Fast Atom Bombardment Mass Spectrometry of Underivatized Phosphatidylcholines, Lysophosphatidylcholines, and Diglycerides

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Fast atom bombardment mass spectrometry was used for the direct semi-quantitative analysis of underivatized biological mixtures of phosphatidylcholines, lysophosphatidylcholines, and diglycerides.

Analysis of complex lipids from biological sources has for the most part required derivatization followed by analysis of the derivative mixtures.[1] We report the use of fast atom bombardment (FAB) mass spectrometry[2] for direct semi-quantitative analysis of three lipid classes. From an analytical point of view, FAB analysis of lipids offers a number of important advantages: (i) analysis is done directly on lipids without derivatization; (ii) lipids with high molecular weights, which include most complex lipids, can be analysed directly (for example, m/z 810 was detected in this study, and based on our studies with peptides, masses of several thousand can be measured); (iii) it gives the molecular weight of each molecular species; however, each molecular weight may represent more than one molecular species; (iv) based on work by Katakuse and Desiderio[3] on FAB spectra of peptides, molecular structural information is available by FAB-collision-induced-decomposition followed by linked-scanning (B/E); (v) computerized identification and quantitation are possible; (vi) finally, FAB lipid analysis and h.p.l.c. are complementary methods.

Phosphatidylcholine (PC) was used to generate lysophosphatidylcholine (LPC) mixtures and diglyceride (DG) mixtures by hydrolysis with phospholipases A_2 and C, respectively. The FAB mass spectra of these mixtures are shown in Figure 1. We have written a computer program to identify species of each class by total acyl chain length and total carbon–carbon double bonds.

In Figure 1A m/z 760 and 758 ions were identified as protonated molecular ions $(M + 1)^+$ of PC 34:1 (total acyl chain length:total C=C double bonds) and 34:2, respectively, and correspond to 47.8 and 27.7% of total relative intensities, respectively. Masses 788, 786, 784, 782, and 810 correspond to PC 36:1, 36:2, 36:3, 36:4, and 38:4, respec-

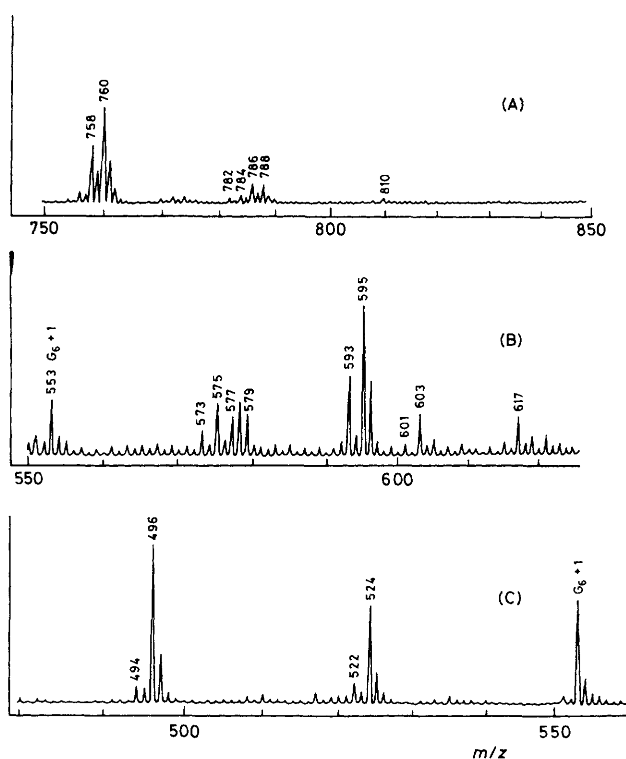

Figure 1. FAB mass spectra of lipid mixtures. (A): Phosphatidylcholines from an egg yolk. (B): Diglycerides. (C): Lysophosphatidylcholines.

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tively, with percentages of total relative intensity ranging from 2 to 10%.

Gas chromatographic analysis of the methyl esters of the PC showed the following quantitative composition: 16:0 (42.2%); 16:1 (1.6%); 18:0 (11.3%); 18:1 (27.6%); 18:2 (14.0%); 20:4 (2.7%); 22:6 (0.4%). The FAB analysis of PC was used to predict the fatty acid composition as follows. Based on the fatty acids present in the original sample, PC 34:1 can only contain 16:0/18:1 and/or 16:1/18:0. Thus the minimum contribution by PC 34:1 to the percentage of 16:0 in fatty acid analysis is (%PC 34:1)/2 −%16:1 or 22.3%. Also, from the fatty acids present, PC 34:2 can only contain 16:0/18:2 and 16:1/18:1. By a similar calculation, the minimum contribution by PC 34:2 to the 16:0 is 13.9%. The only other PC, found by FAB, that could contain 16:0 is PC 36:4, a minor component (1.8%), which would contribute a maximum of 0.9% to the total 16:0 content. Thus FAB PC composition predicts the 16:0 content of the sample to be (22.3 + 13.9 + 0.9) = 37.1%, compared to 42.2% determined by methyl ester analysis. Taking into account the fatty acid analysis, PC 36:3, 36:1, and 38:4 could only be 18:1/18:2, 18:0/18:1, and 18:0/20:4, respectively. PC 36:2 could be 18:0/18:2 and/or 18:1/18:1. For the purpose of accounting for the fatty acid analysis, rather than being an unequivocal analysis, the calculated amounts of other fatty acids are: calculated (actual) 16:1, 1.6 (1.6); 18:0, 9.9 (11.3); 18:1, 31.1 (27.6); 18:2, 18.6 (14.0); 20:4, 1.8 (2.7); 22:6, 0 (0.4).

The diglycerides exhibited FAB-induced dehydration in the mass spectrometer such that in most cases both $(M + 1)^+$ and $(M + 1 - H_2O)^+$ were present. For example, the two major species, DG 34:1 and 34:2, gave the pairs of ions 595/577 and 593/575, respectively (Figure 1B).

The FAB spectrum of LPC (Figure 1C) was simple as expected. Masses 494, 496, 522, and 524 correspond to LPC 16:1, 16:0, 18:1, and 18:0, respectively.

As can be seen, background noise was very low in the mass range used (480—850 a.m.u.) except when low amounts ($\leqslant 10\ \mu g$) of sample were used (Figure 1B). Polymers of glycerol provide convenient mass markers at 92 a.m.u. intervals according to the formula $(\text{glycerol})_n + 1$. Relatively low background in this region makes it probable that a total mixture containing all three lipid classes would have given a composite of spectra 1A + 1B + 1C with no overlap amongst the classes. The results also indicate that there is no molecular fragmentation that would interfere with analysis of PC and LPC. Dehydration of diglycerides does not present a problem for diglyceride analysis. One peak in a FAB spectrum may or may not contain more than one molecular species; however, a recent development in high performance liquid chromatography (h.p.l.c.), by Patton and co-workers, makes it hopeful that such mixtures can be resolved.[4] These workers resolved rat liver PC into 28 h.p.l.c. peaks and found by fatty acid analysis of each peak that only 6 contained two molecular species and none contained more than two molecular species.

To our knowledge this is the first report of FAB spectra of these lipid classes. Rinehart has recently reported the FAB spectrum of lactosylceramide.[5] We believe that the ability to analyse complex mixtures of underivatized and normally non-volatile lipids with high molecular weights, namely PC, LPC, and DG, by FAB represents a major methodological advancement for the lipid chemist and the biochemist. Both platelet-activating-factor[6] and anti-hypertensive renomedullary polar lipids[7] are known to be *O*-alkyl-2-acetyl-3-phosphorylcholines and the latter has been analysed by FAB.[8]

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