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Short note

MALDI-TOF MS of phosphatidylethanolamines: Different adducts cause different post source decay (PSD) fragment ion spectra

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Abstract

Matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) is increasingly applied to lipids. However, positional acyl chain analysis of lipids by MALDI was so far scarcely described.

In this paper, the fragmentation behavior of phosphatidylethanolamine (PE) is investigated by using post-source decay (PSD) MS. In dependence on the investigated adduct, significant differences could be obtained. It will be shown that in particular the negative ion spectra enable the determination of the individual acyl chains as well as their positions (*sn*-1 or *sn*-2). Therefore, MALDI-TOF PSD spectra are a real alternative to more sophisticated MS/MS methods.

Keywords: MALDI-TOF MS; Phosphatidylethanolamine; Post source decay; Acyl chain composition

The interest in lipids and particularly phospholipids (PL) is continuously increasing because they represent important disease markers and second messengers. Consequently, besides "proteomics" and "genomics" the term "lipidomics" was recently established [1].

Unequivocally, mass spectrometry (MS) is a powerful tool to characterize the composition of unknown lipid mixtures [2]. Nowadays, different "soft" ionization techniques are available, whereby electrospray (ESI) [3] and atmospheric pressure chemical ionization (APCI) [4] were most successfully used in lipidomics so far. Although matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) MS was less frequently used, there is growing evidence that this is also a very useful technique [5].

The overall acyl chain composition of a lipid can be derived directly from its monoisotopic mass weight (i.e. the *m/z* ratio), but more detailed information cannot be obtained so easily. For instance, a PL with the acyl chain composition 18:1/18:1 gives the same monoisotopic mass as a PL that consists of 18:0/18:2 or 18:2/18:0. There are different sophisticated methods of positional acyl chain analysis of lipids. However, the majority of these techniques is based on MS/MS methods [3]. Unfortunately, however, many mass spectrometers do not provide MS/MS capability.

However, if MALDI-TOF devices with a reflectron are available, it is possible to record "post source decay" (PSD) spectra: A given parent ion penetrates to the back of the reflectron and may be easily focused onto the detector. Fragment ions of the parent ion do not penetrate as deeply and consequently are not as well focused. This problem can be overcome by acquiring several spectra (known as segments) at reduced reflectron voltages. The different mass regions are then stitched together to form a single PSD spectrum representing principally a MS/MS spectrum. Surprisingly, only a very limited number of lipid PSD studies is available so far [6] with the clear focus on phosphatidylcholine (PC) [7].

MALDI-TOF mass spectra are characterized by molecular adducts generated by the addition of ions to the analyte. In the majority of cases, H^+ and Na^+ adducts are observed [8]. From the PSD spectra of different PC adducts it is known that the yield as well as the type of fragment ions differ significantly [7] and the Na^+ adducts give a more complex fragmentation pattern than the corresponding H^+ adducts. Although phosphatidylethanolamines

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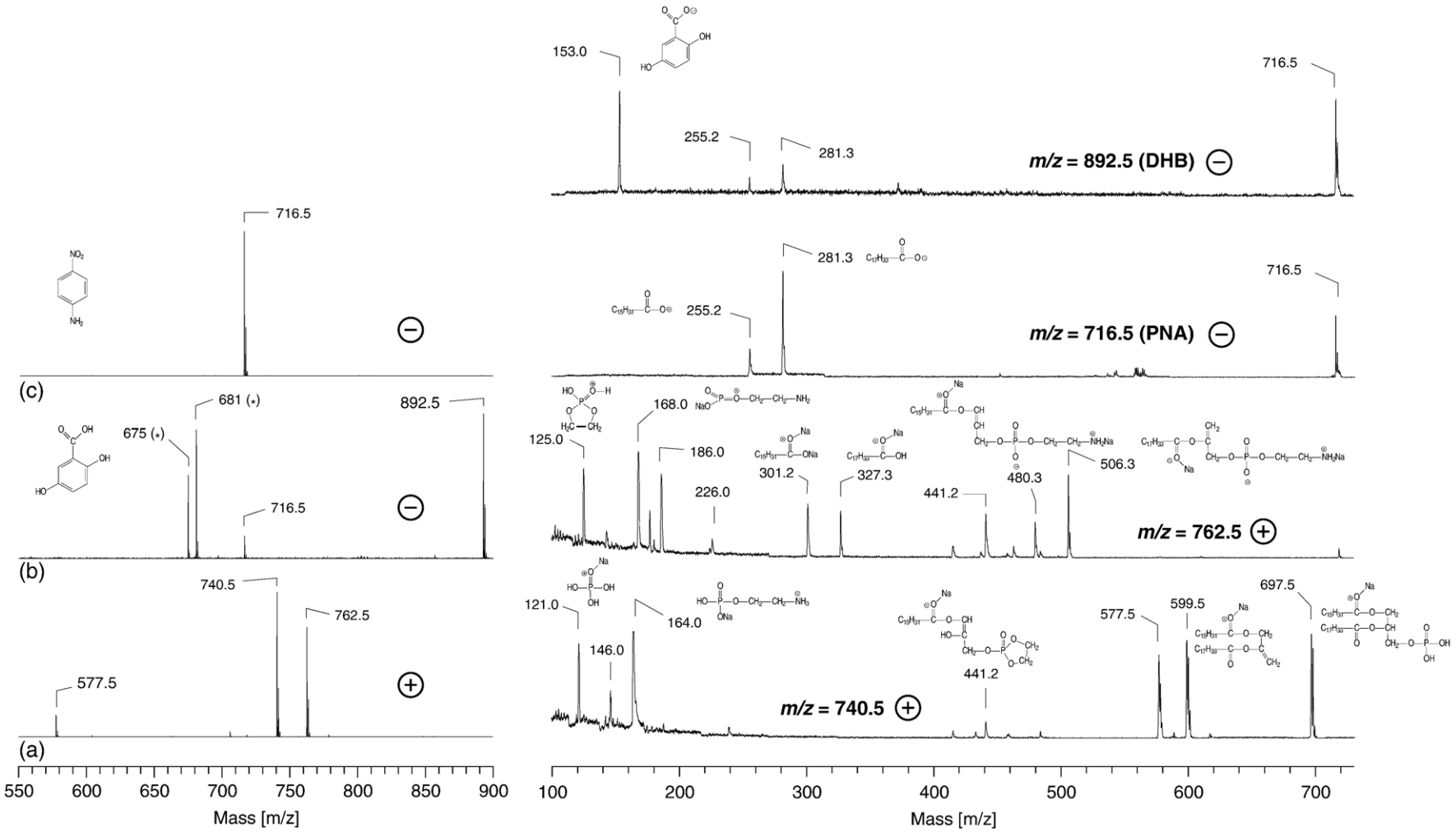

Fig. 1. Left: Positive (**a**) and negative (**b**, **c**) ion MALDI-TOF mass spectra of 1-palmitoyl-2-oleoyl-*sn*-phosphatidylethanolamine (POPE). A POPE solution in $CHCl_3$ was purchased from AVANTI Polar Lipids and used as supplied. Spectra (**a**) and (**b**) were recorded with DHB and (**c**) with PNA as matrix. DHB was applied as 0.5 mol/l solution in CH_3OH [8] and PNA as 0.17 mol/l solution in $CHCl_3/CH_3OH$ (2:1, v/v) [10]. Each matrix was mixed 1:1 (v/v) with POPE (1 mg/ml) and this mixture deposited onto the target. All spectra were acquired on a Bruker Autoflex device (Bruker Daltonics, Bremen, Germany). This system utilizes a pulsed nitrogen laser, emitting at 337 nm. The extraction voltage was 20 kV and gated matrix suppression was applied to prevent the saturation of the detector by matrix ions [5]. Spectra represent the average of 128 individual laser shots. In order to enhance the spectral resolution all spectra were acquired in the reflector mode using delayed extraction conditions. A more detailed methodological description of MALDI-TOF MS is available in [5,11]. All peaks are labeled according to their *m/z* ratio and peaks of the DHB matrix are marked with asterisks. PSD spectra of all detected ions are shown at the right side and the *m/z* values of the selected parent ions are indicated. The same laser intensities were used in all cases. The precursor ions were isolated using a timed ion selector. The fragment ions were refocused onto the detector by stepping the voltage applied to the reflectron in appropriate increments. This was done automatically by using the "FAST" ("fragment analysis and structural TOF") subroutine of the Flex Analysis Program provided by Bruker Daltonics. Peak assignments are given in the figure. Please note that the position of charges was assigned according to Al-Saad et al. [6,7].