A fast atom bombardment-mass spectrometric method to quantitate lysophosphatidylserine in rat brain

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Abstract A method to quantitate lysophosphatidylserine by fast atom bombardment-mass spectrometry using 1-hexadecanoyl-*sn*-glycero-3-phospho-L-serine as internal standard is described. The standard curve is linear with a correlation coefficient r^2 = 0.999 from 10 to 1000 ng. This curve has been used to quantitate LPS in rat brain using phosphorus assay as a test control. We found 475 ± 70 ng of LPS in 1 mg of tissue (n = 3). This method presents advantages due to its sensitivity and its capability to give molecular information of the unmodified compound. —**Benfenati, E., G. De Bellis, S. Chen, L. Bettazzoli, R. Fanelli, M. T. Tacconi, G. Kirschner, and G. Pege.** A fast atom bombardment-mass spectrometric method to quantitate lysophosphatidylserine in rat brain. *J. Lipid Res.* 1989. **30:** 1983–1986.

Supplementary key words cortex • 1-octadecanoyl-*sn*-glycero-3-phospho-L-serine

Considerable evidence has been accumulated concerning the important role of phosphatidylserine (PS) in membrane function. In recent years, bovine cortical PS has aroused interest on account of its potential as a therapeutic agent (1). Several pharmacological effects have been identified. These include the redistribution of carbohydrate reserves (2), increased catecholamine turnover in the hypothalamus (3), release of acetylcholine from the brain cortex (4), and reduction of brain energy metabolism (5). Further studies indicated that PS may not be active by itself. A phospholipase-mediated conversion to lysophosphatidylserine (LPS) occurs and the lysoderivative, a less abundant lipid class, may be the active metabolite (6).

Interest has thus focused on developing a sensitive and specific method to detect nanograms of LPS in biological samples. A procedure of quantitating LPS by high performance liquid chromatography gave unsatisfactory results (7). Mass spectrometry has played an important role in qualitative and quantitative analysis of phospholipids (8). Since its first appearance (9), fast atom bombardment (FAB) mass spectrometry has been developed as a powerful tool for analyzing intact phospholipids. It has been demonstrated that the relative intensities of analyte-related ions obtained by FAB mass spectrometry are as reproducible as electron impact mass spectrometry (10). Although the sensitivity of FAB cannot yet compete with gas-liquid chromatography-mass spectrometry, this technique does present some advantages: *1*) avoidance of working procedures requiring chemical modification of the compounds of interest for gas-liquid chromatographic analysis and the selection of optimal gas-liquid chromatographic conditions; and *2*) the shorter measurement time. Published reports have clearly stated that it is feasible to use FAB for the quantitation of underivatized phospholipids if abundant and steady ions, characteristic of the analyte of interest and of the internal standard, can be obtained (11–13).

In this report, we describe a method for quantitative analysis of LPS 18:0 by negative ion FAB mass spectrometry, using LPS 16:0 as internal standard, and its application to the quantification of LPS in cortex of rat brain.

MATERIALS AND METHODS

Analytical grade solvents obtained from commercial sources were used. TLC plates, silica gel 60, with concentration zone were from Merck (Darmstadt, F. R. G.). LPS (synthetic 16:0; 18:0 obtained with phospholipase A_2 from bovine brain PS) were from Fidia Research Laboratories, Abano Terme, Italy. Diethanolamine was purchased from Fluka Chemie AG, Buchs, Switzerland.

Abbreviations: FAB, fast atom bombardment; LPS, lysophosphatidylserine; PS, phosphatidylserine; TLC, thin-layer chromatography.

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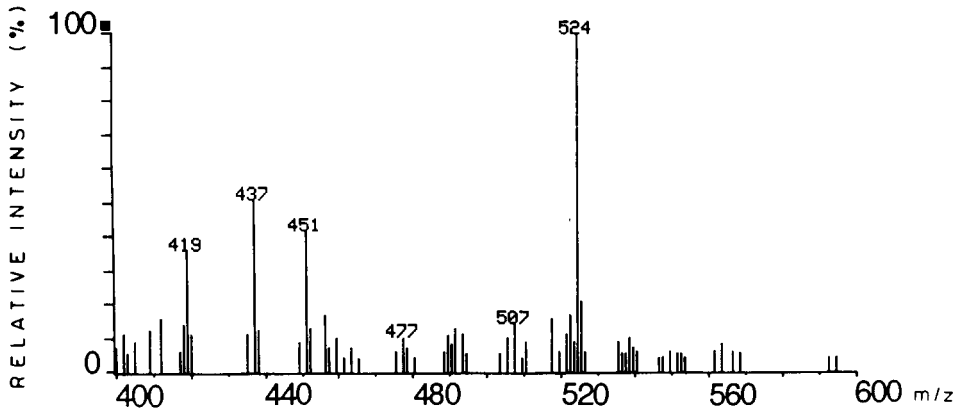

Fig. 1. Negative ion FAB mass spectrum of rat brain cortical extract after purification without addition of LPS 16:0 as internal standard. The ion at *m/z* 524 corresponds to the $[M-H]^-$ ion of LPS 18:0. About 400 ng of LPS was used.

Mass spectrometry

FAB mass spectrometry was performed on a VG 70-250 with standard FAB gun and target operated at 8 kV (1mA). Xenon was used as bombarding gas. All analyses were carried out in negative mode with the source at room temperature and 6 kV acceleration voltage. Diethanolamine was used as liquid matrix and applied on the surface of the target probe. The samples were dissolved in chloroform-methanol-water 10:1:0.1 and from 10 to 1000 ng of LPS standard with 250 ng of LPS 16:0 as internal standard were transferred over the surface of diethanolamine without mixing. About 40 scans were acquired from *m/z* 450 to *m/z* 600 at 4 s per decade and the peak heights of the $(M-H)^-$ ions for LPS 18:0 (*m/z* 524) and LPS 16:0 (*m/z* 496) were measured. Average values from 30 scans (from 5th to 35th) of absolute peak heights were used to plot the calibration curve.

Preparation of biological samples

Lipids from rat brain (cortex) were extracted with chloroform-methanol-HCl 10 M in water 100:50:1, according to Folch, Lees, and Sloane Stanley (14). Portions of lipid extract corresponding to 40 mg of fresh tissue, to which 25 μg of LPS 16:0 was added as internal standard, were spotted on TLC plates which were then developed with chloroform ethanol triethylamine water, 30:34:35:8. TLC fractions corresponding in R_f to authentic LPS were extracted with methanol and the extracts were diluted in 100 μl of methanol and used for FAB analysis. Each determination was repeated three times using 2 μl of the solution.

Portions corresponding to 10 mg of fresh tissue, without the internal standard, run on TLC and extracted as described above, were used for phosphorus assay after acidic digestion (15). When an authentic LPS atandard was chromatographed in parallel, we measured, after extrac-

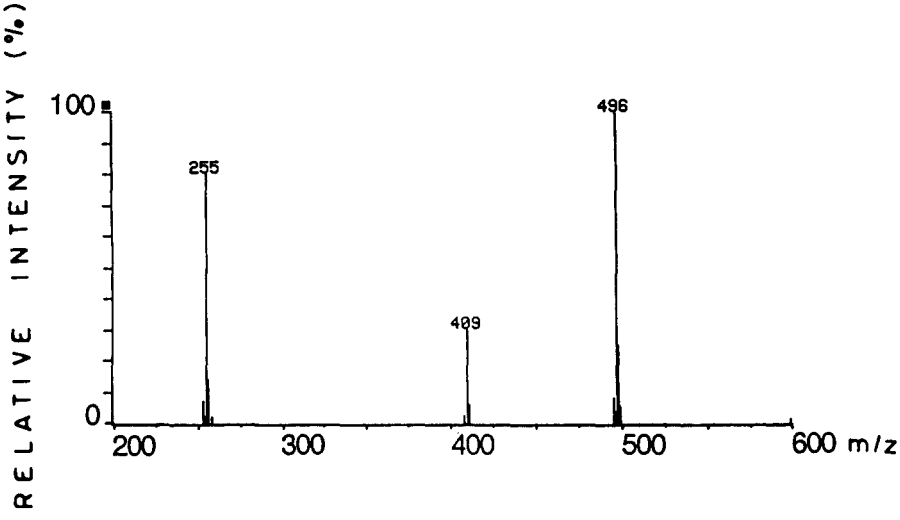

Fig. 2. Negative ion FAB mass spectrum of LPS 16:0. About 1 μg of LPS was used.

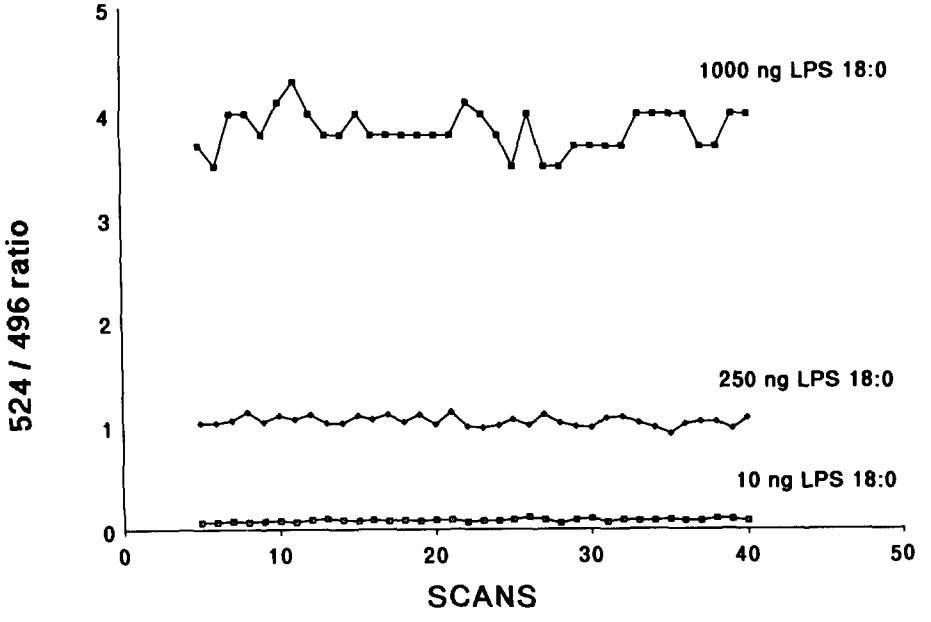

Fig. 3. Absolute intensity ratio (LPS 18:0/16:0) during 40 scans at different concentrations (250 ng of LPS 16:0 added).

tion from silica gel, a recovery of 85-90% as inorganic phosphorus.

RESULTS

Rat brain PS does not contain more than 1% of palmitic acid at the *sn*-1 position (16); it may therefore be assumed that rat brain LPS is also very poor in the 16:0 species. In fact, no significant signals corresponding to LPS 16:0 were found in negative ion FAB mass spectra of rat brain cortex, the main species being LPS 18:0 (**Fig. 1**). For this reason we decided to use LPS 16:0 as internal standard. **Fig. 2** presents the negative ion mass spectrum of LPS 16:0.

Considering the mass spectrometric properties of LPS 16:0 and 18:0, we found that the ratio of the ion current of ions 524 (for LPS 18:0) and 496 (for LPS 16:0) was stable in a wide range of sample concentrations (**Fig. 3**), and that the standard curve for LPS 18:0 was linear with a correlation coefficient r^2 = 0.999 from 10 to 1000 ng (**Fig. 4**). We observed a signal-to-noise ratio of 10 when we placed 10 ng of LPS standard on the FAB target.

Using the standard curve of LPS 18:0, we measured LPS content in rat brain from three animals; in cortex we found 475 ± 70 ng/mg fresh tissue of LPS 18:0. This value is close to that obtained by phosphorus assay (597 ± 79 ng/mg fresh tissue) on the same brain extract after similar TLC separation, as described in the Methods section. By FAB analysis, a lower amount of LPS is expected because some minor species of LPS are globally quantified only with the phosphorus assay, but not with our FAB method.

Another difference between phosphorus and FAB analyses is the sensitivity. As reported in the Methods section,

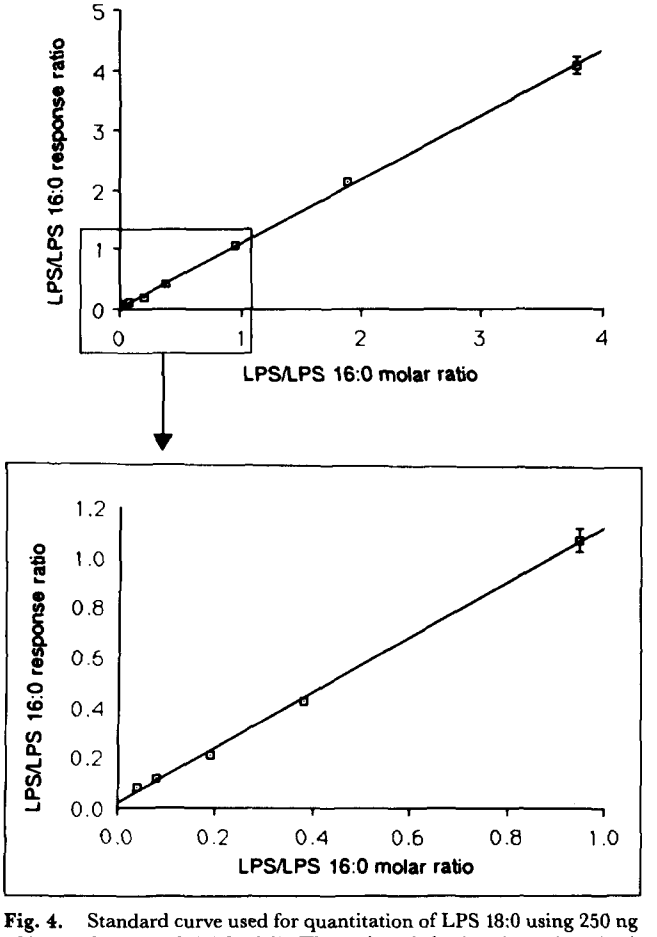

Fig. 4. Standard curve used for quantitation of LPS 18:0 using 250 ng of internal standard (LPS 16:0). The ratios of absolute intensity of *m/z* 524 (LPS 18:0) and *m/z* 496 are plotted against molar ratio of LPS 18:0/LPS 16:0. Error bars are smaller than symbols if not shown. Each determination was repeated three times and the mean value of ratios was plotted. The linear equation obtained was y = 0.0381 + 1.0737x with r^2 = 0.999.

we used the equivalent of 0.8 mg of fresh tissue to perform a single FAB analysis. On the other hand, the larger amount (10 mg) we used for the phosphorus assay was the minimum necessary to detect LPS.

DISCUSSION

Quantitation of LPS by negative ion FAB mass spectrometry offers several advantages. *1*) It appears to be at least 10 times more sensitive than phosphorus assay, so it may be suitable for the analysis of smaller samples. *2*) It is based on the direct intensity measurement of the molecular ion of the compound of interest, so it is more specific than a phosphorus assay. *3*) It gives direct information on the molecular species, without further analysis, since a single quasi-molecular ion corresponds to a single chain. However, from such mass spectrometric data no information could be obtained about the position of the chain. *4*) It uses a reliable internal standard by which it is possible to keep account of any losses during the analytical procedure, thus improving the accuracy.

We have demonstrated here that in some selected cases it is possible to use a structural analog as an internal standard for quantitative FAB. Studies are now under way to extend this method to the analysis of other phospholipids.

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