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MEMBRANE LIPID MODIFICATIONS: BIOSYNTHESIS AND IDENTIFICATION OF PHOSPHATIDYL-*N*-METHYL-*N*-ISOPROPYLETHANOLAMINE IN RAT LIVER MICROSOMES

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In previous studies on the modification of polar head groups of membrane phospholipids with the unnatural base analog, *N*-isopropylethanolamine, we reported an unidentified phospholipid in addition to phosphatidyl-*N*-isopropylethanolamine in the various membrane fractions of rat liver. The structure of this phospholipid has now been identified as phosphatidyl-*N*-methyl-*N*-isopropylethanolamine by nuclear magnetic resonance spectroscopy, and by chromatographic and enzymic analysis. In addition, we found that when rats were injected intraperitoneally with the *N*-methyl-*N*-isopropylethanolamine, 19% of the liver microsomal phospholipid was phosphatidyl-*N*-methyl-*N*-isopropylethanolamine.

I. Introduction

To what extent can the polar head groups of membrane lipids be modified and how does such manipulation alter membranes and cell properties in mammalian cells? These questions have recently been approached experimentally by using analogs of amino alcohols as phospholipid precursors [1–7]. We have previously shown that N-isopropylethanolamine (IPE) could be incorporated into membrane phospholipids in rat livers in vivo [1] and in mouse L-M cells grown as monolayers [2,3]. In rat liver, IPE was not only incorporated into phosphatidyl-IPE, but also into a second unidentified phospholipid [1]. In this communication, we establish that the unidentified lipid is phosphatidyl-*N*-methyl-*N*-isopropylethanolamine (phosphatidylmethyl-IPE) and show that the methyl group on the nitrogen is derived from methionine. Our studies also demonstrate that methyl-IPE itself can be incorporated into this unnatural phospholipid in rat liver.

II. Experimental

The preparation of IPE has been described elsewhere [2]. We synthesized *N*-

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methyl-*N*-isopropylethanolamine (methyl-IPE) by a modification of the method of Bowman and Stroud [8]. Four grams of 10% palladium on carbon was added to a mixture of 21 g of IPE, 50 ml of formaldehyde (40%), and 20 ml of formic acid in a hydrogenator bottle. Hydrogen gas was added at 60 psi and left overnight. The mixture was suctioned and filtered; 36 ml of concentrated HCl was then added to the filtrate. After remaining at room temperature for 15 min, the mixture was concentrated by means of a rotary evaporator and 28 ml of a 50% NaOH solution was added to the residue. The mixture was extracted three times with 200 ml of ether; the ether was removed and the residual oil was repeatedly extracted with hexane until homogeneous as determined by thin-layer chromatography. Both the IPE and methyl-IPE were converted to the hydrochloride.

Charles River (CD strain, males, 250–300 g) rats were maintained on a Purina Lab Chow diet until the night before injecting IPE or methyl-IPE. All rats were then fasted throughout the experimental period. Animals were injected daily with the amino alcohol analogs for 4 days (20 mg/100 g body weight intraperitoneally). The phosphatidyl-IPE and phosphatidylmethyl-IPE were isolated from rat liver microsomes as reported earlier [1].

Microsomal phospholipids were analyzed by 2-dimensional thin-layer chromatography; first in system I, chloroform–methanol–acetic acid (50:25:8 v/v) and then, after drying the plate for 1 hr in a vacuum desiccator, in system II, chloroform–methanol–ammonium hydroxide (65:35:5 v/v). Each spot visualized after spraying with concentrated H_2SO_4 and charring at 180–200°C was analyzed for phosphorus [9].

Phospholipase C (EC 3.1.4.3) (*Bacillus cereus*, from Grand Island Biological Corp.) hydrolysis of the phospholipids was done as reported elsewhere [10]. After extraction of the diacylglycerols and any unreacted phospholipid by the method of Bligh and Dyer [11], the aqueous methanol layer containing the phosphobases was lyophilized and then analyzed by nuclear magnetic resonance spectroscopy. NMR spectroscopic data of the phosphobases were obtained in the FT mode on two different samples in D_2O solution ($\delta_{DSS} = 0$ ppm). For the first sample 6370 scans were collected and for the second sample 4000 scans were collected. The delay time between data acquisition was 6 sec.

In the labeling studies, fasted rats were injected intraperitoneally with IPE (20 mg/100 g body weight) daily for 3 days. On the second and third day, the rats were injected intraperitoneally with 10 μCi of [$^{14}CH_3$] methionine (specific activity, 48.36 mCi/mmol in 100 μl of ethanol–water (7:3), New England Nuclear). One hour after the last ^{14}C injection, the rats were sacrificed and the microsomal phospholipids isolated as described above. The phospholipids were separated by thin-layer chromatography in System I and the distribution of radioactivity determined from zonal scans by liquid scintillation spectrometry [12].

III. Results and discussion

Nuclear magnetic resonance demonstrated that the hydrocarbon portion of the

phosphobase released after phospholipase C hydrolysis of the unidentified phospholipid isolated from the IPE-injected rats was identical to authentic methyl-IPE. Each spectrum showed a broadened methyl doublet at 1.33 ppm and an *N*-methyl singlet at 2.70 ppm. Other unresolved weak multiplets were visible in the regions near 3.3 and 3.6 ppm (corresponding to a *N*-CH) and near 3.9 ppm (corresponding to an *O*-CH_2).

Analysis of the phospholipids from livers of rats administered the IPE and [$^{14}CH_3$] methionine demonstrated that a significant amount of label chromatographed with the phosphatidylmethyl-IPE. Under these conditions, the percentage of ^{14}C in the various lipid classes was: lysophosphatidylcholine, 1.8%; sphingomyelin, 5.4%; phosphatidylcholine, 83.5%; and phosphatidylmethyl-IPE, 9.1%. These data indicate that phosphatidyl-IPE is methylated in the rat liver to form phosphatidylmethyl-IPE by a pathway similar to that responsible for the synthesis of phosphatidylcholine from phosphatidylethanolamine [13]. The phosphatidylmethyl-IPE isolated from rats injected intraperitoneally with methyl-IPE was also found to chromatograph in both system I and system II with the previously unidentified phospholipid (phosphatidylmethyl-IPE) isolated from rats injected with IPE. The per cent of each phospholipid class in liver microsomes after the injections of methyl-IPE was: phosphatidylcholine 41%, phosphatidylethanolamine 33%, phosphatidylmethyl-IPE 19%, sphingomyelin 4.5%, and phosphatidylserine/phosphatidylinositol 3.0%.

The results of this study demonstrate that IPE can cause two modifications in membrane phospholipids in rat liver microsomes: first phosphatidyl-IPE is formed via incorporation of the intact amino alcohol analog and secondly phosphatidylmethyl-IPE is formed by *N*-methylation via methyl transfer from methionine. The effect that such modifications in polar head groups of membrane phospholipids have on membrane-bound enzyme properties, cell cycle, and cell transformation is under investigation in our laboratory.

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