Detection of phosphatidylserine with a modified polar head group in human keratinocytes exposed to the radical generator AAPH

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Abstract

Phosphatidylserine (PS) is preferentially located in the inner leaflet of the cell membrane, and translocation of PS oxidized in fatty acyl chains to the outside of membrane has been reported as signaling to macrophage receptors to clear apoptotic cells. It was recently shown that PS can be oxidized in serine moiety of polar head-group. In the present work, a targeted lipidomic approach was applied to detecting OxPS modified at the polar head-group in keratinocytes that were exposed to the radical generator AAPH. Glycerophosphoacetic acid derivatives (GPAA) were found to be the major oxidation products of OxPS modified at the polar head-group during oxidation induced by AAPH-generated radicals, similarly to previous observations for the oxidation induced by $OH^{\bullet}$ radical. The neutral loss scan of 58 Da and a novel precursor ion scan of *m/z* 137.1 ($HOPO_3CH_2COOH$) allowed the recognition of GPAA derivatives in the total lipid extracts obtained from HaCaT cells treated with AAPH. The positive identification of serine head group oxidation products in cells under controlled oxidative conditions opens new perspectives and justifies further studies in other cellular environments in order to understand fully the role of PS polar head-group oxidation in cell homeostasis and disease.

Keywords

Phosphatidylserine, Keratinocytes, AAPH, oxidative stress, mass spectrometry, shotgun lipidomic

Introduction

Phosphatidylserine (PS) is a phospholipid that has been identified to be a preferential target of *in vivo* oxidation. PS is located preferentially in the inner leaflet of cell membranes, but PS oxidation products are translocated to the outside of membrane. These products are considered to be markers of the early stages of apoptosis. It is known that one of the first steps of cellular apoptosis involves PS oxidization in the fatty acyl chains that are recognized by macrophages receptors for the clearance of apoptotic cells [1-3]. Several studies showed that oxidized PS is preferentially recognized by macrophage scavenger receptors over non-oxidized PS [4-7]. The oxidation products of PS identified *in vivo* consisted of oxidative modifications in the fatty acyl chains, such as PS hydroxide and hydroperoxide derivatives and truncated *sn*-2 fatty acyl species [4, 8, 9]. These types of oxidation products retained an intact PS head-group and are usually identified by the typical fragmentation pathways under MS/MS conditions, which involved the loss of the serine group [8, 10, 11]. However, in a recent study of oxidation of PS standards using the Fenton reagent, it was observed that the PS head-group can also undergo oxidative modifications leading to the formation of modified polar head-groups [10, 12]. Among these oxidation products, the derivatives with a polar head-group containing an acetic acid linked to the phosphate group, called glycerophosphoacetic acid (GPAA), were found to be the most abundant [10]. These products showed a distinct fragmentation and neutral loss under MS/MS conditions and the typical loss of the serine group is absent. This behavior explains why this type of PS oxidation product has been overlooked. As yet, PS modified in the serine polar head-group has only been found in mitochondria from brain of rats treated with tacrine, which is associated with neurotoxicity and oxidative stress conditions [13]. Evaluation of pro-inflammatory activities of PS oxidation products with modifications in serine polar head-group was tested through cytokine production, and it was found that GPAA had no pro-inflammatory activity [12]. Until now, no other efforts have been made to detect these species with oxidative modifications on the polar head-group of PS. Nevertheless, it is likely that they can occur *in vivo*, in cells exposed to oxidative conditions.

To give new insight into this subject, the present work aimed to evaluate the formation of GPAA species in keratinocytes (HaCaT cells) after exposure to a radical generator. Keratinocytes were selected as a cell model since externalized PS and oxidation of fatty acyl

chains in PS were identified in human keratinocytes during oxidative stress [14, 15]. These cells are frequently used in oxidation studies, because they are susceptible to modifications under oxidative stress conditions, such as UV, organic peroxides, or radical generators such as AAPH [16-19]. The immortal human keratinocyte line HaCaT is frequently employed for studies of skin keratinocytes *in vitro*, since they retain their differentiation capacity [20]. Keratinocytes were incubated with the water-soluble azo-initiator (AAPH), which is frequently used *in vitro* for oxidation studies. The GPAA derivatives were detected in total lipid extracts using a targetted lipidomic approach involving neutral loss and precursor ion scanning modes, following optimization of this strategy with commercial 1-palmitoyl-2-oleoyl-phosphatidylserine (POPS) as a model system.

Materials and Methods

Materials

2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was from Sigma Aldrich. Trypsin and Dulbecco's Modified Eagle Medium (DMEM) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Foetal calf serum, streptomycin and penicillin were purchased from Invitrogen (Paisley, UK). 1-palmitoyl-2-oleoyl-phosphatidylserine (POPS) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AI, USA). Chloroform (Analytical reagent grade), and methanol (HPLC grade) were from Fisher (UK).

Oxidation of Phosphatidylserine with AAPH

Vesicles of POPS were prepared in ammonium bicarbonate buffer 5mM (pH 7.4). In a typical experiment, 250 µg of phospholipid dissolved in chloroform was evaporated to dryness, the buffer was added and the mixture was vortexed for 2 min and then sonicated for 1 minute in a sonicating water bath. The oxidation was performed by addition of 15 µL of AAPH to a final concentration of 30 mM and 50 mM in a volume of 250 µL. The mixture was incubated at 37°C in the dark for 24 hours. The phospholipid oxidation products were extracted using a modification of the Folch method [21] with chloroform-methanol (2:1, v/v).

Cell Culture

The human keratinocyte cell line HaCaT was obtained from DKFZ (Heidelberg, Germany). HaCaT is a spontaneously transformed immortalized human epithelial cell line from adult skin that maintains full epidermal differentiation capacity [22]. The cells were used after reaching 70–80% confluence, which occurs approximately every 3 days after initial plating. Cells beyond passage 45 were discarded. The cells were cultured in Dulbecco's Modified Eagle Medium (high glucose) supplemented with 4 mM glutamine, 10% heat inactivated foetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37ºC in a humidified atmosphere of 95% air and 5% $CO_{2.}$

Chemical treatment

HaCaT cells (15×10^6) were cultured in 150- cm^2 flasks and subjected to AAPH exposure for 24 h, at 37 °C. The AAPH was dissolved in culture medium to obtain a final concentration of 30 mM and 50 mM. Control experiments consisted of untreated cells.

Cell viability/metabolic activity assay

The effects of AAPH exposure on cell viability/metabolic activity were evaluated by the resazurin assay [23]. Briefly, cells were plated in triplicate in a 96 well plate at a density of $0.4x10^6$ cells/mL, in a final volume of 0.2 mL/well and exposed to 30 mM or 50mM AAPH. After 20 h, the cells were washed with PBS and fresh culture medium containing 50 µM of resazurin was added to each well. After 4 h (in a total of 24 h AAPH treatment) absorbance was read at 570 and 600 nm with a standard spectrophotometer (Multiskan GO, Thermo Scientific). Viable and therefore metabolic active cells are able to reduce resazurin (a blue dye) into resorufin (pink coloured) and, hence, their number correlates with the magnitude of dye reduction.

Assessment of cell PS externalization/Apoptosis

PS externalization following AAPH-cell treatment was analysed by fluorescent microscopy using the FITC-Annexin V apoptosis detection kit with 7-AAD from Biolegend (San Diego, CA, USA). Briefly, $0.4x10^6$ cells were plated in each well of a µ-Slide 8 well plate (IBIDI GmbH, Germany), in a final culture medium volume of 0.2 mL. The cells were then treated with 30 mM or 50mM AAPH for 24 h, or with 1µM Staurosporine for 4h (as a positive control for induction of apoptosis). Cells were washed twice with sterile PBS and incubated for 20 minutes in fresh

culture medium containing 2 µg/ml Hoescht 33258 (Molecular Probes, Invitrogen, Paisley, UK). After this, medium was removed and the wells washed twice with PBS, following by 15 minutes incubation in the dark with FITC-Annexin V / 7-AAD staining solution. After three washing steps, slides were analyzed with a fluorescent microscope (Nikon Corporation, Japan) at 630X magnification. Images were captured with a DS-Fi2 High-definition digital camera and analyzed in NIS-Elements Imaging Software (Nikon Corporation, Japan).

Lipid extraction

For lipid extraction, untreated cells and AAPH-treated cells (after 24 h stimulation) were washed twice with ice-cold phosphate-buffered saline (PBS), scraped into 5 mL of ice-cold PBS and the cells pelleted by centrifugation at 200 g for 4 min. The pellet was resuspended in 1 mL of milli-Q ddH_2O. Thereafter, total lipids were extracted using the Bligh and Dyer [24]. Lipid extracts were evaporated to dryness under nitrogen and resuspended in 500 µL of chloroform.

ESI-MS conditions

Oxidation products of phosphatidylserine standards and HaCaT total lipid extracts were diluted in methanol (1:10, v/v) and detected using a 5500 QTrap mass spectrometer (ABSciex, Warrington, UK) operating in negative ion detection mode with direct infusion at a flow rate of 5 $\mu L.min^{-1}$. Mass spectra were acquired over a mass range of 400-1000 Da. Turbo spray source temperature was set at 150°C, spray voltage was set at -4.5 kV , declustering potential was set at -50 eV and nominal curtain gas flow was set at 20. Enhanced mass spectra were acquired at 10,000 Da/s in dynamic fill. Targeted detection of GPAA was performed by neutral loss scans (NLS) for 58 Da at a scan speed of 10,000 Da/s, with collision energy set at -45 eV, Q1 set to low resolution and Q3 set to unit resolution. Targeted detection of GPAA by precursor ion scanning (PIS) at *m/z* 137.1 were collected at 1000 Da/s scan speed with step size of 0.5 Da, collision energy at -45 eV and with Q1 and Q3 set to unit resolution. Enhanced product ion (EPI) spectra were acquired for the ion of interest with collision energy varying between 40-50 eV. Dynamic fill time was used with a maximum fill time of 250 ms and all other parameters optimized to give maximum signal. There was a small error in the calibration of the instrument, resulting in masses slightly higher than the theoretical mass of the lipids by 0.1-0.2 Da, especially in targetted scan modes.

Results and discussion

To observe whether PS polar head-group oxidation occurred in HaCaT cells after AAPH incubation, as observed after radical oxidation of PS by hydroxyl radical [10, 25], cells were subjected to oxidation with AAPH (30 and 50 mM). The oxidative stimuli used were the same as reported in a previous study that mimicked oxidative stress injury in keratinocytes as a model of inflammation [18]. AAPH was chosen as it is a water-soluble azo-initiator frequently used for oxidation studies, using in *in vitro*, model systems. AAPH after activation lead to alkylperoxyl radicals and akylperoxides that are capable of initiating radical lipid peroxidation in liposomes and cells [18].

As can be observed in figures 1 A and B, the concentrations of AAPH used effectively induce apoptosis in keratinocytes, as demonstrated by PS externalization and a marked decrease in cell metabolic activity. Moreover, apoptosis induction was clearly dependent on AAPH concentration, as keratinocytes treated with 50 mM were uniformly in the late stage of apoptosis, characterized by massive externalization of cell membrane PS (in green) and nuclear binding of the 7-AAD probe (in red). These findings support the AAPH-treated HaCaT cell model as a suitable one to investigate the oxidation of PS in apoptosis.

The analysis of the lipid extracts obtained from keratinocytes was first undertaken using ESI-MS in negative mode (Figure S1). PS species were observed as $[M-H]^-$ in low abundance since they are minor PL in total extracts, and thus oxidation products were not detectable. In order to achieve the sensitivity required to identify PS oxidation species, either in the fatty acyl chain or the polar head-group, a targeted shotgun lipidomic approach involving neutral loss scanning of 87 Da was performed on control and oxidized cell extracts (Figure 2). Loss of 87 Da is a well known and typical fragmentation of phosphatidylserine under MS/MS conditions. The most abundant phosphatidylserine species observed were at *m/z* 788.7, identified as PS-(18:0/18:1)), and at *m/z* 760.7, identified as PS-(16:0/18:1). It should be noted that the observed masses of the phospholipids were slightly above their theoretical masses (e.g. 788.54 for PS-(18:0/18:1), owing to small deviations in the calibration of the instrument. The composition of non-modified PS species was confirmed by MS/MS. The full list of PS species observed are summarized in Table 1; many of these species have been identified previously in keratinocytes and cell cultures [26].

Comparing the spectra obtained by NLS of 87 Da from the total lipid extract of HaCaT cells treated with AAPH (30 mM and 50 mM) with control HaCat cells, no differences between spectra were observed, suggesting that PS modified exclusively in fatty acyl chains are not formed or were formed at very low abundance and not detected under the experimental conditions used. However, we used direct infusion of a total lipid extract and it is possible that ion suppression of oxidized PS with oxygenated acyl chains occurred. For complete confidence in the absence of these oxidation products, an LC-MS analysis would be required. In contrast, using NLS of 58 Da, which is selective for GPAA as a polar head-group, clear evidence for the oxidation of the PS head-group in cells treated with 50mM AAPH was obtained (Figure 3). The neutral loss of 58 Da is a characteristic fragmentation of GPAA, which contains an acetic acid functional group as a polar head-group (Scheme 1), as described previously [10, 25, 27]. In GPAA fragmentation the neutral loss of 87 Da does not occur. In Figure 3, ions at *m/z* 731.6, 757.5, 759.5 and 807.7 were observed and identified as GPAA derivatives, and their identity was confirmed by further MS/MS studies. The MS/MS spectrum of the most abundant ion at *m/z* 759.5 is shown as an example in Figure 4. This precursor ion is 29 Da smaller than an un-modified PS at *m/z* 788.6, and fragments to yield product ions at *m/z* 281.3 and 283.3, corresponding to the deprotonated oleic acid and stearic acid, respectively, and an ion at *m/z* 419.3 corresponding to lyso-phosphatidic acid, LPA-(18:0). The ion at *m/z* 253.3 also indicates the presence of a GPAA-(16:1/20:0) species. This enabled the identification of *m/z* 759.5 as the product of PS-(18:0/18:1) oxidation in the serine polar head-group.

Interestingly, in all the MS/MS spectra of GPAA derivatives (Figure 4) it was possible to observe a product ion at *m/z* 137.1, which was assigned as $HOPO_3CH_2COO^-$ following fragmentation studies. This product ion is absent in the MS/MS spectra of un-modified PS and it was not observed in our previous work that identified GPAA derivatives for the first time, because those experiments were conducted in a linear ion trap mass spectrometer; these instruments have a cut off of 30% of the *m/z* value of the precursor ion, so the ion at *m/z* 137 could not be detected. This demonstrates the advantages of a Q-trap for the identification of novel diagnostic ions for lipid oxidation products. To test the value of this diagnostic marker, PIS at *m/z* 137.1 was used to confirm the occurrence of GPAA modifications in cells exposed to AAPH (Figure 5), and lipids containing GPAA were clearly observed in samples treated with 30

and 50 mM AAPH. These novel results show that PIS for this diagnostic ion is more sensitive than NLS for 58 Da, which only allows detection of GPAA under the higher stress conditions, but its selectivity is not as good, as several ions that did not correspond to the GPAA derivatives were also observed, such as ions at *m/z* 746.9 and 818.8/818.9. The combined information from NLS of 58 Da and precursor ion scan (PIS) 137.1 allowed confirmation of the presence of species that correspond to the GPAA derivatives in HACT cells. Table 2 summarizes the ions corresponding to GPAA that were observed in the NLS of 58 and PIS *m/z* 137 spectra obtained from total lipid extract of HaCaT cells incubated with 30 mM and 50 mM of AAPH.

To confirm and corroborate our results from HaCaT cells, further characterization of oxidation and fragmentation pathways following AAPH-induced oxidation of a PS standard, 1-palmitoyl-2-oleoylphosphatidylserine (POPS (16:0/18:1), was performed. The reaction was monitored by ESI-MS and MS/MS in negative ion. POPS was selected since PS (18:0/18:1) and PS-(16:0/18:1) are known to be the most abundant species of PS in HaCaT cells [26]. Analysis of the ESI-MS spectrum obtained for POPS after treatment with AAPH (Figure 6A) allows several deprotonated molecular ions ($[M-H]^-$) to be identified, corresponding to the PS oxidation products. The ions at higher *m/z* than the native POPS (*m/z* 760.6) were found to be oxidation products of the unsaturated fatty acyl chain (*m/z* 776.6, PS+16 Da (hydroxy), 792.6, PS+32 Da (hydroperoxy), *m/z* 774.6, PS+14 Da (keto /epoxy derivatives). These are typical oxidation products of PS observed previously both *in vitro* and *in vivo* [8, 10, 28-30]. Other ions at *m/z* values smaller than the non-modified PS were also detected and were identified as oxidation products with modifications in the serine polar head-group. These products were identified as PS with a glycerophosphoacetic acid linked to the phosphate group (GPAA), (*m/z* 731.6), confirming that the PS modified at the polar head-group can be formed during AAPH-induced radical attack, as previously reported during the oxidation of PS induced by hydroxyl radical [10]. Oxidation products at the serine polar head-group and the fatty acyl chain (*m/z* 745.6 (GPAA+14 Da), 747.6 (GPAA+16 Da) and 763.6 (GPAA+32 Da) were also identified (Figure 6). Tandem mass spectrometry (MS/MS) was used to confirm the previous assignments. In the ESI-MS/MS spectra of GPAA in negative ion mode a neutral loss of 58 Da was observed, corresponding to the loss of CH_3COOH (acetic acid). These PS oxidation products are easily differentiated from the native PS and PS oxidized at the fatty acyl chains, as the latter show a

typical neutral loss of 87 Da (loss of aziridine-2-carboxylic acid). Also, comparing the ESI-MS/MS spectra of non-modified POPS and GPAA, it can be seen that the product ion at *m/z* 137.1 (assigned as $HOPO_3CH_2COO^-$) is only present in the GPAA MS/MS spectrum. Figures 5 B and 5C show that by using neutral loss scanning (NL of 87Da and NL of 58Da) precursor ion scan (PIS) at *m/z* 137.1 based on these distinct fragmentation profiles of PS oxidation products, it was possible to identify GPAA derivatives in the total lipid extracted obtained from HaCaT cells incubated with AAPH.

AAPH is a water-soluble azo-initiator which generates peroxyl radicals (Scheme 2, equation 1) at a constant rate and at a given temperature by thermal decomposition [31] and is independent of the cellular metabolism. The AAPH peroxyl radical intermediate is the reactive oxygen species responsible for the oxidation of biomolecules such as lipids and peptides and proteins. The formation of the GPAA derivatives occurs due to the AAPH-induced oxidation at the serine polar head-group, similar to the mechanism that occurs in amino acids and peptides [32]. This reaction is proposed to be initiated by the AAPH peroxyl radical, which causes the abstraction of the hydrogen linked to the α-carbon, generating a tertiary radical that is stabilized by the amine nitrogen and carbonyl group [31]. This carbon-centered radical reacts with an oxygen molecule and decomposes further leading to other oxidation products. Oxidative decarboxylation with formation of an additional keto group is usually observed during amino acid oxidation [33]. This occurs due to the loss of CO_2 from C terminal via β-scission of an alkoxyl radical at the C terminal α-carbon (scheme 2 equations 2 and 3) [34].

In spite of the important role of PS oxidation, there is limited knowledge of the modifications that can be generated in this phospholipid under oxidative stress, particularly in the serine polar head-group. The possibility of formation of several oxidation products makes it important to define exactly which are being formed during stress, so that biological effects can be correctly linked to the precise products. PS is normally maintained in the inner leaflet membrane by the action of aminophospholipid translocase; it seems probable that GPAA derivatives in membranes, like PS oxidized at the fatty acyl chains, would not be recognized by this enzyme, thus promoting the presence of PS modified in polar head-group in outer leaflet membrane and contributing to the recognition of apoptotic cells. Interestingly, a recent study evaluated the capacity of GPAA to stimulate monocytes and dendritic cells to produce pro-inflammatory

cytokines and the results showed that GPAA has no pro-inflammatory activity [12]. Nevertheless, the specific role of these species in remains to be elucidated and more studies are needed. PS oxidized at the polar head-group may also have a significant role in these processes and should be explored further in the future.

Conclusions

This study highlights that oxidation of the serine polar head group, as well as fatty acyl chains, in phosphatidylserines in keratinocytes can occur after radical attack by the azo-initiator AAPH, and is likely to be missed by conventional techniques for identifying PS oxidation that depend on detection of the PS head-group. The GPAA derivatives formed due to serine head-group modification can be identified using an improved targetted lipidomic approach, based on the observation that GPAA oxidative products have a specific fragmentation pathway producing a product ion at *m/z* 137 ($HOPO_3CH_2COOH$) and a neutral loss of 58 Da. Further studies are needed to investigate the possible formation of these species in other cell and tissues.

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Caption tables

Table 1: Identification of major PS species identified by NLS of 87 Da in the HaCaT cells lipid extract, with the indication of the *m/z* values of the [M-H]- ions observed

PS species (fatty acyl composition)	NLS of 87Da $[M-H]^-$ m/z
16:0/18:1	760.7
18:0/18:2	786.7
18:0/18:1 16:1/20:0	788.7
18:0/18:0	790.7
18:0/20:2	814.7
18:0/22:6	834.7
18:0/22:5	836.7

Table 2: The major GPAA species observed in HaCaT cells incubated with 30 mM and 50 mM AAPH and the *m/z* values of the $[M-H]^-$ ions observed in the 58 Da of HaCaT cells incubated with 50mM AAPH, and in the PIS at *m/z* 137.1 for HaCaT cells incubated with 30mM and 50mM AAPH.

	NLS 58 Da		PIS m/z 137	
GPAA-species	30mM	50mM	30mM	50mM
16:0/18:1	-	731.6	-	731.7
18:0/18:2	-	757.5	757.7	757.7
18:0/18:1 16:1/20:0	-	759.6	759.7	759.7
18:0/18:0	-	-	-	761.6
18:0/22:5	-	807.6		807.7

Captions Figures

Figure 1: Effect of AAPH on cell metabolic activity and PS externalization. A) Cells were treated with 30 mM or 50 mM AAPH during 24h and the viability was assessed by resazurin assay. In this assay metabolic conversion of resazurin into resorufin will be proportional to cell viability. B) Keratinocytes were treated with 30 mM or 50 mM AAPH during 24h, and the apoptosis stage was analyzed by fluorescent microscopy using Annexin V, 7-AAD and Hoescht 33258 probes. Intact cells appear with just blue nuclei, early apoptotic cells present blue nuclei and green membranes (PS externalization) and finally late apoptotic cells show marked green fluorescence and red nuclei. Images representative of different fields were acquired with a DS-Fi2 High-definition digital camera coupled to a Nikon fluorescent microscope (magnification 630x) and analyzed in NIS-Elements Imaging Software (Nikon Corporation, Japan). Bar scale: 10 µm.

Figure 2: The lipid profile of control and cells exposed to AAPH. Spectra of neutral loss of 87 Da scanning of lipid extracts of HaCaT cells. A) Control lipid extracts. B) Lipid extracts obtained from HaCaT cells incubated with 30 mM AAPH. C) Lipid extracts obtained from HaCaT cells incubated with 50 mM AAPH.

Figure 3: PS oxidized in the serine headgroup was observed in HaCaT cells after oxidative stress. Spectrum of neutral loss scan of 58 Da obtained from HaCaT cells incubated with 50 mM AAPH.

Figure 4: Tandem mass spectrometric analysis of GPAA species. MS/MS spectrum of the ion $[M-H]^-$ at *m/z* 759.5 corresponding to the GPAA species formed by PS headgroup oxidation.

Figure 5: Use of the diagnostic marker at *m/z* 137.1 to detect oxidized PS in cells exposed to AAPH. A) Spectrum of precursor ion scanning of the ion at *m/z* 137.1 obtained from control HaCaT cells. B) Spectrum of precursor ion scanning of the ion at *m/z* 137.1 obtained from

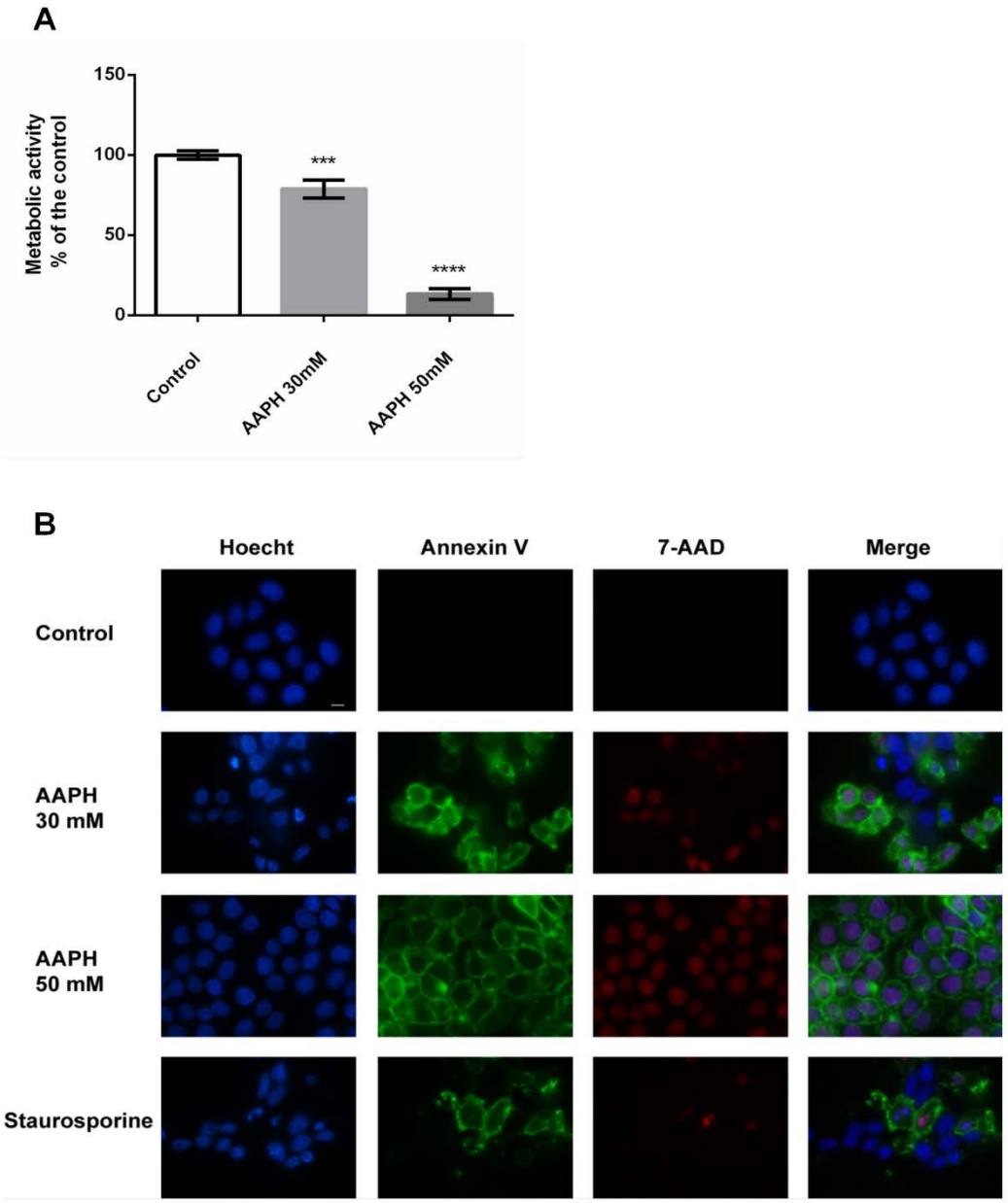
HaCaT cells incubated with 30 mM AAPH during 24h. C) Spectrum of precursor ion scanning of the ion at *m/z* 137.1 obtained from HaCaT cells after incubation with 50mM AAPH during 24h.

Figure 6: Mass spectrometric analysis of oxidized POPS by different scanning routines. A) MS spectrum obtained in negative ion mode of an oxidized mixture of POPS showing the $[M-H]^-$ ions. Oxidation of POPS was induced after incubation with 30 mM AAPH. (B) Spectrum of neutral loss scanning of 87 Da obtained from an oxidized mixture of POPS. C) Spectrum of neutral loss scanning of 58 Da obtained from an oxidized mixture of POPS. D) Spectrum of precursor ion scanning of the ion at *m/z* 137.1 obtained from an oxidized mixture of POPS. Mass spectra were acquired using a 5500 QTrap mass spectrometer.

Captions Schemes

Scheme 1: Phosphatidylserine and glycerophosphoacetic acid (GPAA) structures. Specific neutral loss of 87 Da from non-modified PS and neutral loss of 58 Da from GPAA. Formation of an ion at *m/z* 137 observed during GPAA fragmentation.

Scheme 2: Reaction pathways that occurred during PS polar head oxidation by AAPH radical generator: Decomposition of AAPH produces molecular nitrogen and two carbon centered radicals. The carbon radicals may react with molecular oxygen to give peroxyl radicals (1). There are two possible pathways for the formation of GPAA derivatives from PS oxidation. PS polar head group may be deaminated and carboxylated to yield an aldehyde one carbon shorter than the original and the aldehyde may be oxidized to a carboxylic acid (2). Alternatively PS polar head group may be transaminated, resulting in the formation of an α-keto acid which can be oxidatively decarboxylated to yield a carboxylic acid one carbon shorter than the original PS polar head (3).



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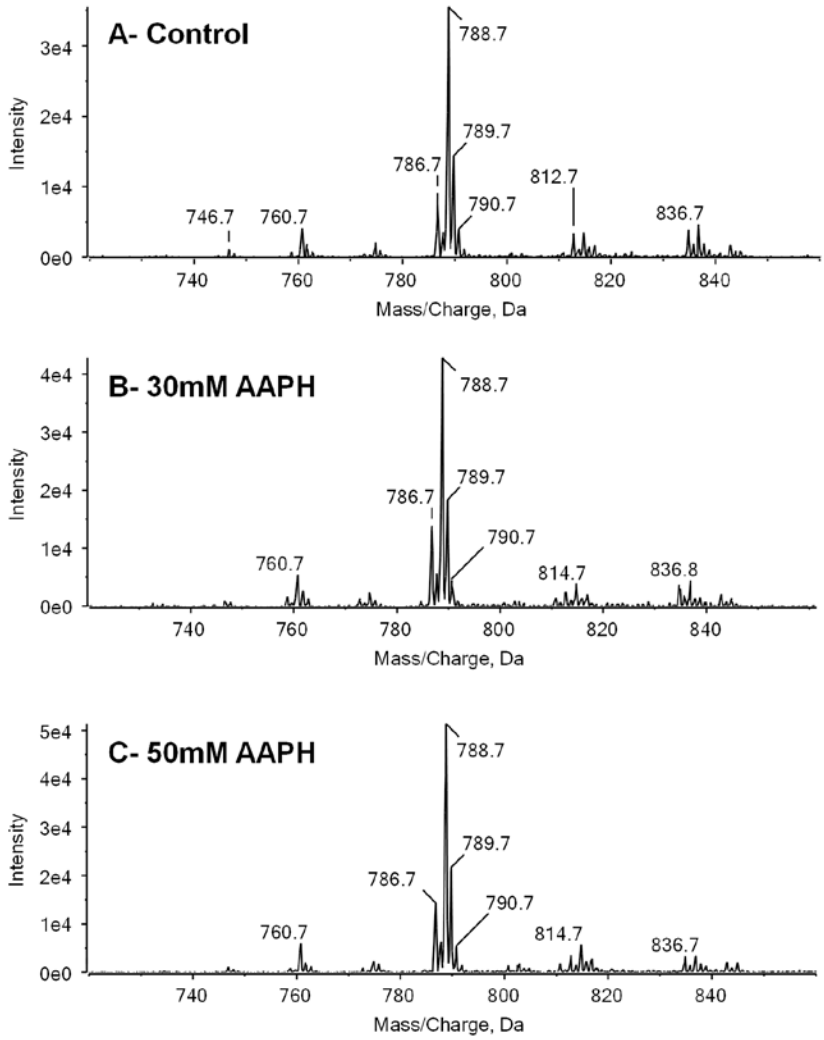

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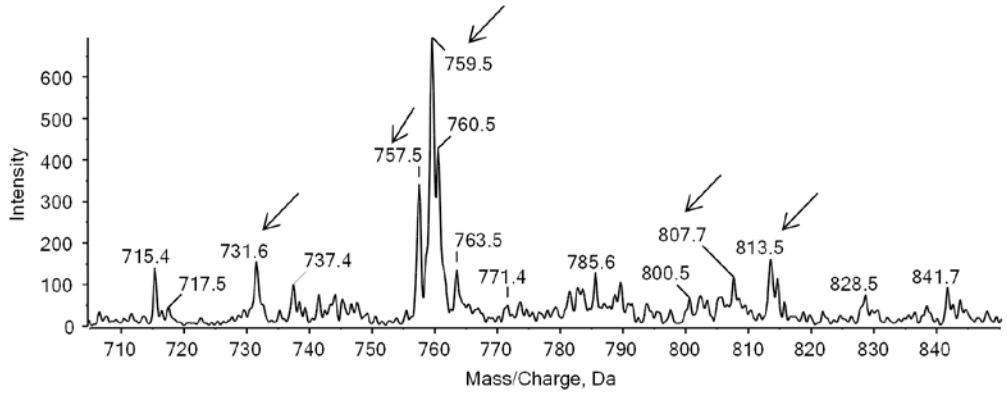

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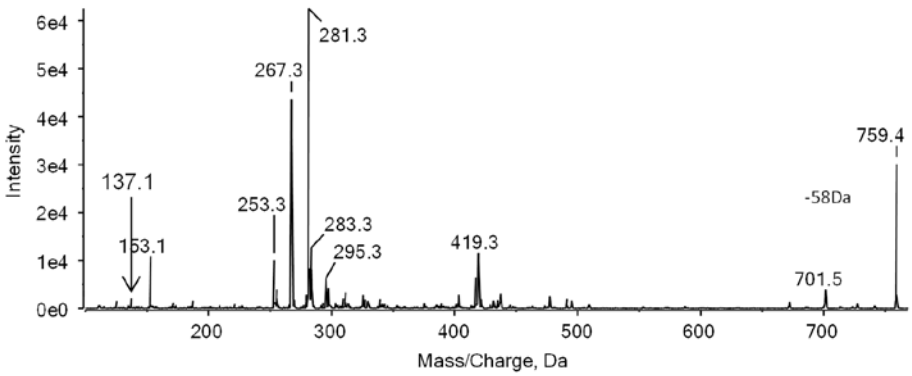

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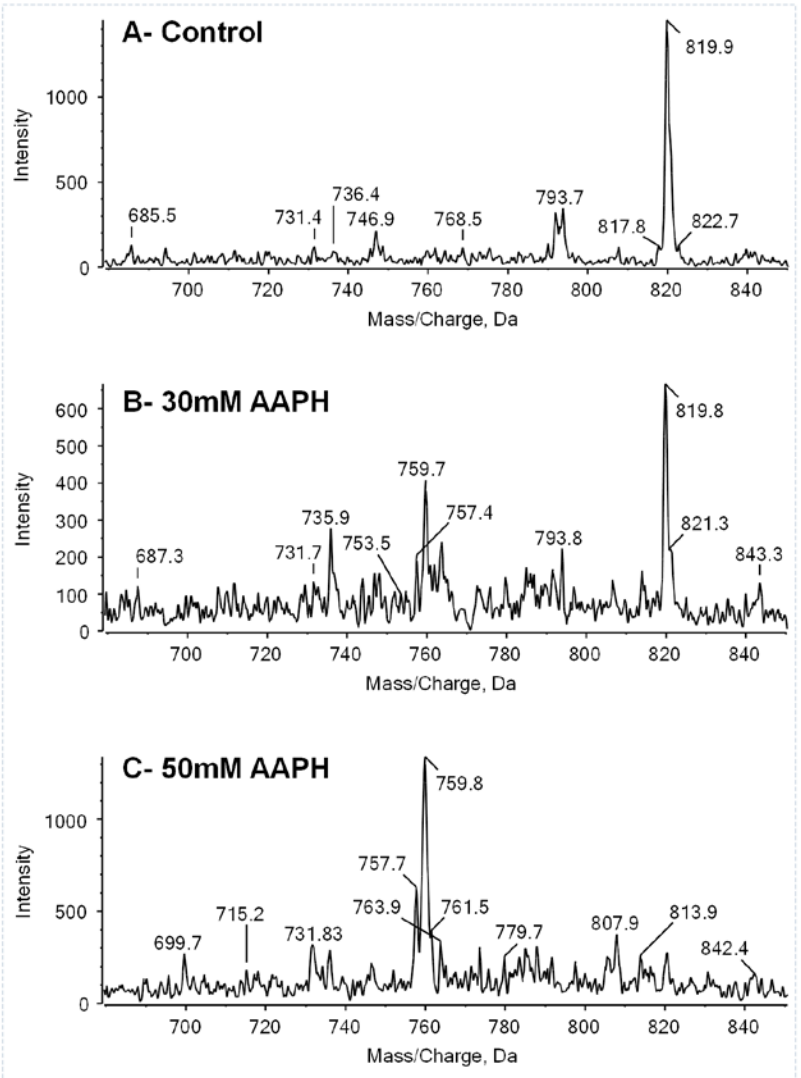

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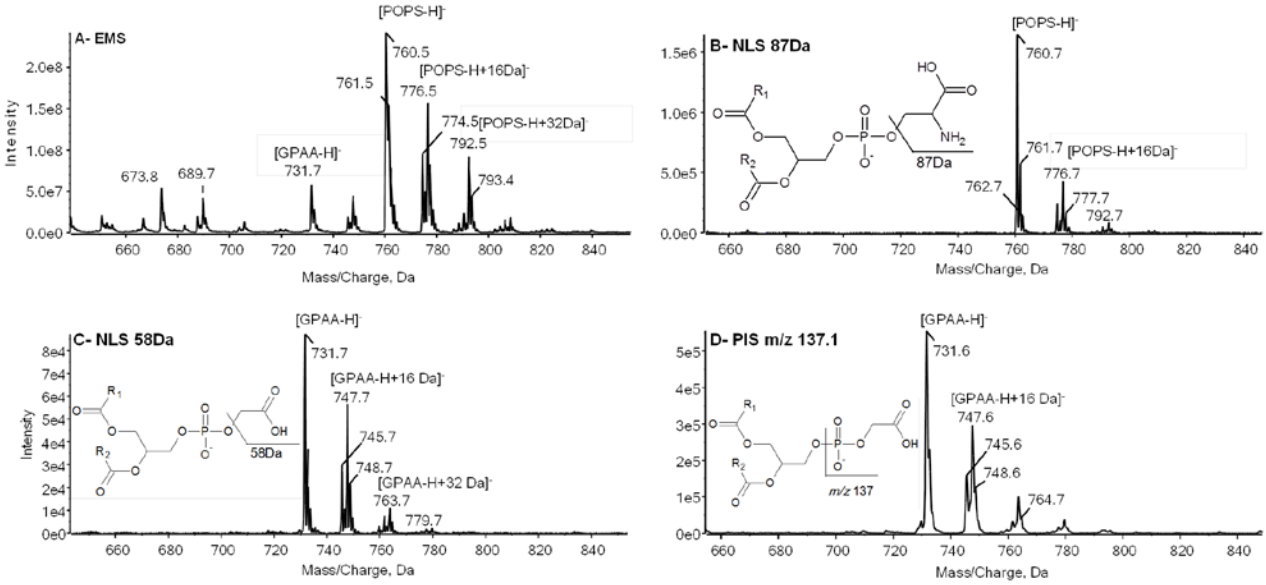

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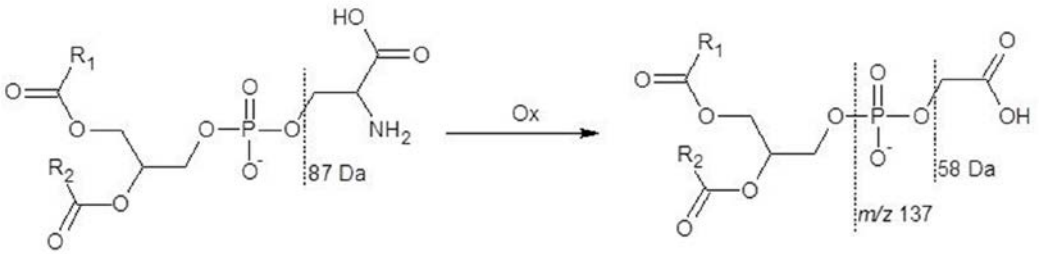

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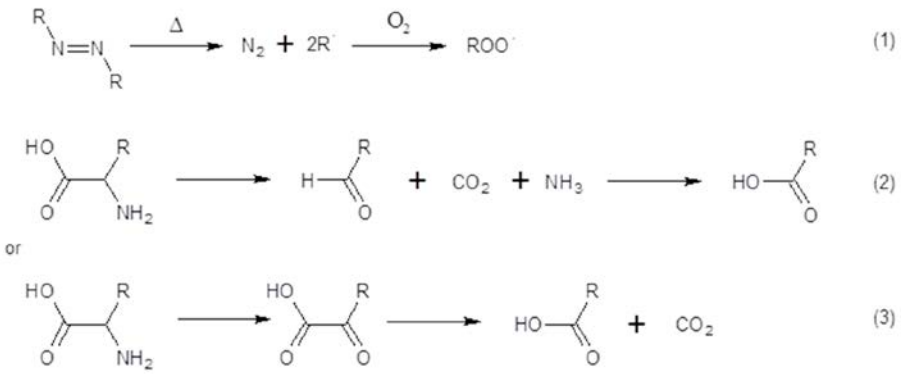

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