Vol. 101, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 16, 1981 Pages 237-242

ACYL-COA: sn-GLYCEROL 3-PHOSPHATE ACYLTRANSFERASE IN MITOCHONDRIA AND MICROSOMES OF ADULT AND FETAL GUINEA PIG LUNG

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Received June 1, 1981

SUMMARY: Guinea pig lung mitochondrial and microsomal glycerophosphate acyltransferase (GAT) was measured using palmityl- or oleyl-CoA as acyl donor. The activities of the two fractions differed in substrate specificity and sensitivity to N-ethylmaleimide, trypsin, and acetone. In comparison to adult lung, the fetal lung had markedly higher subcellular GAT activity. The results demonstrate a bimodal distribution of GAT in lung and suggest that this enzyme may plan an important role in lung development.

It is generally agreed that the synthesis of phosphatidylcholine (PC) in lung primarily occurs via CDP-choline pathway (1). The initial requirement in the de novo synthesis of PC is the formation of phosphatidic acid (PA). An important route for the synthesis of PA, as established in liver, involves stepwise acylation of sn-glycerol 3-phosphate by CoA thioesters of fatty acids in reactions catalyzed by two different enzymes, glycerophosphate acyltransferase (GAT) and 1-monoacylglycerophosphate acyltransferase (MGAT) (2,3). The GAT and MGAT are believed to be at least partly responsible for the selective positioning of saturated and unsaturated fatty acids found in most naturally occurring phosphoglycerides (4). Studies on substrate and positional specificity of liver mitochondrial GAT have shown strong preference of this enzyme for saturated fatty acids in position 1 of sn-glycerol 3-phosphate (5,6). Investigations with liver microsomes, however, have led to disagreement on the substrate and the positional specificity of the two acyltransferases (7-9).

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0006-291X/81/130237-06$01.00/0

Synthesis of PA from glycerophosphate has been shown to be important in both whole lung (10-12) and isolated type II cells (13). However, it is not clear if PA synthesis from glycerophosphate proceeds in two successive acylation steps in lung as in liver. Futhermore, the studies conducted on the subcellular distribution of GAT in lung (12) have not established a clear difference in properties between mitochondrial and microsomal enzymes.

In this paper, we report the subcellular distribution and properties of GAT in guinea pig lung. Guinea pig has been used as an animal model because of its close similarity with the human in lung development (14).

MATERIALS AND METHODS: Pregnant guinea pigs (Hartley strain) of timed gestation were purchased from William Cavies Co., Kentucky. The sources of chemicals were described elsewhere (15). Groups of fetuses with gestational age of 55 days were delivered by Caesarean section. The lungs of these fetuses, and also those from the mother guinea pigs (approximately 300 gm body wt) were removed, washed with cold saline and with 0.25M sucrose and finally homogenized in 0.25M sucrose/0.01M Tris, pH 7.4 (5ml/g lung) with a Potter Elvehjem homogenizer. The homogenate was centrifuged at 1,000 g for 10 min. The supernatant was centrifuged at 20,000 g for 20 min. The pellet was resuspended in sucrose-tris and resedimented at 6,500 g. The sediment (6,500 g) was washed three times with sucrose tris buffer. It was finally resuspended in the same medium and used as mitochondrial preparation. The 20,000 g supernatant was centrifuged at 105,000 g for 60 min. to sediment the microsomes. The microsomal pellets were washed two times and resuspended in sucrose-tris buffer. Protein was estimated according to the method of Lowry et al (16). Mitochondrial and microsomal fractions were checked for cross-contamination by marker enzymes. Citrate synthase (17) and NADPH-cytochrome c reductase (18) served as mitochondrial and microsomal markers respectively. GAT assays were performed as described in previous studies (15). All assays contained an optimal concentration of acyl-CoA (unless otherwise stated), 1.5mM sn-glycerol 3-phosphate and approximately 0.1 mg subcellular protein. N-ethylmaleimide (NEM), trypsin and acetone were added as described in individual experiments. The reaction was initiated by the addition of subcellular fraction and terminated after 3 min. by the addition of 1.0 ml of 1-butanol. Aliquot (0.7 ml) of the washed butanol extract was taken in a scintillation vial, evaporated under nitrogen and mixed with aquasol (5 ml) and counted in a Beckman LS-355 scintillation counter. All other methods are described previously (15).

RESULTS AND DISCUSSION: As reported for other mammalian organs (15,19), the microsomal GAT activity of lung was inhibited by NEM (Fig 1a). With palmityl-CoA as substrate, the inhibition was approximately 60% and 90% at NEM concentrations of 2.0 and 8.0mM respectively. However, greater inhibition was observed at all NEM concentrations when oleyl-CoA was used as an acyl donor. The mitochondrial GAT activity, on the other hand, was resistant even to 8.0mM NEM, regardless of whether palmityl- or oleyl-CoA was used as substrate. It is not

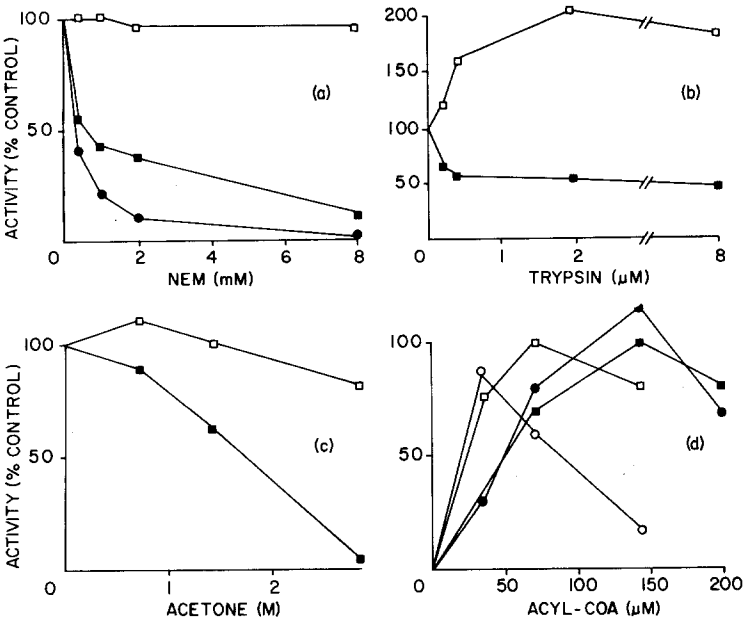

FIG. 1. Differential Properties of Adult Guinea Pig Lung Mitochondrial and Microsomal GAT. Assay conditions were described under "Methods". The control specific activities (n mole/min/mg) of mitochondrial GAT in the presence of optimal concentrations of palmityl- and oleyl-CoA were 0.56 and 0.47 respectively; the corresponding values for microsomal GAT were 4.50 and 5.11. In (d), the specific activities of GAT at optimal concentration of palmityl-CoA were considered 100%. The open symbols represent mitochondrial and the closed symbols are for microsomal activity. The squares and circles are for palmityl- and oleyl-CoA respectively.

known if the differential substrate dependent inhibitory action of NEM on the microsomal GAT is due to the substrates themselves or some of their products. Fig 1(b) documents the action of trypsin on mitochondrial and microsomal GAT activity. The microsomal GAT activity was inhibited by trypsin, the maximum inhibition being 56% at a trypsin concentration of 8μM. The mitochondrial GAT activity, by contrast, was stimulated by the addition of trypsin. Differential response to acetone provided another criteria by which lung mitochondrial and microsomal GAT could be distinguished. Fig. 1(c) documents that acetone markedly inhibited the microsomal activity while not substantially affecting the mitochondrial enzyme. At an acetone concentration of 2.8M, the microsomal activity was almost completely inhibited, while the mitochondrial retained over 80% of the activity.

Table 1

Glycerophosphate Acyltransferase Activity in Adult and Fetal Guinea Pig Lung Mitochondria and Microsomes

	sn-Glycerol 3-phosphate incorporated (nmole/min/mg)	
Source	Mitochondria	Microsomes
Adult	0.78 ± 0.02	1.97 ± 0.04
Fetus	3.05 ± 0.05	3.47 ± 0.05

Lungs from five 55 days old fetuses and those from their mothers were used to prepare the subcellular fractions. Assay conditions for GAT are described under "Methods". Palmityl-CoA at concentrations of 72 μM for mitochondria and 144 μM for microsomes was used as the acyl donor. Values are the mean ± S.E. for five samples.

Previous studies on rabbit lung microsomes showed lack of acyl group specificity in the incorporation of fatty acids into PA (11). Fig. 1(d) confirms this observation in guinea pig lung microsomes at all concentrations of palmityl- and oleyl-CoA. The mitochondrial GAT showed preference toward palmityl-CoA over oleyl-CoA only at higher concentrations of the acyl donors. Moreover, the optimal acyl-CoA concentrations in mitochondria were substantially lower than those found in the microsomes. Analysis of acylation products showed a striking difference in the amounts of mono-(LPA) and diacylated (PA) products formed by the mitochondria and microsomes (results not shown). In the mitochondrial fraction, the mono- and diacylated products were present in equal amounts (LPA 0.85, PA 0.86 nmole/mg/3min) whereas, in the microsomes, the diacylated derivative was predominant (LPA 3.15, PA 10.17 nmole/mg/3min).

To our knowledge no study has been previously undertaken on the subcellular distribution of GAT in fetal lung. Table 1 shows that the GAT activity in both mitochondrial and microsomal fractions was higher in fetal lung than that in adult lung. The ratio of fetal to adult GAT activity was higher in mitochondria (3.91) than in microsomes (1.76). In the fetal lung the mitochondrial and microsomal activity was comparable. However, in adult

lung, the microsomal activity was about 2.5 fold higher than the mitochondrial activity.

This investigation shows that GAT activity is associated with both mitochondrial and microsomal fractions of lung. The mitochondrial preparations had no detectable NADPH-cytochrome c reductase activity but contained highest citrate synthase activity (51.2 $\pm$ 2.7 μmol/min/mg protein; 3 determination). At the same time, the microsomal preparation had highest specific activity of NADPH-cytochrome c reductase (40% of the total) and no detectable citrate synthase activity. The extent of purity of the mitochondrial and microsomal fractions together with the differential response of the fractions to NEM, trypsin, acetone, and the acyldonors (Fig. 1) demonstrate that the GAT activity in the mitochondrial fraction is a genuine mitochondrial property and not due to microsomal contamination. Thus, like liver, GAT has a bimodal distribution in lung.

The overall action of acetone, NEM and trypsin in the lung and liver (15) mitochondrial and microsomal GAT is similar. However, the lung mitochondrial GAT remained unaffected by acetone (Fig. 1c) whereas the liver mitochondrial activity is stimulated in the presence of 0.35-2.8M acetone (15). Also, the lung microsomal GAT is less susceptible to NEM and trypsin compared to that of liver. For example, in liver 80-90% inhibition was achieved at 2mM NEM or 8μM trypsin as opposed to only 50-60% inhibition in lung. Furthermore, unlike liver, the lung mitochondrial GAT showed over 100% stimulation in the presence of trypsin. The strong activation of mitochondrial GAT by trypsin is noteworthy. In liver, mitochondrial GAT has been shown to be located in the inner side of the outer membrane (20,21). Further studies are needed to determine if trypsin has a direct or an indirect effect on lung mitochondrial GAT in causing the observed activation.

Although the microsomal GAT activity was three fold higher than mitochondrial activity in adult lung, the activities of these two subcellular fractions were comparable in fetal lung (Table 1). Also, the fetal lung

mitochondria and microsomes had approximately 4 fold and 2 fold higher activity than the corresponding fractions in adult lung. These results suggest that GAT may plan an important role in the development of lung.

ACKNOWLEDGEMENTS: This work was supported by NIH grant No. RR-08037-10 funded by the Division of Research Resources and the National Heart, Lung and Blood Institutes.

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