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Photolysis of incorporated benzophenone derivatives inside compressed lipid monolayers

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The goal of this work was to study the possibility of the occurrence of radical-type lipid peroxidation of the lipid constituents on biomembranes, in compressed monolayers, having lipoidal benzophenone photosensitizers incorporated. The triplets of the photosensitizer abstract allylic and doubly-allylic hydrogen atoms from anti-conjugated moities of the lipid molecules. The results simultaneously confirmed the occurrence of H-abstraction (and so the initiation of the peroxidizing chain mechanism), and the absence of the formation of lipid peroxides. The reason lies in "cage effect": the highly restricted spacial area of compressed lipid monolayers limits the mobility of the created radicals (lipid radicals and ketyl radicals) and leads to their recombination, thus preventing the propagation step of the chain mechanism. With certain reservations it may be concluded that these results have a clear implication on real biomembranes: the structure of which is one of the main factors preventing the spread of the chain reaction, and the formation of lipid peroxides.

Keywords: free radicals, lipid peroxidation, photosensitizers, benzophenone, monolayers.

INTRODUCTION

Due to the enormous importance of biomembranes for the functioning of cells and tissues, lipid peroxidation studies have always attracted a lot of attention. According to a broader definition, the phenomenon of lipid peroxidation implies the oxidative destruction of polyunsaturated lipids,[1] which as a consequence leads to the production of numerous pathological effects. The primary consequences include lipo-protein and protein-protein crosslinking,[2] which may cause changes in the structure of enzymes and their activities.[3] The more specific consequences include: damage on subcellular organelles (such as the swelling of mitochondria; fast ribosomes leaking from the endoplasmtic reticulum; hemolysis of erythrocites), inhibition of proteins synthesis by peroxidation nus-products (such as malonaldehyde), and the inhibition of DNA and RNA synthesis in the liver and lungs, as a result of acrolein action.[4] On a macro-level, lipid peroxidation effects are included in number of widely spread diseases, from ath-

erosclerosis[5] to carcinogenesis.[6] Moreover, lipid peroxidation has been seen as "a common pathogenetic mechanism".[7]

Lipid peroxidation can be initiated photolitically through two different mechanisms. A photosensitizer in its excited state may transfer energy to ground state oxygen to produce reactive singlet oxygen ($^1O_2$), which in turn attacks lipids to generate peroxide species *via* a non-radical pathway (Type II). Alternatively, direct reaction between a photosensitizer and lipid may occur to generate reactive lipid free radicals (Type I).[8,9] The presence of polyunsaturated hydrophobic moieties in lipid constituents of biological membranes renders them particularly vulnerable to peroxidative degradation.[9] This vulnerability may be ascribed largely to the presence of double bonds in the hydrocarbon moieties, and the adjacent allylic and doubly-allylic sites from which hydrogen abstraction may be facilitated[10,11] (Fig. 1). While unsaturated sites may be directly attacked by singlet oxygen to produce lipid peroxides (Type II), more extensive damage occurs *via* the free-radical mechanism (Type I). The extent of such chain degradation depends on the concentration, packing and other parameters of the lipid environment.

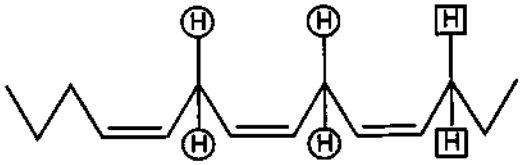


Fig. 1. Doubly-allylic (circled) and allylic (squared) hydrogen atoms in a typical anticonjugated structure of lipid hydrophobic moieties.

The Type II sensitized-reaction has been very widely studied and is relatively well understood. On the other hand, kinetic data of the radical-chain mechanism (Type I) are to a large extent absent.

While the chain peroxidation effects have been studied extensively *via* autooxidation,[12] quantitative characterization of the degradation requires *controlled* initiation of H-abstraction from the allylic and doubly-allylic sites. Several radiation chemical studies carried out using $^{\cdot}OH$ or $O^{2-\cdot}$ radicals as the H-abstraction agents suffered from non-selectivity as to the site of attack in the complex environments.[13–15] On the other hand, the use of benzophenone (BZP), already well known as a very efficient initiator of polymerization processes in which it reacts as a typical Type I photosensitizer,[16,17] permits very selective abstraction from allylic and doubly-allylic sites by its triplet excited state (^{3}BZP)[18] and so appeared to be a promising approach for further quantitative chain peroxidation studies.

There are two possible approaches to the study of the mechanisms of photosensitized peroxidation in biological membranes. The first one implies conducting experiments *in vivo*. The large number of related variables appears to be a very limiting factor for such approach. The second one includes experiments on membrane models, with increasing degree of molecular organization (micelles, compressed monolayers, vesicles), providing better control of the chain process inside. The latter approach was used in our previous studies.[18–20] The choice of benzophenone as the sensitizer is based on the well known organic photochemistry reaction, H-abstraction by longer-lived triplet

states of aromatic ketones.[21] To obtain basic data, significantly lacking, of the influence of the surrounding molecular organization, a series of BZP interactions with unsaturated lipoidal fatty acids was performed in benzene solution.[18] Then the same reaction was studied in micelles of sodium dodecyl sulphate (SDS),[20] and linoleic acid (LA)[19] to observe the influence of structure and molecular organization, by comparing the two sets of kinetic data from the two media. This report is a step forward, since it describes BZP-sensitized peroxidation in compressed lipid monolayers at the air-water interface, representing a more organized medium, even more imitative and similar to real biomembranes.

MATERIALS AND METHODS

Synthesis of benzophenone derivatives

Benzophenone-4-heptyl-4-undecanoic acid (BHUA) was synthesized according to the procedure described in a former report.[20] The second BZP-derivative, diphenyl-1-*o*-hexadecyl-2-*o*-(benzophenone-4-heptyl-4'-pentanoil)-*sn*-glycero-3-phosphate (DBP), was synthesized using a procedure described for the synthesis of very similar compounds.[22,23] The structures of BZP, BHUA and DBP are shown in Fig. 2.

The lipid used to create the monolayers, 1,2-dilinoleoyl-phosphatidyl-choline (1,2-DLPC) was purchased from Avanty Polar Lipids (Birmingham, AL).

Photolysis experiments on monolayers

The photolysis experiments on the lipid monolayers with incorporated BZP-derivatives as photosensitizers were performed using a specially constructed, self-made experimental set-up.

A teflon trough 15 cm 54 cm 3 mm (depth) was filled with water. An immovable rod was fixed parallel to the longer edge of the trough. The rod served as a support for two parallel teflon barriers 2–2.5 mm immersed in the water subphase. The two barriers, parallel to the shorter edge of the trough, glide along the rode and, on approaching each other, compress the lipid monolayer already created on the water surface. The compression speed of the monolayers, in the range of 0.016– 1.6 cm/min, was controlled by a four-phase Airpax stepper motor model K82954-MS (North American Phillips Controls Corp.). To prevent heating during operation the motor was cooled by water flowing through a copper tubing coiled around its cylindrical surface. A little box with the scales of a Cahn 2000 Electrobalance was fixed at the top of the experimental set-up. On the left arm of the scales, a nichrome wire was attached, with a 0.95 cm square filter paper plate at the end. The paper plate was immersed 1–2 mm into the water subphase, straight into the center of the trough. It served to register the changes in the surface pressure resulting from the monolayer compression. The right arm was fixed to the Cahn Electrobalance which transforms the stretching of the balance arms (caused by the surface pressure changes) into millivolts, mV. A calibration was performed prior to the experiments. The electrobalance is connected to a Hewlett Packard chart recorder, model 745A.

After creation of the lipid (1,2-DLPC) monolayer and the start of the compression, the surface pressure changes were registered on a chart. In this manner typical - isotherms were recorded, with the Y-axis ( ) giving the values of the surface pressure in mN/m, and the X-axis ( ) expressing the values of the molecular packing in $(Å)^2$/molecule. The values were easily calculated, knowing the exact valume and the concentration of the 1,2-DLPC solution (used for the monolayer creation), as well as the trough dimensions.

The monolayers samples were mixtures of the lipid (1,2-DLPC) and the photosensitizer (BHUA, DBP). The lipid/sensitizer ratios used in the experiments were 4:1 and 6:1. Usually, aliquots of 5 – 50 10^{-6} dm^{-3} were used with an approximate total (lipid + sensitizer) concentration of 1.5 mmol dm^{-3}.

Low pressure filament mercury lamps (manufactured by the Southern New England Ultraviolet Co., Hamden, CONN.) with phosphor coatings were used in the photolysis experiments. The lamps were packed into two separated sets, fixed about 15 cm above the water on a solid adjustable rack bearer. Each lamp housing contained 10 individually water-jacketed lamps, spaced 3.7 cm apart and in a position spanning beyond the entire monolayer area (each set covering approximately half of the area). To prevent the lamps heating (which can destroy the film), distilled water was circulated *via* tygon tubing coiled around the lamps. This provided an internal temperature control of ºC. Before the photolysis experiments, the lamps were turned on and thermostated for about 10 min. The emission, directed towards the film, was blocked until the start of the experiments.

Since measurement of surface pressure is highly temperature dependent,[24,25] the entire trough and the lamp apparaturs was placed on a solid rectangular block haused inside a 92 cm 69 cm 61 cm plexiglass glove box. Since some experiments needed a particular atmosphere inside the plexiglass box (N_2, O_2, air), gas channels for the box interior were provided.

Post-photolysis procedure

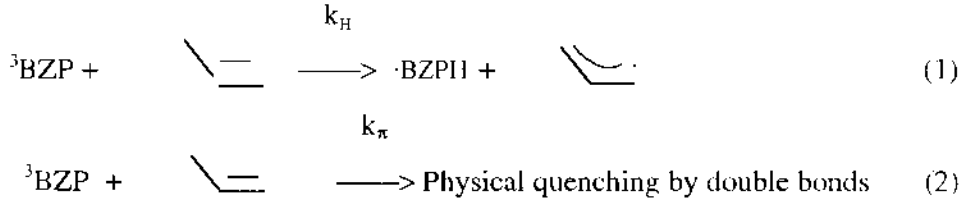
At the end of the photolysis, the monolayers were aspirated by Pasteur pipette using a strong vacuum, into an especially designated cylindrical flask. A few cm^3 of the water phase was usually aspirated too, despite the efforts to dimenish the quantity. The collected water phase was then evaporated under vacuum in a small round-bottom flask. 1 cm^3 of CH_3CN was then added to dissolve the post-photolytical monolayer products. The solution was then analyzed by HPLC. The water subphase was also analyzed by this procedure periodically, to detect an eventual (undesirable) presence of post-photolytical monolayer products. About 30 % of the post-photolytical monolayer products were lost during the procedure from aspiration to HPLC analysis (estimated by a standard comparative method).

HPLC detection

A -bondapak C-18 column 8 mm 10 cm (especially designed to work under high pressures) and a 90 % aq. MeOH as the mobile phase were used for HPLC analysis (Waters Associates, Milford, MA) of the postphotolytical monolayer products, by analogy with report of Crawford and coworkers.[26] The total concentration was in the 10^{-5} mol dm^{-3} range. The peroxides were detected at 234 nm, at the maximal absorption ($A_{max.}$) of the created dienes structures.[27] The photosensitizers were detected in the 250–270 nm range.

RESULTS AND DISCUSSION

The triplet benzophenone chromophore, ^{3}BZP, reacts with olefinic structures *via* two competing mechanisms[28]:



The first mechanism represents H-abstraction of the allylic, $H_{all.}$, and doubly allylic, $H_{dbl.all.}$, hydrogen atoms by ^{3}BZP with the consequential formation of two radicals: BZP ketyl-radical (·BZPH), and the lipid radical ( ). According to the second mechanism (2), BZP does not diminish as a chemical species (as it does by the first

mechanism): its triplet being physically quenched by the olefinic double bonds. Eventually, additional mechanisms are also included, depending on the employed medium: BZP interaction with solvent molecules in homogeneous solution,[29] the recombination of the created radical pairs in micelles.[30]

In an earlier report,[18] the fraction of the quenching events leading to ketyl-radical (·BZPH) generation, F_k, by H-abstraction from a series of olefinic compounds with increasing number of double bonds (unsaturated fatty acids and the two triglycerides): $k_H = F_k \quad k_r$, was calculated. k_H is the rate constant for H-abstraction, and k_r is the rate constant for the overall reactivity of ^{3}BZP toward the olefinic compounds. The calculated F_k values ranged from 0.36 (oleic acid) to 0.85 (arachidonic acid). If was very important to obtain these basic data, since only the related mechanism (1) – lipid radicals creation – is of relevance for the chain peroxidizing mechanism. In an other paper,[19] the behaviour of the created radical pairs (BZPH – lipid radicals) was studied in a very stericially restricted medium that mimics more closely real biomembranes. The use of BZP and its synthesized derivative, benzophenone-4-heptyl-4'-pentanoic acid (BHPA), as the photosensitizers in linoleic acid micelles (where LA plays the role of the lipid and of the medium simultaneously) resulted in F_k values of 0.41 (BZP) and 0.58 (BHPA). The somewhat higher F_k value found for BHPA was explained by the better alignment of the BZP-chromophore towards the main potential targets (H_{all} and $H_{dbl.all}$ atoms) caused by the better incorporation of the BHPA hydrophobic "tail". Furthermore, the study of BZP and BHPA interaction with LA in sodium dodecyl sulphate (SDS) micelles suggested a crucial role of the micellar "cage" in controlling the behaviour of the created radical pairs. They mostly undergo recombination inside the "cage", and only a small fraction (7–14 %) escapes to the surrounding aqueous phase.[20] These escaped lipid radicals are the only ones relevant for the peroxidation process, since they undergo the propagation step in the presence of O_2. The very small fraction (calculated in relation to initial number of radicals) suggested the significance of the "cage effect" on the efficiency of the whole process, and needed further supporting evidence. For this reason, a photolysis study using compressed lipid monolayers (with photosensitizers incorporated inside) emerged as a logical continuation of the previous work, since the molecular organization is even more spatially restricted and comparable to real biomembranes.

For these studies, 1,2-dilinoleoyl phosphatidylcholine (1,2-DLPC) was used as the lipid substrate spread in a monolayer at the air-water interface. The two choosen BZP-sensitizers were benzophenone-4-heptyl-4'-undecanoic acid (BHUA), and diphenyl-1-*o*-hexadecyl-2-*o*-(benzophenone-4-heptyl-4'-pentanoil)-*sn*-glycero-3-phosphate (DBP). The choice of the lipid and the sensitizers was not accidental. 1,2-DLPC is naturally present in certain types of biomembranes. The two double bonds located in the two hydrophobic "branches" (the two LA chromophores) should permit high reactivity with the incorporated photosensitizers, primarily by H-abstraction. On the other hand, BHUA and especially DBP (which is a lipoidal benzophenone – Fig. 2) should permit a more suitable incorporation inside the 1,2-DLPC monolayer, and the best possible alignment of the BZP-chromophore towards the main H-abstraction targets: the two doubly-allylic and 4 allylic H-atoms. The as-

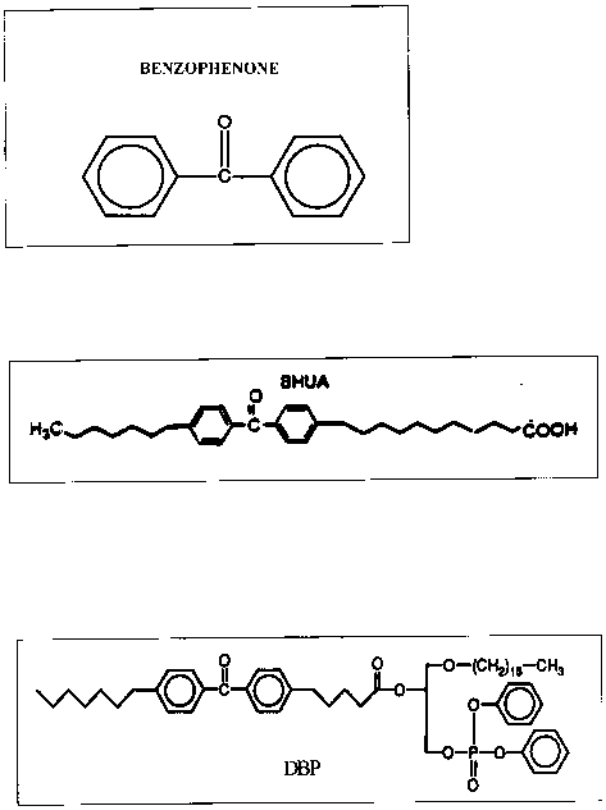


Fig. 2. Structure of benzophenone (top), as well as its synthesized derivatives: BHUA (middle), with hydrocarbon "tails", and DBP (bottom), representing actually a lipid with an incorporated benzophenone chromophore.

sumed optimal position of the BZP-chromophore inside the compressed 1,2-DLPC monolayer is shown in Fig. 3.

The idea of incorporating a sensitized chormophore into a lipid ("lipoidal chromophore") used for monolayers studies has already been exploited. Bohorquez and Patterson[31] used lipoidal pyrene for the study of monolayers by following the excimer fluorescence of the probe. Or, more recently and more relevant to this report, Maziere and coworkers[32] used diphenylhexatriene (DPH)-labeled lipids as a potential tool for studying lipid peroxidation in monolayer films; DPH was previously known as a fluorescent probe for monitoring lipoprotein peroxidation.[33] However, whereas DPH fluorescence served as a probe to follow lipid peroxidation started by another agent,[32] DBP used in this report itself initiates the peroxidation process.

However, before starting the photolysis experiments on the monolayers, it was necessary to consider "the blank" in solution: LA was used as the lipid substrate in solution and micelles,[18-20] not 1,2-DLPC itself. LA and 1,2-DLPC are not significantly different (1,2-DLPCmay be represented as two LA "branches" plus a polar phosphate head – Fig. 3). Still, the presence of the polar head (totally irrelevant for the peroxidation process) might cause (especially in the monolayer "cage") additional steric re-

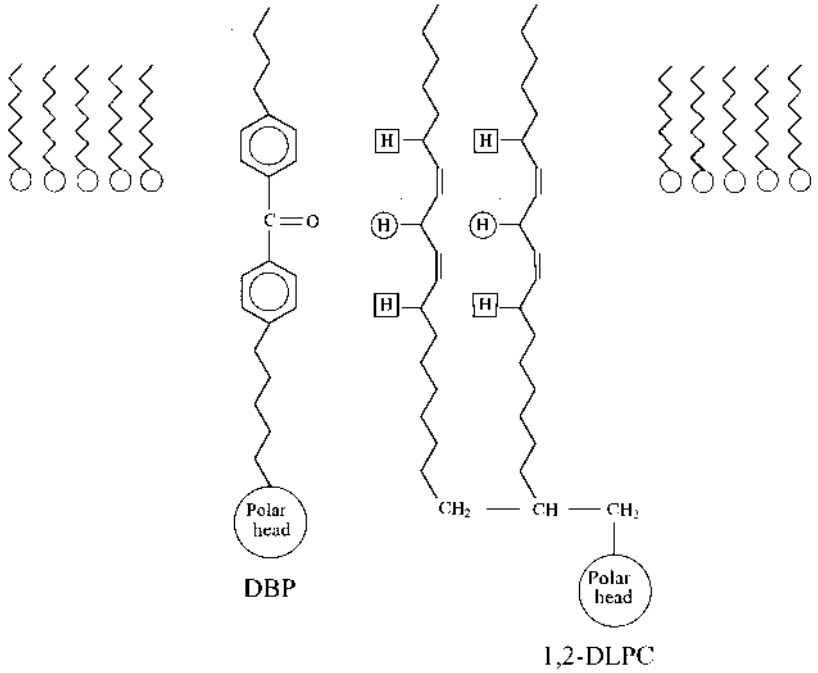


Fig. 3. The anticipated optimal position of the carbonyl chromophore of the benzophenone derivatives (BHUA and DBP) in compressed 1,2-DLPC monolayers, towards the main potential H-abstraction targets: allylic(squared) and doubly-allylic (circle) H-atoms.

strictions for the initiation of the process. The 1,2-DLPC peroxidation with BHUA as the sensitizer was performed in benzene with continuous photolysis. The result is shown in Fig. 4. The left part of the picture represents the HPLC chromatogram before photolysis (the photosensitizer peak only). The right part represents the HPLC chromatogram after two minutes of UV-irradiation: two peroxides peaks, and the photosensitizer peak in the middle can be seen, with the same absorption scale (to make comparison easier). The two peroxides peaks probably indicate incomplete peroxidation, otherwise only one peak is to be expected. The yield of BHUA photolysis has been calculated, based on chromatograms obtained for several irradiation periods. The calculated value of 0.1 is about 5 times smaller compared to the F_k value for the BZP-LA reaction in benzene. The difference is expected and can be attributed not only to the differences between the sensitizers (BZP and BHUA) and the substrates (LA and 1,2-DLPC), but to the presence of oxygen too: oxygen was depleted in the first (BZP + LA) case, but it is essential in the second (BHUA + 1,2-DLPC) for the production of peroxides. The rate constant for ^{3}BZP quenching with O_2 is very high, 2.3 ^{9} dm^3 mol^{-1} s^{-1},[34] and this is the main reason for the very low BHUA photolysis (*i.e.,* H-abstraction) yield. The choice of the UV-lamps with insignificant emission under 300 nm (A_{max} 350 nm – not shown) assured the prevention of the destruction of the formed peroxides, with A_{max} at 234 nm.[27] The same lamps were used in the monolayer photolysis experiments.

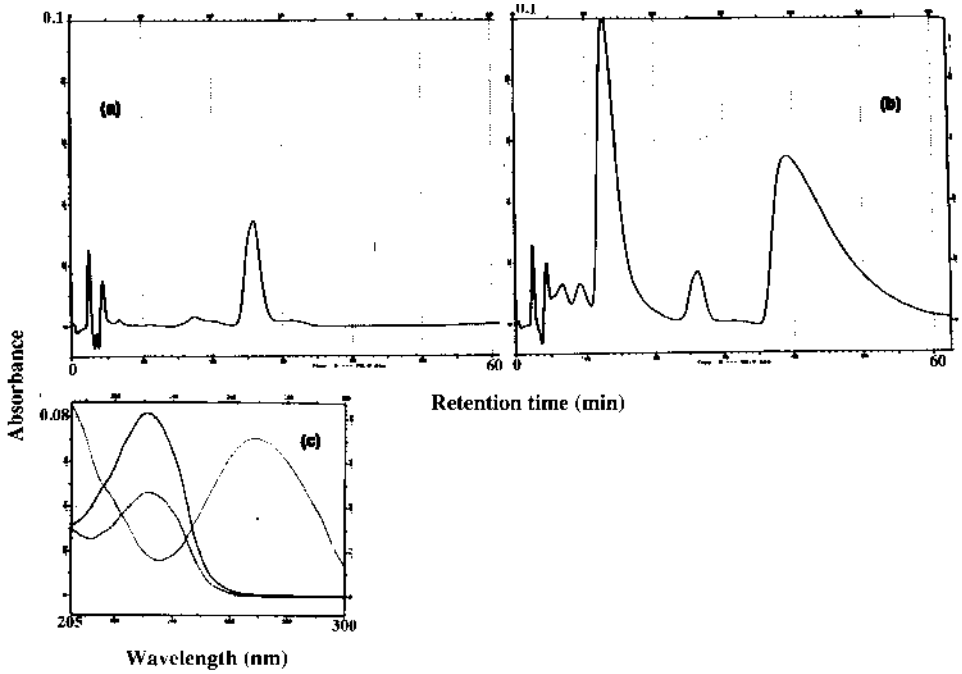


Fig. 4. (a) HPLC chromatogram, representing 1.26 mmol dm^{-3} 1,2-DLPC with 0.1 mmol dm^{-3} BHUA in benzene, before photolysis; (b) HPLC chromatogram of the same material, after two minutes of UV-irradiation, at the same absorption scale (for easier comparison); (c) Spectra of the separated compounds: (———) and (--------), 1,2-DLPC peroxides; (……..), BHUA.

Since the photosensitized peroxidation of 1,2-DLPC with BHUA is evidently possible (Fig. 4), the implication of the possibility of its occurrence in the compressed monolayers must be considered next: if the 1,2-DLPC peroxides do not appear in the HPLC chromatogram (under the same separation and detection conditions as those in Fig. 4) the reason could be, (1) in the prevention or at least significant suppression of initiation caused by steric limitations in the compressed monolayer "cage", or, (2) in the recombination of the formed radical pairs due to the "cage effect", logically expected to be even more pronounced, compared to SDS and LA micelles.

The photolysis experiments were performed with lipid (1,2-DLPC)/photosensitizers (BHUA, DBP) ratios of 4:1 and 6:1, over a broad range of surface ( ) pressures (5–30 *m*N/m), and for various irradiation periods (1–10 min). The ratios were not accidentally choosen. With the two ratios, the monolayers demonstrated complete stability before irradiation over the whole -range. This was necessary as a "blank" to be able to attribute the appearance of eventual (photolitically induced) changes to the lipid-sensitizer reaction itself, and to exclude any other factors. Furthermore, the relationships (4:1 and 6:1) prevented triplet self-quenching (or "fusion"), already detected in solution,[35] and so reasonably expected in the compressed monolayer "cages". The experiments were performed in a N_2 atmosphere (when the initiation step was in particular to be studied), and in O_2 or air atmosphere (when the accent was on the observance of eventual formation of lipid peroxides).

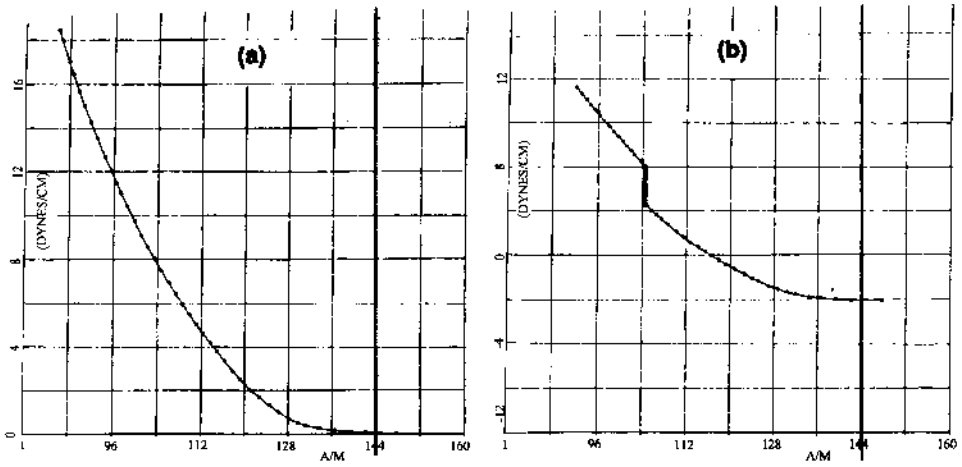


Fig. 5. isotherm of a (1,2-DLPC + BHUA, 4:1) monolayer, (a) before, and (b) after 10 min photolysis. The compressing teflon barriers were in a fixed position (*i.e.,* constant -value) during the photolysis. The -values (y-axes) are expressed in *m*N/m, and the -values (x-axes) show square angstroms, $(Å)^2$, per molecule.

Indirect evidence for the (lipid/sensitizer) reaction is obtained from Fig. 5, which shows isotherms of a (1,2-DLPC + BHUA, 4:1) monolayers, (a) before, and (b) after photolysis for 10 min. The isotherm itself has a shape typical for olefinic compounds, where the presence of double bonds prevents sharp (phase) changes during monolayer compression. A simple visual comparison of the isotherms given in Fig. 5(a,b) shows an increase of the surface pressure during the photolysis, performed at a constant value (the compressing teflon barriers were in a fixed position). However, more solid evidence for the occurrence of the lipid / sensitizer reaction is given in Fig. 6, which shows the decrease of the BHUA concentration in a (1,2-DLPC + BHUA, 6:1) monolayer during photolysis. The BHUA photolysis yield, calculated on the basis of an actinometer,[19] was 0.59, which is very close to the F_k value for BZP-LA interaction in benzene.[18] This is reliable evidence for ^{3}BHUA H-abstraction from 1,2-DLPC, since there are *no* other types of interaction between BHUA and 1,2-DLPC in the monolayers (and in a general sense) which would diminish the BHUA concentration (Fig. 6), *i.e.,* which would lead to its disappearance as a chemical species. Physical quenching by the 1,2-DLPC double bonds *does not* transform ^{3}BHUA into the corresponding ketyl-radical.

So, not surprisingly, the HPLC chromatogram recorded after photolysis (not shown) *did not show* traces of 1,2-DLPC peroxides. This was to be expected, based on results obtained in LA and SDS micelles.[19,20] The effect of radical pairs recombination is even more pronouced in the "cage" of the 1,2-DLPC compressed monolayer: the "escape event" is even less probable. Certainly, this last statement does not exclude, in an absolute sense, the possibility of lipid peroxide production in the investigated monolayers.

Certainly, it must not be forgotten that the detection of peroxides is limited by the HPLC conditions established for the "blank" (BHUA plus 1,2-DLPC, in benzene). So,

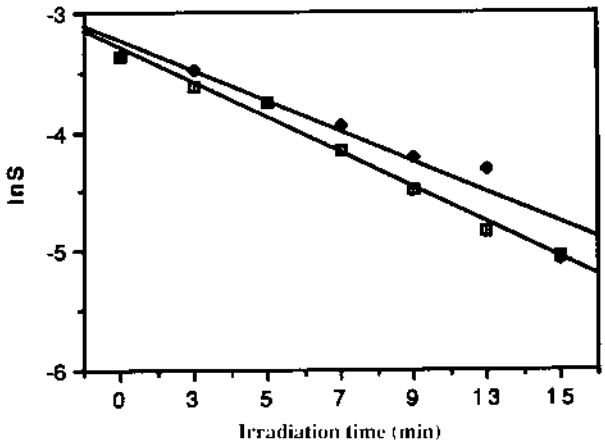


Fig. 6. Kinetics of BHUA reduction in a (1,2-DLPC + BHUA, 6:1) monolayers. The opened squares indicate the actinometer (BHUA in EtOH); S – HPLC integrated area of the BHUA peaks at 270 nm.

a limited peroxides production cannot be excluded as a possibility. To quantitatively establish the HPLC detection limit, the following procedure was employed. 1,2-DLPC peroxides were sythesized in benzene with BHUA, as for the "blank". After evaporation of benzene, $CHCl_3$ was added, and the peroxides concentration adjusted to be 1.3 mmol dm^{-3} (the concentration used for the formation of the monolayers). Aliquots of this solution, in the 5–50 10^{-3} cm^3 range, were spread over the water, thus forming surface, a set of monolayers containing known concentrations of peroxides. Then the post-photolytical procedure (described in the Materials and Methods) was applied. This time 1,2-DLPC peroxides *were detected* by HPLC. The chromatograms (not shown) contained only one peak, with a retention time close to the first peak in the chromatogram shown in Fig. 4.

The integrated peaks areas were used as the X-axis values. The second (Y-axis) set of data came from the same (5–50 10^{-3} cm^3) aliquots. The $CHCl_3$ was evaporated and the peroxides were then dissolved in 1 cm^3 CH_3CN (the same amount of the same solvent as was used for the final dissolution of the post- photolytical monolayer material - see the procedure in Materials and Methods). The samples absorbances were then measured on a UV-VIS spectrophotometer at 234 nm (the A_{max} value) to get the Y-axis values (it must be emphasized that at this wavelength the measured values reflect mostly the absorbance of the peroxides, and the absorbance of the unoxidized 1,2-DLPC molecules contributing only to a negligible amount).

The calibration plot is given in Fig. 7. From the smallest, but still detectable peak on the HPLC chromatogram (the lower detection limit), and the integrated area value, the corresponding absorbance was determined, and the concentration of the 1,2-DLPC peroxides calculated. This value was then expressed in %, calculated on the basis of the known concentration of the unoxidized 1,2-DLPC. The final result of 1–3 % defines the detection limit. If the post-photolytical peroxides concentration does not exceed 1–3 % of the total lipid (1,2-DLPC) monolayer material, they *will not be detected* under the given HPLC conditions. This small percentage value is very strong evidence in support of the

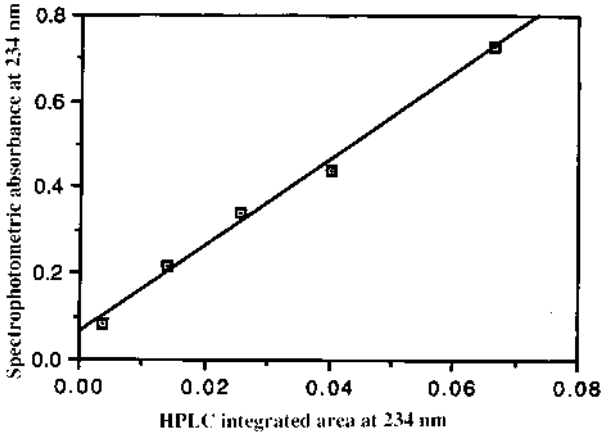


Fig. 7. Calibration diagram to determine the minimum detectable 1,2-DLPC peroxides concentration under the chosen HPLC conditions.

basic conclusion concerning the importance of the influence of the "cage" effect on the inhibition of the peroxidation process. It is reasonable to further conclude that the percentage of the photolitically induced 1,2-DLPC peroxides in the monolayers is probably less than 1 %: the presence of 1–3 % lipid peroxides in freshly isolated or synthesized unoxidized lipid material is considered normal, due to autooxidation.

Finally, additional proof in support of the absence of a significant extent of peroxidation in compressed lipid monolayers came from experiments performed at constant -pressures. This time the photolitically induced change was expressed in

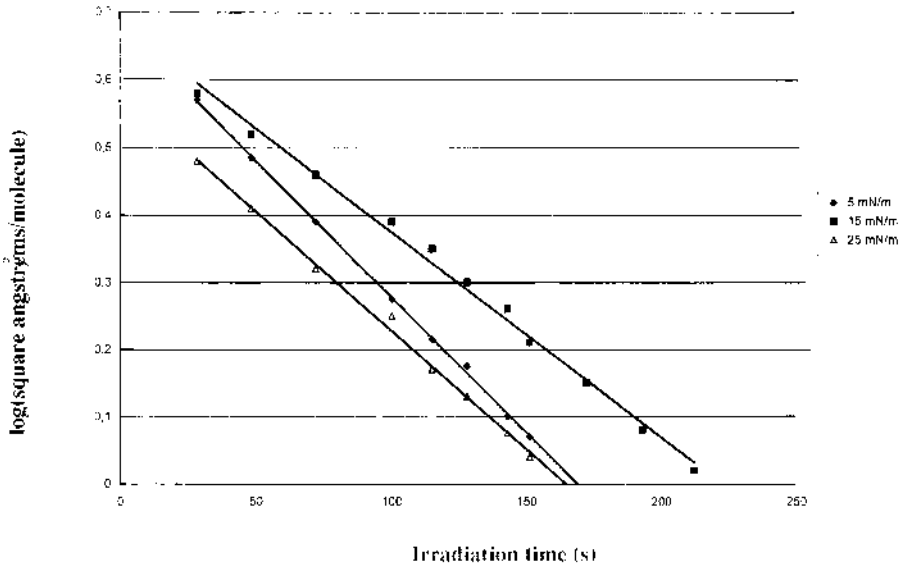


Fig. 8. Plots of temporal changes of molecular packing in (1,2-DLPC + DBP, 6:1) monolayers during photolysis (expressed in square angstroms per molecule), performed at various surface pressures.

terms of the number of square angstroms $(Å)^2$ per molecule. The calculation of these values was based on the knowledge of all the necessary data: the area encompassed by the compressing teflon barriers, the concentrations as well as the volumes of the aliquots used for the formation of the monolayers. The log plots representing temporal changes in the molecular packing of the (1,2-DLPC + DBP, 6:1) monolayers during photolysis are shown in Fig. 8, for three different -pressures.

Two clear facts may be seen from the plots. First, the ^{3}DBP –1,2-DLPC reaction is of pseudo-first order, reflecting the significantly smaller concentration of *triplet* sensitizer, compared to the lipid concentration: the reaction rate is dependent on the dynamics of the disappearance of the triplets only. Second, the calculated anti-log values (derived from the Y-axis in Fig. 8), giving the range of the molecular packing change during photolysis, do not exceed 5 $(Å)^2$/molecule, for all the experimental -pressures (5, 10, 15, 20, and 25 *m*N/m). For the lipid/senzitizer ratio of 6:1 used in the experiments, the maximum range change was about 30 $(Å)^2$/molecule. This last number is clear and additional proof of the absence of significant peroxidation in 1,2-DLPC compressed monolayers. Otherwise, the number would be much higher.

CONCLUSIONS

1. Production of peroxides in monolayers of 1,2-DLPC with the incorporated lipoidal photosensitizers was not detected up to 1–3 %, based on the total amount of lipid used for the formation of the monolayers. The result was obtained from experiments performed at different surface pressures. The reason is not because the photochemical reaction inside the monolayers does not occur; a clear decrease of the photosensitizer concentration with increasing irradiation time was found. Hence, H-abstraction inside the 1,2-DLPC monolayers (by ^{3}BHUA and ^{3}DBP) certainly occurs, but 1,2-DLPC peroxides were not formed because the radical pairs predominantly recombine inside the "cage" of compressed monolayers, in which their mobility is extremely limited. Additional proof for this statement lies in the proportionally small change of square angstroms per molecule in the investigated monolayers. The change would certainly be bigger if the lipid peroxidation chain reaction occurred to a significant extent.

2. Although compressed monolayers are only artificial models for biomembranes, the basic conclusion concerning the possibility of the occurrence of lipid peroxidation inside monolayers can be applied to biomembranes, with certain reservations. At least one of the factors which prevents the propagation of the lipid peroxidation chain mechanism inside biomembranes is their structure. It clearly appears that biomembranes, in the case of radical pair events, have some kind of self-protection mechanism on at least one part of the total peroxidation content. Certainly, this does not exclude the action of other factors preventing propagation of the chain mechanism (such as anitoxidants).

ABBREVIATIONS

BZP – benzophenone

BHUA – benzophenone-4-heptyl-4'-undecanoic acid

DBP – diphenyl-1-*o*-hexadecyl-2-*o*-(benzophenone-4-heptyl-4'-pentanoil)-*sn*-glycero-3-phosphate

1,2-DLPC – 1,2-dilinoleoyl-phosphatidyl-choline

BHPA – benzophenone-4-heptyl-4'-pentanoic acid

SDS – sodium dodecyl sulphate

LA – linoleic acid

И З В О Д

ФОТОЛИЗА ИНКОРПОРИСАНИХ ДЕРИВАТА БЕНЗОФЕНОНА У САБИЈЕНИМ МОНОМОЛЕКУЛСКИМ СЛОЈЕВИМА ЛИПИДА

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Циљ рада је проучавање могућности одигравања липидне пероксидације слободно-радикалског типа на липидним конституентима биомембрана, у сабијеним мономолекулским слојевима, са инкорпорисаним липоидалним фотосензибилизаторима бензофенонског типа. Њихови триплети апстракују алилне и двоструко-алилне атоме водоника из антикоњугованих целина липидних молекула. Резултати истовремено потврђују одигравање апстракције водоникових атома (и према томе иницијацију ланчаног механизма пероксидације) и одсуство формирања липидних пероксида. Разлог је у "ефекту кавеза": ограничени простор сабијених липидних мономолекулских слојева ограничава покретљивост створених радикала (липидни и кетил-радикали) и води до њихове рекомбинације, спречавајући пропагациони корак ланчаног механизма. Са извесном апроксимацијом може се закључити да ови резултати имају јасну импликацију на реалне биомембране: сама њихова структура је један од главних фактора за спречавање ланчане реакције и формирање липидних пероксида.

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