UV-induced reaction kinetics in dilinoleoylphosphatidylcholine monolayers with incorporated photosensitizers

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Abstract: Mixed insoluble monolayers (Langmuir films) of 1,2-di-O-linoleoyl-3-sn-phosphatidylcholine (1,2-DLPC) and incorporated benzophenone-type photosensitizers at an air-water interface were exposed to prolonged UV-irradiation. The irradiation was initiated at a particular fixed molecular packing value. Changes of the surface pressure during the UV-induced photolysis of the sensitizers were plotted against the irradiation time and the results were interpreted in terms of the molecular lipid / sensitizer ratios inside the monolayers.

Keywords: monolayers, UV-irradiation, kinetics, lipids, photosensitizer.

INTRODUCTION

Spread lipid monolayers at an air-water interface (also known as monomeric Langmuir films) are biomimetic systems enabling the study of numerous chemical reactions and processes occurring in vivo, but in a much more controlled manner. One of these processes is lipid peroxidation, which damages all kinds of membrane lipids, leading to several physiopathological events (e.g. atherosclerosis, cancer, ageing, neurodegenerative diseases, etc.). Lipid peroxidation may also be studied in other biomimetic systems, such as micelles, bilayers, liposomes, isolated natural membranes as well as artificial membranes. Lipid peroxidation may occur via a free-radical chain mechanism (Type I) or non-radical pathway – Type II. Usually, in the former case, lipid peroxidation is sensitized through a variety of external stresses, enabling the production of peroxidation initiating agents, photosensitized initiation is one of them.

Monolayers submitted to gradual compression appear to be a useful tool for providing insight into changes of molecular organization, starting from a “liquid-expanded” and finishing with a “solid-condensed” phase, which will certainly affect not only the geometry of the system but also the accompanying kinetics. When Type I – sensitized
lipid peroxidation is operative, it will certainly affect the mutual position of a chosen photosensitizer and the lipid host, as well as the kinetics of the initiating step.

Our previous reports were related to the interconnection between the lipid peroxidation process initiated by the benzophenone triplet (3BZP) and the degree of molecular organization in the studied systems, ranging from homogeneous solution,\textsuperscript{16} \textit{via} SDS\textsuperscript{17} and linoleic acid micelles\textsuperscript{18} to compressed monolayers of unsaturated phosphatidylcholine.\textsuperscript{19} This work is a continuation of the last system, since it deals with the same lipid (1,2-di-O-linoleoyl-3-sn-phosphatidylcholine – 1,2-DLPC) and the same BZP-type photosensitizers. However, while the former paper was more focused on the detection of lipid peroxides, paying less attention to the kinetics of the steps involved, this work is primarily devoted to the initiation event. It deals with the changes in the monolayer surface pressure resulting from different UV-irradiation periods.

**EXPERIMENTAL**

11-[4-(4-Heptylbenzoyl)phenyl]undecanoic acid (BHUA) was synthesized according to a described procedure.\textsuperscript{17} The second BZP derivative ( “BZP-lipid”), diphenyl 1-O-hexadecyl-2-O-[5-[4-(4-heptylbenzoyl)phenyl]pentanoyl]-sn-glycerol-3-phosphate (DBP) was synthesized according to a recipe for the synthesis of very similar compounds.\textsuperscript{20,21}

The lipid used to form monolayers, 1,2-di-O-linoleoyl-3-sn-phosphatidylcholine (1,2-DLPC) was purchased from Avanty Polar Lipids (Birmingham, Alabama).

**Photolysis experiments on monolayers**

The photolysis experiments on lipid monolayers with BZP-derivatives incorporated 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. A stationary rod was fixed parallel to and above the longer trough edge. It served as a support for two parallel teflon barriers immersed 2–2.5 mm below the surface of the aqueous subphase. The barriers, in a parallel position to the shorter edge of the trough, glide along the rod, approaching each other and compressing the lipid monolayer already created on the water surface. The monolayer compression speed, 1.3 cm/min, was controlled by a four-phase Airpax stepping motor model K82954 (North American Phillips Controls Corp.). To prevent heating during the operation, the motor was cooled by water flowing through copper tubing coiled around its cylindrical surface. A small box with the scales of a Cahn 2000 electrobalance was fixed at the top of the experimental set-up. A nichrome wire was hooked to the left arm of the scales, ending with a 0.95 cm square filter paper, immersed 1–2 mm into the aqueous subphase, straight into the center of the trough. It served for registration of changes of the surface pressure resulting from the compression of the monolayer. The right arm was fixed to the Cahn electrobalance, which converts stretching of the balance arms (caused by surface pressure changes) into millivolts. A calibration was performed prior to the experiments.

Following the beginning of the formation and compression of the lipid (1,2-DLPC) and mixed lipid (1,2-DLPC) / sensitizers (BHUA, DBP) monolayers, changes in the surface pressure (π) were registered on a chart (the Y-axis in mN/m), together with changes in the molecular packing (area per molecule – σ), expressed in square angstroms (Å\textsuperscript{2}) (the X-axis). The σ-values were easily calculated, knowing the exact volume (50–60 × 10\textsuperscript{-3} cm\textsuperscript{3}) and the concentration (1.5–1.6 × 10\textsuperscript{-3} mol dm\textsuperscript{-3}) of the 1,2-DLPC solution (used for the monolayer formation), as well as the trough dimensions.

The lipid/sensitizers ratios used in the experiments were 4:1 and 6:1 (1,2-DLPC/BHUA) and 6:1 (1,2-DLPC/DBP). Again, volumes of the samples were in the 50–60 × 10\textsuperscript{-3} cm\textsuperscript{3} range, and the concentrations were 1.5 – 1.6 × 10\textsuperscript{-3} mol dm\textsuperscript{-3}.  

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Low-pressure mercury lamps (manufactured by the Southern New England Ultraviolet Co., Hamden Connecticut) were used for irradiation in the photolysis experiments. The lamps were packed into two separated sets, fixed about 15 cm above the water level on a solid adjustable rack bearer. Each lamp housing contained 10 individually water-jacketed lamps, spaced 3.7 cm apart and positioned beyond the whole monolayer area (each set covering approximately half of the area). To prevent the lamps heating and possible film destruction, distilled water was circulated via tubing coiled around the lamps. This provided temperature control within \( \pm 0.5 \) °C.

Before the photolysis experiments, the lamps were turned on and thermostated for about 10 min. The emission, directed toward the film, was blocked until the start of the experiment. The lamps emission had a maximum at 350 nm and negligible intensities below 300 nm.

Since the surface pressure is highly dependent on temperature, the whole trough and the lamps assembly were placed on a solid rectangular block and housed inside a 92 cm acrylic glass glove box. The experiments were performed under an inert N\(_2\) atmosphere. The relative humidity was kept below 65 %, to avoid any changes in the molecular organization induced by high relative humidity.

RESULTS

The structures of the photosensitizers, BHUA and DBP, and the lipid host, 1,2-DLPC, are shown in Fig. 1. The benzophenone chromophore, in the middle of hydrophobic chains, is responsible for H-abstraction and initiation of the lipid peroxidation chain mechanism. The long tails of the sensitzers contribute to a better positioning of the central carbonyl chromophore toward the targets, allylic and doubly-allylic H-atoms.

The \( \pi-\sigma \) curves of a 1,2-DLPC monolayer, and a \((1,2\text{-DLPC} + \text{BHUA}, 4:1)\) mixed monolayer before UV-irradiation are shown in Fig. 2a. Under compression, the involved molecules align vertically with the polar heads of the lipid touching

![Fig. 1. Structures of 1,2-DLPC (top), as well as of BHUA and DBP, with incorporated benzophenone (BZP) chromophores.](image-url)
the water surface; the sensitizers maintain the same vertical position with orientation of the crucial carbonyl chromophore toward the major targets, allylic and doubly-allylic H-atoms. The $\pi$–$\sigma$ curve of the same mixed monolayer, photolysed for 15 min at a fixed molecular packing value of $\approx 100 \text{ Å}^2$ per molecule (the compressing barriers were stopped in a defined position), is shown in Fig. 2b. The rise of the $\pi$-pressure indicates the occurrence of the photochemical reaction of H-abstraction by the triplet of BHUA, initiating the peroxidation chain mechanism.

The $\pi$–$\sigma$ curves of a 1,2-DLPC monolayer, a sensitizer (DBP) monolayer, and a (1,2-DLPC + DBP, 6:1) mixed monolayer before UV-irradiation are shown in Fig. 3a. The $\pi$–$\sigma$ curve of the same mixed monolayer, UV-photolysed for 7 min at a fixed molecular packing value of $\approx 108 \text{ Å}^2$ per molecule, is shown in Fig. 3b. The rise of the $\pi$-pressure again indicates the photochemical reaction, i.e., initiation of the chain mechanism.

Fig. 2. $\pi$ (surface pressure) – $\sigma$ (area per molecule) curve of (a) 1,2-DLPC monolayer, and (1,2-DLPC + BHUA, 4:1) mixed monolayer before UV-irradiation; (b) the same (1,2-DLPC + BHUA, 4:1) mixed monolayer exposed for 15 min to UV-irradiation at a fixed molecular packing value of $\approx 100 \text{ Å}^2$ per molecule.

Fig. 3. $\pi$ (surface pressure) – $\sigma$ (area per molecule) curve of (a) 1,2-DLPC monolayer, sensitizer (DBP) monolayer, and (1,2-DLPC + DBP, 6:1) mixed monolayer before UV-irradiation; (b) the same (1,2-DLPC + DBP, 6:1) mixed monolayer exposed for 7 min to UV-irradiation, at a fixed molecular packing value of $\approx 108 \text{ Å}^2$ per molecule.
Kinetic plots of the surface pressure changes ($\Delta \pi$ in mN/m) as a result of UV-irradiation ($t_{irr}$ in min) of (a) (1,2-DLPC + BHUA, 6:1) mixed monolayer at a molecular packing value of $\approx 120$ Å$^2$ per molecule; (b) (1,2-DLPC + BHUA, 4:1) mixed monolayer at a molecular packing value of $\approx 100$ Å$^2$ per molecule.

Kinetic plots of the surface pressure changes ($\Delta \pi$) as a function of the irradiation time for the two mixed monolayers are shown in Fig. 4a,b. Evidently, some changes occurred even before the irradiation was started. The equations of the lines are indicated at the bottom of the plots.

DISCUSSION

Phospholipids (phosphatidylcholines among them) are widely recognized as major chemical components of biomembranes, which significantly determine their physicochemical properties and play an important role in cellular activities. Therefore, analysis of the isotherms of phospholipid monolayers could provide information about their orientation and arrangement (packing) at the interface, their stability and compressibility.25 Bearing this in mind, as well as the goal of this paper, 1,2-DLPC was again chosen as the target of UV-induced attack mediated via incorporated photosensitizers.19 The choice was based on two criteria, (a) solid mechanical stability easily controlled by a gradual compression, (b) potential high reactivity – toward UV-irradiation – provided by the presence of double bonds in the hydrocarbon moieties.26,27 There are reports dealing with UV-induced polymerization of linoleic acid monolayers.28–30 In the case of 1,2-DLPC with an incorporated sensitizer (BHUA, DBP), UV-irradiation is indirectly involved: Thus, UV-irradiation creates sensitizers triplets ($^3$BHUA, $^3$DBP) which abstract allylic and doubly allylic H-atoms from hydrophobic branches of DLPC, forming radical-pairs (DLPC-radicals – BHUA, DBP ketyl radicals) which mainly undergo a recombination “cage effect”, whereby termination of the chain mechanism and peroxides formation are prevented.19 The reaction kinetics follow a pseudo-first order law, since the sensitizers triplets are present in a minor amount compared to the other reactant.19

DLPC monolayers express a smooth, gradual compressibility (Figs. 2a and 3a), excluding sharp transition points among the corresponding states. This behaviour is the very opposite of the one of saturated dipalmitoylphosphatidylcholine...
(DPPC), which has at least three well-recognized states: liquid-expanded, liquid-condensed and solid-condensed. On the other hand, DLPC monolayers appear much more similar to the ones of linoleic acid (LA), obtained on subphases with lower pH values. This is not surprising, for the two mixed monolayers with the same incorporated photosensitizer (BHUA) as the two branches of 1,2-DLPC represent, in fact, LA molecules. This is a proof that the compressibility of DLPC monolayers is predominantly determined by the presence of 4 double bonds in the two LA branches. Naturally, the molecular packing values are different since the size of the two molecules (DLPC and LA) are quite different. Thus, the initial compression point for DLPC molecules at about 140 Å² (Figs. 2a and 3a) is much higher than for LA monolayers (from 30–70 Å², depending on the pH values of the subphase). Finally, the DLPC π–σ curves are similar to that of dillinoleoylphosphatidylethanolamine (DLPE), with respect to the whole compressibility behaviour as well as to the particular points.

The π–σ curves of the mixed monolayers (1,2-DLPC + BHUA, 4:1; 1,2-DLPC + DBP, 6:1) appear very similar to those of DLPC (Figs. 2a and 3a). This was also expected, bearing in mind: (a) both BHUA and DBP are quite lipoidal – their structures are in fact projected to match the structure of DLPC as much as possible, enabling them to be incorporated among the DLPC molecules; (b) the molecular ratios (4:1 and 6:1) ensure a DLPC type of behaviour. With BHUA, the compression slope is a little smoother (Fig. 2a), while with DBP (which is actually “BZP-DLPC”) the compression slope is a little steeper (Fig. 3a) than those of DLPC and DBP: the bigger DBP molecules have a somewhat denser packing, thus a higher surface pressure is required for the same σ values.

The π–σ curves of the mixed monolayers (Figs. 2a and 3a) served as “blanks” for the UV-irradiation experiments. The π–σ curves of the same but photolysed mixed monolayers are shown in Figs. 2b and 3b.

Kinetics studies of the photoinduced changes in the monolayers can be performed by following the changes of (a) molecular packing (Δσ), or (b) surface pressure (Δπ), as a function of the irradiation time (t_irr). The first approach was used in polymerization studies of LA monolayers, as well as in the author’s former report. The second approach was applied in this work. The Δπ vs. t_irr plots but with two different lipid / sensitizers ratios (6:1 and 4:1), are shown in Fig. 4a, b.

The plots follow pseudo-first order kinetics for both monolayers, which is in accordance with the same conclusion from the former report when Δσ vs. t_irr plots were employed. “Pseudo” is related to the minor concentration of triplets (3BHUA, 3DBP) and not to non-excited sensitizers species. This kinetics pattern was also seen in the frame of other molecular organizations, i.e., in homogeneous solution, SDS micelles, and linoleic acid micelles, the initial step, H-abstraction from lipids by BZP-sensitizers triplets, depends on the lipid concentration only.

Two facts emerged from a comparison of the two linear plots (Fig. 4a, b): the intercept is the same for both plots (close to 1), while the slopes are remarkably different:
for (1,2-DLPC + BHUA, 6:1) monolayer it is 0.346, while for the (1,2-DLPC + BHUA, 4:1) monolayer it is 0.106. The first fact is easier to explain, since a Δσ oscillation of 1 mN/m can be ascribed to a relaxation of the spread monolayers at a particular σ value (this was confirmed in the stability control experiment performed before the irradiation). The fact that the ratio of the two slopes is more than 3 is more complicated to explain. Photolysis of the (1,2-DLPC + BHUA, 4:1) monolayer was performed over a broader t \text{ irr.} range, and the deviation of the points from the line are evidently bigger (Fig. 4b), compared to other plot (Fig. 4a), when the photolysis was performed over a shorter irradiation range (14 vs. 40 min). Still, this is probably of marginal significance. It is difficult to estimate how much the difference in the DLPC / sensitizer ratios (4:1 and 6:1) affected the values of the slopes. The ratios themselves are molecular (number) relationships, not implying any homogeneous distribution of the involved molecular species (DLPC, the sensitizers) inside the frame of a particular mixed monolayer, at any defined molecular packing value. Consequently, there is no proof that BHUA will react with every 6\textsuperscript{th} or 4\textsuperscript{th} DLPC molecule. Such circumstances may lead to a possible "fusion" of 3BHUA triplets inside the monolayer (triplet self-quenching) – already detected in solution.\textsuperscript{31} Probably this would occur more frequently when the ratio is 4:1 (compared to 6:1), and this could certainly result in a smaller slope (0.106 – Fig. 4b). However, this is just a possible explanation; some other experimental techniques should be employed to provide other supporting evidence.

**CONCLUSION**

1. This study of the changes in the surface pressure (Δσ) induced by UV-irradiation of mixed monolayers of 1,2-DLPC with incorporated lipoidal benzophenone-type photosensitizers confirmed pseudo-first order reaction kinetics, already found in a former investigation,\textsuperscript{19} when the Δσ values (changes in molecular packing) were plotted against the irradiation time.

2. It appears that the lipid / sensitizer molecular ratio in the involved mixed monolayers affects the reaction rate. The possible self-annihilation of sensitizer triplets (triplet self-quenching) inside the compressed monolayers may be one of the governing factors.

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kog tipa, na površini voda-vazduh, izloženi su produženom dejstvu ultraljubičastog zraka. Zrake je započinjano na tачно određenoj fikiranoj vrednosti molekularnog pakovanja. Pраћene su promene površinskog pritiska u monomolekulskom sloju tokom fotoizlaze senzibilizatora izazvane ultraljubičastim zračenjem, u funkciji vremena ovrživanja. Rezultati su interpretirani u svetlu molekularnog odnosa lipida/fotosenzibilizator unutar dатог monomolekulskog sloja.

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REFERENCES