Acyl and alk-1-enyl group compositions of the alk-1'-enyl acyl and the diacyl glycerophosphoryl ethanolamines of mouse and ox brain

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ABSTRACT The ethanolamine phosphoglycerides were prepared from lipid extracts of ox and mouse brains by preparative thin-layer chromatography. The cyclic acetal derivatives of the alk-1-enyl groups were made by treating the ethanolamine phosphoglycerides with 1,3-propanediol. The resulting monoacyl glycerophosphoryl ethanolamines were separated from the unchanged ethanolamine phosphoglycerides by preparative thin-layer chromatography. Methyl ester derivatives of the acyl groups from both of these fractions were prepared by alkaline methanolysis. The cyclic acetal and methyl ester derivatives were analyzed by gas-liquid chromatography.

Substantial differences were found in the composition of the side chains when the combined alk-1-enyl and acyl side chains of the alk-1'-enyl acyl glycerophosphoryl ethanolamines were compared with the side chains of the diacyl glycerophosphoryl ethanolamines. The side chains from the 1-position of these two ethanolamine phosphoglycerides are different in chain length and unsaturation as well as in chemical bonding. The acyl groups from the 2-position of the alk-1'-enyl acyl glycerophosphoryl ethanolamines were predominantly unsaturated. Therefore, acyl group compositions of the total ethanolamine phosphoglyceride from brain are of limited value and individual types should be analyzed.

SUPPLEMENTARY KEY WORDS plasmalogens . phosphoglycerides . dimethyl acetals . cyclic acetals . 1,3-dioxolanes . thin-layer chromatography

APPRECIABLE QUANTITIES of plasmalogens are found in the brain, heart, and some other tissues of mammals (1, 2). Since the plasmalogens differ from the corresponding diacyl phosphoglycerides in their acyl group compositions (2), the compositions of the two types should be determined separately. The most practical approach for this kind of analysis entails removal of the alk-1-enyl groups from the plasmalogens, followed by separation of the resulting mixture of monoacyl and diacyl phosphoglycerides (3). Although this removal and separation can be simply achieved by a two-dimensional separation-reaction-separation TLC procedure (4), the resultant amounts of material are too small for GLC. 1,3-Propanediol derivatives of the alk-1-enyl groups (cyclic acetals) can be prepared quantitatively from phosphoglyceride mixtures that contain plasmalogens (5). In the present investigation, we have extended the cyclic acetal method to include the quantitative analysis of the acyl groups from plasmalogens, and compared the side-chain compositions of the diacyl and the alk-1'-enyl acyl glycerophosphoryl ethanolamines from mouse and ox brains.

MATERIALS AND METHODS

Phosphoglycerides
Ox heart lecithin was purchased from Sylvana Chemical Company (Orange, N.J.). Three ox brains were obtained within 30 min of death and transported to the laboratory on ice. Most of the meninges and external blood vessels were removed and a lipid extract from each brain was prepared (6).

Mouse brain lipids were prepared from 10 males and 10 females (preparation A) and from 30 females (preparation B). The mice, strain C57BL/10, were 3 months of age. The procedures for lipid extraction, separation of

Abbreviations: CA, cyclic acetal; DMA, dimethyl acetal; EPG, ethanolamine phosphoglycerides; GPE, sn-glycero-3-phosphoryl ethanolamine; GLC, gas-liquid chromatography; TLC, thin-layer chromatography. Fatty acids designated by chain length: number of double bonds.

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the phospholipid fraction by column chromatography with Unisl. and separation of the major phospholipids by preparative TLC on Silica Gel G have been described previously (7). Phosphorus (8) and alk-1-enyl group (9) contents were determined.

The content of alk-1'-enyl acyl glycerophosphoryl ethanolamines was determined by separation-reaction-separation two-dimensional TLC (4). The acid-stable EPG is a mixture of diacyl GPE, alkyl acyl GPE, and possibly dialkyl GPE. The area containing this mixture was scraped off the TLC plate and subjected to alkaline methanolysis as described below. Portions of the upper and lower phases were taken for phosphorus assay. The remaining portions of the lower phases were pooled, concentrated, and applied to a TLC plate. Ox brain phospholipids were applied to an adjacent lane as a reference. After development, the areas corresponding to intact EPG and lyso-EPG were scraped from the plate and assayed for phosphorus.

Preparation of Cyclic Acetals

The method of Rao, Ramachandran, and Cornwell (5) for the preparation of cyclic acetal (CA) derivatives was modified slightly. Solutions containing about 15-50 μmoles of phospholipid and 0.5-1.0 mg of ρ-toluenesulfonic acid were placed in a Teflon-lined screw-cap culture tube and taken to dryness with a stream of nitrogen. After the addition of 5 ml of CHCl₃ that contained 50 μl of 1,3-propanediol, the tube was sealed and heated in a water bath at 80°C for 2 hr. When the tube had cooled, 3 ml of CH₃OH, 1 ml of CH₃COONa, and 3 ml of H₂O were added and mixed thoroughly. The upper phase that contained the excess 1,3-propanediol was discarded. The lower phase was then taken to dryness and applied to several lanes of a TLC plate that was developed with toluene. The methyl ester bands, Rf 0.60, were scraped from each lane and eluted with CHCl₃. The CHCl₃ eluates were taken to dryness and dissolved in hexane for GLC.

Preparation of Dimethyl Acetals

Phospholipids were subjected to acidic methanolysis, and dimethyl acetals were isolated from the reaction products as described previously (7).

Gas-Liquid Chromatography

An Aerograph model 204-B gas chromatograph (Varian Aerograph, Walnut Creek, Calif.) equipped with hydrogen flame detectors was used. Columns, 6 ft long and 1/8 inch i.d., packed with 10% EGSS-X (an ethylene glycol succinate-silicone copolymer) on 100-120 mesh Gas-Chrom P, were purchased from Applied Science Laboratories Inc. (State College, Pa.). Flow rates for nitrogen carrier gas and hydrogen were approximately 20 and 37 ml/min, respectively. A temperature program from 165 to 200°C at 2°C/min was used for the methyl esters and isothermal temperatures of 186 and 200°C were used for the dimethyl and cyclic acetals, respectively. Temperatures of the injection port and the detector compartment were approximately 330 and 280°C, respectively.

Methyl esters and dimethyl acetals were identified as described previously (7) except that polyunsaturated fatty acid methyl ester standard 66B (Supelco, Inc., Bellefonte, Pa.) was added for the identification of long-chain polyunsaturated fatty acids. A mixture of synthetic cyclic acetals was given by Dr. V. Panganamala. Uncorrected peak areas measured by triangulation were used to calculate the composition of the mixture. Quantitative results with fatty acid standard D (National Heart Institute) differed from the stated composition with a relative error of less than 1.5% for major components (>10% of mixture) and less than 4% for minor components (<10% of mixture). The per cent relative error was the mean from 11 determinations.

RESULTS

Methodology

The disappearance of alk-1-enyl groups from a CA reaction mixture was monitored with an iodine addition reaction (9). The reaction mixture contained 2.4 μmoles of alk-1-enyl groups, 50 μl of 1,3-propanediol, and 0.5 mg of ρ-toluenesulfonic acid in 5 ml of CHCl₃. After this mixture has been heated for 30 min in a sealed tube at 80°C, 0.1 μ mole of alk-1-enyl groups was found. No alk-1-enyl groups were detected after 60 min. When the result-
ing reaction mixture was applied to a TLC plate that was developed with toluene, only one spot migrated from the origin. This spot has the same mobility as cyclic acetal that were run on the same plate as marker compounds. No aldehydes were detected. The phospholipid products from the CA reaction were examined by two-dimensional TLC (4). No alk-1-enyl groups were detected. Table 1 gives the alk-1-enyl group compositions of two phosphoglyceride preparations as obtained with dimethyl acetals and cyclic acetals.

**Compositions**

The ox brain EPG were composed of 62.4% alk-1'-enyl acyl GPE, 23% diacetyl GPE, and 5% alkyl radyl GPE. TLC of the acid- and base-stable EPG (see Methods) gave equal quantities of phosphorus in the areas corresponding to intact EPG and lyso-EPG. Since dialkyl glycerols could not be detected among the products of phospholipase C hydrolysis (Horrocks, unpublished data) the two areas may represent alkyl acyl GPE and alkyl GPE. The alk-1'-enyl acyl GPE accounted for 46.7% of the EPG from mouse brain preparation A. Although the alkyl GPE content of this preparation was not measured, the EPG from similar preparations have had alkyl radyl GPE contents of about 4%.

Compositions of the CA derivatives of the alk-1-enyl groups and the methyl ester derivatives of the acyl groups from brain EPG are given in Tables 2 and 3. In addition, the acyl group composition found for the total EPG is compared with the composition calculated from the compositions of the monoacetyl and diacetyl GPE.

**DISCUSSION**

The procedures described for analysis of the acyl groups from alk-1'-enyl acyl GPE are quantitative and rapid, and do not require unusual equipment or large amounts of lipid. Other methods are required for the separate analysis of the acyl groups from alkyl acyl GPE (11). The modified CA procedure is more convenient than the original procedure (5). In comparison with the reactions for formation of the dimethyl acetals, the conditions for formation of cyclic acetals have no effect on acyl groups and give a quantitative conversion of alk-1-enyl groups to the CA derivatives (2-alkyl-1,3-dioxolanes).

The determined alk-1-enyl group compositions were the same whether DMA or CA derivatives were used (Table 1). In addition, the alk-1-enyl group compositions of ox heart choline phosphoglycerides were quite similar to those previously reported (5, 12).

The acyl group compositions given for the diacetyl GPE include a small contribution of acyl groups from the 2-position of alkyl acyl GPE. We estimate the latter as 5% of the ox brain diacetyl GPE and 2% of the mouse brain diacetyl GPE. With the present methods, the EPG acyl groups were subjected to two incubations and two thin-layer separations. The possibility of oxidative losses of polyunsaturated acyl groups was examined by comparing the acyl group compositions that were found for the total EPG with the compositions calculated for the total EPG from the compositions of the component parts. The calculated 22:6 content was slightly lower than the 22:6 content of the total EPG mixtures. The values for other acyl groups differed by less than the estimated experimental error.

The EPG alk-1-enyl groups from the female mouse brain had a slightly higher proportion of 18:1 than the same fraction from mixed male and female mouse brains. In the brain EPG alk-1-enyl groups from older mice (7), the proportion of 18:1 is higher and the proportion of

**TABLE 1** COMPARISON OF THE USE OF DIMETHYL ACETAL (DMA) AND CYCLIC ACETAL (CA) DERIVATIVES FOR THE DETERMINATION OF ALK-1-ENYL GROUP COMPOSITIONS

<table>
<thead>
<tr>
<th>Chain Length and Unsaturation</th>
<th>Ox Heart CPG</th>
<th>CA Hydrogenated (3)</th>
<th>Mouse Brain EPG*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMA (3)</td>
<td>CA (10)</td>
<td></td>
</tr>
<tr>
<td>Unident.†</td>
<td>1.4 ± 0.07</td>
<td>1.2 ± 0.32</td>
<td>0.5 ± 0.07</td>
</tr>
<tr>
<td>15:0</td>
<td>2.0 ± 0.10</td>
<td>1.9 ± 0.05</td>
<td>2.0 ± 0.19</td>
</tr>
<tr>
<td>br 16:0</td>
<td>1.1 ± 0.02</td>
<td>0.9 ± 0.04</td>
<td>0.8 ± 0.08</td>
</tr>
<tr>
<td>18:0</td>
<td>66.5 ± 0.55</td>
<td>66.9 ± 0.22</td>
<td>66.3 ± 0.86</td>
</tr>
<tr>
<td>br 17:0</td>
<td>5.8 ± 0.14</td>
<td>5.9 ± 0.22</td>
<td>5.2 ± 0.08</td>
</tr>
<tr>
<td>17:0</td>
<td>1.8 ± 0.05</td>
<td>2.8 ± 0.09</td>
<td>2.8 ± 0.15</td>
</tr>
<tr>
<td>18:9</td>
<td>17.7 ± 0.66</td>
<td>16.8 ± 0.27</td>
<td>22.3 ± 0.74</td>
</tr>
<tr>
<td>18:1</td>
<td>5.4 ± 0.03</td>
<td>4.3 ± 0.21</td>
<td>30.2 ± 0.54</td>
</tr>
<tr>
<td>Unident.†</td>
<td>0.7 ± 0.15</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

CPG, choline phosphoglycerides. Number of determinations in parentheses.

* Preparation A.
† Unidentified, carbon numbers below 15.0 and above 18.5.
‡ The alkyl chain, originally attached by 1-enyl linkage to C-1 of glycerol, is designated by No. of carbons: No. of double bonds; br, branched.

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18:0 is lower. The cause of these age- and sex-related differences is not known although it may be significant that the EPG alk-1- enyl groups have a higher proportion of 18:1 in myelin than in gray matter (13) or in other mouse brain subcellular fractions (Sun and Horrocks, unpublished data). The amount of myelin increases with age in the mouse brain (14).

The bovine diacyl GPE composition is quite similar to that calculated from the molecular species data of Renkonen (15). The alk-1- enyl and acyl group compositions of the total EPG of mouse and bovine brain are in good agreement with previous results from other species (13, 16). The acyl group composition of mouse EPG is very similar to that of human gray matter EPG (16), whereas the bovine composition resembles that of human white more than that of human gray matter (16).

Comparisons of the side chains of alk-1'-enyl acyl GPE with those of diacyl GPE show marked differences for both mouse and ox brain. For example, in ox brain the plasmalogen has 13.8% 16:0 and 6.1% 22:6(n-3) (mean of acyl and alk-1'-enyl groups) whereas the diacyl GPE has 7.4% and 14.9%, respectively. The small amount of 22:6(n-3) in alk-1'-enyl acyl GPE when compared to diacyl GPE is due, at least in part, to the relatively high concentration of plasmalogen in myelin. Myelin EPG have a very low content of the (n-3) polyunsaturated acyl groups when compared with the EPG of other brain fractions (16; Sun and Horrocks, unpub-
lished data). More significant comparisons can be made when analyses are available for the molecular species (11, 15, 17) of subcellular fractions.

It is apparent that the diacyl GPE and alk-1'-enyl acyl GPE differ in composition, occurrence, physical properties (18, 19), and metabolism (20, 21). The present work emphasizes the importance of analyzing the individual components of the EPG for meaningful evaluations of brain phosphoglycerides.

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