Long-chain fatty acids and their acyl-CoA esters cause the translocation of phosphatidate phosphohydrolase from the cytosolic to the microsomal fraction of rat liver

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A translocation of phosphatidate phosphohydrolase from the cytosolic to the microsomal fraction was promoted in cell-free extracts of rat liver by oleate and palmitate and their CoA esters. Oleate was more potent in this respect than palmitate and the CoA esters were more effective than the unesterified acids. Octanoate, octanoyl-CoA and CoA did not cause the translocation. It is proposed that the interaction of phosphatidate phosphohydrolase with the membranes that synthesize glycerolipids causes it to become metabolically active. This enables the liver to increase its capacity for triacylglycerol synthesis in response to an increased supply of fatty acids.

Acyl-CoA ester *Fatty acid* *L-α-Phosphatidate phosphohydrolase* *Translocation* *Triacylglycerol synthesis*

1. INTRODUCTION

Phosphatidate phosphohydrolase is thought to be important in regulating the synthesis of glycerolipids, particularly of triacylglycerols in the liver [1]. Normally its activity changes to a greater extent under different physiological conditions than do the other enzyme activities involved in triacylglycerol synthesis. For example, glucocorticoids [2,3] and cyclic AMP [4] stimulate the synthesis of the phosphohydrolase, whereas insulin suppresses the glucocorticoid effect [5,6]. Vasopressin (and probably other hormones that mobilize Ca^{2+}) stimulate the phosphohydrolase activity within a few minutes [7].

In diabetes and stress conditions the liver is exposed to high concentrations of glucocorticoids relative to insulin, and the hepatocytes contain increased concentrations of cyclic AMP. The increase in activity of phosphatidate phosphohydrolase that results provides the liver with an increased potential to synthesize triacylglycerols [1,4–6]. However, for this potential to be expressed the phosphohydrolase needs to be translocated from the cytosol and onto the membranes on which the phosphatidate is synthesized [8,9]. On its own, cyclic AMP has the opposite action of displacing the phosphohydrolase from the membranes [9] and of inhibiting the synthesis of triacylglycerols [10]. These effects, however, can be reversed by relatively high concentrations of fatty acids such as would normally occur in diabetes and in stress [8–10]. The increased triacylglycerol synthesis limits the increase in the concentrations of fatty acids and their acyl-CoA esters and therefore prevents them from becoming toxic. This can often result in the formation of a fatty liver [11]. The secretion of very low density lipoproteins can also be increased and these are used preferentially by cardiac and skeletal muscle where lipoprotein lipase activity is increased relative to adipose tissue in metabolic stress and in diabetes [12].

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Previous work on the translocation of the phosphohydrolase between the cytosol and membrane-associated compartment was performed using rat hepatocytes in monolayer culture [8,9]. The present studies were performed to investigate whether fatty acids and acyl-CoA esters can effect this translocation directly in cell-free systems.

2. MATERIALS AND METHODS

The sources of the rats and most of the materials have been described previously [3,7]. Rotenone and cytochrome *c* were from the Sigma (London) Chemical Co., Poole, England.

The mixed anhydrides of palmitic, oleic and octanoic acids were prepared as described in [13]. These were then incubated at a molar ratio of 2.5:1 with CoA in tertiary butanol/0.26 M $KHCO_3$ (2:1, v/v) for a few minutes at room temperature until a test for thiol groups with 5,5′-dithiobis(2-nitrobenzoate) was essentially negative. The pH was adjusted to about 2 with 6 M HCl and water and tertiary butanol were removed by rotary evaporation under reduced pressure at room temperature. The residues containing palmitoyl-CoA and oleoyl-CoA were washed three times with ice-cold 01.22 M $HClO_4$, twice with acetone and four times with diethylether. The residue containing octanoyl-CoA was washed twice with diethylether/acetone (4:1, v/v), three times with diethylether and once with ice-cold 0.2 M HCl. The acyl-CoA esters were dissolved in water, the pH was adjusted to 4.5 with $KHCO_3$ and the samples were stored at −20°C until required. Concentrations were determined from the decrease in A_{232nm} after alkaline hydrolysis by using an extinction coefficient of 4500 $M^{-1} \cdot cm^{-1}$. Yields of the palmitoyl-CoA, oleoyl-CoA and octanoyl-CoA were 68, 78 and 83% respectively based upon CoA.

2.1. *Preparation and treatment of subcellular fractions*

Livers from the rats were suspended at 4°C in 4 vols of 0.25 M sucrose containing 0.2 mM dithiothreitol and adjusted to pH 7.4 with $KHCO_3$. They were homogenized in a Teflon-stainless steel homogenizer with five strokes up and down. The homogenates were then centrifuged at 4°C for 10 min at 18000 × *g* (r_{max} = 10.7 cm) and the supernatant was collected. This had a protein concentration of 20–25 mg/ml.

In some experiments (fig.1; table 1) Hepes adjusted to pH 7.4 with KOH was added to the supernatant to give a final concentration of 20 mM. Solutions of acyl-CoA esters or of the fatty acids (in a 20% molar excess of KOH) were added as indicated in a final volume of 1.5 ml to 20–25 mg of protein from the 180000 × *g*·min supernatant. The mixtures were incubated for 10 min at 37°C and then cooled on ice. The microsomal and soluble fractions were then collected after centrifugation at 90000 × *g* (r_{av} = 6.3 cm) for 90 min at 4°C and the microsomal fraction was resuspended in 0.25 M sucrose containing 0.2 mM dithiothreitol and 20 mM Hepes buffer, pH 7.4.

In other experiments (table 2), the 180000 × *g*·min supernatant was centrifuged at 190000 × *g*

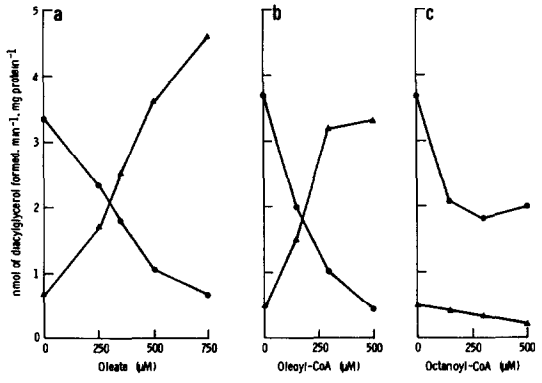

Fig.1. Effects of oleate, oleoyl-CoA and octanoyl-CoA on the activity of phosphatidate phosphohydrolase in the microsomal and soluble fraction of rat liver. The 180000 × *g*·min supernatant of rat liver was incubated for 10 min at 37°C with the concentrations of fatty acids or acyl-CoA esters that are indicated. It was then separated into microsomal (▲) and soluble fractions (●) by centrifugation (section 2). The experiments were performed with four independent preparations and two independent sets of results for oleate, oleoyl-CoA, palmitate, palmitoyl-CoA and octanoyl-CoA were obtained. A typical result is shown for oleate, oleoyl-CoA and octanoate. The effects of palmitate and palmitoyl-CoA were similar to those of oleate and oleoyl-CoA (not shown) except that the latter two compounds were more effective (table 1).

Table 1

Effects of fatty acids and acyl-CoA esters on the recovery of phosphatidate phosphohydrolase in the microsomal and soluble fractions of rat liver

Additions		Relative distribution of phosphatidate phosphohydrolase activity (%)		Total activity of PAP relative to control	
		Soluble fraction	Microsomal fraction		
(A)	None (control)	92 ± 1	8 ± 1	1	(4)
	Palmitate	86 ± 0	14 ± 0	0.89 ± 0.04	(2)
	Palmitoyl-CoA	65 ± 12	35 ± 12	0.70 ± 0.08	(4)
	Oleate	75 ± 3	25 ± 3	0.81 ± 0.05	(2)
	Oleoyl-CoA	55 ± 12	45 ± 12	0.71 ± 0.09	(4)
	Octanoate	93 ± 0	7 ± 0	0.86 ± 0.04	(2)
	Octanoyl-CoA	93 ± 2	7 ± 2	0.60 ± 0.08	(4)
	CoA	94 ± 1	6 ± 1	0.98 ± 0.04	(2)
(B)	None (control)	97 ± 0	3 ± 0	1	(2)
	Oleate	89 ± 1	11 ± 1	0.94 ± 0.02	(2)
	Oleoyl-CoA	78 ± 1	22 ± 1	0.84 ± 0.09	(2)

The 180000 × g·min supernatant was incubated for 10 min at 37°C (A), or left at 4°C (B) in the presence of 300 μM of the various compounds indicated. The microsomal and soluble fractions were then separated by centrifugation (section 2). The relative PAP activities are quoted as means ± SD or ± ranges for four or two independent experiments, respectively, as shown in parentheses. The absolute PAP activities were 2.27 ± 0.29 and 1.84 ± 0.05 nmol of diacylglycerol formed·min^{-1}·mg of protein^{-1} in sections A and B, respectively

(r_{av} = 6.3 cm) for 60 min at 4°C and the microsomal pellet was resuspended in 0.25 M sucrose containing 0.2 mM dithiothreitol. The supernatant fraction was collected from beneath the lipid layer and it was recentrifuged at 190000 × g for 60 min to ensure that it was essentially free from contaminating membranes. In fact, the final supranatant contained less than 1.0% of the rotenone-insensitive NADH cytochrome c reductase activity of the 180000 × g·min supernatant. The microsomal and soluble fractions were then incubated alone or together for 10 min at 37°C with acyl-CoA esters or fatty acids in 20 mM Hepes buffer at pH 7.4 (see above). After cooling the fractions were centrifuged at 90000 × g for 90 min and the cytosolic and microsomal fractions were collected (table 2).

2.2. *Analytical methods*

The method for the determination of protein was as described in [14] except that PAGE Blue G-90 was employed. Phosphatidate phosphohydrolase was assayed as described in [15] by using a final concentration of 5 mM $MgCl_2$. Rotenone-insensitive NADH cytochrome c reductase was employed as a marker for endoplasmic reticulum membranes and it was measured by the method described in [16]. The cytosolic marker was lactate dehydrogenase and its activity was determined by the method described in [17].

3. RESULTS AND DISCUSSION

The addition of oleate, oleoyl-CoA, palmitate and palmitoyl-CoA (fig.1; table 1) to the 180000 × g·min supernatant of rat liver caused a progressive increase in the recovery of phosphatidate phosphohydrolase in the microsomal fraction and a decrease in the soluble fraction. Palmitate was less effective than palmitoyl-CoA in this respect,

Table 2

The translocation of phosphatidate phosphohydrolase activity between the microsomal membranes and the soluble fraction by oleate and oleoyl-CoA

Additions	Relative activity of phosphatidate phosphohydrolase (%)					
	180000 × g·min supernatant		Particle-free supernatant		Microsomal fraction + particle-free supernatant	
	Pellet	Supernatant	Pellet	Supernatant	Pellet	Supernatant
None	11 (11)	89 (89)	7 (6)	93 (94)	8 (7)	92 (93)
Oleate (750 μM)	79 (58)	21 (42)	3 (6)	97 (94)	68 (41)	32 (59)
Oleoyl-CoA (200 μM)	41 (50)	59 (50)	1 (10)	99 (90)	67 (53)	33 (47)

The fractions indicated above were incubated at 37°C for 10 min in the presence or absence of oleate or oleoyl-CoA and the membrane fractions (pellets) were separated from the soluble proteins by centrifugation (see section 2). The combined microsomal and particle-free supernatant fractions contained the same relative concentrations of those components as were present in the 180000 × g·min supernatant. The results in parentheses are from a second independent experiment

and the unesterified acids were less effective than their CoA esters in the four independent experiments for each compound (fig.1, table 1, section A). These differences were still apparent after compensating for the slightly lower recoveries of the phosphohydrolase that were obtained after incubating with the CoA esters (table 1). The translocation of the phosphohydrolase was increased by incubating the 180000 × g·min supernatant with oleate or oleoyl-CoA at 37°C for 10 min. This can be seen by comparison with table 1, section B, where parallel samples were stored on ice. The temperature dependence probably reflects differences in fluidity of oleate, oleoyl-CoA and the membranes which would influence their abilities to interact. It is less likely that oleate was undergoing an enzymic modification since ATP and CoA were not added to these incubations, although such a conversion cannot be completely excluded.

Octanoyl-CoA was used as an example of a medium-chain acyl-CoA ester and it decreased the phosphohydrolase activity in both the microsomal and soluble fractions (fig.1c; table 1). Octanoic acid also failed to promote the translocation of the phosphohydrolase at concentrations of 300 μM (table 1) and at 1 mM (not shown). CoA had no significant effect on the recovery of the phosphohydrolase activity or its distribution between the soluble and microsomal fraction (table 1).

It was possible that the apparent translocation of the phosphohydrolase could have resulted if fatty acids and their CoA esters were able to cause its aggregation such that the phosphohydrolase could be recovered as a pellet after centrifugation. The results in table 2 test this possibility. Treatment of the particle-free supernatant with oleate or oleoyl-CoA failed to decrease significantly the relative activity of the phosphohydrolase that was recovered in the soluble fraction after centrifugation. Oleate and oleoyl-CoA did not have a large effect on the absolute activity of the phosphohydrolase since averages of 80 and 87% of this activity were recovered in the supernatant fraction respectively in the two experiments described in table 2.

The addition of the microsomal fraction to the particle-free supernatant restored the ability of oleate and oleoyl-CoA to translocate the phosphohydrolase activity to the pellet. Oleate did not cause the translocation of the cytosolic marker, lactate dehydrogenase to the pellet (not shown). Control experiments were also performed in which 750 μM of either palmitate or oleate were added to the microsomal membranes in the absence of the particle-free supernatant and this failed to increase the phosphohydrolase activity.

The present work confirms in a cell-free system the results obtained using isolated hepatocytes [8,9]. Namely that phosphatidate phosphohydrolase is an ambiquitous enzyme [18] that can trans-

locate between the cytosolic and membrane-bound compartments. In particular the present work demonstrates that long-chain fatty acids and their CoA esters have a direct rather than an indirect effect in this respect. The membranes involved in this translocation are probably those of the endoplasmic reticulum where glycerolipid synthesis occurs. Our initial experiments to investigate this by using centrifugation on Percoll gradients show that the peak of the phosphohydrolase activity coincides with that of rotenone-insensitive cytochrome *c* reductase. However, more work is required to prove that the phosphohydrolase is associated entirely with membranes of the endoplasmic reticulum.

The control of the translocation of phosphatidate phosphohydrolase by fatty acids and cyclic AMP resembles that of CTP:phosphocholine cytidylyltransferase [10, 19, 20]. This latter enzyme is important for regulating the synthesis of phosphatidylcholine. It is believed that the co-ordinated translocation of the phosphohydrolase and the cytidylyltransferase from cytosol to the membranes on which glycerolipid synthesis occurs enables the enzyme activities to be expressed metabolically. This co-ordinated control matches the rates of synthesis of triacylglycerols and phosphatidylcholine to the fatty acid supply of the liver. In particular, it should facilitate the synthesis of glycerolipids and the secretion of very low density lipoproteins in diabetes and in conditions of metabolic stress. This controls the rise in the concentrations of fatty acids and acyl-CoA esters in hepatocytes which might otherwise become toxic. The triacylglycerols that are secreted can be used by muscles as a source of energy.

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