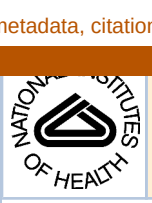



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# *Macrobrachium borellii* Hepatopancreas Contains a Mitochondrial Glycerol-3-Phosphate Acyltransferase Which Initiates Triacylglycerol Biosynthesis

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## Abstract

Mammals express four isoforms of glycerol-3-phosphate acyltransferase (GPAT). The mitochondrial isoform GPAT1 may have been the acyltransferase that appeared first in evolution. The hepatopancreas of the crustacean *Macrobrachium borellii* has a high capacity for triacylglycerol (TAG) biosynthesis and storage. In order to understand the mechanism of glycerolipid biosynthesis in *M. borellii*, we investigated its hepatopancreas GPAT activity. In hepatopancreas mitochondria, we identified a GPAT activity with characteristics similar to those of mammalian GPAT1. The activity was resistant to inactivation by SH-reactive N-ethylmaleimide, it was activated by polymyxin-B, and its preferred substrate was palmitoyl-CoA. The reaction products were similar to those of mammalian GPAT1. A 70-kDa protein band immunoreacted with an anti-rat liver GPAT1 antibody. Surprisingly, we did not detect high GPAT specific activity in hepato-pancreas microsomes. GPAT activity in microsomes was consistent with mitochondrial contamination, and its properties were similar to those of the mitochondrial activity. In microsomes, TAG synthesis was not dependent on the presence of glycerol-3 phosphate as a substrate, and the addition of monoacylglycerol as a substrate increased TAG synthesis 2-fold. We conclude that in *M. borellii* the de novo triacylglycerol biosynthetic pathway can be completed in the mitochondria. In contrast, TAG synthesis in the ER may function via the monoacylglycerol pathway.

### Keywords

Triacylglycerol; Crustacean; Glycerolipid synthesis

## Introduction

The most important organ for crustacean metabolism is the hepatopancreas, which is analogous to the vertebrate liver and insect fat body because it synthesizes and secretes digestive enzymes, absorbs digestive dietary products, stores mineral reserves and organic substances, metabolizes lipids and carbohydrates, and aids in distributing stored reserves during the intermoult cycle and xenobiotic metabolism [1]. The hepatopancreas of *Macrobrachium borellii*, a Decapod Crustacean, is active in triacylglycerol (TAG) biosynthesis. During the winter season the TAG content of hepatopancreas comprises more than 80% of the total lipid mass of the organ [2], and in vivo incubation of *M. borellii* with [$^{14}$C]palmitic acid revealed that the hepatopancreas

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is the principal organ for TAG synthesis [3]. The hepatopancreas mobilizes TAG when the animal is deprived of food or subjected to thermal stress [4,5]. These facts suggest that, in addition to the hepatopancreas' digestive and biosynthetic roles, it functions to synthesize and store TAG, analogous to adipose tissue in vertebrates.

The first steps in the de novo biosynthesis of glycerolipids include the activation of fatty acids by acyl-CoA synthetases and their subsequent esterification by sn-glycerol-3-phosphate acyltransferases (GPAT, EC 2.3.1.15). Historically two types of GPAT have been characterized in mammals [6]: (1) a microsomal form, which comprises 90% of total GPAT activity in most organs, is sensitive to sulfhydryl reagents like *N*-ethylmaleimide (NEM), and lacks long-chain acyl-CoA substrate preference, and (2) a mitochondrial isoform that is resistant to NEM inactivation and prefers to use saturated acyl-CoA. The mitochondrial NEM-resistant isoform (GPAT1) was cloned twenty years ago from mouse and rat liver [7,8]. More recently, a second mitochondrial NEM-sensitive isoform (GPAT2) [9] and two endoplasmic reticulum (ER) isoforms (GPAT3 and GPAT4) were cloned [10–12]. Although the specific roles of each isoform are not yet well understood, several studies [13,14] strongly suggest that the mitochondrial NEM-resistant isoform (GPAT1) initiates the synthesis of TAG in rat liver under conditions of high carbohydrate feeding and caloric excess.

We previously reported that microsomes from *M. borellii* hepatopancreas have a significant acyl-CoA synthetase activity [15] and that the entire synthesis of TAG may occur in the ER of crustacean hepatopancreas [3]. Moreover, a mitochondrial type of acyl-CoA synthetase activity was described in this organ [16]. However, little is known about the acyltransferases that are present in invertebrates. Taking into account the preponderant role of *M. borellii* hepatopancreas in TAG synthesis, we hypothesized that glycerolipid biosynthesis compartmentalization in this crustacean would be similar to that of mammalian liver, so at least two GPAT isoforms would be present in both mitochondria and ER. Our aim was to characterize GPAT activity in these two cellular compartments in order to understand the mechanism of TAG synthesis in an organ intimately connected with versatile aspects of lipid metabolism.

## Experimental Procedures

### Animals

Adult specimens of *M. borellii* were collected in a freshwater stream near Rio de la Plata, Argentina. They were kept in the laboratory in glass aquaria containing dechlorinated tap water at room temperature until they were used for experiments. All animal protocols were approved by the Committee for Care and Use of Laboratory Animals, School of Medicine, University of La Plata.

### Isolation of Subcellular Fractions from Hepatopancreas of *M. borellii*

Approximately 60 animals were used in each isolation experiment. Specimens were kept on ice for 20 min and the hepatopancreas were quickly dissected and submerged in pre-cooled buffer H (10 mM Hepes-KOH, pH 7.4, 0.25 M sucrose, 1 mM EDTA, and 1 mM dithiothreitol (DTT)) with 0.002% v/v protease inhibitor cocktail (general use, Sigma). The total homogenate was prepared with 10 up-and-down strokes in a motor-driven Teflon-glass homogenizing vessel. Large debris and nuclei were removed by centrifuging twice at 600×*g* at 4°C for 5 min, and the supernatant (post-nuclear homogenate) was centrifuged for 15 min at 10,000×*g* at 4° C in a Sorvall refrigerated centrifuge. The resulting pellet was resuspended and recentrifuged twice with ice-cold buffer H to obtain the mitochondrial fraction. Additional purification of the mitochondrial fraction was performed by density-gradient centrifugation as previously described [17]. The combined supernatants were centrifuged for 1 h at 100,000×*g* (Beckman

LE-80K ultracentrifuge, rotor 70.1 Ti) to obtain microsomes. All steps were performed at 4° C and the fractions were aliquoted and frozen at −70°C. The total protein content of each fraction was determined with bovine serum albumin (BSA) as the standard [18].

## GPAT Activity

GPAT activity was assayed in each fraction (100 μg of total protein) [19]. The incubation buffer contained 0.8 mM glycerol-3-phosphate (0.5 μCi [$^{14}$C]glycerol-3-phosphate, Amersham Biosciences), 75 mM Tris–HCl pH 7.4, 4 mM MgCl2, 2 mg/ml BSA, 8 mM NaF, 1 mM DTT, and the indicated concentrations of palmitoyl-CoA or oleoyl-CoA. The reaction was incubated at 30°C for 10 min and total radioactivity from the organic phase was quantified by liquid scintillation counting. In order to determine the effect of *N*-ethylmaleimide (NEM) or polymyxin B on GPAT activity, samples were pre-incubated for 30 min on ice in the presence of varying concentrations of NEM or polymyxin B. Products of the GPAT reaction were analyzed by thin-layer chromatography on silica-gel G plates. The solvent systems used to separate the products were hexane/ethyl ether/acetic acid (80:20:1.5; v/v) and chloroform/methanol/acetic acid/water (65:25:1:4; v/v) for neutral and polar lipids, respectively.

## Marker Enzyme Activities

Arylesterase activity (ER marker) was measured as described previously [20], NADPH cytochrome c reductase (ER marker), and cytochrome c oxidase (inner mitochondrial membrane marker) were determined using cytochrome c oxidase and cytochrome c reductase (NADPH) assay kits (Sigma).

## Immunoblotting

Fifty or one hundred microgram of total protein for each subcellular fraction were separated on an 8% SDS-PAGE, transferred to a polyvinylidene difluoride membrane (Bio-Rad), and probed with a 1/10,000 dilution of anti-GPAT1 antibody in phosphate buffer saline −0.05% Tween-20. Anti-GPAT1 antibody was obtained from rabbits immunized with recombinant rat liver GPAT1 expressed in insect cells and purified from inclusion bodies [21]. The membranes were then washed extensively and probed with horseradish peroxidase-conjugated goat anti-rabbit IgG (Pierce). For chemiluminescent detection, the membranes were incubated with Super Signal detection kit (Pierce).

## Lipid Biosynthesis, Extraction and Separation

[$^{14}$C]palmitate (3.5 nmol; 0.2 μCi) dissolved in 10 μl of propylene glycol was incubated with 300 μg of total microsomal or mitochondrial protein at 30°C for 60 min. The incubation mixture contained 75 mM Tris–HCl pH 7.4, 2 mM $MgCl_2$, 2 mg/ml BSA, 5 mM DTT, 5 mM ATP, and 0.2 mM CoASH (lithium salt) in a final volume of 0.35 ml. The reactions were carried out in either the presence or the absence of 20 mM glycerol-3-phosphate in the incubation mixture. 1-Monostearoyl-*rac*-glycerol dissolved in ethanol was added to the reaction in the absence of glycerol-3-phosphate at a final concentration of 100 μM. Lipids were extracted by the Folch procedure [22] and the radioactive lipid products were separated by thin-layer chromatography on silica-gel G plates using the same solvent systems described above. The radioactivity associated with each spot was quantified using a STORM 840 scanner (Amersham, Biosciences). Appropriate standards, run simultaneously, were visualized by exposure to iodine vapor.

## Results

### Characterization of the Cell Fractions Obtained from *M. borellii* Hepatopancreas

In this study we used total cellular homogenate, mitochondrial and microsomal subcellular fractions from *M. borellii* hepatopancreas. To confirm the purity of these fractions, we tested them for marker enzymes that are widely used in mammals. The purity of the fractions was good (Table 1). Cytochrome c oxidase activity indicated some mitochondrial contamination of the microsomal fraction (near 20% of the total protein). Two different ER marker enzymes were tested: NADPH cytochrome c reductase and arylesterase activities. Both activities were present in the microsomal fraction but not in mitochondria. The mitochondrial fraction (10,000 g pellet) was further purified in a Percoll gradient. This technique is widely used to separate mitochondrial membranes from ER contaminants in lipid-biosynthetic organs like rat liver, and yields highly purified mitochondria and a separate band of ER-derived membranes [23, 24]. When *M. borellii* hepato-pancreas mitochondria were purified by this method, no band corresponding to ER-derived membranes could be visualized (results not shown). These results are consistent with the absence of ER-markers in the 10,000 g mitochondrial pellet.

### GPAT Activity was Primarily Recovered in the Mitochondrial Fraction

GPAT activity was assayed in hepatopancreas total homogenate, and in mitochondrial and microsomal fractions (Table 1, Fig. 1a). Because *M. borellii* GPAT is unstable after long freezing periods, all experiments were performed within two weeks of fractionation. GPAT activity was measured either in fresh and frozen-thawed samples, and no differences were observed in the specific activity values (results not shown). GPAT activity was highly enriched in the mitochondrial fraction (21-fold higher than homogenate) and 47% of total GPAT activity was recovered. In microsomes only 9% of the total GPAT activity was recovered and the enrichment was only 1.4-fold, similar to that of cytochrome c oxidase activity. These results suggest that all the GPAT activity present in microsomes was due to mitochondrial contamination.

### The Main Product of GPAT Activity in Mitochondria was Phosphatidic Acid

Radioactive lipids were extracted from mitochondrial GPAT enzymatic assays and analyzed by thin-layer chromatography (Fig. 1b). The distribution of radioactivity associated with the glycerolipids synthesized from [$^{14}$C]glycerol-3-phosphate was consistent with the products of the mammalian GPAT1 reaction [20]. In the mitochondrial fraction, the major product was phosphatidic acid (56%). The presence of diacylglycerols (11%) and TAG (32%) suggests that the enzymatic activities acyl-glycerol-3-phosphate acyltransferase (AGPAT), phosphatidic acid homogenate phosphohydrolase (PAPase), and diacylglycerol acyltransferase (DGAT) are also present in mitochondria. The amount of radioactivity obtained from GPAT assays in microsomes was not sufficient to analyze the reaction products.

### Mitochondrial GPAT Preferred Saturated Fatty Acyl-CoA

The substrate specificity of GPAT was determined using palmitoyl-CoA and oleoyl-CoA as acyl-CoA donors. In the mitochondrial fraction, palmitoyl-CoA was the preferred substrate (Fig. 2a). The Vmax value calculated with oleoyl-CoA as a substrate was 4-fold lower than the value obtained when the substrate was palmitoyl-CoA. Since higher concentrations of palmitoyl-CoA inhibited the GPAT activity, it was not feasible to determine a $K_m$ value for this substrate. In contrast, oleoyl-CoA did not inhibit GPAT activity at a concentration as high as 120 μM and the apparent $K_m$ value for this substrate, calculated by Lineweaver-Burk transformation, was 31.2 μM. The same experiments were performed using microsomes and identical substrate specificities were obtained, although the specific activities were 80% lower (Fig. 2b).

### Mitochondrial GPAT Activity was NEM Resistant and Activated by Polymyxin B

Mitochondrial GPAT activity was resistant to inhibition by NEM at concentrations as high as 10 mM (Fig. 3). In contrast, preincubation of the mitochondrial fraction with the antibiotic polymyxin B markedly stimulated GPAT activity; at 0.5 mg/ml polymyxin B GPAT activity increased 2.7-fold (Fig. 3). The microsomal GPAT activity was also resistant to NEM-inactivation (results not shown).

### Mitochondria from *M. borellii* Hepatopancreas Contained an Anti-GPAT1 Immunoreactive Protein

The polyclonal anti-GPAT1 antibody raised against rat-liver GPAT1 recognized an immunoreactive protein with a molecular mass of 70 kDa in the mitochondrial fraction from hepatopancreas (Fig. 4). No immunoreactive protein was detected when 50 μg of total protein from microsomes were loaded on the gel, but a weak band was detected with 100 μg. Consistent with the low GPAT specific activity found in microsomes, this band might be the result of mitochondrial contamination. The presence of this immunoreactive band suggests that hepatopancreas mitochondria contain a protein homologous to GPAT1, consistent with NEM-resistant GPAT activity. The molecular weight of this protein was lower than that of rat liver GPAT1 (90 kDa). However, ER-GPAT 3 and 4 both have molecular weights of approximately 40 kDa [10,12], indicating that smaller GPAT proteins are also active.

### Microsomes from *M. borellii* Hepatopancreas Esterified [$^{14}$C]Palmitate in the Absence of Glycerol-3-Phosphate

We had previously reported that microsomes from *M. borellii* hepatopancreas actively synthesized TAG [3]. To investigate the TAG synthesis pathway, experiments were performed under previously optimized experimental conditions in either the presence or absence of glycerol-3-phosphate and monoacylglycerol. When glycerol-3-phosphate (20 mM) was added to the reaction mixture, 54% of the [$^{14}$C]palmitate was esterified (104 pmol of esterified palmitate/min mg protein) (Table 2 and Fig. 5). Radioactivity was incorporated primarily into neutral glycerolipids (83.8%) and to a lesser extent into phospholipids (14%). The major neutral lipid product was TAG (about 65%). When glycerol-3-phosphate was omitted, 40% of the [$^{14}$C]palmitate was esterified, (78 pmol of esterified palmitate/min mg protein) and the distribution of the radioactivity among lipid classes was similar; under this condition adding 100 μM monostearoylglycerol increased TAG synthesis nearly 2-fold (Fig. 6).

In mitochondria, TAG synthesis required glycerol-3-phosphate as a substrate. [$^{14}$C]palmitate esterification into lipids was higher than in microsomes (74%, 144 pmol of esterified palmitate/min mg protein) (Table 2 and Fig. 5) and 70% of the radioactive palmitate was detected in the TAG fraction.

## Discussion

In crustaceans, the hepatopancreas carries out essential functions of energy metabolism. We reported that the hepatopancreas of the fresh-water shrimp *M. borellii* is the major lipid-biosynthetic organ, and that the microsomal fraction synthesizes TAG [3]. Thus, we focused our studies on the enzyme that catalyzes the first and committed step in glycerolipid synthesis, GPAT, hypothesizing that, as in mammalian cells, more than one isoform might be present in different subcellular compartments to exert a specific metabolic function.

We measured GPAT activity following a method widely used in mammalian tissues [17]. Under these conditions, GPAT activity can be detected in microsomes and mitochondria from rat liver. GPAT activities can be distinguished by their varying sensitivities to sulfhydryl reagents like NEM. In liver the NEM-sensitive activity comprises 50–60% of total GPAT

activity whereas in most other organs it comprises about 90% of total GPAT activity [6]. In rat liver, the NEM-sensitive activity is attributed to microsomal GPAT3 and GPAT4 because expression of the mitochondrial NEM-sensitive isoform GPAT2 is very low [9].

Surprisingly, since microsomal fractions synthesize TAG [3], GPAT activity was recovered primarily in the mitochondrial fraction. Kinetic properties of the *M. borellii* mitochondrial activity were similar to those of rat and mouse GPAT1 [25]. The activity was NEM-resistant, it showed a preference for saturated-acyl-CoA substrates, and like mammalian GPAT1, it was activated by polymyxin B [26], an antibiotic that interferes with the glycerolipid packing in both bacteria and synthetic membranes. Activation of GPAT by polymyxin B was more striking in shrimp hepatopancreas than in rat liver mitochondria, suggesting that in the crustacean, membrane fluidity plays an important role in regulating GPAT activity. Finally, an anti-GPAT1 polyclonal antibody, raised against full-length recombinant rat GPAT1, recognized a single 70 kDa protein present in the mitochondrial fraction. These data suggest that GPAT1 from rat-liver mitochondria and the mitochondrial GPAT from *M. borellii* hepatopancreas may be homologous. Cloning and sequencing the *M. borellii* GPAT gene will be required to fully address this issue.

When hepatopancreas mitochondria were incubated with [$^{14}$C]glycerol-3-phosphate under the conditions optimized to measure GPAT activity, the major reaction product was phosphatidic acid, as observed in membranes from rat liver that contain both GPAT and acyl-glycerol-3-phosphate acyltransferase. An additional 43% of the radiolabel was found in diacylglycerol and TAG. Under different conditions that were optimized for TAG synthesis, and which depended on the activities of several enzymes, the major reaction product was TAG (74%), indicating that mitochondria can perform the full pathway of de novo TAG synthesis.

Our results are consistent with the interpretation that the GPAT activity detected in microsomes is due to mitochondrial contamination. First, enrichment of GPAT activity in the microsomal fraction was equivalent to that of the mitochondrial marker enzyme cytochrome c oxidase. Second, the GPAT activity in microsomes had the same kinetic parameters and substrate preference as the mitochondrial activity. Finally, only a very faint band reactive to the anti-GPAT1 antibody was detected when 100 μg of microsomal protein was analyzed by western blot. Nevertheless, the possibility remains open that an independent microsomal GPAT isoform exists, that this activity has different kinetic parameters, and that the activity is not detectable under our experimental conditions.

The low GPAT activity in the microsomal fraction was unexpected because we had previously reported that the microsomal fraction incorporates [$^{14}$C]palmitate into TAG as the major product [3]. In that study, assays were performed in the presence of glycerol-3-phosphate, and we had assumed that TAG was synthesized de novo via the glycerol-3-phosphate pathway. In the present study, we showed that in the absence of glycerol-3-phosphate, microsomes retain 75% of their capacity to esterify [$^{14}$C]palmitate into glycerolipids. We attribute the difference in microsomal specific activity values for palmitate esterification in the presence and absence of high concentrations of glycerol-3-phosphate (104 and 78 pmol of esterified palmitic acid/min mg, respectively) to mitochondrial GPAT activity that is contaminating the microsomal fraction (about 20%).

Taking into account the absorptive function of the hepatopancreas and the TAG-lipase activity that has been reported in hepatopancreas cells from *M. borellii* [16] and other crustaceans [1], we considered the possibility that an alternative substrate, different from glycerol-3-phosphate, might account for the high rate of TAG synthesis observed in the microsomal fraction. Replacing glycerol-3-phosphate with 100 μM monoacylglycerol caused a 2-fold increase in

TAG synthesis, suggesting that in microsomes, TAG synthesis occurs mainly via the monoacylglycerol pathway.

The initial step in glycerolipid synthesis in bacteria is catalyzed by a single gene product, *plsB* (GPAT) in Gram-negative bacteria and by *plsY* (acyl-ACP:phosphate transacylase) in Gram-positive bacteria. In contrast, both lower and higher eukaryotes express more than one GPAT isoform and at least one of the isoforms is located in the ER. Information about TAG synthesis in invertebrates is scarce. Only a single report from more than three decades ago, described the incorporation of [$^{14}$C]glycerol-3-phosphate into the lipids of the insect *Ceratitis capitata* and demonstrated that TAG could be synthesized in mitochondria, although a higher rate of TAG synthesis was observed in microsomes [27].

We conclude here that de novo synthesis of TAG is initiated in mitochondria, whereas in microsomes TAG synthesis occurs by an alternate pathway, probably the monoacylglycerol pathway. Although the *M. borellii* GPAT gene has not yet been cloned, its properties suggest that it could be homologous to *PlsB* and to mitochondrial GPAT1, members of a highly conserved acyltransferase family.

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## Abbreviations

| | |
|---|---|
| BSA | Bovine serum albumin |
| DGAT | Diacylglycerol acyltransferase |
| DTT | Dithiothreitol |
| EDTA | Ethylenediaminetetracetic acid |
| ER | Endoplasmic reticulum |
| GPAT | Glycerol-3-phosphate acyltransferase |
| NEM | *N*-ethylmaleimide |
| TAG | Triacylglycerols |

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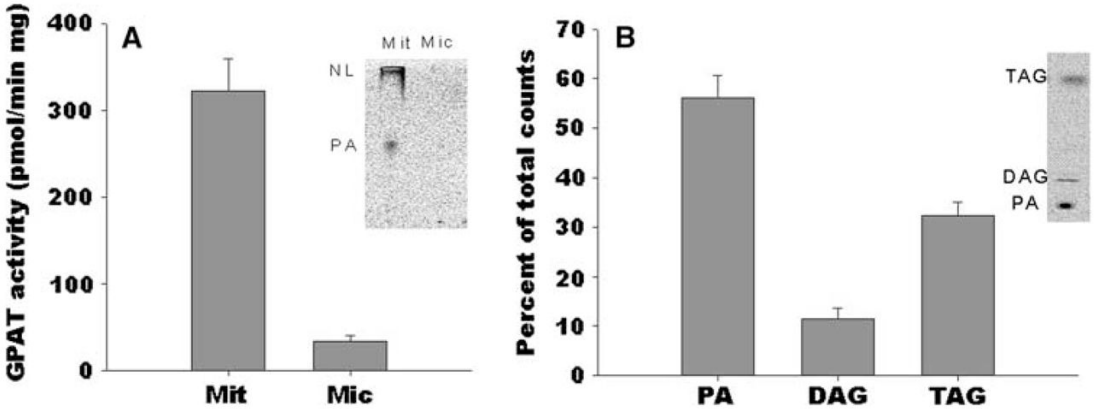


**Fig. 1.**

**a** GPAT specific activity in mitochondrial (Mit) and microsomal (Mic) fractions from *M. borellii* hepatopancreas. Results are the means ± S.E from three independent experiments. **b** Lipid classes distribution of [$^{14}$C]glycerol-3-phosphate esterified by mitochondria. Results are representative of two independent experiments. The *inset* show the products of GPAT reaction resolved in TLC plates for polar (**a**) and neutral (**b**) lipids: PA phosphatidic acid; *NL* neutral lipids; *DAG* diacylglycerol; *TAG* triacylglycerol

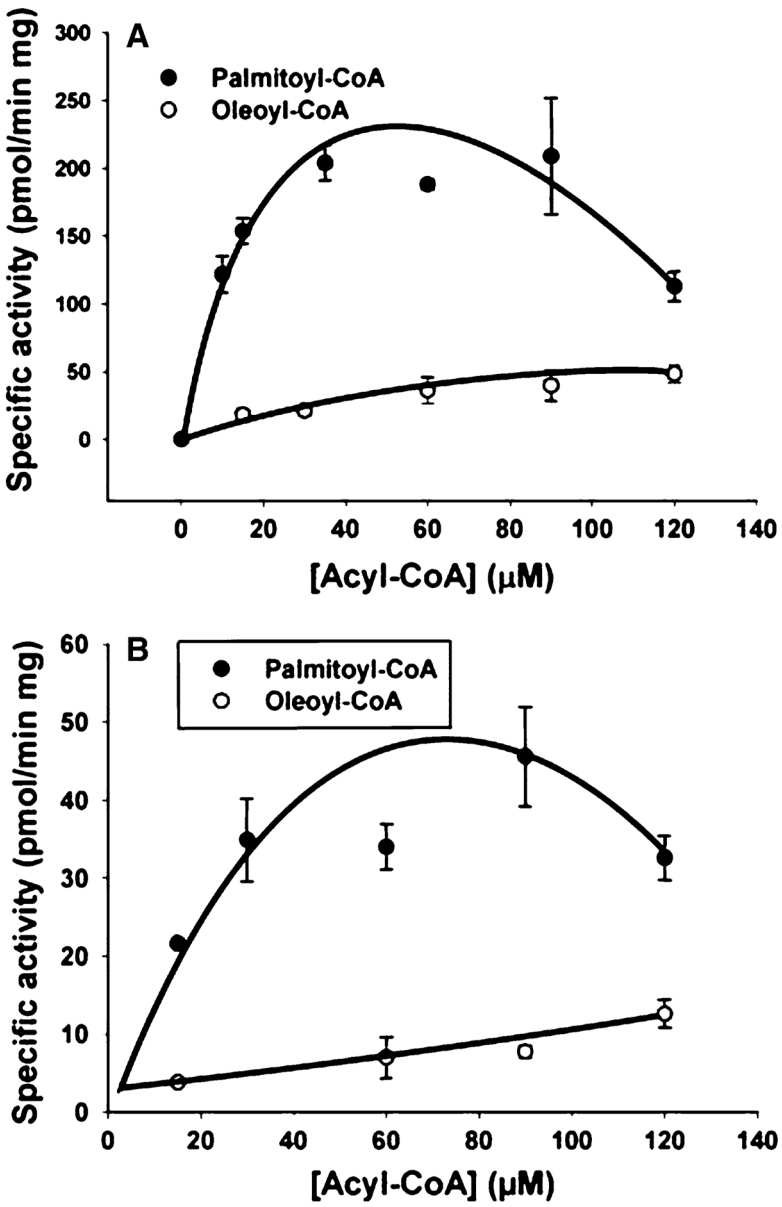


**Fig. 2.**

Acyl-CoA substrate specificity of GPAT reaction in mitochondria (**a**) and microsomes (**b**). All assays contained 4 mM $MgCl_2$, 2 mg/ml BSA, 8 mM NaF, 1 mM DTT, 0.8 mM glycerol-3-phosphate (0.5 μCi [$^{14}$C]glycerol-3-phosphate per reaction) and the indicated concentrations of palmitoyl-CoA (*filled circle*) or oleoyl-CoA (*open circle*). Data points represent the means ± SE of an experiment performed in duplicate and are representative of two independent experiments

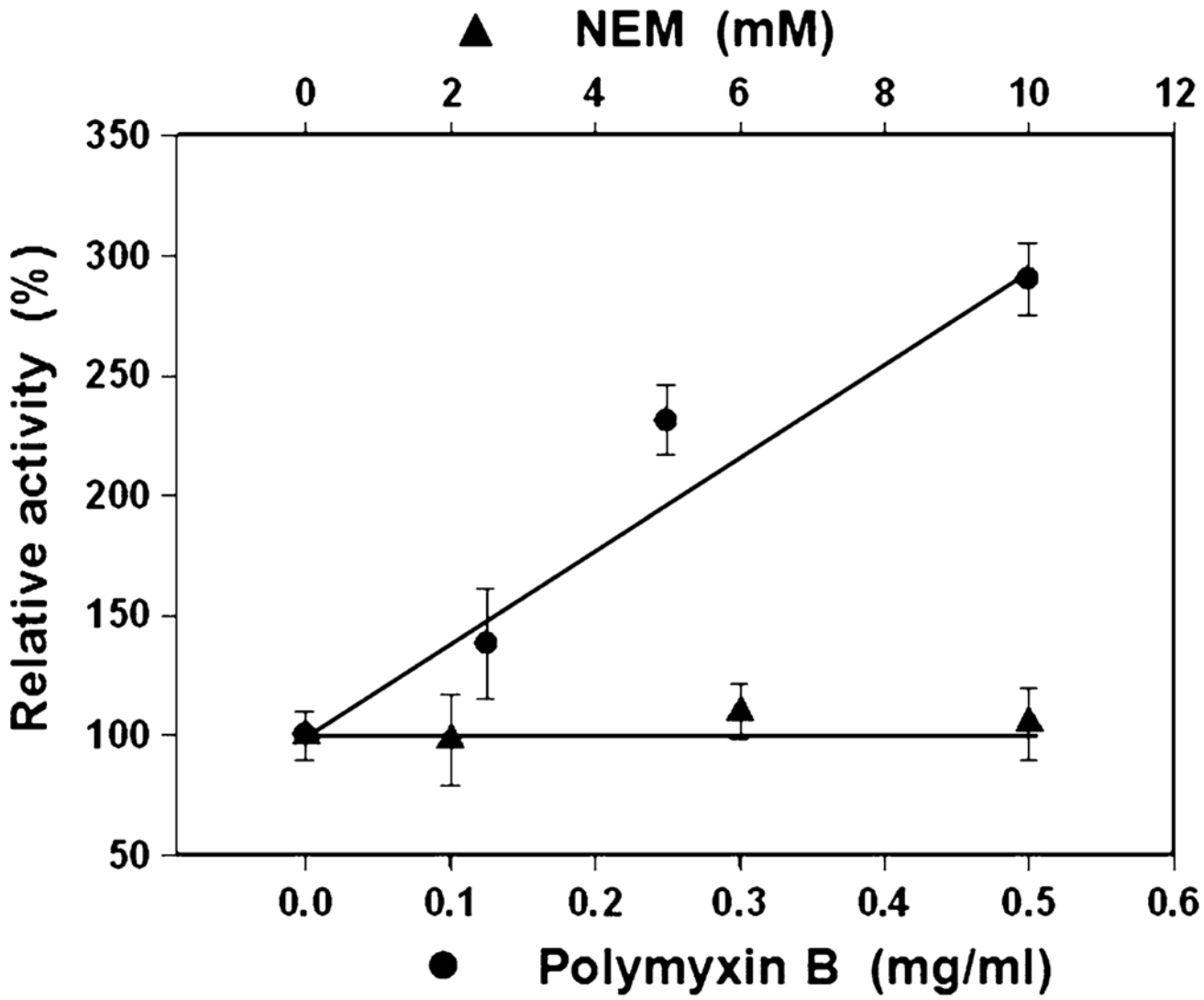


**Fig. 3.**

Effect of NEM and polymyxin B on GPAT activity. Mitochondrial samples were pre-incubated for 30 minutes on ice in the presence of the indicated concentration of NEM (*filled triangle*) or polymyxin B (*filled circle*) in the incubation mixture. The results represent the means ± SD from three independent experiments. The baseline specific activity was 322 ± 38 pmol/min mg protein

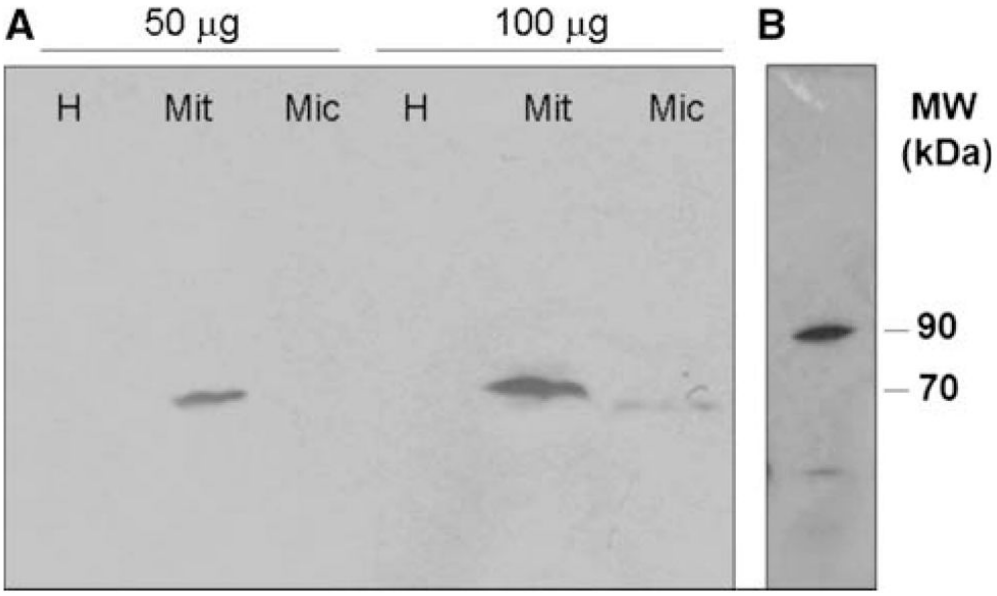


**Fig. 4.**

Mitochondria from *M. borellii* contain an immunoreactive protein against GPAT1 antibody. **a** 50 μg or 100 μg of total protein from total homogenate (*H*), mitochondria (*Mit*), and microsomes (*Mic*) were loaded on SDS-PAGE 8%, transferred to PVDF membranes, probed with a polyclonal anti-rat liver GPAT1 antibody and visualized by ECL. The immunoreactive band molecular mass is 70 kDa. **b** 50 μg of rat-liver mitochondria were probed with the same antibody. The immunoreactive band molecular mass is 90 kDa

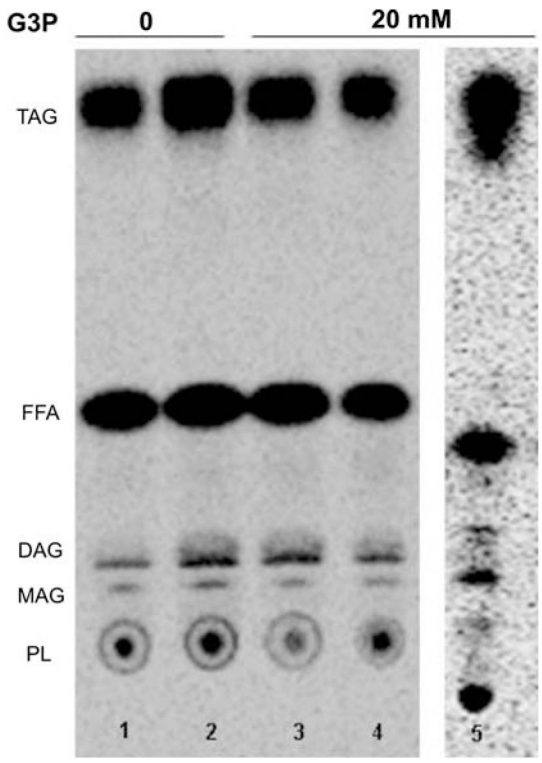


**Fig. 5.**

Microsomal and mitochondrial glycerolipid biosynthesis from [$^{14}$C]-palmitate. About 300 μg of hepatopancreas microsomal (lanes 1–4) or mitochondrial (lane 5) protein was incubated using [$^{14}$C]palmitate (0.2 μCi) as substrate either in the presence or in the absence of 20 mM glycerol-3-phosphate (G3P). Reaction products were analyzed by TLC and the radioactivity quantified by scanning proportional counting. *TAG* triacylglycerol, *FFA* free fatty acids, *DAG* diacylglycerol, *MAG* monoacylglycerol, *PL* phospholipids

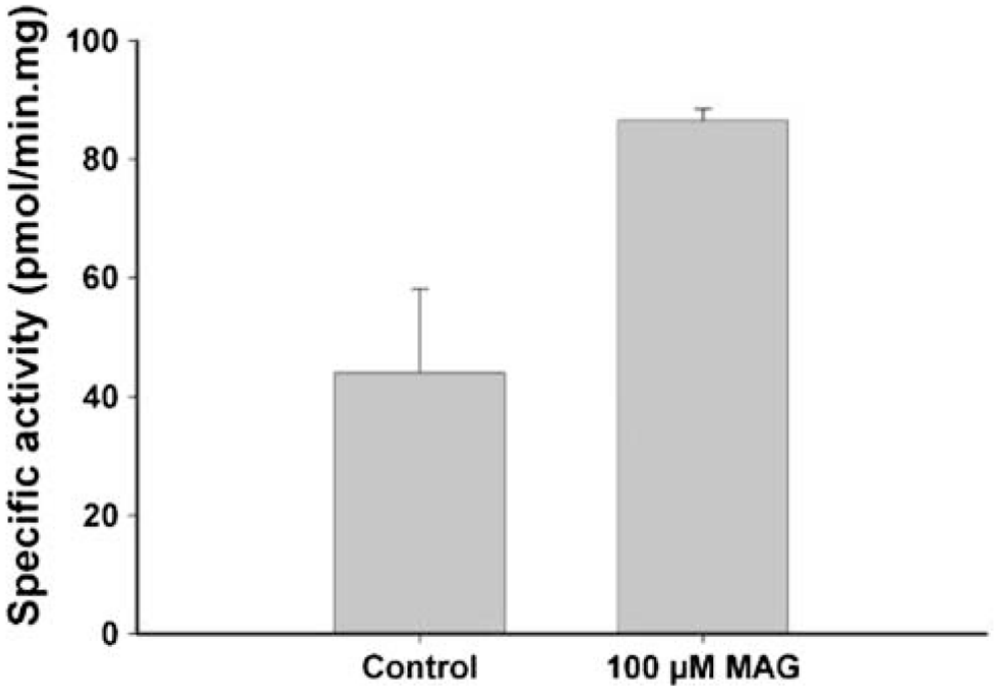


**Fig. 6.**

Microsomal triacylglycerol biosynthesis is increased in the presence of monostearoylglycerol. About 300 μg of hepatopancreas microsomal protein was incubated using [$^{14}$C]palmitate (0.2 μCi) as substrate without any external source of glycerol (Control) and 100 μM monostearoylglycerol (MAG). Reaction products were analyzed by TLC and the radioactivity present in the TAG fraction quantified by scanning proportional counting. Specific activity values correspond to pmoles of synthesized triacylglycerol/min. mg of microsomal protein. Values represent the means ± S.E of two independent experiments

**Table 1**

GPAT activity was enriched in the mitochondrial fraction of *M. borellii* hepatopancreas

| | Cytochrome c oxidase | | | | GPAT | | | | Arylesterase | | Cyt c Red | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Specific activity (mU/mg) | Total activity (U) | Fold enrichment | Recovery (%) | Specific activity (pmol/ mg min) | Total activity (pmol/min) | Fold enrichment | Recovery (%) | Specific activity(μmol/mg min) | Fold enrichment | Specific activity (mU/mg) | Fold enrichment |
| Total Homogenate | 8.5 ± 1.9 | 2.07 | – | | 23 ± 7 | 5,377 | – | | 148 ± 16 | – | 3.6 ± 0.6 | – |
| Mitochondria | 181 ± 14 | 1.43 | 21.3 | 69 | 322 ± 38 | 2,524 | 14 | 47 | – | – | – | – |
| Microsomes | 11.9 ± 1.0 | 0.44 | 1.4 | 21.2 | 34 ± 7 | 515 | 1.5 | 9.6 | 519 ± 32 | 3.5 | 10.9 ± 0.7 | 3.0 |

GPAT specific activity was measured in total homogenate and in mitochondrial and microsomal fractions from *M. borellii* hepatopancreas. The purity of the fractions was monitored assaying the marker enzymes cytochrome c oxidase (mitochondria), NADPH cytochrome c reductase (Cyt c Red) and arylesterase (endoplasmic reticulum). Results are expressed as the means ± SD of three independent experiments. Fold enrichment was calculated with respect to total homogenate

## Table 2

Microsomal and mitochondrial esterification of [$^{14}$C]palmitate

| | Microsomes | | Mitochondria |
|---|---|---|---|
| G3P addition | 0 | 20 mM | 20 mM |
| Specific activity (pmol/min mg)[a] | 78 ± 4 | 104 ± 14 | 144.3 ± 10 |
| Neutral lipids (%) | 81.1 ± 7.3 | 83.8 ± 2.7 | 80.0 ± 8.2 |
| Triacylglycerols (%) | 56.6 ± 18.2 | 66.5 ± 11.3 | 70.7 ± 2 |
| Phospholipids | 18.4 ± 7.4 | 13.9 ± 4.2 | 17.8 ± 1.4 |

Esterification of [$^{14}$C]palmitate by microsomes and mitochondria from *M. borellii* hepatopancreas was measured either in the presence or absence of 20 mM glycerol-3-phosphate in the incubation mixture. Lipids were separated by TLC and the radioactivity quantified. Neutral glycerolipids include triacylglycerol, diacylglycerol, and monoacylglycerol. Triacylglycerol values are distinguished

[a]Specific activity indicates the pmol of [$^{14}$C]palmitate esterified into lipids/min. mg protein