



Soybean phosphatidylcholine liposomes as model membranes to study lipid peroxidation photoinduced by pterin



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ABSTRACT

Oxidized pterins, efficient photosensitizers under UVA irradiation, accumulate in the skin of patients suffering from vitiligo, a chronic depigmentation disorder. Soybean phosphatidylcholine (SoyPC) liposomes were employed as model membranes to investigate if pterin (Ptr), the parent compound of oxidized pterins, is able to photoinduced lipid peroxidation. Size exclusion chromatography and dialysis experiments showed that Ptr is not encapsulated inside the liposomes and the lipid membrane is permeable to this compound. The formation of conjugated dienes and trienes, upon UVA irradiation, was followed by absorption at 234 and 270 nm, respectively. The photoproducts were characterized by mass spectrometry and oxygenation of SoyPC was demonstrated. In addition, analysis of MS/MS spectra suggested the formation hydroperoxides. Finally, the biological implications of the findings are discussed.

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1. Introduction

Lipid peroxidation is involved in many physiological and pathological events, and is usually due to oxidative stress [1–3]. This process can proceed by three different mechanisms: free radical-mediated oxidation, which occurs by chain reaction mechanism (initiation, propagation and termination), free radical-independent, non-enzymatic oxidation, that involves singlet oxygen (1O_2), and enzymatic oxidation [4]. The most important one is the free radical mechanism, where the initiation phase includes hydrogen atom abstraction, and the most abundant compounds of lipid membranes, phospholipids containing polyunsaturated fatty acids (PUFAs), are the main targets.

A photosensitized reaction is defined as a photochemical alteration occurring in one molecular entity as a result of initial absorption of radiation by another molecular entity called photosensitizer [5]. Photosensitized oxidations can take place through two different pathways: type I mechanism, which involves the generation of radicals via electron transfer or hydrogen abstraction, or type II mechanism where there is an energy transfer from the triplet state of the photosensitizer to the molecular oxygen to form 1O_2 [6]. PUFAs do not absorb solar radiation and artificial light, but they can undergo photosensitized oxidation by endogenous or exogenous photosensitizers. Photosensitized lipid peroxidation by different molecules has been studied for a few decades and it is known that PUFAs can react by both photosensitized mechanisms (type I and type II), leading to different products [7–10].

Pterins are heterocyclic compounds which occur in a wide range of living systems and participate in relevant biological functions [11]. The most common pterin derivatives are 6-substituted compounds and they can exist in different oxidation states: oxidized or aromatic pterins and reduced pterins (dihydropterins and tetrahydropterins). Several pterins are present in human epidermis, in particular, 5,6,7,8-tetrahydrobiopterin (H_4Bip) is an essential cofactor in the hydroxylation of the aromatic amino acids [12] and participates in the regulation of melanin biosynthesis [13]. Vitiligo is a skin disorder characterized by the acquired loss of constitutional pigmentation manifesting as white macules and patches [14]. In this disease, the H_4Bip metabolism is altered, the protection against UV radiation fails due to the lack of melanin, the main pigment of skin, and unconjugated oxidized pterins accumulate in the affected tissues [15].

The absorption spectrum of unconjugated oxidized pterins has an intense band in the UVA region (320–400 nm) (Fig. 1), the main component of the solar UV energy incident on the Earth. Under UVA excitation these compounds can fluoresce, undergo photooxidation to produce different photoproducts, generate reactive oxygen species (ROS) [16], and can also photosensitize biomolecules. In the presence of oxygen, pterins act as photosensitizers through both type I [17] and type II mechanisms [18]. In the context of our investigations on the photosensitizing properties of pterins, we have recently demonstrated that pterins are able to damage biomolecules involved in the pigmentation of the skin, such as tyrosinase [19], the α -melanocyte-stimulating hormone [20] and amino acids [21]. Even more, in a study performed using cervical cancer cells (HeLa), we ascertained that pterins are readily incorporated into the cells, that cell death takes place upon UVA

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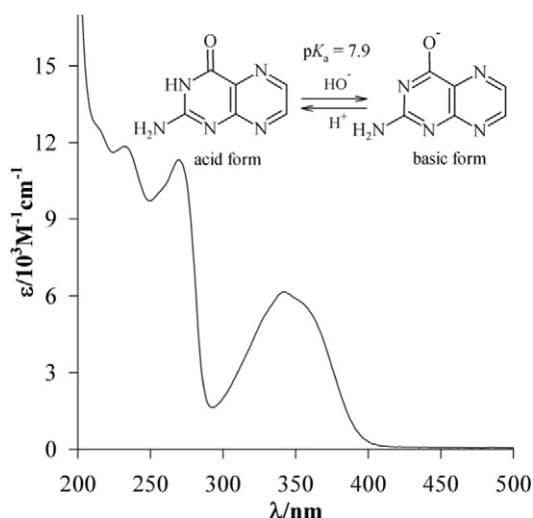


Fig. 1. Molecular structure of Ptr and the absorption spectra in buffer Tris pH 7.4.

irradiation of pterins, and that the integrity of the cell membrane is affected, among other alterations undergone by the cells [22].

Taking into account these results and to go further with the investigation of the photosensitizing properties of pterins, we set out to study the damage of membranes photoinduced by pterin (Ptr, parent and unsubstituted compound of oxidized pterins). In particular, in the present work we investigate the lipid peroxidation of phospholipids. As a model membrane we employed liposomes of phosphatidylcholine from soybean (SoyPC), mainly for two reasons: phosphatidylcholine is the most abundant phospholipid in eukaryotic cellular membrane and SoyPC has a high amount of PUFAs, with a fatty acid distribution of approximately 13% palmitic (C16:0), 4% stearic (C18:0), 10% oleic (C18:1), 64% linoleic (C18:2), and 6% linolenic (C18:3), with other fatty acids being minor contributors, according to the company's specifications [23]. Therefore, around 70% of fatty acids are polyunsaturated and can undergo lipid peroxidation. In addition, the association to the membrane or entrapment of Ptr in the liposomes was explored.

2. Materials and methods

2.1. Chemicals and reagents

Pterin (purity >99%) was acquired from Schircks Laboratories (Switzerland). L- α -Phosphatidylcholine from soybean (SoyPC, $\geq 99\%$ (TLC), lyophilized powder), fluorescein (FL), 3,5-di-tert-4-butylhydroxytoluene (BHT), bromochlorophenol blue and Sepharose® CL-4B were bought from Sigma-Aldrich. Tris(hydroxymethyl)aminomethane (Tris) was provided by Genbiotech. Chloroform and methanol used were HPLC grade and were purchased from U.V.E. and J. T. Baker, respectively. Deionized water further purified in a Milli Q Reagent Water System apparatus. The specific electrical resistance of water measured was ~ 10 M Ω cm.

2.2. Preparation of liposomes

SoyPC liposomes were prepared from stock solution of SoyPC 1 mg/ml in chloroform and were dried under nitrogen stream to form lipid films. Then the films were hydrated either in Tris buffer (20 mM, pH 7.4), Ptr (60 μ M), bromochlorophenol blue (4 μ M) or FL (20 μ M) dissolved in Tris buffer (20 mM, pH 7.4), to have a final phospholipid concentration of 250 μ M. The samples were vortexed for a few minutes and subsequently, the dispersions were sonicated using a probe sonicator (Sonics Vibra Cell, VCX750). SoyPC liposomes were kept at 4 °C until used. Dynamic light scattering measurements were performed with a Malvern Zetasizer Nano-ZS to

estimate the size of SoyPC liposomes formed. Results showed a diameter of 132 ± 54 nm. The polydispersity index was 0.4.

2.3. Permeability studies

2.3.1. Molecular exclusion chromatography

The Sepharose® CL-4B was equilibrated in 20 mM Tris pH 7.4 and poured into a 1.5×9 cm or 0.8×10 cm column. The gel was left sedimenting overnight. Samples were eluted with the same buffer and collected in 1 ml fractions.

2.3.2. Dialysis

SoyPC liposomes were placed in dialysis tubing (Spectra/Por® 4) with molecular weight cut off (MWCO) of 12–14 kDa and dialyzed against buffer Tris pH 7.4 at 4 °C for 72 h, with buffer change at 8, 24 and 48 h.

2.3.3. UV/vis analysis

Electronic absorption spectra were recorded on a Shimadzu UV-1800 spectrophotometer. Measurements were made using quartz cells of 0.4 or 1 cm optical path length.

2.3.4. Fluorescence spectroscopy

Fluorescence measurements were performed using a Single-Photon-Counting equipment FL3 TCSPC-SP (Horiba Jobin Yvon). The equipment has been previously described in detail [24]. Briefly, in steady-state measurements the sample solution in a quartz cell was irradiated with a 450 W Xenon source through an excitation monochromator. The fluorescence, after passing through an emission monochromator, was registered at 90° with respect to the incident beam using a room-temperature R928P detector. The total fluorescence intensities (I_F) were calculated by integration of the fluorescence spectra.

2.4. Steady-state irradiation

The continuous photolysis of SoyPC liposomes in the presence or absence of Ptr was carried out irradiating in quartz cells (0.4 cm optical path length). Two Rayonet RPR 3500 lamps (Southern N.E. Ultraviolet Co.) with emission centered at 350 nm (band width (fwhm) 20 nm) were employed as radiation source. Photolysis experiments were performed in air-equilibrated aqueous dispersions, unless stated otherwise. Aberchrome 540 (Aberchromics Ltd.) was used as an actinometer for the measurement of the incident photon flux density ($q^{0,V}_{n,p}$) at the excitation wavelength [25]. The obtained value was $2.5 (\pm 0.2) \times 10^{-5}$ Einstein $L^{-1} s^{-1}$.

2.5. Measurements of lipid peroxidation by detection of conjugated dienes and trienes

To determine conjugated dienes and trienes production, absorption spectra were recorded in a spectrophotometer (see Section 2.3), in the range between 200 and 300 nm. Lipid peroxidation was assessed continuously by measuring the increase in absorbance at 234 nm (formation of conjugated dienes) and 270 nm (formation of conjugated trienes).

2.6. Mass spectrometry

2.6.1. Lipid extraction

Lipids were extracted from the reaction mixture (with previously addition of 0.01% BHT) using a modified Bligh–Dyer method [26]. Briefly, chloroform (1 ml) and methanol (2 ml) were added to 0.8 ml of mixture (1:2:0.8) and shaken vigorously for 20 min to form a single phase. Soon after, 1 ml of chloroform was added (proportions 2:2:0.8) to form a biphasic mixture and again was strongly shaken for another 20 min. After resting for 20 min, the lower (chloroform-rich) phase was

recovered into a glass vial, dried under a stream of nitrogen gas, and stored at $-20\text{ }^{\circ}\text{C}$ before MS analysis.

2.6.2. Equipment

A quadrupole time-of-flight mass spectrometer, Xevo G2-QToF from Waters, was operated in positive ion mode with a capillary voltage of 3 kV, the cone voltage of 50 V, the cone gas flow of 50 l/h, the source temperature set to $110\text{ }^{\circ}\text{C}$ and the desolvation temperature set to $300\text{ }^{\circ}\text{C}$. For the tandem MS experiments the collision energies varied between 15 and 35 eV. The scan range was from 50 to 1000 amu (Da).

3. Results and discussion

3.1. Permeability of liposomes to pterin

To investigate the permeability of the phospholipid bilayer to Ptr, SoyPC liposomes prepared in Tris buffer containing Ptr ($60\text{ }\mu\text{M}$) were passed through a molecular exclusion chromatography column. Experiments were performed using Tris pH = 7.4 as elution buffer and fractions of 1 mL were collected and analyzed independently. Knowing that the inner volume of liposomes is very small and that Ptr is highly fluorescent [16], fluorescence measurements with appropriate conditions were performed to improve the sensitivity of Ptr detection. Therefore, absorption and fluorescence spectra of each fraction were recorded. Taking advantage of the light scattering of liposomes, their detection was carried out measuring the absorbance of the fractions at 540 nm, where Ptr does not absorb. Ptr was determined by integrating the emission spectra of each fraction by excitation at 340 nm (Ptr presents an intense emission band centered at 440 nm). In Fig. 2a, the absorbance elution profile shows a large peak (fractions 3 to 6) associated with the void volume of the column, which reflects the elution of liposomes, also indicated by the turbidity of the solution. The fluorescence elution profile shows a peak between fractions 10 and 15 that corresponds to the elution of the free Ptr (Fig. 2a). Noticeably and as it can be observed on the inset of Fig. 2a, no fluorescence of Ptr was detected at fractions where liposomes were collected. These results suggest that Ptr is not associated to the lipid bilayer, and at the same time, it shows that Ptr is not encapsulated in the inner compartment of the liposomes and can freely cross the membrane.

Control experiments were performed using fluorescein (FL) instead of Ptr. FL was chosen because it is not permeable to the lipid membrane [27] and therefore, after preparation of liposomes in FL solution it remains encapsulated inside the liposomes. This compound is also highly fluorescent ($\Phi = 0.93$) with an emission band centered at 515 nm [28], so that it can be easily detected by emission measurements. As shown in Fig. 2b, and in contrast to what was observed for Ptr, emission of FL was detected not only as free FL (fractions 10 to 15), but also in the fractions where liposomes are eluted. This result confirms that fluorescence emission can be detected if the compound is encapsulated in the inner part of the liposomes.

Similar experiments were performed with bromochlorophenol blue, which presents an intense absorption band centered at 590 nm. Therefore samples of liposomes prepared in aqueous solutions of bromochlorophenol blue were separated by molecular exclusion chromatography and the elution profiles were obtained by registering the absorbance at 590 nm of each fraction. Bromochlorophenol blue was detected in the fractions where liposomes eluted (Fig. S1) confirming that this compound stayed inside the liposomes. The experiments performed with FL and bromochlorophenol blue demonstrated that liposomes do not suffer damage and that their permeability properties are not affected when manipulated and analyzed by chromatography.

To further investigate the permeability of the phospholipid bilayer, dialysis experiments were also performed with Ptr and FL as a control. Absorbance and fluorescence spectra of the inner aliquot

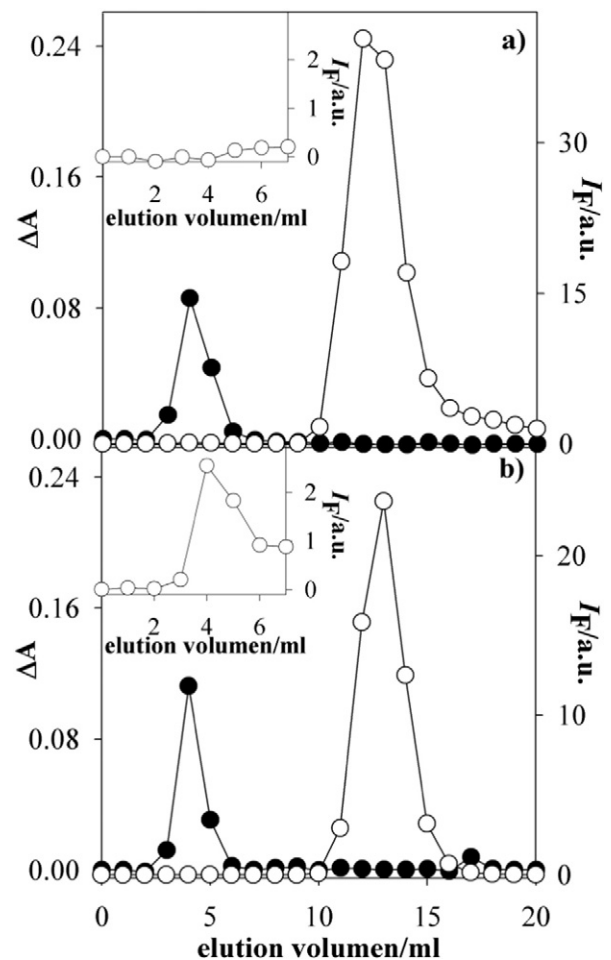


Fig. 2. Elution profiles of the molecular exclusion chromatography performed to separate liposomes from free (a) Ptr and (b) FL. The absorbance at 540 nm (\bullet) and I_F (\circ) was registered for each fraction to detect the liposomes and the solute (Ptr or FL), respectively. Inset: detailed I_F of the fractions corresponding to the elution of the liposomes. In fluorescence measurements the excitation wavelength was 340 nm for Ptr and 470 nm for FL.

and the dialysates (buffers) were registered. After the buffer was replaced 3 times (B1–B4), the fluorescence of the inner aliquot of the experiment carried out with Ptr was not detectable, indicating that no remaining Ptr was present in the liposomes. On the contrary, typical fluorescence spectrum of FL was observed in the inner aliquot of the experiment carried out with this compound (Fig. 3), thus confirming that the membrane of the liposomes is not permeable in this case. Two controls dialysis were made: the first one, with a solution of the compound (Ptr or FL) to ensure that it passes through the dialysis membrane, and the second one, with SoyPC liposomes without compound to guarantee that they do not cross the dialysis membrane.

These experiments clearly showed that Ptr is able to pass through phospholipids bilayer and very likely cell membranes. This fact might be relevant from a biological point of view. Unconjugated oxidized pterins, compounds able to act as photosensitizers, are not present in cells under physiological conditions, but they are generated in the cytoplasm of skin cells affected by some pathologies like vitiligo. Therefore the results presented in this section imply that unconjugated oxidized pterins, once produced in the cytoplasm, can diffuse and reach any cellular compartment. In this way, processes photosensitized by pterins, studied in cell-free systems, such as the photoinactivation of tyrosinase [19], enzyme that catalyzes the first and rate limiting step of the biosynthesis of melanin, and the photoinduced DNA damage [29] could take place in vivo by diffusion of pterins to the melanosomes and nucleus, respectively.

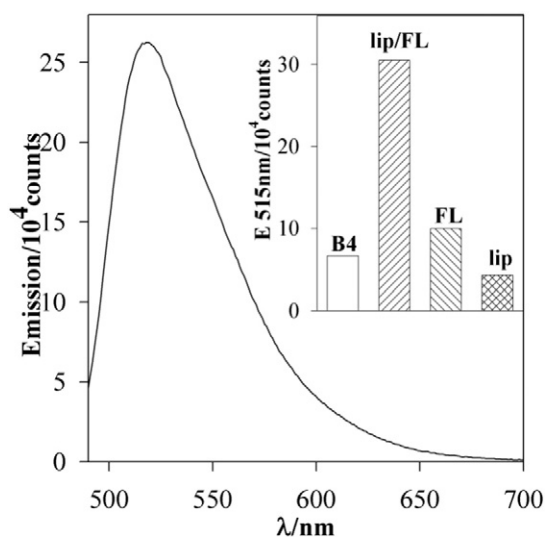


Fig. 3. Fluorescence spectra of the inner aliquot of dialysis experiment performed for liposomes with FL (excitation wavelength 470 nm). Inset: fluorescence emission at 515 nm detected for buffer 4 (B4) and the inner aliquots of liposomes with FL (lip/FL), only FL (FL) and liposomes without FL (lip).

3.2. Lipid peroxidation photosensitized by Ptr under UVA irradiation

Although it is known that Ptr produces cell death and alterations of cytoplasmic membrane integrity by photosensitization under UVA excitation [22], the mechanism involved has not been yet identified neither the kind of damage that the membrane undergoes. Therefore, SoyPC liposomes prepared in buffer Tris pH 7.4 in the presence and absence of Ptr, were exposed to UVA irradiation (see Section 2.4) during different periods of time and lipid peroxidation was investigated.

The samples analyzed by UV-vis spectrophotometry revealed a significant increase in the absorbance at 234 nm (A234), where it is known that conjugated dienes absorb. As shown in Fig. 4a, the production of conjugated dienes was clearly enhanced when Ptr was present in the liposome suspension. In our irradiation conditions, a maximum was reached at around 50–60 min of irradiation and afterwards, A234 decreased, suggesting possible breaks of fatty acid chains of SoyPC after conjugation. Moreover, the time evolution of the absorbance at 270 nm (A270), where conjugated trienes absorb, is shown in Fig. 4b. Clearly, conjugated trienes are formed only when Ptr is present in the liposome suspension, at least at these irradiation times. Controls of non-irradiated liposomes with and without Ptr were also performed, and no significant conjugated dienes and trienes were produced. In addition, negligible changes in absorption of Ptr were detected during the irradiation, indicating that no significant photodegradation of this photosensitizer occurred. These experiments clearly show that Ptr is able to photoinduced lipid peroxidation.

Photoinduced lipid peroxidation can take place by two mechanisms: type I, with radical formation or type II, which involves $^1\text{O}_2$ (see Section 1). Taking into account that pterins are able to act as photosensitizers through both mechanisms [30], several experiments were performed to elucidate which of the two mechanisms is the mayor pathway involved in this case. Firstly, SoyPC liposomes were dissolved in D_2O and irradiated in the presence and absence of Ptr. The formation of conjugated dienes and trienes was followed spectrophotometrically (A234 and A270, respectively) and these results were compared to those obtained using H_2O as a solvent, under otherwise the same experimental conditions. Fig. 5a shows that a very slight increase on the rate of formation of conjugated dienes was registered in D_2O compared to H_2O . This result suggests that $^1\text{O}_2$ is not being involved in the lipid peroxidation of the phospholipids.

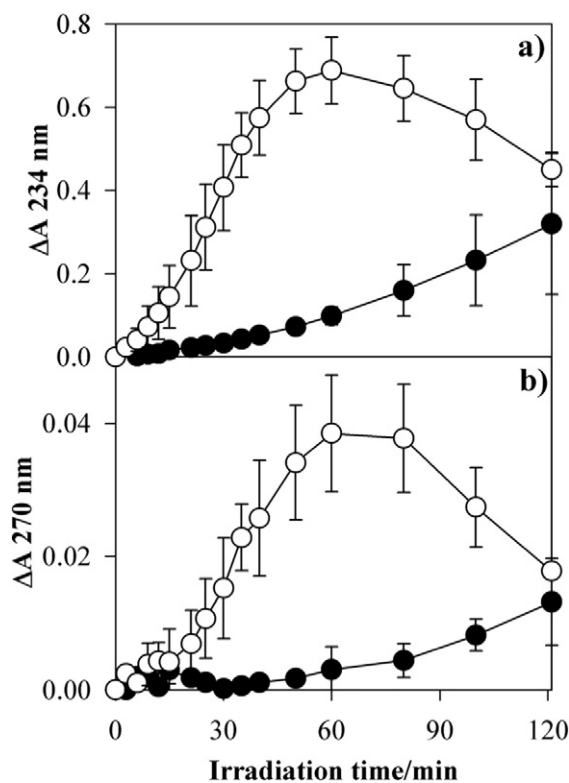


Fig. 4. Time evolution of the absorbance of (a) conjugated dienes (A234) and (b) conjugated trienes (A270) for liposomes in the presence (○) and in the absence (●) of Ptr (60 μM) as a function of the irradiation time. Error bars correspond to SD from 2 to 3 different experiments.

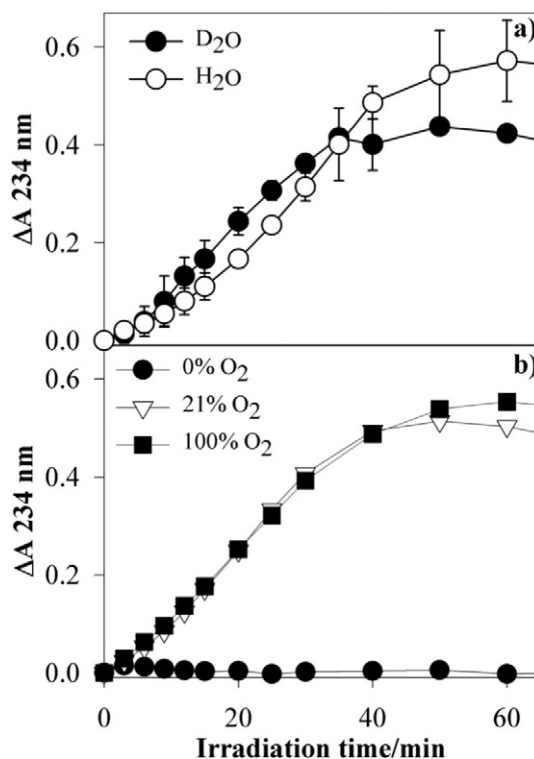


Fig. 5. Time evolution of absorbance of conjugated dienes (A234) for liposomes with Ptr as a function of irradiation time under different conditions. a) Experiments carried out in H_2O and D_2O ; b) experiments carried out in air-equilibrated (21% O_2), O_2 -saturated (100% O_2) and Ar-bubbled (0% O_2) liposome suspension. Error bars correspond to SD from 2 to 3 different experiments.

In another set of experiments, before irradiation, samples of SoyPC liposomes with Ptr were bubbled with argon gas to eliminate the dissolved O_2 , or with O_2 to increase its concentration. Then the deoxygenated and O_2 -saturated samples were irradiated, A234 and A270 were registered as a function of irradiation time and the corresponding curves were compared to those obtained for air-equilibrated samples. As expected, no conjugated dienes (Fig. 5b) and trienes (data not shown) were formed in the absence of O_2 (0% O_2), indicating that O_2 is needed for the process. Also, air-equilibrated (21% O_2) and O_2 -saturated (100% O_2) samples showed no difference in the rate of formation of conjugated dienes.

3.3. Mass spectrometry analysis of photoproducts

Photoinduced lipid peroxidation products were analyzed by mass spectrometry in positive mode, since phosphatidylcholines are naturally positively charged molecules due to the trimethylamine group at the polar head. Before and after UV exposure of SoyPC liposomes with Ptr, lipids were extracted as describe above (see Section 2.6) and injected directly into the mass spectrometer. As shown in Fig. 6a, for the non-irradiated sample, several peaks were observed with m/z values corresponding to the $[M + H]^+$ and $[M + Na]^+$ ions of the different PC species present in the SoyPC liposomes (table in Fig. 6a). Comparison of the MS spectra of the irradiated samples with those of untreated samples allowed the detection of new peaks that were assigned to products of the photosensitized process. Such species presented higher m/z values than those registered for the unmodified PCs (Fig. 6b). The most intense signals corresponded to $[M + H + 2O]^+$, $[M + Na + 2O]^+$, $[M + H + 4O]^+$, $[M + Na + 4O]^+$ (table in Fig. 6b). These data reveal that incorporation of oxygen atoms takes place as a result of the photosensitized process and suggest that the detected species can be assigned to hydroperoxides, since these kinds of compounds are the

most important products generated in photoinduce lipid peroxidation [31,32].

To go further with the investigation about the structure of the photoproducts formed, tandem mass experiments (MS/MS) were performed. The results from the MS/MS analysis will be explained in detail for the oxidation products of PC(18:2/18:2) and PC(16:0/18:2), which are the most abundant PC species in SoyPC. The ion $[M + H + 2O]^+$ with a m/z value of 790.5 Da can be assigned to the hydroperoxy derivatives of PC(16:0/18:2) (PC(16:0/18:2)-OOH). The MS/MS spectrum of this specie (Fig. 7a) showed the characteristic fragment at m/z 184 Da, which corresponds to the phosphocholine polar head ($[H_2PO_4CH_2CH_2N(CH_3)_3]^+$) and the ions at m/z values of 772.5 Da and 756.5 Da formed by loss of one molecule of H_2O and H_2O_2 , respectively from the $[M + H]^+$ ion. The loss of -34 Da (H_2O_2) highly supports the presence of a hydroperoxide derivative [33]. Additional ions at m/z values between 450 Da and 700 Da give structural information about the sites of modification in the PC moiety (inset Fig. 7a) [34,35]. The ion observed at m/z 496.3 Da corresponds to lyso-PC(16:0) and confirms that oxidation occurred to the sn-2 acyl chain (18:2). Also the ions at m/z 650.4 Da and 702.4 Da could correspond to the structures shown in Fig. 7a, where the hydroperoxy group is at position 9 or 13, respectively, in the fatty acid chain of 18:2.

Fig. 7b shows the MS/MS spectrum of the ion $[M + H + 2O]^+$ with a m/z value of 814 Da, which can be assigned to the hydroperoxy derivatives of PC(18:2/18:2) (PC(18:2/18:2)-OOH). As observed for the ion of the product PC(16:0/18:2)-OOH (Fig. 7a), the fragment at m/z 184 Da was detected together with the fragments formed by the loss of H_2O and H_2O_2 , at m/z values of 796.5 Da and 780.5 Da, respectively. The fragment consistent to the loss of -59 Da, which corresponds to the choline head ($N(CH_3)_3$), was also registered. To assess the position of the hydroperoxide, in this case, only one ion was found corresponding to the m/z value of 726.4 Da, which is in agreement with the hydroperoxide at C-13.

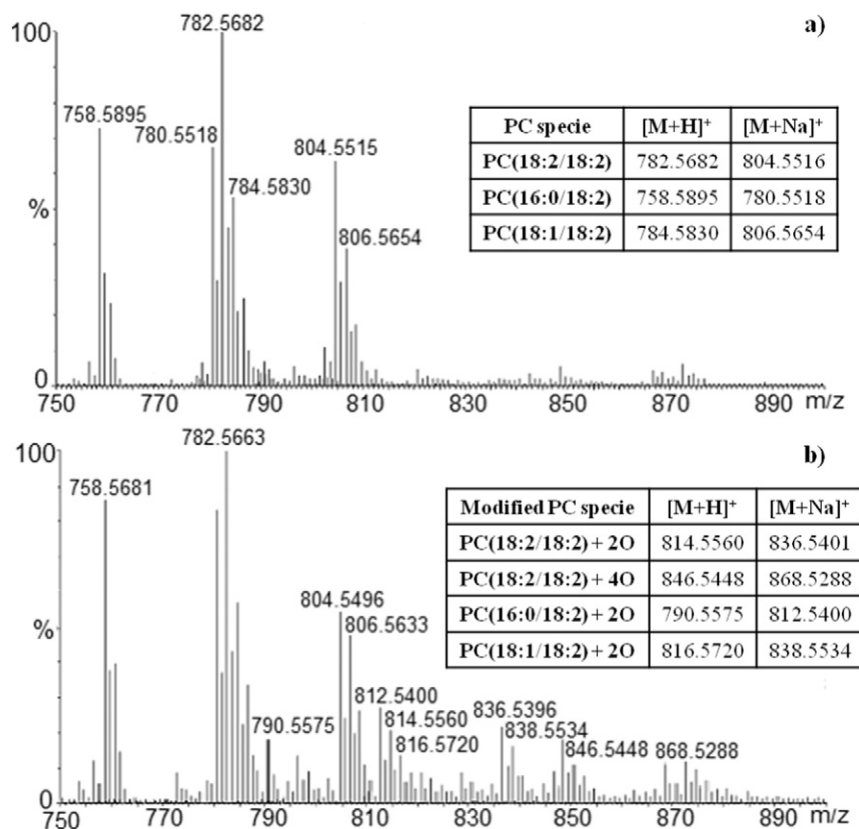


Fig. 6. (a) Mass spectrum of non-irradiated sample, table shows m/z values of the various PC species present in SoyPC liposomes. (b) Mass spectrum of a sample irradiated for 20 min, table shows m/z values of peaks corresponding to photoproducts.

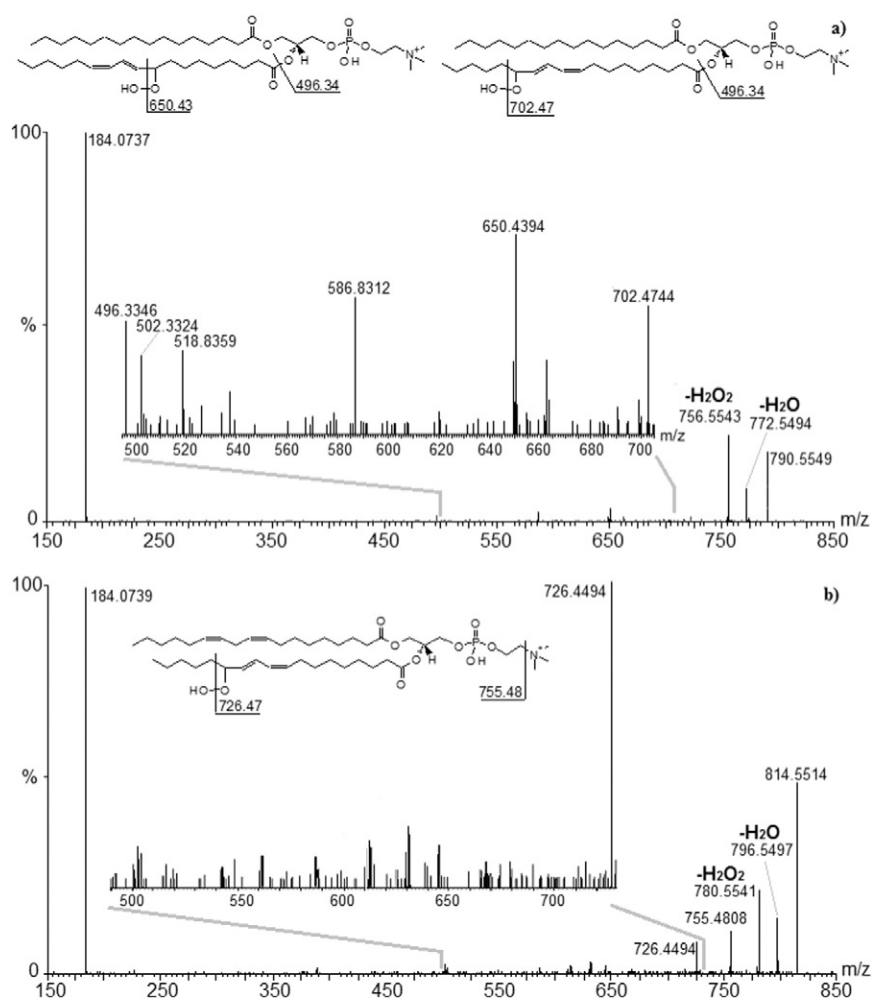


Fig. 7. Tandem mass spectra (MS/MS) acquired for the ions at (a) m/z 790 Da and (b) m/z 814 Da, corresponding to the hydroperoxy derivatives of PC(16:0/18:2) and PC(18:2/18:2), respectively. Zoom areas (m/z 490–730) show fragments that allowed the oxidation sites to be assigned, which are shown in the structures.

The decrease of A234 observed at long irradiation times (Fig. 4) indicated a decrease in the concentration of conjugated dienes, which suggests further reactions leading to secondary products. Several studies have reported that, after conjugation, breaks of fatty acid chains take place and secondary oxidation products containing terminal aldehydic and carboxylic groups are formed [36,37]. Therefore, to investigate this point, samples containing SoyPC and Ptr, irradiated for more than 50 min, were analyzed by mass spectrometry and the corresponding mass spectra were compared to those registered for untreated samples. To simplify the study, only products derived from PC(16:0/18:2) (1-hexadecanoyl-2-octadecadienyl-GPC) were investigated. As shown in Table 1, four major m/z ions were detected in the mass spectra of the irradiated sample, which were not present in the mass spectra of sample before irradiation. Those ions correspond to products containing oxidized fragmented acyl chains formed by alkoxyl rearrangement in linoleyl chains [38,39]. These results suggested that conjugated dienes underwent further Ptr-photosensitized reactions involving oxidation

and cleavage of fatty acid chains to yield molecules of lower molecular weights bearing aldehyde, ketone and carboxy functional groups.

4. Conclusions

In this work we have investigated the oxidation of liposomes of phosphatidylcholine from soybean (SoyPC) photoinduced by pterin (Ptr), the parent and unsubstituted compound of unconjugated oxidized pterins. Upon UVA irradiation (320–400 nm) of SoyPC liposomes with Ptr, conjugated dienes and trienes were detected, showing that Ptr is able to photoinduce lipid peroxidation. The process would be initiated by an electron transfer step, whereas singlet oxygen (1O_2) would not be involved as the main pathway. Additionally, various hydroperoxides corresponding to the oxidation of different PC derivatives were found as photoproducts, which later on suffered cleavages in the carbon chain generating short-chain secondary oxidation products. Finally, permeability studies revealed that Ptr is not encapsulated in the inner

Table 1

Molecular formula, observed and calculated mass, mass error and name of the short-chain secondary products detected in MS spectra.

| Product name | Molecular formula | Calculated mass (Da) | Observed mass (Da) | Δ (mDa) |
|---|--|----------------------|--------------------|----------------|
| 1-hexadecanoyl-2-(9-Oxo-nonanoyl)-GPC | C ₃₃ H ₆₅ NO ₉ P | 650.4397 | 650.4408 | -1.1 |
| 1-hexadecanoyl-2-(9-Carboxy-nonanoyl)-GPC | C ₃₃ H ₆₅ NO ₁₀ P | 666.4346 | 666.4346 | 0 |
| 1-hexadecanoyl-2-(11-Oxo-9-undecenyl)-GPC | C ₃₅ H ₆₇ NO ₉ P | 676.4553 | 676.4545 | 0.8 |
| 1-hexadecanoyl-2-(9-Keto-12-oxo-10-dodecenyl)-GPC | C ₃₆ H ₆₇ NO ₁₀ P | 704.4502 | 704.4450 | 5.2 |

fraction of liposomes and that Ptr is able to freely cross the phospholipid bilayer.

Unconjugated oxidized pterins accumulate in the skin of patients suffering from vitiligo, a depigmentation disorder in which the protection against the UV radiation fails due to the lack of melanin. Therefore the results presented in this work are relevant from a biomedical point of view because they suggest that pterins are able to damage cell membranes. Moreover, the fact that pterins can cross phospholipid bilayers suggests that these compounds, generated in a given cellular compartment, can migrate to different compartments and, upon irradiation, oxidize other membranes and biomolecules.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.bbamem.2015.11.002>.

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