A genuine acyl donor activity of oleyl-CoA for mitochondrial glycerophosphate acyltransferase

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1. INTRODUCTION

Both mitochondria and microsomes of mammalian organs contain glycerophosphate acyltransferase (EC 2.3.1.15), the first enzyme in the pathway of glycerolipid biosynthesis from *sn*-glycerol 3-phosphate [1–4]. The mitochondrial glycerophosphate acyltransferase shows 4–6 times preference for palmityl-CoA over oleyl-CoA as acyl donor. By contrast, the microsomal enzyme is highly active with either acyl-CoA [1,2]. With both mitochondrial and microsomal glycerophosphate acyltransferase, the acylation takes place in position 1 of *sn*-glycerol 3-phosphate molecule [1,5,6]. Thus, the properties of the mitochondrial enzyme provide the basis of an excellent mechanism for the selective positioning of saturated fatty acids in position 1 found in most naturally occurring glycerophospholipids [5,6]. To determine the degree of acyl-CoA specificity of the mitochondrial glycerophosphate acyltransferase, we have tested whether the small amount of activity associated with the mitochondrial fraction in the presence of oleyl-CoA is indeed a mitochondrial property or a reflection of microsomal contamination. With oleyl-CoA as the acyl donor, the mitochondrial acyltransferase was unaffected by *N*-ethylmaleimide and trypsin and, was stimulated by acetone and polymyxin B. All of these reagents strongly inhibited the microsomal enzyme. These distinguishing properties together with an analysis of the acylation products establish that oleyl-CoA is a true acyl donor for mitochondrial glycerophosphate acyltransferase.

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Abbreviations: glycerophosphate acyltransferase, acyl-CoA: *sn*-glycerol 3-phosphate acyltransferase; polymyxin B, polymyxin B sulfate

2. MATERIALS AND METHODS

sn-[2-^{3}H]Glycerol 3-phosphate was synthesized enzymatically from [2-^{3}H]glycerol, and purified as described previously [5]. [2-^{3}H]Glycerol and Biofluor were purchased from New England Nuclear, Boston, Mass.; trypsin, *N*-ethylmaleimide and polymyxin B sulfate from Sigma Chemical Co., St. Louis, MO. The origin of all other chemicals was as described elsewhere [1].

Liver mitochondrial and microsomal fractions were prepared [5] from male Sprague–Dawley rats weighing between 100–125 g and fed ad libitum. The subcellular fractions, suspended in 0.3 M sucrose, were either used fresh or stored at −70°C for a period not exceeding one month. Once thawed, the unused portion of a given sample was discarded. There was virtually no difference in activity between fresh and frozen samples.

Assay of glycerophosphate acyltransferase was performed as described previously [1] using 1.5 mM *sn*-glycerol 3-phosphate and approximately 0.1 mg subcellular protein. Asolectin was omitted from the assay. Optimal concentrations of oleyl-CoA, 36 μM and 144 μM, were used for mitochondrial and microsomal incubations respectively. The reaction was initiated by the addition of subcellu-

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lar fraction and was terminated and the radioactive phospholipids extracted by adding 0.5 ml of 1-butanol as described [5]. Aliquots of 0.1 ml of the washed butanol extract were mixed with 2.9 ml portions of Biofluor in minivials and counted in a Packard Tri Carb liquid scintillation spectrometer, model 3255. The results represent mean of duplicate experiments and are expressed as nanomoles of *sn*-glycerol 3-phosphate acylated. The products of acylation of glycerophosphate were separated by thin-layer chromatography and were identified by comigration with authentic standards and analyzed as described by Monroy et al. [5]. Other methods were as described previously [1].

3. RESULTS AND DISCUSSION

The mitochondrial fractions used in this investigation were tested for their purity by assaying marker enzymes such as NADPH-cytochrome *c* reductase and, trypsin and *N*-ethylmaleimide sensitivity of palmityl-CoA induced glycerophosphate acyltransferase activity. The results indicated only 0–4% microsomal contamination of the mitochondrial fractions as reported earlier [1].

The specific activity of glycerophosphate acyltransferase at optimal concentration of oleyl-CoA ranged in our experiments between 0.47–0.87 and 6.10–7.10 nmol · min^{-1} · mg^{-1} for mitochondrial and microsomal fractions, respectively. Since the mitochondrial activity was approximately one tenth that of the microsomes, we looked for distinguishing properties of the activity associated with the two subcellular fractions. Figure 1 documents the effects of *N*-ethylmaleimide, trypsin, acetone and polymyxin B on mitochondrial and microsomal glycerophosphate acyltransferase using oleyl-CoA as substrate.

Figure 1a demonstrates that the mitochondrial activity was resistant to up to 12 mM *N*-ethylmaleimide while the microsomal activity was inhibited approximately 80% in the presence of as low as 0.4 mM of the sulfhydryl group reagent. Further increase in the concentration of *N*-ethylmaleimide did not increase the extent of inhibition of the microsomal system. Similar results were obtained previously [1,5] with mitochondrial and microsomal enzyme in the presence of palmityl-CoA. Thus the microsomal, but not the mitochondrial enzyme requires free sulfhydryl group(s) for its function.

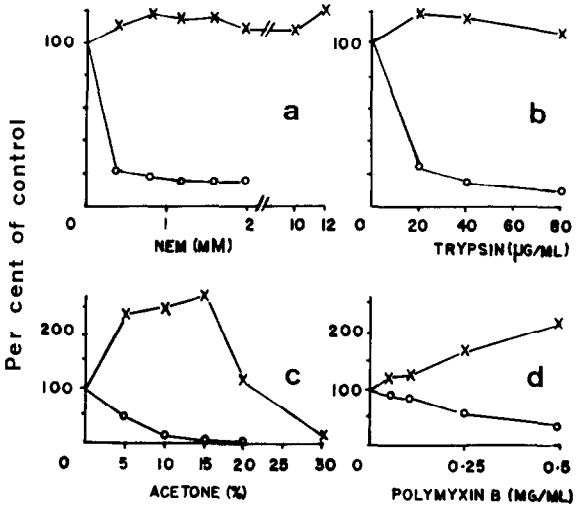

Fig.1. Distinguishing properties of mitochondrial and microsomal glycerophosphate acyltransferase using oleyl-CoA as acyl donor. The assay system contained (a) *N*-Ethylmaleimide (NEM), (b) trypsin, (c) acetone and (d) polymyxin B. Total incubation time was 3 min at 37°C. The results represent mean of duplicate experiments. The amount of glycerophosphate acylated in the absence of any activators or inhibitors was taken as 100%; mitochondria (×); microsomes (○).

The micromosal inhibition due to *N*-ethylmaleimide was always more with oleyl-CoA than when palmityl-CoA was used as the acyl donor [3]. Although in previous studies [1], *N*-ethylmaleimide was used on mitochondrial and microsomal glycerophosphate acyltransferase in the presence of oleyl-CoA, no conclusions could be reached because only one comparatively low concentration of the reagent was used and, furthermore, varying degree of inhibition by *N*-ethylmaleimide was observed with the mitochondrial fraction isolated from different mammalian organs [1].

Some proteolytic enzymes have been recently found to inhibit palmityl-CoA dependent microsomal glycerophosphate acyltransferase [3,7,8], but not the mitochondrial enzyme [3,8,9]. Figure 1b shows that with oleyl-CoA as the acyl donor, the glycerophosphate acyltransferase activity associated with the mitochondria was resistant to trypsin (up to 80 μg/ml) added to the incubation medium. Under similar conditions the microsomal enzyme

was inhibited over 90% by trypsin. These results are consistent with the previous suggestions that the mitochondrial enzyme is located in the inner side of the outer membrane [8,9] whereas the microsomal acyltransferase is located on the outer surface of the endoplasmic reticular membrane [7].

The effect of different concentrations of acetone on the mitochondrial and microsomal glycerophosphate acyltransferase in the presence of oleyl-CoA is documented in fig.1c. Acetone markedly stimulated the mitochondrial activity while inhibiting the corresponding microsomal enzyme. In the presence of 15% acetone, the mitochondrial activity was about 175% higher than control whereas the microsomal activity was completely inhibited. Previously, we reported similar differential effect of acetone on palmityl-CoA dependent acyltransferase activity of mouse liver mitochondria and microsomes [1].

Treatment with polymyxin B sulfate provided another criterion to distinguish between mitochondrial and microsomal glycerophosphate acyltransferase with oleyl-CoA as a substrate. The rationale for using polymyxin B is as follows: the antibiotic alters bacterial membrane structure by combining with phospholipids [10]. Furthermore, mitochondrial glycerophosphate acyltransferase activity is affected by its phospholipid environment [11]. The effect of different concentrations of polymyxin B on mitochondrial and microsomal glycerophosphate acyltransferase using oleyl-CoA as acyl group donor is shown in fig.1d. The mitochondrial activity was stimulated whereas the microsomal activity was inhibited. At polymyxin B concentration of 0.5 mg/ml, the mitochondrial activity was approximately double whereas the corresponding microsomal activity was inhibited 60%. The effect of polymyxin B on mitochondrial and microsomal acyltransferase is in keeping with the idea that the phospholipids present in the microenvironment of the enzyme molecules play an important role in regulating the enzymatic activity [8].

In the acylation of *sn*-glycerol 3-phosphate by palmityl-CoA, 1-palmityl-*sn*-glycerol 3-phosphate is the main mitochondrial reaction product, whereas the microsomal system accumulates, 1,2-dipalmityl-*sn*-glycerol 3-phosphate as the principal product [1,2,5]. However, the ratio of mono- to diacylglycerophosphate shows wide variability with different types of mitochondria [1]. With oleyl-CoA as acyl donor, the rat liver microsomal system produced diacylglycerophosphate as the major product (mono- to diacylglycerophosphate ratio, 0.23). Table 1 documents the products of acylation formed with liver mitochondria using oleyl-CoA as acyl donor. The monoacylated compound was always the major reaction product. However, the accumulation of the diacylated compound increased as the time of incubation increased. The maximal steady state concentration of the monoacylated compound presumably reached within 1.5 min (cf [5]) after which increasing amounts of the diacylated product accumulated with time. Previous studies with Ehrlich ascites tumor cells, which possess no mitochondrial glycerophosphate acyltransferase activity, showed that their microsomal enzyme might produce more mono- than diacylated product under some experimental conditions [1]. Thus, manipulation of the experimental condition and reaction environment can significantly change the product distribution of the same mitochondrial or microsomal enzyme.

Table 1

Ratio of mono- to diacylated glycerophosphate in rat liver mitochondria using oleyl-CoA as substrate

Time of incubation	Total incorporation (nmol)	mono-/diacyl glycerophosphate
1.5 min	0.18	16.1
3.0 min	0.27	11.5
5.0 min	0.36	7.0
10.0 min	0.67	5.2

Each of the essay mixtures contained 0.138 mg mitochondrial protein. The samples were incubated at 37°C for the time indicated. The reactions were stopped and the phospholipids extracted by the addition of 0.5 ml of 1-butanol as described in Materials and Methods. The acylation products were separated by thin-layer chromatography and analyzed as described [5]. The results represent mean of duplicate experiments.

The above results, taken together, demonstrate that the activity of the mitochondrial glycerophosphate acyltransferase in the presence of oleyl-CoA is a genuine mitochondrial property and is not a reflection of microsomal contamination. The question arises whether, at certain concentrations, oleyl-CoA can be an equally efficient or better

substrate in comparison with palmityl-CoA for mitochondrial glycerophosphate acyltransferase. Intracellular acyl-CoA concentration normally varies between 15–144 μM depending on nutritional condition [12,13]. We found that between 7.2–144 μM, and, regardless of the concentration used for either acyl-CoA, mitochondrial preparations always showed a strong preference for palmityl- over oleyl-CoA. Therefore, although mitochondrial enzyme does not show absolute specificity for palmityl-CoA, its property is in keeping with the selective positioning of saturated fatty acids in position 1 of glycerolipids. Indeed, recent experiments with Ehrlich ascites tumor cells [1] and cells maintained in tissue culture [14] suggest that the mitochondrial glycerophosphate acyltransferase may be involved in regulating the ultimate distribution of fatty acids in cellular phosphoglycerides. The possibility which remains to be explored is whether or not the mitochondria contain two or more glycerophosphate acyltransferases of different acyl-CoA specificities.

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