Biochimica et Biophysica Acta 861 (1986) 243–250
Elsevier

BBA 73299

Structural simulation of free radical damage in a model membrane system: a small-angle X-ray diffraction study

John Katsaras [a], Robert H. Stinson [a], Edward J. Kendall [b] and Bryan D. McKersie [b]

[a] *Department of Physics and* [b] *Department of Crop Science, Biophysics Interdepartmental Group, University of Guelph, Guelph, Ontario, N1G 2W1 (Canada)*

(Received 26 May 1986)

Key words: Membrane damage; Free radical; Deesterification; Small-angle X-ray diffraction; Differential scanning calorimetry; Radiolysis

Membrane injury is an important factor contributing to cellular death following environmental stress. Recent evidence suggests that in plants, changes in membrane phase properties after exposure to a lethal environmental stress may be a result of lipid deesterification mediated by free radicals. To test this hypothesis, the effects of primary free radicals, produced by the radiolysis of water in the absence of oxygen, upon the structure of a dipalmitoylphosphatidylcholine (DPPC) model membrane system were studied. Small-angle X-ray diffraction and differential scanning calorimetry were used to examine DPPC bilayers exposed to increasing doses of gamma radiation. The electron density distribution of the bilayer did not change with increasing irradiation, but the thickness of the water layer between adjacent bilayers increased significantly. The upper limit of the broadened gel to liquid-crystal transition was increased by 4 to 10 Cdeg by the radiation treatment. Compositional analysis of the DPPC bilayer after irradiation indicated that a substantial portion of the phospholipid had been deesterified. Lysophosphatidylcholine was not detected. The changes in structural properties of the bilayer were simulated when the sodium salt of palmitic acid was incorporated into the DPPC bilayers but not by the addition of the acid itself. A mixture of lysophosphatidylcholine, sodium palmitate and DPPC did not simulate the structural changes. The free radicals generated by irradiation promoted deesterification at both the 1 and 2 positions of the glycerol backbone of DPPC, and the resultant accumulation of fatty acid salt in the bilayer altered its structural properties.

Introduction

Various environmental stresses such as freezing [1] or dehydration [2] can induce significant membrane injury. This damage is expressed as elevated gel to liquid-crystalline phase transition temperatures and increased free fatty acid concentrations in microsomal membranes [3,4]. In dehydration, the deesterification events have been attributed to free radical activity.

There are many reports of free radical damage to biomembranes [5–7] and phosphatidylcholine bilayers [8]. In some cases the free radicals were generated by radiolysis [7,8], as in this paper, and in others they were produced by chemical means [5,6]. The effects of free radicals on bilayers of saturated lipids have received little attention; however, the peroxidation of unsaturated lipids by free radicals has been extensively documented [6,9].

Radiolysis of water in the absence of free

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; lysoPC, lysophosphatidylcholine; GPC, *sn*-glycerol-3-phosphorylcholine.

Correspondence address: Dr. R.H. Stinson, Department of Physics, Biophysics Interdepartmental Group, University of Guelph, Guelph, Ontario, N1G 2W1, Canada.

oxygen results in the formation of primary free radicals such as e^-_{aq}, H', 'OH, and H_2O_2 [7,10]. The 'OH radical damages the head group of 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine [8] with the result that there is increasing water penetration of the bilayer and increased rigidity of the interior of the bilayer.

Free radicals can promote deesterification in complex lipid mixtures. For example, Niehaus [11] shows deesterification of membrane lipids using the superoxide anion radical (O_2^-). Senaratna et al. [4], using xanthine oxidase as a free radical generator, report deesterification of soybean microsomal membrane. Peroxidation of lipids in these systems is very slight. Kong and Davison [7] propose that relatively little membrane damage occurs following radiolysis of water since the free radicals produced are either charged or polar and thus are poorly soluble in the membrane matrix.

These findings prompted us to examine the efficacy of free radical mediated deesterification. Structural changes in the membrane were determined by small-angle X-ray diffraction, differential scanning calorimetry (DSC) and compositional changes by gas chromatography (GC).

In the present study, we report that increased levels of free 16 : 0 chains are present following exposure of the bilayer to free radicals. These chemical changes were accompanied by increases in phase transition temperature and water layer thickness. Similar changes could also be observed in simulations consisting of DPPC model membranes into which the salt of the fatty acid was incorporated.

Materials and Methods

1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC)was obtained from Calbiochem and used as supplied. The quality was checked by DSC and thin-layer chromatography (TLC, $CHCl_3$/CH_3OH/H_2O, 65 : 25 : 5 by volume). The free fatty acid salt used in all experiments was sodium palmitate, which was obtained from Sigma, and its purity was checked by TLC (petroleum ether/diethyl ether/acetic acid, 70 : 30 : 2). The 1-palmitoyl-*sn*-glycero-3-phosphatidylcholine (lyso-PC) was used as supplied by Sigma, as was *sn*-glycero-3-phosphorylcholine (GPC) from Calbiochem. Palmitic acid was obtained from Calbiochem. A 0.05 M potassium phosphate monobasic sodium hydroxide buffer (pH 7.0) was used in all bilayer sample preparations.'

All samples were prepared in an argon environment and all attempts were made to avoid exposure of the chemicals to air. The buffer solution was degassed by repeated freezing and thawing in vacuum.

DPPC bilayers were prepared by adding excess buffer to DPPC in 6 ml polycarbonate centrifuge tubes. These were centrifuged at $180000 \times g$ for 1.5 h, following which the supernatant was removed and the remaining pellet of saturated bilayers was distributed into 2.0 mm o.d. diameter glass capillary tubes which were then sealed and weighed. The weight was monitored throughout the experiments to confirm that there was no loss of water. Each sample tube contained about 3.5 mg of DPPC. The amount of buffer incorporated was between 50 and 60% by weight.

DPPC samples were irradiated using a γ-source (Gammacell 220, Atomic Energy of Canada, Ltd.) for periods ranging from 2 to 10 days. The dose rate was 99350 rad/h ($6.21 \cdot 10^{21}$ eV $\cdot$ kg$^{-1} \cdot$ h^{-1}) and the temperature approx. 25°C. In addition, to achieve very high doses in a reasonable time, a few samples were placed in a high dose rate (1.1 Mrad $\cdot$ h^{-1}) cobalt-60 γ-source.

When additional components were added to the bilayers, a different procedure was adopted to ensure adequate mixing and to prevent loss of any component which might partition into the supernatant. Two- and three-component mixing was achieved by dissolving the desired amount of each component in methanol (Caledon Laboratories). The solution was dried using a rotary evaporator (Buchi Rotavapor) at 35°C and then placed under vacuum for 24 h at room temperature. The powder mixture was then placed in a porcelain dish and suspended in an excess of buffer. The buffer was consequently evaporated to a point whereby the sample weight was between 50 and 50% buffer. Subsequent preparation was the same as for pur DPPC. All samples were stored at −10°C and before use, were annealed by cycling from −10°C to room temperature several times. In addition, to determine the influence of buffer content on *d*-spacing, a series of DPPC/sodium palmitate sam-

ples (28.6 mol% sodium palmitate) of buffer content ranging from 40 to 90% was prepared.

All DSC scans were obtained with a Microcal MC-1 (Amherst, MA) calorimeter at a scan rate of 14.4 Cdeg per h.

Small-angle X-ray diffraction patterns were obtained using a line source from a Franks' camera [12] equipped with a nickel coated mirror. A Philips generator operating at 40 kV and 20 mA provided the copper Kα X-rays. Patterns were detected with a Tennelec (Oak Ridge, TN) linear position-sensitive detector and collected using 512 channels in a Tracor-Northern (Middleton, Wi.) multichannel analyzer. Data were transferred to an IBM PC micro-computer for analysis. The system was calibrated to yield *d*-spacings (Bragg spacings) for various reflections from the location (channel number) of peaks for the constant sample to detector distance of 28.9 cm. To obtain reflection intensities, smooth background curves were drawn on printouts of the patterns and peak areas measured with an electronic planimeter (Numonics Corp.).

The diffraction pattern is the convolution of the Fourier transforms of the bilayer electron density distribution and of the one-dimensional lattice on which the bilayers are located. In a somewhat disorganized system the lattice transform will have an amplitude large enough for a significant sampling of the bilayer transform only at or near those points in reciprocal space corresponding to integral multiples of the reciprocal of the lattice spacing. As the water thickness between bilayers increases, the *d*-spacing increases and the bilayer transform is sampled at locations closer together in reciprocal space. This results in variations of the relative intensities of the diffraction peaks even when the bilayer itself does not change significantly. The measured powder pattern intensities were increased by the Lorenz correction of h, where h is the order number of reflection [13]. In all cases the phases assigned to the first 4 reflexions (where detected) where π, π, 0 and π. These are the accepted phases for DPPC [14] and tests with other phase assignments at all spacings led to very improbable electron density distributions.

Circulating water baths regulated the sample holder on the X-ray diffraction camera to ± 0.5 Cdeg.

Total fatty acid and free fatty acid compositions were determined by gas chromatography (Shimadzu GC-5 with DB-17 megabore column) following methylation using the methods of Morrison and Smith [15] and Walker et al. [16], respectively.

Results

Specific analyses of selected samples indicated that the relative concentration of free 16 : 0 chains rose with increasing irradiance (Table I). TLC and GC performed on the irradiated samples listed in Table I revealed not only phospholipid and free 16 : 0 chains, but also low molar mass components. Diacylglycerols and lysophosphatide were not detected in any sample.

Samples of saturated DPPC bilayers gave small-angle X-ray diffraction patterns (Fig. 1C)

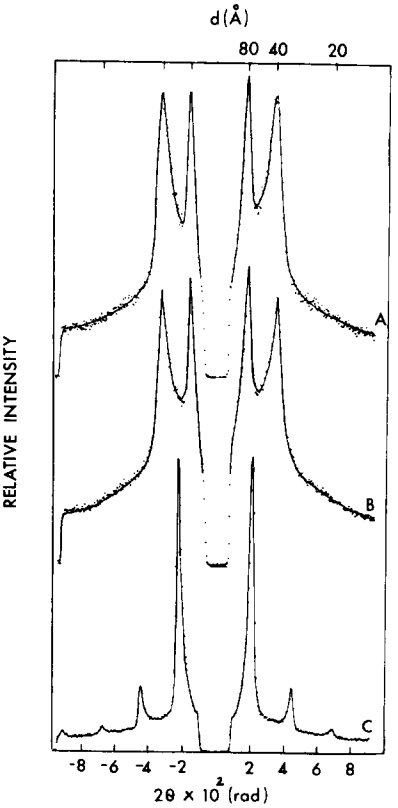

Fig. 1. Comparison of small-angle X-ray diffraction patterns at 4.0 °C for (A) an irradiated DPPC sample (23.8 Mrad), (B) simulation using DPPC and 28.6 mol% of sodium palmitate and (C) DPPC in excess buffer.

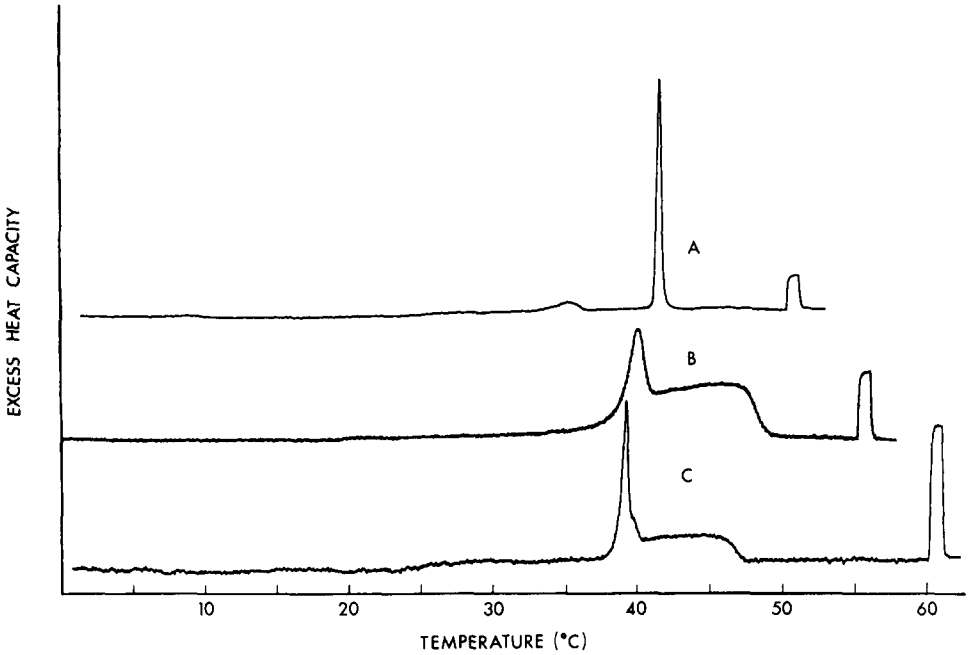

Fig. 2. Comparison of calorimetric transition curves for three systems. (A) DPPC, (B) irradiated DPPC (23.8 Mrad) and (C) simulation using DPPC and 22.2 mol% each of sodium palmitate and GPC. Each scan finishes with a calibration pulse ($1.27 \cdot 10^{-2}$ cal). In all cases shown, the scan rate was 14.4 degC/h.

with 4 orders and low background indicative of a well-ordered system. Corresponding DSC scans exhibited the characteristic pretransition and main transition (Fig. 2A).

Following γ-irradiation of DPPC samples, there were significant changes in small-angle X-ray diffraction patterns (Fig. 1A) and DSC traces (Fig. 2B). At 5.7 Mrad the diffraction pattern (not

TABLE I

SMALL-ANGLE X-RAY DIFFRACTION (4.0°C) AND GC DATA FOR IRRADIATED MODEL MEMBRANES, SIMULATIONS AND DPPC

Sample	*d*-space (Å)	*d*-aq (Å)	*d*-bilayer (Å)	I_1/I_2 [d]	*A* Total palmitate (μmol)	*B* Non-esterified palmitate (μmol)	*B*/*A* (%)
DPPC	63	23	40	5.58	11.0	0	0
DPPC + 5.7 Mrad	66	25	41	4.0	17.1	0.8	4.7
DPPC + 9.4 Mrad	78	36	42	0.53	13.3	0.8	6.0
DPPC + 23.8 Mrad	79	38	41	0.34	6.14	0.67	10.9
DPPC + 185 Mrad	97	45	52	0.56	7.4	3.0	40.5
DPPC/sodium palmitate [a]	80	38	42	0.45	9.9	1.4	14.1
DPPC/sodium palmitate/GPC [b]	79	36	43	1.1	–	–	–
DPPC/palmitic acid [c]	67	27	40	6.08	–	–	–

[a] 28.6 mol% of sodium palmitate.
[b] The molar ratio of DPPC/sodium palmitate/GPC was 75 : 25 : 12.5.
[c] 27.6 mol% of palmitic acid.
[d] I_1/I_2, intensity ratio of first order to second order.

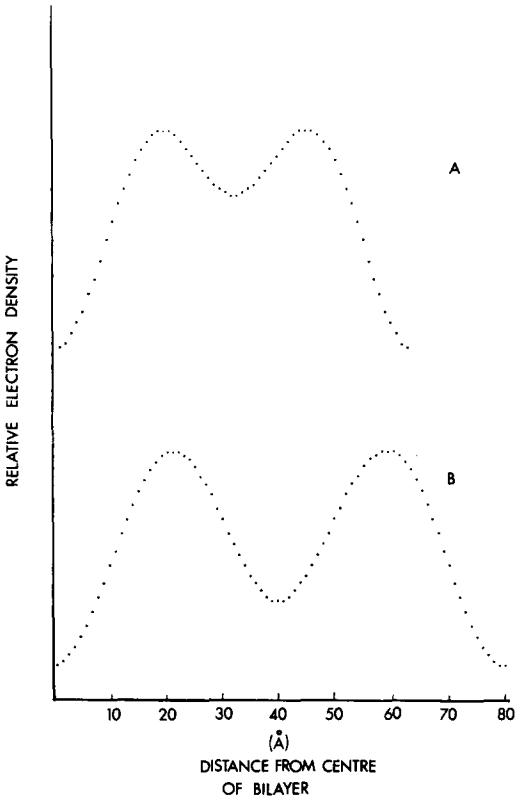

Fig. 3. Comparison of one-dimensional electron density distribution maps of (A) DPPC, from Fig. 1(C), and (B) irradiated DPPC from Fig. 1(A). The difference in *d*-spacing between the two samples can be attributed to the increase in hydration between the polar head-groups. Peaks (high electron density) represent choline head-groups. The electron density map from the simulated sample (Fig. 1B) is indistinguishable from the irradiated sample at this scale.

shown) resembled that of pure DPPC, except that peaks were considerably broader, *d*-spacing increased by 2.5 to 3.0 Å, the background was somewhat elevated and the 4th order could not be observed. These observations are consistent with some membrane disorganization.

At 9.4 Mrad (not shown) and above, the diffraction patterns were qualitatively similar to each other, in that they always showed two broad peaks and elevated background (Fig. 1A), indicating even greater disorganization than at 5.7 Mrad.

Electron density distributions of non-irradiated and irradiated DPPC samples (Fig. 3) showed relatively little change in bilayer thickness, but a significant increase in the thickness of the adjacent water layer following irradiation. Since it was not possible to accurately determine the precise thickness of the water layer, we have arbitrarily recorded the separation between the centres of the high-density peaks to indicate the thickness of the water layer. The influence of irradiation on structural dimensions at 4°C is shown in Table I.

Saturated DPPC (37% buffer) [17,18] has a bilayer thickness very close to 40 Å based on calculations using 1 as the approximate specific gravity of the lipid. The electron density distribution for such a sample also shows a 40 Å bilayer thickness based on peak to peak measurement (Fig. 3A). A series of DPPC/sodium palmitate (28.6 mol%) samples with buffer contents ranging from 40 to 90% by weight showed that saturation occurred by 70% buffer at which point the *d*-spacing was 110 Å. Below saturation the bilayer thickness, as determined by calculations based on relative compositions and densities and also by peak to peak measurements from the electron density distributions, stayed essentially constant at 40 to 45 Å. For example, at 50% buffer (and *d*-spacing of 80 Å) the bilayer thickness calculates as 40.8 Å and the measurement from the electron density distribution (not shown) was 42 Å. The calculation includes a correction for the slight decrease in bilayer density due to the addition of the sodium palmitate. We therefore regard the water layer thickness, as determined from the electron density maps, to be quite reliable.

Precise intensity measurements of the orders (Fig. 1A, B) are difficult, because of uncertainties created by peak overlap and the location of the background curve. A number of approaches were tried, but they all led to electron density profiles that were remarkably similar. The peak to peak separations in the profiles were always the same for a given experiment and only small changes in the depth of the 'valleys' were observed. In all cases only 2 orders were used to generate the electron density profiles, thus producing profiles of comparable resolution. X-ray diffraction patterns and consequently electron density profiles virtually identical to those of the irradiated bilayers could be obtained with samples composed of DPPC and appropriate amounts of sodium palmitate (Fig. 1B) or of DPPC, sodium palmitate and GPC (not shown). Parameters for the simu-

TABLE II

EFFECT OF TEMPERATURE ON SMALL-ANGLE X-RAY DIFFRACTION RESULTS FOR DPPC TREATED WITH 23.8 Mrad

Temperature (°C)	*d*-space (Å)	*d*-aq (Å)	*d*-bilayer (Å)	I_1/I_2 [a]
4.0	92	44	48	0.19
40.0	97	47	50	0.45
50.0	85	39	46	1.32
65.0	80	36	44	2.11

[a] Intensity ratio of first order to second order.

lated systems are shown in Table I.

Table II shows the changes in bilayer properties of a radiation damaged sample as temperature increases. Table III indicates that the transition from the gel phase for this irradiated (23.8 Mrad) sample would begin at less than 40°C and be completed at 49.2°C. As melting progresses there is a slight decrease in bilayer thickness and a significant decrease in the thickness of the adjacent water layer. The degree of order in the sample, as indicated by diffraction peak width, showed very little change with temperature.

DSC results for DPPC are compared with those of irradiated samples and simulations (Fig. 2; Table III). In all samples irradiated to more than 5.2 Mrad and in simulations, the main transition peak (T_p) occurred at a lower temperature compared to DPPC. In some cases, the beginning of the transition (T_0) could not be accurately determined. Pretransitions were not observed in irradiated membranes or in binary or tertiary mixtures. T_f, which corresponded to the completion of the gel to liquid-crystal transition, increased with increasing doses of radiation and was in all cases elevated with respect to that of DPPC.

TABLE III

DSC DATA SHOWING TRANSITION PROPERTIES OF DPPC, IRRADIATED DPPC AND DPPC MIXTURES

Abbreviations: T_0, start of gel to liquid crystal transition; T_p, main transition peak; T_F, end of gel to liquid-crystal transition.

Sample	T_0 (°C)	T_P (°C)	T_F (°C)
DPPC	41	41.5	43.5
DPPC + 5.2 Mrad	40.2	42.5	47.6
DPPC + 23.8 Mrad	–	40	49.2
DPPC + 185 Mrad	–	38.8	53.8
DPPC/sodium palmitate [a]	–	38.9	46.6
DPPC/sodium palmitate/GPC [b]	37	38.7	45.5
DPPC/palmitic acid [c]	42.5	–	52.5
DPPC/sodium palmitate/GPC [d]	37.7	39.4	47.6

[a,b] and [c] See footnotes to Table I.

[d] The molar ratio of DPPC : sodium palmitate : GPC was 77.8 : 22.2 : 22.2.

A DPPC/palmitic acid/GPC sample (28.6 mol% of the acid) and a DPPC/palmitic acid sample (27.6 mol% of the acid) gave DSC scans (not shown) very similar to that of Mabrey and Sturtevant [19] who used a DPPC/palmitic acid (27.8 mol% of the acid) mix with a 0.01 M sodium phosphate buffer (pH 7.0). The scan showed a broad transition extending from about 42.5°C to about 52.5°C and did not resemble the scan of the irradiated material (Fig. 2B). The small-angle X-ray diffraction pattern of the DPPC/palmitic acid sample was virtually indistinguishable in general appearance from that of pure DPPC (Fig. 1C) although the *d*-spacing increased slightly to 67 Å.

Alternative mixtures tested to see if they would simulate the radiation damaged systems consisted of DPPC plus varying amounts of lysoPC plus sodium palmitate with the mole ratio of the latter two components always 1 : 1. Neither the small-angle X-ray diffraction patterns nor the DSC scans (not shown) resembled those from the radiation damaged material or the DPPC/sodium palmitate or DPPC/sodium palmitate/GPC mixtures.

Discussion

These results support the hypothesis that deesterification of membrane lipids by free radicals can cause some of the membrane lipid to remain in the gel phase at physiologically high temperatures. Thus, it seems likely that a similar mechanism will account for the elevated phase-transition temperatures demonstrated by stress-damaged membranes [3,4].

In this study, the fully saturated phospholipid DPPC ws chosen to avoid the complications of peroxidation side-reactions. Free radicals were generated by gamma irradiation to avoid problems associated with adding other materials (e.g.,

biochemical induction of free radicals) to the samples.

The results indicate that the free radicals generated possess significant deesterification activity in the absence of oxygen. The characteristic small-angle X-ray diffraction pattern of DPPC underwent a profound change as a result of even moderate irradiation and this changed pattern could be closely simulated by the inclusion of sodium palmitate in a mixture with DPPC.

Simulations with DPPC, lysoPC and sodium palmitate did not yield diffraction patterns resembling those of the damaged material and lysoPC was not detected with TLC. Thus, it seems that a first deesterification may leave a lysoPC which is much more susceptible to a further deesterification than is an integral DPPC. If this occurred, samples would then contain GPC which might enter the water phase and consequently have little effect on bilayer structural changes, GPC consists of only the backbone and headgroup of the phospholipid.

Addition of GPC to binary DPPC/sodium palmitate samples gave results which were similar to those from just DPPC/sodium palmitate (Tables I and III), which supports the suggestion that GPC is in the water phase.

Schullery et al. [20] report differential thermal analysis data for mixtures of DPPC with sodium palmitate. Their results like ours, show a depression of the main transition temperature but do not show the broad transition at higher temperatures until much higher sodium palmitate content than used in our experiments. However, there were differences in procedure. They used excess water, whereas, we used buffer. Our DSC scans were at a much lower rate (14.4 Cdeg $\cdot$ h^{-1}) than their differential thermal analysis thermograms (5 Cdeg $\cdot$ min^{-1}). Our scans were not made until samples had equilibrated sufficiently to yield the characteristic X-ray diffraction pattern, whereas they approached equilibrium by repeated heating in a jet of steam, etc. until their sample was visually homogeneous.

Kohn and Schullery [21] present a complex phase diagram of DPPC and palmitic acid in excess water. At concentrations similar to those used in our experiments they propose that, at low temperatures, a gel phase of palmitic acid/DPPC (2 : 1) forms with the excess DPPC in a gel $L_{\beta'}$ phase. On heating, the mixture exhibits a sharp endotherm at about 44°C due to melting of the $L_{\beta'}$ portion to an L_{α} phase followed by a broad transition corresponding to the melting of the palmitic acid/DPPC (2 : 1) mix. A somewhat similar process may occur in our samples.

Small-angle X-ray diffraction patterns and DSC scans of membranes incorporating palmitic acid rather than the sodium salt of the acid did not resemble those of the radiation-damaged material. Palmitic acid has a pK_a of 10.2 in a DPPC bilayer [20] and thus would be protonated in our samples. The protonated acid is less hydrophilic than the sodium salt; consequently, the water layer thickness and DSC scans would be different for DPPC membranes containing palmitic acid than those containing the sodium salt.

The crucial point is that increasing radiation caused increasing free radical formation leading to increasing deesterification and an increase in the maximum temperature at which some gel phase remained.

The unusually broad peaks in the diffraction patterns (Fig. 1A, B), indicated significant disorganization in the damaged bilayers. Fourier transforms of these patterns yielded low resolution electron density distributions (Fig. 3) that indicated relatively little change in the bilayer but a significant increase in the thickness of the adjacent water layers (Table I).

All DPPC samples, prior to irradiation, possessed an excess of buffer. Consequently there was buffer available to move into the space between bilayers as radiation damage occurred. There was essentially no change in the bilayer thickness either in the irradiated samples or those simulations where sodium palmitate was added to DPPC. The increase in *d*-spacing for these samples was almost entirely due to an increase in the water layer thickness. While these samples were not fully saturated, all water in excess to DPPC only had been drawn into the interbilayer space.

It is important to have buffer in excess of that required to saturate DPPC for two reasons. First, radiation induced deesterification is probably optimized when the buffer thickness in the DPPC samples is at a maximum, and second, it is necessary to have some buffer free to move into the

interbilayer region. Excess buffer sufficient to saturate the irradiated (or simulated) samples was not used, except for calibration purposes, because of the poor quality of the diffraction patterns from such samples. Buffer in the range of 50–60% by weight satisfied all these requirements.

We speculate that the insertion of sodium palmitate in the DPPC bilayer has separated the choline head-groups and has led to a net increase in hydrophilic groups per unit area, thus contributing to the increased interbilayer hydration. Also, soap charge-induced bilayer repulsion may be contributing to the increase in hydration.

Acknowledgements

We wish to thank Drs. Jim H. Davis, Mike R. Morrow and J.B.M. Rattray for helpful discussions. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada.

References

1 Kendall, E.J., McKersie, B.D. and Stinson, R.H. (1985) Can. J. Bot. 63, 2274–2277
2 McKersie, B.D. and Stinson, R.H. (1980) Plant Physiol. 66, 316–320
3 Senaratna, T., McKersie, B.D. and Stinson, R.H. (1984) Plant Physiol. 76, 759–762
4 Senaratna, T., McKersie, B.D. and Stinson, R.H. (1985) Plant Physiol. 77, 472–474
5 Cohen, G. (1978) Photochem. Photobiol. 28, 669–675
6 Kellogg, E.W. and Fridovich, I. (1975) J. Biol. Chem. 250, 8812–8817
7 Kong, S. and Davison, A.J. (1981) Biochim. Biophys. Acta 640, 313–325
8 Barber, D.J.W. and Thomas, J.K. (1978) Radiat. Res. 74, 51–65
9 Bland, J. (1978) J. Chem. Educ. 55, 151–155
10 Swallow, A.J. (1973) Radiation Chemistry, John Wiley & Sons, New York
11 Niehaus, W.G., Jr. (1978) Bioorg. Chem. 7, 77–84
12 Franks, A. (1955) Proc. Phys. Soc. B 68, 1054–1064
13 McIntosh, T.J., Simon, S.A., Ellington, J.C., Jr. and Porter, N.A. (1984) Biochemistry. 23, 4038–4044
14 Torbet, J. and Wilkins, M.H.F. (1976) J. Theor. Biol. 62, 447–458
15 Morrision, W.R. and Smith, L.M. (1964) J. Lipid Res. 5, 600–608
16 Walker, M.A., Roberts, D.R. Dumbroff, E.B. (1982) J. Chromatogr. 241, 390–391
17 Powers, L. and Pershan, P.S. (1977) Biophys. J. 20, 137–152
18 Inoku, Y. and Mitsui, T. (1978) J. Phys. Soc. Japan 44, 1918–1924
19 Mabrey, S. and Sturtevant, J.M. (1977) Biochim. Biophys. Acta 486, 444–450
20 Schullery, S.E., Seder, T.A., Weinstein, D.A. and Bryant, D.A. (1981) Biochemistry, 20, 6818–6824
21 Kohn, A.B. and Schullery, S.E. (1985) Chem. Phys. Lipids 37, 143–153