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pH-Sensitive liposomes as a carrier for oligonucleotides: a physico-chemical study of the interaction between DOPE and a 15-mer oligonucleotide in excess water

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Abstract

The cytoplasmic delivery of drugs encapsulated into pH-sensitive liposomes is under the control of a lamellar-to-hexagonal transition. In a previous study, under anhydrous conditions, oligonucleotides (ODN) encapsulated in pH-sensitive liposomes composed of dioleoylphosphatidylethanolamine (DOPE)/oleic acid (OA)/cholesterol (CHOL) were shown to modify the phase behaviour of DOPE. In the present study, the lipid/ODN interactions were evaluated in fully hydrated samples by surface tension measurements, differential scanning calorimetry, X-ray diffraction and turbidimetry. Concerning the lipids, it was shown that OA provoked a disorganisation of DOPE lamellar phases and led to the complete disappearance of hexagonal transition along with heating. The addition of CHOL further decreased the lipid packing in the bilayers. Concerning ODN, these molecules provoked an increase

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in the surface pressure of a DOPE/OA/CHOL monolayer, indicating the existence of molecular interactions with the lipids. At a supramolecular level, ODN induced a more ordered organisation of DOPE molecules in the lamellar and hexagonal phases, and completely abolished the disorganisational effect of OA and CHOL.

Keywords: pH-Sensitive liposomes; Antisense oligonucleotide; Dioleoylphosphatidylethanolamine; Differential scanning calorimetry; X-Ray diffraction

1. Introduction

pH-Sensitive liposomes have been proposed to improve the intracellular delivery of nucleic acids, especially ODN [1–4]. They actually represent an interesting alternative to cationic lipids, especially because their cytotoxicity is less pronounced.

pH-Sensitive liposomes have been designed to exploit the endosomal acidification process, which may lead to the destabilisation of the liposomes with the subsequent release of their contents into the cell cytoplasm. Most pH-sensitive liposomes take advantage of the fusogenic properties of phosphatidylethanolamines compounds (PE) which preferentially adopt hexagonal rather than lamellar phases under physiological conditions [5]. At neutral pH, the use of titratable amphiphiles, such as oleic acid (OA) [6] or cholesterylhemisuccinate (CHEMS) [7] is required to allow the stabilisation of PE into lamellar structures by electrostatic repulsions. However, once in acidic environment, the protonation of OA or CHEMS suppresses charge repulsion in the bilayer and promotes liposome destabilisation and/or fusion with the endosome. Hence, the occurrence of a lamellar-to-hexagonal transition phase may play an important role in the subcellular delivery of drugs by such pH-sensitive liposomes.

Recently, biophysical investigations have demonstrated the importance of the hexagonal phase of DOPE in inducing the fusion of cationic liposome–DNA complexes with model anionic lipid membranes [8]. Moreover, electron microscopy observations have clearly shown the destabilisation of endosomal membranes by DNA/liposomes containing DOPE occurring, but not by its structural analogue, dioleoylphosphatidylcholine (DOPC) [9].

In a previous study, we investigated the phase behaviour of DOPE in the presence of a 15-mer antiviral ODN under low hydration conditions [10]. It was found that ODN interacted with DOPE, bringing the molecules closer and, thus, modifying the thermal behaviour of the phospholipid. In the presence of OA and CHOL, the interactions of DOPE with ODN even led to the stabilisation of lamellar structures. Although of interest, the study was, however, far from reality, because it was performed under low hydration conditions and in the absence of vesicles. Thus, it urged us to investigate, under conditions of excess water, the possible modification that ODN could bring to the self-assembly of DOPE/OA/CHOL liposomes, also because such a formulation was shown to be very efficient against infection by the Friend retrovirus [1].

In the present study, the interactions between DOPE and ODN were investigated both at the molecular and supramolecular levels. Surface tension measurements of ODN and pH-sensitive liposomes components containing monolayers were first carried out. Then, the phase behaviour of DOPE with ODN was examined under different conditions by time-resolved synchrotron X-ray diffraction as a function of temperature (XRDT) and differential scanning calorimetry (DSC), using a newly developed instrument allowing both determinations from the same sample. Temperature-controlled turbidity measurements were also required to give additional information.

2. Materials and methods

2.1. Materials

1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanol-

amine (DOPE), oleic acid (OA) and cholesterol (CHOL) as well as ethylenediamine tetraacetic acid (EDTA) and tris-(hydroxymethyl)aminomethane (TRIS) were obtained from the Sigma Chemical Company (St. Louis, MO, USA). Lipids were used without further purification. The 15-mer 5′-phosphorylated oligonucleotide (ODN), 5′TGAACACGCCATGTC3′ (complementary sequence to the region of the initiation codon AUG of Friend virus *env* gene) was purchased from Eurogentec S.A. (France). The water used in surface tension experiments was obtained by osmosis from a Milli-RO6 Plus Millipore apparatus, and then doubly distilled from permanganate solution in an all-Pyrex apparatus. Its pH was 5.5, and its surface tension was typically above 72 mN/m.

2.2. Sample preparation

DOPE based liposomes were prepared by mixing chloroform solutions of DOPE, OA and CHOL at the required molar fractions (1:0:0; 1:1.3:0; and 1:1.3:0.4) and the resulting mixtures were then evaporated to dryness under a vacuum. After initial vortexing, the lipidic film was hydrated with 50 mM TRIS/5 mM EDTA (pH 8.5). The vesicles were calibrated by extrusion through 0.4 and 0.2-μm polycarbonate filters. In the case of pure DOPE liposomes, hydration was carried out at 4°C below the lamellar to hexagonal phase transition temperature, and was not followed by calibration. In the case of ODN-containing liposomes, an aqueous solution of the ODN 15-mer was added to the extruded liposomes up to 130 μM, then the mixture was subjected to three cycles of freeze (liquid N_2) and thaw (34°C). The liposomes were separated from non-entrapped ODN by ultracentrifugation ($150\,000 \times g$, 40 min, 4°C), and washed twice with fresh TRIS buffer. The resulting pellet was found to have a water weight fraction of approximately 0.9 and was directly used for X-ray diffraction analysis coupled to differential scanning calorimetry (DSC). For all other experiments, the pellets were resuspended in TRIS buffer. The ODN entrapment ratio was evaluated using the competitive enzyme hybridisation assay described previously by Deverre et al. [11] and was found to be 15% of the initial concentration.

2.3. Surface tension measurements

Surface tension measurements were performed using the Wilhelmy plate method, in which a thin platinum plate was attached to a system consisting of a force transducer, an amplifier, and a chart recorder [12]. The measurement cell (delimiting a 10.4-cm^2 surface area) was mounted on a magnetic stirrer and a Teflon-coated stir bar was slowly rotated for 15 s after injection of the ODN solution into the water or the 50 mM TRIS/5 mM EDTA buffer sub-phase (adjusted to pH 8.5) to obtain the desired ODN concentration (19 nmol/ml). When needed, HCl was added to the buffer substrate to decrease its pH from 8.5 to 7.4 or 5.5. The surface tension was continuously measured as a function of time until equilibrium was reached. The precision of the force transducer of the apparatus was ± 0.1 mN/m. The surface pressure was deduced from the $\pi = \gamma_0 - \gamma$ relationship, in which γ_0 is the surface tension of the water or buffer solution and γ is the surface tension observed in the presence of a spread DOPE/OA/CHOL (1:1.3:0.4 ratio, 0.17 mM) or adsorbed ODN monolayer. The change in surface pressure ($\Delta\pi$) is the difference between the surface pressure recorded when the ODN sample was injected beneath the lipid monolayer, and the surface pressure due to the lipid monolayer spread over a clean water or buffer sub-phase. The lipids forming the monolayers were previously dissolved in chloroform/methanol 9:1, so that less than 20 μl were allowed to reach a surface pressure equal to 20 mN/m.

2.4. Synchroton small angle X-ray diffraction scattering (XRDT) and DSC

Samples, prepared as described above, were added to thin glass capillaries (GLAS, Muller, Berlin, Germany) (0.01-mm wall thickness, diameter $1.3 < \phi < 1.5$ mm), by means of soft centrifugation at approximately $1000 \times g$. The capillaries were filled with argon gas, sealed with a drop of

melted paraffin, and stored at 4°C until analysis. A laboratory-made calorimeter allowing accurate temperature monitoring through simple computer commands with a 0.01°C resolution in the −30°C to +130°C temperature range was used [13]. DSC recordings were performed on a microcalorimeter of the heat-flux type at a heating rate of 1.0°C/min. Highly purified lauric acid was used for temperature and enthalpy calibration [13,14]. Scanning, from either −30–20°C or 0–50°C, was performed. The data acquisition time interval was 3 s for DSC and 60 s for XRDT. The transition temperatures were considered by the position of the peak maximum. The recording of X-ray diffraction data as a function of temperature was carried out through one-dimensional position-sensitive proportional detectors (512 or 1024 channels). A computer program, originally written in Visual Basic and compiled, was running under Microsoft Windows and monitored DSC and X-ray data simultaneously in order to avoid any time or temperature lag between XRDT and DSC measurements. Diffraction patterns were taken every minute at a 1°C/min heating rate. The D22 and D24 benches of the DCI synchrotron of LURE were used successively (λ = 1.377 Å and λ = 1.489 Å, respectively). The experiments were repeated at least twice.

2.5. Turbidity measurements

Turbidity was monitored as a function of temperature by continuous recording of optical density (OD) at 350 nm using a Perkin-Elmer Lambda 2 double beam spectrophotometer monitored by an IBM PC. A volume of 320 μl of liposomal suspensions containing varying molar ratios of DOPE, OA and CHOL (1:0:0; 1:0.05:0; 1:1.3:0; and 1:1.3:0.4) was added to 1680 μl of 50 mM TRIS/5 mM EDTA (pH 8.5), and introduced in a 1-cm optical cuvette. The suspensions were maintained under constant agitation using a rotation speed-controlled paddle stirrer that did not interfere with the light path. The samples were heated by means of a temperature-controlled bath at a rate of 1°C/min [15].

3. Results

3.1. Interfacial behaviour of the ODN in the absence and in the presence of a spread DOPE / OA / CHOL monolayer

Data of the interfacial behaviour of ODN after injection into a water (pH 5.5) or a TRIS/EDTA buffer (pH 8.5) sub-phase are presented in Fig. 1a. The effect of the amphiphilic buffer ions on the surface tension has been taken into account and the data in Fig. 1a were corrected accordingly. In the presence of the ODN in the aqueous subphase, an increase in the surface pressure with time was observed, corresponding to its spontaneous adsorption at the air/water or the air/buffer interface. The lowering of the pH of the buffer sub-phase from pH 8.5 to 7.4 and then to 5.5 did not induce any marked change in the surface pressure. It seems, therefore, that the pH did not affect ODN adsorption. Conversely, oligonucleotide adsorption was enhanced in the presence of buffer ions in the sub-phase compared to that in water.

When a DOPE/OA/CHOL monolayer was spread onto a buffer sub-phase (pH 8.5) at an initial surface pressure of 20 mN/m, the buffer ions did not affect the surface pressure which remained stable for more than 25 h after lipid spreading. The ODN injection into the sub-phase produced a significant change in the surface pressure ($\Delta\pi$) which increased with time (Fig. 1b). If the ODN adsorption to the lipid monolayer was slower than that at the monolayer free interface, when equilibrium was reached, the increase in surface pressure (Fig. 1b) corresponded exactly to the maximum surface pressure in Fig. 1a. The $\Delta\pi$-values did not vary significantly upon HCl addition into the sub-phase. Indeed, the observed slight increase at pH 7.4 and pH 5.5 most probably corresponded to an injection effect. When the sub-phase was pure water, the $\Delta\pi$-values increased for 5 h, and then decreased to zero. This discrepancy between the observed surface pressure changes at the lipid/water and lipid/buffer interfaces evidenced the influence of buffer ions on the adsorption of the oligonucleotide to a lipid monolayer.

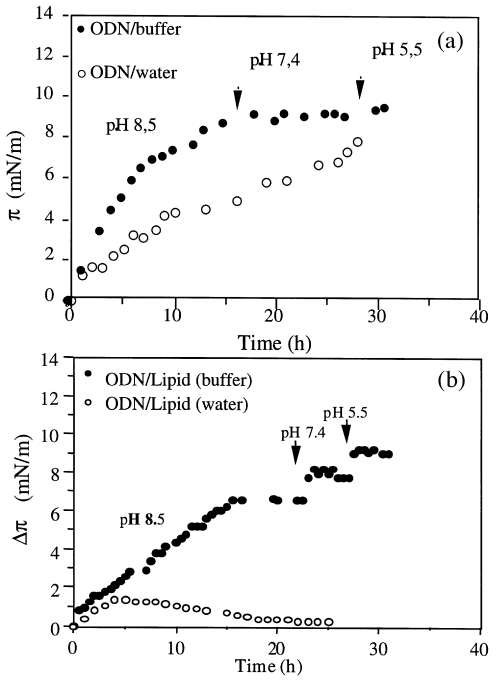

Fig. 1. π-Time (a) and Δπ-time (b) relationships for oligonucleotide adsorption at the monolayer free interface and in the presence of a spread lipid monolayer. Arrows indicate HCl injections into the buffer sub-phase.

3.2. XRDT and DSC

Fig. 2 gives an example of the X-ray diffraction patterns obtained and the simultaneous calorimetric traces recorded in our experimental conditions. In the case of pure DOPE (Fig. 2a), a first order reflection could be observed, with larger spacings below −9.8°C (approx. 56–59 Å) than above −8.8°C (approx. 52.5–54.3 Å). These reflections could be attributed, respectively, to L_β and L_α lamellar phases. Also noted, during the phase transition itself, was the appearance of a second broad diffraction peak at approximately 75 Å, which could not be assigned. Above 7.2°C, the diffraction patterns revealed Bragg spacings at ratios of 1:1, 1:√3, and 1:2, indicating the transition to a hexagonal phase. The addition of ODN to DOPE samples (Fig. 2b) did not significantly modify the temperature at which both lamellar and hexagonal transitions occurred, but a more accurate analysis of the traces revealed a slight decrease of the peak width (Fig. 3b) for all of the three phases (L_β, L_α, H_{II}), which was most visible in the case of the hexagonal phase, and an increase of the lattice spacing in the hexagonal phase (Fig. 3a). The presence of ODN also induced the occurrence of a sharp and reproducible

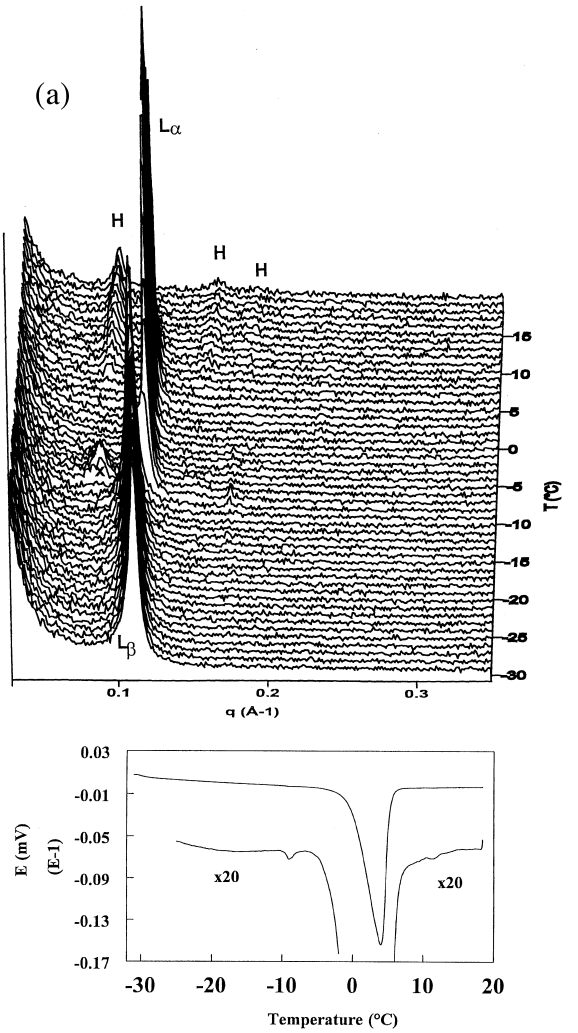

Fig. 2. X-Ray diffraction patterns and calorimetric traces (insert) simultaneously recorded for DOPE (a) and DOPE/ODN (b) samples, as a function of temperature (1.0°C/min heating rate). ODN was added at a DOPE/ODN ratio of 1:0.002. Lower traces of calorimetric recordings represent initial upper traces magnified in the *y* direction by a factor of 20.

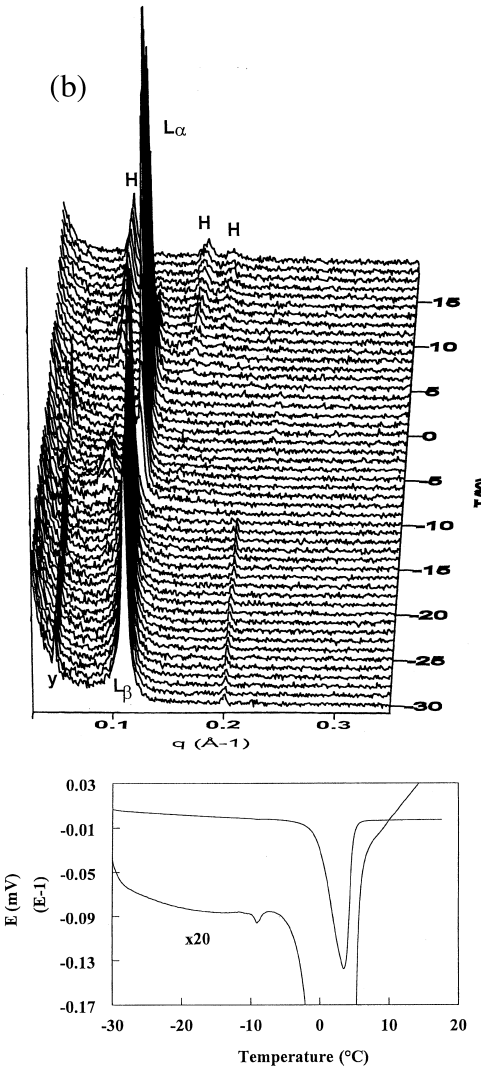

Fig. 2. (*Continued*).

diffraction peak at 126 Å. The exact attribution of this peak has not yet been clarified. In addition to X-ray diffraction, Fig. 2 also gives information about the thermal events occurring in DOPE samples. Besides the main peak at approximately 0°C, attributed to ice melting, it was particularly easy to distinguish an endotherm centred at −8.8°C in both DOPE (Fig. 2a, insert) and DOPE/ODN (Fig. 2b, insert), well correlated to the L_β/L_α-transition simultaneously detected by XRDT. In the case of pure DOPE, an approximately twice less energetic endotherm was also detected at approximately 10.9°C corresponding to the $L_\alpha - H_{II}$ transition.

Fig. 4 summarises XRDT results obtained for DOPE/OA and DOPE/OA/ODN samples. As previously, a first order reflection could be seen before 0°C, with a transition occurring at −23.8°C, corresponding to the L_β/L_α-transition. No diffraction could be detected above 0°C. Concerning the X-ray spacings (Fig. 4a), lower values were recorded compared to DOPE samples (approximately 53.4–54.3 Å for L_β and 47.4 for L_α), but with a much higher peak width (Fig. 4b). Indeed, whereas the peak width for DOPE samples was approximately 3–4 Å for L_β and 1.5–2 Å for L_α, the values reached 9 Å for L_β and 3–5 Å for L_α in the case of the DOPE/OA sample. It could also be noted that the X-ray spacings of the L_α phases increased with temperature in the case of DOPE/OA samples (Fig. 4a) (from 47.4 to 56.7 Å), whereas it was constant in the case of DOPE.

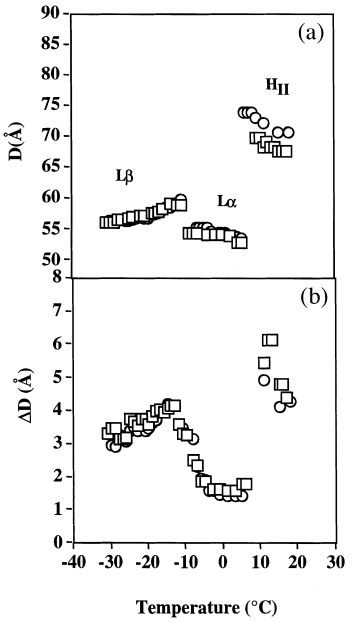

Fig. 3. Evolution of X-ray spacings (a) and diffraction peak half widths (b) for DOPE (squares) and DOPE/ODN (circles) samples as a function of temperature. ODN was added at a DOPE/ODN ratio of 1:0.002.

Again, the addition of ODN to DOPE/OA samples did not modify the transition temperature, but led to a slight decrease of the lattice spacings of the L_α phase (Fig. 4a) and to a clear decrease of the peak widths of both L_β and L_α phases (Fig. 4b).

Finally, in the case of DOPE/OA/CHOL samples (Fig. 5), it was much more difficult to precisely localise the L_β/L_α transition (approx. −22°C). The effect of ODN addition was, however, particularly clear in the L_α state, with a deep decrease of the diffraction peak width from 4 to 9 Å in the absence of ODN to 3–2 Å in its presence (Fig. 5b). Moreover, the temperature-dependence of the line width was found to be the opposite: increasing without ODN and decreasing in their presence (Fig. 5b).

3.3. Turbidity measurements

Because XRDT/DSC failed to give information about structural changes above 0°C in DOPE/OA samples, turbidity measurements were carried out to investigate the possible effects of OA on the occurrence of a L_α/H_{II} transition.

Fig. 6 reports the variation of the optical density at 350 nm as a function of temperature. In the case of DOPE, a first inflection point could be detected at approximately 13°C, followed by a deeper break of the curve at 18°C (Fig. 6a). When 5 mol.% OA was added to DOPE (Fig. 6b), the optical density remained stable until a temperature of approximately 19°C was reached. However, it should be mentioned that the repeatability of the experiments was poor in the presence of OA, regarding the temperature at which OD started to increase. When the OA concentration was still increasing up to the molar ratio used for pH-sensitive liposomes (48 mol.%) (Fig. 6c), the optical density remained stable all along the temperature range (until 65°C). The presence of cholesterol did not significantly modify the comportment of the liposomes with regard to turbidimetry measurements up to 45°C.

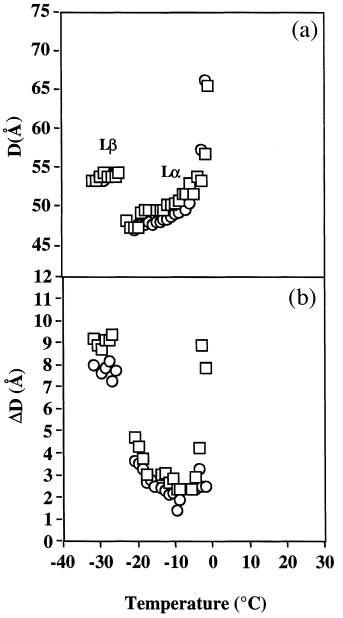

Fig. 4. Evolution of X-ray spacings (a) and diffraction peak half widths (b) for DOPE/OA (1:1.3) (squares) and DOPE/OA/ODN (circles) samples as a function of temperature. ODN was added at a DOPE/ODN ratio of 1:0.002.

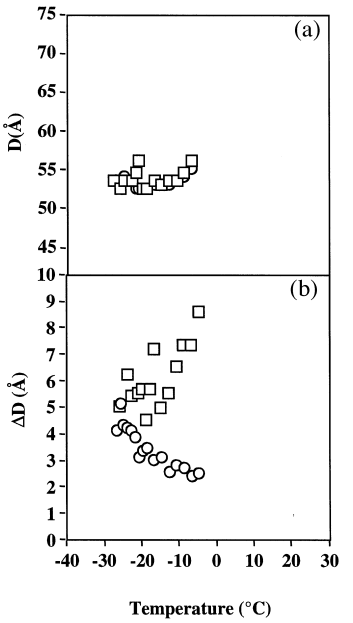

Fig. 5. Evolution of X-ray spacings (a) and diffraction peak half widths (b) for DOPE/OA/CHOL (1:1.3:0.4) (squares) and DOPE/OA/CHOL/ODN (circles) samples as a function of temperature. ODN was added at a DOPE/ODN ratio of 1:0.002.

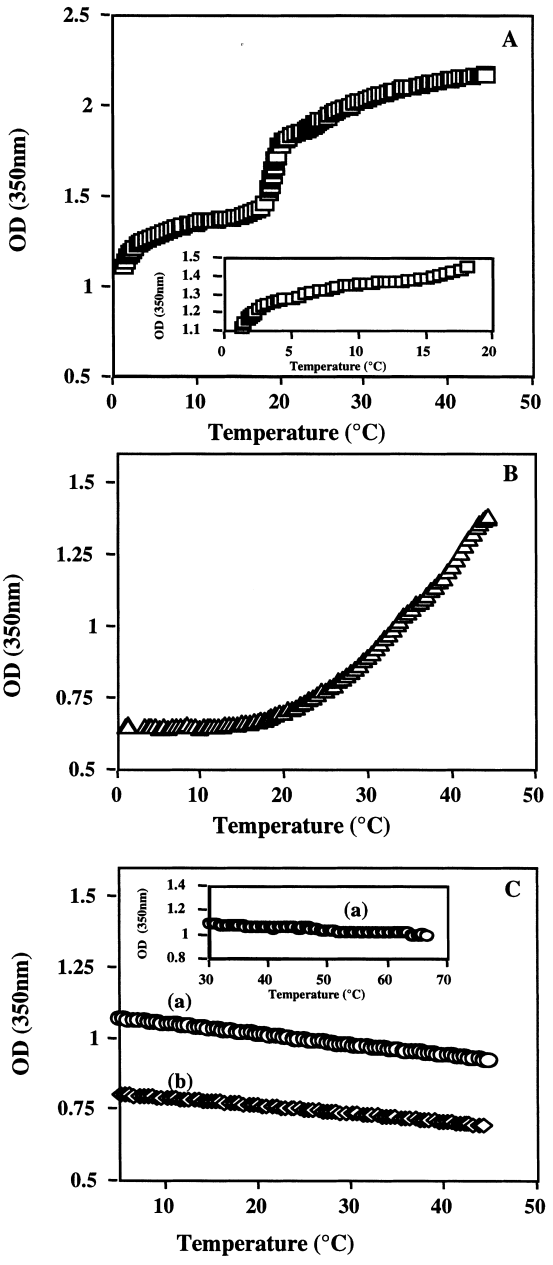

Fig. 6. The effect of the presence of OA on turbidity measurements at 350 nm of DOPE samples as a function of temperature (1°C/min heating rate): DOPE/OA (1:0) (a); DOPE/OA (1:0.05) (b); and DOPE/OA/CHOL (c); with ratios of (1:1.3:0) (a) and (1:1.3:0.4) (b). Insert in (a) shows the magnification of the optical density between 1.1 and 1.5. Insert in (c) shows the further evolution of the optical density between 30 and 70°C for sample (a).

4. Discussion

The results of the experiments described above have evidenced the effects resulting from a possible interaction between ODN and the lipids forming pH-sensitive liposomes.

4.1. Investigations at the molecular level

Surface tension measurements revealed the surface active character of the oligonucleotide. Whereas ODN adsorption was found to be dependent both on time and on the presence of buffer ions, it was not affected by the pH of the sub-phase. This was probably due to the low value of ODN pK_a (pK_a lower than 1).

In the presence of a DOPE/OA/CHOL monolayer, the adsorption of ODN was slower compared to that at the interface free of lipids. Thus, the lipid monolayer acted as a barrier, probably due to its condensed state at the initial surface pressure of 20 mN/m [16,17]. The $\Delta\pi$-time relationship at the lipid/buffer interface clearly differed from that at the lipid/water interface. Whereas ODN adsorption profiles were identical at both interfaces during the first 5 h, a progressive desorption of the oligonucleotide from the lipid/water interface then occurred, indicating an unfavourable interaction between ODN and the lipids. Conversely, at the lipid/buffer interface, the hindering effect of the lipid monolayer seemed to be counterbalanced by the effect of buffer ions, which favoured ODN adsorption and its durable insertion into the monolayer. The discrepancy between the results obtained at the lipid/water and the lipid/buffer interface may be explained by the difference in the degree of dissociation of hydrophilic lipid moieties in water and in buffer. In water, pH 5–6, the somewhat limited dissociation degree would bring about less repulsion between charged lipid headgroups, resulting in a weak interaction between ODN and lipid molecules [18]. On the contrary, the repulsion of charged OA headgroups at the lipid/buffer interface, at pH 8.5, despite the charge screening due to the presence of buffer salts, might favour oligonucleotide pene-

tration into the monolayer by enlarging the available free space between lipid molecules.

4.2. DOPE / ODN mixtures: supramolecular investigations

It is well known that phosphatidylethanolamines derivatives give rise to the formation of either lamellar or hexagonal structures [19]. XRDT allows us to precisely determine in the DOPE samples two phase transitions, from gel to liquid–crystalline lamellar and from lamellar to hexagonal phases. The transition temperatures (−8.8 and 7.2°C, respectively) and the structural dimensions measured were in good agreement with the literature data [5,16,20]. What we have observed is that the addition of ODN modified the structural dimensions of the hexagonal phase only, but had no effect on the transition temperatures, showing that ODN located in the water compartment of the liposomes, and likely near the headgroups, had no effect on chain packing and did not penetrate into the lipid lattices. Nevertheless, more regular packing of DOPE in the presence of ODN could be noticed when looking at the reduction of the width of the diffraction peaks. This effect could be the result of DOPE/ODN interactions, particularly electrostatic interactions. As previously proposed by de Oliveira et al. [10], linear ODN molecules may lie along the lipid layers of the liposomes and then interactions between several neighbouring DOPE amine groups and the ODN phosphate groups would take place, leading to a more ordered structure of DOPE. At this stage, it should be mentioned that DSC ran simultaneously with XRDT, despite the low energies involved, gave complementary and well-correlated information.

4.3. Effect of OA on the lipid packing

OA was added to DOPE liposomes in order to both give a pH-sensitivity character and to stabilise the lamellar structure at 37°C and neutral pH. As OA molecules inserted in-between DOPE hindered the headgroups packing, the L_β/L_α-transition was consequently lowered and the lattice was partly disorganised. This last effect could be explained by the higher hydration of DOPE/OA bilayers compared to DOPE ones because of the additional presence of negatively charged carboxyl groups due to OA. This was especially sensitive above 0°C. Upon ice melting, water inserts between DOPE/OA bilayers resulted in a large interbilayer distance increase by almost 10 Å (Fig. 4b). A similar behaviour has already been described with bilayers including dipalmitoylphosphatidylglycerol (DPPG), a negatively charged phospholipid [21]. Above the melting temperature of water, the bilayer swelling must even increase because of a suddenly increased hydration, thus explaining the disappearance of any diffraction in XRDT above 0°C.

Since the lamellar–to-hexagonal phase transition is supposed to play a crucial role in drug delivery using pH-sensitive liposomes, it was interesting to determine the effect of OA on L_α/H_{II} transition temperature. Because XRDT above 0°C could no longer be useful, turbidity measurements were carried out. It was then possible to follow the stability of the vesicles over a large range of temperature. Indeed, if the vesicle size suddenly increased because of membrane destabilisation and fusion, the turbidity should increase. The curve obtained with DOPE clearly showed that such events occurred at approximately 13 and 18°C. The addition of a low amount of OA smoothened these variations and tended to shift them towards higher temperatures. However, the most interesting result was that with the pH-sensitive liposome composition, the vesicles appeared to be perfectly stable up to 45°C and even higher (65°C). It could then be assessed that OA completely abolished the L_α/H_{II} transition under heating since no aggregation of the vesicles could be evidenced by turbidimetry. Such an observation might have implications for cytoplasmic delivery. However, the present experiments were carried out along with temperature variations at a given pH (8.5). Consequently, no assumptions can reasonably be made about the final comportment of these liposomes in the acidic subcellular medium. Nevertheless, this point deserves to be clarified and further investigations will deal with the comportment of the

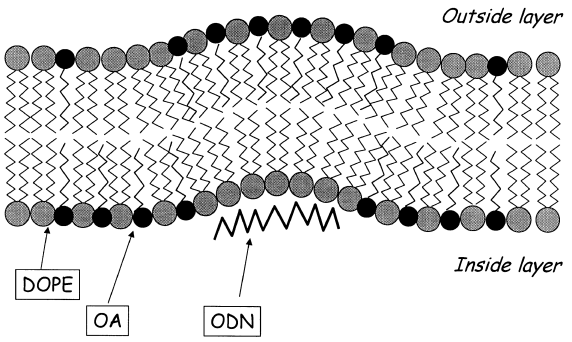

Fig. 7. Schematic representation of ODN interacting with DOPE/OA bilayer. The presence of ODN interacting with DOPE headgroups probably leads OA to separate in neighbouring areas because of charge repulsion between the ODN and the anionic OA molecules.

DOPE/OA/CHOL liposomes under continuous acidification.

4.4. ODN / DOPE mixtures in the presence of OA

The addition of ODN in DOPE/OA (1:1.3 molar ratio) liposomes reduced the hydration of the bilayers as reflected by a higher packing of both L_β and L_α lamellar phases. It was previously shown in quasi-anhydrous samples [10] that ODN molecules counteracted the effect of OA, leading to a possible segregation of DOPE and OA in two different phases. In diluted samples, ODN retained this ability to compete with OA, resulting to a higher structural organisation of the bilayers. However, the presence of water in very large excess probably explains the reason why this competition remained limited and only led to an increased order of the lamellar phases. Fig. 7 gives a schematic illustration of the possible arrangement of ODN molecules besides the inner layer of the vesicle membrane, showing the redistribution of OA in neighbouring areas.

4.5. Effect of CHOL

As already described, the addition of cholesterol in DOPE/OA samples smoothened the L_β/L_α phase transitions, as regards to lattice spacings. This may result in the reduction of molecular motion by cholesterol above the DOPE phase transition, as has been reported by New [22]. In addition, the presence of cholesterol induced highly irregular packing of the lipids in both lamellar phases, as demonstrated by the width of diffraction peaks. The addition of ODN markedly restored the organisation of the lipids, especially in the L_α phase. Here again, the interactions between the ODN and DOPE headgroups at low temperatures were strong enough to counteract the effect of cholesterol. Such an observation might be explained by the likely high ordering effect due to the simultaneous 15 functional groups of each ODN molecule, able to interact with 15 different polar DOPE headgroups, thus forced to range at equal distances from each other.

These studies, performed both at the molecular and supramolecular level, have clearly highlighted the existence of interactions between ODN molecules and DOPE in fully hydrated conditions, which are sufficiently high to induce some significant structural changes. ODN molecules were even shown to be able to compete with OA and CHOL molecules at low temperatures. Owing to these results, further investigations should concentrate on the impact of ODN/DOPE interactions on the pH sensitivity of the liposomes, by following the structural changes as a function of the pH.

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