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Topological distribution of choline phospholipid fatty acids in trout intestinal brush-border membrane

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The transbilayer distribution of choline phospholipids in trout intestinal brush-border membrane has been investigated using phospholipase C (from *Clostridium welchii*). In the middle intestine, 84% of phosphatidylcholine (PC) and 60% of sphingomyelin (SP) are located in the outer membrane leaflet. In the posterior intestine, 89% of PC and 52% of SP are located in the outer membrane leaflet. The externally located PC molecular species are ($n-3$) fatty acid-rich in both parts of the intestine. While the sphingomyelin molecular species containing 24:1($n-9$) are exclusively located in the outer leaflet in the middle intestine, those containing 14:0 are more abundant in the same leaflet but in the posterior intestine. This strongly asymmetric distribution of both choline phospholipids may have numerous consequences on the brush-border membrane characteristics.

Introduction

Phospholipids play an important role in the properties of most biological membranes of eukaryotic cells, such as fluidity [1], permeability [2] and enzyme activities [3].

Easy to purify [4] and leading to closed vesicles remaining in the original orientation of the membrane [5], the intestinal brush-border membrane is responsible for the digestive and absorption functions of the enterocytes. Hence, the intramembrane distribution of the different phospholipids may participate in the control of the vectorial processes occurring through this particular membrane.

In a previous paper [6], we reported the asymmetric distribution of aminophospholipids between the two leaflets of trout intestinal brush-border membrane. To complete this work, we investigated the topological location of choline phospholipids in this membrane, using phospholipase C from *Clostridium welchii*, a useful tool for that kind of study [7].

Material and Methods

Fish and membrane preparations

Rainbow trout (250–350 g) were fed for 3 months a linolenic acid-rich diet, prepared as previously described [4]. Intestinal brush-border membranes were prepared from the middle and the posterior intestine according to an original procedure, yielding pure and closed vesicles with low amounts of contaminating organelles and therefore suitable for biochemical studies [4,8]. For the experiments, membranes of similar purity were selected according to the alkaline phosphatase enrichment (13–18-fold).

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Phospholipase C treatment of the brush-border vesicules

The hydrolysis of the brush-border phospholipids by phospholipase C from *C. welchii* (type X, Sigma) was carried out as described by Freysz et al. [9]. Brush-border membranes (0.6 mg protein/assay) were suspended in 20 mM Tris-HCl, 0.87% NaCl (pH 7.4). 1 mM CaCl2 in a final volume of 0.45 ml. The reaction started with the addition of 2 U/ml phospholipase C. An aliquote was immediately extracted corresponding to the zero-time of hydrolysis. The reaction was performed at 37 °C and stopped by lipid extraction [10].

Phospholipid analysis

Phospholipids were purified on silica gel column and separated by thin-layer chromatography [11]. Fatty acid methyl esters were then prepared from each phospholipid according to Morrison and Smith [12], and analyzed by gas chromatography [4]. The data are expressed as percent molar distribution. The amount of each phospholipid, before and after hydrolysis, was estimated using heptadecanoate as an internal standard. Their average molecular weight was computed from their fatty acid composition. The hydrolyzed phospholipid quantity is expressed as molar percent of the initial quantity. The fatty acid composition of the outer leaflet phospholipids was calculated by substracting the value obtained after phospholipase C treatment (phospholipid remaining from the inner leaflet) from those obtained for total phospholipids.

Results

Treatment of brush-border vesicles with phospholipase C

The rate of hydrolysis of phospholipids extracted from brush-border membranes and dispersed by sonication in the incubation medium was very similar to that reported for microsomal phospholipids [13], PC, PE and sphingomyelin (SP) were rapidly hydrolyzed whereas the hydrolysis of PI was very slow and PS remained unmodified. Therefore the use of phospholipase C for the study of the asymmetry of brush-border membranes is valuable for PC, PE and sphingomyelin especially when compared with other methods.

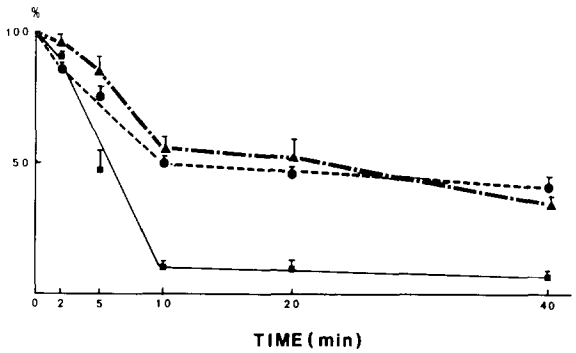

Fig. 1. Time course for the hydrolysis of choline and ethanolamine phospholipids of intestinal brush-border membrane from rainbow trout. The extent of the reaction is expressed as phospholipids (mol%) remaining intact. Incubation at 37 °C with 2 U of phospholipase C/ml. Values are means ± S.E. of three determinations. ■, PC; ●, SP; ▲, PE.

The incubation of brush-border vesicles from the posterior intestine with 2 U phospholipase C led to the hydrolysis of about 55% of total phospholipids (89% of PC, 50% of SP and 49% of PE) in 10 min and did not change up to 20 min (Fig. 1). Slight degradation was observed thereafter suggesting that the treatment of brush-border membranes for long time produced the disruption of the vesicles as it has been reported by Higgins [14] and Van Meer [15] for microsomal vesicles. However, the vesicles remained sealed during the first 20 min of incubation, since only about the half of the membrane phospholipids was hydrolyzed and that the amount of PE hydrolyzed corresponds to the amount reacting with TNBS in non-penetrating conditions [6]. These data indicate that the treatment of brush-border vesicles with phospholipase C for 20 min hydrolyzed only PC, PE and sphingomyelin of the external leaflet and that this hydrolysis is complete.

Fatty acid distribution in PC and sphingomyelin of the two leaflets of brush-border membranes

When brush-border membranes from the middle intestine of trout fed the experimental diet were incubated for 20 min with phospholipase C about 84% of PC, 60% of SP and 49% of PE were hydrolyzed (Table I). In the posterior intestine the same treatment induced the hydrolysis of 89% of PC, 52% of SP and 36% of PE.

TABLE I

DISTRIBUTION OF CHOLINE AND ETHANOLAMINE PHOSPHOLIPIDS (mol%) BETWEEN THE TWO LEAFLETS OF INTESTINAL BRUSH-BORDER MEMBRANE

Results are mean ± S.E. for three preparations. * $P < 0.05$, *** $P < 0.001$ for differences between the two leaflets (Student's t-test)

	Middle intestine		Posterior intestine	
	internal leaflet	external leaflet	internal leaflet	external leaflet
PC	15.6 ± 3.0 ***	84.4 ± 3.0	10.8 ± 3.0 ***	89.2 ± 3.0
SP	39.7 ± 1.1 ***	60.3 ± 1.1	48.4 ± 0.6 *	51.6 ± 0.6
PE	54.0 ± 6.9	46.0 ± 6.9	63.8 ± 3.0 **	36.2 ± 3.0

The analysis of the fatty acid distribution of PC and SP from both inner and outer leaflets of the brush-border membranes from the middle intestine showed that the PC of the internal leaflet was richer in 18 : 0 and 18 : 1($n-9$) and contained less 22 : 6($n-3$) than those of the external one (Table II). The SP of the external leaflet contained also less 16 : 0 than the inner one and all 24 : 1($n-9$) is located in the sphingomyelin of the external leaflet.

In the brush-border membrane of the posterior intestine PC of the inner leaflet contained higher amount of 14 : 0 and less 20 : 5($n-3$) and 22 : 6($n-3$) than those of the outer one whereas SP of the external leaflet is richer in 14 : 0 than those of the inner one.

Due to the asymmetrical distribution of both PC and SP, the asymmetrical location of fatty acids between both leaflets is emphasized, when considering the contribution of each leaflet to the fatty acid composition of the PC and sphingomyelin of the whole membrane (Fig. 2). In both intestinal regions, the major contribution to most of the fatty acids from PC is that of the external leaflet while, for sphingomyelin, this contribution is identical for both leaflets except that 14 : 0 is

TABLE II

CHOLINE PHOSPHOLIPID FATTY ACID COMPOSITION (mol%) OF THE INTERNAL AND EXTERNAL LEAFLETS FROM MIDDLE INTESTINE BRUSH-BORDER MEMBRANE

Values are means ± S.E. of three preparations. * $P < 0.05$ for differences between both leaflets (Student's t-test). Fatty acids contributing less than 1% were omitted. In brackets, sum of all the fatty acids of the indicated species.

Fatty acid	PC		SP	
	internal leaflet	external leaflet	internal leaflet	external leaflet
14 : 0	2.3 ± 1.5	–	23.8 ± 10.7	51.8 ± 22
16 : 0	17.0 ± 3.3	23.8 ± 1.4	28.5 ± 4.8 *	9.0 ± 4.6
18 : 0	10.4 ± 1.4 *	3.9 ± 0.4	15.3 ± 2.5	9.0 ± 2.9
(sat)	33.0 ± 2.9	28.4 ± 1.7	67.6 ± 5.7	71.9 ± 4.6
16 : 1	2.7 ± 1.2	–	3.3 ± 1.7	2.0 ± 1.1
18 : 1	21.3 ± 1.9 *	12.5 ± 0.5	26.2 ± 4.5	12.5 ± 6.2
24 : 1	–	–	–	9.8 ± 7.8
($n-9$)	24.7 ± 2.2 *	13.9 ± 1.5	31.0 ± 4.5	22.7 ± 14
18 : 2	9.9 ± 1.9	7.7 ± 0.8	–	4.3 ± 3.0
20 : 4	3.8 ± 1.9	–	–	–
($n-6$)	13.7 ± 2.7	9.8 ± 0.7	–	4.3 ± 3.0
18 : 3	13.5 ± 1.3	18.1 ± 2.0	–	–
20 : 5	1.9 ± 1.0	6.2 ± 1.6	–	–
22 : 6	10.6 ± 0.3 *	15.7 ± 1.2	–	–
($n-3$)	28.6 ± 3.5 *	48.1 ± 2.7	–	–
Unsat/sat	2.1 ± 0.3	2.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.3
($n-3$)/($n-6$)	2.1 ± 0.5 *	4.9 ± 0.8	–	–

TABLE III

CHOLINE PHOSPHOLIPID FATTY ACID COMPOSITION (mol%) OF THE INTERNAL AND EXTERNAL LEAFLETS FROM POSTERIOR INTESTINE BRUSH-BORDER MEMBRANE

Values are means ± S.E. on three preparations. * $P < 0.05$, *** $P < 0.001$ for differences between both leaflets (Student's t-test). In brackets, sum of all the fatty acids of the indicated species.

Fatty acid	PC		SP	
	internal leaflet	external leaflet	internal leaflet	external leaflet
14:0	4.8±0.9 *	1.0±0.5	19.4± 5.0 *	47.6±6.3
16:0	34.1±5.9	26.8±2.4	26.7± 9.6	18.1±0.7
18:0	13.4±4.5	4.9±0.7	8.9± 0.4	6.0±2.4
(sat)	52.3±5.3 *	32.8±2.3	55.0± 5.0	71.8±9.5
16:1	7.2±3.0	2.3±1.0	5.4± 3.1	8.2±2.0
18:1	17.2±5.2	17.2±1.0	23.1±11	9.1±1.1
24:1	–	–	16.5± 3.2	10.3±6.8
($n-9$)	24.4±3.9	20.6±0.8	45.0± 5.0	28.2±9.5
18:2	3.4±0.5	4.7±1.0	–	–
($n-6$)	8.0±2.5	7.9±1.2	–	–
18:3	4.7±0.8	7.8±2.4	–	–
20:5	2.8±1.4 *	7.9±1.1	–	–
22:6	7.7±2.2 ***	15.7±0.3	–	–
($n-3$)	15.3±2.2 ***	38.6±0.3	–	–
Unsat/sat	1.0±0.2 *	2.1±0.2	0.8± 0.2	0.4±0.2
($n-3$)/($n-6$)	1.9±0.5 *	4.9±0.7	–	–

mainly located in the external leaflet and 24:1($n-9$) is exclusively external in the middle intestine.

Discussion

Brush-border membranes from middle and posterior intestine of trout have already shown differences in their biophysical properties as well as in their fatty acid composition [4], their enzyme activities [8] and their absorption properties [16]. Moreover, we have recently demonstrated on these membranes an asymmetrical distribution of aminophospholipids [6] with regard to the polar head group as well as to the fatty acyl tails. This study has been completed by the determination of the topological distribution of choline phospholipids using phospholipase C. The phospholipase C of *C. welchii* was used previously to investigate the phospholipid asymmetry of brain and liver microsomes [13,17]. The kinetic study of this enzyme on brush-border vesicles indicates that it is also a valuable tool for the study of the distribution and fatty acid composition of PC and sphingomyelin in both leaflets of this membrane.

The present results indicate for the first time differences in the topological distribution of the choline phospholipids in the brush-border membranes from middle and posterior intestines. PC, which represents about 39% and 35% of the brush-border membrane phospholipids from the middle and posterior parts, respectively, is mostly located in the external leaflet (84% and 89% external, respectively). On the opposite, sphingomyelin (13 and 18% of the brush-border membrane phospholipids from the middle and posterior intestine, respectively), has a nearly symmetric distribution in both leaflets of the posterior intestine and is more aboundant in the external leaflet of the middle intestine (52% vs. 60% external leaflet, respectively).

A similar asymmetrical distribution of PC has already been suggested in rat liver microsomes [17], in chick brain microsomes [15] and in rat enterocyte plasma membrane [18]. While in hu-

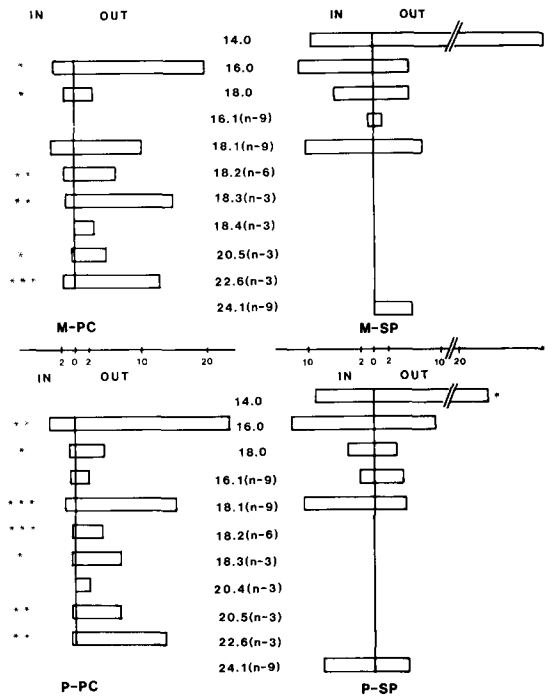

Fig. 2. Fatty acids distribution (mol%) between inner and outer leaflets of trout intestinal brush-border membrane. Fatty acids contributing less than 2% in the whole membrane phospholipid are omitted. M, brush-border membrane from the middle intestine; P, brush-border membrane from the posterior intestine. IN, inner leaflet; OUT, outer leaflet. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for differences between both leaflets (Student's t-test).

man platelet plasma membranes, PC is mainly located in the inner membrane leaflet [19], in rabbit intestinal brush-border membrane, more than 90% of PC is located in the outer leaflet [20].

In contrast to the reported randomized distribution of PC molecular species in the human [21] and rat [22] erythrocyte membrane and in the mouse LM cell plasma membrane [23], the two pools of PC in the trout microvillus membrane are different in their fatty acid composition. The noteworthy asymmetric distribution of $22:6(n-3)$ between the two PC pools, mainly in the posterior intestine, is also associated with a preferential outer location of all fatty acids of the $(n-3)$ series. This distribution is compensated by an opposite asymmetry of saturated and/or $(n-9)$ fatty acids. The reversed situation previously reported [6] for the differential distribution of $22:6(n-3)$-rich PE and PS species allows to estimate that no major differences exist at the level of the overall transbilayer distribution of $22:6(n-3)$ in both intestinal part. The important fluidizing role played by this highly unsaturated fatty acid [24] suggests it should regulate and possibly equalize the fluidity of each membrane leaflet.

The preferential location of SP molecules with a 14 carbon acyl chain in the outer membrane leaflet might have a considerable effect on the bilayer organization [25]. Since the distribution of sphingomyelin molecular species in any liposomal bilayer or biological membrane is yet unknown, one can only speculate about the possible relationship between the asymmetrical distribution of sphingomyelin molecular species and microvillus shape.

Choline phospholipids, which contribute for 52–53% of all brush-border membrane phospholipids, are external phospholipids while aminophospholipids, which represent 35–38% of all membrane phospholipids, are rather internal [6]. At present, one may only speculate about the physiological significance of this lipid asymmetry at the level of both polar head group distribution and fatty acid composition but it might have numerous consequences on the membrane structure [26,27] as well as on its function [28,29] and physical properties [30].

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References

1 Stubbs, C.D. and Smith, A.D. (1984) Biochim. Biophys. Acta 779, 89–137.
2 Coleman, R. (1973) Biochim. Biophys. Acta 300, 1–30.
3 Brenner, R.R. (1984) Prog. Lipid Res. 23, 69–96.
4 Pelletier, X., Duportail, G. and Leray, C. (1986) Biochim. Biophys. Acta 856, 267–273.
5 Klip, A., Grinstein, S. and Semenza, G. (1979) FEBS Lett. 99, 91–96.
6 Pelletier, X., Mersel, M., Freysz, L. and Leray, C. (1987) Biochim. Biophys. Acta 902, 223–228.
7 Etemadi, A.H. (1980) Biochim. Biophys. Acta 604, 423–475.
8 Di Costanzo, G., Florentz, A., Leray, C. and Nonnotte, L. (1983) Mol. Physiol. 4, 111–123.

9 Freysz, L., Dreyfus, H., Vincendon, G., Binaglia, L., Roberti, R. and Porcellati, G. (1982) in Phospholipids in the Nervous System, Vol. I, Metabolism (Horrocks, L.A., Ansell, G.B. and Porcellati, G., eds.), pp. 37–47, Raven Press, New York.
10 Folch, J., Lees, M. and Sloane-Stanley, G.M. (1957) J. Biol. Chem. 226, 497–509.
11 Leray, C., Pelletier, X., Hemmendinger, S. and Cazenave, J.P. (1987) J. Chromatogr. 420, 411–416.
12 Morrison, W.R. and Smith, L.M. (1964) J. Lipid Res. 5, 600–608.
13 Dominski, J., Binaglia, L., Dreyfus, H., Massarelli, R., Mersel, M. and Freysz, L. (1983) Biochim. Biophys. Acta 734, 257–266.
14 Higgins, J.A. (1982) J. Cell. Sci. 53, 211–225.
15 Van Meer, G. (1986) Trends Biochem. Sci. 11, 194–195.
16 Dabrowski, K., Leray, C., Nonnotte, G. and Colin, D.A. (1986) Comp. Biochem. Physiol. 83A, 27–39.
17 Higgins, J.A. and Dawson, R.M.C. (1977) Biochim. Biophys. Acta 470, 342–356.
18 Renooij, W., Van Golde, L.M.G., Zwaal, R.F.A. and Van Deenen, L.L.M. (1976) Eur. J. Biochem. 61, 53–58.
19 Perret, B., Chap, H.J. and Douste-Blazy, L. (1979) Biochim. Biophys. Acta 556, 434–446.
20 Barsukov, L.I., Hauser, H., Hasselbach, H.J. and Semenza, G. (1980) FEBS Lett. 115, 189–192.
21 Renooij, W., Van Golde, L.M.G., Zwaal, R.F.A., Roelofsen, B. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 363, 287–292.
22 Renooij, W., Van Golde, L.M.G., Zwaal, R.F.A. and Van Deenen, L.L.M. (1976) Biochim. Biophys. Acta 61, 53–58.
23 Sandra, A. and Pagano, R.E. (1978) Biochemistry 17, 332–338.
24 Conroy, D.M., Stubbs, C.D., Belin, J., Pryor, C.L. and Smith, A.D. (1986) Biochim. Biophys. Acta 861, 457–462.
25 Barenholz, Y. and Gatt, S. (1982) in New Comprehensive Biochemistry (Neuberger, A. and Van Deenen, L.L.M., eds.), Vol. 4, Phospholipids (Hawthorne, J.N. and Ansell, G.B., eds.), pp. 129–177, Elsevier, Amsterdam.
26 Geiger, B. (1985) Trends Biochem. Sci. 10, 456–461.
27 Op den Kemp, J.A.F., Roelofsen, B. and Van Deenen, L.L.M. (1985) Trends Biochem. Sci. 10, 320–323.
28 Rothman, J.E. and Kennedy, E.P. (1977) J. Mol. Biol. 110, 603–618.
29 Bergelson, L.D. and Barsukov, L.I. (1977) Science, 197, 224–230.
30 Seigneuret, M., Zachowski, A., Hermann, A. and Devaux, P.F. (1984) Biochemistry 23, 4271–4275.