuum, it was dissolved in 3 ml of chloroform and purified by chromatography on a silicic acid column (5 g; 8 mm × 100 mm) which was equilibrated with chloroform. The column was first eluted with 75 ml of chloroform and then with 150 ml of chloroform–methanol (96:4, by vol.). The latter eluate was evaporated and the residue redissolved in 2 ml chloroform. Final purification of *S*-palmityl pantetheine was achieved by thin-layer chromatography on Analtech silica gel G plates. Partially purified *S*-palmityl pantetheine was applied to three plates and the plates developed with chloroform–methanol (9:1, by vol.). The area containing the palmityl pantetheine (R_F 0.45–0.55) was scraped off and

placed in a column. The column was washed with 50 ml of chloroform and then the palmityl pantetheine was eluted with 50 ml of chloroform–methanol (9:1, by vol.). After evaporation, the purified palmityl pantetheine (yield about 120 mg) was recrystallized from *n*-pentane. The melting point of this material was 60–62°, and it showed a characteristic ultraviolet absorption at 229 mμ. On thin-layer chromatography, in a variety of systems, the synthetic palmityl pantetheine chromatographed identically to the material obtained by the incubation of palmityl-CoA with membranes.

RESULTS

Identification of S-acyl pantetheine

As shown in the preceding paper (ref. 1: Fig. 1) substantial amounts of [^{14}C]acyl-CoA were converted by the membrane preparations into a lipid-soluble material which was eluted from a silicic acid column with 2% methanol in chloroform. Using 1.0 μmole of [^{14}C]palmityl-CoA as a precursor, we could recover, after purification, from 40 to 60 mμmoles of the unknown lipid from incubation mixtures which contained the isolated plasma membranes from 40 g of rat liver. The isolated lipid was purified by repeated chromatography on silicic acid and by thin-layer chromatography. The ultraviolet spectrum showed a single, sharp maximum at 229 mμ in *n*-pentane. Infrared spectrometry of KBr micropellets gave a broad band at 3340 cm^{-1} (–OH); well defined signals for $-CH_2-$ at 2915 and 2842 cm^{-1} and a fairly sharp peak at 1685 cm^{-1} (thioester). Two amide bands were apparent at 1638 and 1530 cm^{-1} and several smaller peaks at 1450 ($-CH_2-$) and 1375 cm^{-1}. The purified material yielded an acid to ester ratio of near unity, using the method of RAPPORT AND ALONZO[12] and chromatography of the hydroxamic acid after treatment with NH_2OH

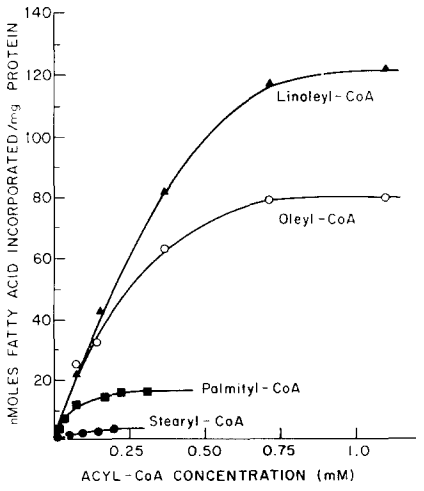

Fig. 1. Formation of acyl pantetheine from several acyl-CoA compounds. Liver plasma membranes (180 μg protein) were incubated with Tris–HCl (pH 7.4) (140 mM) and [1-^{14}C]acyl-CoA as indicated, in a final volume of 220 μl for 0.5 h at 37°. The specific activities of the acyl-CoA compounds were as follows: [1-^{14}C]palmityl-CoA, $5.29 \cdot 10^7$ disint./min per μmole; [1-^{14}C]stearyl-CoA, $4.34 \cdot 10^7$ disint./min per μmole; [1-^{14}C]oleyl-CoA, $1.02 \cdot 10^7$ disint./min per μmole; [1-^{14}C]linoleyl-CoA, $7.53 \cdot 10^6$ disint./min per μmole.

(ref. 13) gave a material which was identical with synthetic palmityl hydroxamic acid. Measurement of liberated –SH groups by the use of the reagent of ELLMAN[15] after mild acid hydrolysis indicated the presence of –SH but the yields were not quantitative relative to the ester determinations. Low resolution mass spectrometry gave a signal for an apparent parent ion with the mass of 514. Larger amounts of ions with the respective masses of 444, 386, 315, 256, 239 and 71 were detected and it was found that occurrence of these ions was consistent with the fragmentation expected from palmityl pantetheine. The elution patterns and R_F-values obtained with the biosynthetic palmityl pantetheine by column or thin-layer chromatography were identical in all respects with the chemically synthesized material.

Studies on the formation of acyl pantetheine in vitro

Various subcellular fractions were incubated with palmityl-CoA and it is apparent from the data in Table I that the formation of acyl pantetheine was most active with the isolated membranes. Addition of liver subcellular fractions to membrane preparations led to an apparent inhibition of acyl-pantetheine formation (Table II). *S*-Acyl pantetheine was not formed in significant yields when the membranes were incubated with free fatty acid, ATP and CoA (Table III) which led us to conclude that the thiokinase activity of the membranes was low or due to microsomal contamination.

Attempts to solubilize the enzymes which are responsible for the cleavage of acyl-CoA to form acyl pantetheine have not met with success. Addition of various

TABLE I

FORMATION OF PALMITYL PANTETHEINE FROM [1-^{14}C]PALMITYL-CoA BY LIVER CELL FRACTIONS

In both experiments membranes were isolated by the procedure of NEVILLE[4]. The Schneider–Hogeboom technique[5] was utilized to obtain the fractions in Expt. 1. In Expt. 2 the whole homogenate was prepared in 1 mM $NaHCO_3$. After centrifugation at 78000 × *g* (60 min) in the Spinco No. 30 rotor the resulting pellet was resuspended in 1.15% KCl. This was centrifuged at 10000 × *g* (10 min) to yield the 'mitochondrial' fraction. The combined supernatants were centrifuged at 78000 × *g* (60 min) to yield the 'microsomal' fraction. The final supernatant was discarded. The incubation medium contained 130 mM Tris–HCl (pH 7.4), 0.36 mM [1-^{14}C]palmityl-CoA ($6.2 \cdot 10^6$ disint./min per μmole) and 200–400 μg protein in 225 μl. Incubation was for 0.5 h at 37°. When indicated the cell fraction was boiled for 5 min.

Fraction	*Palmityl pantetheine liberated (mμmoles/mg protein)*	
	Boiled fraction	*Untreated fraction*
Expt. 1		
Homogenate	1.34	5.09
Nuclear	2.49	4.91
Mitochondrial	1.22	4.50
Microsomal	3.24	11.50
Supernatant	2.39	2.33
Membranes	1.23	60.90
Expt. 2		
Homogenate	0.58	0.58
Mitochondrial	0.58	0.57
Microsomal	0.91	1.10
Membranes	2.43	70.47

TABLE II

EFFECT OF LIVER FRACTIONS ON FORMATION OF PALMITYL PANTETHEINE BY LIVER PLASMA MEMBRANES

In Expt. 1 subcellular fractions were isolated by the procedure of SCHNEIDER AND HOGEBOOM[5] from 5 g of fresh liver; in Expt. 2 the homogenate was prepared in 1 mM $NaHCO_3$ according to NEVILLE[4]. The incubation medium in Expt. 1 contained 130 mM Tris–HCl (pH 7.4), 0.36 mM [1-^{14}C]-palmityl-CoA ($6.2 \cdot 10^6$ disint./min·μmole) and protein as indicated in a final vol. of 225 μl. In Expt. 2 the concentration of [1-^{14}C]palmityl-CoA was 0.46 mM ($4.74 \cdot 10^7$ disint./min·μmole). Incubation was for 0.5 h at 37°. Succinic dehydrogenase and glucose-6-phosphatase were assayed as described in the text. Plasma membranes had succinic dehydrogenase and glucose-6-phosphatase specific activities of 4.6 (3) and 0.58 (2) respectively.

Membrane protein (μg)	*Additions*	*Enzymic properties of added fractions*		*Palmityl pantetheine liberated* (mμmoles *per reaction mixture*)		
		*Succinic dehydrogenase**	*Glucose-6-phosphatase***	*Boiled control*	*Untreated*	*Inhibition* (%)
Expt. 1						
170	None	—	—	0.21	10.47	—
170	Sucrose liver homogenate, 240 μg protein	14.8 (2)***	1.54 (1)	1.11	4.75	55
170	Nuclei, 273 μg protein	14.3 (1)	2.29 (1)	0.22	3.97	62
170	Mitochondria, 405 μg protein	28.3 (2)	1.55 (2)	0.32	4.52	57
170	Microsomes, 174 μg protein	2.1 (2)	4.43 (2)	0.51	4.39	58
170	Supernatant, 200 μg protein	0 (1)	0.19 (1)	1.23	4.49	57
Expt. 2						
214	None	—	—	0.60	8.50	—
214	Liver homogenate, 24 μg protein	—	—	0.60	2.31	73
214	Liver homogenate, 118 μg protein	—	—	0.62	1.84	78
214	Liver homogenate, 236 μg protein	—	—	0.62	1.58	81
214	Liver homogenate, 590 μg protein	—	—	0.65	1.32	84
214	Boiled liver homogenate, 590 μg protein	—	—	—	1.63	81

* mμmoles dichlorophenolindophenol reduced/mg protein per min at 25°.

** μmoles P_i formed/mg protein per h at 37°.

*** The number in parentheses represents the number of preparations assayed, each in duplicate or triplicate.

TABLE III

FORMATION OF PALMITYL PANTETHEINE FROM [1-^{14}C]PALMITYL-CoA AND [1-^{14}C]PALMITIC ACID BY LIVER PLASMA MEMBRANES

The incubation mixtures contained 130 mM Tris–HCl (pH 7.4), 214 μg membrane protein and either 0.46 mM [1-^{14}C]palmityl-CoA ($4.74 \cdot 10^7$ disint./min·μmole) or 0.22 mM [1-^{14}C]palmitic acid ($2.56 \cdot 10^7$ disint./min·μmole) as indicated in a final volume of 225 μl. When indicated 1 mM $MgCl_2$, 1 mM CoA or 1 mM Na_2ATP were added. Incubations were for 0.5 h at 37°.

Additions to incubation mixture	*Palmityl pantetheine formed (mμmoles/mg protein)*
[^{14}C]Palmitic acid	0.87
[^{14}C]Palmitic acid + boiled membranes*	0.73
[^{14}C]Palmitic acid + $MgCl_2$ + CoA + ATP	2.09
[^{14}C]Palmitic acid + $MgCl_2$	1.51
[^{14}C]Palmitic acid + CoA	0.69
[^{14}C]Palmitic acid + ATP	1.18
[^{14}C]Palmitic acid + ATP + CoA	2.08
[^{14}C]Palmityl-CoA	28.19
[^{14}C]Palmityl-CoA + boiled membranes*	1.61

* Boiled for 5 min in buffer before addition of other component to the reaction vessel.

detergents to isolated membranes solubilized up to 50% of membrane protein, but the enzymatic activity remained in the sedimentable fractions after such treatment. The pH maximum for acyl-pantetheine formation was about 7.5 in Tris buffer and yields fell off sharply at more acid or more alkaline pH. Since the reaction involves several enzymes, it is probable that this pH value represents an optimal composite for acyl-pantetheine formation by membranes.

Initially, all incubations were carried out exclusively with freshly prepared membranes and it was observed that the yield of acyl pantetheine, though linear at first, fell off markedly if the incubation was extended for more than 30 min. Later observations made on membrane preparations which had been used after storage in the cold (Table IV) indicated that the enzyme which degraded S-acyl pantetheine to free fatty acid and pantetheine may be labile and attenuated by storage. Precise data on the conditions under which acyl pantetheine is degraded so far have not been

TABLE IV

EFFECT OF STORAGE OF MEMBRANES ON FORMATION OF PALMITYL PANTETHEINE FROM [1-^{14}C]PALMITYL-CoA

Assay conditions as in Table I with 200 μg of membrane protein.

Storage conditions	*Storage time*	*Palmityl pantetheine liberated (mμmoles/mg protein)*	
		Membranes boiled	*Membranes untreated*
None	None	4.86	33.5
0°	48 h	5.67	55.7
−20°	27 h	6.48	62.7
−20°	48 h	3.25	62.7
−20°	16 days	6.48	64.8
−20°	41 days	3.73	55.1

obtained, because this substrate is difficult to solubilize in an aqueous incubation mixture. Preliminary results indicate, however, that enzymatic cleavage of the thioester bond does occur and that this reaction is pH dependent (Table V).

The nature of the acyl moiety of acyl-CoA markedly affected the yields of acyl pantetheine. Considerably higher yields were obtained with [^{14}C]oleyl- and [^{14}C]-linoleyl-CoA compared with the fully saturated substrates (Fig. 1). When these data are compared with the liberation of free fatty acid from these substrates[1] it becomes apparent that the yield of *S*-acyl pantetheine and free fatty acid liberation are inversely related.

TABLE V

DEACYLATION OF PALMITYL PANTETHEINE BY LIVER PLASMA MEMBRANES

Palmityl pantetheine (labeled from [1-^{14}C]palmityl-CoA) was isolated and purified as described in the text. Aliquots (29090 disint./min) were pipetted into conical centrifuge tubes and were blown to dryness under N_2. A 25-μl aliquot of Triton X-100 (1 mg/ml) was added, followed by buffer, water and membranes (180 μg protein) to yield a final vol. of 220 μl. When indicated membranes were first boiled for 5 min before addition to the reaction vessel. The system was buffered with one of the following systems (180 mM): Tris-acetate (pH 5.7); Tris-acetate (pH 6.6); Tris–HCl (pH 7.5); Tris–HCl (pH 8.2). The reaction mixture was incubated at 37° for 0.5 h. Figures represent disint./min per reaction mixture.

pH of incubation	*Palmityl pantetheine*		*Free fatty acid*	
	Boiled membranes	*Untreated membranes*	*Boiled membranes*	*Untreated membranes*
5.7	26 000	21 226	0	2594
6.6	29 946	21 526	194	2370
7.5	29 588	17 218	0	4092
8.2	28 222	15 860	0	3888

Addition of a variety of classical inhibitors to the reaction mixture showed that the most powerful inhibitors were DFP and Zn^{2+} (Table VI). Both DFP and Zn^{2+} produced their effects at concentrations which are relatively high in comparison to their effects in a number of other systems. Sulfhydryl inhibitors had no effect on the reaction. The addition of –SH compounds could be inhibitory. This latter effect is probably due to the occurrence of transacylation reactions (E. G. TRAMS, unpublished data). Prolonged dialysis of the isolated membranes against water or dilute buffer does not lead to an appreciable decrease in enzymatic activity, which would argue against an ion requirement for the reaction. Since some inhibition was obtained by the addition of EDTA, however, it is conceivable that dialysis against water was not sufficient to remove the required ion. Further studies on this aspect will be necessary.

The addition of materials with some detergent action (Triton, deoxycholate, lysolecithin) did not affect the system when only low levels were introduced. Anaerobic conditions or agents known to interfere with oxidative reactions (KCN, 2,4-dinitrophenol) had no effect. Some agents or hormones which at one time or another were implicated in transport reactions have been tested. All of the following were found to be without effect on the synthesis of *S*-acyl pantetheine: insulin, epinephrine, and norepinephrine, carnitine, theophylline, ouabain, acetylcholine (with or without eserine) and tetrodotoxin. We have noted that the addition of about 3 mM UDPG

TABLE VI

EFFECTS OF VARIOUS AGENTS ON THE FORMATION OF PALMITYL PANTETHEINE FROM [1-^{14}C]PALMITYL-CoA

The incubation mixtures contained 130 mM Tris–HCl (pH 7.4), 100–200 μg membrane protein and 0.2–0.4 mM [1-^{14}C]palmityl-CoA ($6.2 \cdot 10^6$ disint./min·μmole) in a final volume of 220 μl. Incubations were for 0.5 h at 37°. When indicated membranes were boiled for 5 min in buffer.

Additions	*Palmityl pantetheine liberated (mμmoles per mg protein)*	*Control (%)*
Expt. 1		
None	36.5	100
None, membranes boiled	1.1	3
Diisopropylfluorophosphate, 4.5 mM	24.3	67
Diisopropylfluorophosphate, 23 mM	1.9	5
Diisopropylfluorophosphate, 111 mM	0.6	2
Tris–arsenite, 4.5 mM	31.1	85
2.2 mM BAL–2.2 mM arsenite	31.4	86
Triton X-100, 1.0 mg/ml	30.6	84
$Zn(NO_3)_2$, 0.1 mM	24.1	66
$Zn(NO_3)_2$, 1.0 mM	1.3	4
β-Mercaptoethanol, 16.0 mM	14.8	41
Iodoacetamide, 11 mM	44.5	122
Expt. 2		
None	16.6	100
None, membranes boiled	1.4	8
Tris-BAL, 0.2 mM	14.5	87
Tris-BAL, 5.3 mM	5.3	32
Dithioerythritol, 5.3 mM	13.4	81
Cysteine, 8.9 mM	9.2	55
Expt. 3		
None	30.0	100
None, membranes boiled	4.4	15
2,4-Dinitrophenol, 9 mM	26.5	88
UDPG, 2.7 mM	12.0	40
p-Chloromercuribenzoate, 9 mM	22.8	76
EDTA, 1.8 mM	12.6	42
Expt. 4		
None	22.6	100
None, membranes boiled	5.4	24
EDTA, 0.6 mM	13.7	61
EDTA, 5.6 mM	7.4	33
$MgCl_2$, 0.1 mM	20.8	92
$MgCl_2$, 1.1 mM	16.1	71
$MgCl_2$, 11.1 mM	7.7	34
Expt. 5		
None	28.9	100
None, membranes boiled	1.4	5
DL-Carnitine, 6 mM	24.9	86
Lysolecithin, 1.0 mg/ml	15.0	52

produced more than 50% inhibition. This may be related to a competitive reaction, since UDPG is broken down by membrane preparations, liberating glucose (E. G. TRAMS AND J. ROBINSON, unpublished observations). Acyl-pantetheine formation

TABLE VII

FORMATION OF PALMITYL PANTETHEINE AND LIBERATION OF FREE FATTY ACID FROM [1-^{14}C]PALMITYL-CoA BY FRACTIONS FROM VARIOUS TISSUES

The incubation media contained 150 mM Tris–HCl (pH 7.4), 0.236 mM [1-^{14}C]palmityl-CoA ($2.2 \cdot 10^7$ disint./min·μmole) and *E. electricus* electroplax membranes (stored 4 months at -195°) (1.88 mg protein), erythrocyte ghosts (0.92 mg protein), adipose tissue homogenate (2.18 mg protein), isolated fat cells (0.84 mg protein), or fat cell ghosts (0.99 mg protein) in a final volume of 1.6 ml. Incubations were for 0.5 h at 37°. The zero time figures have been subtracted.

Preparation	*mμmoles liberated/mg protein*	
	Palmityl pantetheine	*Free fatty acid*
Electroplax membranes	31.3	5.1
Erythrocyte ghosts	0	15.3
Adipose tissue homogenate	5.8	43.0
Fat cells	20.6	125.6
Fat cell ghosts	31.6	186.6

and free fatty acid release, from palmityl-CoA by various fractions from other tissues have also been studied (Table VII). Acyl pantetheine was formed by a membrane preparation from the electric organ of *E. electricus*, but no formation could be detected using erythrocyte ghosts. However both preparations released free fatty acid from palmityl-CoA. A comparison of palmityl-pantetheine formation by adipose tissue

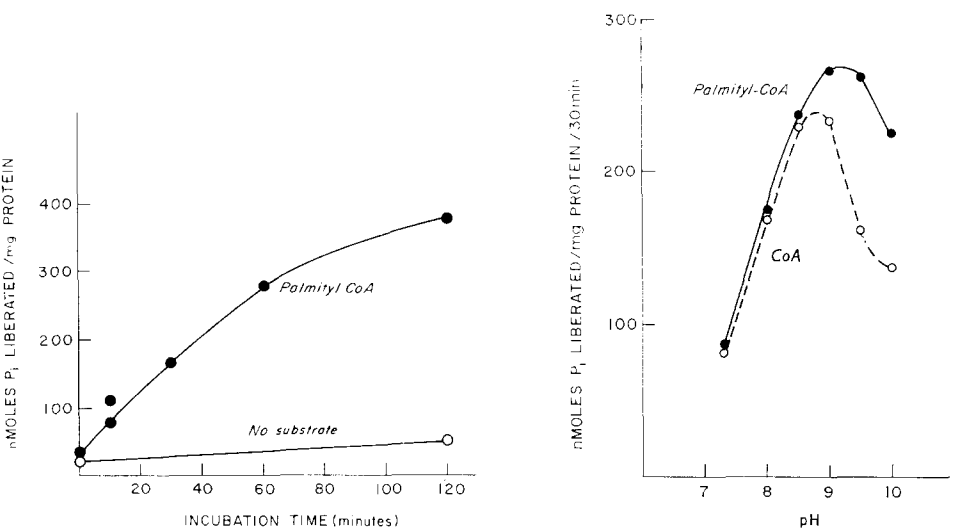

Fig. 2. Time course of the release of P_i from palmityl-CoA by liver plasma membranes. The incubation medium contained 3 mg membrane protein, 67 mM Tris–HCl (pH 7.4) and 1.0 mM palmityl-CoA in a final volume of 1.33 ml. Incubation was at 37°. The reaction was terminated with 30% trichloroacetic acid.

Fig. 3. The effect of pH on release of P_i from palmityl-CoA and CoA by liver plasma membranes. The incubation media contained 240 μg membrane protein, 0.244 mM palmityl-CoA or CoA and 100 mM 2-amino-2-methyl-1,3-propanediol buffer of the pH indicated, in a final volume of 205 μl. Incubation was for 0.5 h at 37°. The reaction was terminated with 30% trichloroacetic acid. Controls were run in the absence of substrate and have been subtracted.

homogenate, isolated fat cells and fat cell ghosts showed that membrane preparations gave the highest yield, as had been found with liver.

The mechanism of the reaction forming S-acyl pantetheine

Feeding rats with pantothenic acid which had been labeled with tritium by the technique of WILZBACH[16,17] led to incorporation of tritium into the protein as well as the lipids of membranes isolated from liver, adipose tissue and intestinal mucosa. Incubation of these labeled membranes with unlabeled palmityl-CoA led to a small but consistent increase in radioactivity eluted from a silicic acid column in the position where acyl pantetheine was eluted. Thus it is conceivable that an acyl carrier protein or a pantetheine source other than CoA possibly contributed pantethenyl fragments in this reaction or that *S*-acyl pantetheine occurs in trace amounts in the membrane.

The formation of acyl pantetheine is accompanied by the liberation of P_i (Fig. 2). Conceivably, acyl-CoA is attacked by a nucleotide pyrophosphatase (EC 3.6.1.9) and then a phosphomonoesterase cleaves P_i from 4′-phospho-*S*-acyl pantetheine. The pH maximum for P_i liberation is about 9.0 depending somewhat on the nature of the substrate (Fig. 3). Since the ratio of P_i liberated to acyl-CoA added is near unity or exceeds unity slightly, we assume that either the 3′-phosphate or the α-5′-phosphate, or both, are contributing to the P_i yield.

DISCUSSION

If acyl-CoA is incubated with liver plasma membranes, electroplax membranes or with adipose tissue cell ghosts, the CoA derivative is cleaved to form *S*-acyl pantetheine, P_i and a nucleotide residue. The amount of *S*-acyl panthetheine formed appears to be related to the purity of the membrane preparations, since addition of other subcellular particles decreased the yield. The increased acyl-pantetheine yield, with increased storage time in the cold, is thought to be due to a decrease in the thioester hydrolase (palmityl-CoA hydrolase, EC 3.1.2.2). Similarly, failure to obtain *S*-acyl pantetheine from other subcellular fractions may be the result of excessive hydrolase activity. There appears to be some differences, however, between the palmityl-CoA hydrolase described previously[18] and the activity observed here. The enzyme of PORTER AND LONG[18] was found to be soluble and stable for months in the frozen state; pH optimum was at 7.0. The membrane-bound thioester hydrolase appears to be quite unstable and pH optimum appeared to be between 7.5 and 8. Some preliminary experiments have indicated that the deacylation of *S*-acyl pantetheine may be accompanied by a transacylation reaction in which the acyl group is transferred. Our evidence only suggests an *S*-acyltransferase reaction, possibly analogous to the thioltransacylase reaction described by BRADY AND STADTMAN[19].

Our main interest has been focused on the nucleotide pyrophosphatase activity, though our evidence for this reaction at the present time is circumstantial. The product of the proposed reaction, other than phospho-acyl pantetheine, would be adenosine 3′,5′-diphosphate. Our yields of P_i indicated that the membrane preparations would break the adenosine 3′,5′-diphosphate down further. Whether or not the long chain *S*-acyl pantetheine obtained from acyl-CoA in the membrane fractions has any significance in transacylation reactions will have to be demonstrated. Another

possibility, namely that this compound may be involved in acyltranslocation reactions is presently under study.

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