# THE ACYLATION OF GLYCEROPHOSPHATE IN RAT LIVER MITOCHONDRIA AND MICROSOMES AS A FUNCTION OF FATTY ACID CHAIN-LENGTH

Ludvig N.W. DAAE
*Institute of Clinical Biochemistry, University of Oslo, Rikshospitalet, Oslo, Norway*

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## 1. Introduction

In 1953 Kornberg and Pricer [1] described the enzymatic acylation of glycero-3-phosphate. They found the saturated straight-chain $C_{16}$–$C_{18}$ acids to be the best substrates, and phosphatidic acid was the reaction product. Glycerophosphate acylation takes place both in the endoplasmatic reticulum and in the outer membranes of mitochondria (for references, see [2]). Recent work in this laboratory has shown that the fatty acid specificity of the mitochondrial glycerophosphate acylation is different from that of the endoplasmatic reticulum. The mitochondrial enzyme shows a distinct preference for palmityl-CoA, and 1-palmitylglycerophosphate (lysophosphatidic acid) is the main reaction product [3]. In incubations with microsomes unsaturated $C_{18}$ acids are the most efficient substrates and phosphatidic acids are their main reaction products. These new observations have recently been confirmed [4]. In the present paper we report that there are also specificity differences depending on the chain-length of the saturated fatty acids in the two systems for glycero-3-phosphate acylation.

## 2. Methods

Materials and methods are described in details elsewhere [2,3].

## 3. Results and discussion

Table 1 shows that two activity maxima depending on chain-length are found with rat liver mitochondria, one with palmitate, the other with laurate. An identical picture was found with isolated outer mitochondrial membranes. Also with microsomes a maximum corresponding to palmitate was found. However, the maximum corresponding to laurate was not present. A reproducible 'dip' in the microsomal values corresponding to the $C_{15}$ acid was obtained. Possibly therefore, there are two activity maxima also with microsomes.

Fig. 1 shows that both laurate and palmitate gave monoacylglycerophosphate (lysophosphatidic acid) as the chief reaction product in mitochondria while both gave diacylglycerophosphate (phosphatidic acid) in microsomes. Thin-layer chromatography on boric acid impregnated silica gel GF-covered glass plates developed with chloroform–acetone (92:8, by vol.), as previously described [2,5], indicated that both the monolauryl- and the monopalmitylglycerophosphate were acylated in the 1-position.

The reason for two activity maxima depending on chain-length in the incubations (especially with mitochondria) is uncertain: The 'dips' in activity (table 1) most likely is not caused by varying detergent effects of the $C_{13}$–$C_{18}$ acylcarnitines since the detergent effect probably increases with chain-length of the fatty acid. Fig. 2 shows that in the presence of a high concentration of albumin, increasing concentrations of $C_{12}$, $C_{14}$ and $C_{16}$ acylcarnitines showed very similar optima and substrate inhibition curves. The double optimum most likely cannot be explained by a corresponding specificity for chain-lengths by the auxilliary carnitine acyltransferase used; the same auxilliary enzyme was used both in the microsomal and in the mitochondrial experiments,


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Table 1
Glycerophosphate acylation by rat liver subcellular fractions with carnitine esters of saturated fatty acids as the acyl donors.

| Fatty acid | Mitochondria | | Microsomes | |
|---|---|---|---|---|
| | (nmoles/mg protein × min) | % of $C_{16}$ activity | (nmoles/mg protein × min) | % of $C_{16}$ activity |
| $C_8$ | 0.90 | 16 | 0.37 | 5 |
| $C_{10}$ | 1.74 | 30 | 0.94 | 12 |
| $C_{11}$ | 4.87 | 84 | 2.53 | 32 |
| $C_{12}$ | 5.34 | 93 | 3.34 | 43 |
| $C_{13}$ | 4.60 | 80 | 4.77 | 61 |
| $C_{14}$ | 3.07 | 53 | 6.04 | 77 |
| $C_{15}$ | 3.23 | 56 | 4.16 | 53 |
| $C_{16}$ | 5.77 | 100 | 7.81 | 100 |
| $C_{17}$ | 3.54 | 61 | 6.39 | 82 |
| $C_{18}$ | 0.80 | 14 | 4.26 | 55 |

2.7 mg of mitochondrial protein, and 4.7 mg of microsomal protein were used in the assays. To an incubation volume adjusted to 1.0 ml were added: 0.125 mM CoA; 5 mM GSH; 5 mM *rac*-glycero-1-phosphate, to which had been added *sn* [1(3)-$^3$H] glycero-3-phosphate to give an activity of about 120000 cpm/μmole of the biologically active isomer (in the reaction product studies 800000 cpm/μmole); 15 mM TES [*N*-Tris (hydroxy-methyl)-methyl-2-aminoethane sulfonic acid] buffer, pH 7.4; 0.5% (w/v) bovine serum albumin; carnitine palmityl-transferase, 1.8 mg of protein, fatty acylcarnitines in amounts varying 0–1.0 mM (the value obtained with optimal concentration of each acylcarnitine is given in the table). The reaction was started with the particulate fractions used. The reaction was carried out at 30° for 7 min and was stopped with 1.5 ml n-butanol and 2.0 ml of water. After shaking, the reaction mixture was centrifuged and the butanol phase was pipetted off and washed once with 1.0 ml of butanol-saturated water. Then aliquots were taken for the radioactivity measurements and for thin-layer chromatography. Checks showed that even the $C_8$- and $C_{10}$-reaction products were efficiently extracted by butanol.

and addition of increased amounts of auxilliary enzyme did not increase the activity with $C_{13}$–$C_{15}$ acylcarnitines in the mitochondria. A reasonable explanation of the two activity maxima depending on chain-length may be the presence of two different acylating enzymes. Overlapping in the specificity of the two enzymes then probably exists (table 1). Further studies on the possibility of more than one enzyme, having monoacylglycerophosphate as the primary reaction product, but differing in their chain-length specificity, are complicated by the membrane-bound and detergent-sensitive nature of the enzyme system.

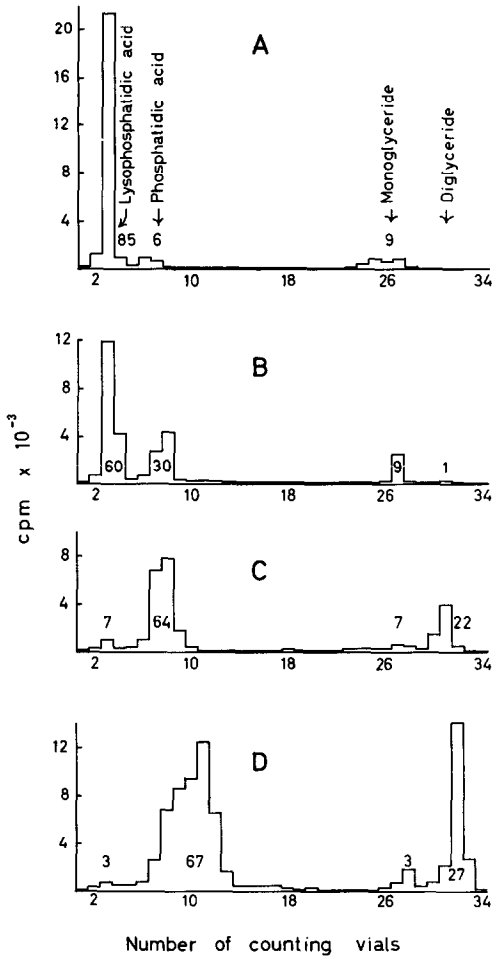


Fig. 1. Glycerophosphate acylation products by rat liver subcellular fractions with saturated $C_{12}$- and $C_{16}$-fatty acid carnitine ester as the acyl donor. Mitochondria incubated with laurylcarnitine (A); with palmitylcarnitine (B); microsomes incubated with laurylcarnitine (C) and with palmitylcarnitine (D). Assay conditions as described in legend to table 1. The chromatography was performed on silica gel H-covered glass plates and developed with chloroform–methanol–acetic acid–water (85:15:2:2, by vol.). 5-mm bands were scraped into counting vials for localization of the radioactive reaction products, iodine-spotted commerical standards were used for comparison. The per cent of the total cpm is indicated on each peak.

The $C_{10}$–$C_{15}$ saturated fatty acids are minor constituents of triglycerides from rats fed on a conventional diet [6]. However, in suckling rats [6] and in rats fed e.g. a diet rich on $C_{11}$ saturated acid [7] considerable amounts of these fatty acids are incor-

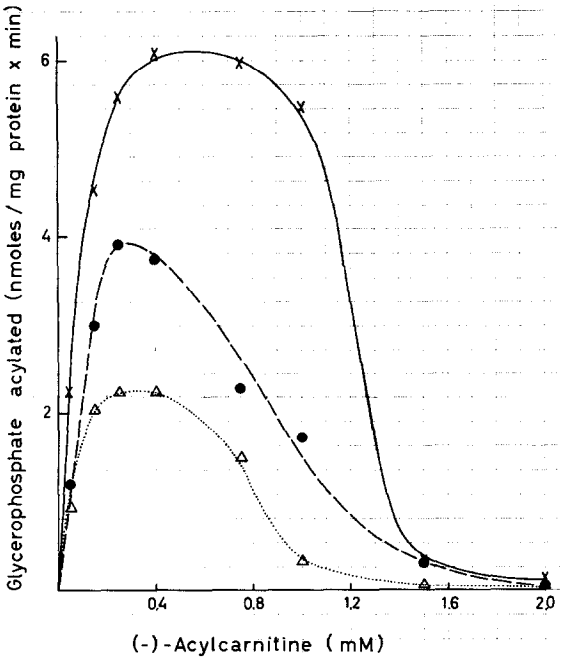


Fig. 2. Glycerophosphate acylation by rat liver mitochondria as a function of carnitine ester concentration. (●--●--●) $C_{12}$ acid, (Δ...Δ...Δ) $C_{14}$ acid, (X–X–X) $C_{16}$ acid. Incubation conditions were as listed in table 1 except that 1.5% albumin and 0.25 mM CoA were used. Increased addition of albumin to the assay system decreased the inhibition by high carnitine ester concentrations without changing the relative activities depending on fatty acid chain-length.

porated into the adipose tissue (in the undecanoate experiment 28% on the average of the total fatty acids was the $C_{11}$ acid). Thus a great capacity for the acyltion of glycero-3-phosphate must exist also for the intermediary-chain fatty acids. Kornberg and Pricer [1] obtained a very low activity with fatty acids shorter than $C_{15}$. Our findings, however, are in better agreement with the *in vivo* observations mentioned above.

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