THE FORMATION OF ALKYL PALMITATE BY RAT LIVER MICROSOMES

Anne M. BAKKEN, Mikael FARSTAD and Rolf BERGE
University of Bergen, Laboratory of Clinical Biochemistry, N-5016 Haukeland Sykehus, Norway

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1. Introduction

During our work with a method for the determination of diacylglycerol acyltransferase [1,2], we observed an unexpected spot in the thin-layer chromatogram (TLC) in addition to the triacylglycerol spot. The unexpected spot was only found when ethanol was present in the incubation mixture, and was identified as ethyl palmitate.

Recently methyl palmitate has been found in kidneys from patients with Reyes syndrome [3], a rare disorder of metabolism [4]. Formation of ethyl palmitate or other alkyl esters of palmitic acid have not been reported in the literature. This paper reports evidence of formation of ethyl palmitate and propyl palmitate in rat liver.

2. Materials

Bovine serum albumin (essentially fatty acid free), palmitoyl-coenzyme-A (grade II), methyl palmitate, ethyl palmitate, propyl palmitate, cholesteroyl palmitate, Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) were from Sigma. 1,2-dioleoyl-*sn*-glycerol, tripalmitoylglycerol, trioleoyl-glycerol were from Supelco [1-^{14}C]palmitic acid, [1-^{14}C]-palmitoyl-CoA and tri [1-^{14}C]palmitoylglycerol were from New England Nuclear. Scintillation liquid (Scint Hei III) was from Koch-Light. TLC plates, silica gel 60 were from Merck.

2.1. *Liver homogenates and subfractions*

Livers from fasting rats of a local Wislar-Møll strain were homogenized in 9 vol. 0.25 M sucrose, and the mitochondria were isolated from the homogenate as in [5]. The supernatant was centrifuged at 27 000 × g_{max} for 30 min, the pellet containing the lysosomes was discarded. The resulting supernatant was then centrifuged at 130 000 × g_{max} for 60 min, and the pellet was the microsomes and the final supernatant was the cytosol. Protein was determined by Lowry procedure [6].

3. Methods and results

The activity of diacylglycerol acyltransferase in rat liver microsomes was determined as in [1] with the following modifications: 50 mM Hepes buffer (pH 7.5), 50 μM [1-^{14}C]palmitoyl-CoA, 50 μM 1,2-dioleoyl-*sn*-glycerol (or 1,2-dipalmitoyl-*sn*-glycerol) in absolute ethanol and 100–200 μg microsomal protein in total vol. 200 μl were used. The reaction was started by addition of 50 μM [1-^{14}C]palmitoyl-CoA. Incubation was for 5 min at 35°C. The final extracts dissolved in heptane were evaporated in N_2 gas, the residue dissolved again in 20 μl heptane.

TLC was done to check the identity of triacylglycerol in the following solvent system: petroleum ether (boiling range 40–60°C)/diethyl ether/acetic acid, 75/25/1 (v/v/v). Sample extracts and standards of trioleoylglycerol, tripalmitoylglycerol, ethyl palmitate, cholesteroyl palmitate, [1-^{14}C]palmitoyl-CoA and diacylglycerols were run. Then silica gel plates were sprayed with I_2 in petroleum ether, the gel removed in sections of 0.5 or 0.25 cm from the point of application (fig.1A) and counted in a Packard Tricarb Model 2450.

The R_F value of the spot with the highest radio-

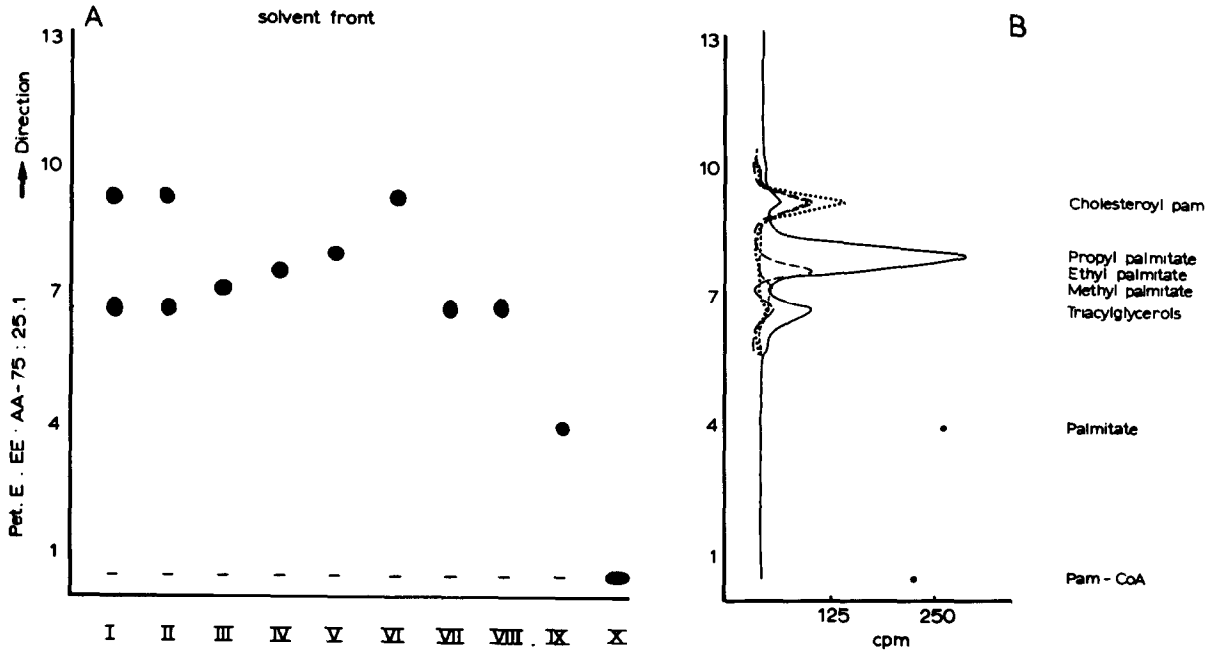

Fig.1. Thin-layer chromatography of lipids from heptane extracts of incubated samples (I, II) and appropriate standards (III–X). (A) Solvent system: (Pet.E) petroleum ether, b.p. 40–60°C; (EE) diethyl ether; (AA) acetic acid; 75/25/1 (v/v/v); (I) 1,2-dioleoyl-*sn*-glycerol as substrate or 1,2-dipalmitoyl-*sn*-glycerol as substrate; (II) methanol, ethanol or propanol as substrate; (VI) cholesteroyl palmitate; (VII) tri-[1-^{14}C]palmitoylglycerol; (VIII) trioleoylglycerol; (IX) [1-^{14}C]palmitate; (X) [1-^{14}C]palmitoyl-CoA. (B) Radioactivity diagram corresponding to spots on the chromatogram in A from samples run without added diacylglycerol: (————) with propan-1-ol; (– – – –) with ethanol; (- - -) with methanol; (· · · · · · · · ·) no alcohol.

activity corresponded to the R_F value of ethyl palmitate standard, and not to the R_F value of triacylglycerol as expected [1,2].

TLC was also done using a different solvent system: benzene/diethyl ether/ethanol/acetic acid, 100/80/4/0.4 (v/v/v/v). Again a radioactive spot with a R_F value corresponding to ethyl palmitate was found. Both the triacylglycerol and the ethyl palmitate had different R_F values in this solvent system (not shown). No radioactivity with R_F corresponding to palmitoyl-CoA or palmitate was found in either system.

Omitting the washing procedure of the heptane layer in 0.5 M NaOH, ethanol and water did not change the amount of ethyl palmitate.

When the assay was performed with 1,2-dioleoyl-*sn*-glycerol dissolved in acetone instead of ethanol, no ethyl palmitate was formed, whereas the formation of triacylglycerol and cholesteroyl palmitate was about the same (table 1). Addition of ethanol to the incubation mixture in the absence of diacylglycerol also gave a radioactive spot with a R_F value identical to ethyl palmitate (table 1).

Experiments were also carried out with methanol or propan-1-ol as substrate, omitting diacylglycerol. The incubation mixture contained 2.5% (v/v) alcohol, and the assays were performed as described for ethanol. Higher alcohols are increasingly insoluble in water and were therefore not tested.

TLC of the heptane extract was also done after incubation with methanol and propan-1-ol (fig.1). Radioactive spots with R_F values identical with those of propyl palmitate and methyl palmitate standards were found. The formation of propyl palmitate was higher than the formation of ethyl palmitate (table 1). The methyl palmitate peak was, however, small and could not be properly quantified.

For further investigation we used a Hewlett

Table 1
The formation of triacylglycerol, ethyl palmitate, propyl palmitate and cholesteroyl palmitate from [1-^{14}C]palmitoyl-CoA in the presence and absence of 1,2-dioleoyl-*sn*-glycerol in acetone and 2.5% v/v ethanol (0.43 mol/l) or propan-1-ol (0.33 mol/l)

Dioleoyl-glycerol	Ethanol	Propanol	Triglycerols	Ethyl palmitate	Propyl palmitate	Chol. palmitate
+	+		0.10	0.20		0.15
+	–		0.10	0		0.10
–	+		0.06	0.15		0.15
–	–		0.05	0		0.20
–		+	0.15		0.85	0.03
Heat-inactivated tissue			0	0	0	0

Assay conditions were as stated in the text. Activities: nmol. min^{-1}. mg protein^{-1}. Mean values from 3 experiments with 3 different microsomal preparations

Packard 5720 A gas–liquid chromatograph equipped with a FID detector, and a coiled glass column (3 mm i.d., 6 ft long) containing 10% EGSS-S on diatomite 'C' 100–120 mesh (Pye Unicam). Carrier gas was N_2, flow rate 30 ml/min; air flow rate 240 ml/min; H_2 flow rate 30 ml/min. Temperatures: oven 180°C, injector 200°C, detector 225°C. Ethyl palmitate (0.2 nmol) was applied as a standard. Extract from the spot on silica gel plate tentatively identified as ethyl palmitate gave the same retention time as ethyl palmitate standard.

The extract plus ethyl palmitate standard gave one single peak on the chromatogram indicating identity between the spot and ethyl palmitate.

The heptane phase after incubation with methanol and propan-1-ol as substrates were also run on the gas–liquid chromatograph. Methyl palmitate and propyl palmitate in the extracts, tentatively identified by thin-layer chromatography, gave retention times identical with standards of methyl and propyl palmitate.

When protein was omitted from the reaction mixture, or heat-inactivated protein was used, no radioactive products were formed (table 1).

Assays with methanol, ethanol or propan-1-ol as substrate were run with isolated mitochondria, microsomes and cytosol, and the radioactive products extracted by heptane were counted, omitting the chromatography step. Table 2 shows the formation of radioactive products by subfractions of rat liver. The activity was highest in the microsomes, and increasing with increasing chain length of the added alcohol.

Table 2
The formation of total heptane extractable radioactivity formed from [1-^{14}C]palmitoyl-CoA in the presence of methanol, ethanol and propan-1-ol by subfractions of rat liver

Substrate	(mol/l)	Mitochondria	Microsomes	Cytosol
Methanol	0.62	0.085	0.29	0.16
Ethanol	0.43	0.025	0.34	0.05
Propan-1-ol	0.33	0.085	1.00	0.16

Diacylglycerol was omitted. Assay conditions otherwise as stated in the text. Activities are nmol. min^{-1}. mg protein^{-1}

Both in mitochondria and cytosol the activity was lower, and no significant effect of the chain length was found.

4. Discussion

These results are in accordance with the findings of increased formation of triacylglycerol in the presence of ethanol [1,2]. Our results indicate, however, that the effect of ethanol in the incubation mixture is formation of ethyl palmitate, and not stimulation of triacylglycerol formation.

The formation of alkyl palmitate was dependent on the presence of an alcohol and protein, and was not observed when the microsomal protein was heat inactivated. It seems probable that the formation

is a result of an activity that is catalyzed by a native protein, viz. of an enzymatic nature. At present, knowledge of both the specificity of the reaction, and its physiological significance are lacking. However, in the light of the report of isolation of a fatty acid alkyl ester (methyl palmitate) from patients with Reyes syndrome [3], the formation of such esters may be of considerable interest.

Acknowledgements

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References

[1] Coleman, R. and Bell, R. M. (1976) J. Biol. Chem. 251, 4537–4543.
[2] Bell, R. M. and Miller, Y. (1976) Anal. Biochem. 71, 436–443.
[3] Sudo, M., Tanioka, K.-I., Momoi, T., Akaishi, K. and Suzuki, Y. (1978) Clin. Chim. Acta 84, 179–184.
[4] Reye, R. D. K., Morgan, G. and Baral, J. (1963) Lancet 2, 749–752.
[5] Romslo, I. and Flatmark, T. (1973) Biochim Biophys. Acta 305, 29–40.
[6] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.