Acylation of 1-alkenyl-glycerophosphocholine and 1-acyl-glycerophosphocholine in guinea pig heart

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The deacylation–reacylation process has been shown to be an important pathway for phospholipids to attain the desired acyl groups at the C-2 position. The acylation of 1-acyl-glycerophosphocholine (-GPC) in mammalian hearts has been well documented, but the acylation of 1-alkenyl-GPC has not been described. In this paper, we demonstrate the presence of acyl-CoA:1-alkenyl-GPC acyltransferase for the acylation of 1-alkenyl-GPC in mammalian hearts; the highest activity is found in guinea pig heart. The guinea pig heart 1-alkenyl-GPC acyltransferase has only 10–40% of the 1-acyl-GPC acyltransferase activity, and both activities are located in the microsomal fraction. However, these two enzymes respond differently to cations, detergents and heat treatment, and the two enzymes also display different acyl specificity. Kinetic studies indicate that both reactions could not be accommodated by the same catalytic site. The results provide strong evidence that the two activities are from separate and distinct proteins. The specificity of 1-alkenyl-GPC acyltransferase for unsaturated species of acyl-CoA may play an important role in the maintenance of the high degree of unsaturated acyl groups found in guinea pig heart plasmalogens.

INTRODUCTION

In the preceding paper we reported the absence of plasmalogenase activity towards plasmenylcholine in the guinea pig heart [1]. We also demonstrated that the major catabolic route of plasmenylcholine is initiated by the hydrolysis of the C-2 fatty acid to produce lysoplasmenylcholine [1]. Lysoplasmenylcholine, like other lysophospholipids, is cytolytic and has to be actively metabolized to prevent its intracellular accumulation [2–5]. The hydrolysis of the vinyl ether group of lysoplasmenylcholine is catalysed by lysoplasmalogenase, which has been found to be very active in the guinea pig heart microsomal and mitochondrial fractions [1]. Another means of control is the reacylation of the lysophospholipid back to the parent phospholipid. This mechanism is thought to play an important role in achieving the desired C-2 acyl composition of phospholipids [6,7].

The ability to acylate choline lysophosphoglycerides containing 1-acyl groups [8,9], 1-*O*-alkyl groups [10] and 1-*O*-alkenyl groups [9] has been detected in a number of tissues and cells, but it was not known if these different classes of choline lysophosphoglycerides were acylated by the same or separate acyltransferases. The acylation of 1-acyl-GPC has been shown to be present in most mammalian tissues [11]; however, the acylation of 1-alkenyl-GPC may be limited to specific mammalian organs. Although enzyme activity for the acylation of 1-alkenyl-GPC was reported for rabbit skeletal muscle, this activity was not observed in rabbit brain and heart [9]. In view of the high plasmenylcholine content in rabbit heart compared with skeletal muscle [12], the absence of 1-alkenyl-GPC:acyl-CoA acyltransferase would require alternative metabolic routes in cardiac tissue to achieve

the desired fatty acid pattern of the phospholipid. As a prerequisite to studying any such alternative routes, we re-examined the presence of acyl-CoA:1-alkenyl-GPC acyltransferase activity in mammalian hearts. We found the activity to be present in all the mammalian hearts tested. The highest enzyme activity was present in guinea pig heart, whereas the lowest (but detectable) enzyme activity was found in rabbit heart. Our study also provides strong evidence that the acyl CoA:1-alkenyl-GPC acyltransferase and acyl CoA:1-acyl-GPC acyltransferase in guinea pig heart are separate and distinct enzymes.

MATERIALS AND METHODS

Materials

Palmitoyl-, stearoyl-, oleoyl-, linoleoyl- and arachidoyl-CoA and choline chloride were obtained from Sigma Chemical Co. All detergents except Chaps (Pierce Chemical Co.), Miranol H2M (Miranol Chemical Co.) and Zwittergent TM 314 (Calbiochem), were also obtained from Sigma. Arachidonyl chloride was the product of NuChek Prep, Elysian, MN, U.S.A. Santoquin and 5,5'-dithiobis-(2-nitrobenzoic acid) were purchased from ICN Pharmaceuticals. CoA was obtained from Pharmacia. [1-¹⁴C]Palmitoyl-glycerophosphocholine, [1-¹⁴C]oleoyl-CoA, [1-¹⁴C]stearoyl CoA and [1-¹⁴C]palmitoyl-CoA were obtained from Amersham International. [methyl-³H]Choline chloride was purchased from New England Nuclear. Lysophosphatidylcholine and phosphatidylcholine (pig liver) were purchased from Serdary Laboratories. Other chemicals and solvents were obtained as described previously [1].

Abbreviation used: GPC, *sn*-glycero-3-phosphocholine; Chaps, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonic acid

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Preparation of 1-alkenyl-glycerophospho[methyl-³H]-choline

Guinea pig hearts were isolated and perfused with Krebs-Henseleit buffer containing 10 μ Ci of [methyl-³H]choline (2 mCi/ml) for 2 h in the Langerdorff mode as described by Zelenski *et al.* [13]. Coronary flow rate was maintained at 8 ml/min. After the perfusion, lipids were extracted from the heart and lysoplasmenylcholine was obtained as described previously [1].

Preparation of acyl-CoA

Arachidonyl-CoA was synthesized chemically from arachidonyl chloride and CoA according to the procedure of Reitz & Lands [14]. The arachidonyl-CoA synthesized was found to be 100% reactive as assessed by the procedure of Lands *et al.* [15].

Acyl-CoA:1-alkenyl-GPC acyltransferase assay

The assay mixture (0.7 ml) contained 75 mM-Tris/succinate, pH 6.5, 100 nmol of 1-alkenyl-GPC, 46 nmol of acyl-CoA and the appropriate amount of protein from the enzyme source. Labelled 1-alkenyl-GPC or labelled oleoyl-CoA was used for the assay. The reactants were mixed and the reaction was initiated by the addition of the subcellular fraction. Incubation was for 30 min at 25 °C in a shaking water bath. The reaction was stopped by the addition of 6 ml of chloroform/methanol (2:1, v/v). This was followed by the addition of 2.3 ml of water to form a biphasic mixture. The upper phase was removed and the lower phase was washed with 2 ml of methanol/water (1:1, v/v). An aliquot of the lower phase was removed and the solvent was evaporated under N₂. The residue was analysed by t.l.c. with a solvent system of chloroform/methanol/water/acetic acid (35:15:2:1, by vol.). The choline phosphoglyceride was visualized by exposure to I₂ vapour and the radioactivity associated with this band was determined by scintillation counting. Enzyme activity was also determined without the addition of 1-alkenyl-GPC, which served as the control. In some experiments, the product formed from the reaction was further evaluated in the following manner.

Procedure A. The choline phosphoglyceride fraction was visualized with 0.2% dichlorofluorescein in ethanol, and the phospholipid was eluted from the silica gel and washed according to the method of Arvidson [16]. The choline phosphoglycerides were subjected to mild acid hydrolysis [17] in order to cleave the 1-alkenyl bond. The resultant 2-acyl-GPC was separated from the remaining diacyl-GPC by t.l.c. with the solvent system described above. The amount of radioactivity associated with the 21-acyl-GPC was determined.

Procedure B. The choline phosphoglyceride fraction was visualized, and subjected to mild alkaline treatment [17] for the hydrolysis of all acyl groups. The resultant fatty acids and choline lysophosphoglycerides were separated by t.l.c. and radioactivity in these fractions were determined.

Acyl-CoA:1-acyl-GPC acyltransferase assay

The assay mixture consisted of 75 mM-Tris/HCl, pH 8.5, 100 nmol of 1-acyl-GPC (pig liver) and 86 nmol of acyl-CoA in a total volume of 0.7 ml. Labelled 1-acyl-GPC or labelled oleoyl-CoA was used in the assay. All other conditions of the reaction and subsequent

analysis of the products were similar to those described for the 1-alkenyl-GPC acyltransferase assay. The choline phosphoglyceride fraction was separated by t.l.c. and the enzyme activity was calculated from the radioactivity associated with this fraction. Enzyme assays without 1-acyl-GPC were used as controls. All other experimental procedures are described in the preceding paper [1].

RESULTS

The plasmenylcholine content of mammalian hearts has been shown to be species-specific. In guinea pig heart, plasmenylcholine formed 34% of the choline phosphoglyceride fraction. For comparative purposes, the plasmenylcholine content in several mammalian hearts were determined and the results are shown in Table 1.

In an attempt to demonstrate the presence of acyl CoA:1-alkenyl-GPC acyltransferase in guinea pig heart, subcellular fractions of the heart were incubated with 1-alkenyl-GPC and labelled oleoyl-CoA at 25 °C for 30 min, and the radioactivity associated with the choline phosphoglyceride fraction after the incubation was determined. At least 90% of total enzyme activity was located in the microsomal fraction, and about 9% in the mitochondrial fraction (Table 2). Since the mitochondrial fraction contained 8–9% of microsomal enzymes (see [1]), the enzyme activities in the mitochondrial fraction can be attributed to microsomal contamination. For comparative purposes, the subcellular distribution of acyl CoA:1-acyl-GPC acyltransferase in guinea pig heart subcellular fractions was determined and the results are also tabulated in Table 2.

To demonstrate that the product of the reaction was 1-alkenyl-2-oleoyl-GPC, the choline phosphoglyceride fraction was eluted and subjected to mild acid treatment to hydrolyse the alkenyl bond. The hydrolytic products were separated by t.l.c. and the radioactivity in the choline phosphoglyceride and lysophosphoglyceride fractions were determined. Over 95% of the radioactivity was found associated with the lysophosphoglyceride fraction. In another set of experiments, the choline phosphoglyceride fraction was eluted and subjected to mild alkaline treatment to hydrolyse the acyl bond. This resulted in the complete loss of radioactivity in both choline phosphoglyceride and lysophosphoglyceride fractions. All radioactivity was recovered in the neutral lipid fraction. When microsomes were omitted from the assay there was no incorporation of radioactivity into the choline phosphoglyceride fraction. These results show quite conclusively that acyl CoA:1-alkenyl-GPC acyltransferase was present in guinea pig heart microsomes. The ability of other mammalian heart microsomal fractions to acylate 1-alkenyl-GPC was investigated. As depicted in Table 1, the highest activity of acyl CoA:1-alkenyl-GPC acyltransferase was found in the guinea pig heart. Of all the mammalian hearts used in this study, the rabbit heart showed the lowest enzyme activity. The specific activity of rabbit heart enzyme was less than 20% of that found in the guinea pig heart.

The optimal conditions for the assay of the 1-alkenyl-GPC acyltransferase activity were determined. The rate of the reaction was linear with up to 750 μ g of microsomal protein. At 37 °C, linearity of the reaction was maintained only for a maximum of 4 min, regardless of substrate concentration used. However at 25 °C the reaction was linear for at least 40 min. Hence, the enzyme

Table 1. 1-Alkenyl-2-acyl-GPC content and acyl-CoA:1-alkenyl-GPC acyltransferase activity in mammalian hearts

1-Alkenyl-2-acyl-GPC (choline plasmalogen) content in the heart was determined and is expressed as a percentage of total choline phosphoglyceride. Acyl-CoA:1-alkenyl-GPC acyltransferase activity was assayed in the tissue homogenate. The results are expressed as means $\pm$ S.D. of the values obtained from four individual sets of experiments, each of which was determined in duplicate.

Heart	1-Alkenyl-2-acyl-GPC content (% of choline phosphoglyceride)	Acyl-CoA: 1-alkenyl-GPC acyltransferase (nmol/30 min per mg of protein)
Guinea pig	34.2 $\pm$ 1.0	56.0 $\pm$ 3.5 (100%)
Pig	34.7 $\pm$ 5.0	39.0 $\pm$ 2.4 (70%)
Dog	43.6 $\pm$ 1.4	34.5 $\pm$ 2.1 (62%)
Hamster	2.2 $\pm$ 1.0	21.6 $\pm$ 1.9 (36%)
Rabbit	38.7 $\pm$ 2.5	11.8 $\pm$ 1.2 (20%)

Table 2. Subcellular distribution of acyl CoA:1-alkenyl-GPC acyltransferase and acyl-CoA:1-acyl-GPC acyltransferase activities in guinea pig heart subcellular fractions

1-Alkenyl-GPC acyltransferase and 1-acyl-GPC acyltransferase activities were determined in the subcellular fractions of the guinea pig heart. The results are means $\pm$ S.D. of the values obtained from two individual sets of experiment, each of which was determined in duplicate. Enzyme activities are expressed as nmol of product formed/30 min per mg of protein.

Subcellular fraction	1-Alkenyl-GPC acyltransferase	1-Acyl-GPC acyltransferase
Mitochondria	5.8 $\pm$ 1.3	93.4 $\pm$ 8
Microsomes	56.0 $\pm$ 5.0	893.0 $\pm$ 25
Cytosol	1.1 $\pm$ 0.5	14.4 $\pm$ 3

in the guinea pig microsomes was assayed at 25 °C for 30 min unless otherwise stated. The enzyme exhibited a broad pH profile for activity, with an optimum around pH 6.5. Maximum enzyme velocity was obtained with a 1-alkenyl-GPC concentration of 100 nmol and oleoyl-CoA concentration of 30 nmol (Fig. 1). Higher concentrations of 1-alkenyl-GPC had no effect on enzyme activity, but oleoyl-CoA concentrations above 30 nmol appeared to inhibit the activity. A similar effect of oleoyl-CoA on the 1-alkyl-GPC:acyl-CoA transferase activity from rat brain microsomes has been reported [18].

The acyl-CoA specificity of the enzyme was determined under the optimal conditions reported above. Labelled acyl-CoA or labelled 1-alkenyl-GPC were used as substrates in the enzyme assay. No difference in enzyme activity was observed between these two labelled substrates. After the reaction, the choline phosphoglycerides formed were subjected to acid hydrolysis as described above and the radioactivity in choline lysophosphoglyceride was measured. The effect of increasing concentrations of different acyl-CoA species on the rate of acylation of labelled 1-alkenyl-GPC is shown in Fig. 1. The enzyme showed very little activity for the saturated acyl-CoA species as compared with the unsaturated ones. All acyl-CoA species used for this study were found to be inhibitory at high concentrations.

1-Acyl-GPC acyltransferase has been reported to be

Fig. 1. Acyl-CoA specificity of 1-alkenyl-GPC acyltransferase

The activities of acyl CoA:1-alkenyl-GPC acyltransferase in the presence of various concentrations of acyl-CoA were determined. The assay mixture (0.7 ml) contained 100 nmol of 1-alkenyl-glycerophospho[methyl-³H]choline (choline lysoplasmalogen) and 0.08 mg of microsomal protein, and the enzyme was assayed at pH 6.5 for 30 min at 25 °C. The acyl-CoA used in the assays were palmitoyl-CoA (▲), stearoyl-CoA (■), arachidonyl-CoA (●), linoleoyl-CoA (□), oleoyl-CoA (○) and arachidonyl-CoA (△). Each point in the Figure is the mean of two separate sets of experiment, each of which was determined in duplicate. The S.D. for each point is less than 5% of the mean value.

very active in mammalian heart [19,20]. However, the subcellular distribution, optimal assay conditions and acyl specificity of this enzyme have not been established in the guinea pig heart. Hence, these parameters of the enzyme in guinea pig heart were studied and compared with the 1-alkenyl-GPC acyltransferase. Although the pH optimum for 1-acyl-GPC acyltransferase was at 8.5, only

small changes in activity could be detected between pH 6 and 10. The reaction was also temperature-sensitive, and at 25 °C the reaction was linear up to 40 min and up to 400 μg of microsomal protein used. The saturating concentrations of 1-acyl-GPC and oleoyl-CoA required for maximum activity were determined and optimal conditions (as described in the Materials and methods section) were used in all subsequent assays.

The acyl specificity of the acyl CoA:1-acyl-GPC acyltransferase at pH 8.5 was determined (Fig. 2). The specific activity of the acylation of 1-acyl-GPC in guinea pig heart microsomes was 2.5–12-fold higher than the acylation of 1-alkenyl-GPC, depending on the species of acyl-CoA used. Oleoyl-CoA, linoleoyl-CoA and arachidonyl-CoA were preferred substrates, and enzyme activity was markedly decreased when saturated acyl-CoA was used. The preference of the enzyme for unsaturated acyl-CoA was unchanged when assayed at pH 6.5. At this pH, enzyme activity with oleoyl-CoA was unchanged, but a 40% lower activity was obtained with linoleoyl- or arachidonyl-CoA. It is clear that substantial differences exist between the reaction rate and acyl-CoA specificity of the two acyltransferases. 1-Acyl-GPC acyltransferase activity was not inhibited by high concentrations of acyl-CoA.

Guinea pig heart microsomes were assayed for 1-alkenyl-GPC and 1-acyl-GPC acyltransferase activities in the presence of Mg^{2+} (0–10 mM) and Ca^{2+} (0–10 mM).

Fig. 2. Acyl-CoA specificity of 1-acyl-GPC acyltransferase

The activities of acyl CoA:1-acyl-GPC acyltransferase in the presence of various concentrations of acyl-CoA were determined. The assay mixture (0.7 ml) contained 100 nmol of 1-acyl-glycerophospho-[methyl- ^3H]choline (lysophosphatidylcholine) and 0.04 mg of microsomal protein, and the enzyme was assayed at pH 8.5 for 30 min at 25 °C. The symbols for the acyl-CoA species used are the same as in Fig. 1. Each point in the Figure is the mean value of two separate sets of experiment, each of which was determined in duplicate. The S.D. for each point is less than 5% of the mean value.

As depicted in Fig. 3, the two enzyme activities responded very differently to both Ca^{2+} and Mg^{2+} . At 10 mM, Ca^{2+} and Mg^{2+} enhanced the 1-alkenyl-GPC acyltransferase activity by 50% and 30%, respectively. At the same concentration, the two cations inhibited the 1-acyl-GPC acyltransferase activity by 50% at pH 6.5 and 8.5.

The effect of anionic, cationic, nonionic and zwitterionic detergents on both enzyme activities was investigated. As shown in Table 3, the effect of many detergents on the two acyltransferase activities were distinctly different, and the differential effect was maintained when 1-acyl-GPC acyltransferase was assayed at pH 6.5. For example, when 0.05% Chaps was present in the assay, a 38% loss of 1-alkenyl-GPC acyltransferase activity was observed as compared with a 67% loss in 1-acyl-GPC acyltransferase activity. Differential effects to these two activities were also observed with Miranol. Nevertheless, some detergents, such as Tyloxapol, Tweens 20, 40, and 80 and Brij 35 affected both activities in similar fashion. Both enzyme activities were completely inhibited by 0.05% hexadecyltrimethylammonium bromide, SDS and deoxycholate.

The effect of heat treatment on the two acyltransferase activities was studied. Microsomal preparations were heated at 55 °C for different periods of time and subsequently assayed for acyltransferase activities. As shown in Fig. 4, the two acyltransferase activities were inactivated to different extents by this treatment.

Fig. 3. Effect of Ca^{2+} and Mg^{2+} on 1-alkenyl-GPC acyltransferase and 1-acyl-GPC acyltransferase activities

Acyl CoA:1-alkenyl-GPC acyltransferase activity (●, ○) and acyl-CoA:1-acyl-GPC acyltransferase activity (▲, △) were determined in the presence of Ca^{2+} (closed symbols) and Mg^{2+} (open symbols). The results are expressed as percentages of enzyme activities assayed in the presence of 2 mM-EGTA (no Ca^{2+} ; 100% activity) or in the presence of 2 mM-EDTA (no Mg^{2+} ; 100% activity). Each point is the mean value obtained from two separate sets of experiments, each of which was determined in duplicate. The S.D. for each point is less than 10% of the mean value.

Table 3. Effects of detergents on acyl-CoA:1-alkenyl-GPC acyltransferase and acyl-CoA:1-acyl-GPC acyltransferase activities from guinea pig heart

1-Alkenyl-GPC acyltransferase and 1-acyl-GPC acyltransferase activities in guinea pig heart microsomes were assayed in the presence of 0.05% detergent. The values are expressed as a percentage of activity in the absence of detergent, and are the means $\pm$ S.D. of two separate sets of experiment, each of which was assayed in duplicate.

Detergent used in assay	1-Acyl-GPC acyltransferase	1-Alkenyl-GPC acyltransferase
None	100	100
Chaps	33 $\pm$ 3	62 $\pm$ 6
Miranol H2M	68 $\pm$ 7	27 $\pm$ 8
Sodium deoxycholate	2 $\pm$ 1	1 $\pm$ 0.5
Zwittergent TM-314	0	4 $\pm$ 2
Tyloxapol	51 $\pm$ 5	50 $\pm$ 5
Tween 40	37 $\pm$ 7	31 $\pm$ 2
Brij 35	26 $\pm$ 1	18 $\pm$ 3
Triton X-100	1 $\pm$ 1	5 $\pm$ 1
Hexadecyltrimethylammonium bromide	0	2 $\pm$ 1

Fig. 4. Effect of heat treatment on 1-alkenyl-GPC acyltransferase and 1-acyl-GPC acyltransferase in guinea pig heart microsomes

Guinea pig heart microsomes were incubated at 55 °C for various time periods. The activities of 1-alkenyl-GPC acyltransferase (●) and 1-acyl-GPC acyltransferase (▲) after heat treatment were determined. The results are expressed as percentages of enzyme activities without heat treatment (= 100%). Each point is the mean value of two separate sets of experiments, each of which was determined in duplicate. The vertical bars are S.D. values.

1-Alkenyl-GPC acyltransferase activity was extremely heat-labile. After 1 min at 55 °C only 5% of the original activity remained. Under the same conditions, 37% of the 1-acyl-GPC acyltransferase activity was recovered.

The rate of acylation of 1-alkenyl-GPC (15–60 μ M) at different concentrations of 1-acyl-GPC was determined. Labelled oleoyl-CoA was used in this study. The labelled choline phosphoglyceride formed was subjected to acid

Fig. 5. Lineweaver-Burk plot of acyl CoA:1-alkenyl-GPC acyltransferase activity and 1-alkenyl-GPC concentrations in the presence of 1-acyl-GPC

Enzyme activity was determined with varying concentrations (15–60 μ M) of 1-alkenyl-GPC at 0 μ M (●), 7 μ M (■) and 28 μ M (▲) of 1-acyl-GPC. The activities were determined at 25 °C for 20 min with 0.08 mg of microsomal protein. Enzyme activity is expressed as nmol of product formed. Each point is the mean of two separate sets of experiment, each of which was assayed in duplicate. The S.D. for each point is less than 10% of the mean value. The lines were fitted by least squares analysis.

hydrolysis and the radioactivity in the resultant choline lysophosphoglyceride was determined. In another set of experiments, the rate of acylation of 1-acyl-GPC (10–60 μ M) at different concentrations of 1-alkenyl-GPC was studied. The choline phosphoglyceride formed in the reaction was again subjected to acid hydrolysis, but radioactivity remaining in the choline phosphoglyceride fraction was determined. The Lineweaver-Burk plots of the results are depicted in Figs 5 and 6. 1-Acyl-GPC was found to inhibit the acylation of 1-alkenyl-GPC in a non-competitive manner. In contrast, 1-alkenyl-GPC inhibited the acylation of 1-acyl-GPC in an uncompetitive fashion.

DISCUSSION

The hydrolysis of plasmalogencholine by phospholipase A₂ produces lysoplasmalogencholine. To prevent the accumulation of this cytolytic agent, the lysolipid has to be degraded or reacylated to the parent molecule. We have demonstrated the degradation of lysoplasmal-

Fig. 6. Lineweaver-Burk plot of acyl-CoA:1-acyl-GPC acyltransferase activity and 1-acyl-GPC concentrations in the presence of 1-alkenyl-GPC

Enzyme activity was determined with varying concentrations (10–60 μM) of 1-acyl-GPC at 0 μM (●), 10 μM (■) and 40 μM (▲) of 1-alkenyl-GPC. The activities were determined at 25 °C for 20 min with 0.04 mg of microsomal protein. Other conditions for the determination were identical with those described for Fig. 5.

choline by lysoplasmalogenase in the heart. Reacylation of lysoplasmenylcholine is dependent on the presence of acyl CoA:1-alkenyl-GPC acyltransferase, an enzyme that was reported to be absent from the rabbit heart [9]. In the present study, we have shown that the enzyme is present in all the mammalian hearts assayed, including rabbit heart. The fact that the enzyme was not detected previously in rabbit heart [9] was probably due to the relatively low specific activity of the enzyme in this tissue, coupled with the use of tissue homogenates for the assay. Our analysis also showed that there is no correlation between the cardiac plasmenylcholine content of various mammalian species and their specific activity (or total activity) of the 1-alkenyl-2-acyl-GPC acyltransferase. The reasons for this species variation in activity is presently unknown.

The relatively low 1-alkenyl-GPC acyltransferase activity in the cardiac tissue in comparison with 1-acyl-GPC acyltransferase led to the speculation that these two activities might originate from the same enzyme, and the acylation of 1-alkenyl-GPC might arise from the non-specific nature of the 1-acyl-GPC acyltransferase. The subcellular distribution of the two acyltransferases apparently supports this supposition. However, the differences in behaviour of the two acyltransferases with respect to acyl-CoA specificity, cation requirements, detergent and heat treatments all suggest that they are two separate and distinct enzymes. The non-competitive inhibition of the acylation of

1-alkenyl-GPC by 1-acyl-GPC and the uncompetitive inhibition of the acylation of 1-acyl-GPC by 1-alkenyl-GPC give further support that both reactions cannot be accommodated by the same catalytic site.

Although the nature of inhibition by 1-acyl-GPC of the reacylation of 1-alkenyl-GPC (and vice versa) is not clear, it may provide the tissue with a regulatory mechanism for the selective control of the two acylation reactions, since the acylation of 1-alkenyl-GPC and 1-acyl-GPC compete for the same pool of acyl-CoA. The fact that Ca²⁺ and Mg²⁺ enhanced 1-alkenyl-GPC acyltransferase activity and inhibited 1-acyl-GPC acyltransferase activity may provide the tissue with an additional mechanism for controlling both activities.

Guinea pig heart microsomal and mitochondrial fractions contain considerable amounts of plasmenylcholine [18]. Phospholipase A₂ hydrolysis of choline plasmalogen in both subcellular fractions will yield lysoplasmenylcholine. The presence of 1-alkenyl-GPC:acyl-CoA acyltransferase activity in the microsomes, but not in the mitochondria, implies that lysoplasmenylcholine can be metabolized by two separate routes in the microsomes, whereas in the mitochondria hydrolysis by lysoplasmalogenase is currently the only known metabolic route.

Both acyltransferases favour the utilization of unsaturated acyl-CoAs. This is not surprising since both the diacyl- and 1-alkenyl-2-acyl-GPC in the guinea pig heart have a preponderance of unsaturated fatty acids at the C-2 position [18]. However, 1-alkenyl-GPC acyltransferase was more reactive towards linoleoyl-CoA than other unsaturated acyl-CoA species, whereas the 1-acyl-GPC acyltransferase showed little preference between the unsaturated species. Although it is tempting to correlate the acyl specificity of these two enzymes to the C-2 acyl composition of the diacyl- and 1-alkenyl-2-acyl-GPC in the guinea pig heart [18], it is obvious that a meaningful correlation cannot be made with such simplistic approach. It should be noted that the acyl compositions of these two choline-containing phospholipids are also regulated by other undefined factors such as the availability of intracellular acyl-CoAs and the turnover rate of the C-2 acyl groups. Nevertheless, it can be concluded that the acylation of 1-alkenyl-GPC plays an important role in the maintenance of the high degree of unsaturated acyl groups in the guinea pig heart plasmalogens. The importance of the acylation for the resynthesis of diacyl-GPC with the appropriate acyl groups at the C-2 position in the heart has been well documented [19].

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