Positional distribution of fatty acids in glycerophosphatides of bovine gray matter

HYAKUJI YABUUCHI* and JOHN S. O'BRIEN

Department of Pathology, University of Southern California School of Medicine, Los Angeles, California 90033

ABSTRACT Glycerophosphatides were isolated from ox brain gray matter by column chromatography. The fatty acid compositions of ethanolamine glycerophosphatides (EGP), serine glycerophosphatides (SGP), and choline glycerophosphatides (CGP) were determined by gas–liquid chromatography. The positional distribution of fatty acids in these glycerophosphatides were determined by phospholipase A hydrolysis (*Habu habu* venom). C_{20} and C_{22} polyunsaturated acids were confined almost exclusively to the 2-position of these lipids, where they comprised the majority of 2-substituents in EGP and SGP (oleic acid predominated in this position in CGP). In the 1-position, palmitoyl was the major substituent in CGP, stearoyl in SGP, and stearoyl or the corresponding alk-1-enyl group in EGP.

KEY WORDS bovine brain · gray matter · glycerophosphatides · fatty acids · positional distribution · predominant species of phospholipid

THE AMINO GLYCEROPHOSPHATIDES from mammalian gray matter contain an extraordinarily high content of C_{20} and C_{22} polyunsaturated fatty acids; 40–60% of the fatty acids of ethanolamine glycerophosphatides (EGP) and serine glycerophosphatides (SGP) from human brain gray matter are of this type (1). The distribution of fatty acids on each carbon of the diglyceride moiety of each lipid has not been determined. This paper reports the positional distribution of fatty acids in glycerophosphatides from bovine cerebral gray matter.

Abbreviations: Fatty acids are denoted by chain length: number of double bonds. ω6 denotes a double bond six carbons removed from the methyl end of the fatty acid chain. EGP, ethanolamine glycerophosphatides; SGP, serine glycerophosphatides; CGP, choline glycerophosphatides.

* Present address: Department of Pediatrics, University of Osaka, School of Medicine, Osaka, Japan.

MATERIALS AND METHODS

The extraction of tissues, the isolation of each glycerophosphatide, and the determination of the purity of each lipid by thin-layer chromatography are described in our previous publications (1, 2). Gray matter was dissected from ox brains (mature animals) obtained immediately after death. After extraction of gray matter, individual phosphatides were isolated by a combination of DEAE-cellulose chromatography and silicic acid chromatography (2). The purity of each lipid (and its hydrolysis products) was determined by thin-layer chromatography. Silica Gel H was the adsorbent and chloroform–methanol–concentrated ammonia 18:6:1 and chloroform–methanol–water 65:25:4 were the solvents.

EGP, SGP, and choline glycerophosphatides (CGP) were subjected to phospholipase A hydrolysis by using habu venom (*Trimeresurus flavoviridis*). Each lipid was incubated in ethyl ether and habu venom according to the procedure of Okuyama and Nojima (3). 5 ml of a solution of phospholipid in diethyl ether (3 mg/ml) was mixed with 0.4 ml of a venom solution (4 mg/ml venom in 0.1 M borate buffer at pH 7 which contained $CaCl_2$ in a concentration of 2.5×10^{-3} M). The reaction was carried out at 27°C for 2 to 4 hr with constant shaking. For the SGP to be hydrolyzed completely, it was necessary to make the reaction mixture alkaline with a small amount of 0.1 N KOH.

After hydrolysis, the products were examined by thin-layer chromatography. Only two products were observed: the lysophosphatide and free fatty acids. The fatty acids were then separated from the lysoderivatives by chromatography on an acidic silicic acid column. Fatty acids were eluted with chloroform and the lysophosphatide was eluted with methanol. Both the fatty acids of

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the lysophosphatide and the released fatty acids were methylated in 5% methanolic HCl for 1 hr at 60°C (1).

Fatty acid methyl esters were quantified by gas–liquid chromatography on a 10% diethylene glycol succinate column as previously described (1, 2). Purified fatty esters (National Heart Institute Standard F and a laboratory standard) were used as standards for identification and quantification. The quantitative results with both standards agreed with the stated composition with a relative error of less than 5% for major components (more than 10% of the total mixture) and less than 7% for minor components (less than 10% of the total mixture). Fatty aldehyde dimethyl acetals obtained after methylation of EGP were also examined by gas–liquid chromatography on 10% diethylene glycol succinate columns as described (1). The fatty aldehyde content and composition of each glycerophosphatide were determined by gas–liquid chromatography as reported previously (1).

RESULTS

The fatty acid compositions (Tables 1–3) of EGP, SGP, and CGP from ox brain gray matter were similar to those of the corresponding lipids from human gray matter (1, 4). The major fatty acids of EGP were 18:0, 18:1, 20:4, and 22:6, the last-named comprising somewhat more than 27% of the total fatty acids. The fatty acids of SGP were similar to those of EGP except for lower concentrations of 16:0 and 20:4. SGP also contained high proportions of polyunsaturated fatty acids but much lower proportions of 16:0 and 16:1 than EGP.

Aldehydes comprised 24.2% of the total aliphatic groups of EGP. SGP and CGP contained only trace amounts of aldehydes. All the aldehydes initially present in EGP were recovered in the lyso-EGP obtained after venom hydrolysis and were, therefore, linked to the 1-position of the diglyceride moiety.

The distributions of fatty acids in the 1- and 2-positions of these three phosphatides (Tables 1–3) confirm the general rule that polyunsaturated fatty acids are preferentially esterified to the 2-position of glycerophosphatides. Only traces of C_{20} and C_{22} polyunsaturates were found in the 1-position. In EGP and SGP, the 22:6 fatty acid comprised 44.4 and 59.6% of the total fatty acids at the 2-position. Although CGP contained much lower percentages of C_{20} and C_{22} polyunsaturated acids, they were again exclusively linked to the 2-position.

The fatty acids in the 1-position differed widely from one lipid to another. The percentage of 1-linked 16:0 was very low in SGP, higher in EGP, and highest in CGP, whereas 1-linked 18:0 predominated in SGP and was least prominent in EGP. Although 18:1 was present in high proportions in the 2-position, significant proportions of this fatty acid were found in the 1-position as well.

TABLE 1 FATTY ACIDS OF ETHANOLAMINE GLYCEROPHOSPHATIDES

	1-FA	2-FA	Total	Expected
14:0	0.8	0.2	0.3	0.4
15:0	1.0		0.5	0.3
16:0	17.5	3.6	8.4	8.3
16:1	1.6	0.6	0.8	0.9
17:0	1.8		0.2	0.6
18:0	65.0	3.4	28.5	24.4
18:1	12.3	12.6	13.2	12.5
18:2		0.2	0.2	0.1
20:1		0.6	0.2	0.4
20:4		20.5	13.2	13.6
22:5ω6		12.4	6.7	8.2
22:5ω3		1.5	0.5	1.1
22:6		44.4	27.3	29.2
Polyunsaturates		79.0	47.9	52.2

Values are expressed as a percentage of the fatty acids (FA) in each position (or of the total). Expected values are calculated by averaging the values for fatty acids in the 1- and 2-positions, taking into account that 25.2% of the aliphatic groups in the 1-position are aldehydes. Values are the results of duplicate determinations.

TABLE 2 FATTY ACIDS OF SERINE GLYCEROPHOSPHATIDES

	1-FA	2-FA	Total	Expected
14:0	1.0	0.3	0.2	0.6
15:0	0.7		tr.	0.3
16:0	3.1	2.3	1.3	2.7
16:1	1.7	0.5	0.3	1.1
17:0		1.0	0.1	0.5
18:0	80.7	1.2	44.4	41.1
18:1	12.8	24.7	19.5	18.8
18:2		0.2	tr.	0.1
20:1		0.3	0.4	0.2
20:4		1.2	1.1	0.6
22:5ω6		5.6	3.1	2.8
22:5ω3		2.9	1.2	1.4
22:6		59.6	28.4	29.8
Polyunsaturates		69.5	33.8	34.7

Values are expressed as a percentage of the fatty acids (FA) in each position (or of the total). Expected values are calculated by averaging the values for fatty acids in the 1- and 2-positions. Values are the results of duplicate determinations.

DISCUSSION

Snake venom phospholipase A is a highly specific esterase for the 2-linked fatty acids of glycerol 3-phosphatides. The chain length or degree of unsaturation of the fatty acids in each position on the diglyceride moiety does not affect the specificity of enzymic hydrolysis (5).

The results obtained here indicate that C_{20} and C_{22} fatty acids are almost exclusively confined to the 2-position of these glycerophosphatides from gray matter. From this finding, one can deduce the predominant species of each glycerophosphatide class. For EGP, the major molecular species is 1-stearoyl (or octadec-1′-en-1′-yl) 2-docosahexaenoyl glycerophosphoryl ethanolamine; for SGP, 1-stearoyl 2-docosahexaenoyl glycerophosphoryl-serine; and for CGP, 1-palmitoyl (or stearoyl) 2-oleoyl glycerophosphoryl choline.

TABLE 3 FATTY ACIDS OF CHOLINE GLYCEROPHOSPHATIDES

	1-FA	2-FA	Total	Expected
14:0	1.7	0.8	1.3	1.3
15:0	0.3	0.2	0.3	0.3
16:0	37.6	32.9	36.5	35.3
16:1	5.3	3.8	4.6	4.6
17:0	0.7	0.2	0.5	0.5
18:0	32.4	0.3	13.4	16.3
18:1	21.4	47.8	33.6	34.6
18:2	0.6	1.3	1.1	1.0
20:4		8.8	5.2	4.4
22:6		4.1	3.5	2.0
Polyunsaturates	0.6	14.2	9.8	7.4

See footnote to Table 2.

In each of these lipids, the composition and positional distribution of fatty acids is different. EGP and SGP resemble one another closely in their 2-linked fatty acids, and these two are known to be interconverted (6). Yet the 1-linked fatty acids of these two lipids are quantitatively different. There are also major differences in aldehyde content. One-half of the groups in the 1-position of EGP are aldehydes, whereas SGP contains only traces of aldehydes. Thus, a simple precursor–product relationship is not plausible.

These glycerophosphatides are major constituents of biological membranes in the brain, especially mitochondria (7). It has been proposed that the fatty acid moieties of glycerophosphatides interact with mitochondrial proteins to give macromolecular aggregates of globular membrane lipoproteins (8,9). The high proportions of docosahexaenoic acid in EGP and SGP and the localization of the acid in the 2-position of the diglyceride moiety should be kept in mind when lipid–protein interactions in neural membranes are visualized.

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REFERENCES

1. O'Brien, J. S., D. L. Fillerup, and J. F. Mead. 1964. *J. Lipid Res.* **5:** 329.
2. O'Brien, J. S., and E. L. Sampson. 1965. *J. Lipid Res.* **6:** 537.
3. Okuyama, H., and S. Nojima. 1965. *J. Biochem.* (*Tokyo*). **57:** 529.
4. O'Brien, J. S., and E. L. Sampson. 1965. *J. Lipid Res.* **6:** 545
5. Van Deenen, L. L. M., and G. H. de Haas. 1963. *In* Advances in Lipid Research. R. Paoletti and D. Kritchevsky, editors. Academic Press, Inc., New York. **2:** 168.
6. Borkenhagen, L. F., E. P. Kennedy, and L. Fielding. 1961. *J. Biol. Chem.* **236:** PC 28.
7. OBrien, J. S. 1967. *J. Theoret. Biol.* **15:** 307.
8. Green, D. E., and Fleischer, S. 1963. *Biochim. Biophys. Acta.* **70**: 554.
9. Benson, A. A. 1966. *J. Am. Oil Chemists' Soc.* **43**: 265.