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LIPID OXIDATION AND GEL TO LIQUID-CRYSTALLINE TRANSITION TEMPERATURES OF SYNTHETIC POLYUNSATURATED MIXED-ACID PHOSPHATIDYLCHOLINES

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Synthetic preparations of the polyunsaturated phosphatidylcholines 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (SLPC) and 1-stearoyl-2-α-linolenoyl-*sn*-glycero-3-phosphocholine (SLnPC) were observed to undergo autooxidation sometimes during synthesis and also on storage. Oxidation was also induced by treatment of unoxidized SLPC with ultraviolet irradiation. Oxidation was estimated by thin layer chromatographic, fatty acid and ultraviolet spectral analyses. With limited oxidation, the gel to liquid-crystalline transition temperatures of aqueous dispersions of these lipids were seen to increase. Extensive oxidation led to a reduction in the enthalpies of the transitions. The increases in transition temperatures were consistent with the presence of conjugated double bonds, as shown by increased absorption at 230 nm, and *trans* double bonds, in the oxidized lipids leading to the creation of more rigid domains within the bilayer. Some of the changes in the transitions, especially the decreasing enthalpy after extensive oxidation, may have occurred because of the presence of small amounts of lysophosphatidylcholine and other oxidation intermediates or breakdown products seen by thin layer analysis. Thermograms of mixtures of unoxidized SLPC with amounts of lysostearoylPC found in the oxidized samples showed, however, that lysoPC likely did not contribute significantly to the increase in transition temperatures. Thin layer analysis suggested that the presence of cross-linked products could have contributed to the observed thermotropic properties.

Lipid peroxidation is deleterious to membrane function, particularly in senescent membranes [1,2] and may affect the integrity of pulmonary surfactant [3]. Recent reports on autoxidation in biological and model membranes have shown this process to be associated with changes in permeability [4–7], with an increase in transbilayer movement of lipids [8,9], with the promotion of vesicle fusion [10], and in some instances, with decreases in lipid fluidity or increases in order as measured by fluorescence and spin probes [11–16] and X-ray diffraction [17]. An increase in proportion of gel-phase lipid or a decrease in fluidity at physiological temperature has also been observed in senescing membranes and erythrocyte membranes from old donors [2,18–22]. For bean cotyledon membranes and rat liver plasma membranes the increased rigidity has generally been associated with decreases in polyunsaturated fatty acids concomitant with increases in the relative amounts of cholesterol in the membrane lipids [2,20]. For human

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Abbreviations: DSC, differential scanning calorimeter(ry); GLC, gas liquid chromatography; PC, phosphatidylcholine; SLPC, 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine; SLnPC, 1-stearoyl-2-α-linolenoyl-*sn*-glycero-3-phosphocholine; T_c, gel to liquid-crystalline transition temperature (in degrees Celsius); TLC, thin layer chromatography; T_{max}, temperature of maximum heat flow during a thermal event (in degrees Celsius).

erythrocyte membranes, Hegner [2] observed a decrease in phospholipids in older cells but no change in cholesterol, whereas Shiga et al. [22] found both phospholipid and cholesterol to be decreased so that there was no change in the phospholipid to cholesterol ratio. Barrow and Lentz [23] have observed that fluorescence intensity of diphenylhexatriene in egg yolk liposomes was decreased by oxidation but that the decrease was caused by chemical modification of the probe, so that caution is necessary not to underestimate microviscosity based upon relative intensity measurements of diphenylhexatriene. Using chlorophyll *a* as an endogenous fluorescent probe, van Ginkel and Fork [24] have observed a decrease in the transition temperature of thylakoid membranes on aging. Here we present findings on the thermotropic properties of dispersions of synthetic polyunsaturated mixed-acid phosphatidylcholines which have undergone autoxidation. The gel to liquid-crystalline transition temperatures of these lipids initially increased after limited oxidation, but with extensive oxidation the transitions progressively disappeared. These findings suggest that the increased rigidity observed in peroxidized and possibly aged membranes, may involve initial changes in unsaturated fatty acids precedent to their ultimate loss from the membrane.

Materials and Methods

Two preparations (Batches I and II) of 1-stearoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (SLPC) were synthesized using the procedure of Roseman et al. [25] with minor modifications as outlined elsewhere [26]. Batch IV of SLPC was obtained from Avanti Polar Lipids, Birmingham, AL. The sample of 1-stearoyl-2-α-linolenoyl-*sn*-glycero-3-phosphocholine (SLnPC) and one batch of SLPC (III) were prepared by a small modification of the method of Gupta et al. [26,27]. With the exception of a preliminary batch and Batch I of SLPC, precautions were taken to avoid autoxidation during syntheses. Syntheses were carried out in the presence of small amounts (25 mg) of hydroquinone or *N'*-tetramethylenephenylenediamine dihydrochloride. All solvents and vessels were flushed with N_2, and as much as possible, all manipulations were made in a special chamber which was flushed with N_2. Unsaturated fatty acids and unsaturated mixed-acid phosphatidylcholines were stored at $-20°C$ or $-70°C$, either in $CHCl_3$ under N_2, or dry and in vacuo. Lysostearoylphosphatidylcholine used in the mixing study was made by the action of phospholipase A_2 (*Crotalus adamanteus* venom, Miami Serpentarium, Miami, FL) on distearoylphosphatidylcholine (Sigma Chemical Co., St. Louis, MO) followed by two or four washings with ether [28]. These materials contained only traces of phosphatidylcholine and free fatty acids by TLC and GLC. Hematin was a product of Sigma Chemical Co., and H_2O_2 was from British Drug Houses, Toronto, ON.

Purity of the fatty acids was assessed by GLC [28], and by ultraviolet absorption spectrometry [29]. Purity of the synthetic phosphatidylcholines was assessed by TLC, GLC, positional analyses [28] and ultraviolet absorption of samples in absolute or 95% ethanol, usually at 0.1–1 $mg \cdot ml^{-1}$ [29].

Lipid dispersions were made and differential scanning calorimetry was performed as described before [28]. All materials for which DSC thermograms are reported in Fig. 2 were analysed immediately after calorimetry and were found to contain only a phosphatidylcholine spot on TLC. Some contained oxidation products as indicated by increased absorbance near 230 nm, and by abnormal fatty acid ratios. Samples of extracts of dispersions of SLPC used for ultraviolet-induced oxidation showed other spots on TLC besides PC (see results).

Ultraviolet-induced oxidation was carried out by drying SLPC IV from solvent to a residual film in a small beaker. The film was exposed to ultraviolet irradiation from a UVSL-13 Mineral light (Ultra-Violet Products, Inc., San Gabriel, CA) at a distance of 11 cm. Samples were exposed to the direct rays for various times and then under the cover of a glass microscope slide (1 mm thick) for 16 h afterwards. The lipid was dissolved in $CHCl_3$, transferred to a small vial, dried under N_2 gas and evacuated over P_2O_5 for 1–1.5 h. Dispersions were then made in the usual way at room temperature in appropriate solvents.

Results

Changes in lipids and thermograms during synthesis and on storage. In a preliminary synthesis of SLPC with no special precautions against oxidation, the material which was isolated after 24 h as a unique phosphatidylcholine from a TLC plate was found to have a high stearate to linoleate ratio (18 : 0/18 : 2 = 4/1) indicating that extensive oxidation had occurred. Incubation of pure linoleic acid under identical conditions of reacylation [25,26] resulted in a product which gave the ultraviolet spectrum shown in Fig. 1a. The high absorbance in the region of 230 nm was consistent with the presence of hydroperoxides and conjugated double bonds [29,30], while the more diffuse absorbance in the region of 270 nm was probably due to decomposition products of the hydroperoxides, e.g., aldehydes and ketones [31]. The spectrum of an unoxidized linoleic acid sample is shown for comparison in Fig. 1b. Despite the presence of autoxidation as indicated by the ultraviolet spectrum, the methyl esters of the oxidized fatty acid showed only one peak corresponding to methyl linoleate on GLC with ethylene succinate-methyl silicone copolymer-LS (EGSS-X, Applied Science, State College, PA) as stationary phase.

Further preparations of SLPC (Batches I and II) yielded products with varying amounts of oxidation as indicated by the ultraviolet spectra in Fig. 1c and d. The material which gave the spectrum in Fig. 1c showed an excess of 18 : 0 (18 : 0/18 : 2 = 65/35) while the apparently oxidation-free lipid (Batch II, Fig. 1d) showed a 1 : 1 mole ratio of fatty acids (Table Ia). However, oxidation in this latter sample was observed to occur within 4 days upon storage in $CHCl_3$ at −20°C under N_2 (Table Ib). After three months under these conditions, breakdown of this phosphatidylcholine was indicated by the appearance on TLC of a lysoPC and a highly mobile spot (free fatty acid-like) together with a PC spot. The mobile spot was highly ultraviolet-quenching and it gave an ultraviolet spectrum similar to that in Fig. 1a but with a more intense absorption in the 260–280 nm region. The PC spot showed an almost equimolar ratio of fatty acids (18 : 0/18 : 2 = 48/52). In this case oxidation of some lipid appeared to have proceeded completely to leave relatively pure, intact SLPC. This repurified SLPC gave a DSC thermogram very similar to the one for the original sample (Fig. 2a). The SLnPC which was initially pure by all criteria (Table Id) also underwent limited oxidation on storage for approx. 2 weeks in vacuo at −20°C (Table Ie). The lipids in the

TABLE I

EFFECT OF SPONTANEOUS OXIDATION ON THE THERMOTROPIC PROPERTIES AND SOME ANALYTICAL PROPERTIES OF POLYUNSATURATED PHOSPHATIDYLCHOLINES

Data was obtained from differential scanning calorimetry of dispersions of polyunsaturated lipids which were either pure or had undergone varying amounts of natural autoxidation. Fatty acid mole ratios and molar extinction coefficients are used as indices of peroxidation. Roman numerals refer to batch numbers of the lipid products (see text for details). T_c, gel to liquid-crystalline transition temperature (°C) determined as the intersection of the tangent to the leading edge of the transition and the baseline. T_{max}, temperature (°C) of maximum heat flow into or out of the sample. n.d., not determined.

Lipid sample	Heating runs		Cooling runs		ΔH (kcal·mol^{-1})	Fatty acid mole ratio (sat : unsat)	E_{mol}^{230}
	T_c	T_{max}	T_c	T_{max}			
(a) SLPC II	−18.5	−16.5	−14.5	−18.0	2.24	50 : 50	~ 60 [a]
SLPC III	−19.0	−16.0	−13.0	−18.0	n.d.	50 : 50	~ 60 [a]
(b) SLPC II	−16.0	−14.0	n.d.	−15.0	1.70	53 : 47	726
(c) SLPC III	−11.0	−9.0	−4.0	−8.0	0.73	68 : 32	5860
(d) SLnPC	−13.0	−11.0	−11.0	−13.0	6.6	50 : 50	~ 60 [a]
(e) SLnPC	−3.5	−1.5	0	−3.0	1.02	64 : 36	4590

[a] These values were essentially no different from that of DPPC where $E_{mol}^{230} = 60$.

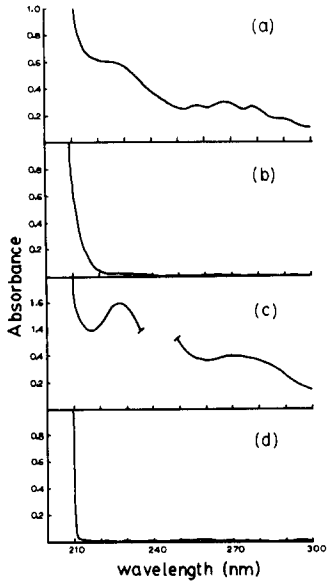


Fig. 1. Ultraviolet spectrophotometric scans of linoleic acid samples and synthetic SLPC samples. (a) oxidized linoleic acid; (b) unoxidized linoleic acid; (c) oxidized SLPC (I); (d) unoxidized SLPC (II).

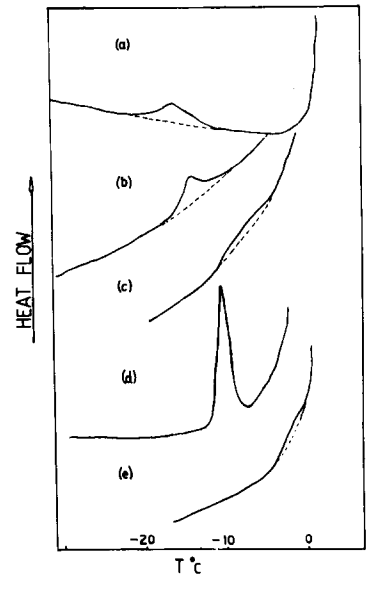


Fig. 2. Differential scanning calorimetric thermograms (heating) of variously oxidized dispersions of polyunsaturated mixed-acid phosphatidylcholines. Scan rates were between 1.25 and 5°/min while sensitivities full scale were 1 mcal/s for SLPC II (a), SLPC II (b), SLPC III (c) and SLnPC (d) and 0.5 mcal/s for SLnPC (e). The letters correspond to the materials described in Table I.

dispersions shown in Fig. 2 gave only phosphatidylcholine when analyzed by TLC after the DSC runs, but other analyses (Table I) showed the presence of oxidation intermediates in the phosphatidylcholine.

DSC thermograms for apparently pure SLPC (II) and SLnPC are shown in Fig. 2a and d. The thermograms of these two materials after they had undergone limited oxidation are shown in Fig. 2b and e, respectively. Also shown in Fig. 2c is the thermogram for another SLPC preparation (III) which had undergone more extensive oxidation (Table Ic) 4 weeks after isolation as a pure compound (Table Ia). As oxidation proceeded three parameters associated with the gel to liquid-crystalline transitions were seen to change. The transition temperatures and the transition widths increased while the transition enthalpies decreased.

Changes caused by experimentally-induced oxidation. A more detailed attempt to study the changes caused by oxidation was carried out by treating another preparation of SLPC (SLPC-IV) with various procedures to induce fatty acid oxidation. The results of these experiments are summarized in Table II and in Fig. 3. These clearly demonstrate that as ultraviolet-induced oxidation proceeded there was a rise in T_c and T_{max}. There was no significant change in enthalpy until oxidation had become extensive (Fig. 3(6) and Table II(6)) when a reduction in enthalpy was observed. The values of the enthalpy of 3.7 to 5.0 kcal · mol^{-1} are within the range of variability found for SLPC-IV under conditions of little or no oxidation [26]. Small amounts of hematin in the presence of O_2 or H_2O_2 did not promote extensive oxidation. All DSC samples were extracted and analyzed and the fatty acid analyses of the materials which had been treated with ultraviolet irradiation indicated a progressive increase with ultraviolet exposure in the ratio of stearate to linoleate and in E_{mol}^{230}. We feel this is due to loss of linoleate during oxidation, and to the breakdown of chains with oxidation intermediates during the transmethylation process [26]. The fatty acid ratios and the values of E_{mol}^{230} for the samples in Table III(1) and III(3) suggest they had oxidized slightly by the time of analysis. These had been kept at room temperature for 3–4 days, however, before analysis. Other measure-

TABLE II

EFFECT OF INDUCED OXIDATION ON THE THERMOTROPIC PROPERTIES AND SOME ANALYTICAL PROPERTIES OF SLPC

Analytical data obtained on the lipid extracts of the dispersions after DSC runs.

Sample label	Sample treatment	Heating runs T_c	Heating runs T_{max}	$\Delta T_{1/2}$ (degrees)	ΔH (kcal·mol^{-1})	Fatty acid mole ratio (18:0/18:2)	E_{mol}^{230}
(1)	SLPC control dispersed in H_2O	−15.1	−12.8	4.0(6.9) [a]	3.7	55:45	97
(2)	SLPC dispersed in 5 μM hematin bubbled with O_2 for 3 min	−15.9	−13.8	5.2	5.0	53:47	50
(3)	SLPC dispersion from (2) dried slowly in air on bench overnight, redispersed in 5 μM hematin plus 1 mM H_2O_2	−16.1 [b]	−13.2	5.1	3.0	55:45	159
(4)	SLPC treated with ultraviolet under glass overnight. No direct exposure. Dispersed in 5 μM hematin plus 1 mM H_2O_2	−15.9 [c]	−11.4 [c]	5.4	4.4	64:36	69000
(5)	SLPC as in (4) with 1 h direct exposure to ultraviolet. Dispersed in H_2O	−13.4	−10.5	3.6	3.7	70:30	128000 (6130) [d]
(6)	SLPC as in (5) except 2 h direct exposure to ultraviolet	−11.3	−7.9	6.7	1.8	86:14	129000 (6110) [d]
(7)	SLPC as in (5) except 4 h direct exposure	−7.8	−4.9	5.0	— [e]	— [e]	— [e]
(7b)	Sample 7 after 3 days at room temperature	(−11.7) [f]	(−6.3) [f]	(—) [f,g]			

[a] Sample gave wider transition with less intense low temperature peak after 3 days at room temperature. Samples in hematin plus O_2 or in hematin plus H_2O_2 did not show systematic changes during 1 to 4 days storage at room temperature in DSC pans.

[b] Sample showed a slight 'premelting' beginning at −22°C.

[c] Very small 'premelt' beginning at −17.5°C. This sample had a low temperature shoulder with $T_{max} = -13.6$°C on the first day of dispersion, but no shoulder was present in the same sample when it was analyzed after storage for 4 days at 25°C.

[d] Values in brackets were obtained 26 days after the original extracts were made.

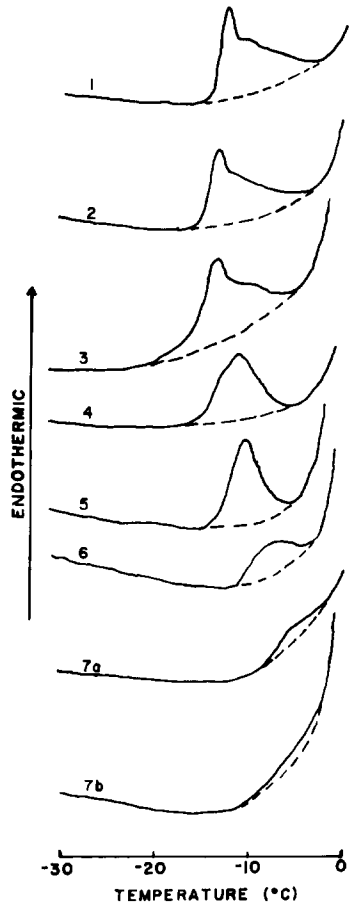
[e] Sample was dropped before analytical data obtained.

[f] Estimates are very approximate because of such a low enthalpy, broad peak on the water melt.

[g] Transition of too small an excess heat capacity for proper estimate.

ments have shown that for samples of SLPC (IV) having values of E_{mol}^{230} in the range of those in Table III(1) to III(3), had some variability in DSC properties which were more likely correlated to the physical nature of the dispersion than with the extent of oxidation [26]. Correlation between increased T_c, altered fatty acid ratio, increased E_{mol}^{230} and decreased enthalpy were apparent when oxidation was greater (Table III(4) to III(7)).

TLC analysis of these lipids showed that a number of bands appeared after ultraviolet treatment (Fig. 4). A major PC spot was seen, but multiple banding occurred in the PC region, something also seen by Shaw and Thompson (1981). When small loads of the samples which had received between 1 and 2.5 h of direct ultraviolet



exposure were analyzed, four bands were seen in the PC region. In order of decreasing mobility they contained 38–40%, 50–53%, 7–8% and trace–5% of the PC phosphorus. The ultraviolet-treated samples also showed some lysophosphatidylcholines – these being 5, 9, and 16% for the lipids treated for 0, 1 and 2 h with direct ultraviolet exposure, respectively. Samples not treated with ultraviolet showed 0–2% lysoPC in the extracts. Samples treated with ultraviolet for 1 and 2 h showed, on charring with 70% H_2SO_4, spots at the origin and just ahead of the origin, but these contained little or no phosphorus (0–3%). Small amounts of P (0.5–3.0%) were observed at or near the solvent front.

The effect of lysostearoylphosphatidylcholine on the phase transition of SLPC. Since there was some lysoPC in the extracts of samples treated with ultraviolet irradiation, samples of SLPC-IV were mixed with lysostearoylPC for analyses. The results of these experiments are shown in Fig. 5 and Table III. The samples containing 8% or 16% lysoPC were designed to nearly correspond to the

Fig. 3. Differential scanning calorimetric thermograms of various dispersions of SLPC (IV). Heating rates were 5 degrees/min and full scale sensitivity was 1 mcal/s. Sample numbers correspond to dispersions in Table II. Baseline was difficult to estimate in 7b.

TABLE III

THERMOTROPIC PROPERTIES OF SLPC: LYSOSTEAROYLPC MIXTURES IN AQUEOUS DISPERSIONS

The samples correspond to the mixtures shown in the thermogram in Fig. 5. Samples in series A were made with lysostearoylPC which was washed twice with diethyl ether. Those in series B were made with lysostearoylPC which was washed four times with ether.

Sample label	Ratio SLPC/lysoPC	T_c	T_{max}	$\Delta T_{1/2}$	ΔH (kcal·(mol P)$^{-1}$)	E_{mol}^{230}
(A1)	100: 0	−15.3	−13.4	4.1	n.d. [a]	n.d. [a]
		(−15.1) [b]	(−12.8)	(4.0)	(3.7)	(97)
(A2)	92: 8	−14.4 [c]	−11.2	4.9	3.1	526
(A3)	84:16	−15.9	−11.7	7.6	1.6	1214
(B1)	100: 0	−15.9	−13.9	4.9	4.3	570
(B2)	92: 8	−15.1 [d]	−10.7	8.7	3.7	420
(B3)	84:16	−13.4 [e]	−10.1	6.4	2.5	640 [f]

[a] Not determined.
[b] Values in brackets from Table II. The same batch of SLPC was used for both control dispersions.
[c] This sample had a 'premelting' region starting at −20.7°C.
[d] This sample had a 'premelting' region starting at −20.0°C.
[e] This sample had a 'premelting' region starting at −20.8°C.
[f] This sample had an A_{mol}^{265} which was about 4–5-times greater than the other B samples.

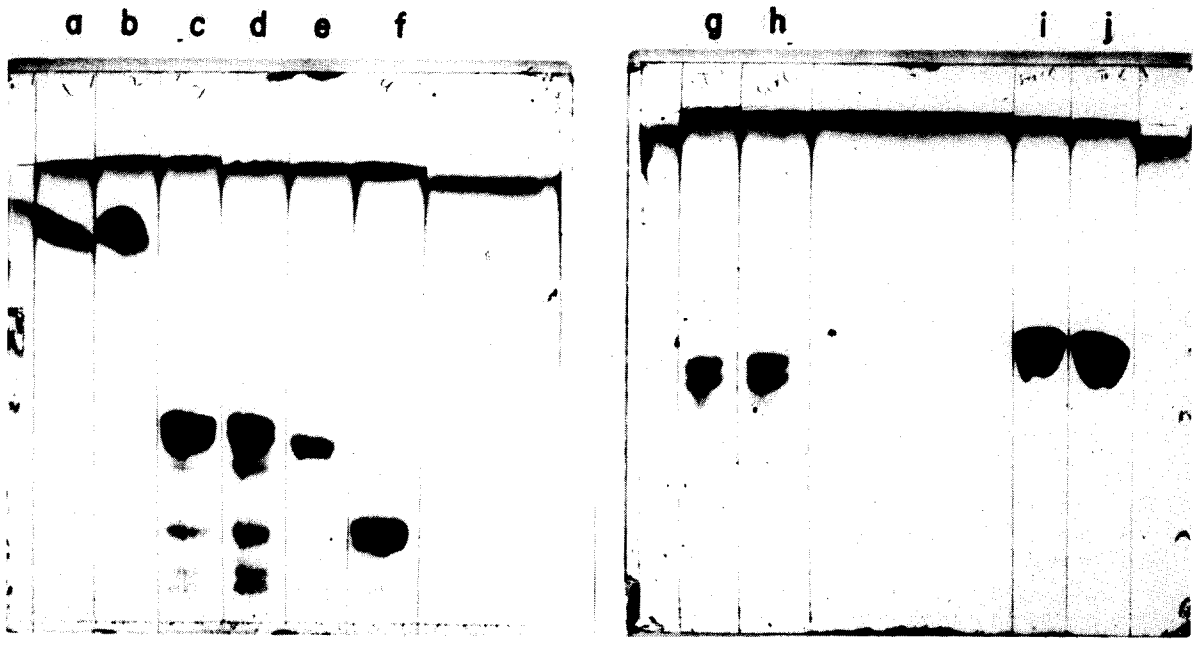


Fig. 4. The analysis of SLPC (IV) treated with ultraviolet irradiation. Silicagel G plates using chloroform/methanol/water 65 : 25 : 4. Plates were charred overnight after spraying with 70% H_2SO_4. a, stearic acid; b, palmitic acid; c, SLPC after 1 h direct ultraviolet, 17 μg P; d, SLPC after 2 h direct ultraviolet, 18 μg P; e, dipalmitoylPC; f, lysoPC; g and h, SLPC after 2.5 h direct ultraviolet (phosphatidylcholine spot isolated by TLC and 6 μg P rechromatographed); i and j, SLPC from stock solution, 21 μg P.

samples treated for 1 and 2 h with direct ultraviolet exposure (see Table II(5) and II(6)). In one experiment (A) there was a very slight rise in the T_c and T_{max} induced by the lysoPC, but in neither case was it as great as that observed for the corresponding ultraviolet-treated sample (Table II and Fig. 3). The lysoPC itself promoted oxidation as is evident by the increased extinction at 230 nm. There was a decrease in enthalpy induced by the lysoPC as well as peak broadening. In a second experiment (B) with more extensively ether-washed lysoPC, there was more pronounced peak broadening in the presence of lysoPC. A comparison of the traces in Fig. 5 with those in Fig. 3 shows that the effects of lysoPC alone were not comparable to the effects of ultraviolet irradiation. The analysis of the extracts of the dispersions of SLPC-lysoPC used in the DSC suggested that a slight additional amount of breakdown to free fatty acids and lysoPC occurred during the DSC runs.

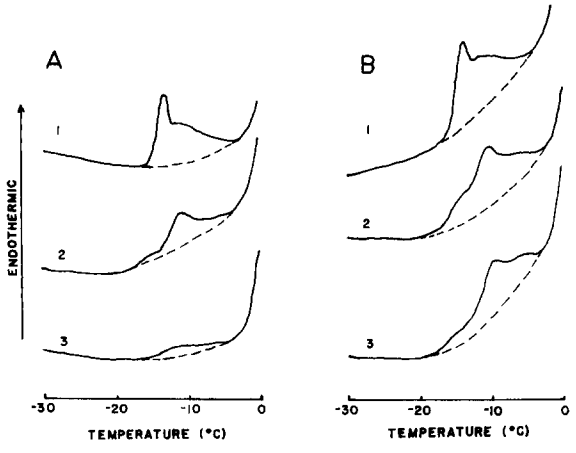


Fig. 5. Differential scanning calorimetric thermograms of dispersions of SLPC (IV) plus lysostearoylPC. Scan rate was 5°/min and full scale sensitivity was 1 mcal/s. Scan numbers refer to dispersions in Table III. A and B represent different experiments with different lysoPC.

Discussion

The relationship between transition temperatures of the two pure polyunsaturated mixed-acid phosphatidylcholines has been discussed elsewhere

[26]. We report here that the gel to liquid crystalline phase transition temperatures of these lipids increased with oxidation. It has been observed in this laboratory, and elsewhere [30] that synthetic preparations of pure polyunsaturated phosphatidylcholines can undergo autoxidation. This is evidenced by the appearance of an absorption maxima near 230 nm. In synthetic lipids made in our laboratory the oxidized material showed only PC on TLC, but fatty acid analysis of the material from the TLC plate showed an apparent loss of unsaturated fatty acid. This is consistent with breakdown of partially oxidized chains in the PC during the methylation procedure [26]. Presumably the linoleate and linolenate had undergone limited changes to produce various conjugated double bond systems, hydroperoxides and possibly epoxy and hydroxy fatty acids [3,32]. On longer storage, e.g., during storage of SLPC-II for 3 months, decomposition of the oxidation products to shorter chains [31,33] and lysoPC had occurred. Such decomposition prior to or during transmethylation could account for the low proportions of linoleate and linolenate found in the oxidized phosphatidylcholines. Also, short-chain fission products which were still attached at the 2-position were likely not detected under the GLC conditions used. Thus, while our synthesized lipids appeared pure by TLC, they could have contained some modified phosphatidylcholines as evidenced by the altered molar ratios of fatty acids.

Ultraviolet irradiation of dried SLPC samples produced effects on the thermotropic properties of lipid dispersions which were similar to those seen during spontaneous oxidation in our synthetic preparations. The transition profiles moved to higher temperatures with increasing oxidation. There was a gradual peak-widening and a reduction in enthalpy when oxidation became extensive. The presence of small amounts of hematin either with O_2 or H_2O_2 did not cause significant changes in thermotropic properties of the SLPC dispersions, nor did they cause significant changes in oxidation as evidenced by fatty acid and TLC analysis. While it was intended that O_2 or H_2O_2 be present in catalytic amounts only, they may have been in too low concentrations to cause significant amounts of lipid to become oxidized in a short time.

In the studies on the ultraviolet-induced oxidation some lysoPC accumulated in the samples which had received substantial irradiation. Adding external lysostearoylPC to unoxidized SLPC produced a marginal increase in T_c or T_{max}, but this was attributable to increased oxidation in the presence of the lysoPC. Addition of 16% lysoPC (equivalent to 2 h ultraviolet exposure) produced a decrease in enthalpy comparable to that seen in the ultraviolet-treated sample. These observations are in keeping with those of van Echteld et al. [34], who observed that the addition of small amounts of lysopalmitoylPC to dipalmitoyl and dioleoylPC caused little change in T_c but a reduction in enthalpies of transition. The increases in transition width and, the decreases in transition enthalpy, but not the increase in T_c, after extensive oxidation may be attributable at least in part to the production of lysoPC, or other breakdown products, in the oxidized lipid samples.

Autoxidation of methyl linoleate has been found to produce four major hydroperoxy and up to six isomeric diperoxide fatty acids, all with conjugated double bond systems [32,35]. Some chains can have one or both double bonds in the *trans* configuration. It can be envisaged that conjugated double bonds would restrict the number of carbon-carbon rotamers more so than would the methylene-interrupted double bond systems of the unoxidized chains. A third double bond may also be present in some of these products [35,36]. Multiple double bonds would restrict the number of conformers available and raise the T_c as has been observed for a series of polyunsaturated phosphatidylcholines with increasing numbers of double bonds [26]. The introduction of *trans* double bonds would allow for tighter packing of the unsaturated chains in comparison to those which are all *cis*. It has been noted that the transition temperatures of PC containing *trans* bonds are substantially higher than those of the corresponding lipids with *cis* double bonds [37,39]. There is a possibility that cross-linked lipids might be present in the ultraviolet-treated samples (represented by one or more of the spots shown in Fig. 4). Shaw and Thompson [9] have also seen multiple bands in the PC region after TLC analysis of oxidized egg yolk PC. Such products may have transition temperatures higher than the corresponding PC.

Eichenberger et al. [16] have suggested such cross-linking might cause the increase in order seen in the acyl chains of microsomal membranes after NADPH-mediated peroxidation. The presence in the bilayers of free, long-chain, fatty acids with melting points, or the creation of mixtures of lysoPC plus long chain fatty acids could conceivably cause a small increase in transition temperature [40–42]. However, any free chains created by autoxidation may be expected to be short scission products which would lower the transition temperature. Also there is little evidence from TLC for substantial amounts of long chain acids (Fig. 4). Eichenberger et al. [16] also observed that there was little fatty acid released during enzymatically-catalyzed peroxidation of liver microsomes.

Whether the changes in T_c seen in these samples would lead to significant changes in order at higher temperatures cannot be stated currently. Pauls and Thompson [17] have shown a dramatic increase in the T_c of isolated bean cotyledon microsomal membranes subjected to ozone-induced lipid peroxidation. After extensive ozone-treatment, the T_c increased from 12°C in the untreated membranes to 52°C in the peroxidized membranes. Such increases in T_c have also been observed for extensively senescing membranes [19,20]. Although peroxidation in the former case was accompanied by loss of phospholipid, the alterations in bonds of the unsaturated chains outlined above could partially account for the increased rigidity observed in these, and other natural membranes [11,12] especially in early stages of autoxidation. The results of van Ginkel and Fork [24] on the change in transition temperatures of ageing thylakoid membranes by monitoring the relative intensity of fluorescence of endogenous chlorophyll *a*, suggest that the temperature of peak fluorescence moved transiently upward and then down with increasing time of storage (see especially Fig. 1d of van Ginkel and Fork [24]). It is of interest that there is some evidence for a similar trend in the sample of SLPC treated with direct ultraviolet exposure for 4 h (Fig. 2(7) and Table II(7)).

These results are consistent with the initial occurrence on autoxidation of more rigid lipids through the formation of conjugated and *trans* double bonds (and possibly cross-linked lipids) which raise the T_c. There may be countervailing, although lesser, destabilizing effects of peroxy, diperoxy, hydroxy and epoxy groups on packing. More substantial destabilization of packing may arise with the production of lysoPC and other breakdown products. Besides changing the physical chemical properties of lipids, oxidation could lead to abnormal membrane activity because of direct modification of proteins by the lipids through covalent interaction with highly reactive oxidation intermediates.

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