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A universal product ion nomenclature for $[M-H]^-$, $[M+H]^+$ and $[M+nNa-(n-1)H]^+$ (n = 1–3) glycerophospholipid precursor ions based on high-energy CID by MALDI-TOF/RTOF mass spectrometry

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ABSTRACT

The fragmentation behavior of various types of precursor ions ($[M-H]^-$, $[M+H]^+$, $[M+Na]^+$, $[M+2Na-H]^+$ and $[M+3Na-2H]^+$) of the six major classes of glycerophospholipids (glycerophosphatidic acid, glycerophosphatidylglycerol, glycerophosphatidylinositol, glycerophosphatidylserine, glycerophosphatidylethanolamine and glycerophosphatidylcholine) was studied in detail by high-energy collision-induced dissociation (CID)-TOF/RTOF mass spectrometry. Whereas negative-ion CID-spectra of the $[M-H]^-$ ion of these lipids primarily exhibited the molecular mass of the fatty acid substituents, protonated and monosodiated precursor ions showed abundant loss of the polar head group identifying the class of glycerophospholipid. In contrast, doubly and triply sodiated singly charged glycerophospholipid precursor ions not only yielded charge-remote fragmentation of the fatty acid substituents, but also several abundant low-mass product ions of diagnostic value for the identification of the phospholipid class as well as specific phosphate-related sodium adduct ions for determining the number of sodium cations attached to the precursor ion. These findings finally lead to the establishment of common fragmentation rules as well as a universal product ion nomenclature for all types of glycerophospholipid precursor ions investigated (applicable for low- and high-energy CID). This nomenclature is based on the **A**-, **B**-, **C**-, **D**-, **E**-, **F**-. and **G**-product ion nomenclature previously published for sodiated triacylglycerol precursor ions [C. Cheng, M.L. Gross, E. Pittenauer, Anal. Chem. 70 (1998) 4417–4426] with adaptations for the type of precursor ion and class of glycerophospholipid investigated.

1. Introduction

For many common classes of biomolecules composed of well-known small building blocks (e.g., amino acid, monosaccharides, nucleotides, fatty acids, isoprenes, etc.) distinct product ion nomenclatures facilitating the interpretation of collision-induced dissociation (CID)-mass spectra have been described partly in the past. The first, meanwhile generally accepted nomenclature developed during the 80s of the last century was the peptide product ion nomenclature described by Roepstorff and Fohlman [1] later modified by Biemann and co-workers [2–6] to include side-chain losses and side-chain fragmentations as typically observed under high-energy CID-conditions (i.e., keV-collisions) obtained on (tandem) magnetic sector instruments. This nomenclature was originally developed for singly charged protonated precursor ions but few examples were also shown for singly sodiated precursor ions [7,8]. For the second important class of biomolecules, oligosaccharides and glycoconjugates, Domon and Costello published a commonly applicable and accepted product ion nomenclature for positively and negatively charged precursor ions [9]. This nomenclature was later improved by two different groups utilizing high-energy CID on a tandem time-of-flight (TOF/RTOF) instrument [10,11]. A partly accepted product ion nomenclature for oligonucleotides was first published by Cerny et al. for singly charged negative precursor ions at high collision energy [12] and later refined by McLuckey et al. by low-energy CID of multiply charged negative precursor ions utilizing an ion trap instrument [13].

Among lipids, especially for negatively charged steroid precursor ions (sulfated, sulfonated or glucuronidated molecules) a product ion nomenclature was reported by Griffiths et al. [14–16] involving typically two-bond ring cleavages. This nomenclature was later also developed and utilized for positive precursor ions of Girad P-derivatized steroids [17–21]. The same research group also published a nomenclature for product ions of deprotonated precursor ion of (multiply) unsaturated fatty acids for the first time although previous researchers investigated the fragmentation behavior of deprotonated as well as cationized fatty acid precursor ions in detail by high-energy CID [22–32] which was also described in one review [33]. Later on, a product ion nomenclature

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for ceramides and sphingomyelins, the first one for complex lipids comprising at least of a sphingoid base and one amide-linked fatty acid, was published by Ann and Adams [34–36].

One of the last product ion nomenclatures of common biomolecules (the triacylglycerols) reported in the literature by the end of the last century was done by Cheng and co-workers enabling positional isomer differentiation between s*n*1-/s*n*3- and s*n*2-fatty acid substituents, respectively, based on different fragmentation pathways by high-energy CID utilizing (tandem) magnetic sector instrumentation [37–39] in combination with FAB and ESI. These fragmentations and the described product ions were later fully reproduced by vacuum MALDI in combination with high-energy CID and TOF/RTOF-instrumentation [40]. An alternative product ion nomenclature for sodiated triacylglycerol precursor ions based on FAB tandem MS was published by Kim et al. [41].

Interestingly, for the chemically related class of lipids, namely the various classes of glycerophospholipids, which are composed of a diglyceride-moiety, a phosphate group and a polyalcohol or aminoalcohol, respectively, no stringent nomenclature for product ions has been published up to now. Although several groups attempted to establish for a limited number of fragment ions obtained by negative-ion FAB-MS and/or product ions based high-energy CID in combination with tandem mass spectrometry (MS/MS) a suggested fragment/product ion nomenclature was never widely used [42–49]. Alternatively, Hsu et al. investigated the fragmentation behavior of deprotonated, protonated as well as (multiply) lithiated precursor ions of various classes of glycerophospholipids in detail by low-energy CID utilizing either a triple quadrupole or an ion trap instrument combined with ESI but without postulating a product ion nomenclature based on common fragmentation rules [50–58]. Several reviews dealing with the fragmentation behavior of various classes of glycerophospholipids also appeared in literature [59–63] but without defining a systematic and universal nomenclature.

Due to this situation and our findings we here present a universal product ion nomenclature for the six major classes of glycerophospholipids [glycerophosphatidic acid (A), glycerophosphatidylglycerol (G), glycerophosphatidylinositol (I), glycerophosphatidylserine (S), glycerophosphatidylethanolamine (E) and glycerophosphatidylcholine (C)] for all major types of precursor ions available from individual lipid species based on high-energy CID MALDI-TOF/RTOF mass spectrometry. The selected precursor ions include **[M−H]⁻**, **[M+H]⁺**, **[M+Na]⁺**, **[M+2Na−H]⁺** and **[M+3Na−2H]⁺** molecular ions. The product ion identity for all ions observed was correlated with the triacylglycerol product ion nomenclature published previously [37] with some additional product ion types. Although several papers are published on MALDI-TOF/RTOF MS of various precursor ions of glycerophospholipids including deprotonated, (multiply) lithiated and (multiply) sodiated molecular ions, none of these papers exhibit a complete and detailed structural study on the above mentioned analytes as either only metastable ions or CID-generated ions at E_{LAB} = 1 keV, which are different from conditions at E_{LAB} = 20 keV on the instrument used for this study, were investigated [64–68].

2. Experimental

2.1. Chemicals

The glycerophospholipid standards including 1-palmitoyl-2-arachidonoyl-glycerophosphatidic acid, 1-palmitoyl-2-arachidonoyl-glycerophosphatidylglycerol, 1-palmitoyl-2-arachidonoyl-glycerophosphatidylserine and 1-palmitoyl-2-arachidonoyl-glycerophosphatidylcholine were purchased from Avanti Polar Lipids (Alabaster, AL, USA). All other glycerophospholipid standards including 1-, 2-dipalmitoyl-glycerophosphatidylglycerol, 1-, 2-dioleoyl-glycerphoshosphatidylserine, 1-palmitoyl-2-linoleoyl-glycerophosphatidylethanolamine, 1-, 2-dipalmitoyl-glycerophosphatidylcholine and a phosphatidylinositol mixture from bovine brain were obtained from Sigma–Aldrich (St. Louis, MO, USA). Chemicals used for sample preparation on MALDI-MS target as sodium chloride (pro analysis) and methanol (gradient grade for HPLC) were delivered by Merck (Darmstadt, Germany) and 2-, 4-, 6-trihydroxyacetophenone by Fluka (Buchs, Switzerland).

2.2. MALDI mass spectrometry

All measurements were performed using a prototype Axima TOF^2 MALDI time-of-flight (TOF)/reflectron (RTOF) mass spectrometer (Shimadzu Biotech—Kratos Analytical, Manchester, UK) described in detail elsewhere [39,69]. Briefly, the instrument consisted of a linear TOF analyzer as MS1 and a wide energy-acceptance curved field reflectron (RTOF) as MS2 for product ion analysis, a grounded gas collision cell with differential pumping of the collision region and a double Bradbury–Nielsen wire ion gate for narrow mass-window precursor ion selection (3–4 Dalton [Da] at *m*/*z* 700–1000 as experimentally determined). The accelerating voltage was set to 20 kV.

All glycerophospholipid samples were dissolved at roughly 1 mg/1000 μl methanol. 2-, 4-, 6-Trihydroxyacetophenone was selected as MALDI-MS matrix for all experiments [70]. The matrix solution was prepared by dissolving 10 mg matrix in 1000 μl methanol or alternatively in methanol saturated with sodium chloride to suppress peak splitting of analyte ions between sodium and potassium adduct ions (the latter ones are frequently present in low abundance) and to promote doubly (triply) sodiated singly charged precursor ions. For final sample preparation analyte solution and matrix solution were mixed 1:1 (v/v) immediately prior to applying 0.8 μl of the resulting mixture onto the stainless steel MTP target surface (dried droplet preparation) yielding a final amount of approximately 400 ng glycerophospholipid on target.

MALDI-RTOF-mass spectra of the analytes were acquired near threshold laser irradiance to avoid/reduce peak broadening of deprotonated, protonated and sodiated ions and possible *in-source* fragmentation resulting in a mass spectrometric resolution of approximately 7000–8000 FWHM (full width at half maximum). All displayed MALDI-RTOF-mass spectra were based on 1000–2000 single and unselected laser pulses (λ = 337 nm). This number of shots was acquired to obtain a satisfactory ion statistics.

Metastable or seamless post-source decay (PSD) experiments were performed at nearly the same laser irradiance as used for to standard MALDI-mass spectra to keep mass resolution for product ions (typically between 500 and 2000 at FWHM depending on the observed *m*/*z*-value of the product ion) as high as possible. A total of 2500 unselected laser shots were acquired for optimum ion statistics for PSD fragment ion analysis. Precursor ion selection was done by selecting a 4 Da-window centered symmetrically around the monoisotopic precursor ion of interest by the ion gate.

All CID-experiments were performed at a collision energy of E_{LAB} = 20 keV by introducing helium into a differentially pumped grounded collision cell at an approximate pressure of $p = 5 \times 10^{-6}$ mbar to attenuate the selected precursor ion signal by roughly 50–70%. A total of 5000 unselected single laser pulses was acquired in this case at nearly unchanged laser irradiance to avoid unwanted PSD outside the grounded collision cell for the high-energy CID spectrum of the selected analyte precursor ions. Typical mass spectrometric resolution values obtained in resulting CID-spectra for product ions were at least comparable to PSD-data. The ion gate was also set to a 4 Da-mass window as described above for PSD-experiments.

3. Results and discussion

Previously, we could successfully demonstrate that sodiated triacylglycerol precursor ions exhibit identical product ions (including **A**-, **B**-, **C**-, **D**-, **E**-, **F**-, **G**- and **J**-type ions) by high-energy CID MALDI-TOF/RTOF mass spectrometry [39,40] as originally described for high-energy CID utilizing (tandem) sector instrumentation by FAB or ESI as desorption/ionization technique [37,38]. Based on these findings by high-energy CID MALDI-TOF/RTOF the CID-behavior of the six major classes of glycerophospholipids (A, G, I, S, E and C) was investigated with special emphasis to all types of precursor ions generally formed (**[M−H]⁻**, **[M+H]⁺**, **[M+Na]⁺**, **[M+2Na−H]⁺**, **[M+3Na−2H]⁺** depending on the chemical nature of the glycerophospholipid). Finally, the product ion-nomenclature

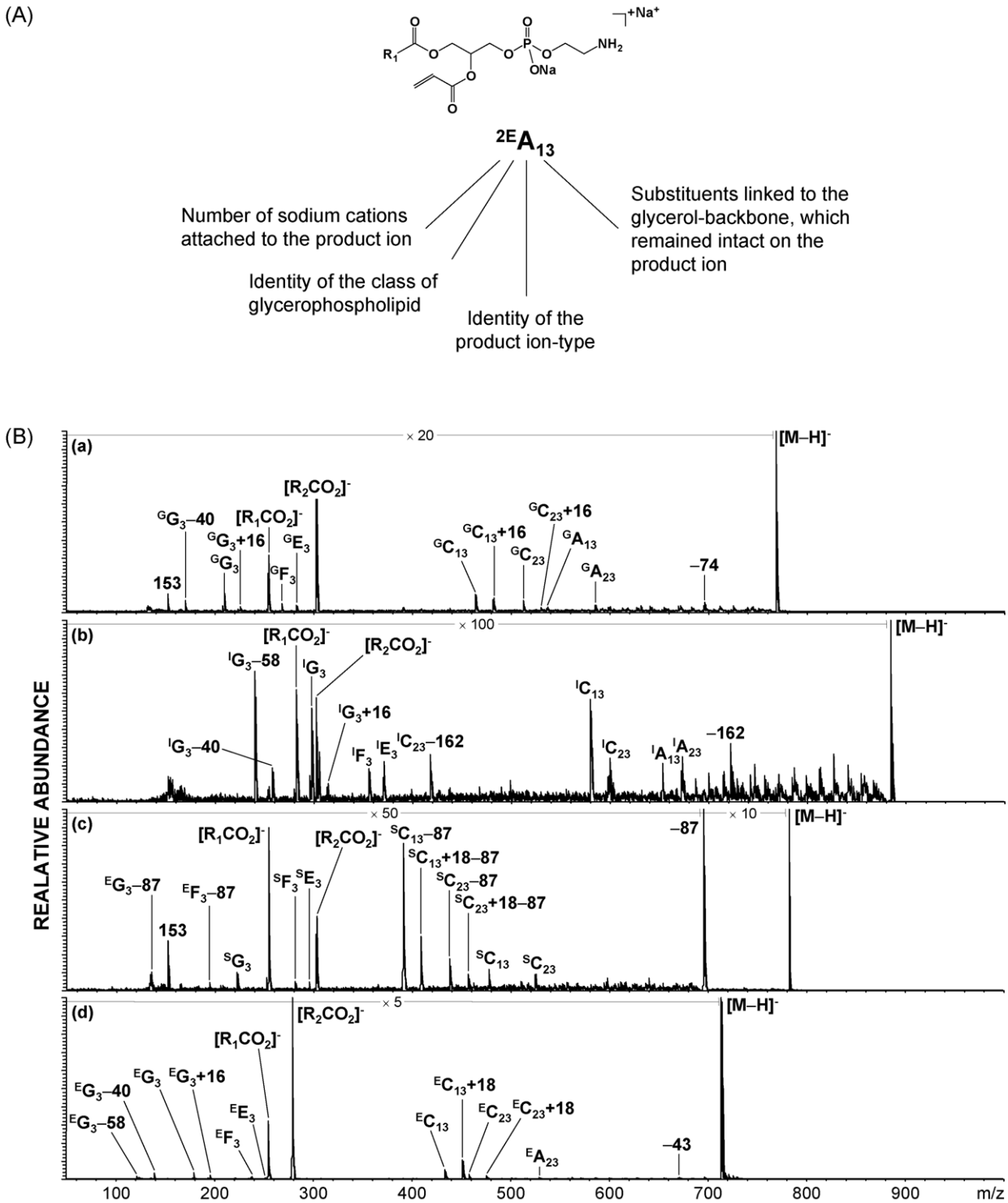

Fig. 1. (A) Example of the application of a systematic and universal product ion nomenclature for product ions of glycerophospholipid precursor ions. (B) Negative-ion high-energy CID MALDI-TOF/RTOF-spectra of the **[M−H]⁻**-precursor ions of 1-palimtoyl-2-arachidonoyl-phosphatidylglycerol (*m*/*z* 747.5) (a), of 1-stearoyl-2-arachidonoyl-phosphatidylinositol from bovine liver phosphatidylinositol-mixture (*m*/*z* 885.5) (b), of 1-palmtioyl-2-arachidonoyl-phosphatidylserine at (*m*/*z* 782.5) (c) and of 1-palmitoyl-2-linololeoyl-phosphatidylethanolamine (*m*/*z* 714.5) (d).

for sodiated triacylglycerol precursor ions was adapted for all types of negatively and positively charged glycerophospholipid precursor ions with some important modifications/additions (number of sodium cations attached to the product ions; identity of the glycerophospholipid class).

One typical glycerophospholipid product ion could have, for example (Fig. 1A) the notation $^{2E}\mathbf{A_{13}}$, where the preceding superscripted "2" explains, how many sodium cations are attached to the type of singly charged product ion under investigation. This is only important for (multiply) sodiated precursor ions. In contrast, deprotonated and protonated precursor ions do not need any preceding arabic number as the subtraction and addition, respectively, of a proton is given by the ion polarity of instrumental parameters during the measurement (e.g. $^{E}\mathbf{G_3}$, see Fig. 1 and Fig. 3). Further, the identity of the glycerophospholipid class is written as superscripted capital letter (A, G, I, S, E or C) – giving the identity of the glycerophospholipid class – before the bold capital letter identifying the type of product ion (**A**, **B**, **C**, **D**, **E**, **F** or **G**). As **B**- and **C**-type triacylglycerol product ions only differ by 22 Da (i.e., the mass difference between a sodiated and protonated dehydrated diglyceride cation), all product ions which have lost the substituent of the glycerol backbone containing the *sn*3-substitutent (i.e., the variable phosphate-containing substituent) are all termed as $\mathbf{B_{12}}$- and $\mathbf{C_{12}}$-type ions sharing the same structure with triacylglycerol product ions. All successive subscripted arabic number(s) (either one or two) identify(ies) the substituent(s) linked to the glycerol backbone, which remained intact during CID-fragmentation of the precursor ion. Finally, some product ions can retain one molecule of water, which is simply assigned by a **"+18"**, and some others exhibit additional loss of small neutrals (e.g., "−**43**"). Both, additions to or additional losses of small neutrals compared to typical product ions are added after the subscripted arabic number(s). Several other types of low-mass product ions not observable among triacylglycerol product ions are either simply identified by their nominal mass value or (multiply) sodiated phosphate ions are identified by a capital letter "**P**" with a preceding superscripted arabic number (for the numbers of sodium cations attached to the product ion) and, if necessary, "−**18**" for an additional loss of water.

3.1. High-energy CID-spectra of $[M-H]^-$-precursor ions (A, G, I, S, E)

Based on their chemical nature all glycerophospholipids exhibited abundant $[M-H]^-$-ions without any significant *in-source* fragmentation at threshold laser irradiance. The only exception was found for glycerophosphatidylcholines, which contain a fixed positive charge, a quarternary ammonium residue. In this case, the only adduct ion-type detectable in the negative-ion MALDI reflectron mass spectrum was a very low abundant $[M+THAP-H]^-$-adduct ion, an adduct with the particular

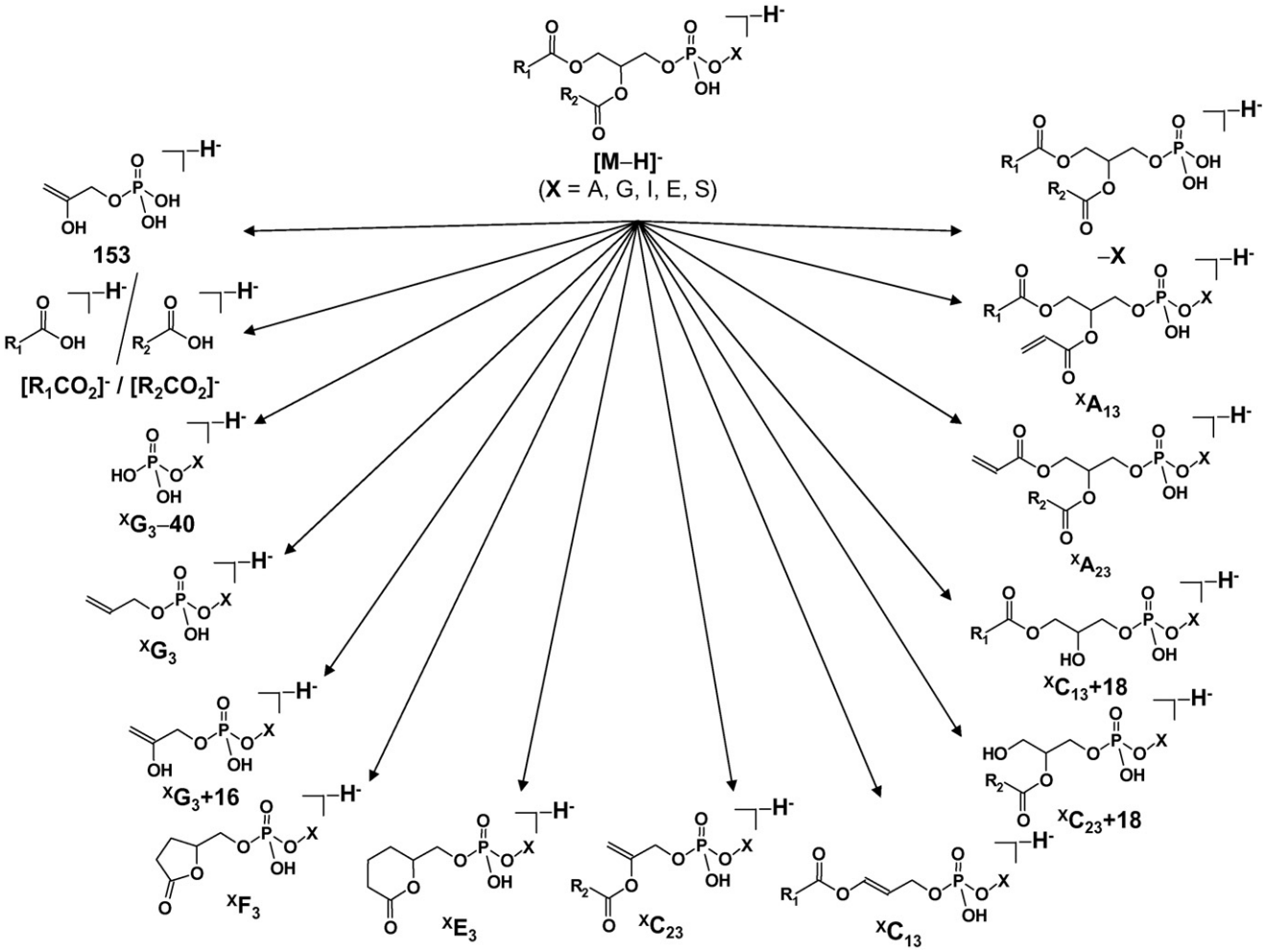

Fig. 2. General fragmentation scheme of $[M-H]^-$-precursor ions obtained by high-energy CID MALDI-TOF/RTOF mass spectrometry identifying the structure of the major product ions (abbreviations used: A = glycerophosphatidic acid, G = glycerophosphatidylglycerol, I = glycerophosphatidylinositol, S = glycerophosphatidyl-serine, E = glycerophosphatidylethanolamine).

MALDI-MS matrix, which was used in all experiments (data not shown). Typical negative-ion high-energy CID-TOF/RTOF-spectra of the **[M−H]**$^-$-precursor ions of 1-palmitoyl-2-arachidonoyl-glycerophosphytidylglycerol, 1-stearoyl-2-arachidonoyl-glycerophosphatidyl-inositol, 1-palmitoyl-2-arachidonoyl-glycerophosphytidylserine and 1-palmitoyl-2-linoleoyl-glycerophosphytidylethanolamine are displayed in Fig. 1B.

The high-mass regions of the CID-spectra of all other glycerophospholipids investigated were filled by low-abundant charge-remote fragmentations of the two fatty acid substituents, namely the $^{X}\mathbf{A_{13}}$ and $^{X}\mathbf{A_{23}}$-type ions (for product ion structures see Fig. 2) being the most abundant (X = G, I and E), and by loss of the variable polyalcohol- or aminoalcohol-residues (G: −**74**, I: −**162**, S: −**87**, E: −**43**; for product ion structures see Fig. 2). Further product ions are formed by loss of ketene and one free fatty acid differing in mass by 18 Da for one given fatty acid-substituent leading to $^{X}\mathbf{C_{13}+18}/^{X}\mathbf{C_{23}+18}$ (X = A, G or E) and $^{X}\mathbf{C_{13}}/^{X}\mathbf{C_{23}}$ (X = A, G, I, S or E) product ions, respectively (for all product ion structures see Fig. 2). In contrast, phosphatidylserine exhibited more prominent product ions of the type $^{X}\mathbf{C_{13}+18-87}/^{X}\mathbf{C_{23}+18-87}$ and $^{X}\mathbf{C_{13}-87}/^{X}\mathbf{C_{23}-87}$, which is due to the ease of the cleavage of the labile phosphoserine ester bond. In the low mass range, product ions corresponding to the two fatty acid substituents, the carboxylate anions, are labeled as $\mathbf{[R_1CO_2]^-}$- and $\mathbf{[R_2CO_2]^-}$-product ions (for product ion structures see Fig. 2). Typically glycerophospholipids, which directly fragment from the intact deprotonated precursor ions to form carboxylate anions exhibit the following rough estimation for the intensity ratio of these two product ions: $\mathbf{[R_1CO_2]^-}$ from the s*n*1-position < $\mathbf{[R_2CO_2]^-}$ from the s*n*2-position. This estimated ratio reverses for formation of the carboxylate anions from glycerophosphatidic acid and from glycerophosphatidylserine precursor ions. The latter exhibits a very abundant loss of serine (−**87**) leading to a glycerophosphatidic acid-structure for this product ion which further leads to the same intensity ratios for the carboxylate anions as the glycerophosphatidic precursor ion itself [46]. Other low-mass product ions include very low-abundant $^{X}\mathbf{E_3}$- [X = G (*m*/*z* 283.1), I (*m*/*z* 371.1), S (*m*/*z* 296.0) and E (*m*/*z* 252.1)] and $^{X}\mathbf{F_3}$-ions [X = G (*m*/*z* 269.0), I (*m*/*z* 357.1), S (*m*/*z* 282.0) and E (*m*/*z* 238.0)] separated by 14 Da keeping only the s*n*3-substituent (i.e., the polar head group) intact (for all product ion structures see Fig. 2). The mechanism of formation of these important ions was already published for sodiated triacylglycerol precursor ions as these product ions – based on their mechanism of formation – exclusively identify the s*n*1- and the s*n*3-substiuents of the analyte ion investigated [37]. Unfortunately, as in this case the only ionic site of the lipid molecules is located at the phosphate-containing s*n*3-substituent of the molecules and these findings do not assist in positional isomer differentiation of the fatty acid substituents because neither the corresponding ion for the s*n*1- nor for s*n*2-position is formed. The next product ions detected in the low-mass range could be identified as $^{X}\mathbf{G_3}$-type ions [X = A (*m*/*z* 137.0), G (*m*/*z* 211.0), I (*m*/*z* 299.1), S (*m*/*z* 224.0) and E (*m*/*z* 180.0)] according to an O-allyl-derivative of the polar head group. Several other related product ions, the $^{X}\mathbf{G_3+16}$- [X = A (*m*/*z* 153.0), G (*m*/*z* 227.0), I (*m*/*z* 315.0), E (*m*/*z* 197.0)], the $^{X}\mathbf{G_3-40}$- [X = G (*m*/*z* 171.0), I (*m*/*z* 259.0), E (*m*/*z* 140.0)] and the $^{X}\mathbf{G_3-58}$-product ions [X = G (*m*/*z* 153.0), I (*m*/*z* 241.0), E (*m*/*z* 122.0)], are also detected (for all product ion structures see Fig. 2). No product ions with significant abundance could be found for typical negative phosphate-related ions at *m*/*z* 97.0 ($\mathbf{[H_2PO_4]^-}$) or at *m*/*z* 79.0 ($\mathbf{[HPO_3]^-}$) with significant abundance. A general fragmentation scheme of **[M−H]**$^-$-precursor ions of glycerophospholipids under high-energy CID-conditions is shown in Fig. 2.

3.2. High-energy CID of $\mathbf{[M+H]^+}$-precursor ions (S, E, C)

Protonated ions are only possible for aminophospholipids, i.e., glycerophospholipids which contain either a primary amino group (S and E) or a quarternary ammonium group (C). All three classes of lipid compounds exhibit such protonated precursor ions but only if the original sample is free of salts or if a special rinsing procedure within a specific MALDI-MS sample preparation is performed (rinsing of the already crystallized matrix-/analyte-surface with pure water and removal with a kim wipe). **[M+H]**$^+$-ions of glycerolphosphatidylserines and glycerophosphatidylethanolamines show very abundant loss of their polar head group (a **[M+H−185]**$^+$- for S and (**[M+H−141]**$^+$-product ion for E). In contrast, this type of fragmentation is absent for glycerophosphatidylcholines.

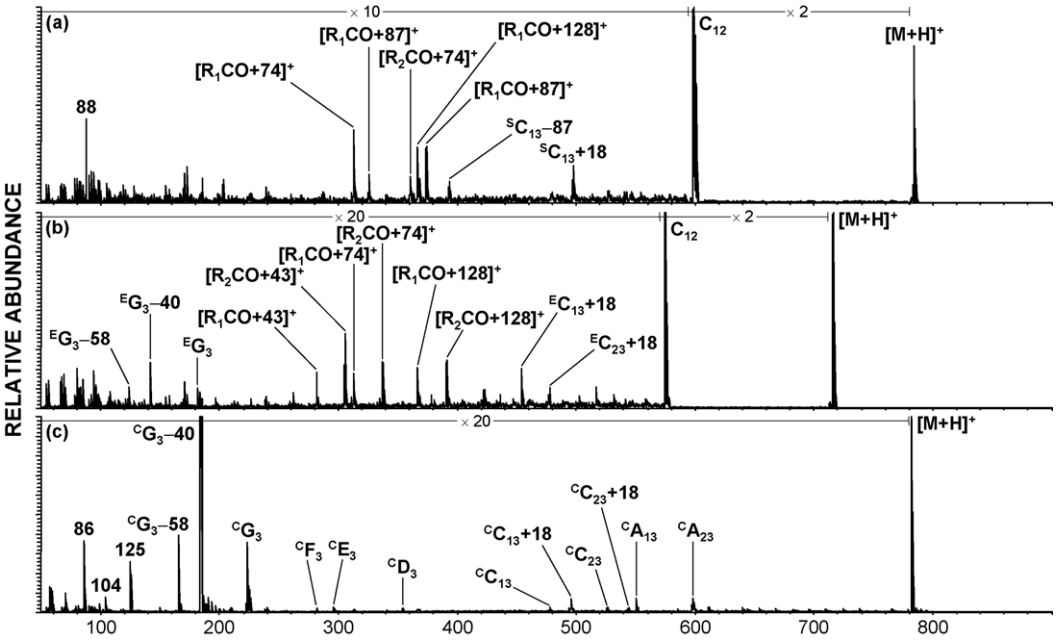

Fig. 3. Positive-ion high-energy CID MALDI-TOF/RTOF-spectra of the **[M+H]**$^+$-precursor ions of 1-palimtoyl-2-arachidonoyl-phosphatidylserine (*m*/*z* 784.5) (a), of 1-palmitoyl-2-linoleoyl-phosphatidylethanolamine (*m*/*z* 716.5) (b) and 1-palmitoyl-2-arachidonoyl-phosphatidylcholine (*m*/*z* 782.6) (c).

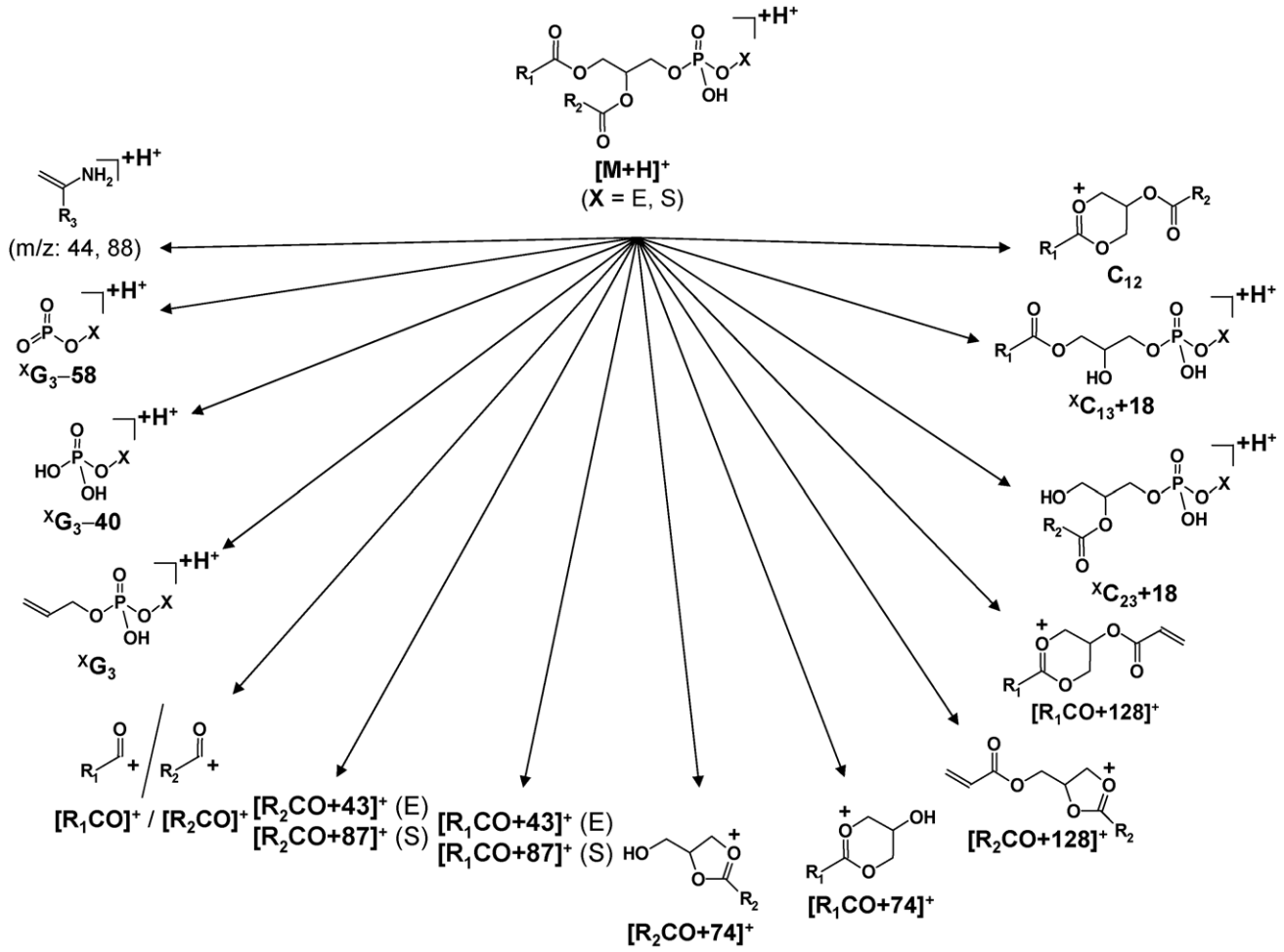

Fig. 4. General fragmentation scheme of **[M+H]$^+$**-precursor ions obtained by high-energy CID MALDI-TOF/RTOF mass spectrometry identifying the structure of the major product ions (abbreviations used: S = glycerophosphatidylserine, E = glycerophosphatidylethanolamine, C = glycerophosphatidylcholine).

Representative high-energy CID-spectra of the **[M+H]$^+$**-precursor ions of 1-palmitoyl-2-arachidonoyl-glycerophosphatidylserine, 1-palmitoyl-2-linoleoyl-glycerophosphatidylethanolamine and 1-palmitoyl-2-arachidonoyl-glycerophosphatidylcholine are shown in Fig. 3.

This type of ion obtained by neutral loss of the corresponding polar head group structurally corresponds to a sodium-free dehydrated diacylgylcerol product ion also observed among product ions of sodiated triacylglycerols and named **C_{12}**-type ion (for product ion structure see Fig. 4) [37]. Other, low-mass product ions include **$^X C_{13}+18$**- (X = S, E) and **$^X C_{23}+18$**-ions (X = E); **$[R_1CO+128]^+$**-, **$[R_2CO+128]^+$**- (only observed under CID-conditions, but never under PSD-conditions; data not shown), **$[R_1CO+74]^+$**-, **$[R_2CO+74]^+$**-ions (all: X = S, E); **$[R_1CO+87]^+$**-/**$[R_1CO+43]^+$**- and **$[R_2CO+87]^+$**-/**$[R_2CO+43]^+$**-ions (all ion types: X = S/E; for all product ion structures see Fig. 4). This already established product ion nomenclature for **$[RCO+128]^+$**- and **$[RCO+74]^+$**-ions ($n = 1$ or 2) of triacylglycerol product ions [37] was not changed for product ions of protonated glycerophospholipid precursors because there is no more clear structural relationship to glycerophospholipids. The other two pairs of low-mass product ions (**$[R_nCO+87]^+$** for S and **$[R_nCO+43]^+$** for E; $n = 1$ or 2) were subject of a structural misinterpretation in the past [47,48] and are therefore renamed. The mechanism of formation of these rather unusual rearrangement product ions will be subject of a future paper [E. Pittenauer, G. Allmaier, to be published]. In the case of glycerophosphatidylethanolamine low-abundant **$^E G_3$**- (m/z 182.1), **$^E G_3 - 40$**- (m/z 142.0) and **$^E G_3 - 58$**-ions (m/z 124.0) are detected (for all product ion structures see Fig. 4). Finally, the smallest product ions observed are found at m/z 88.0 (protonated dehydrated serine) and at m/z 43.0 (protonated dehydrated ethanolamine).

Protonated molecules of glycerophosphatidylcholine behaved completely different from the corresponding precursor ions of the other aminophospholipids, glycerophosphatidylserine and glycerophosphatidylethanolamine, upon high-energy CID MALDI-TOF/RTOF MS. In the high-mass region, only very low abundant product ions corresponding to charge-remote fragmentations of the fatty acid substituents including **$^C A_{12}$**- and **$^C A_{23}$**-type ions as well as product ions corresponding to the loss of fatty acid ketenes (**$^C C_{12}+18$** and **$^C C_{23}+18$**) and to the loss of one neutral fatty acid (**$^C C_{12}$** and **$^C C_{23}$**), respectively, were found (for corresponding ion structures to common negative-ion product ion structures see Fig. 2). Product ions, where both fatty acid substituents are at least partially fragmented, include the **$^C D_3$**- (m/z 354.1), the **$^C E_3$**- (m/z 296.1) and the **$^C F_3$**-type ions (m/z 282.1), which are again in structural analogy to sodiated triacylglycerol product ions [37] (for corresponding ion structures to common negative-ion product ion structures see Figs. 6 and 8). In the low-mass region several product ions of diagnostic value for the identification of glycerophosphatidylcholines are detected including the **$^C G_3$**- (m/z 224.1), the **$^C G_3 - 40$**- (m/z 184.1 being the base-peak for protonated glycerophosphatidylcholine product ions) and **$^C G_3 - 58$**-ion (m/z 166.1) (for corresponding ion structures to common negative-ion product ion structures see Fig. 6). Other product characterizing the polar

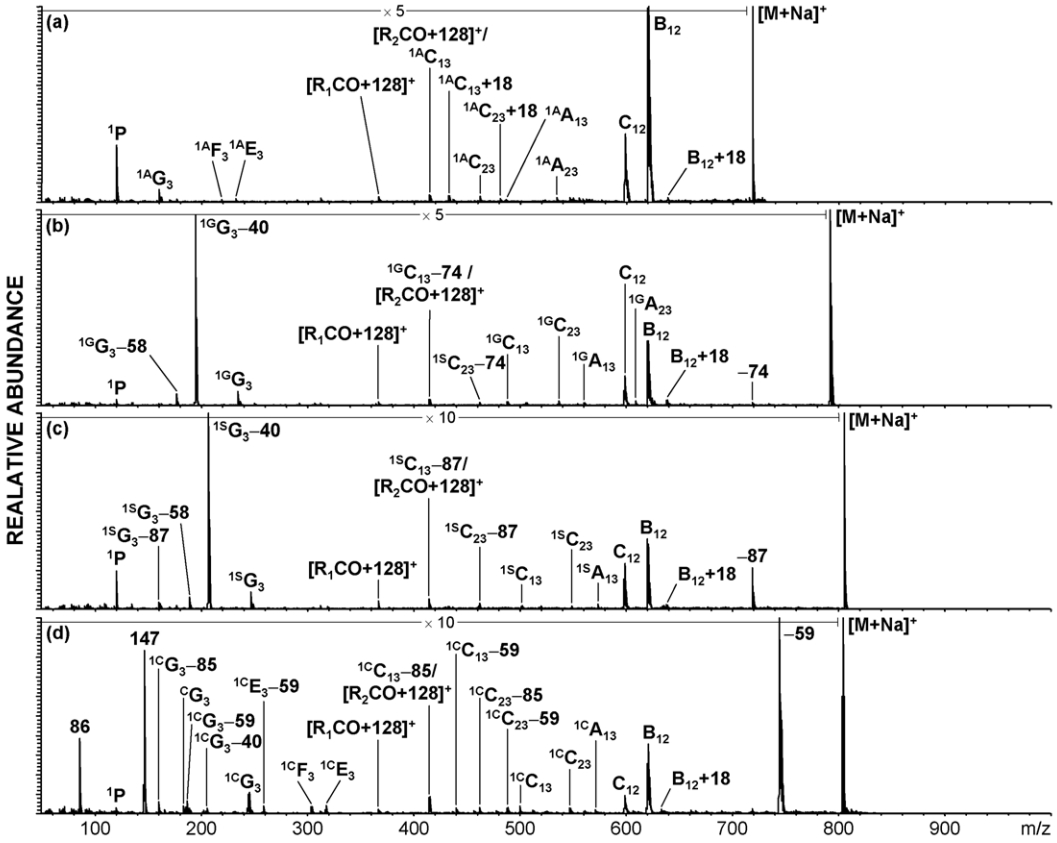

Fig. 5. Positive-ion high-energy CID MALDI-TOF/RTOF-spectra of the **[M+Na]⁺**-precursor ions of 1-palmitoyl-2-arachidonoyl-phosphatidic acid (*m/z* 747.5) (a), of 1-palmitoyl-2-arachidonoyl-phosphatidylglycerol (*m/z* 793.5) (b), of 1-palmitoyl-2-arachidonoyl-phosphatidylserine (*m/z* 806.5) (c) and of 1-palmitoyl-2-arachidonoyl-phosphytidylcholine (*m/z* 804.5) (d).

head-group includes *m/z* 125.0 (protonated O-vinyl-phosphate or a cyclized form thereof), *m/z* 104.1 (protonated choline) and *m/z* 86.1 (protonated dehydrated choline). A fragmentation scheme only valid for **[M+H]⁺**-precursor ions of glycerophosphatidylserines and glycerophosphatidylethanolamines is displayed in Fig. 4 due to the fact that this type of precursor ion of glycerophosphatidylcholine exhibits different fragmentation pathways as described above.

3.3. High-energy CID of *[M+Na]⁺* precursor ions (A, G, I, S, E, C)

In contrast to deprotonated and protonated glycerophospholipid precursor ions, all six glycerophospholipid classes investigated exhibited abundant singly sodiated precursor ions. A selection of high-energy (E_{LAB} = 20 keV) CID-TOF/RTOF-spectra of **[M+Na]⁺**-precursor ions including 1-palmitoyl-2-arachidonoyl-glycerophosphatidic acid, 1-palmitoyl-2-arachidonoyl-glycerophosphatidylglycerol, 1-palmitoyl-2-arachidonoyl-glycerophosphatidylserine and 1-palmitoyl-2-arachidonoyl-glycerophosphatidylcholine is shown in Fig. 5.

With the exception of glycerophosphatidic acid, all other compounds (G, I, S, E and C) showed significant loss of the variable aminoalcohol- or polyalcohol-substituent (G: **–74**, I: **–162**, E: **–43**, S: **–87**; C: **–59**, i.e., loss of trimethylamine instead of dehydrated choline) in the high-mass region (for product ion structures see Fig. 6). Another significant loss observed for all six classes of glycerophospholipids was the loss of the entire polar head group leading to the **B_{12}**- [sodiated dehydrated diacylglycerol cations for A: **[M+Na–98]⁺**, for G: **[M+Na–172]⁺**, for I: **[M+Na–232]⁺**, for S: **[M+Na–185]⁺**, for E: **[M+Na–141]⁺** and for C: **[M+Na–183]⁺**] and the **C_{12}**-product ions (sodium-free dehydrated diacylglycerol cations; for product ion structures see Fig. 6). As these two types of product ions are structurally identical to product ions obtained from sodiated triacylglycerols these ions have the same nomenclature as corresponding product ions from these neutral lipid molecules [37]. A very low abundant ion accompanying the **B_{12}**-ion, the **B_{12} + 18**-ion (sodiated diacylglycerol cation), is observed for all six glycerophospholipid classes. Further, charge-remote fragmentations of the fatty acid substituents are generally not detected, but product ions of the type **$^{1X}A_{13}$** and also **$^{1X}A_{23}$**, end products of charge-remote fragmentation of long chain fatty acid substituents, are found at very low abundance for all six compound classes (for product ion structures see Fig. 6). Similar observations were made for **$^{1X}C_{13}$** and **$^{1X}C_{23}$** product ions, where one neutral fatty acid substituent either from the *sn*1- or the *sn*2-position is lost (for product ion structures see Fig. 6). These types of **C**-ions exhibit in all cases additional loss of the aminoalcohol- or the polyalcohol-residue [G: **$^{1G}C_{13}$–74/$^{1G}C_{23}$–74**; I: **$^{1I}C_{13}$–162/$^{1I}C_{23}$–162**; S: **$^{1S}C_{13}$–87/$^{1S}C_{23}$–87**; E: **$^{1E}C_{13}$–43/$^{1E}C_{23}$–43**; C: **$^{1C}C_{13}$–59/$^{1C}C_{23}$–59** (loss of trimethylamine) and **$^{1C}C_{13}$–85/$^{1C}C_{23}$–85** (loss of dehydrated choline)]. The product ions **$[R_1CO+128]^+$/$[R_2CO+128]^+$** (present for all six lipid classes), which represent sodium-free ions, were already described in the section of protonated precursor ions. **$^{1X}E_3$**- and **$^{1X}F_3$**-type ions are only found under high-energy CID-conditions but not under PSD-conditions (data not shown). As for glycerophospholipids only the phosphate-containing *sn*3-substituent yields **$^{1X}E_3$**- [X = A (*m/z* 233.0), G (*m/z* 307.0), I (*m/z* 395.1), S (*m/z* 320.1), E (*m/z* 276.1) and C (*m/z* 318.1)] and **$^{1X}F^3$**-type ions [X = A (*m/z* 219.0), G (*m/z* 293.0), I (*m/z* 381.1), S (*m/z* 306.0), E (*m/z* 262.1) and C (*m/z* 304.1)], but this fragmentation cannot be used for differentiation of isomeric glycerophos-

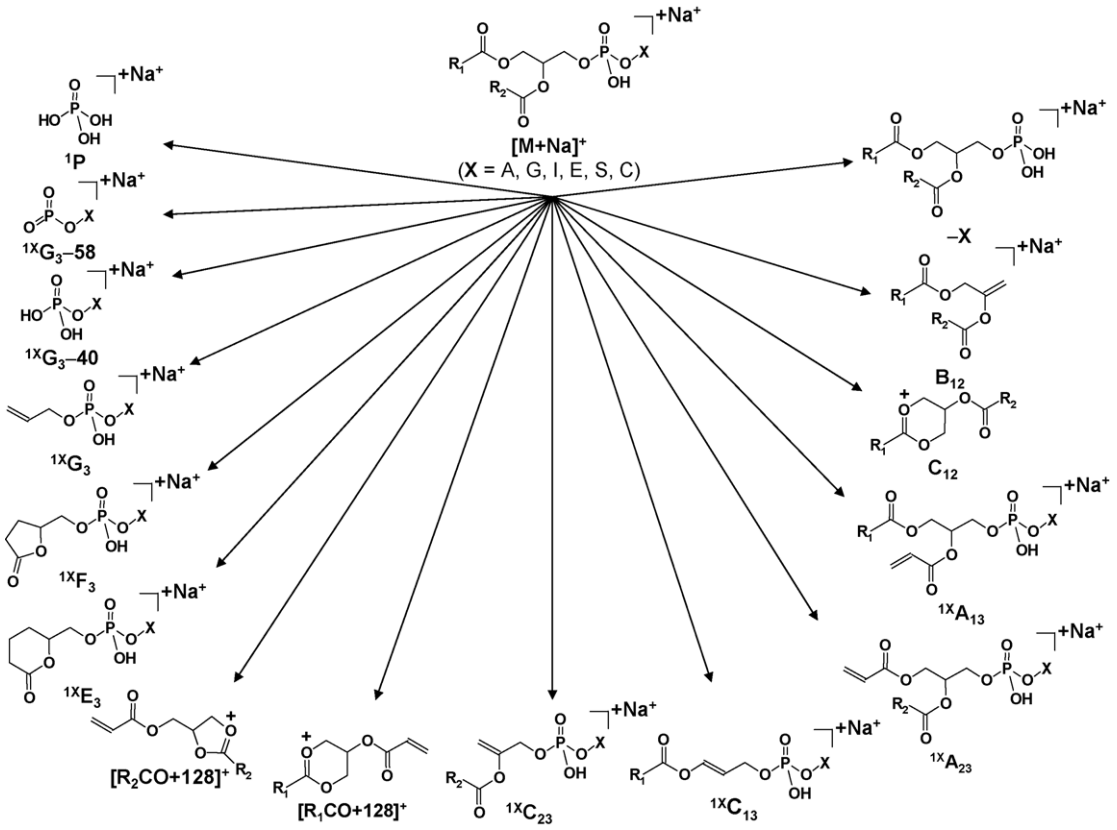

Fig. 6. General fragmentation scheme of **[M+Na]$^+$**-precursor ions obtained by high-energy CID MALDI-TOF/RTOF mass spectrometry identifying the structure of the major product ions (abbreviations used: A = glycerophosphatidic acid, G = glycerophosphatidylglycerol, I = glycerophosphatidylinositol, S = glycerophosphatidylserine, E = glycerophosphatidylethanolamine, C = glycerophosphatidylcholine).

pholipids of the same class (for all product ion structures see Fig. 6).

Only in the case of glycerophosphatidylcholine additional loss for the **$^{1C}E_3$**- and **$^{1C}F^3$**-type ions could be found [**$^{1C}E_3$** – **59** (*m*/*z* 259.0), **$^{1C}E_3$** – **85** (*m*/*z* 233.0), **$^{1C}F_3$** – **59** (*m*/*z* 245.0) and **$^{1C}F_3$** – **85** (*m*/*z* 219.0)]. Other low-mass ions found for all six different glycerophospholipid classes include the **$^{1X}G_3$**-ions [X = A (*m*/*z* 161.0), G (*m*/*z* 235.0), I (*m*/*z* 323.1), S (*m*/*z* 248.1), E (*m*/*z* 204.1) and C (*m*/*z* 246.1)]. The **$^{1X}G_3$** – **40**-ions (loss of the O-allyl-substituent) were detected for all lipid classes except for glycerophosphatidic acid [X = G (*m*/*z* 195.0), I (*m*/*z* 283.0), S (*m*/*z* 208.0), E (*m*/*z* 164.0) and C (*m*/*z* 206.1)] and the **$^{1X}G_3$** – **58**-ions (loss of the O-allyl-substituent plus water) for all except for glycerophosphatidic acid and glycerophosphatidylcholine [X = G (*m*/*z* 177.0), I (*m*/*z* 265.0), S (*m*/*z* 190.0) and E (*m*/*z* 146.0)] (for all product ion structures see Fig. 6). Additionally, in the case of glycerophosphatidylcholine **$^{1C}G_3$** – **59**- (loss of trimethylamine, *m*/*z* 187.0) and **$^{1C}G_3$** – **85**-ions (loss of dehydrated choline, *m*/*z* 161.0) as well as in the case of glycerophosphatidylinositol **$^{1I}G_3$** – **120**-ions (sodiated inositol, *m*/*z* 203.0) were observed. Two other ions of minor abundance found for phosphatidylcholine, the ions at *m*/*z* 184.1 and *m*/*z* 166.1, represent sodium-free product ions of the type **$^{C}G_3$** – **40** and **$^{C}G_3$** – **58** (for product ion structures see Fig. 6). Their appearance at low intensity in the high-energy CID-spectrum of the corresponding **[M+Na]$^+$**-precursor ion is in good agreement with previous findings from high-energy CID with tandem sector instrumentation combined with FAB [49]. The ions at *m*/*z* 147.0 and *m*/*z* 86.1 in the CID-spectrum of sodiated glycerophosphatidylcholine are due to sodiated cyclized O-vinyl-phosphate and protonated dehydrated choline, respectively. The ion of lowest *m*/*z* value detected for all six glycerophospholipids could be detected at *m*/*z* 121.0, the sodiated phosphate- or **^{1}P**-ion (for product ion structure see Fig. 6). A general fragmentation scheme applicable for all six types of glycerophospholipids investigated in this study is summarized in Fig. 6.

3.4. High-energy CID of *[M+2Na–H]$^+$- (A, G, I, S, E) and [M+3Na–2H]$^+$- (A, S) precursor ions*

Due to the zwitterionic structure of glycerophosphatidylcholine, this analyte can only carry one sodium cation and therefore this type of molecule does not form either doubly or triply sodiated singly charged precursor ions under vacuum MALDI-MS conditions. The remaining five classes of glycerophospholipids (A, G, I, S and E) all formed abundant **[M+2Na–H]$^+$**-ions, especially after doping the MALDI-MS matrix solution with sodium chloride. Examples of corresponding high-energy CID-TOF/RTOF-spectra of the **[M+2Na–H]$^+$**-precursor ions of 1-palmitoyl-2-arachidonoyl-glycerophosphatidylglycerol, 1-stearoyl-2-arachidonoyl-glycerophosphatidylinositol and 1-palmitoyl-2-linoleoyl-glycerophosphatidylethanolamine as well as of the **[M+3Na–2H]$^+$**-precursor ion of 1-palmitoyl-2-arachidonoyl-glycerophosphatidylserine are displayed in Fig. 7.

The five glycerophospholipid classes mentioned above all exhibited abundant loss of the polyalcohol- or aminoalcohol-substituent (G: –**74**, I: –**162**, E: –**43**, S: –**87** except glycerphosphatidic acid, which does not contain such substituent) as well as significant charge-remote fragmentation of the fatty acid-residues, the most abundant ions to be the **$^{2X}A_{13}$**-/**$^{2X}A_{23}$**-product ions (for all product ion structures see Fig. 8). Glycerohosphatidylserine additionally showed some additional ions corresponding to **$^{2S}A_{13}$**–**87**-/**$^{2S}A_{23}$**–**87**-product ions demonstrating the weakness of the phosphoserine bond under high-energy CID-conditions.

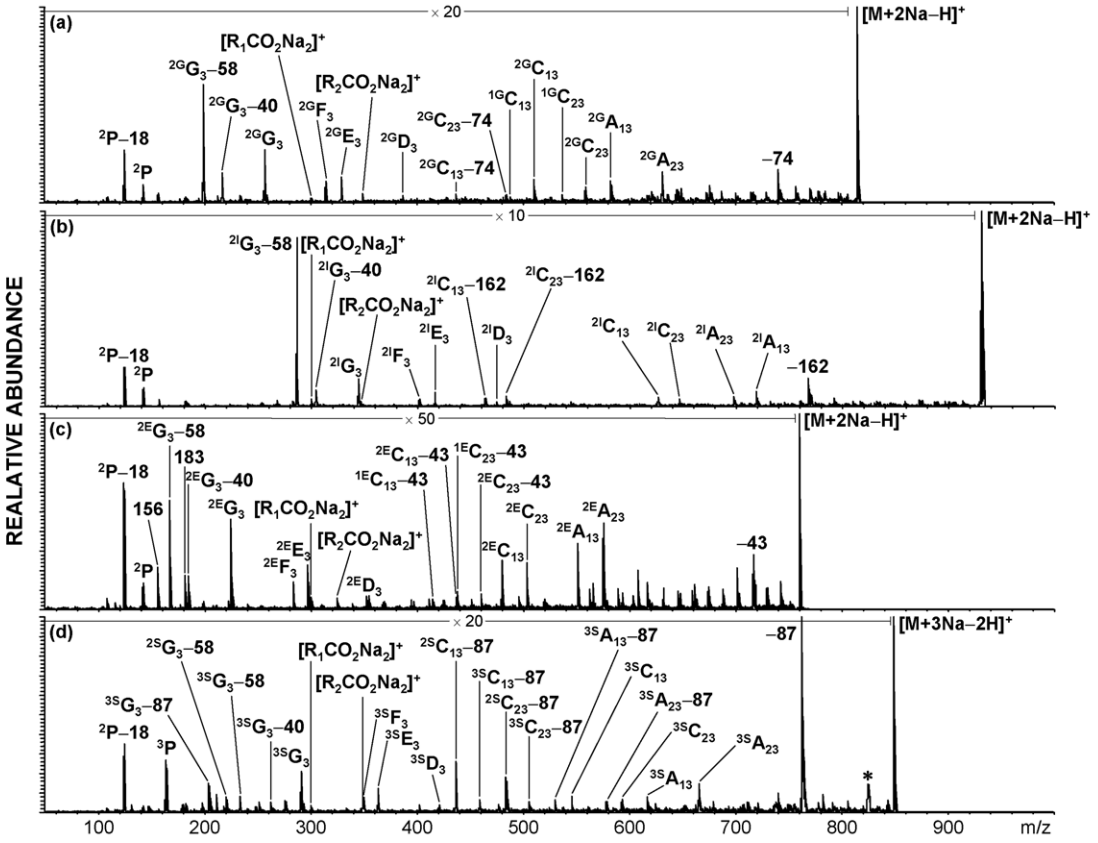

Fig. 7. Positive-ion high-energy CID MALDI-TOF/RTOF-spectra of the **[M+2Na−H]⁺**-precursor ions of 1-palmitoyl-2-arachidonoyl-phosphatidylglycerol (*m/z* 815.5) (a), of 1-stearoyl-2-arachidonoyl-phosphatidylinositol (*m/z* 931.5) (b), of 1-palmitoyl-2-linoleoyl-phosphatidylethanolamine (*m/z* 760.5) (c), and of the **[M+3Na−2H]⁺**-precursor ion of 1-palmitoyl-2-arachidonoyl-phosphatidylserine (*m/z* 850.5) (d).

Corresponding **B_{12}**- and **C_{12}**-type product ions, as observed for monosodiated glycerophospholipid precursor ions, were not observed in high-energy CID-spectra of doubly sodiated singly charged glycerophopholipid precursor ions. Instead, **$^{2X}C_{13}$**- and **$^{2X}C_{23}$**-product ions accompanied by **$^{2X}C_{13}$**−**X**- and **$^{2X}C_{23}$**−**X**-ions (X for G: −**74**, I: −**162**, E: −**43**, S: −**87**) are dominating the spectra (for all product ion structures see Fig. 8). Interestingly, glycerophosphatidylethanolamines also show **$^{1E}C_{13}$**−**43**- and **$^{1E}C_{23}$**−**43**-ions, where one sodium cation is lost during fragmentation. One type of product ion already found in high-energy CID-spectra of sodiated triacylglycerol precursor ions, the low abundant **$^{2X}D_3$**-type ions [X = A (*m/z* 313.0), G (*m/z* 387.0), I (*m/z* 475.1), S (*m/z* 400.0), E (*m/z* 356.0)], where both fatty acid substituent are partially fragmented, were detected for all mentioned compound classes (for product ion structure see Fig. 8). An interesting type of product ion was detected for the intact fatty acid substituents, the **$[R_nCO_2Na_2]^+$**-product ion (*n* = 1 or 2; for product ion structure see Fig. 8). These ions are only observed for doubly sodiated singly charged glycerophospholipid precursor ions containing one negative charge-site, i.e., glycerophosphatidylglycerol, glycerophosphatidylinositol and glycerophosphatidylethanolamine. Their formation seems not to depend on activation of the particular precursor ion by (high-energy) CID as these ions are also observed under metastable (post-source decay) conditions [65]. **$^{2X}E_3$**- [X = A (*m/z* 255.0), G (*m/z* 329.0) I (*m/z* 417.1), S (*m/z* 342.0) and E (*m/z* 298.0)] and **$^{2X}F_3$**-type ions [X = A (*m/z* 241.0), G (*m/z* 315.0) I (*m/z* 403.0), S (*m/z* 328.0) and E (*m/z* 284.0)] are detected as doubly sodiated product ions for all five glycerophospholipid classes. Glycerophosphatidylserine also exhibit loss of serine related to these two ion types at *m/z* 255.0 (**$^{2S}E_3 - 87$**) and *m/z* 241.0 (**$^{2S}F_3 - 87$**). **$^{2X}G_3$**-product ions [X = A (*m/z* 183.0), G (*m/z* 257.0), I (*m/z* 345.0), S (*m/z* 270.0) and E (*m/z* 226.0)] are product ions of significant abundance for all five different **[M+2Na−H]⁺**-glycerophospholipid precursor ions (for all product ion structures see Fig. 8). In contrast, **$^{2X}G_3$**-product ions exhibiting additional loss of either 40 Da [X = G (*m/z* 217.0), I (*m/z* 305.0), S (*m/z* 230.0) and E (*m/z* 186.0)] as well as 58 Da [X = G (*m/z* 199.0), I (*m/z* 287.0), S (*m/z* 212.0) and E (*m/z* 168.0)], respectively are observed for all analytes except glycerophosphatidic acid. Finally, all **$^{2X}G_3$**-product ions except those for glycerophosphatidic acid also exhibit loss of the polyalcohol- or aminoalcohol-residue (**$^{2G}G_3 - 74$**, **$^{2I}G_3 - 162$**, **$^{2S}G_3 - 87$** and **$^{2E}G_3 - 43$**) yielding all one common product ion at *m/z* 183.0. An interesting low-mass product ion was detected at *m/z* 156.0 for practically all five groups of analytes investigated with the highest abundance for the doubly sodiated singly charged precursor ion of glycerophophatidylethanolamine. Based on our calculations and previous findings by others [49], the structure of this ion most likely corresponds to a disodiated O-methylene phosphate radical cation. A disodiated phosphate product ion (**^{2}P**: *m/z* 143.0) and a dehydrated disodiated phosphate ion (**^{2}P**−**18**: *m/z* 124.9) were the typical product ions at the low mass end of the high-energy CID-spectra of all five glycerophospholipids forming disodiated precursor ions (for all product ion structures see Fig. 8).

The only glycerophospholipid species, which allowed the generation of **[M+3Na−2H]⁺**-adduct ions after doping the matrix solution with sodium chloride, were glycerophosphatidic acid and glycerophosphatidylserine. These two glycerophospholipids contain two acidic sites, so that two sodium cations neutralize the two acidic hydrogens and one sodium cation acts as the charging species. Product ions typically observed for triply sodiated singly charged glycerophospholipid precursor ions are practically analogous to those observed for doubly sodiated precursor ions with

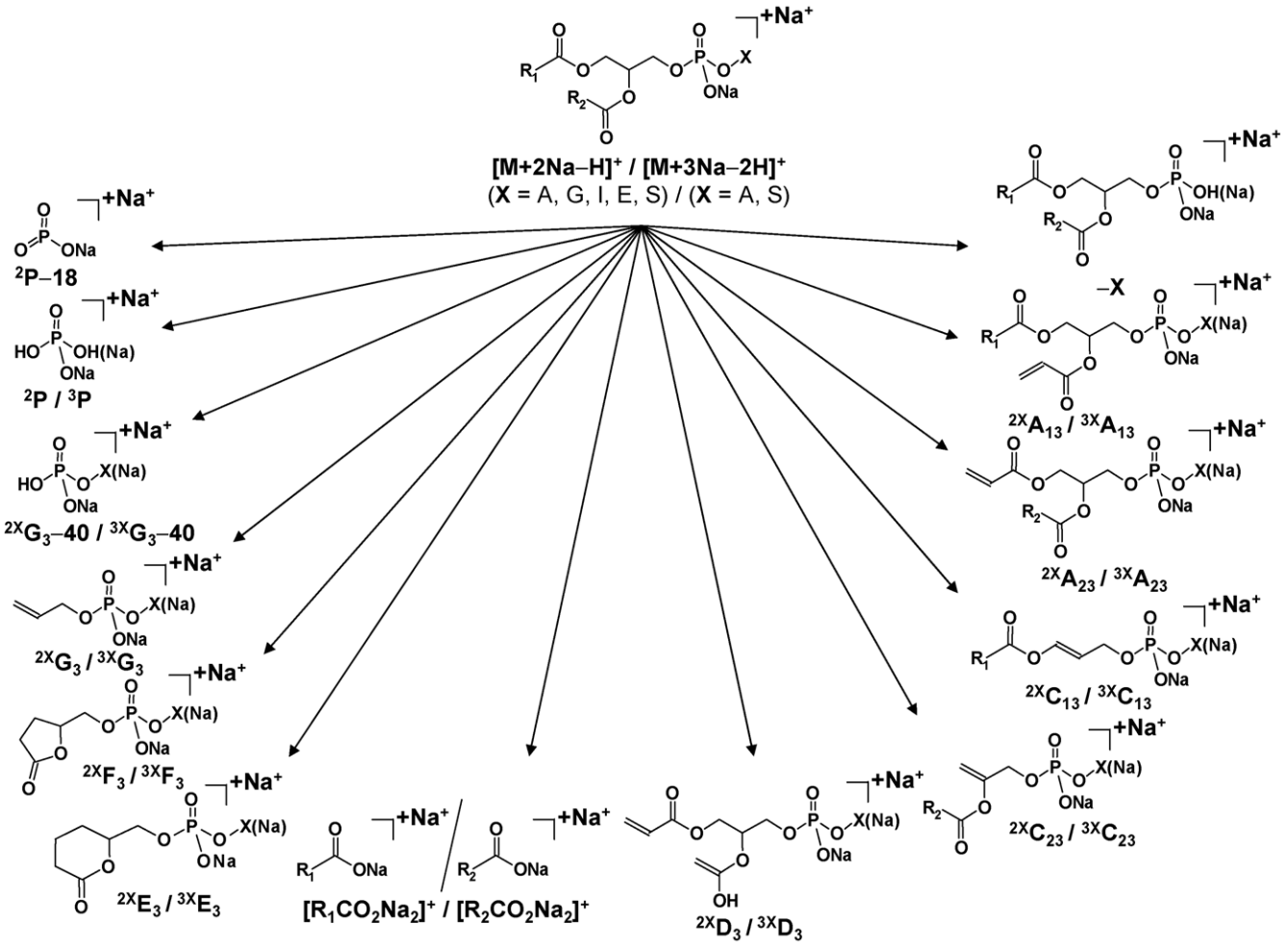

Fig. 8. General fragmentation scheme of **[M+2Na–H]**$^+$- and of **[M+3Na–2H]**$^+$-precursor ions, respectively, obtained by high-energy CID MALDI-TOF/RTOF mass spectrometry identifying the structure of the major product ions (abbreviations used: A = glycerophosphatidic acid, G = glycerophosphatidylglycerol, I = glycerophosphatidylinositol, S = glycerophosphatidylserine, E = glycerophosphatidylethanolamine).

some minor differences. Several product ions in the middle to low *m*/*z* region do not only occur as triply sodiated (**$^{3X}C_{13}$**, **$^{3X}C_{23}$**, **$^{3X}C_{13}$ – 87**, **$^{3X}C_{23}$ – 87**; X = A, S), but also as doubly sodiated product ions (**$^{2X}C_{13}$**, **$^{2X}C_{23}$**, **$^{2X}C_{13}$ – 87**, **$^{2X}C_{23}$ – 87**; X = A, S). The product ions corresponding to doubly sodiated fatty acids (**$[R_nCO_2Na_2]^+$**, *n* = 1 or 2) are only formed from triply sodiated singly charged precursor ions (glycerophosphatidic acid, glycerophosphatidylserine), but were never generated from doubly sodiated precursor ions of these two analytes. Additionally, **$^{3X}D_3$**- [X = A (*m*/*z* 335.0), S (*m*/*z* 422.0)], **$^{3X}E_3$**- [X = A (*m*/*z* 277.0), S (*m*/*z* 364.0)], **$^{3X}F_3$**- [X = A (*m*/*z* 263.0), S (*m*/*z* 350.0)] and **$^{3X}G_3$**-ions [X = A (*m*/*z* 205.0), S (*m*/*z* 292.0)] were observed for these two glycerophospholipid species. The most prominent ions at the low mass end of CID-spectra of triply sodiated singly charged glycerophospholipid precursor were the triply sodiated phosphate ion (**^3P**: *m*/*z* 164.9) and the dehydrated doubly sodiated phosphate ion (**$^2P - 18$**: *m*/*z* 124.9) (for all product ion structures see Fig. 8). The common product ions of doubly and triply sodiated glycerophospholipid precursor ions are displayed in Fig. 8. With respect to the number of sodium cations attached to the lipid molecules.

4. Conclusion

Selecting different precursor ions (**[M–H]**$^-$-, **[M+H]**$^+$-, **[M+Na]**$^+$-, **[M+2Na–H]**$^+$- and **[M+3Na–2H]**$^+$-ions) of six different classes of glycerophospholipids (glycerophosphatidic acid, glycerophosphatidylglycerol, glycerophosphatidylinositol, glycerophosphatidylserine, glycerophosphatidylethanolamine and glycerophosphatidyl-choline) differing in the type of polyalcohol (glycerol, inositol) and aminoalcohol (serine, ethanolamine, choline) linked to the polar phosphate-moiety yields a wide variety of different fragmentation pathways. Independently of the polarity (negative or positive ions) and the type of the selected glycerophospholipid precursor ions a precise and stringent product ion nomenclature could be established by adapting our previously published product ion nomenclature for sodiated triacylglycerol precursor ions [37] for different classes of glycerophospholipids: (1) a preceding superscripted arabic number (1, 2 or 3) indicates the number of sodium cations attached to the lipid molecule (if absent, either deprotonated or protonated precursor ions have to be considered), (2) a preceding superscripted capital letter identifies the class of glycerophospholipid (A, G, I, S, E or C), (3) a bold capital letter identifies the type of product ion (**A**, **B**, **C**, **D**, **E**, **F** or **G**), (4) (a) successive subscripted arabic number(s) identify (e.g., 12, 13, 23, 3) substituents, which remained intact on the product ion and (5) additional loss of small neutrals are indicated by, e.g., "–**40**". This system of universal product ion nomenclature allows full description of product ions obtained by high-energy CID- as well as by metastable ion (post-source) decay and also of other types of precursor ions as, e.g., other types of

adduct ions with alkali cations as lithium or potassium, the latter being frequently present in biological preparations of brain tissue [68]. Finally, this product ion nomenclature can easily be adapted for product ion spectra obtained by low-energy CID utilizing either ion trap-, triple quadrupole- or Q-RTOF-instrumentation and should simplify/assist in complex data interpretation as typically obtained from imaging mass spectrometry (IMS)- [68] or lipidomics-experiments [71].

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References

[1] P. Roepstorff, J. Fohlman, Proposal for a common nomenclature for sequence ions in mass spectra of peptides, Biomed. Mass Spectrom. 11 (1984) 601.
[2] S.A. Martin, K. Biemann, A comparison of keV atom bombardment mass spectra of peptides obtained with a two-sector mass spectrometer with those from a four-sector tandem mass spectrometer, Int. J. Mass Spectrom. Ion Proc. 78 (1987) 213–228.
[3] J.T. Stults, J.T. Watson, Identification of a new type of fragment ion in the collisional activation spectra of peptides allows leucine/isoleucine differentiation, Biomed. Environ. Mass Spectrom. 14 (1987) 583–586.
[4] R.S. Johnson, S.A. Martin, K. Biemann, J.T. Stults, J.T. Watson, Novel fragmentation process of peptides by collision-induced decomposition in a tandem mass spectrometer: differentiation of leucine and isoleucine, Anal. Chem. 59 (1987) 2621–2625.
[5] R.S. Johnson, S.A. Martin, K. Biemann, Collision-induced fragmentation of $(M+H)^+$ ions of peptides. Side chain specific sequence ions, Int. J. Mass Spectrom. Ion Proc. 86 (1988) 137–154.
[6] K. Biemann, Contributions of mass spectrometry to peptide and protein structure, Biomed. Environ. Mass Spectrom. 16 (1988) 99–111.
[7] K.B. Tomer, L. Deterding, C. Guenat, Collisionally activated dissociation spectra of sodiated peptides and peptide amides, Biol. Mass Spectrom. 20 (1991) 121–129.
[8] S.C. Summerfield, V.C.M. Dale, D.D. Despeyroux, K.R. Jennings, Charge remote losses of small neutrals from protonated and group 1 metal-peptide complexes of peptides, Eur. Mass Spectrom. 1 (1995) 183–195.
[9] B. Domon, C.E. Costello, A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates, Glycoconjug. J. 5 (1988) 397–409.
[10] E. Spina, L. Sturiale, D. Romeo, G. Impallomeni, D. Garozzo, D. Waidelich, M. Glueckmann, New fragmentation mechanisms in matrix-assisted laser desorption/ionization time-of-flight/time-of-flight tandem mass spectrometry of carbohydrates, Rapid Commun. Mass Spectrom. 18 (2004) 392–398.
[11] S.L. Maslen, F. Goubet, A. Adam, P. Dupree, E. Stephens, Structure elucidation of arabinoxylan isomers by normal phase HPLC-MALDI-TOF/TOF-MS/MS, Carbohydr. Res. 342 (2007) 724–735.
[12] R.L. Cerny, K.B. Tomer, M.L. Gross, L. Grotjahn, Fast atom bombardment combined with tandem mass spectrometry for determining structures of small oligonucleotides, Anal. Biochem. 165 (1987) 175–182.
[13] S. McLuckey, G.J. Van Berkel, G.L. Glish, Tandem Mass Spectrometry of small, multiply charged oligonucleotides, J. Am. Soc. Mass Spectrom. 3 (1992) 60–70.
[14] W.J. Griffiths, J. Zhang, J. Sjövall, Charge-remote fragmentation of sulfated and glucuronidated bile acids and their 2-aminoethanesulfonic acid derivatives, Rapid Commun. Mass Spectrom. 8 (1994) 227–236.
[15] W.J. Griffiths, A. Brown, R. Reimendal, Y. Yang, J. Zhang, J. Sjövall, A comparison of fast atom bombardment and electrospray as methods of ionization in the study of sulphated- and sulphonated-lipids by tandem mass spectrometry, Rapid Commun. Mass Spectrom. 10 (1996) 1169–1174.
[16] W.J. Griffiths, S. Liu, Y. Yang, R.H. Purdy, J. Sjövall, Nano-electrospray tandem mass spectrometry for the analysis of neurosteroid sulphates, Rapid Commun. Mass Spectrom. 13 (1999) 1595–1610.
[17] W.J. Griffiths, S. Liu, G. Alvelius, J. Sjövall, Derivatisation for the characterisation of neutral oxosteroids by electrospray and matrix-assisted laser desorption/ionisation tandem mass spectrometry: the Girard P derivative, Rapid Commun. Mass Spectrom. 17 (2003) 924–935.
[18] W.J. Griffiths, G. Alvelius, S. Liu, J. Sjövall, High-energy collision-induced dissociation of oxosteroids derivatized to Girard hydrazones, Eur. J. Mass Spectrom. 10 (2004) 63–88.
[19] Y. Wang, M. Hornshaw, G. Alvelius, K. Bodin, S. Liu, J. Sjövall, W.J. Griffiths, Matrix-assisted laser desorption/ionization high-energy collision-induced dissociation of steroids: analysis of oxysterols in rat brain, Anal. Chem. 78 (2006) 164–173.
[20] W.J. Griffiths, Y. Wang, G. Alvelius, S. Liu, K. Bodin, J. Sjövall, Analysis of oxysterols by electrospray tandem mass spectrometry, J. Am. Soc. Mass Spectrom. 17 (2006) 341–362.
[21] W.J. Griffiths, Y. Yang, J.A. Lindgren, J. Sjovall, Charge remote fragmentation of fatty acid anions in 400 eV collisions with xenon atoms, Rapid Commun. Mass Spectrom. 10 (1996) 21–28.
[22] K.B. Tomer, F.W. Crow, M.L. Gross, Location of double bond position in saturated fatty acids by negative ion MS/MS, J. Am. Chem. Soc. 105 (1983) 5487–5488.
[23] N.J. Jensen, K.B. Tomer, M.L. Gross, Gas-phase ion decomposition occurring remote to a charge site, J. Am. Chem. Soc. 107 (1985) 1863–1868.
[24] N.J. Jensen, K.B. Tomer, M.L. Gross, Collisional activation decomposition mass spectra for locating double bonds in polyunsaturated fatty acids, Anal. Chem. 57 (1985) 2018–2021.
[25] K.B. Tomer, N.J. Jensen, M.L. Gross, Fast atom bombardment and tandem mass spectrometry for determining structural modification of fatty acids, Anal. Chem. 58 (1986) 2429–2433.
[26] N.J. Jensen, M.L. Gross, Fast atom bombardment and tandem mass spectrometry for determining iso and anteiso-fatty acids, Lipids 21 (1986) 362–365.
[27] J. Adams, M.L. Gross, Tandem mass spectrometry for collisional activation of alkali-metal-cationized fatty acids: a method for determining double bond location, Anal. Chem. 59 (1987) 1576–1582.
[28] J. Adams, M.L. Gross, Structural determination of modified fatty acids by collisional activation of cationized molecules, Org. Mass Spectrom. 23 (1988) 307–316.
[29] J.S. Crockett, M.L. Gross, W.W. Christie, R.T. Holman, Collisional activation of a series of homoconjugated octadecanoic acids with fast atom bombardment and tandem mass spectrometry, J. Am. Soc. Mass Spectrom. 111 (1989) 183–191.
[30] J. Adams, M.L. Gross, Charge-remote fragmentations of closed-shell ions. A thermolytic analogy, J. Am. Chem. Soc. 1 (1989) 435–440.
[31] E. Davoli, M.L. Gross, Charge remote fragmentation of fatty acids cationized with alkaline earth metal ions, J. Am. Soc. Mass Spectrom. 1 (1990) 320–324.
[32] M.J. Contado, J. Adams, N.J. Jensen, M.L. Gross, A Charge-remote allylic cleavage reaction: mechanistic possibilities, J. Am. Soc. Mass Spectrom. 2 (1991) 180–183.
[33] N.J. Jensen, M.L. Gross, Mass spectrometry methods for structural determination and analysis of fatty acids, Mass Spectrom. Rev. 6 (1987) 497–536.
[34] Q. Ann, J. Adams, Structure determination of ceramides and neutral glycosphingolipids by collisional activation of $[M+Li]^+$ ions, J. Am. Soc. Mass Spectrom. 3 (1992) 260–263.
[35] Q. Ann, J. Adams, Structure-specific collision-induced fragmentations of ceramides cationized with alkali-metal ions, Anal. Chem. 65 (1995) 7–13.
[36] Q. Ann, J. Adams, Collision-induced decomposition of sphingomyelins for structural elucidation, Biol. Mass Spectrom. 22 (1993) 285–294.
[37] C. Cheng, M.L. Gross, E. Pittenauer, Complete structural elucidation of triacylglycerols by tandem sector mass spectrometry, Anal. Chem. 70 (1998) 4417–4426.
[38] E. Pittenauer, W. Welz, Free fatty acids and triacylglycerols, in: M.L. Gross, R.M. Caprioli (Eds.), The Encyclopaedia of Mass Spectrometry, Vol. III: Biological Applications Part A: Carbohydrates, Nucleic Acids and other Biological Compounds, Elsevier, Oxford, UK, 2005, pp. 397–415.
[39] E. Pittenauer, G. Allmaier, High-energy collision-induced dissociation of biomolecules by means of MALDI-TOF/RTOF mass spectrometry in comparison to tandem sector mass spectrometry, Comb. Chem. High Throughput Screen. 12 (2009) 137–155.
[40] E. Pittenauer, G. Allmaier, The renaissance of high-energy CID for structural elucidation of complex lipids: MALDI-TOF/RTOF-MS of alkali cationized triacylglycerols, J. Am. Soc. Mass Spectrom. 20 (2009) 1037–1047.
[41] Y.H. Kim, K.-Y. So, J.-K. Limb, G.-J. Jhon, S.-Y. Han, Identification of triacylglycerols containing two short-chain fatty acids at sn-2 and sn-3 positions from bovine udder by fast atom bombardment tandem mass spectrometry, Rapid Commun. Mass Spectrom. 14 (2000) 2230–2237.
[42] H. Münster, J. Stein, H. Budzikiewicz, Structure analysis of underivatized phospholipids by negative ion fast atom bombardment mass spectrometry, Biomed. Environ. Mass Spectrom. 13 (1986) 423–427.
[43] N.J. Jensen, K.B. Tomer, M.L. Gross, Fast atom bombardment and tandem mass spectrometry of phosphatidylserine and phosphatidylcholine, Lipids 21 (1986) 580–588.
[44] N.J. Jensen, K.B. Tomer, M.L. Gross, FAB MS/MS for phosphatidylinositol, -glycerol, -ethanolamine and other complex phospholipids, Lipids 22 (1987) 480–489.
[45] H. Münster, H. Budzikiewicz, Structural and mixture analysis of glycerophosphoric acid derivatives by fast atom bombardment tandem mass spectrometry, Biol. Chem. Hoppe-Seyler 369 (1988) 303–308.
[46] A. Hayashi, T. Matsubara, M. Morita, T. Kinoshita, T. Nakamura, Structural analysis of choline phospholipids by fast atom bombardment mass spectrometry and tandem mass spectrometry, J. Biochem. 106 (1989) 264–269.
[47] S. Chen, K.W. Li, Structural analysis of underivatized aminophospholipids and phosphatidic acid by positive ion liquid secondary ion and collisionally induced dissociation tandem mass spectrometry, J. Biochem. 116 (1994) 811–817.
[48] S. Chen, Tandem mass spectrometric approach for determining structure of molecular species of aminophospholipids, Lipids 32 (1997) 85–100.
[49] Y.H. Kim, J.S. Yoo, M.S. Kim, Structural determination of fatty acyl groups of phospholipids by fast atom bombardment tandem mass spectrometry of sodium adduct molecular ions, Bull. Korean Chem. Soc. 18 (1997) 874–880.

[50] F.-F. Hsu, A. Bohrer, J. Turk, Formation of lithiated adducts of glycerophosphocholine lipids facilitates their identification by electrospray ionization tandem mass spectrometry, J. Am. Soc. Mass Spectrom. 9 (1998) 516–528.
[51] F.-F. Hsu, J. Turk, Charge-remote and charge-driven fragmentation processes in diacyl glycerophosphoethanolamine upon low-energy collisional activation. A mechanistic proposal, J. Am. Soc. Mass Spectrom. 11 (2000) 892–899.
[52] F.-F. Hsu, J. Turk, Charge-driven fragmentation processes in diacyl glycerophosphatidic acids upon low-energy collisional activation. A mechanistic proposal, J. Am. Soc. Mass Spectrom. 11 (2000) 797–803.
[53] F.-F. Hsu, J. Turk, Characterization of phosphatidylethanolamine as a lithiated adduct by triple quadrupole tandem mass spectrometry with electrospray ionization, J. Mass Spectrom. 35 (2000) 596–606.
[54] F.-F. Hsu, J. Turk, Characterization of phosphatidylinositol, phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate by electrospray ionization tandem mass spectrometry: a mechanistic study, J. Am. Soc. Mass Spectrom. 11 (2000) 986–999.
[55] F.-F. Hsu, J. Turk, Electrospray ionization/tandem quadrupole mass spectrometric studies on phosphatidylcholines: the fragmentation process, J. Am. Soc. Mass Spectrom. 14 (2003) 352–363.
[56] F.-F. Hsu, J. Turk, Studies on phosphatidylserine by tandem quadrupole and multiple stage quadrupole ion-trap mass spectrometry with electrospray ionization: structural characterization and the fragmentation process, J. Am. Soc. Mass Spectrom. 16 (2005) 1510–1522.
[57] F.-F. Hsu, J. Turk, T. Williams, R. Welti, Electrospray ionization multiple stage quadrupole ion-trap and tandem quadruple mass spectrometric studies on phosphatidylglycerol from Arabidopsis leaves, J. Am. Soc. Mass Spectrom. 18 (2007) 783–790.
[58] F.-F. Hsu, J. Turk, Structural characterization of unsaturated glycerophospholipids by multiple-stage linear ion-trap mass spectrometry with electrospray ionization, J. Am. Soc. Mass Spectrom. 19 (2008) 1681–1691.
[59] N.J. Jensen, M.L. Gross, A comparison of mass spectrometry methods for structural determination and analysis of phospholipids, Mass Spectrom. Rev. 7 (1988) 41–69.
[60] R.C. Murphy, K.A. Harrison, Fast atom bombardment of phospholipids, Mass Spectrom. Rev. 13 (1994) 57–75.
[61] R.C. Murphy, J. Fiedler, J. Hevko, Analysis of nonvolatile lipids by mass spectrometry, Chem. Rev. 101 (2001) 479–526.
[62] M. Pulfer, R.C. Murphy, Electrospray mass spectrometry of phospholipids, Mass Spectrom. Rev. 22 (2003) 332–364.
[63] J. Schiller, R. Süß, J. Arnhold, B. Fuchs, J. Leßig, M. Müller, M. Petkovic, H. Spalteholz, O. Zschörnig, K. Arnold, Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry in lipid and phospholipid research, Prog. Lipid Res. 43 (2004) 449–488.
[64] S.N. Jackson, H.-Y. Wang, A.S.J. Woods, *In situ* structural characterization of phosphatidylcholines in brain tissue using MALDI-MS/MS, J. Am. Soc. Mass Spectrom. 16 (2005) 2052–2056.
[65] B. Fuchs, C. Schober, G. Richter, R. Süß, J. Schiller, MALDI-TOF MS of phosphatidylethanolamines: different adducts cause different post source decay (PSD) fragment ion spectra, J. Biochem. Biophys. Meth. 70 (2007) 689–692.
[66] G. Stübiger, E. Pittenauer, G. Allmaier, MALDI seamless postsource decay fragment ion analysis of sodiated and lithiated phospholipids, Anal. Chem. 80 (2008) 1664–1678.
[67] J. Gidden, J. Denson, R. Liyanage, D.M. Ivey, O.L. Lay Jr., Lipid composition in *Escherichia coli* and *Bacillus subtilis* during growth as determined by MALDI-TOF and TOF/TOF mass spectrometry, Int. J. Mass Spectrom. 283 (2009) 178–184.
[68] Y. Sugiura, M. Setou, Selective imaging of positively charged polar and nonpolar lipids by optimizing matrix solution composition, Rapid Commun. Mass Spectrom. 23 (2009) 2369–2378.
[69] O. Belgacem, A. Bowdler, I. Brookhouse, F.L. Brancia, E. Raptakis, Dissociation of biomolecules using a ultraviolet matrix-assisted laser desorption/ionisation time-of-flight/curved field reflectron tandem mass spectrometer equipped with a differential-pumped collision cell, Rapid Commun. Mass Spectrom. 20 (2006) 1653–1660.
[70] G. Stübiger, O. Belgacem, Analysis of lipids using 2-, 4-, 6-trihydroxyacetophenone as a matrix for MALDI mass spectrometry, Anal. Chem. 79 (2007) 3206–3213.
[71] P.L. Ivanova, S.B. Milne, D.S. Myers, H.A. Brown, Lipidomics: a mass spectrometry based systems level analysis of cellular lipids, Curr. Opin. Chem. Biol. 13 (2009) 526–531.