SHORT COMMUNICATION

Characterization of 1-Alkenyl-sn-glycero-3-phosphorylcholine Acyltransferase Activity in the Microsomes from Rat Parotid Gland

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Introduction

The alkyl and alk-1-enyl (plasmalogen) types of ether-linked phospholipids are present in mammalian cells almost exclusively as structural analogues of phosphatidylcholine and phosphatidylethanolamine, which are major membrane phospholipid compositions[1]. The alkyl type is found predominantly as choline phosphoglycerolipids, whereas plasmalogen occurs mainly as ethanolamine phosphoglycerolipids[1]. Recently, many specific physiological functions of alkyl and alkenyl-type phospholipids have been reported[2]. Not only have significant discoveries and advancements in the studies of alkyl type phospholipid like platelet activating factor (PAF: 1-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine)[3] and antitumor ether lipids (AELs)[4,5] been reported, but also the alkenyl type of phospholipids have been shown to play a role in the regulation of certain protein kinase C isoforms[6,7], a possible role in membrane-membrane fusion events[8,9] and the ability to serve as endogenous antioxidants[10,11]. It has also been suggested that alkenyl glycerophospholipids serve as a reservoir for polyunsaturated fatty acids[12,13] which, when released by a calcium-independent, plasmalogen-specific, phospholipase A2, can form bioactive molecules such as prostaglandins and leukotrienes[14]. In the submandibular and sublingual salivary glands, the analyses of ether-linked phospholipids and the characterization of their synthesizing enzymes have been reported[15]. However, there is very little information about ether-linked phospholipids in the parotid glands. Recently, analyses of membrane phospholipid compositions and their physical properties were reported, and it was shown that there are three types of phosphatidylcholines, 1-acyl-2-acyl, 1-alkenyl-2-acyl and 1-alkyl-2-acyl, in the rat parotid gland[16-18]. In the present study, a phospholipid-remodelling enzyme, acyltransferase, was characterized and the possible mechanism of enriching polyunsaturated fatty acid in choline plasmalogen was suggested.

Materials and Methods

1. Chemicals and reagents
[1-14C] Oleoyl-CoA (1.94 GBq/mmol) was obtained from Amersham Pharmacia Biotech, U. K. Egg yolk phosphatidylcholine and bovine serum albumin (fraction V) were purchased from Sigma Chemicals (St. Louis, MO). Linoleic acid and coenzyme A (sodium salt) were from Nacalai Tesque (Kyoto, Japan) and Kyowa Hakko Kogyo (Tokyo, Japan), respectively. 1-Alkenyl-sn-glycero-3-phosphorylcholine (1-alkenyl-GPC) and arachidonic acid, and 1-O-hexadecyl-sn-glycero-3-phosphorylcholine (1-alkyl-
GPC) were from Serdary Research Laboratories, Inc. (London, Canada) and BACHEM AG (Bubendorf, Switzerland), respectively. All other reagents were of the highest reagent grade available.

2. Animals
Male Wistar rats (8–11 weeks old) were maintained on Oriental MF solid chow (Oriental Yeast Co., Tokyo, Japan) and water ad libitum. After fasting overnight, rats were killed by exsanguination under light diethyl ether anaesthesia. Immediately, the parotid glands were isolated and trimmed off connective and adipose tissues and blood vessels. The obtained gland tissues were stored at -60°C until use.

3. Preparation of microsomes
All procedures were carried out at 0–4°C. Tissues were homogenized with 0.1 M Tris–HCl buffer (pH 7.2) containing 0.25 M sucrose. Microsomes were obtained as a precipitate of centrifugation (105,000 × g, 60 min) as described previously19). The resulting pellet was suspended to approx. 10 mg microsomal protein per ml in 0.1 M Tris–HCl buffer (pH 7.2) without sucrose, using a glass pestle homogenizer, and was stored at -85°C until use.

4. Preparation of substrates
1-Acyl-sn-glycero-3-phosphorylcholine (1-acyl-GPC) was prepared from egg yolk phosphatidylcholine by hydrolysis with snake venom phospholipase A219). Phospholipid phosphorus content was determined according to Bartlett20), as modified by Marinetti21). Linoleoyl and arachidonoyl-CoAs were synthesized by means of the coupling reaction of acylchloride derived from fatty acid and coenzyme A according to Seubert's procedure22), as modified by Okuyama et al.23). Other acyl-CoAs used here were of the highest grade available from commercial sources as described24).

5. Assays of lysophosphatidylcholine acyltransferase activity
Lysophospholipid acyltransferase activities were measured as the rate of coenzyme A release from acyl-CoA using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB)24). The reaction mixture contained 20 μM acyl-CoA, lysophospholipid (150 μM 1-acyl-GPC, 1-alkyl-GPC or 1-alkenyl-GPC), 1 mM DTNB and 0.1 mg/ml of microsomal protein in 90 mM Tris–HCl buffer (pH 7.2) unless otherwise mentioned. The reaction was initiated by the addition of acyl-CoA and carried out at 37°C in duplicate. To eliminate the effects of nonspecific release of coenzyme A and of other SH groups reacting with DTNB, all values were obtained by subtraction of the values given in the complete mixture without the acyl acceptor. The acylation activity was expressed as nmol acyl transferred/min per mg of microsomal protein.

Acyltransferase activities were also measured using radiolabelled acyl-CoA. The assay conditions were identical to those of the spectrophotometric assay described above, except that [14C]oleoyl-CoA (0.23 kBq/nmol) was used instead of unlabelled oleoyl-CoA and DTNB was omitted. After incubation at 37°C for 1 min, reaction products were extracted using the method of Bligh and Dyer26) and separated by thin layer chromatography (TLC)19). Areas corresponding to phosphatidylcholine or free fatty acid were scraped off the TLC plate into counting vials and 7.5 ml of scintillant (3.0 g of 2,5-diphenyloxazole and 230 mg of 1,4-bis(2-(5-phenyloxazolyl)) benzene in 1,000 ml of toluene/Triton X-100/water (16 : 4 : 1, v/v)) was added. The radioactivities were measured by an Aloka LSC-900 liquid scintillation system. Counting efficiency was more than 92% and each count was corrected to dpm for its counting efficiency.

Protein concentration was estimated according to Lowry et al.27), using bovine serum albumin as a standard.

Results

1. Characterization of lysophosphatidylcholine acyltransferase activities from rat parotid gland microsomes
There are three types of phosphatidylcholine, 1-acyl-2-acyl, 1-alkyl-2-acyl and 1-alkenyl-2-acyl, according to the binding types of the long carbohydrate to the glycerol backbone. The incorporation of fatty acids into the C-2 position of those three types

Table 1 Comparison of assay methods for acyltransferase activity in rat parotid gland microsomes

<table>
<thead>
<tr>
<th>Lysophosphatidylcholine</th>
<th>Acyltransferase activity (nmol/min/mg of protein) assayed</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Spectrophotometrically</td>
</tr>
<tr>
<td>1-acyl-GPC</td>
<td>21.2</td>
</tr>
<tr>
<td>1-alkyl-GPC</td>
<td>—*</td>
</tr>
<tr>
<td>1-alkenyl-GPC</td>
<td>14.3</td>
</tr>
</tbody>
</table>

*not detectable

Each value represents the mean of two separate experiments.

GPC : glycerophosphorylcholine

of phosphatidylcholines is accomplished by the acyltransferases using various types of lysophosphatidylcholines as substrates. Table 1 shows the 1-acyl-GPC, 1-alkyl-GPC and 1-alkenyl-GPC acyltransferase activities in the rat parotid gland microsomes, comparing the two different assay methods. The spectrophotometric assay is a very convenient method but not for direct determination of reaction products. However, using the labelled precursor for phosphatidylcholine biosynthesis, the procedure of estimation of the enzyme activity is complicated but exact. As shown in Table 1, the 1-acyl-GPC acyltransferase activity obtained from the spectrophotometric assay was 1.4-fold higher than that from the radio-isotope assay, and the 1-alkenyl-GPC acyltransferase was 1.3-fold greater. However, the 1-alkyl-GPC acyltransferase activity was one thirtieth lower than the 1-acyl-GPC acyltransferase activity in the radioisotope assay, and was not detected by the spectrophotometric assay. The spectrophotometric assay was adequate to assay the 1-acyl-GPC and 1-alkenyl-GPC acyltransferase activities.

Figure 1 shows the effects of the concentration of 1-alkenyl-GPC on the acyltransferase activity in the presence of oleoyl-CoA as a fatty acyl donor. The 150—300 μM 1-alkenyl-GPC was required for maximal activity of 1-alkenyl-GPC acyltransferase. Using the same assay conditions except that the 150 μM 1-acyl-GPC instead of 1-alkenyl GPC was used, the activity of 1-acyl-GPC acyltransferase was 36.9 nmol/min/mg of protein, which was approximately 1.4-fold higher than that of 1-alkenyl-GPC acyltransferase. These observations were similar to the findings given in Table 1. The apparent $K_m$ value for 1-alkenyl-GPC, obtained from Lineweaver-Burk plots from the findings in Fig. 1, was shown to be 29.7 μM, and the $V_{max}$ using oleoyl-CoA was 33.3 nmol/min/mg of microsomal protein. The dependency of 1-alkenyl-GPC acyltransferase activity on oleoyl-CoA concentration is shown in Fig. 2. The concentration of oleoyl-CoA required to obtain maximal activity was greater than 10 μM for 1-alkenyl-GPC acyltransferase. The apparent $K_m$ value of 1-alkenyl-GPC acyltransferase for oleoyl-CoA was 0.91 μM. The $K_m$ and $V_{max}$ values obtained in the parotid gland microsomes are summarized in Table 2, and were
compared with those from the rat submandibular gland. The $K_m$ values for the two different substrates were very similar between the two salivary glands.

2. Specificity of various acyl-CoAs by 1-alkenyl-GPC and 1-acyl-GPC acyltransferases from rat parotid gland microsomes

Under the optimal conditions established above, acyl-CoA specificities of 1-alkenyl-GPC and 1-acyl-GPC acyltransferases in rat parotid gland microsomes were studied using various saturated and unsaturated fatty acyl-CoAs. The findings are summarized in Table 3. Oleoyl-CoA was the best substrate for both 1-alkenyl-GPC and 1-acyl-GPC acyltransferase activities in which the former activity was slightly lower than the latter. In 1-alkenyl-GPC acyltransferase, the activity was apparently higher for unsaturated fatty acyl-CoAs than for saturated ones. These tendencies for acyl-CoA specificities in 1-alkenyl-GPC acyltransferase were also observed in the 1-acyl-GPC acyltransferase activity. However, when the reacylation rates of various acyl-CoAs were

![Graph](image)

**Fig. 2** Effect of oleoyl-CoA concentrations on 1-alkenyl-GPC acyltransferase activity in rat parotid gland microsomes.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Kinetic constants of 1-alkenyl-GPC acyltransferase in rat parotid gland microsomes</th>
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<tbody>
<tr>
<td>Salivary gland</td>
<td>$K_m$ ($\mu$M) for 1-alkenyl-GPC</td>
</tr>
<tr>
<td>Parotid gland</td>
<td>29.7</td>
</tr>
<tr>
<td>Submandibular gland*</td>
<td>26.5</td>
</tr>
</tbody>
</table>


GPC : glycerophosphorylcholine

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Specificity for acyl-CoAs in 1-alkenyl-GPC acyltransferase from rat parotid gland microsomes</th>
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</thead>
<tbody>
<tr>
<td>Acyl-CoA</td>
<td>1-Alkenyl-GPC acyltransferase</td>
</tr>
<tr>
<td></td>
<td>Specific activity</td>
</tr>
<tr>
<td></td>
<td>(nmol/min/mg of protein)</td>
</tr>
<tr>
<td>Palmitoyl-CoA</td>
<td>1.8</td>
</tr>
<tr>
<td>Stearoyl-CoA</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Oleoyl-CoA</td>
<td>27.5</td>
</tr>
<tr>
<td>Linoleoyl-CoA</td>
<td>18.2</td>
</tr>
<tr>
<td>Arachidonoyl-CoA</td>
<td>21.9</td>
</tr>
</tbody>
</table>

Each value represents the mean of two separate experiments. The relative activity is expressed as the percent of the specific activity to that for oleoyl-CoA.

GPC : glycerophosphorylcholine
compared between 1-alkenyl-GPC and 1-acyl-GPC acyltransferases in the relative activity expressed as the percent of the specific activity to that for oleoyl-CoA, polyunsaturated fatty acyl-CoAs, especially arachidonoyl-CoA, showed a two-fold higher rate for 1-alkenyl-GPC acyltransferase than for 1-acyl-GPC acyltransferase. In contrast, palmitoyl-CoA for 1-alkenyl-GPC acyltransferase showed half the 1-acyl-GPC acyltransferase activity.

**Discussion**

In mammalian tissues, the reacylation of lysophospholipids plays important roles in establishing molecular species of membrane phospholipids and regulation of the free polyunsaturated fatty acids level\(^{29}\). In this communication, we showed that 1-alkenyl-GPC acyltransferase activity is present in rat parotid gland microsomes as well as in the guinea pig heart\(^ {30} \) and rat submandibular and sublingual glands\(^ {19}\). In general, the 1-alkenyl-GPC acyltransferase activity is thought to be normally less than the activity of 1-alkyl-GPC acyltransferase from the same tissue\(^ {31}\). Indeed, the content of 1-alkyl-type phosphatidylcholine was higher than that of the 1-alkenyl type phosphatidylcholine (choline plasmalogen) in the rat parotid gland\(^ {18}\). However, when the activities of both acyltransferases for 1-alkenyl-GPC and 1-alkyl-GPC were compared in the same rat parotid gland microsomes, the former’s activity was significantly higher than that of the latter (Table 1). Similar findings have also been reported in rat submandibular and sublingual glands\(^ {19}\). These observations suggest that there may be no direct correlation between the specific activity of the enzyme and the content of ether-linked phosphatidylcholines. This suggestion is also adopted not only in the ether-linked phospholipids but also the acyl-type phospholipids. It is thought that one of the rate-limiting steps for phosphatidylcholine biosynthesis is the supply of CDP-choline synthesized by cytidylyltransferase\(^ {22}\). Furthermore, the selectivity of various types of diglycerides, containing not only the acyl type but also ether-linked types, by cholinephosphotransferase is very important to determine the composition of phosphatidylcholine analogues\(^ {23}\). Therefore, the complex biosynthetic pathways may influence the final composition of various analogues of phosphatidylcholine.

Despite the differences in the \( V_{\text{max}} \) activities in the 1-alkenyl-GPC acyltransferase between the parotid and submandibular glands, the \( K_m \) values for the acyl acceptor substrate, 1-alkenyl-GPC, and the acyl donor type, oleoyl-CoA, resembled each other (Table 2). These findings imply that similar enzymes in the parotid and submandibular gland microsomes catalyze the reacylation of 1-alkenyl-GPC, which are formed from the ether-linked phosphatidylcholine by the deacylation reaction of phospholipase A\(_2\)\(^ {26}\).

The acyl-CoA selectivity of acyltransferases, especially reacylation enzymes, is very important to enrich the unsaturated fatty acids in the C-2 position of phospholipids. However, the contribution of acyltransferase for attaining and maintaining the observed molecular compositions of the choline-containing various types of phospholipids in the tissue is unclear. Recently, the multiple pathways for syntheses of various types of choline-containing phospholipids and their biological functions have been becoming clear\(^ {2} \). The majority of the newly synthesized phosphatidylcholines undergo remodelling via the deacylation-reacylation cycle. Indeed, it was observed that there were three different types of reacylation enzyme activity in the parotid gland microsomes (Table 1), and the specificity of 1-alkenyl-GPC acyltransferase activity showed very high levels of polyunsaturated fatty acyl-CoAs such as arachidonoyl-CoA (Table 3). Apart from the intrinsic acyl-CoA specificities of the enzyme, the intracellular flux and composition of 1-acyl-GPC, 1-alkenyl-GPC and 1-alkyl-GPC, as well as the pool size of acyl-CoA, totally contribute to the composition of the molecular species of the choline-containing phospholipids. However, the transacylation reaction is also widely distributed in the microsomes of various tissues\(^ {29}\), but the presence in the parotid gland has not been clarified. Therefore, these reacylation enzymes affect each other and are involved in the remodelling of fatty acids in phosphatidylcholines, and contribute to the regulation of the physicochemical properties of membranes such as membrane fluidity, and the distribution
and accumulation of physiologically important fatty acids such as arachidonic acid.

It is still unclear how the plasmalogen phospholipids function in the salivary glands. However, the results obtained here may provide a specific function in which to study the mechanism of saliva secretion involving the production of eicosanoids and membrane fusion.

References


