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Note

Ionic alkoxylipids from the liver of elasmobranch fishes

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Deep-sea fish are characterized by the presence of unusual lipids[1,2]. For example, lipids containing ether bonds, *i.e.*, alkoxylipids, small proportions of which occur widely in nature, are abundant in the liver of elasmobranch fishes[3]. Much work has been carried out on the analysis of neutral alkoxylipids in the liver of chimaeras and sharks[2,3], but the ionic alkoxylipids, which are believed to be the metabolically active ether lipids[4], have not been isolated from fish liver oils and analyzed.

We have found that the liver of *Hariotta raleighana* and *Rhinochimaera atlantica*, which are chimaeras indigenous to the deep waters of the North Atlantic, contains little neutral lipids but fairly large proportions of phospholipids including ionic alkoxylipids. This paper describes the isolation of alkylacylglycerophosphorylethanolamines (II), alk-1-enylacylglycerophosphorylethanolamines (III) and the corresponding diradylglycerophosphorylcholines from these tissues (see Fig. 1). In addition, we report the composition of the alkyl moieties and of the alk-1-enyl moieties of each of the two pairs of ionic alkoxylipids in comparison with the fatty acid composition of the total liver lipids.

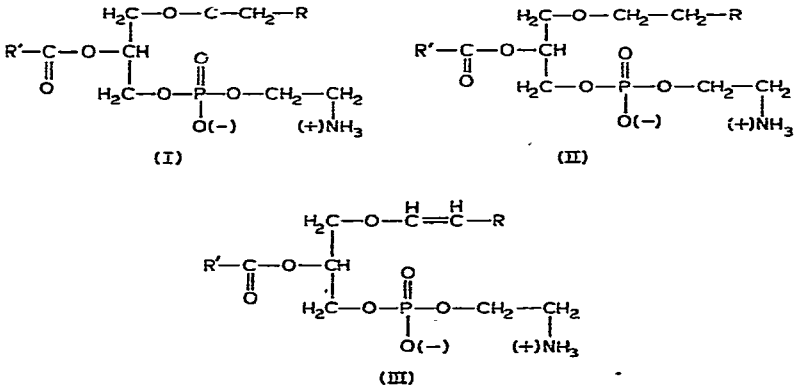

Fig. 1. The three types of diradyl-glycero-3-phosphorylethanolamines: I = 1,2-diacyl-glycero-3-phosphorylethanolamines; II = 1-O-alkyl-2-acyl-glycero-3-phosphorylethanolamines; III = 1-O-alk-1′-enyl-2-acyl-glycero-3-phosphorylethanolamines ("ethanolamine plasmalogens").

The results of such analyses contribute to a better understanding of the substrate specificity of enzymes that catalyse the formation of the alkyl and alk-1-enyl moieties in alkoxylipids.

EXPERIMENTAL

Materials

H. raleighana and *R. atlantica*, which had been caught off Rockall Bank at 1200 m and in the Bay of Biscay at 2060 m, respectively, were obtained from the Bundesforschungsanstalt für Fischerei, Hamburg, G.F.R. Air-free water and freshly distilled solvents were used. Reference compounds were purchased from Nu Check Prep., Elysian, Minn., U.S.A.

Extraction of lipids

The total lipids from the livers were extracted following established procedures[5].

Preparative chromatography

The total lipids (*ca.* 150 mg) were fractionated on layers of silica gel H (E. Merck, Darmstadt, G.F.R.), 0.5 mm in thickness, 50 mg per 20 × 20 cm plate. *n*-Hexane–diethyl ether (60:40) was used as the developing solvent for separating neutral lipids from the phospholipids. The material remaining on the starting line, which consisted of phospholipids and other ionic lipids, was eluted from the adsorbent with chloroform–methanol–water–acetic acid (50:30:10:1)[6] and filtered through sintered glass. The constituents of these fractions, diradylglycerophosphorylethanolamines and diradylglycerophosphorylcholines, were separated by chromatography on layers of silica gel H, 0.5 mm in thickness, with chloroform–methanol–water (65:25:4)[7]. The fractions were detected in UV light after spraying the plates with a 0.2% solution of 2′,7′-dichlorofluorescein in ethanol.

The two classes of phospholipids were eluted separately from the adsorbent with chloroform–methanol–water–acetic acid (50:30:10:1)[6]. The purity of the two classes of phospholipids isolated, each of which contained the respective diacyl, alkylacyl and alk-1-enylacyl forms, was confirmed by analytical thin-layer chromatography under the conditions described above.

Derivatization

Each of the two phospholipid fractions was subjected to reaction with lithium aluminium hydride in diethyl ether[8,9]. The resulting alcohols, alkylglycerols and alk-1-enylglycerols were dissolved in *n*-hexane and separated on thin layers of silica gel H, 0.5 mm in thickness, using *n*-hexane–diethyl ether (20:80) as the developing solvent[8]. The fractions of alkylglycerols and alk-1-enylglycerols were eluted from the adsorbent with diethyl ether which had been saturated with air-free water, and filtered through sintered glass. The solvent was evaporated and the lipids were re-dissolved in *n*-hexane. The mixture of alkylglycerols and alk-1-enylglycerols was subjected to acid-catalysed hydrolysis[10,11].

The aldehydes derived from the alk-1-enylglycerols were separated from the alkylglycerols by adsorption chromatography on layers of silica gel H, 0.3 mm in thickness, with *n*-hexane–diethyl ether (90:10) as the developing solvent. The frac-

tions were detected in UV light after spraying the plates with a 0.2% solution of 2',7'-dichlorofluorescein in ethanol. The aldehydes were eluted from the adsorbent with dry diethyl ether and the alkylglycerols with diethyl ether saturated with air-free water. The eluates were filtered through sintered glass. The aldehydes were reduced immediately to alcohols with lithium aluminium hydride and acetylated[10]. The resulting alkyl acetates were analysed by gas chromatography[10]. The fraction of alkylglycerols was converted into isopropylidene derivatives by reaction with acetone in the presence of small amounts of perchloric acid[12] and analysed by gas chromatography[12].

Gas chromatography

The instrument used was a Perkin-Elmer F7 gas chromatograph equipped with a hydrogen flame detector. The columns, 6 ft. × 1/8 in. I.D., were packed with 10% Silar 5CP on Gas-Chrom Q, 80–100 mesh (for analysing the alkyl acetates) and with 10% EGSS-X on Gas-Chrom P, 100–120 mesh (for analysing the isopropylidenealkylglycerols). The temperatures were 200° for the isopropylidenealkylglycerols and 180° for the alkyl acetates. Nitrogen at a flow-rate of 30 ml/min was used as the carrier gas. The temperature of the injection port was 250° and that of the detector 270°. Fractions were identified by comparison with reference substances and quantitated by triangulation.

RESULTS AND DISCUSSION

The liver of *H. raleighana* and *R. atlantica* contained 1.8% and 2.85% of total lipids, respectively. Over 80% of the total lipids consisted of phospholipids and other ionic lipids, the ratio of diradylglycerophosphorylethanolamines to diradylglycerophosphorylcholines being approximately 1:3. Table I gives the composition of the alkyl moieties in the alkylacylglycerophosphorylethanolamines and in the alkylacylglycerophosphorylcholines from the liver of the two chimaeras. The chain lengths of the alkyl moieties range from C-14 to C-22, but the alkylacylglycerophosphorylethanolamines contain larger proportions than the alkylacylglycerophosphorylcholines of alkyl chains with more than 18 carbon atoms. All of the alkyl moieties are saturated or monounsaturated; polyunsaturated chains occur only in trace amounts, if at all. Significant proportions of a series (A) of branched-chain alkyl moieties occur in both types of phospholipids.

Table II gives the composition of the alk-1-enyl moieties in the alk-1-enylacylglycerophosphorylethanolamines and in the alk-1-enylacylglycerophosphorylcholines from the liver of the two chimaeras. The chain lengths of the alk-1-enyl moieties range from C-14 to C-20. It is surprising that in both types of phospholipids the 18:0 and 18:1 alk-1-enyl moieties comprise roughly half of the total. All of the alk-1-enyl moieties are saturated or monounsaturated; polyunsaturated chains occur only in trace amounts, if at all. Significant proportions of two series (A) and (B) of branched chain alk-1-enyl moieties occur in both types of phospholipids.

The alkyl and alk-1-enyl moieties in the ionic alkoxylipids of the liver of *H. raleighana* and *R. atlantica* have in common that they are devoid of polyunsaturated constituents, although the total lipids contain 26.7% and 24.1% of polyunsaturated acyl moieties, respectively, almost half of which consist of 22:6 acyl chains. It is

TABLE I

ALKYL MOIETIES IN THE ALKYLACYLGLYCEROPHOSPHOLIPIDS OF *HARIOTTA RALEIGHANA* AND *RHINOCHIMAERA ATLANTICA*

The alkyl moieties were analysed by gas chromatography of the isopropylidenealkylglycerols (see text). Values are given as a percentage of the total peak areas.

Chain length: No. of double bonds	*Hariotta raleighana*		*Rhinochimaera atlantica*	
	*AGP-ethanolamines**	*AGP-cholines**	*AGP-ethanolamines**	*AGP-cholines**
14:0	3.9	4.3	0.3	0.1
15:0 br (A)	—	1.9	2.1	1.0
15:0 br (B)	—	—	—	—
15:0	2.3	2.5	0.8	0.5
16:0 br (A)	2.9	2.5	—	0.2
16:0 br (B)	—	—	—	—
16:0	4.4	13.6	14.7	0.1
16:1	2.0	13.6	2.6	3.2
17:0 br (A)	—	—	—	—
17:0 br (B)	—	—	—	—
17:0	tr	0.6	1.0	3.7
17:1	2.9	0.5	tr	—
18:0 br (A)	—	—	—	—
18:0 br (B)	—	—	—	—
18:0	6.2	1.9	tr	2.1
18:1	1.6	35.8	29.2	82.4
19:0 br (A)	5.1	—	—	—
19:0 br (B)	—	—	—	—
19:0	11.6	4.3	5.1	1.2
19:1	4.9	5.1	9.8	1.1
20:0	—	tr	—	—
20:1	—	9.7	6.3	0.3
21:0	—	—	tr	3.0
21:1	8.6	—	tr	0.4
22:0	—	—	5.8	—
22:1	43.7	—	14.9	—

* AGP = alkylacylglycerophosphoryl.

known the alkyl moieties in alkoxylipids are derived from fatty acids, and that the substrate specificity of the fatty acid reducing enzym(s) is responsible for the fact that the alkyl chains are all saturated and monounsaturated[13,14]. It is also established that alk-1-enyl moieties are derived from the alkyl moieties, which explains that the alk-1-enyl residues also consist exclusively of saturated and monounsaturated chains[4].

It is striking that the alkylacylglycerophosphorylethanolamines in the liver of both *H. raleighana* and *R. atlantica* contain high levels of 22:1 alkyl chains (43.7 and 14.9%, respectively), whereas the corresponding acyl moieties comprise only 1.3 and 5.4%, respectively, of the constituent fatty acids in the total lipids.

It is surprising that both the alkyl and alk-1-enyl moieties contain large proportions of branched chains, whereas the acyl moieties of the total lipids are virtually free of such constituents. It is also difficult to explain why the alkyl moieties contain one series (A) of saturated branched chains whereas the alk-1-enyl moieties contain two series (A) and (B) of saturated branched chains. A comparison of our data with

TABLE II

ALK-1-ENYL MOIETIES IN THE ALK-1-ENYLGLYCEROPHOSPHOLIPIDS OF *HARIOTTA RALEIGHANA* AND *RHINOCHIMAERA ATLANTICA*

The alk-1-enyl moieties were analysed by gas chromatography of the alkyl acetates that were obtained from the aldehydes after acid-catalysed hydrolysis (see text). Values are given as a percentage of the total peak area.

Chain length: No. of double bonds	*Hariotta raleighana*		*Rhinochimaera atlantica*	
	*AeGP-ethanolamines**	*AeGP-cholines**	*AeGP-ethynolamines**	*AeGP-cholines**
14:0	1.6	5.1	7.9	1.7
15:0 br (A)	1.2	3.9	—	—
15:0 br (B)	0.1	0.4	5.2	2.3
15:0	1.8	5.9	9.9	5.9
16:0 br (A)	0.5	1.1	tr	0.3
16:0 br (B)	0.8	1.5	tr	1.2
16:0	12.9	18.6	19.1	12.5
16:1	0.3	4.6	6.1	3.9
17:0 br (A)	6.0	—	—	—
17:0 br (B)	1.7	1.0	—	tr
17:0	1.3	2.4	3.6	3.7
17:1	3.4	1.8	—	tr
18:0 br (A)	—	—	—	—
18:0 br (B)	0.2	3.2	5.6	1.2
18:0	10.9	18.5	14.1	6.6
18:1	42.7	22.7	28.6	51.1
19:0 br (A)	—	tr	—	—
19:0 br (B)	—	—	—	—
19:0	1.8	5.8	—	3.6
19:1	0.4	3.7	—	tr
20:0	tr	tr	tr	tr
20:1	12.6	—	tr	8.4
21:0	—	—	—	1.1
21:1	—	—	—	—
22:0	—	—	—	—
22:1	—	—	—	—

* AcGP = alk-1-enylacylglycerophosphoryl.

the results of gas chromatographic[15,16] and mass spectrometric studies[16] on branched-chain aldehydes leads to the conclusion that the alkyl and alk-1-enyl moieties of the (A) series are the *iso* compounds whereas those of the (B) series are the *anteiso* compounds.

It is expected that these results will contribute to a better understanding of the biosynthesis of alkoxylipids, in particular to the recognition of substrate specificities in the metabolic interconversions of various alkoxylipid classes.

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