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# PHOSPHATIDIC ACID OF RETINAL MICROSOMES CONTAINS A HIGH PROPORTION OF DOCOSAHEXAENOATE

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SUMMARY. Microsomes obtained from the bovine retina contain phosphatidic acid enriched in polyenoic fatty acids. A relatively high proportion of docosahexaenoate, not reported previously in phosphatidic acid of any subcellular fraction, is the main feature of this phospholipid. It is suggested that acylation by docosahexaenoyl-CoA takes place prior to the synthesis of phosphatidic acid in retinal microsomes.

INTRODUCTION. Phosphatidic acid is the first diacylated glycerolipid formed in the neobiosynthetic pathway of phospholipids. Docosahexaenoate as well as other polyunsaturated fatty acids is distributed among phospholipid classes in relatively high proportions in the retina (1-3), notably in rod outer segment membranes (1). In this tissue, the presence of such unusual lipids as dipolyunsaturated phosphatidylcholine and phosphatidylethanolamine as supraenoic molecular species has been noted (4). Since in the toad retina more than 40% of the acyl groups of diglycerides consist of docosahexaenoate, it has been suggested that this highly unsaturated fatty acid is introduced during biosynthesis (5,6). Thus, it was of interest to survey the acyl groups in phosphatidic acid to decide whether docosahexaenoate was already present in the immediate precursor of diacylglycerol. This report describes the fatty acid composition of phosphatidic acid of microsomes from bovine retinas.

METHODS. Bovine retinas were homogenized with 0.32 M sucrose buffered in 50 mM Tris-HCl, pH 7.4 containing $10^{-4}$M EDTA by a Potter-Elvejhem homogenizer and a motor driven teflon pestle. After centrifuging 20 min at 11,500 x g and two washings and resuspensions at each step microsomes were obtained by spinning 50 min at 140,000 x g. All these steps were carried out at 2-5°C.

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Lipid extraction was begun by homogenization of microsomal pellets in chloroform-methanol 2:1 (v/v) and then by following the procedure of Folch et al as described elsewhere (7). Phosphatidic acid was isolated by thin-layer chromatography (8) and methanolyzed with 14% $BF_3$-Me OH without prior elution from the silica gel scrapings. Fatty acid methyl esters were separated in a 5700 model Varian Aerograph gas-liquid chromatograph. Columns 1,5 m by 2 mm inside diameter packed with 6% diethyleneglycol succinate on Diatoport S and flushed with $O_2$-free $N_2$ (30 ml/min) as carrier were used. The operating conditions were injector (220°C), oven (195°C) and flame ionization detector (240°C).

RESULTS AND DISCUSSION. Table 1 gives the fatty acid composition of phosphatidic acid of retinal microsomes. Stearate, docosahexaenoate and oleate are in that order the predominant acyl groups. About 50% of the fatty acids are unsaturated and a relatively high unsaturation degree occurs (Table 1). Moreover, about 21% of the acyl groups consist of docosahexaenoate. This is, to the best of our knowledge, the highest relative proportion of this fatty acid in phosphatidic acid found in a subcellular fraction. Table 1 also includes a comparison with the fatty acid profiles of phosphatidic acid in the whole

TABLE I

COMPOSITION AND UNSATURATION OF ACYL CHAINS OF PHOSPHATIDIC ACID IN MICROSOMES FROM BOVINE RETINA.

| ACYL CHAINS | MICROSOMES (Bovine retina) N=10 | WHOLE RETINA (Bovine)[a] | MICROSOMES (Rat brain)[b] |
|---|---|---|---|
| 16:0 | 15.34 ± 1.44 | 19.0 | 10.9 |
| 16:1 | 1.49 ± 1.21 | 1.8 | - |
| 18:0 | 28.23 ± 3.94 | 26.1 | 39.8 |
| 18:1 | 16.92 ± 2.51 | 11.4 | 16.3 |
| 18:2 | 1.55 ± 0.62 | 4.0 | 1.9 |
| 20:1 | 0.18 ± 0.09 | - | 4.6 |
| 20:4 ω6 | 6.87 ± 0.95 | 9.6 | 13.7 |
| 22:4 | - | - | 2.5 |
| 22:5 | 1.93 ± 0.23 | 1.8 | - |
| 22:6 ω3 | 21.14 ± 2.55 | 16.7 | 10.3 |
| UNSATURATION DEGREE | 186 | 169 | 151 |

a, data taken from Aveldaño and Bazán (1977);

b, data taken from Su and Sun (1978).

bovine retina and in brain microsomes. The latter contain only 10.3% of 22:6 and also differ from phosphatidic acid of retinal microsomes when other fatty acids are compared as well (9). Although brain also contains other membranes richly endowed with highly unsaturated fatty acids, in the retina there is a relatively higher content of phospholipids containing such acyl groups. Therefore the presence in the retina of at least 40% of its phosphatidic acid as an hexaenoic molecular specie, if one assumes that in this lipid the unsaturated fatty acid is esterified to the C 2 of glycerol, implies that the docosahexaenoate is introduced prior to its synthesis. Moreover since radioactive glycerol uptake shows that in the retina there is an active de novo biosynthesis of phosphatidic acid (10,11) and of other phospholipids, a rapid flow of docosahexaenoate-containing phosphatidic acid may take place to synthesize highly unsaturated phospholipids. Because phosphatidic acid of whole retina has a fatty acid pattern similar to that of retinal microsomes (Table 1) phosphatidic acid from microsomes may serve as precursor to other lipids and also may be distributed to other subcellular sites. Alternatively phosphatidic acids with composition similar to that of microsomes may also be formed in other organelles. Following up this study should provide data to differentiate the proportion of 22:6 originating with phosphatidic acid de novo biosynthesis and that, if any, derived by acylation-deacylation reactions.

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