N-ACYLPHOSPHATIDYLETHANOLAMINE IN THE FISH BRAIN

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It has been shown that a minor phospholipid isolated from the brain of the mackerel Hexagrammos octogrammus is a N-acylphosphatidylethanolamine a considerble part of which is present in the plasmalogen form. This lipid was detected with the aid of a specially developed method of high-performance micro-TLC in the brains of 15 species of bony fishes, but it could not be detected in the brains of the shark and the ray.

In 1975, Hack and Helmy [1] found in the brain of the mudfish Amia calva a weakly polar phospholipid to which the structure of a bisphosphatidic acid (BPA) was assigned since it was chromatographically identical with a lipid from the heart of an infarcted dog described previously by the same authors [2]. As M. Hack has reported, he has found a similar lipid in the brains of several more species of fresh-water and marine fish. In 1980, Schmid et al. showed that the phospholipid from the infarcted zones of dog heart was not a BPA but a N-acylphosphatidylethanolamine (APE) [3]. Subsequently, Schmid's group, on the basis of the metabolism of APE and the properties of its metabolic product – N-acylethanolamine (AEA) – came to the conclusion that the formation of these N-substituted lipids may form the basis of the mechanism of the protection of the cells of the heart from the harmful action of ischemia [4, 5].

In the present investigation we have shown by comparing the chromatographic properties, the results of chemical transformations, and IR spectra that the weakly polar phospholipids from the brain of bony fish is also an APE and not a BPA. It was detected in the brains of all the species of bony fish investigated but was not found in the brains of the shark and the ray.

We have given preliminary results of the investigation [6, 7]. When our investigations were in the concluding stage, a new report appeared from Hack and Helmi [8] in which, on the basis of paper chromatography and literature analogies they reconsidered their views on the weakly polar phospholipid of fish brains as a BPA and identified it as an APE.

When a lipid extract of cod brain was subjected to TLC in the usual systems, a weakly polar phospholipid was detected. A comparison of this lipid with standard known polar phospholipids in system 5 and then in the specially developed systems 2 and 4 showed that it was similar in its chromatographic behavior to an APE but differed from BPA, semilyso-BPA, N-acetylphosphatidylethanolamine, a N-diacetone derivative of phosphatidyl-ethanolamine, and phosphatidylmethanol. The IR spectrum of the weakly polar phospholipid isolated in a large-scale experiment from mackerel brain was almost completely identical with the spectrum of APE with the exception of the fact that the peak at 1732 cm^{-1} (–COO–) had an intensity equal to the intensity of the peak at 1654 cm^{-1} (–CONH–), and not a higher intensity as in the synthetic standard and as reported in the literature – for example, in [3]. This may indicate a higher content of the plasmalogen form than in the standard form. In fact, when the plasmalogens in the initial lipid were decomposed a large part of it (about 80% in terms of phosphorus) passed into a product chromatographically identical with lyso-APE. On mild alkaline saponification fo the lipid under investigation two substances were formed, one of which was identical in its chromatographic behavior with lyso-APE and the other with N-acylglycerophosphoethanolamine (AGPE). On the successive saponification of the lipid and decomposition of the plasmalogens a substance containing phosphorus and chromatographically indistinguishable from APE was formed. The methanolysis of this gave the methyl esters of fatty acids, α-glycerophosphate, inorganic phosphate, and 2-aminoethanol. The same products were obtained on the similar treatment of synthetic APE.

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A characteristic feature of APE is the formation of AEA on its hydrolysis with glacial acetic acid [9]. In fact, having performed this procedure we obtained from the weakly polar phospholipid of fish brain a substance chromatographically indistinguishable from AEA. On the basis of all the results obtained, we concluded that the weakly polar phospholipid of fish brain was in fact APE.

Of the 17 species of fish that we studied (14 species of marine bony fish, one species of fresh-water bony fish, and two species of marine cartilaginous fish) the lipid was found in the brains of all the bony fish but was not found in the brains of the cartilaginous fish. We were unable to detect APE in the other organs of the bony fish (gills, skin, viscera). Denaturing the enzymes by boiling before the extraction of the lipids did not change the pattern of distribution of the APE in the brains of the bony fish.

EXPERIMENTAL

IR spectra were taken on a Specord 75IR instrument in chloroform.

Solvent Systems. Chloroform–methanol (95:5) (1). Chloroform–methanol–benzene–7 N aqueous ammonia (60:15:10:1) (2);(65:30:10:6) (3) [10]. Acetone–benzene–glacial acetic acid–water (20:30:4:1) (4). Chloroform–methanol–7 N aqueous ammonia (80:20:2) (5) [3]. Hexane–diethyl ether–glacial acetic acid (85:15:1) (6). Diethyl ether–acetone–hexane–glacial acetic acid (70:20:20:1) (7) [11]. Methanol–water–7 N aqueous ammonia (6:3:1) (8) [12].

Micro Thin-Layer Chromatography. Plates 6 × 6 cm with a layer of silica gel firmly fixed with a silicic acid sol [13]. The weakly polar phospholipids were separated in systems 2-5. The fatty acid methyl esters and dimethyl acetals were chromatographed in system 6 and in toluene. AEA was revealed in system 7. The TLC of the polar products of hydrolysis was performed in system 8. The substances on the chromtograms were detected by nonspecific reagents (water, iodine vapor, 10% sulfuric acid in methanol followed by carbonization) and specific reagents (0.2% of ninhydrin in acetone, phospholipid reagent [14], and reagent for phosphorus-containing compounds [15]).

Isolation of the Weakly Polar Phospholipid from Fish Brain. The lipids from the brain of freshly caught or frozen fish were extracted by a modification of the Bligh–Dyer method [16]. In a number of cases, live fish were previously kept for 3-5 min in boiling water. For preparative isolation, 4.5 g of the brain lipids of the mackerel Hexagrammos octogrammus was taken. Chromatography was performed on a column containing 90 g of KSK silica gel. The column was washed with chloroform and the weakly polar phospholipid was eluted with solvent system 1. Of the fraction obtained, 250 mg was transferred to a column containing 6.5 g of KSK silica gel, which was washed with chloroform and acetone, after which the phospholipids were eluted with methanol. Rechromatography of the fraction collected on a column of the same size in system 2 gave a phospholipid fraction which was purified by supplementary preparative TLC on KSK silica gel (plates 13 × 18 cm, solvent system 2). As a result, 23 mg of chromatographically individual phospholipid was obtained. The product consisted of a yellow viscous mass. IR spectrum, λ_{max}^{NaCl}, cm^{-1}: 1732 (–COO–), 1654 (–CONH–), 1245 (P=O), 1070 (P–O–c), 3380 (N–H). R_f 0.55 (2), 0.92 (3), 0.33 (4).

Liver Standards. APE was isolated from the lipids of wheaten flour [17] by preparative TLC in system 2. APE was also synthesized from ox heart phosphatidylethanolamine and stearoyl chloride [18]. N-acetylphosphatidylethanolamine was obtained similarly from phosphatidylethanolamine and acetyl chloride. Lyso-APE and AGPE were obtained, respectively, by the partial (room temperature, 15 min) and complete (56°C, 30 min) hydrolysis of authentic APE with the Clarke–Dawson reagent [19]. Synthetic BPA and synthetic semi-lyso-BPA were obtained from phosphatidylglycerol and stearoyl chloride [18] and also from 1,2-diglycerides and $POCl_3$ [20], followed by partial saponification. Phosphatidylmethanol was obtained from egg phosphatidylcholine and methanol by the action of cabbage phospholipase D [21]. N-Palmitoyl- and N-stearoylethanolamines were obtained by the reaction of the corresponding acids with 2-aminoethanol by a modification of the method of Rowe et al. [22]. The N-diacetone derivative of phosphatidylethanolamine was obtained by the method of Ando et al. [23].

Degradative Reactions. The mild alkaline saponification of lipids was carried out by treating the substances with a 1% solution of sodium methanolate in methanol or with the methylamine reagent [19]. The plasmalogens were decomposed with a 10% solution of hydrochloric acid in methanol or by the method of Vaskovsky and Dembitzky [24]. Severe acid hydolysis was performed in a sealed tube with a 5% solution of HCl in methanol at 130°C for

4 h. The fatty acid methyl esters were extracted with hexane after the addition of water to the hydrolysis mixture. Hydrolysis with glacial acetic acid [9] was carried out in a sealed tube at 100°C for 2 h.

SUMMARY

1. It has been shown that a weakly polar phospholipid from the brain of the mackerel Hexagrammos octogrammus is a N-acylphosphatidylethanolamine with a high (or the order of 80%) content of the plasmalogen form.

2. A high-performance micro-TLC method has been developed for seeking N-acylphosphatidylethanolamine in lipid extracts.

3. N-Acylphosphatidylethanolamine was found in the brain of all the species of bony fish studied but was not detected in the brains of cartilaginous fish.

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