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Fe^{2+} promoted peroxidation of 1,2-diacyl-*sn*-glycero-3-phosphocholine liposomes in the presence of calf thymus DNA

Paolo Bruni,*[a] Michela Pisani,[a] Marco Iacussi[a] and Oriano Francescangeli[b]

[a] *Dipartimento di Scienze dei Materiali e della Terra, Università Politecnica delle Marche, Via Brecce Bianche, 60126 Ancona, Italy. E-mail: p.bruni@univpm.it; Fax: +39 071 2204714*

[b] *Dipartimento di Fisica e Ingegneria dei Materiali, Università Politecnica delle Marche, Via Brecce Bianche, 60126 Ancona, Italy*

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The peroxidation reaction of some liposomes, namely egg yolk phosphatidylcholine (PC), dioleoyl- (DOPC) and dilinoleoyl- (DLPC) phosphocholines, promoted by ferrous ions (Fenton reaction) has been studied at the physiological pH value, in the absence and in the presence of calf thymus DNA. A catalytic effect of DNA, where the lag time reduces or is completely annihilated, together with an increase in both the yields and the rates of the reactions, has been observed. This effect of DNA has been attributed to the ability of the three components, liposomes, DNA and Fe^{2+}, to form a stable ternary complex, which produces a reduction of the undulatory fluctuations of the hydrocarbon tails of liposomes and strengthens the packing between the acyl chains in the lipid bilayers, with the consequence of enhancing the liposome crystallinity.

Introduction

We have recently investigated the structure and morphology of some ternary complexes obtained by 1,2-diacyl-*sn*-glycero-3-phosphocholine liposomes (L), calf thymus DNA and various bivalent metal cations (Me^{2+}),[1–5] using synchrotron X-ray diffraction and freeze-fracture transmission electron microscopy (TEM). When buffered aqueous solutions (buffer HEPES, pH = 7.2) of the neutral zwitterionic liposomes, DNA and metal ions are mixed, supramolecular structures are formed which consist of ordered multilamellar assemblies (L_α^c phases) where the helices of DNA are sandwiched between the liposome bilayers and the hydrated metal ions act as a bridge between the negative liposome headgroups and the negative phosphate groups of DNA. The structure and morphology of the ternary complex with Mn^{2+} have already been reported.[1] The hydrated Mn^{2+} ions that lie between the liposome bilayers and DNA in the ternary complex strengthen the interactions between the latter two components. This structure is similar to the ones obtained by the condensation of DNA with cationic liposomes (CL–DNA complexes or lipoplexes).[6–11]

It was observed[1] that the Bragg's peaks associated with the ternary complex with Fe^{2+} are weaker and less resolved than the ones obtained with other cations, namely Mn^{2+}, Mg^{2+} and Co^{2+}. Such different behaviour could be due to the ability of Fe^{2+} to catalyse the oxidation of the phospholipids (Fenton reaction) in experimental conditions. Among the numerous papers that have been published on lipid peroxidation, many deal with the damage that the products or the intermediates of peroxidation (namely malondialdehyde and peroxy radicals) cause to DNA,[12–22] whereas only two have dealt specifically with the effect that DNA induces in liposome peroxidation. It has been reported[23,24] that while incubating a mixture of methyl arachidonate enriched samples of egg yolk phosphatidylcholine and DNA in the presence of oxygen, at buffered pH = 7.5, DNA is able to act as an antioxidant, giving rise to a concentration dependent retarding, but not an inhibiting effect on the rate of oxidation. This behaviour was interpreted as due to the ability of DNA to act as a scavenger of $^{\bullet}OH$ and other radicals ($HO_2^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$) that form during liposome peroxidation, a chain radical reaction initiated by any radical able to abstract a hydrogen atom from an allylic or bisallylic methylene of the phospholipid hydrocarbon chain.[25] We show in this paper that DNA is able to influence also the Fe^{2+} induced peroxidation of phospholipids, but in a different way, namely by increasing both the rate and the yield of the reaction. In view of the possibility, which is presently under investigation, of using the ternary complexes obtained by zwitterionic liposomes, DNA and Me^{2+} as DNA transfection agents in gene therapy, we thought it was useful to study such peculiar behaviour of DNA. As a matter of fact, even if iron in biological material is stored in specific proteins that prevent any effect on lipid peroxidation, some evidence exists[26,27] that hydroperoxide groups (LOOH), which are normally present in commercial liposomes[28] or can originate during the preparation and storage of the complexes, react with haemoglobin to release iron, which can promote DNA degradation and stimulate lipid peroxidation by a Fenton reaction.

Results and discussion

The Fe^{2+} catalysed peroxidation of liposomes has been carried out, unless stated otherwise, on multilamellar vesicles (MLV) at the buffered pH of 7.2 and at room temperature. As reported in the experimental section, the reactions have been followed through a procedure described by Ohyashiki *et al.*,[29] which consists of the colorimetric determination at 532 nm, by means of 2-thiobarbituric acid, of the whole of the carbonyl end products (or thiobarbituric acid reactive species, TBARS) formed in the oxidative degradation of the phospholipids. No alternative methods, such as oxygen uptake or chemiluminescence determinations, have been checked, being demonstrated[30] that the different methods normally used by authors in determining the extent of lipid peroxidation lead to substantially comparable results. Before doing peroxidation experiments it was checked that no TBARS were determined at the pH of 7.2 and within the time of the experiments described (6 to 8 hours), either in the multilamellar vesicles of phosphatidylcholine (PCMLV) incubated with oxygen in the absence of Fe^{2+}, or in the samples of DNA incubated with oxygen in the presence or absence of Fe^{2+}.

In order to have a consistent comparison of the results of the present work with the ones reported in the literature cited,[23] a preliminary peroxidation of multilamellar vesicles of egg yolk phosphatidylcholine (PCMLV) was performed in the presence of Fe^{2+}, as shown in Fig. 1 (curve a). An induction time of only 3 hours was observed, which is much lower than the one of 21 hours for the uncatalysed oxidation. This remarkable

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effect on the induction time is certainly the consequence of a Fenton type reaction, that probably starts on LOOH groups already present in some phospholipid molecules of commercial samples. This well known reaction has been reported as LOOH-dependent lipid peroxidation[28] for which Scheme 1 represents a simplified pathway. Peroxides are transformed into alkoxy and peroxy radicals that are responsible for hydrogen abstraction from the allylic or bisallylic methylenes and then for the initiation of a well known chain reaction.[31] In order to check if a mechanism like the one described above could be operating in the conditions of the present work, we incorporated on the PC some triphenylphosphine (TPP), which is able to cleave the O–O bond,[32,33] with reduction of peroxides to alcohols; under these conditions no TBARS were detected, within a time of 6 to 8 hours (the time scale normally used for Fe^{2+} catalysed peroxidation experiments) either from PCMLV or from PCMLV–DNA. Fig. 1 shows also the results obtained when increasing amounts of calf thymus DNA are added to PCMLV. A gradual disappearance of the induction time and an increase of the rate and yields of the oxidative degradation are achieved (Fig. 1, curves b, c, d, e). This striking effect made by the DNA in liposome peroxidation could be related to the finding that ternary complexes are formed when neutral liposomes, DNA and Me^{2+} ions are mixed: this suggests that a ternary complex with Fe^{2+} is the reaction intermediate.

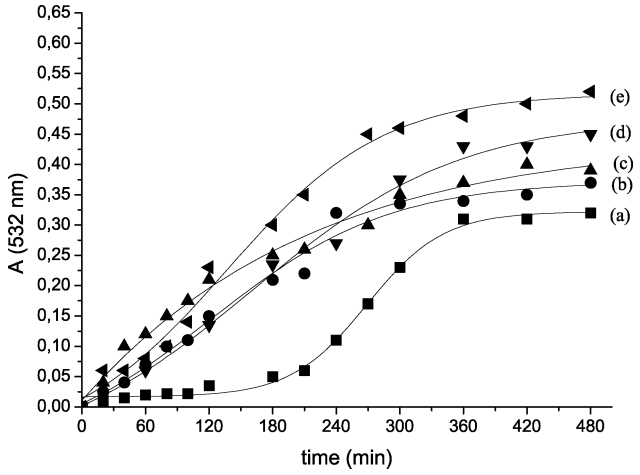

Fig. 1 Effect of calf thymus DNA on the Fe^{2+} induced peroxidation of egg yolk PCMLV. (a) PCMLV; (b) [DNA] : [PCMLV] = 0.5; (c) [DNA] : [PCMLV] = 0.75; (d) [DNA] : [PCMLV] = 1; (e) [DNA] : [PCMLV] = 1.5. The starting solution is 4 mM on PCMLV and 10 mM on Fe^{2+}.

$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO^{\cdot} + OH^{-}$$

$$Fe^{3+} + LOOH \rightarrow Fe^{2+} + LOO^{\cdot} + H^{+}$$

$$LO^{\cdot} + LH \rightarrow L^{\cdot} + LOH$$

$$LOO^{\cdot} + LH \rightarrow L^{\cdot} + LOOH$$

Scheme 1 Pathway of the Fe^{2+} induced LOOH dependent lipid peroxidation (from ref. 32).

Considering that the phosphatidylcholine from egg yolk used in this work is a mixture of different saturated and unsaturated lipids, as reported in Table 1, and that the hydrogen abstraction by alkoxy and peroxy radicals is easier on allylic (C–H bond energy equal to 368 kJ mol^{-1}) and bisallylic carbon atoms (C–H bond energy equal to 320 kJ mol^{-1}) than on the saturated ones (C–H bond energy equal to 422 kJ mol^{-1}), we have repeated the peroxidation with Fe^{2+}, in the presence and absence of DNA, of liposomes originated both from 1,2-dioleoyl (DOPC) and 1,2-dilinoleoyl (DLPC)-*sn*-glycero phosphatidylcholine, which are the main unsaturated components of egg yolk PC. Using the same conditions as for PCMLV, the results described in Figs. 2 and 3 were obtained, which are consistent with the ones from egg yolk PCMLV and confirm the positive effect of DNA on liposome peroxidation. It must be observed that in the oxidation of the two unsaturated components of egg yolk PCMLV, the induction time vanishes completely independently of the addition of DNA. This means that the induction times shown in the peroxidation of both PCMLV–Fe^{2+} and PCMLV–DNA–Fe^{2+} must depend on the 47% of unsaturated phospholipids present on egg yolk PC. This is confirmed by the lack of peroxidation on a saturated liposome like the one from 1,2-dipalmitoyl-*sn*-glycero-phosphatidylcholine (DPPC).

Table 1 Mass composition of the different fatty acids of the commercial egg yolk phosphatidylcholine

Acid	Symbol	Composition (%)
Palmitic	16 : 0	33
Stearic	18 : 0	14
Oleic	18 : 1	30
Linoleic	18 : 2	14
Arachidonic	20 : 4	4

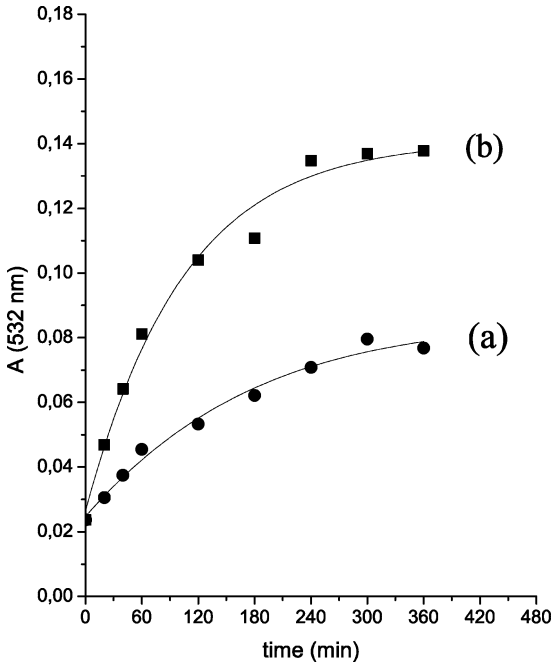

Fig. 2 Fe^{2+} induced peroxidation of DOPC (a) and DOPC–DNA (b). The mole concentrations are: DOPC = 4 mM; DNA = 6 mM; Fe^{2+} = 10 mM.

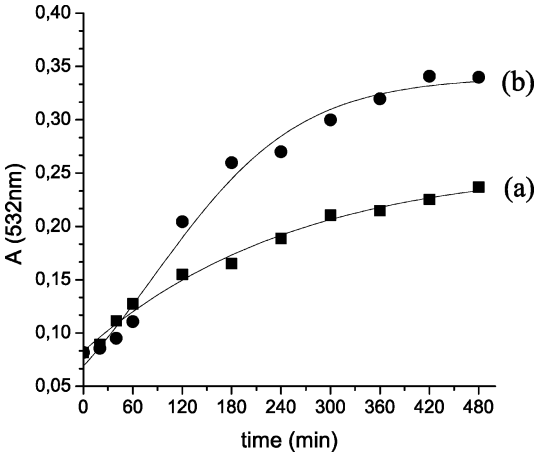

Fig. 3 Fe^{2+} induced peroxidation of DLPC (a) and DLPC–DNA (b). The mole concentrations are: DLPC = 4 mM; DNA = 6 mM; Fe^{2+} = 10 mM.

The hypothesis of a direct correlation between complex formation and the peroxidation pathway can be confirmed from some results obtained by studying the analogous, but more stable and well identified ternary complex DOPC–DNA–Mn^{2+}. The synchrotron XRD study of the model self assembly obtained by DOPC, calf thymus DNA and Mn^{2+} shows (Fig. 4) the coexistence of two different supramolecular structures: a binary assembly L_α, formed by the interaction of DOPC with Mn^{2+}, characterised by three orders of reflection, and the ternary complex $L_\alpha{}^c$, obtained by addition of DNA to a mixture of the two former compounds, which in turn gives four orders of reflection. Assuming that the heights of the X-ray peaks are a measure of the concentrations of the two complexes, their partition depends (i) on the ratio DNA : DOPC, at constant [DOPC] : [Mn^{2+}] (Fig. 5A), (ii) on the amount of Mn^{2+} added, at constant [DNA] : [DOPC] (Fig. 6A) and (iii) on the temperature (Fig. 7). These results can be compared with the ones of the peroxidation experiments: if one plots the values of absorbance at 532 nm due to TBARS of a series of experiments performed on samples obtained using different molar ratios DNA : PCMLV at constant [PCMLV] : [Fe^{2+}], a linear correlation (Fig. 5B), analogous to the one shown in Fig. 5A is observed. The same analogy appears if one compares the results of a series of peroxidations carried out on samples formed using different [Fe^{2+}] : [DNA] ratios at constant [DNA] : [PCMLV] (Fig. 6B) with the partition between ternary (DOPC–DNA–Mn^{2+}) and binary (DOPC–Mn^{2+}) complexes reported in Fig. 6A. Finally, Fig. 8 reports the results of the peroxidation of samples of PCMLV–DNA–Fe^{2+} at three different temperatures: it is clear that the peroxidation extent rises as the temperature increases. Such behaviour is in accord with that reported in Fig. 7, which shows increasing X-ray peak intensities of the ternary complex DOPC–DNA–Mn^{2+} with increasing temperature. Fig. 9 shows that a good linear correlation with temperature is obtained in both experiments.

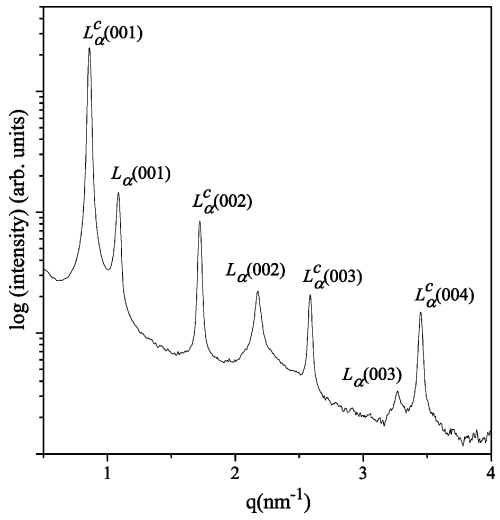

Fig. 4 Synchrotron XRD pattern of DOPC–DNA–Mn^{2+} and DOPC–Mn^{2+} obtained by mixing aqueous solutions of DOPC, calf thymus DNA and Mn^{2+} at a 3 : 4 : 12 mole ratio, at buffered pH = 7.2. The SAXS pattern exhibits a separate set of peaks associated with the ternary complex $L_\alpha{}^c$ and the binary one L_α.

All the results discussed above strongly support the hypothesis that the catalytic effect of DNA on peroxidation of PCMLV must be attributed to the fact that the reaction takes place on the ternary complex. Nevertheless, this statement may be limited by the level of reliability of the comparison between results obtained on complexes of different nature, namely the ones with Fe^{2+}, used on peroxidation measures, against the ones with Mn^{2+}, used on reference X-ray measurements. Unfortunately the X-ray spectrum[1] is not well defined and hence does not allow a detailed structural investigation of the complex with Fe^{2+}. Additional

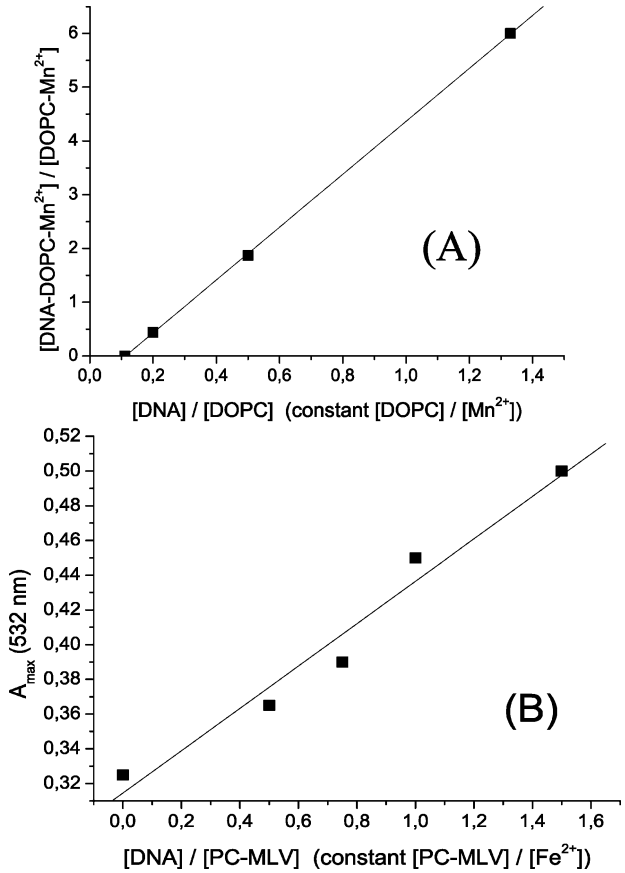

Fig. 5 (A) Plot of the ratio of the peak intensities of DOPC–DNA–Mn^{2+} and DOPC–Mn^{2+} *versus* increasing [DNA] : [DOPC] ratios at constant [DOPC] : [Mn^{2+}]. The DNA : Mn^{2+} mole ratio is equal to 1 : 6. (B) Plot of TBARS production *versus* mixtures of increasing [DNA] : [PCMLV] ratios at constant [PCMLV] : [Fe^{2+}]. The [PCMLV] : [Fe^{2+}] ratio is equal to 1 : 4.

data can help to clarify the situation however. It is known that Mn^{2+} is able to interfere with Fe^{2+} in peroxidation processes[34] and to protect cells[35] significantly decreasing the extent of lipid peroxidation in the liver, spleen and adrenals.[36] The peroxidation with oxygen of the ternary complex PCMLV–DNA–Mn^{2+} has been attempted and, as expected (Fig. 10, curve a) no production of TBARS was observed. To the contrary, some oxidation takes place when Fe^{2+} is added to the preformed complex with Mn^{2+} (Fig. 10, curve b). By comparing this latter result with the one obtained on oxidation of a sample of PCMLV–DNA–Fe^{2+} originally prepared (Fig. 10, curve c), it can be observed that the extent of oxidation of the former sample is lower than the one of the latter, the starting concentrations being identical. This difference could be attributed to a residual negative effect of Mn^{2+}. If we analyse these results in the light of the hypothesis that the ternary complex is the species that undergoes peroxidation, one must conclude that the Fe^{2+} added to the solution of the complex PCMLV–DNA–Mn^{2+} is able to displace, almost partially, the Mn^{2+} ions from the corresponding ternary complex. This process can be assumed possible and demonstrable: Mn is a paramagnetic atom and liquid solutions of its ions exhibit a hyperfine structure corresponding to six lines of equal intensity in the EPR spectrum. It has been demonstrated[37,38] that this hyperfine structure gradually disappears as the Mn^{2+} is encompassed in a complex.

Starting from this observation and assuming that the intensity of an EPR line is a measure of the manganous ion concentration, it is possible to make a series of EPR determinations in order to draw a scale of Mn^{2+} concentrations from 0 (completely bound Mn) to 100 (totally free Mn). Each intermediate value corresponds to a definite concentration of free Mn. If Fe^{2+} is able to displace Mn^{2+} from its own complex to form the new

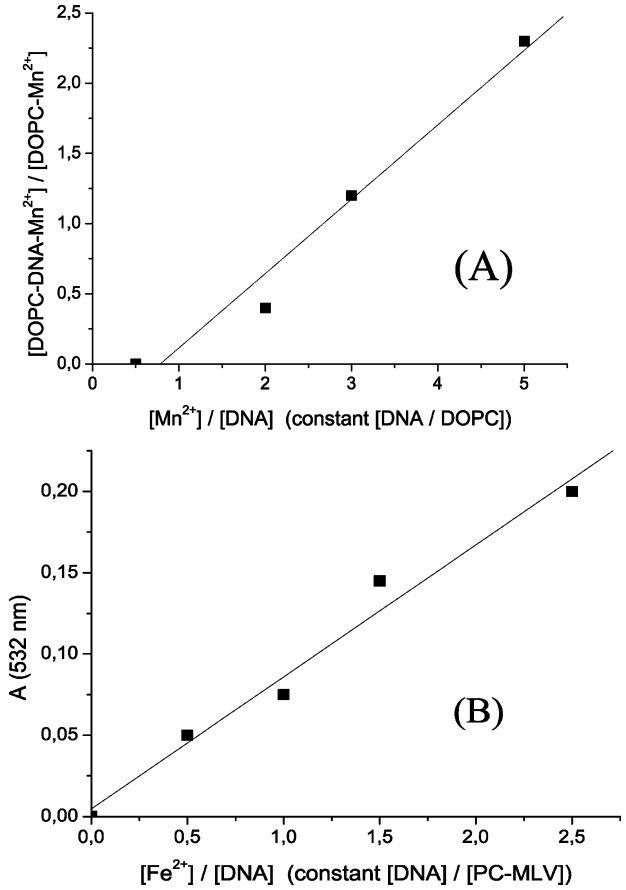

Fig. 6 (A) Plot of the ratios between the peak intensities of DOPC–DNA–Mn^{2+} and DOPC–Mn^{2+} *versus* increasing mole ratios [Mn^{2+}] : [DNA] at constant [DNA] : [DOPC]. (B) Plot of TBARS production *versus* mixtures of increasing Fe^{2+}–DNA mole ratios, at constant [DNA] : [PCMLV]. In both experiments the DNA : liposome mole ratio is equal to 1 : 1.5.

PCMLV–DNA–Fe^{2+} complex, to each amount of Fe^{2+} entering the complex a corresponding amount of Mn^{2+} must be released and can be determined. It is then possible to plot the percentage of the free Mn^{2+} *versus* the ratio [Fe^{2+}] : [Mn^{2+}]. Fig. 11 shows the effect of the addition of different amounts of Fe^{2+} on Mn^{2+} displacement. In the experiment reported in Fig. 10, the amount of Fe^{2+} added to the complex PCMLV–DNA–Mn^{2+} was set to give a ratio [Fe^{2+}] : [Mn^{2+}] of 1 : 1. In these conditions, the yield on TBARS (Fig. 10, curve b) is nearly 30% with respect to the one obtained with complex PCMLV–DNA–Fe^{2+} originally prepared (higher curve). The graph of Fig. 11 indicates that less than 30% of Mn^{2+} should be displaced by Fe^{2+}, at the ratio [Fe^{2+}] : [Mn^{2+}] of 1 : 1; a result that is in good agreement with the one found in the peroxidation experiment and can satisfactorily support the hypothesis that the ternary complex with Fe^{2+} is necessary for the catalytic effect of DNA on liposome peroxidation.† Moreover, an additional argument in favour of a strict relationship between liposome peroxidation and the existence of an intermediate ternary complex comes from the observation that unilamellar vesicles of egg yolk phosphatidylcholine (PCULV) show a different path to oxidation. As shown in Fig. 12, the addition of DNA to the liposome is still able to annihilate the lag time, but the peroxidation yield is higher for PCULV alone than for PCULV in the presence of DNA. In accordance with this result, we could not observe any ternary complex, neither PCULV–DNA–Fe^{2+} nor PCULV–DNA–Mn^{2+}, by X-rays. In these cases an intermediate complex, if it exists, must be very unstable. Finally, as a latter contribution, Fig. 13 reports the results of a peroxidation promoted by Fe^{2+} of linoleic acid and of a mixture of linoleic acid and DNA. Both reactions reach almost identical yields and show an absolutely analogous pathway in the timescale of the typical experiments reported above. The conclusion is that no catalytic effect by DNA is possible if no complexation can take place.

The hypothesis that the effect of DNA on peroxidation is due to its ability to form complexes with PCMLV in the presence of Fe^{2+} seems to be well supported. Therefore, the question is why these complexes are able to realise conditions for an easier oxidizability of liposomes. Data found in existing literature can help: in his work on the Fe^{2+} induced lipid peroxidation in phospholipid liposomes, Ohyashiki[29] found that addition of $AlCl_3$ stimulates peroxidation at physiological pH and shortens the lag phase in a dose dependent fashion. This result has been interpreted as being due to the influence of Al^{3+} in promoting aggregation of the liposomes[39] to form liposomal aggregates. In other words, it is possible that Al^{3+} ions bind

† The result suggests that a dynamic process is operating when Mn^{2+} is replaced by Fe^{2+}. Analogous results, with different displacement ratios, are shown by different cations. Among the ones tested so far, Ni^{2+} shows the higher displacement ability on Mn^{2+}. These results will be published later.

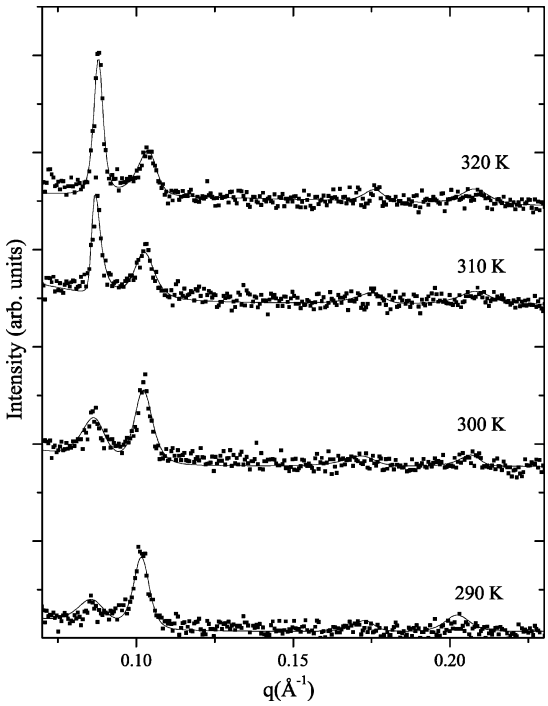

Fig. 7 Dependence of the partition between DOPC–DNA–Mn^{2+} and DOPC–Mn^{2+} complexes on temperature. The peak corresponding to the ternary complex is the one at the lower q value (after ref. 1).

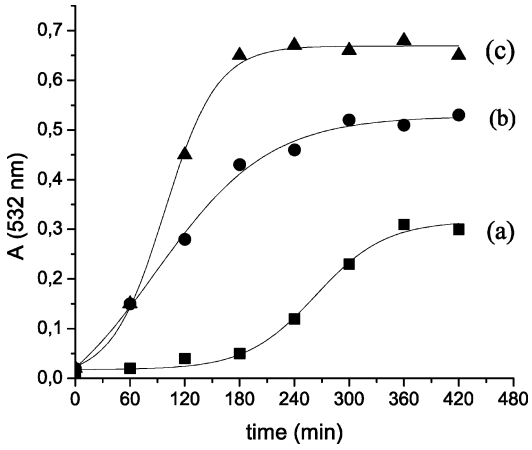

Fig. 8 Peroxidation of samples of the ternary complex PCMLV–DNA–Fe^{2+} at different temperatures. (a) 297 K; (b) 310 K; (c) 323 K.

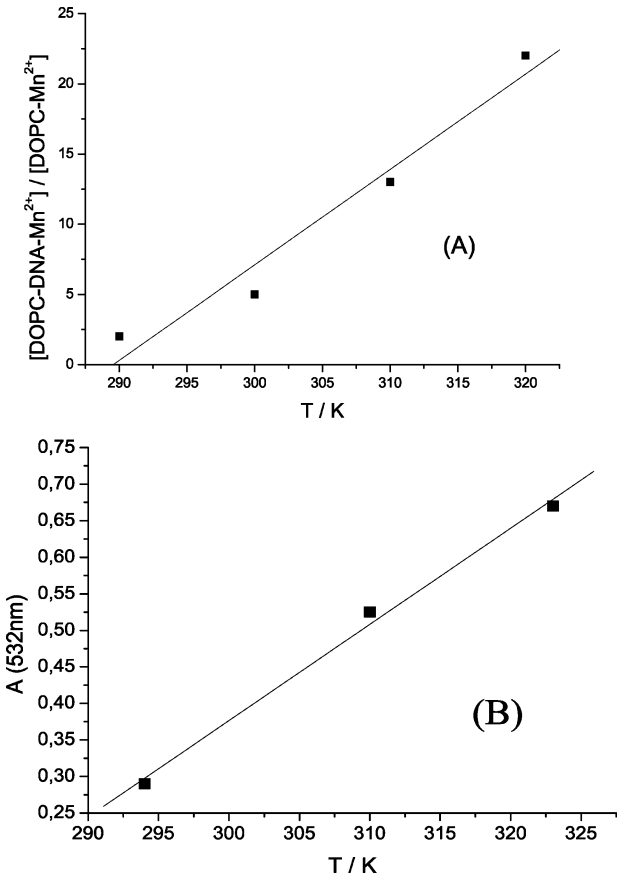

Fig. 9 (A) Plot of the DOPC–DNA–Mn^{2+} X-ray peak intensity *versus* temperature. (B) Plot of TBARS production by Fe^{2+} induced peroxidation of mixtures of PCMLV and DNA *versus* temperature. Both plots show a fair linear correlation.

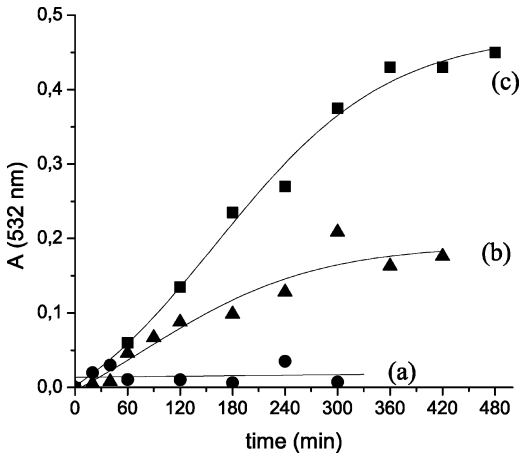

Fig. 10 Peroxidation of complex PCMLV–DNA–Mn^{2+}. (a) No Fe^{2+} added; (b) Fe^{2+} added to reach a ratio [Fe^{2+}] : [Mn^{2+}] equal to 1; (c) peroxidation of the control PCMLV–DNA–Fe^{2+}. The initial mole concentrations for all the experiments are: PCMLV = 4 mM; DNA = 8 mM; Mn^{2+} = 10 mM; Fe^{2+} = 10 mM.

to the liposomal surface[40] making a suitable arrangement of phospholipids in the liposomes, which can suppress the phospholipid chain movements. This concept was later reinforced by the finding that the stimulatory effect of $AlCl_3$ on Fe^{2+} initiated lipid peroxidation in phosphatidylcholine liposomes was more effective if $AlCl_3$ was substituted by a lipophilic aluminium complex, namely aluminium acetylacetonate,[41] which can be incorporated into the liposomal membranes. The result of the new arrangement consists of a strengthening of the packing between the acyl chains in the lipid layer, with the consequence of a higher crystallinity and an implementation of Fe^{2+} induced

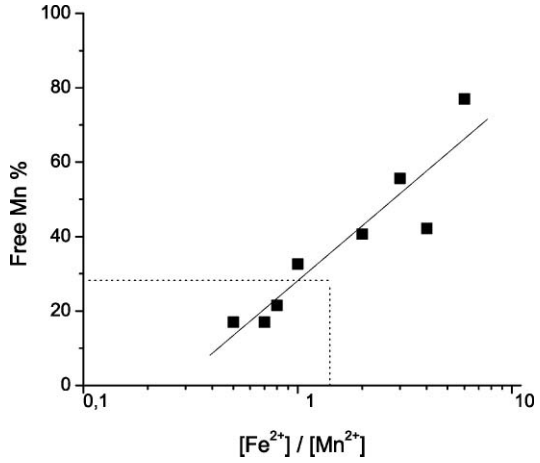

Fig. 11 Displacement of Mn^{2+} from DOPC–DNA–Mn^{2+} by Fe^{2+}. The figure plots the percentage of free Mn^{2+} as a result of displacement due to growing amounts of Fe^{2+}.

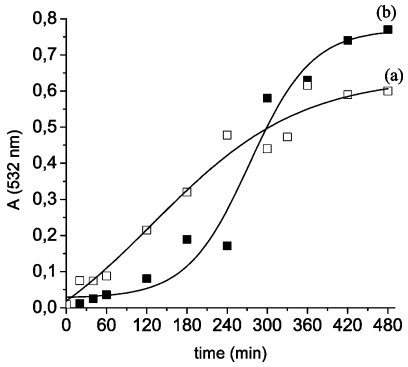

Fig. 12 Fe^{2+} induced peroxidation of mixtures of unilamellar vesicles of egg yolk phosphatidylcholine: (a) no DNA added; (b) DNA added. The mole ratio PCMLV : DNA is equal to 1.

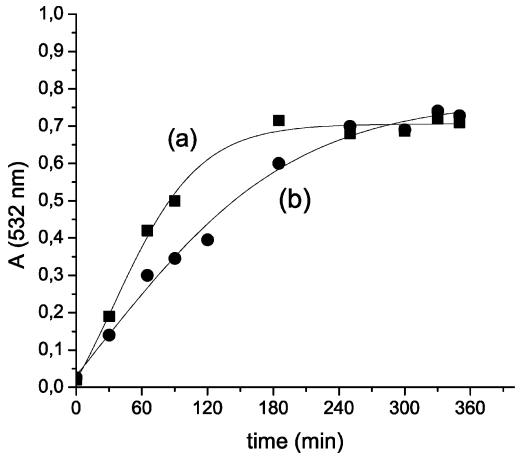

Fig. 13 Fe^{2+} induced peroxidation of linoleic acid (a) and linoleic acid + DNA (b). DNA induces a weak retarding effect.

peroxidation. The finding that lipid peroxidation increases by increasing the concentration of the complex is in accordance with the Ohyashiki result. As a matter of fact, an analogous situation is realised in the PCMLV–DNA–Fe^{2+} complexes. The DNA added to samples of liposomes and Fe^{2+} is able to form stable aggregates in which the hydrocarbon chains of the lipids are able to rearrange giving rise to a regular semicrystalline structure. In addition, it may be considered that a higher packing leads to a stiffening of the lipid bilayers and then to a reduced fluidity of the phospholipid chains, with the consequence of an acceleration of the peroxidation itself. In the case of the model ternary complex DOPC–DNA–Mn^{2+}, it has been

possible to ascertain[2] that a lowering in the bilayer undulations occurs as a consequence of the condensation of DNA within the lipid bilayers. This situation reduces the flexibility of the lamellae in the complex and suppresses almost completely the undulatory fluctuations. X-Ray data allowed us to calculate the mean square amplitude ($|z^2|$) of the layer fluctuation in the DOPC liposome. Assuming that vesicles behave as liquid crystalline systems, one must take into account that two different contributions have a part in the total fluctuation: the thermal term, which is related to the bilayers' mean position and the undulatory effect, which is due to fluctuation of individual bilayers. Calculations led to the conclusion that the latter contribution is almost completely annihilated in the complex by the insertion of DNA helices between the lipid bilayers. In other words, DNA allows only thermal fluctuation, significantly reduces the fluidity and, by consequence, facilitates peroxidation of the hydrocarbon tails. This result agrees also with literature data that demonstrate the existence of a relationship between membrane fluidity and the rate of peroxidation of lipid bilayers.[42] An increase in the rate of peroxidation of arachidonic acid in dipalmitoylphosphatidylcholine[43,44] has been related to the fluidity of the bilayers and it has been demonstrated[45,46] that in relatively rigid bilayers, a minimal decrease in bilayer fluidity results in a large increase of the rate of peroxidation; a result which matches the experimental findings discussed in this paper.

Experimental

Chemicals

3-*sn*-Glycero-phosphatidylcholine from egg yolk, 99% chloroform solution (PC), DNA from calf thymus, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), $FeCl_2$, trichloroacetic acid (TCA), 2-methylthiobarbituric acid (TBA), butylated hydroxytoluene (BHT) and triphenylphosphine (TPP) were purchased from Fluka. DOPC, DLPC and DPPC, 99% chloroform solutions, were purchased from Avanti Polar Lipids. All chemicals were analytical grade and used without further purification. Bidistilled deionized water was used to prepare solutions and emulsions.

Procedures

MLV liposomes were prepared from chloroform solutions of the different phosphocholines, evaporating to dryness with a stream of nitrogen gas and removing the residual solvent under vacuum. The appropriate amount of HEPES solution was added in order to reach a buffered pH = 7.2. The solutions were sonicated in order to have MLV within the range of a 2 μm diameter. The peroxidation experiments were done at a temperature range of 297 to 323 K and followed for six to eight hours. Solutions for peroxidation experiments were prepared as already described[47] by adding 1 ml of a buffered aqueous solution of $FeCl_2$ to 1 ml of a buffered solution of PCMLV, for blank experiments on simple liposomes; and by adding 1 ml of a buffered solution of $FeCl_2$ to a preformed buffered solution made by 1 ml of aqueous DNA and 1 ml of aqueous PCMLV, for experiments on PCMLV–DNA. For typical experiments, the concentration of each reagent was calculated in order to have 4 mM samples both on the MLV liposome and DNA and 10 mM on $FeCl_2$; the concentrations of DNA and the liposome being determined as phosphates. The same molar ratios were normally used in the preparation of complexes of DOPC and DLPC with Mn^{2+}. Different ratios have been used in experiments aimed at studying the influence of DNA concentration on peroxidation: details referring to these experiments are reported in specific figure captions. The extent of peroxidation at different time intervals was assessed using the TBA reactivity method.[29] At the time intervals indicated in the kinetic diagrams, the reaction was stopped by adding 20 μl of BHT (20 mM in ethanol) and to the resulting mixture was added 2.0 ml of an aqueous solution of 0.1 M HCl, containing TBA (0.375%, w/v) and TCA (15%, w/v). The final suspension was vortexed and incubated for 15 min at 363 K, cooled on ice and centrifuged for 7 min at 3000 rev/min. The supernatant was checked at 532 nm on a UV–Vis Uvikon spectrometer and the result expressed as a thiobarbituric acid reactive species (TBARS). The TBA reaction was not influenced by the buffer system. Before doing Fe^{2+} catalysed peroxidations, it was checked that no TBARS were detected from simple PCMLV and PCMLV–DNA incubated with oxygen in the absence of Fe^{2+}, and from simple DNA in the absence and in the presence of Fe^{2+}, within 8 hours.

SAXS experiments

SAXS analysis of the mixture of supramolecular structures shown in Fig. 4 was carried out at ID02 beamline at the European Synchrotron Radiation Facility, Grénoble, France. The wavelength of the incident beam was $\lambda = 0.99$ Å (12.5 KeV), the distance sample-detector was set at 1.2 m. The diffraction patterns were collected by a 2D CCD detector. Measurements were performed at room temperature and a maximum exposure time of 6 sec/frame was used to avoid radiation damage.

EPR and displacement determinations

We started measuring the EPR spectrum of an aqueous solution of Mn^{2+} buffered at pH = 7.2 containing 0.4 moles of Mn^{2+} per liter. To different aliquots of this solution, mixtures of DNA and DOPC were progressively added in order to reach final concentrations equal to 0.92 and 1.30 mM, respectively. At these concentrations, no hyperfine structure for free Mn was detected. The volume of each sample was equal for all the determinations in order to maintain the same initial concentration on Mn^{2+}. Assuming the height of a line is proportional to Mn^{2+} concentration, the value 100 was attributed to the EPR spectrum corresponding to totally free Mn and 0 to the completely bound Mn identified by a lack of any hyperfine structure in the spectrum. Each intermediate intensity of the EPR lines corresponds to a definite value of free Mn^{2+}. The calibration curve for the Fe^{2+} displacement determinations has been drawn by adding increasing amounts of $FeCl_2$ solutions to an aqueous solution of the complex PCMLV–DNA–Mn^{2+} with the Mn^{2+} completely bound (no hyperfine structure shown). Final concentration ratios [Fe^{2+}] : [Mn^{2+}] from 0.5 to 1.5 were checked and the free Mn^{2+} displaced was reported as a corresponding percentage on the total Mn^{2+}present.

References

1 O. Francescangeli, V. Stanic, L. Gobbi, P. Bruni, M. Iacussi, G. Tosi and S. Bernstorff, *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.*, 2003, **67**, 11904/1.
2 O. Francescangeli, V. Stanic, D. E. Lucchetta, P. Bruni, M. Iacussi and F. Cingolani, *Mol. Cryst. Liq. Cryst.*, 2003, **398**, 259.
3 O. Francescangeli, M. Pisani, V. Stanic, P. Bruni and M. Iacussi, *Recent Res. Dev. Macromol. Res.*, 2003, **7**, 247.
4 O. Francescangeli, M. Pisani, V. Stanic, P. Bruni and T. M. Weiss, *Europhys. Lett.*, 2004, **67**(4), 669.
5 M. Pisani, P. Bruni, C. Conti, E. Giorgini, O. Francescangeli, *Mol. Cryst. Liq. Cryst.*, in press.
6 D. D. Lasic, H. Strey, M. C. A. Stuart, R. Podgornik and P. M. Frederik, *J. Am. Chem. Soc.*, 1997, **119**, 832.
7 J. O. Rädler, I. Koltover, T. Salditt and C. R. Safinya, *Science*, 1997, **275**, 810.
8 T. Salditt, I. Koltover, J. O. Rädler and C. R. Safinya, *Phys. Rev. Lett.*, 1997, **79**, 2582.
9 I. Koltover, T. Salditt, J. O. Rädler and C. R. Safinya, *Science*, 1998, **281**, 78.
10 T. Salditt, I. Koltover, J. O. Rädler and C. R. Safinya, *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.*, 1998, **58**, 889.
11 F. Artzner, R. Zantl, G. Rapp and J. O. Rädler, *Phys. Rev. Lett.*, 1998, **81**, 5015.
12 C. E. Vaca, J. Wihlelm and M. Harms-Ringdahl, *Mutat. Res.*, 1988, **195**, 137.

13 R. L. Daniel and D. H. Phillips, *Mutat. Res.*, 1999, **424**, 23.
14 J. W. Park and R. A. Floyd, *Free Radical Biol. Med.*, 1992, **12**, 245.
15 A. M. Hruszkewicz, *Biochem. Biophys. Res. Commun.*, 1988, **153**, 191.
16 K. Stone, M. B. Ksebati and L. J. Marnett, *Chem. Res. Toxicol.*, 1990, **3**, 33.
17 K. Stone, A. Uzieblo and L. J. Marnett, *Chem. Res. Toxicol.*, 1990, **3**, 467.
18 H. Wiseman and B. Halliwell, *Biochem. J.*, 1996, **313**, 17.
19 L. J. Marnett, *Toxicology*, 2002, **181–82**, 219.
20 P. Lim, K. Sadre-Bazzas, J. Shurter, A. Sarasin and J. Termini, *Biochemistry*, 2003, **42**, 15036.
21 G. R. Martinez, A. P. M. Loureiro, S. A. Marques, S. Miyamoto, L. F. Yamaguchi, J. Onuki, E. A. Almeida, C. C. M. Garcia, L. F. Barbosa, M. H. G. Medeiros and P. Di Mascio, *Mutat. Res.*, 2003, **544**, 115.
22 P. Lim, G. E. Wuenschell, L. Dong-Hyun, G. P. Pfeifer, H. Rodriguez and J. Termini, *Biochemistry*, 2004, **43**, 15339.
23 D. D. Pietronigro, M. L. Selisgman and H. B. Demopoulos, *Lipids*, 1976, **11**(11), 808.
24 D. D. Pietronigro, W. B. G. Jones, K. Kalty and H. B. Demopoulos, *Nature*, 1977, **267**, 78.
25 B. Halliwell, J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, 3rd edn., 1999, p. 291.
26 M. C. Gutteridge, *FEBS Lett.*, 1986, **201**(2), 291.
27 J. R. Brandon, D. A. Svitsunenko, C. E. Cooper and M. T. Wilson, *Antioxid. Redox Signaling*, 2005, **6**(6), 954.
28 B. Tadolini and G. Hakim, *Free Radical Res.*, 1996, **25**, 221.
29 T. Ohyashiki, T. Karino and K. Matsui, *Biochim. Biophys. Acta*, 1993, **1170**, 182.
30 T. Noll, H. de Groot and H. Sies, *Arch. Biochem. Biophys.*, 1987, **252**, 284.
31 H. W. Gardner, *Free Radical Biol. Med.*, 1989, **7**, 65.
32 L. Tang, Y. Zhang, Z. Qian and X. Shen, *Biochem. J.*, 2000, **352**, 27.
33 T. Ohyashiki, A. Kadoya and K. Kushida, *Chem. Pharm. Bull.*, 2002, **50**(2), 203.
34 M. Balci, *Chem. Rev.*, 1981, **81**, 91.
35 S. J. Stohs and D. Bagchi, *Free Radical Biol. Med.*, 1995, **18**, 321.
36 G. de Rosa, C. L. Keen, R. M. Leach and L. S. Hurley, *J. Nutr.*, 1980, **110**, 795.
37 M. T. Chen, J. Y. Sheu and T. H. Lin, *J. Toxicol. Environ. Health*, 2000, **61**(A), 569.
38 M. Cohn and J. Townsend, *Nature*, 1954, **173**, 1090.
39 R. M. Farmer and A. I. Popov, *Inorg. Chim. Acta*, 1982, **59**, 87.
40 M. D. Deleers, J. P. Servais and E. Wuffert, *Biochim. Biophys. Acta*, 1985, **813**, 195.
41 T. Ohyashiki, S. Suzuki, E. Satoh and Y. Uemori, *Biochim. Biophys. Acta*, 1998, **1389**, 141.
42 M. A. Acheson, D. N. Munns and R. G. Burau, *Biochim. Biophys. Acta*, 1989, **986**, 33.
43 H. Mowri, S. Nojima and K. Inoue, *J. Biochem. (Tokyo)*, 1984, **95**, 551.
44 G. Cervato, P. Viani, M. Masserini, C. Di Iorio and B. Cestaro, *Chem. Phys. Lipids*, 1988, **49**, 135.
45 P. Viani, G. Cervato, A. Fiorilli, E. Rigamonti and B. Cestaro, *Chem. Phys. Lipids*, 1990, **52**, 49.
46 L. R. McLean and K. A. Hagman, *Free Radical Biol. Med.*, 1992, **12**, 113.
47 P. Bruni, G. Gobbi, G. Morganti, M. Iacussi, E. Maurelli and G. Tosi, *Gazz. Chim. Ital.*, 1997, **127**, 513.