Interaction of amphiphilic chlorin-based photosensitizers with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine monolayers

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A B S T R A C T
The drawbacks of the presently used photosensitizers include their relatively low selectivity toward cancer cells, and long-lasting accumulation in healthy tissues. Our recent results indicate that conjugating a photosensitizer with folic acid both enhances the active uptake by cells, and decreases the accumulation in healthy tissue. Here, the interaction between 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) monolayers used as model membranes, and three different photosensitizers were studied; the derivatives were the non-conjugated meta-tetrahydroxyphenylchlorin (m-THPC, CHL1) and tris(3-hydroxyphenyl)-4-carboxyphenylchlorin (CHL2), as well as a folic acid-conjugated m-THPC-like molecule (CHL3). The results obtained indicate that the folate moiety present in the conjugated derivative CHL3 is involved in the interaction with the phospholipid polar heads. This interaction may be responsible for a better miscibility of CHL3 with the DPPC films compared to CHL1 and CHL2, while elimination of CHL3 from the tissue may be due rather to specific, biological processes and not to its polarity.

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1. Introduction

Photodynamic therapy (PDT) is performed by injecting a photoactivatable molecule that will, after excitation with light of appropriate wavelength, relax to the ground state by transferring energy to surrounding molecules. This process generates highly reactive species, such as radicals and singlet oxygen (Ochsner, 1997). In living organisms, the photoactivatable molecules, termed photosensitizers, can be used to induce necrosis or apoptosis of the host cells (Henderson and Dougherty, 1992; MacDonald and Dougherty, 2001). Used in the treatment of macular degenerations (Ghazi et al., 2001), this strategy has drawn interest in oncology (Kubler et al., 1999; Lam et al., 2001; Sutedja and Postmus, 1996; Weersink et al., 2005). Indeed, photosensitizers display some selectivity toward tumor tissues. On the other hand, in the absence of light, PDT agents generally display negligible to weak cytotoxicity (Stewart et al., 1998). First generation photosensitizers were derivatives of natural products such as hematoporphyrins (HpD) (Dougherty, 1987). The second generation photosensitizers are synthetic compounds with well defined photophysical properties (Jori, 1996; Sternberg et al., 1998). The critical property displayed by good photosensitizing candidates is their ability to absorb light in the red or near-infrared spectrum, because these wavelengths penetrate deep into organic tissues and generate singlet oxygen with high yields. Chlorins appear to be adequate candidates for clinical trials because of their high molar extinction coefficient around 650 nm, and a singlet oxygen production yield generally above 50% (Nyman and Hynninen, 2004). meta-Tetrahydroxyphenylchlorin (m-THPC) is the most extraordinary example of this class of compounds. This second generation chlorin is currently used in clinical applications for the treatment of human mesothelioma and for gynaecological, respiratory and head and neck cancers (Atif et al., 2007; Cramers et al., 2003; Reuther et al., 2001). However, as many other first and second generation photosensitizers, m-THPC shows a relatively low selectivity toward cancer cells. Moreover, accumulation of these molecules in healthy tissues induces side effects, the most important being a week- to month-long skin sensitization to light (Sharma et al., 1999; Vrouenraets et al., 2003).

Recently, a third generation of PDT agents was developed to overcome these problems. The third generation compounds are photosensitizing molecules bound to a targeting moiety, generally a small peptide (Schneider et al., 2006) or specific antibody (Bhatti et al., 2008), which binds to receptors overexpressed on the tumor cell surface (Hanahan and Weinberg, 2000). In this context, folic acid has drawn wide attention (Low and Antony, 2004; Low et al., 2008). The interest in cancer research for folic acid comes from the fact that its high-affinity specific receptor (FR) is overexpressed on the cell surface of several tumor types, such as nasopharyngeal, ovarian or brain cancers. Moreover, experiments proved that the affinity of folic acid toward its receptor is preserved when linked...
to a therapeutic molecule through the γ-carboxylic group (Kamen and Capdevila, 1986). Several studies demonstrated that folate conjugates were preferentially taken up by FR-overexpressing tumor cells in vitro and in vivo when compared to classical compounds (Mathias et al., 1996).

Our group works on the synthesis and evaluation of new chlorin derivatives covalently bound to folic acid. In vitro experiments showed that the uptake of the chlorin conjugates was specifically enhanced in KB cells, which are human nasopharyngeal tumor cells overexpressing FRs (Schneider et al., 2005). A closer attention was brought to poly(ethylene glycol) (PEG). PEG-linked conjugates were carefully studied due to their enhanced accumulation within cells. In a recent study, we demonstrated that 4 h after injection in xenografted mice, the uptake of a m-THPC-like chlorin conjugated to folic acid, CHL3, was higher compared to the non-conjugated CHL2 (Fig. 1).

However, between 4 and 24 h after intravenous injection, the concentration of CHL3 in the tissues decreased, while the non-conjugated CHL2 continued to accumulate; the latter shows a better selectivity but lower uptake of the folate-bound CHL3 (Gravier et al., 2008). Passive targeting predicts that hydrophobic molecules tend to accumulate to a greater extent within cell membranes, especially in tumor cells, compared to hydrophilic compounds (Solban et al., 2006). It can be supposed that conjugating a photosensitizer with folic acid, while enhancing the active uptake by cells, may increase its aqueous solubility.

The experiments aiming to better our understanding of the polar/apolar interactions of the folate conjugate CHL3 with biological membranes must be performed in the absence of specific receptors. Cell membranes are complex structures, of which the major component are phospholipids that form bilayers; phosphatidylcholines are representative of eukaryotic cell membranes.

The Langmuir technique allows preparation of model lipid membranes by spreading phospholipid monolayers at the air/water interface (Davies and Rideal, 1963) and offers a unique way of investigating the interactions between the membranes and different molecules (Corvis et al., 2006a; Hidalgo et al., 2005; Korchowiec et al., 2007, 2006).

In this study, interactions between model membranes and m-THPC (CHL1), CHL2 or CHL3 were investigated by comparing the behavior of pure 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) monolayers with those formed using DPPC/photosensitizer mixtures. The monolayers were studied using surface pressure and potential measurements, Brewster angle microscopy (BAM) and polarization modulation infrared reflection-absorption spectrometry (PM-IRRAS). The results obtained indicate that all three photosensitizers modify the properties of the lipid layers. However, the folate moiety present in the conjugated derivative CHL3 may be involved in the interactions with the phospholipid polar heads. These interactions could be responsible for the better miscibility observed with the conjugate in the DPPC films compared to the other two, more hydrophobic derivatives CHL1 and CHL2.

2. Experimental

2.1. Materials

DPPC (purity >99%) was purchased from Sigma. Spectrophotometric grade chloroform and dimethylsulfoxide (DMSO) (Aldrich, A.C.S.) were used for preparing phospholipid solutions. meta-Tetrahydroxyphenylchlorin (CHL1), tris[3-hydroxyphenyl]-4-carboxyphenylchlorin (CHL2) and folate-conjugated chlorin (CHL3) were prepared as described previously (Gravier et al., 2008). Molar weights of CHL1, CHL2 and CHL3 are 680, 708 and 1261, respectively.
2.2. Compression isotherms and Brewster angle microscopy

The surface pressure (\(\Pi\)) and electric surface potential (\(\Delta V\)) measurements were performed using a KSV 5000 Langmuir balance (KSV Instruments, Helsinki). A Teflon® trough (15 cm × 58 cm × 1 cm) with two hydrophilic Delrin barriers (symmetric compression) was used in compression isotherm experiments. The system was equipped with an electrobalance and a paper plate (perimeter 20.36 mm) as a surface pressure sensor and a surface potential measuring head with a vibrating electrode (KSV SPOT1). A stainless steel plate immersed 4 mm below the water surface was used as a counter electrode. The apparatus was closed in a Plexiglas box, and temperature was kept constant at 20 °C. Before each use, the trough and the barriers were cleaned using cotton soaked in chloroform, gently brushed with ethanol and finally rinsed with MilliQ water. All solvents used for cleaning the trough and the barriers were of analytical grade. Aqueous subphases for monolayer experiments were prepared with MilliQ water, which had a surface tension of 72.8 mN m\(^{-1}\) at 20 °C. Any residual surface-active impurities were removed before each experiment by sweeping and suction of the surface. 0.005, 0.01, 0.05, 0.1 and 0.25 mole fraction photosensitizer:DPPC mixtures were prepared using calibrated solutions of DPPC (concentration 1 mg mL\(^{-1}\), in 1:4 DMSO/chloroform) and CHL1, CHL2 or CHL3 (concentration 0.5 mg mL\(^{-1}\), in 1:4 DMSO/chloroform; 50–100 µL were spread on the subphase). The stability of the surface potential signal was checked before each experiment, after cleaning the subphase surface. After the \(\Delta V\) signal had stabilized, it was zeroed and the film was spread on the subphase. After the equilibration time of 10 min, the films were compressed at the rate of 1.2–1.0 Å\(^2\) molecule\(^{-1}\) min\(^{-1}\) (2.0 mm min\(^{-1}\) barrier\(^{-1}\)). A PC computer and KSV software were used to control the experiments. Each compression isotherm was performed at least three times. The standard error was ±0.5 Å\(^2\) with mean molecular area measurements and ±5 mV with surface potential measurements. The compression isotherms allowed determining the compressibility modulus \((C_\text{coll}^{-1}; C_\text{coll}^{-1} = -\Delta(\partial\Pi/\partial A)_T^\text{coll})\). The collapse parameters, \(\Delta V_\text{coll}\), \(\Pi_\text{coll}\) and \(A_\text{coll}\), corresponding to the highest packing of molecules in the monolayer were determined directly from the compression isotherms.

The morphology of the films was imaged with a computer-interfaced KSV 2000 Langmuir balance combined with a Brewster angle microscope (KSV Optrel BAM 300, Helsinki). The Teflon® trough dimensions were 6.5 cm × 58 cm × 1 cm; other experimental conditions were as described above.

2.3. Polarization modulation infrared reflection-absorption spectroscopy

The PM-IRRAS spectra of pure DPPC and mixed photosensitizer/DPPC monolayers spread on pure water subphase were registered at 20 °C. The Teflon® trough dimensions were 36.5 cm × 7.5 cm × 0.5 cm; other experimental conditions were as described in the preceding paragraph. The PM-IRRAS measurements were performed using a KSV PMI 550 instrument (KSV Instruments Ltd., Helsinki, Finland). The PMI 550 contains a compact Fourier transform IR-spectrometer equipped with a polarization-modulation (PM) unit on one arm of a goniometer, and a MCT-detector on the other arm. The incident angle of the light beam can be freely chosen between 40° and 90°; here, the incident angle was 75°. The spectrometer and the PM-unit operate at different frequencies, allowing separation of the two signals at the detector. The PM unit consists of a photoelectric modulator, which is an IR-transparent, ZnSe piezoelectric lens. The incoming light is continuously modulated between s- and p-polarization at a frequency of 74 kHz. This allows simultaneous measurement of spectra for the two polarizations, the difference providing surface specific information, and the sum providing the reference spectrum. As the spectra are measured simultaneously, the effect of water vapor is largely reduced. The peak retardation wavelength for modulation can freely be selected depending on the wavelength region of interest. The peak retardation wavelength used here was 1500 cm\(^{-1}\). The spectral range of the device is 800–4000 cm\(^{-1}\) and the resolution is 8 cm\(^{-1}\).

3. Results and discussion

3.1. Compression isotherms

To investigate the interactions between photosensitizers and lipid membranes, the interfacial properties of the three chlorin derivatives were first established. Both non-conjugated chlorins CHL1 and CHL2 are small, rigid molecule (Fig. 1a and b) dissimilar from classical amphiphiles forming Langmuir films, and with no structural features in common with phospholipids. The CHL3 derivative, which contains a hydrophobic chlorin moiety connected to the hydrophilic folic acid via a PEG linker (Fig. 1c) is more polar and clearly amphiphilic compared to CHL1 and CHL2. The difference between the three derivatives can be observed with the \(\Delta V-A\) isotherms (Fig. 2, thin curves).

Fig. 3. Brewster angle micrographs of films formed with CHL1 (a), CHL2 (b) and CHL3 (c), taken at 10.0 mN m\(^{-1}\). Scale: the width of the snapshots corresponds to 400 µm.
Fig. 4. Compression isotherms of pure DPPC and mixed DPPC/photosensitizer monolayers. Results obtained with (a) CHL1, (b) CHL2 and (c) CHL3; solid lines: $\Pi - A$ isotherms; dashed lines: $\Delta V - A$ isotherms. The curves (1) to (6) correspond, respectively, to 0, 0.005, 0.01, 0.05, 0.1 and 0.25 mole fraction of the chlorin derivative in DPPC. (d-f) Analysis of the photosensitizer impact on the film properties presented as molecular area shift in function of surface pressure.

Indeed, the CHL3 molecules reorganize and acquire a more upright orientation at higher molecular areas (around 90 Å²) compared to CHL2 (around 70 Å²); the reorganization of CHL1 occurs at the lowest molecular areas (around 60 Å²). Obviously, this behavior of the three derivatives reflects the increasing hydrophobicity \( \text{CHL}_3 < \text{CHL}_2 < \text{CHL}_1 \). On the other hand, the surface pressure–area ($\Pi - A$) isotherms (Fig. 2, bold curves), as well as BAM experiments (Fig. 3) indicate that all three derivatives behave similarly at the air/water interface.

It can be observed that the values of the molecular area at the collapse of the films are low compared to the structures of the three chlorin derivatives. Indeed, the smallest possible area of the projection of any of the three derivatives oriented vertically relative to the water surface can be estimated to be around 55 Å²; CHL1 and CHL2 laying flat on the water surface would occupy around 200 Å² and CHL3 around 350 Å². Comparing the latter values with the experimental results indicates that the chlorin derivatives aggregate at the air/water interface; it can be noticed that no aggregation was detected in the chloroform/DMSO (4:1) solutions studied with UV–vis spectrophotometry (results not shown). This observation is in accordance with the BAM snapshots; the bright zones observed in the snapshots (Fig. 3) suggest that the three derivatives form three-dimensional structures.

The low solubility in water and aggregation at the air/water interface reflect a high hydrophobicity of the chlorin derivatives. Consequently, a high affinity of these photosensitizers for the hydrophobic part of lipid membranes could be expected. To get

Table 1

<table>
<thead>
<tr>
<th>DPPC pure</th>
<th>DPPC + 0.005 CHL1</th>
<th>DPPC + 0.01 CHL1</th>
<th>DPPC + 0.05 CHL1</th>
<th>DPPC + 0.1 CHL1</th>
<th>DPPC + 0.25 CHL1</th>
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<tr>
<td>$\Pi_{\text{coll}}$ (mN m$^{-1}$)</td>
<td>56.4</td>
<td>52.8</td>
<td>53.1</td>
<td>55.9</td>
<td>58.5</td>
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<tr>
<td>$A_{\text{coll}}$ (Å$^2$)</td>
<td>42</td>
<td>43</td>
<td>43</td>
<td>44</td>
<td>46</td>
</tr>
<tr>
<td>$C_1$ (mN m$^{-1}$)</td>
<td>329.8</td>
<td>297.4</td>
<td>286.7</td>
<td>216.5</td>
<td>287.1</td>
</tr>
<tr>
<td>$\Delta V_{\text{coll}}$ (V)</td>
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<td>0.61</td>
<td>0.59</td>
<td>0.47</td>
<td>0.41</td>
</tr>
<tr>
<td>DPPC + 0.005 CHL2</td>
<td>48.2</td>
<td>50.1</td>
<td>55.8</td>
<td>59.7</td>
<td>62.2</td>
</tr>
<tr>
<td>$A_{\text{coll}}$ (Å$^2$)</td>
<td>43</td>
<td>42</td>
<td>43</td>
<td>42</td>
<td>46</td>
</tr>
<tr>
<td>$C_1$ (mN m$^{-1}$)</td>
<td>289.2</td>
<td>256.7</td>
<td>245.7</td>
<td>235.2</td>
<td>222.8</td>
</tr>
<tr>
<td>$\Delta V_{\text{coll}}$ (V)</td>
<td>0.56</td>
<td>0.55</td>
<td>0.48</td>
<td>0.41</td>
<td>0.39</td>
</tr>
<tr>
<td>DPPC + 0.005 CHL3</td>
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<td>59.3</td>
<td>59.4</td>
<td>60.2</td>
<td>62.2</td>
</tr>
<tr>
<td>$A_{\text{coll}}$ (Å$^2$)</td>
<td>43</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>46</td>
</tr>
<tr>
<td>$C_1$ (mN m$^{-1}$)</td>
<td>289.7</td>
<td>256.0</td>
<td>206.4</td>
<td>302.4</td>
<td>320.4</td>
</tr>
<tr>
<td>$\Delta V_{\text{coll}}$ (V)</td>
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<td>0.56</td>
<td>0.55</td>
<td>0.51</td>
<td>0.41</td>
</tr>
</tbody>
</table>
more insight in the interactions of the chlorins with lipid membranes, mixed monomolecular films were prepared. Mixtures with 0.005, 0.01, 0.05, 0.1 and 0.25 photosensitizer mole fraction in DPPC were spread from chloroform/DMSO (4:1) on a pure water subphase. The $\Pi-A$ and $\Delta V-A$ isotherms obtained upon compression of the films formed are shown in Fig. 4a–c; the characteristic parameters of the isotherms are given in Table 1.

The analysis of the film properties is presented as well as a shift of the molecular area of the mixed films relative to the pure DPPC film, as a function of surface pressure (Fig. 4d–f). The impact of the photosensitizer on the film properties can be observed for all concentrations studied. Indeed, even in the case of 0.005 and 0.01 mole fractions, a shift of the $\Pi-A$ isotherms to higher molecular areas is observed with all three derivatives (Fig. 4d–f). This shift is most important in the liquid expanded–liquid condensed (LE–LC) phase transition region. The films containing the photosensitizers are more compressible (less rigid) compared to the pure DPPC film, as indicated by the values of the compressibility modulus (Table 1) (Davies and Rideal, 1963; Korchowiec et al., 2006). However, much more important effects are observed for the concentrations of 0.05 mole fraction and higher. Indeed, for the higher concentrations of the photosensitizers in the film a significant shift of the $\Pi-A$ isotherms to higher molecular areas, together with an increase of the film compressibility is observed (Table 1). On the other hand, the decrease of the surface potential values (Table 1) indicates a less vertical orientation of the molecules forming the film relative

![Brewster angle micrographs of pure DPPC and mixed DPPC/photosensitizer monolayers.](image)

Fig. 5. Brewster angle micrographs of pure DPPC and mixed DPPC/photosensitizer monolayers. The mixed films contained 0.01 mole fraction of CHL1, CHL2 or CHL3. Pure DPPC (a and b), DPPC/CHL1 (c and d), DPPC/CHL2 (e and f) and DPPC/CHL3 (g and h). The micrographs were taken at $\Pi = 5.0 \text{ mN m}^{-1}$ (a, c, e and g) and $10.0 \text{ mN m}^{-1}$ (b, d, f and h). Scale: the width of the snapshots corresponds to 400 µm.
Table 2
Characteristic vibrational wavenumbers of phospholipid and photosensitizer bonds in the pure and mixed films.

<table>
<thead>
<tr>
<th>Film composition</th>
<th>( \nu(\text{CH}_2)(\text{cm}^{-1}) )</th>
<th>( \nu(\text{CH}_3)(\text{cm}^{-1}) )</th>
<th>( \nu(\text{C}=\text{O})(\text{cm}^{-1}) )</th>
<th>( \nu(\text{C}=\text{C})(\text{cm}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 DPPC</td>
<td>2912</td>
<td>2855</td>
<td>1731</td>
<td>–</td>
</tr>
<tr>
<td>DPPC + CHL1</td>
<td>2917</td>
<td>2852</td>
<td>1737</td>
<td>1539</td>
</tr>
<tr>
<td>DPPC + CHL2</td>
<td>2920</td>
<td>2832</td>
<td>1724</td>
<td>1534</td>
</tr>
<tr>
<td>DPPC + CHL3</td>
<td>2916</td>
<td>2856</td>
<td>1736</td>
<td>1540</td>
</tr>
<tr>
<td>5.0 DPPC</td>
<td>2918</td>
<td>2855</td>
<td>1730</td>
<td>–</td>
</tr>
<tr>
<td>DPPC + CHL1</td>
<td>2927</td>
<td>2849</td>
<td>1734</td>
<td>1538</td>
</tr>
<tr>
<td>DPPC + CHL2</td>
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<td>2841</td>
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</tr>
<tr>
<td>DPPC + CHL3</td>
<td>2915</td>
<td>2852</td>
<td>1733</td>
<td>1536</td>
</tr>
<tr>
<td>10.0 DPPC</td>
<td>2920</td>
<td>2853</td>
<td>1728</td>
<td>–</td>
</tr>
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<td>2921</td>
<td>2852</td>
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<td>2850</td>
<td>1728</td>
<td>–</td>
</tr>
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<td>1542</td>
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<tr>
<td>DPPC + CHL3</td>
<td>2917</td>
<td>2851</td>
<td>1728</td>
<td>1537</td>
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</tbody>
</table>

\( ^a \) The mixed films contained 0.01 mole fraction of the photosensitizers.

In accordance with the \( I – A \) isotherms results, the BAM images show that the LE–LC phase transition is shifted to higher surface pressures in the presence of the photosensitizers compared to the pure DPPC film. Indeed, the bright condensed phase domains are observed at 5.0 mN m\(^{-1} \) in the pure DPPC film (Fig. 5a), while only rare bright spots can be observed in the mixed films at this surface pressure (Fig. 5c, e and g). While the condensed phase domains coalesce at 10.0 mN m\(^{-1} \) in the case of the pure DPPC (Fig. 5b), in the mixed films the domains are, on the contrary, clearly seen (Fig. 5d, f and h). The morphology of the condensed phase domains (Fig. 5a) is modified in the mixed films compared to pure DPPC. In general, the condensed phase domains observed in the mixed films are smaller compared to pure DPPC; the domains formed in the DPPC/CHL3 film are significantly smaller compared to DPPC/CHL1.
and DPPC/CHL2. On the other hand, the characteristic shape of the DPPC domains is preserved only in the case of the DPPC/CHL2 mixture. Taken all together, the effects observed with BAM may indicate a better miscibility of CHL3 with DPPC compared to CHL1 and CHL2. The condensed domains observed in the DPPC/CHL3 film would be composed of DPPC and CHL3, while in the case of the DPPC/CHL2 film the photosensitizers would stay in the less condensed phase. The miscibility of CHL1 with DPPC would be situated between those of CHL2 and CHL3.

3.2. PM-IRRAS

In order to better understand the interactions between the photosensitizers and the phospholipid membrane, PM-IRRAS (Blaudez et al., 1993, 1994, 1996) experiments were performed. The values of the characteristic vibrations of the spectra obtained with the pure DPPC and mixed DPPC/photosensitizer films at different surface pressures are given in Table 2; in Fig. 6 are shown spectra obtained at 50.0 mN m\(^{-1}\).

The lipid perturbations induced by the incorporation of the photosensitizers into the monolayers can be investigated through analysis of the lipid symmetric and antisymmetric methylene group stretching vibrations \(\nu(s)(CH_2)\) and \(\nu(a)(CH_2)\) (around 2920 and 2850 cm\(^{-1}\), respectively) and the \(\nu(C=O)\) stretching band (around 1730 cm\(^{-1}\)). The \(\chi_2\) stretching mode region between 2850 and 3000 cm\(^{-1}\) in the infrared spectra of the phospholipid is particularly useful because the frequency and width of the methylene bands are sensitive to the conformation of phospholipid acyl chains (MacPhail et al., 1984; Snyder et al., 1982). Indeed, values lower than 2920 and 2850 cm\(^{-1}\) indicate higher chain ordering in the film, while higher values suggest chain disordering (Bi et al., 2002). On the other hand, the stretching \(\nu(C=O)\) band is sensitive to hydrogen bonding and shifts to higher wavenumbers upon dehydration of the carbonyl groups (Lewis et al., 1994).

The spectra of the pure DPPC monolayers (Fig. 6a) exhibit the characteristic vibrations of phospholipids (Blaudez et al., 1993, 1994, 1996; Corvis et al., 2006b; Du and Wang, 2007; Gromelski and Brezesinski, 2004; Zawisz et al., 2007). Indeed, the symmetric and antisymmetric methylene stretching vibration, as well as the carbonyl stretching vibration are clearly visible in the spectra at around 2850, 2920 and 1730 cm\(^{-1}\), respectively (Table 2). While no significant influence of the photosensitizers on the organization of the hydrocarbon chains can be observed, the shift of the stretching \(\nu(C=O)\) band to higher wavenumbers indicates that a higher number of carbonyl groups is hydrogen bonded in the mixed films compared to pure DPPC. This effect is not surprising, as the hydrogen bond network would be less ordered in the more fluid films (Du and Liang, 2000; Leite et al., 1998; Miranda et al., 1998) containing the photosensitizers. Interestingly, in the case of the most condensed DPPC/CHL3 film, the \(\nu(C=O)\) band appears at the same wavelength as in pure DPPC. This result indicates that hydrogen bonds are established between the polar folic acid moiety of CHL3 and the phospholipid polar heads in the organized film. It can be observed that a conspicuous band is present at around 1540 cm\(^{-1}\) in the mixed films. This band, which could be attributed to the aromatic \(\nu(C=C)\) vibrations of the chlorin derivatives, is a direct indication of the presence of the photosensitizers in the film (Berezin and Nechaev, 2003; Mason, 1958; Wojtkowiak and Chabanei, 1977).

4. Conclusion

The results obtained show that all three chlorin-based photosensitizers interact with the DPPC monolayer and modify its physicochemical properties. Indeed, the monolayer becomes more fluid in the presence of the chlorin derivatives. Importantly, all three photosensitizers are present in the condensed monolayers at biologically relevant surface pressures of around 30 mN m\(^{-1}\). As indicated by the compression isotherms and BAM experiments, the most polar CHL3 shows a better miscibility with DPPC compared to CHL1 and CHL2. This effect may be due to the polar interactions established between DPPC and CHL3. Indeed, while anchoring of CHL1 and CHL2 in the film is due to the chorin moiety, the PM-IRRAS results indicate that in the case of the folate-conjugated CHL3, hydrogen bonding to the phospholipid polar heads occurs. In conclusion, the increased polarity of the folate-conjugated photosensitizer is countered by hydrogen bonding and does not increase its partitioning from the membrane into the aqueous medium. Consequently, the lower accumulation of CHL3 compared to the more hydrophobic chlorin-based photosensitizers observed in vivo after an intravenous injection cannot be attributed to its higher water solubility; it may be due rather to more specific, biological processes. On the other hand, it can be proposed that those photosensitizers showing a lower miscibility with phospholipids, phase separate in the membranes enhancing further accumulation in the photosensitizers–rich phase. In the case of the lipid-miscible photosensitizers, the accumulation would not be possible above the saturation concentration.

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References


