ELSEVIER

Journal of Chromatography B, 661 (1994) 1–5

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Partial characterization of the molecular species of phosphatidylserine from human plasma by high-performance liquid chromatography and fast atom bombardment mass spectrometry

Su Chen

Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK

First received 11 April 1994; revised manuscript received 30 June 1994

Abstract

Phosphatidylserine from human plasma was purified and identified by amino normal-phase and octadecylsilyl reversed-phase high-performance liquid chromatography, respectively. The two major molecular species within the human plasma phosphatidylserine, qualitatively determined by negative-ion fast atom bombardment mass spectrometry, are 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoserine and 1-stearoyl-2-arachidonyl-*sn*-glycero-3-phosphoserine.

1. Introduction

Phosphatidylserine (PS) is an acidic phospholipid class, first identified in the brain in 1941 by Folch and Schneider [1] as a constituent of the cephalin fraction. PS is well known to participate in a number of biological processes, and functions of the lipid appear to be determined by the nature of each of its molecular species components.

The distribution of PS in biological systems depends both on the amount of the lipid compared to other phospholipid classes and on the composition of the fatty acid chains in the molecular species. For example, PS is abundant in the brain (20–25% of total phospholipid), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoserine (PS 18:0–18:1) and 1-stearoyl-2-docosahexaenyl-*sn*-glycero-3-phosphoserine (PS 18:0–22:6) being the major species components in bovine brain [2,3]; PS is found as a minor class in rat (4% of total phospholipid) and rabbit (4% of total phospholipid) kidney, with 1-stearoyl-2-arachidonyl-*sn*-glycero-3-phosphoserine (PS 18:0–20:4) and 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphoserine (PS 18:0–18:2) being the abundant molecular species [4,5]. The amounts of PS have been shown to be rather low in plasma, both in rat and human (less than 1% of total phospholipid), as quantitatively determined by thin-layer chromatography [6] and ^{31}P nuclear magnetic resonance [7], respectively. In the present study, the structural analysis of the molecular species of PS from human plasma by chromatography and negative-ion fast atom bombardment mass spectrometry is reported.

0378-4347/94/$07.00
SSDI 0378-4347(94)00337-8

2. Experimental

2.1. Chemicals

o-Phthalaldehyde, tetrahydrofuran, ethylenediaminotetraacetic acid (EDTA) and the amino acid standards used were obtained from Sigma (St. Louis, MO, USA). HPLC-grade ethanol and acetonitrile, phosphoric acid, chloroform and methanol were purchased from Carlo Erba (Milano, Italy).

2.2. Preparation of human plasma

Two fresh blood samples (sample 1 and sample 2; 5 ml each) were obtained form two healthy male volunteers (25 and 28 years old, respectively). The blood was collected in polypropylene tubes containing potassium EDTA (1 mmol/ml) and separated by centrifugation at 700 *g* at 4°C for 15 min, finally producing 2.5 ml of plasma for each sample.

2.3. Purification of human plasma PS

Total lipids were extracted from the two plasma samples using the method of Folch et al. [8]. The lipids in chloroform–methanol (6:4, v/v) were separated into the two fractions on a short column containing Q-Sepharose anion-exchange resin (Pharmacia, Uppsala, Sweden) using (i) chloroform–methanol (6:4; v/v) for elution of the natural lipids and nonacidic phospholipid classes and (ii) acetic acid–chloroform (5:1, v/v) for elution of the acidic phospholipid classes, according to the reported procedure [9]. The human plasma PS was further purified from the acidic phospholipid fractions on an aminopropyl-bonded normal-phase high-performance liquid chromatography (HPLC) column (Chromapack LiChrosorb NH_2, 10 μm, 250 × 4.6 mm I.D.; London, UK) using a Waters pump combined with a Perkin-Elmer UV detector and a mixture of ethanol–0.9 *M* phosphoric acid (88:12, v/v) as mobile phase [10] at a flow-rate of 1 ml/min. The detection wavelength was 205 nm. The acid in the normal-phase HPLC fraction was removed by the method of Folch et al. [8].

2.4. Structural analysis of the human plasma PS

Reversed-phase HPLC was used for identification of the serine moiety in the purified plasma PS after hydrolysis and derivatization as described previously [11]. The *o*-phthalaldehyde derivatized-serine was separated on a octadecylsilyl (ODS) reversed-phase HPLC column (Ultrasphere-ODS, 5 μm, 250 × 4.6 mm I.D.; Altex Scientific, Berkeley, CA, USA) using a Waters HPLC pump (Model 510) coupled to a Perkin-Elmer fluorescence detector (Model LS4, Perkin-Elmer, Norwalk, CT, USA) at excitation and emission wavelengths of 338 and 450 nm,

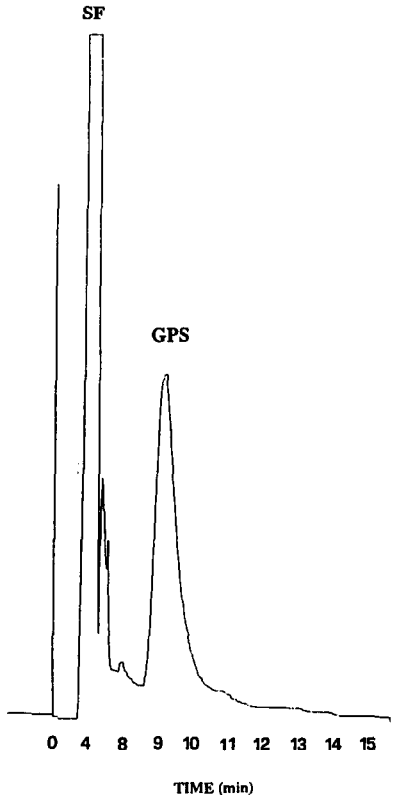

Fig. 1. The normal-phase HPLC separation of phosphatidylserine from human plasma. SF = solvent front; GPS = glycerophoaphatidylserine.

respectively [11]. The negative-ion fast atom bombardment mass spectrum was obtained on a VG ZAB-2F (VG Analytical, Manchester, UK), equipped with a FAB gun, the static FAB ion-source and probe and a PDP-11 data processing system. The bombarding gas used was xenon, with kinetic energy of 7–8 keV. Resolution of the instrument was set at 1000. The sample dissolved in chloroform–methanol–water (58:4:0.2, v/v) was loaded on a diethanolamine matrix without mixing [12]. The FAB probe was introduced into the mass spectrometer after evaporation of the solvent.

3. Results and discussion

Fig. 1 shows the normal-phase HPLC separation of the acidic phospholipid fraction isolated by Q-Sepharose ion-exchange chromatography. The peak eluting between 8.5 and 10.5 min

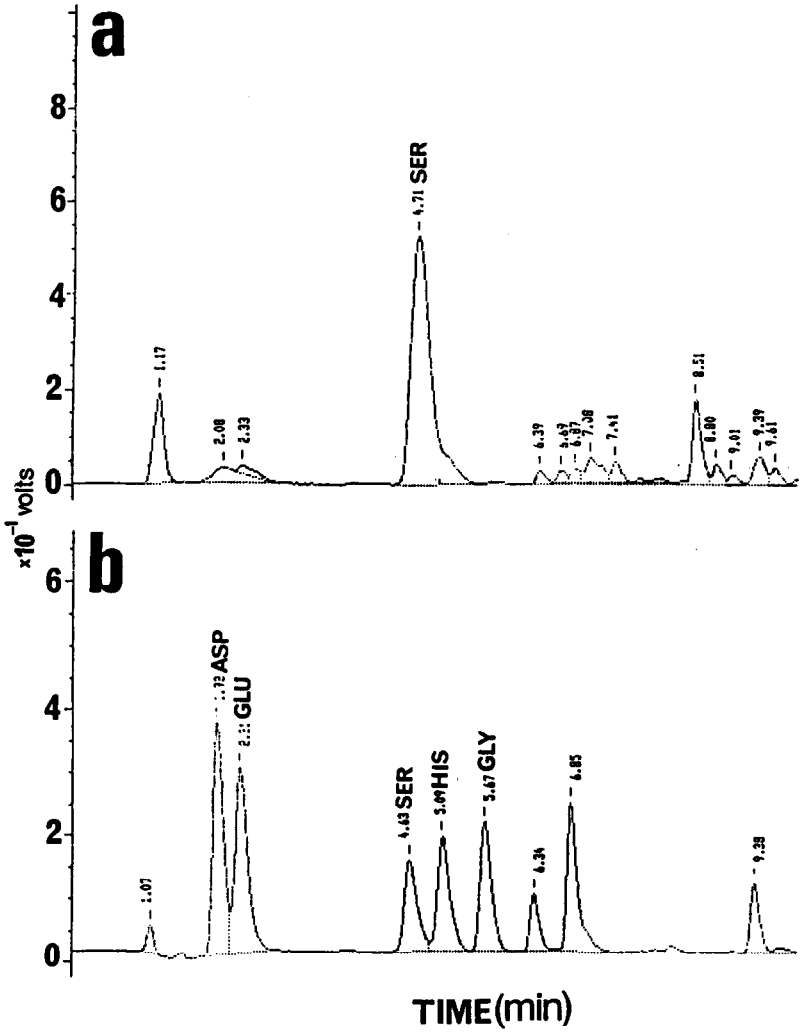

Fig. 2. The reversed-phase HPLC analyses of the *o*-phthalaldehyde derivative of the serine in human plasma phosphatidylserine (a) and the derivatives of amino acid standards (b).

(retention time) was collected and considered to be the PS class [10].

The PS from sample 1, purified by normal-phase HPLC, was hydrolysed, followed by *o*-phthalaldehyde derivatization of the free serine moiety as described [11]. Fig. 2 shows the reversed-phase HPLC separation of the derivative (Fig. 2a), compared with the *o*-phthalaldehyde derivatized-amino acid standards (Fig. 2b). The abundant peak in Fig. 2a is identified as the derivatized-serine, based on the retention times obtained for the standard mixture.

The PS from sample 2, separated on the normal-phase HPLC column was identified negative-ion fast atom bombardment mass spectrometry (shown in Fig. 3). Ions at *m/z* 788 and 810 originate from the deprotonated molecules of PS 36:1 (*m/z* 788) and PS 38:4 (*m/z* 810), respectively [10,12]. Peaks at *m/z* 702 and 724 are formed by the loss of the serine moiety from the two lipid species, as determined by reversed-phase HPLC. Loss of the fatty acids from the ions at *m/z* 788 and 810 gives peaks at *m/z* 524 and 506 [10,12]. The two molecular species within the human plasma PS are identified as 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphorerine (PS 18:0–18:1; *m/z* 788) and 1-stearoyl-2-arachidonyl-*sn*-glycero-3-phosphoserine (PS 18:0–20:4, *m/z* 810).

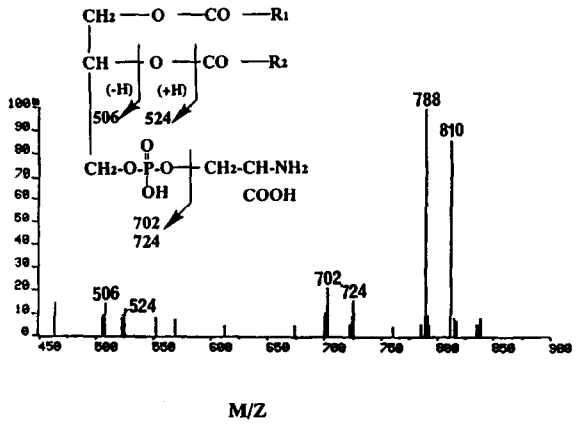

Fig. 3. Negative-ion fast atom bombardment mass spectrum of human plasma phosphatidylserine.

Structural information of phospholipids include: (i) molecular mass of various species; (ii) characterization of the polar head groups; (iii) composition of the fatty acids and location of the acyl chains esterified to the glycerol backbone; and (iv) position of the double bond(s) in the unsaturated fatty acid(s).

The composition of the fatty acids within human plasma PS has not been reported previously. The present study indicates clearly that PS 18:0–18:1 and PS 18:0–20:4 are the two major molecular species in this biological material. The location of the esterified fatty acyl chains could not be detected from the negative-ion fast atom bombardment mass spectrum. However, the unsaturated fatty acids in natural phospholipids are preferentially located at the *sn*-2 position. Positions of the double bonds in the 18:1 and 20:4 fatty acids are still unknown. Quantitative analyses of human plasma PS class and its molecular species have not been carried out. Due to the lack of a chromophore/fluorophore group in PS, the accurate assay of human plasma PS class must be performed by HPLC analysis of the derivatized PS [13,14]. Intensities of the deprotonated molecules of PS 18:0–18:1 (*m/z* 788) and PS 18:0–20:4 (*m/z* 810) in the negative-ion fast atom bombardment mass spectrum do not reflect the ratio of the percentage of the two species. This could be due to suppression of one compound by another under fast atom bombardment ionization [9]. Because of the absence of the 16:0 acyl chain in human plasma PS, it is possible to use a molecular species of PS containing the 16:0 fatty acid at the *sn*-2 position, for example PS 14:0–16:0, as an internal standard in the quantitative determination of the molecular species of human plasma PS by gas chromatographic analysis of the fatty acid methylesters. This work is now in progress.

Acknowledgements

The author would like to thank FIDIA Research Laboratories of Italy for financial support.

This work was carried out in the FIDIA Research Laboratories and Italian Research Council Research Centre at Padua.

References

[1] J. Folch and H.A. Schneider, *J. Biol. Chem.*, 137 (1941) 51.
[2] H. Yabuuchi and J.S. O'Brien, *J. Lipid Res.*, 9 (1968) 65.
[3] F. Hullin, H.Y. Kim and N. Salem, *J. Lipid Res.*, 30 (1989) 1963.
[4] S. Chen, G. Kirschner and E. Benfenati, *Rapid Commun. Mass Spectrom.*, 4 (1990) 495.
[5] S. Chen, O. Curcuruto, S. Catinella, P. Traldi and G. Menon, *Biol. Mass Spectrom.*, 21 (1992) 655.
[6] A. Diagne, J. Fauvel, M. Record, H. Chap and L. Douste-Blazy, *Biochim. Biophys. Acta*, 793 (1984) 221.
[7] S. Bradamante, E. Barchiesi, L. Barenghi and F. Zoppi, *Anal. Biochem.*, 185 (1990) 299.
[8] J. Folch, M. Lees and G.H., Sloane Stanley, *J. Biol. Chem.*, 226 (1957) 497.
[9] S. Chen, G. Kirschner and E. Benfenati, *Rapid Commun. Mass Spectrom.*, 4 (1990) 214.
[10] S. Chen, G. Krischner and P. Traldi, *Anal. Biochem.*, 191 (1990) 100.
[11] D.T. Blankenship, M.A. Krivanek, B.L. Ackermann and A.D. Cardin, *Anal. Biochem.*, 178 (1989) 227.
[12] S. Chen, E. Befenati, R. Fanelli, G. Kirschner and F. Pregnolato, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 1051.
[13] S.S.-H. Chen, A.Y. Kou and H.-H.Y. Chen, *J. Chromatogr.*, 208 (1983) 339.
[14] S. Chen, G. Menon and P. Traldi, *Org. Mass Spectrom.*, 27 (1992) 215.