Polyphosphoinositides Are Derived from Ether-linked Inositol Glycerophospholipids in Rat Brain

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Membrane inositol glycerophospholipid (IGP) is metabolized to phosphatidylinositol-4-phosphate (PIP), phosphatidylinositol-4, 5-bisphosphate (PIP₂), and inositol triphosphate (IP₃) in signaling transduction. This study was carried out to determine the subclasses of IGP involved in signaling pathway. The acyl chain moieties of the phospholipids are easily modulated by dietary fatty acids. We analyzed acyl chain composition of IGP 3-subclasses, PIP and PIP₂ from rat brain after feeding sunflower seed oil enriched with linoleic acid or fish oil high in eicosapentaenoic acid and docosahexaenoic acid. Long chain polyunsaturated fatty acids (LCPUFA) as eicosapentaenoic acid and docosahexaenoic acid were not incorporated into ether-linked IGP (alkenylacyl-glycerophosphoinositol and alkylacyl-glycerophosphoinositol), PIP and PIP₂, while diacyl-glycerophosphoinositol (GPI) contained high LCPUFA. These results suggest that PIP might be phosphorylated from only the ether-linked IGP (alkenylacyl- and alkylacyl species) but not from diacyl subclass for signals to intracellular responses in the plasma membrane of rat brain.

Keywords: Docosapentaenoic acid, Docosahexaenoic acid, Ether-linked inositol glycerophospholipids, Phosphatidylinositol-4-phosphate, Phosphatidylinositol-4,5-bisphosphate, Polyphosphoinositides

Introduction

The functions and biochemical mechanisms involved in cell signalling by inositol glycerophospholipid (IGP) breakdown are now well defined (Low and Saltiel, 1988; Yasutomi, 1992; Berridge, 1993). Hydrolysis of phosphatidylinositol yields inositol triphtosphate, which promotes the release of Ca²⁺ from intracellular stores and diacylglycerol, which activates protein kinase C. The increase in cytosolic Ca²⁺ plays a major role in the physiological responses observed with agonists that promote IGP hydrolysis, although the specific role of the protein kinase C activation still remains unclear. The phosphatidylinositol found to be most important in signal transduction were two phosphorylated derivatives of IGP, phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol-4, 5-bisphosphate (PIP₂), which are thought to be located mainly in the inner leaflet of the plasma membrane. Although PIP, is less plentiful in animal cell membranes than IGP, it is the hydrolysis of PIP, that matters most.

The previous paper has established that the long chain polyunsaturated fatty acids (LCPUFA) profiles of PIP and PIP₂ were little affected by dietary fats, whereas IGP was highly influenced by dietary fats (Alsted and Hoy, 1992). Thus it is important to study the fatty acid profiles of 3-subclasses of IGP affected by dietary lipids to compare the profiles of PIP and PIP₂. In most biological preparations there are usually 3-subclasses of IGP including two types of ether lipids. These ether lipids are alkenyl and alkyl analogs of IGP. The alkenyl moieties have a double bond adjacent to the ether linkage, whereas the alkyl moieties are saturated at this position. The alkenyl, alkyl and acyl chains may have different numbers of carbon atoms and double bonds (Curstedt, 1983).

Although the specific functions of the ether types of IGP have not been well-defined, they might have been associated with IGP pathway in signaling transduction. Thus, it is important to determine the role of ether-linked IGP in IGP hydrolysis during cell signaling.

Materials and Methods

Animal and diets Fourteen weanling female Sprague-Dawley rats (Korea Chemical Research Institute, Taejeon, Korea) weighing average 88.2 g were divided into two groups. One group was fed the diet containing 10% sunflower seed oil enriched in linoleic acid (18:2n-6) and the other was fed the diet containing 9% fish oil containing high eicosapentaenoic acid and docosahexaenoic acid (Table 1), supplemented with sunflower seed oil to satisfy the...
nutritional requirements for 18:2n-6, for 4 weeks and allowed
access to water and diets in a room with controlled
temperature 24-25°C and with 12 h dark-light cycle. The fatty acid
compositions of the diets are shown in Table 2. The rats were killed
by decapitation. The brain from each rat were immediately excised,
comprising, perfused with 0.9% saline solution and weighed. Total lipids
were extracted according to the method of Rahmant et al. (2002).

**Analyses of lipids**  The phospholipid classes were separated by
thin layer chromatography on precoated silica gel 60 plates (E.
Marck, Darmstadt, F.R.G.), using chloroform/methanol/acetic acid/
water (50 : 37.5 : 3.5 : 2, v/v/v/v) as a solvent system (Holub and
Skeaff, 1987) and visualized under ultraviolet light after spraying
with 2',7'-dichlorofluorescein solution. The resulting diacylglycerols were
acylated with 1 ml of acetic anhydride and five drops of pyridine
and diacyl species of the IGP. The resulting diaeacylglycerols were
acylated with 1 ml of acetic anhydride and five drops of pyridine
at 80°C for 1 h and subsequently separated by TLC using a solvent
system of petroleum ether/diethyl ether/acetic acid (90 : 10 : 1, v/v/v/v)
followed by a second development in toluene (Yeo et al., 1989).
The three subclasses of the IGP were visualized under ultraviolet
light after exposure to 2',7'-dichloro-fluorescein solution (Sigma
Chemical Co., St. Louis, USA).

In order to separate phosphatidylinositol-4-phosphate (PIP) and
phosphatidylinositol-4, 5-bisphosphate (PIP$_2$), the TLC plates were
soaked for 2 min in a 0.054 M oxalic acid solution adjusted to pH 7.2 with KOH, allowed to dry at room temperature and activated by
heating at 110°C for 15 min (Chaffoy de Courcelles et al., 1984). The
IGP from brain tissues and standard phospholipids (Sigma
Chemical Co., St. Louis, USA) were spotted on different lanes 1.5
cm from the bottom of the divided plate. The first acidic solvent
system was composed of chloroform/methanol/acetic acid/H$_2$O
(55 : 43 : 3 : 4, v/v/v/v). The development was stopped when the
solvent front reached 25% of the distance of the first solvent front (Medh and Weigel, 1989). The fractions were visualized under ultraviolet light after spraying with 2',7'-dichlorofluorescin solution.

The IGP, 3-subclasses of IGP, PIP and PIP$_2$ fractions were
scraped from the plates and transmethylated with 6% methanolic
sulfuric acid at 90°C for 1 h in the presence of known amounts of
monopentadecanoin (Nu Chek Prep, Elysian, USA) as an internal
standard to give subsequent mass determinations of the fatty acyl
chains. The fatty acid compositions of the three subclasses from the
IGP were analyzed by a Hewlett-Packard Model 5890 A gas
chromatograph equipped with a DB-225 megabore column
(Chromatographic Specialties, Brockville, Canada) and identified
by comparison of their retention times with those of known
standards (Nu Chek Prep, Elysian, USA). The flow rate of the
nitrogen was 36 ml/min and the oven temperature during these
isothermal runs was 210°C. Since the acyl group composition of the
two ether lipid subclasses are derived only from the sn-2 position,
while the acyl groups from the diacyl subclasses represent the sn-1
plus sn-2 positions, the molar amounts of total methyl esters
derived from the two ether lipid subclasses were multiplied by 2 to
calculate them quantitatively with the diacyl subclasses. The results

### Table 1. Composition of the sunflower seed oil (SO) and fish oil (FO) diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition (wt %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SO</td>
</tr>
<tr>
<td>Casein</td>
<td>20.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
</tr>
<tr>
<td>Corn starch</td>
<td>10.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>50.0</td>
</tr>
<tr>
<td>vitamin mix</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>4.5</td>
</tr>
<tr>
<td>Straw</td>
<td>4.0</td>
</tr>
<tr>
<td>Sunflower seed oil</td>
<td>10.0</td>
</tr>
<tr>
<td>Fish oil</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2. Fatty acid composition of the sunflower seed oil (SO) and fish oil (FO) diets

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Composition (mol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SO</td>
</tr>
<tr>
<td>14 : 0</td>
<td>0.4</td>
</tr>
<tr>
<td>16 : 0</td>
<td>10.5</td>
</tr>
<tr>
<td>16 : 1</td>
<td>0.1</td>
</tr>
<tr>
<td>18 : 0</td>
<td>5.7</td>
</tr>
<tr>
<td>18 : 1 (iso)</td>
<td>27.6</td>
</tr>
<tr>
<td>18 : 2n-6</td>
<td>54.5</td>
</tr>
<tr>
<td>18 : 3n-3</td>
<td>tr</td>
</tr>
<tr>
<td>18 : 4n-3</td>
<td>tr</td>
</tr>
<tr>
<td>20 : 1</td>
<td>tr</td>
</tr>
<tr>
<td>20 : 3n-6</td>
<td>0.9</td>
</tr>
<tr>
<td>20 : 4n-6</td>
<td>tr</td>
</tr>
<tr>
<td>20 : 5n-3</td>
<td>tr</td>
</tr>
<tr>
<td>22 : 1</td>
<td>tr</td>
</tr>
<tr>
<td>22 : 6n-3</td>
<td>tr</td>
</tr>
</tbody>
</table>

*Other minor fatty acids have been excluded from the table.
iso means isomers.*
Table 3. Distribution of 3-subclasses of inositolglycerophospholipid in brain of animals fed either the sunflower seed oil (SO) or fish oil (FO) diets

<table>
<thead>
<tr>
<th>Phospholipid subclass</th>
<th>Composition (mol %)</th>
<th>SO</th>
<th>FO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkenylacyl-GPI</td>
<td>22.5 ± 3.2</td>
<td>20.6 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>Alkylacyl-GPI</td>
<td>10.8 ± 0.8</td>
<td>8.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Diacyl-GPI</td>
<td>66.7 ± 4.5</td>
<td>70.7 ± 4.5</td>
<td></td>
</tr>
</tbody>
</table>

*Values represent means ± S.E. for seven animals/group.

were analyzed by using one-way analysis of variance for comparison of the sunflower seed oil or fish oil groups and expressed as mol%.

Results

The relative abundance of the three subclasses of the brain IGP are given in Table 3. The distributions in the sunflower seed oil animals were not significantly different from the fish oil animals. However, the relative distributions of the alkylacyl, alkenylacyl and diacyl subclasses from the IGP were considerably different among the 3-subclasses of IGP from the two dietary group (Table 3).

The compositions of the fatty acyl moieties in the IGP are provided in Table 4. The major unsaturated fatty acid in the IGP from the sunflower seed oil animals was arachidonic acid, with the fish oil animals being relatively more enriched than the sunflower seed oil animals (21.1 versus 26.7 mol%). The fatty acid composition of the IGP was considerably unique compared to CGP and EGP which were very dependent upon dietary fatty acids. Higher levels of arachidonic acid in the fish oil group might be remarkable only in IGP, although there was no enough arachidonic acid or its precursor contained in the fish oil diet. Docosahexaenoic acid level in the fish oil group doubled relative to that in the sunflower seed oil animals.

No docosahexaenoic acid was found in alkenylacyl- and alkylacyl-glycerophosphoinositol (GPI) of brain from both the sunflower seed oil and fish oil group, although the fatty acid was existed in the total IGP fraction (2.2 and 5.6 mol%, respectively) (Fig.2). Whereas markedly higher levels of the polyunsaturated fatty acids as arachidonic acid and docosahexaenoic acid were exhibited in diacyl-GPI subclass of the sunflower seed oil and fish oil animals (Fig. 2). The docosahexaenoic acid provided the greatest contribution to the higher levels of n-3 fatty acids in the diacyl-GPI from the two dietary groups (2.1 and 2.8 mol%, respectively). The docosapentaenoic acid provided for a lesser portion (and docosahexaenoic acid for a greater portion) of the n-3 fatty acids in both the sunflower seed oil and fish oil groups in the diacyl types of IGP. The docosapentaenoic acid and docosahexaenoic acid showed a significant elevation in diacyl subclass of IGP from the brain of the sunflower seed oil and fish oil animals; a greater accumulation was found in the sunflower seed oil (1.4 and 2.1 mol% relative to 1.4 and 2.8 mol% of the fish oil group.

Docosapentaenoic acid and docosahexaenoic acid were not found in the acyl-chain composition of phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol-4, 5-bisphosphate.

![Fig. 1. The possible signaling transduction pathway of ether-linked inositol glycerophospholipids to produce polyphosphoinositides in rat brain.](image-url)

![Table 3. Distribution of 3-subclasses of inositolglycerophospholipid in brain of animals fed either the sunflower seed oil (SO) or fish oil (FO) diets](table-url)

![Table 4. Acyl chain composition of inositol glycerophospholipid in brain of rats fed either a sunflower seed oil (SO) or fish oil (FO) diets](table-url)
Role of Ether-linked Inositol Phospholipids in Signaling Pathway

(PIP<sub>2</sub>) in brain of rats fed either a sunflower seed oil or fish oil diets (Fig. 3) as shown in Fig. 2 for alkenylacyl- and alkylacyl-GPI, respectively.

Discussion

No docosapentaenoic acid and docosahexaenoic acid were found in alkenylacyl- and alkylacyl-GPI of brain from both the sunflower seed oil and fish oil group, though the fatty acid was existed in the total IGP and diacyl-GPI fraction from the two dietary groups (Table 4, Fig. 2). The docosahexaenoic acid, which was found in considerably high levels (both in the sunflower seed oil and fish oil animals) in the diacyl-GPI as compared to the alkenylacyl- and alkylacyl-GPI. This finding is in disagreement with the reported enrichment of the n-3 fatty acids in the alkylacyl and alkenylacyl classes of individual phospholipid in other tissues from animals fed fish oil-based diets (Yeo, Y.K et al., 1988; Yeo et al., 1989; Yeo et al., 1989; Yeo and Holub, 1990; Park et al., 1995; Yeo et al., 1999; Yeo et al., 2000) and suggests that one effect of dietary oil feeding was a different metabolism via bio-availability of docosapentaenoic acid and docosahexaenoic acid in brain IGP.

Interestingly, considerable enrichment of docosapentaenoic acid and docosahexaenoic acid was found in the diacyl-IGP of the sunflower seed oil and fish oil groups. A possible discrimination against docosapentaenoic acid and docosahexaenoic acid release from the diacyl-GPI of membrane IGP via the phospholipase A<sub>2</sub> enzyme might be pronounced in rat brain.

Results from this study indicate that dietary ingestion of the sunflower seed oil and fish oil concentrate to rats was associated with only the diacyl-GPI species of IGP in the brain relative to ether-linked IGP fractions in this tissue. Brains from the two oil-fed rats showed no docosapentaenoic acid and docosahexaenoic acid in alkenylacyl- and alkylacyl-GPI although the oil enriched with docosahexaenoic acid was fed to the animal. The docosapentaenoic acid in the ether-linked IGP fractions seems to be retroconverted from docosahexaenoic acid which was not contained enough in the either dietary sunflower seed oil or fish oil.

This is also apparent from the 22:5n-3/22:6n-3 molar ratios of 0.67 and 0.50 in the diacyl-GPI, respectively, in the sunflower seed oil and fish oil animals. In contrast, the docosapentaenoic acid and docosahexaenoic acid in the ether-linked IGP were not found at all in the two dietary groups.

The inositol phospholipids comprise less than 10% of the total phospholipids in mammalian cell membranes. The IGP, as a minor phospholipid in cell membranes found to be most important in signal transduction, were two phosphorylated derivatives of IGP, PIP and PIP<sub>2</sub>. No polyunsaturated fatty acids (docosapentaenoic acid and docosahexaenoic acid) were
found in the two derivatives of IGP (PIP and PIP₂) in Fig. 3) as
in the ether-linked IGP (alkenylacyl- and alkylacyl-GPI).

A role for inositol phospholipids in signal transduction is
considerably well understood (Darnell et al., 1991; Chilvers et al., 1991; Ohnuma and Mabuchi, 1993; Englund, 1993; Munnik et al., 1994). The chain of events linking extracellular signals to intracellular responses via PIP₂ breakdown begins with binding of the signaling molecules to its receptor in the plasma membrane. Although the details of the activation processes are not as well known as they are in the cyclic AMP pathway, there is increasing evidence that the same type of multistep mechanism operates in the plasma membranes (Yamauchi et al., 1993; Johnso et al., 1993; Sen and Chander, 1994; Palmer et al., 1994; Rameh et al., 1997). The polyphosphoinositides (PIP and PIP₂) are produced by the phosphorylation of IGP. Although all three inositol phospholipids may be hydrolyzed in the signaling responses, it is the breakdown of PIP₂ that is most critical, even though it is the least abundant, constituting less than 10% of the total inositol lipids and less than 1% of the total phospholipids (Granoff, 1987; Chilvers et al., 1991; Darnell et al., 1991).

Of particular interest in the present work are the results showing no docosapentaenoic acid and docosahexaenoic acid content in the ether-linked IGP and polyphosphoinositides (PIP and PIP₂) which are produced by the phosphorylation of IGP from the two dietary oil group. These results are in agreement with Alsted and Hoy (1992) who found little docosahexaenoic acid in PIP and PIP₂ fractions compared to total inositol glycerophospholipid in brain of rat which fed with three different oils including fish oil enriched with docosahexaenoic acid. Their results suggest that PIP and PIP₂ are possibly from only ether-linked IGP because the diacyl-GPI is a major subclass among 3-subclasses (alkenylacyl, alkylacyl and diacyl) of IGP. In view of the result so far achieved, the study strongly suggests that the polyphosphoinositides might be phosphorylated from the ether-linked IGP (alkenylacyl-and alkylacyl species) instead of diacyl subclass for the chain of events linking extracellular signals to intracellular responses in the plasma membrane of rat brain (Fig. 1).

The specific biochemical reactions involved in the biosynthesis and turnover of the alkanylacyl and alkylacyl species of IGP, including those specifically reported in the brain that are phosphorylated to generate the polyphosphoinositides (PIP and PIP₂), remain to be studied. As well, more study is required to determine the effects of dietary fatty acids on the biosyntheses of the phosphoinositides as well as the possible role of these biochemical changes on brain.

References


