Combined Gas Chromatography-Mass Spectrometry of Trimethylsilyl Deacylated Cardiolipin from Rat Brain

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The presence of phospholipids in high concentration in brain has led to considerable speculation regarding their role in neuronal activity. However, the quantitative analysis of brain phospholipids presents a number of problems, not the least of which is the difficulty encountered in separating the lipids from additional organic contaminants in the extract. Although the latter problem may be overcome by employing two-dimensional thin layer chromatography of the intact phospholipids (Rouser, et al., 1968) or ion-exchange chromatography of the deacylated lipids (Dittmer and Douglas, 1969), these methods are cumbersome, time consuming and the assay method (phosphorous determination) lacks specificity. A recent paper from our laboratory (Cicero and Sherman, 1971) reported a method for the identification and measurement of mono-, di- and triphosphoinositide in rat brain by combined gas chromatography-mass spectrometry[1]. In the present paper we wish to report that we have identified and measured cardiolipin, designated as an unknown in our previous report (Cicero and Sherman, 1971), in rat brain by combined GC-MS.

METHODS

Lipid Extraction: The brains of 4 month old rats were rapidly removed following decapitation and frozen in liquid nitrogen. The tissue was weighed while frozen and MPI, cardiolipin, DPI and TPI were extracted as described previously (Cicero and Sherman, 1971). The extracted lipids were then deacylated and converted to their trimethylsilyl derivates with

[1]Abbreviations used: MPI, DPI and TPI: mono-, di- and triphosphoinositide, respectively; GC: gas chromatography; MS: mass spectrometry; TMS: trimethylsilyl.

bis(trimethylsilyl) trifluoroacetamide (+1% trimethylchlorosilane):dry pyridine (2:1, v/v); cardiolipin standards were prepared from commercially available cardiolipin (General Biochemicals) which was first deacylated and then trimethylsilylated (Cicero and Sherman, 1971). Gas chromatograms were obtained on a 2'x 1/4" glass U tube packed with 1% SE-30 on Gas Chrom Q (Applied Science Labs). To obtain optimal separation as the TMS-phospholipids, the GC was temperature programmed from 180° C to 240°C at a rate of 4° C/min. Mass spectra were obtained on a LKB-9000 GC-MS according to procedures described elsewhere (Sherman and Zinbo, 1970).

Results and Discussion

The GC retention time, relative to MPI, for TMS deacyl cardiolipin was 1:38; for TMS deacyl DPI, 1.62 and for TMS deacyl TPI, 2.12. The concentration of cardiolipin in brain (μmoles/g wet weight), determined by a comparison of chromatographic peak heights with a cardiolipin standard, was calculated as 0.62 μmoles/g. The yield of deacyl cardiolipin from the intact phospholipid was quantitative under the conditions of methanolysis used in these experiments. The latter was established by measuring phosphatidic acid, the major breakdown product resulting from the cleavage of the phosphodiester linkage in cardiolipin. Only trace amounts (<0.5%) of phosphatidic acid were found confirming that the procedure used in the present study results in the complete deacylation of phospholipids without significant further hydrolysis of the phospholipid. The concentrations of cardiolipin in brain reported here are in excellent agreement with those determined by Wells and Dittmer (1967) who report values ranging between 0.58-0.63 μmoles/g in rats of approximately the same age.

The mass spectra of standard TMS deacyl cardiolipin and that of cardiolipin extracted from rat brain are shown in Figure 1. It is readily apparent that the mass spectra are virtually identical. Although the molecular ion (M^{+} = 904) is not present the molecular weight of TMS deacyl cardiolipin is confirmed by the presence of several characteristic ions

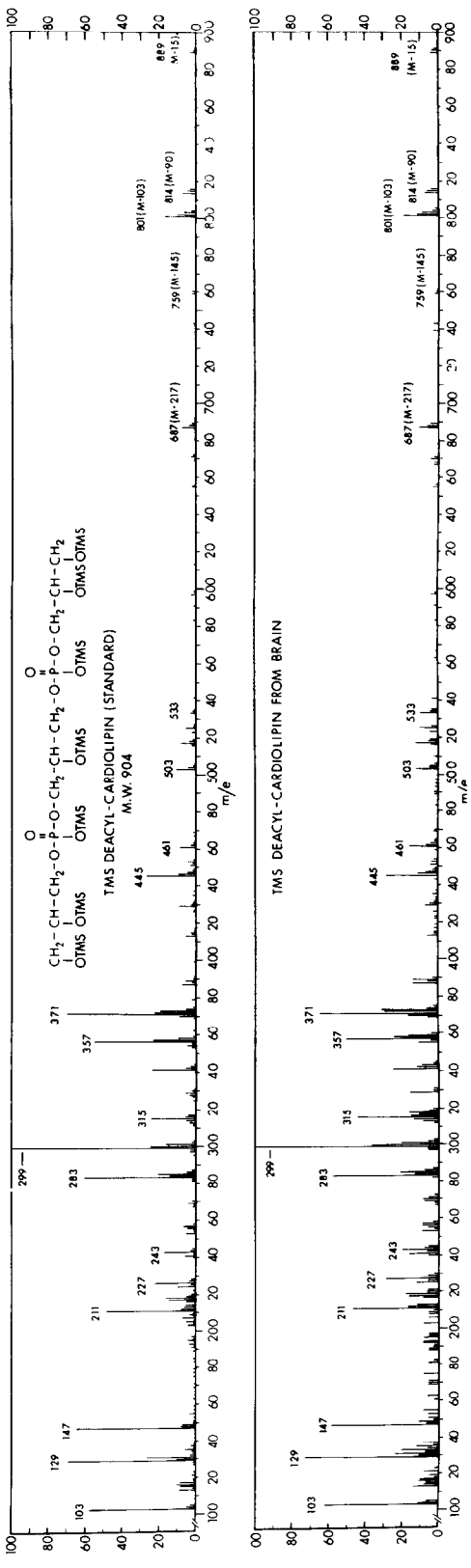

Fig. 1. Mass spectra of a trimethylsilyl (TMS) deacyl cardiolipin standard (top) and TMS deacyl cardiolipin extracted from rat brain (bottom). Ion abundances relative to the base peak, m/e 299, are plotted against the m/e values.

formed by the loss of $CH_3\cdot$(M-15), TMSOH (M-90) and $TMSOCH_2\cdot$(M-103), which were confirmed by labelling with TMS-d_9 groups (McCloskey et al., 1968). The base peak m/e 299 has the structure $[(TMSO)_2PO_2SiMe_2)]^+$, which was confirmed upon labelling with TMS-d_9 groups by a mass shift to m/e 323. The fairly intense ions at m/e 461 $[TMSOCH_2CH(OTMS)CH_2OP(OH)-(OTMS)_2]^+$ and m/e 533 $[TMSOCH_2CH(OTMS)CHOP(OTMS)_3]^+$, whose structures were confirmed upon labelling with TMS-d_9 groups by mass shifts to 497 and 578, respectively, seem to be diagnostic ions for compounds with the terminal sequence $ROPO(OTMS)OCH_2CH(OTMS)CH_2OTMS$ since they appear in a wide range of glycero-phospholipids (Cicero and Sherman, 1971; Duncan et al., 1971). These highly rearranged ions are the counterparts in this series of m/e 315 and m/e 387, respectively, in the spectra of TMS phosphorylated sugars (Zinbo and Sherman, 1970).

It is interesting to note the absence of M-105 (M-TMSOH and $CH_3\cdot$) in the mass spectrum of TMS deacyl cardiolipin. The absence of M-105 is also observed in the spectrum of TMS deacyl TPI, but ions formed by the loss of M-105 are present in TMS deacyl DPI (1.69%) and TMS deacyl MPI (2.13%). In addition the loss of TMSOH and $CH_3\cdot$ is a common event in the mass spectra of TMS derivatives of many phosphorylated sugars. For example, the amounts of M-105 relative to the base peak in the spectra of several carbohydrates follows: TMS glucose-6-P (2.4%), TMS ribose-5-P (5%), TMS fructose-6-P (5%), TMS 6-phosphogluconic acid (6%), (Zinbo and Sherman, 1970); TMS myo-inositol-1-P (4%), (Sherman, Stewart and Zinbo, 1969). It seems logical that, with fewer opportunities to eliminate trimethylsilanol due to increased substitution of TMS deacyl cardiolipin and TMS deacyl triphosphoinositide, the degree of elimination of TMSOH would decline, as observed.

The method reported in this paper appears to offer a simple, highly sensitive and reliable means of simultaneously measuring a number of phospholipids in a single brain extract. The sensitivity of the present method should permit the determination of phospholipids in milligram amounts of tissues.

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