Journal of Chromatography, 343 (1985) 138—142
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2671

Note

Gas chromatographic—mass spectrometric analysis of biologically active phospholipids having an *sn*-2-acetyl group

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(First received January 16th, 1985; revised manuscript received April 15th, 1985)

Platelet activating factor, a mediator of inflammatory and allergic responses, has been shown to be a molecular species of phosphatidylcholine with the unique structure 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine [1, 2]. Subsequent studies on the structure–activity relationships of this interesting phospholipid revealed that the *sn*-2-acetyl group is important for its activity: a variety of *sn*-2-acetyl phospholipids were found to have biological activities [3—10].

The *sn*-2-acetyl group in phospholipids has been analysed by enzymatic hydrolysis of the phospholipids with phospholipase C and then gas chromatography—mass spectrometry (GC–MS) of the dephosphorylated products after derivatization [4, 11]. However, this method has the disadvantage that phospholipase C shows substrate specificity. Acetolysis has also been used as a convenient method for analysis of molecular species of phospholipids

0378-4347/85/$03.30

[12—14]. Unfortunately, acetolysis could not be used to characterize *sn*-2-acetyl-phospholipids, because *sn*-2-acetyl-phospholipids and their lyso-derivatives are converted into the same compounds on acetolysis. Therefore, we modified the acetolysis procedure. In the modified procedure, *sn*-2-acetyl-phospholipids were heated in a mixture of propionic acid and propionic anhydride, and then the reaction products were analysed by GC—MS.

METHODS

1-Palmitoyl-2-lyso-*sn*-glycero-3-phosphocholine (16:0-GPC) was purchased from Sigma (St. Louis, MO, U.S.A.). 1-O-Hexadecyl-2-lyso-*sn*-glycero-3-phosphocholine (O-16:0-GPC) and 1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (O-16:0-2:0-GPC) were obtained from Bachem Feinchemikalien (Bubendorf, Switzerland). 1-Palmitoyl-2-acetyl-*sn*-glycero-3-phosphocholine (16:0-2:0-GPC) was prepared by the reaction of 16:0-GPC with acetic anhydride, as previously described [7]. 1-Palmitoyl-2-lyso-*sn*-glycero-3-phosphate (16:0-GP), 1-palmitoyl-2-acetyl-*sn*-glycero-3-phosphate (16:0-2:0-GP), 1-O-hexadecyl-2-lyso-*sn*-glycero-3-phosphate (O-16:0-GP) and 1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphate (O-16:0-2:0-GP) were prepared by hydrolysis of the corresponding choline phospholipids, as described previously [7]. 1-O-Hexadecyl-2-acetyl-*sn*-glycero-3-phosphoethanol (O-16:0-2:0-GPE) and 1-palmitoyl-2-acetyl-*sn*-glycero-3-phosphoethanol (16:0-2:0-GPE) were prepared from O-16:0-2:0-GPC and 16:0-2:0-GPC, respectively, by the transphosphatidylation reaction with phospholipase D [7].

Phospholipids (10– 100 μg) were heated in 0.5 ml of a solution of propionic acid—propionic anhydride (3:2, v/v) at 130°C for 4 h. The reaction was stopped by addition of 1 ml of distilled water, and lipids were extracted by the method of Bligh and Dyer [15]. The lipid extracts were dissolved in a small volume of ethyl acetate, and aliquots of the solutions were injected into a gas chromatograph in combination with a JEOL JMS-D300 mass spectrometer. The lipids were separated in a 2 m × 2 mm I.D. glass column packed with 2% OV-17 on Chromosorb W (AW, DMCS, 80—100 mesh) at a column temperature of 240°C, an injection temperature of 270°C and a separator temperature of 270°C. Helium gas was used at 1.5 bar/cm^2. The electron impact (EI) mass spectrometer was operated at an ionizing potential of 20 eV, an ionizing current of 300 μA, an accelerating voltage of 3.0 kV and an ion source temperature of 260°C. Chemical ionization (CI)-MS with ammonia gas was performed under the same conditions as those for EI-MS except that the ionizing potential was 200 eV.

RESULTS AND DISCUSSION

16:0-2:0-GP was heated in propionic acid– propionic anhydride and the reaction product was analysed by GC—MS as described. The peak obtained at 6.7 min was due to 1-palmitoyl-2-acetyl-3-propionyl-*sn*-glycerol (16:0-2:0-3:0), because the expected fragment ions were seen in its EI mass spectrum (Fig. 1A) as follows: *m/z* 368, $[M - CH_3COOH]^{+\cdot}$; *m/z* 354, $[M - CH_3CH_2COOH]^{+\cdot}$; *m/z* 239, $[CH_3(CH_2)_{14}CO]^{+}$; *m/z* 173, [M —

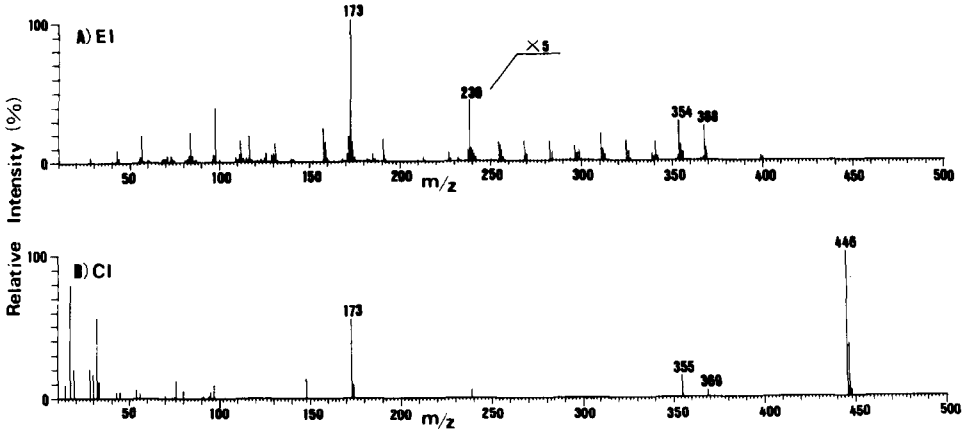

Fig. 1. Typical EI (A) and CI (B) mass spectra of the propionolysis product of 16:0-2:0-GP.

$CH_3(CH_2)_{14}COO]^{+\cdot}$; *m/z* 131, $[CH_3CH_2CO + 74]^{+\cdot}$; *m/z* 117, $[CH_3CO + 74]^{+\cdot}$; *m/z* 57, $[CH_3CH_2CO]^+$ and *m/z* 43, $[CH_3CO]^+$. CI-MS with ammonia gas gave additional proof of the structure of this reaction product (Fig. 1B), where $[M] \cdot NH_4^+$(*m/z* 446) is a base peak. The fragment ions at *m/z* 355 and 369 are assignable to $[M] \cdot H^+ - CH_3CH_2COOH$ and $[M] \cdot H^+ - CH_3COOH$, respectively. The signal at *m/z* 173 would be due to the ion produced by elimination of the palmitic acid moiety from the protonated molecular ion. It was found by GC—MS analysis that 16:0-2:0-GPE and 16:0-2:0-GPC, like 16:0-2:0-GP, were converted into 16:0-2:0-3:0 during heating in propionic acid—propionic anhydride. Thus, this method, tentatively named "propionolysis" can be used to identify *sn*-2-acetyl groups in phospholipids with different head groups.

Next, we examined the reaction products formed by propionolysis of 1-O-hexadecyl-2-acetyl-*sn*-glycero-phospholipids possessing much stronger biological activity than the corresponding 1-palmitoyl-2-acetyl analogues. A peak was seen at 5.4 min in gas chromatograms of the propionolysis products from O-16:0-2:0-GP, O-16:0-2:0-GPE and O-16:0-2:0-GPC, respectively. The EI and CI mass spectra of the products from O-16:0-2:0-GP are shown in Fig. 2. The CI spectrum gave a protonated molecular ion with significant intensity (*m/z* 415) as well as $[M] \cdot NH_4^+$ (*m/z* 432). These data indicate that 1-O-alkyl-2-acetyl-phospholipids were dephosphorylated during heating in propionic acid—propionic anhydride, resulting in formation of 1-O-hexadecyl-2-acetyl-3-propionyl-*sn*-glycerol (O-16:0-2:0-3:0). It should be mentioned that in the CI spectrum of O-16:0-2:0-3:0, the relative intensities of the ions produced by loss of a short-chain carboxylic acid (*m/z* 341 and 355) were more than that of the ion elicited by elimination of the *sn*-1 long-chain hydrocarbon moiety (*m/z* 173), whereas in the CI spectrum of 16:0-2:0-3:0, the reverse was observed. In the EI spectrum of O-16:0-2:0-3:0, the expected ion signals at *m/z* 340 and 354, due to $[M - CH_3CH_2COOH]^{+\cdot}$ and $[M - CH_3COOH]^{+\cdot}$, respectively, could be seen, although the relative intensities of fragment ions with high mass numbers were low in this spectrum. Other representative fragment ions at *m/z* 173, 131, 117, 57 and 43 would be the same as those in the EI spectrum of 16:0-2:0-3:0. The fragment ions at *m/z* 225 and 255 seem to be characteristic

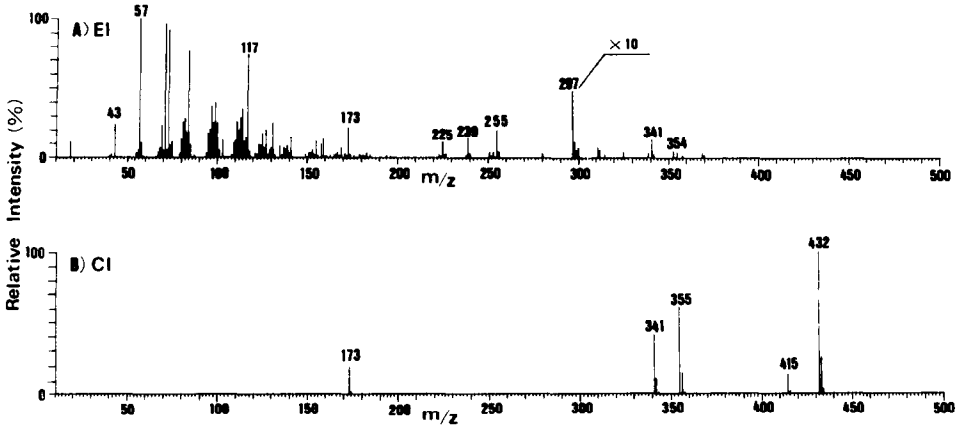

Fig. 2. Typical EI (A) and CI (B) mass spectra of the propionolysis product of O-16:0-2:0-GP.

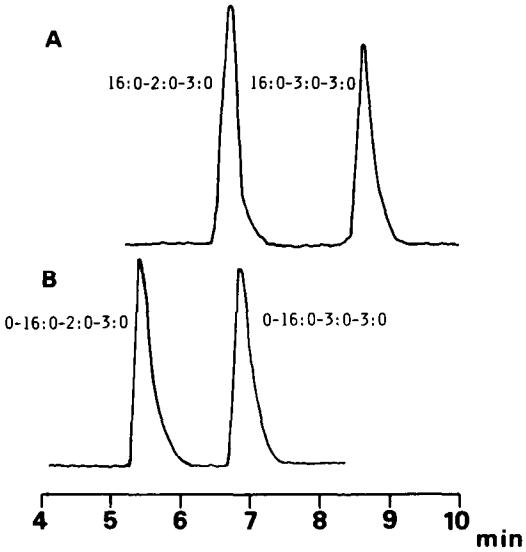

Fig. 3. Gas chromatograms of the propionolysis products of mixtures of 16:0-2:0-GPC and 16:0-GPC (A) and O-16:0-2:0-GPC and O-16:0-GPC (B).

of alkyl ether type glycerides, and can be assigned to $[CH_3(CH_2)_{15}]^{+\cdot}$ and $[CH_3(CH_2)_{15}OCH_2]^{+\cdot}$, respectively. Thus, the fragmentation patterns induced by electron impact of O-16:0-2:0-3:0 seem to be similar to those of 16:0-2:0-3:0.

When 1-acyl-lysophospholipids such as 16:0-GP, 16:0-GPE and 16:0-GPC were subjected to propionolysis, they were converted into 1-palmitoyl-2,3-dipropionyl-*sn*-glycerol (16:0-3:0-3:0). Similarly, 1-O-alkyl-lysophospholipids (O-16:0-GP, O-16:0-GPE and O-16:0-GPC) were degraded to 1-O-hexadecyl-2,3-dipropionyl-*sn*-glycerol (16:0-3:0-3:0). These glycerides were separated well from 16:0-2:0-3:0 and O-16:0-2:0-3:0, respectively, by GC. Typical gas chromatograms of these glycerides are presented in Fig. 3. It is easy to distinguish *sn*-2-acetyl-3-propionyl-glycerides from *sn*-2,3-dipropionyl-

glycerides, because the mass spectra of 1-acyl- and 1-O-alkyl-3:0-3:0 lack the fragment ion of $[M - CH_3COOH]^{+\cdot}$ in the EI spectra and of $[M] \cdot H^+ - CH_3COOH$ in the CI spectra. For example, the ions at *m/z* 368 and 354 corresponding to $[M - CH_3COOH]^{+\cdot}$ and $[M - CH_3CH_2COOH]^{+\cdot}$, respectively, were observed in the EI spectrum of 16:0-2:0-3:0. In the EI spectrum of 16:0-3:0-3:0 the ion corresponding to $[M - CH_3CH_2COOH]^{+\cdot}$ was seen at *m/z* 368, but no significant ion was observed at *m/z* 354.

In summary, the procedure of propionolysis and subsequent analysis of the reaction products by GC—MS is useful for demonstrating the presence of *sn*-2-acetyl groups in phospholipids. CI-MS with ammonia gas gave satisfactory mass spectra, which can be used to determine the structure of propionolysis products derived from different types of parent phospholipid.

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