lized from ethanol: mp 210 °C (sublim); IR (KBr) 3600-2800, 2920, 1620, 1440 cm^{-1}; ^1H NMR ($CDCl_3$) δ 13.6-11.6 (1 H, br s, D_2O-exchange), 7.77 (1 H, s), 7.67 (1 H, d, J = 1.8 Hz), 7.49 (1 H, s), 6.74 (1 H, m), 3.10 (4 H, s); MS, m/e 187 (M^+).

2-Oxo-1,2-dihydrofuro[3,2-*g*]quinoline (20). (a) To a stirred solution of **19** (37 mg, 0.20 mmol) and triethylamine (0.05 mL, 0.38 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise at 0 °C methanesulfonyl chloride (0.02 mL, 0.3 mmol). The resulting mixture was stirred for 20 min at this temperature, diluted with ether, washed with 10% HCl and brine, dried over Na_2SO_4, and condensed under the reduced pressure to give 7-(methanesulfonyloxyimino)-6,7-dihydro-5*H*-1-oxa-*s*-indacene (46 mg, 100%) as a solid which was recrystallized from ethyl acetate/*n*-hexane to give colorless crystals: mp 130 °C dec; ^1H NMR ($CDCl_3$) δ 7.91 (1 H, s), 7.74 (1 H, d, J = 2.2 Hz), 7.55, (1 H, s), 6.79 (1 H, m), 3.26 (3 H, s), 3.19 (4 H, s).

(b) Beckmann rearrangement was performed by the modified method of Yamamoto et al.[16] To a solution of the above mesylate (35 mg, 0.15 mmol) in CH_2Cl_2 was added at -70 °C a 15% hexane solution of diethylaluminum chloride (0.55 mL, 0.45 mmol) and the resulting solution was stirred for 30 min at this temperature. After being warmed up to room temperature, the mixture was stirred for further 1 h and quenched with aqueous 5% NaOH solution (3 mL). Extraction with CH_2Cl_2 and evaporation of the solvent gave **21** as a solid (18 mg, 65%) which was recrystallized from ether to give yellow crystals: mp 214-217 °C; IR ($CHCl_3$) 3400, 3000, 1685, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.8-8.4 (1 H, br s), 7.55 (1 H, d, J = 2.0 Hz), 7.37 (1 H, s), 6.99 (1 H, s), 6.69 (1 H, m), 3.3-2.9 (2 H, m), 2.8-2.5 (2 H, m); MS, m/e 187 (M^+), 159.

(c) A mixture of the above lactam (30 mg, 0.16 mmol) and 10% Pd/C (30 mg) in diphenyl ether (1 mL) was refluxed for 4 h under Ar. The workup as described above and chromatography on silica gel (*n*-hexane/ethyl acetate = 4:1) afforded **20** (9 mg, 28%) as colorless crystals: mp 180-190 °C dec; IR ($CHCl_3$) 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.0-7.4 (1 H, br s), 7.90 (1 H, d, J = 9.5 Hz), 7.78 (1 H, s), 7.66 (1 H, d, J = 2.2 Hz), 7.48 (1 H, m), 6.82 (1 H, dd, J = 2.2, 1.0 Hz), 6.67 (1 H, d, J = 9.5 Hz); high-resolution MS, m/e (M^+) calcd for $C_{11}H_7NO_2$, 185.0476; found: 185.0450.

1-Oxo-1,2,3,4-tetrahydrofuro[3,2-*g*]isoquinoline (22). A mixture of **19** (37 mg, 0.20 mmol) and polyphosphoric acid (PPA) (1 g) was heated at 90 °C for 3 h. After addition of water, the product was extracted with ether, and the organic phases were washed with aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. Evaporation of the solvent in vacuo gave **22** (21 mg, 57%) as a colorless solid: IR ($CHCl_3$) 3410, 2920, 1670 cm^{-1}; ^1H NMR δ 8.27 (1 H, s), 7.74 (1 H, d, J = 2.2 Hz), 7.43 (1 H, s,), 6.76 (1 H, m), 6.2-5.8 (1 H, br s), 3.8-3.4 (2 H, m), 3.2-2.8 (2 H, m).

Registry No. 1, 91798-67-3; **3a**, 41438-24-8; **3b**, 2433-64-9; **3c**, 66434-99-9; **3d**, 2433-57-0; **3e**, 91798-68-4; **3f**, 91164-84-0; **3g**, 91798-69-5; **4**, 21860-46-8; **5**, 91798-70-8; **6**, 91798-71-9; **7a**, 91798-72-0; **7b**, 91798-73-1; **8a**, 91798-74-2; **8b**, 91798-75-3; **9a**, 91798-76-4; **9b**, 91798-77-5; **10a**, 91798-78-6; **10b**, 91798-79-7; **11**, 66-97-7; **12**, 91798-80-0; **13**, 91798-81-1; **14**, 91798-82-2; **15**, 91798-83-3; **16**, 91798-84-4; **17**, 91798-85-5; **18**, 91798-86-6; **19**, 91798-87-7; **20**, 91798-88-8; **21**, 91798-89-9; **22**, 91798-90-2; (*E*)-PhSCH=CHC(O)CH_3, 33944-98-8; (*Z*)-PhSCH=CHC(O)CH_3, 33944-97-7; CH≡CCH_2Br, 106-96-7; $CH_3C(O)CH_2C(O)OEt$, 141-97-9; CH≡$C(CH_2)_2C(O)CH_2C(O)OEt$, 35116-07-5; CH≡$C(CH_2)_2C(O)(CH_2)_2SPh$, 91798-94-6; (*E*)-PhSCH=CHC(O)$(CH_2)_2C$≡CH, 91798-95-7; (*Z*)-PhSCH=CHC(O)$(CH_2)_2C$≡CH, 91798-96-8; furan, 110-009; 2-methylfuran, 534-22-5; 1*H*-pyrrole, 109-97-7; 1-methyl-1*H*-pyrrole, 96-54-8; 1*H*-pyrazole, 288-13-1; 1*H*-imidazole, 288-32-4; 6-(dimethylamino)fulvene, 696-68-4; *trans*-4-(2-furyl)-3-buten-2-ol, 79380-04-4; ethyl 2-[2-(3-butynyl)-1,3-dioxolan-2-yl]acetate, 91798-91-3; 2-(3-butynyl)-2-(2-hydroxyethyl)-1,3-dioxolane, 91798-92-4; 2-(3-butynyl)-2-[2-(phenylthio)ethyl]-1,3-dioxolane, 91798-93-5; neopentyl glycol, 126-30-7; 3,4-dihydropsoralen, 6544-89-4; 7-[[(methylsulfonyl)oxy]imino]-6,7-dihydro-5*H*-1-oxa-*s*-indacene, 91798-97-9.

Autoxidation and Aggregation of Phospholipids in Organic Solvents

L. Ross C. Barclay,*† J. Mark MacNeil,† JoAnn VanKessel,† Bruce J. Forrest,‡ Ned A. Porter,*⊥ Laura S. Lehman,⊥ Karl J. Smith,⊥ and Joe C. Ellington, Jr.⊥

Contribution from the Department of Chemistry, Mount Allison University, Sackville, N.B., Canada E0A 3C0, Department of Chemistry, Dalhousie University, Halifax, N.S., Canada B3H 4J3, and Paul M. Gross Chemical Laboratories, Duke University, Durham, North Carolina 27706. *Received March 9, 1984. Revised Manuscript Received June 27, 1984*

Abstract: The ^{31}NMR Pr^{3+} shift reagent method indicates that phospholipids, dipalmitoylphosphatidylcholine (DPPC), dilinoleoylphosphatidylcholine (DLPC), and egg lecithin (ELPC) aggregate in organic solvents benzene, chlorobenzene, and *o*-dichlorobenzene to form reverse micelles with aggregation numbers in the range 80-100 when the water/phospholipid mole ratio is 20/1. In the presence of lower water/phospholipid ratios (ca. 2 to 16) in these solvents, the ^{31}P NMR method used with both inorganic, Pr^{3+}, and organic-soluble shift reagent $Pr(DPM)_3$ indicates the presence of both monomers and aggregates, the latter increasing regularly with the water content. Sedimentation results on ELPC in *o*-dichlorobenzene show the presence of aggregates in the absence of added water. There was no evidence for aggregation of a phospholipid in the protic solvent *tert*-butyl alcohol (^{31}P method). Product studies of conjugated hydroperoxides from autoxidation of DLPC and 1-palmitoyl-2-linoleoylphosphatidylcholine (1P-2LPC) in organic solvents, compared to these products from methyl linoleate and linoleic acid, indicate that these phospholipids aggregate in organic solvents and this influences the kinetics and product distribution of autoxidation. The kinetics of autoxidation of ELPC and DLPC thermally initiated with di-*tert*-butyl hyponitrite in organic solvents are studied. The rates of photochemically initiated autoxidation of DLPC in organic solvents are accelerated by added water. The increased rate is shown to be related to the fraction of phospholipid aggregated into reverse micelles. The oxidizability of an unsaturated phospholipid (DLPC) in reverse micelles is estimated to be 2 or 3 times higher than that of a homogeneously dispersed substrate. The oxidizability of DLPC in a bilayer is similar to that in *homogeneous* solution.

Lipid peroxidation, the uncontrolled reaction of lipids and molecular oxygen, is a threat to aerobic organisms. The autoxidation of polyunsaturated fatty acids present in phospholipids that make up biomembranes affects cell structure and function, for example, through increased cell membrane permeability.[1]

Recognition of the significance of lipid peroxidation to important pathological events has attracted increased interest in the autoxidation of biologically important molecules[2-4] and the study of

† Mount Allison University.
‡ Dalhousie University.
⊥ Duke University.

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Table I. Parameters for DPPC and DLPC Inverted Micelles at 52 °C

lipid	solvent	lipid concn, mM	D_2O concn, mM	aggregation no.[a]
DPPC	benzene	40.9	829	84
DPPC	benzene	55	1105	92
DPPC	benzene	82	1660	90
DPPC	chlorobenzene	27	552	89
DPPC	chlorobenzene	55	1105	88
DPPC	chlorobenzene	82	1660	96
DPPC	*o*-dichlorobenzene	27	552	47
DPPC	*o*-dichlorobenzene	55	1105	67
DPPC	*o*-dichlorobenzene	82	1660	86
DLPC	chlorobenzene	26	552	90
DLPC	chlorobenzene	51	1105	98
DLPC	chlorobenzene	77	1660	105

[a] The literature value for DPPC in benzene is 92–94.[12]

the mechanism of autoxidation inhibition.[5]

Recent quantitative kinetic studies have been made of the autoxidation of phospholipid bilayers,[6,7] and quantitative studies have also been made on the products (hydroperoxides) of autoxidation of polyunsaturated fatty acids in solution[8] and the products from synthetic phospholipid bilayers.[9] The product studies[8,9] show that the products of autoxidation in solution and in the lipid bilayer are analogous and also indicate that factors affecting product distribution in solution are also important in the bilayer. On the other hand, the kinetic studies[7] indicated that the oxidation of egg lecithin in chlorobenzene is dramatically different from its oxidation in bilayers. In particular, the oxidizability of egg lecithin in chlorobenzene was reported to be some 40-fold that of egg phosphatidylcholine in aqueous emulsions.

It has been known for many years that phospholipids form inverted (reverse) micelles in nonpolar solvents such as benzene, especially in the presence of water.[10] These micelles have also been considered as models for biological systems.[11]

In order to develop a better understanding of the difference of autoxidation in lipid bilayers and in organic solvents, we have carried out a collaborative study of (1) the aggregation of phospholipids in organic solvents, (2) product analysis of autoxidation of unsaturated phospholipids in these solvents compared to that of methyl linoleate and linoleic acid, and (3) the relationship between the phenomena in (1) and (2) and the kinetics of autoxidation in organic solvents.

Results

(1) Aggregation Studies by ^{31}P NMR. ^{31}P NMR coupled with Pr^{3+} shift reagent has proved to be effective in determining the aggregation number for 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) in benzene.[12] In this method, the lanthanide ions (L) inside the micelle induce a downfield hyperfine shift of the ^{31}P head-group resonances of the phospholipid molecules making up the inside surfaces. With use of the Poisson law, the ratio of areas of two adjacent NMR peaks (i.e., a sharp peak with no lanthanide ion and one with one ion) is given by the equation

$$p(L)/p(L-1) = \bar{L}/L$$

(p(L) = the probability of finding a micelle with L lanthanide ions.) Since $\bar{L}$ can be expressed in terms of known stoichiometric concentrations, $\bar{L} = [Ln^{3+}]\bar{n}/[PPC]$, a simple relationship was derived[12] to measure the average aggregation number, $\bar{n}$:

$$\bar{n} = L\frac{p(L)}{p(L-1)}\frac{[PPC]}{[Ln^{3+}]}$$

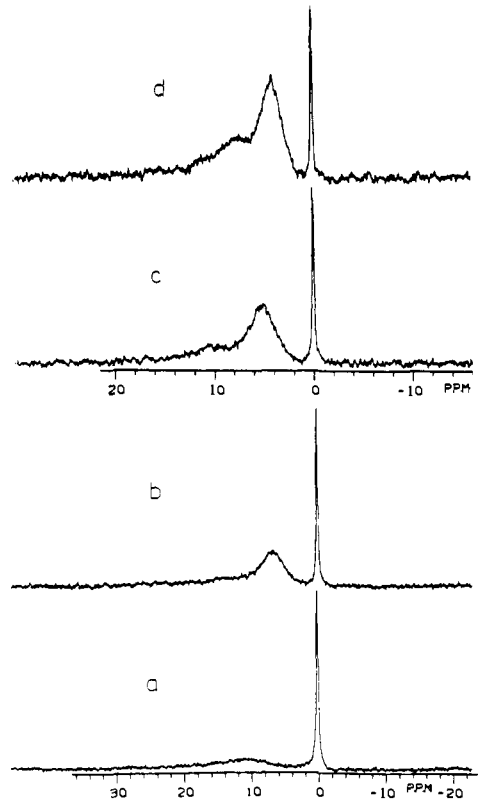

Figure 1. ^{31}P NMR spectra of dilinoleoylphosphatidylcholine (DLPC) (1.44×10^{-2} M) and $Pr(NO_3)_3$ (3.10×10^{-4} M) in H_2O/benzene: (a) H_2O/DLPC = 4.24, (b) H_2O/DLPC = 8.58, (c) H_2O/DLPC = 11.5, (d) H_2O/DLPC = 14.4.

We anticipated that this method would be a sensitive probe for the degree of micellization. This is important for our kinetic studies since the rates of autoxidation in organic solvents proved to be sensitive to the water content of the solvents which in turn affects the micellization (vide infra).

We have determined aggregation numbers for DPPC, 1,2-dilinoleoyl-*sn*-glycero-3-phosphatidylcholine (DLPC), and egg yolk phosphatidylcholine (ELPC) in benzene, chlorobenzene, and *o*-dichlorobenzene by the Pr^{3+} shift method. Aggregation numbers for a relatively high D_2O (or H_2O)/phospholipid molar ratio of 20/1 are given in Table I, it being assumed that the Pr^{3+} ions are randomly distributed among the micelles according to the Poisson distribution[12] for these relatively high $[PC]/[Pr^{3+}]$ ratios of approximately 130–530. We also monitored aggregation number vs. time for DLPC inverted micelles that were undergoing oxidation. Aggregation was maintained in the inverted micelles with as much as 50% of the linoleate acyl chains oxidized. Thus 64 mM DLPC and 1105 mM D_2O in chlorobenzene gave an initial aggregation number of 95 and after 3 days and 50% oxidation (as monitored by reverse phase hplc), the aggregation number was found to be 100 (error of analyses ±7%).

In order to determine the effects of relatively small amounts of water on aggregation, we started with anhydrous (as possible) solutions of phosphatidylcholines in the organic solvents and followed the change in aggregation on addition of water, conditions which approximate the parallel kinetic studies (vide infra). Typical spectra are shown in Figure 1 for DLPC in benzene to illustrate the effect of varying water content (H_2O/PPC 4.0 to ca. 15.0) on the ^{31}P spectra. Similar results were obtained with various combinations of phospholipids with benzene, chlorobenzene, and *o*-dichlorobenzene. In these experiments, the Pr^{3+} concentration was increased to the range $[PPC]/[Pr^{3+}] \simeq 45$–53 to ensure that

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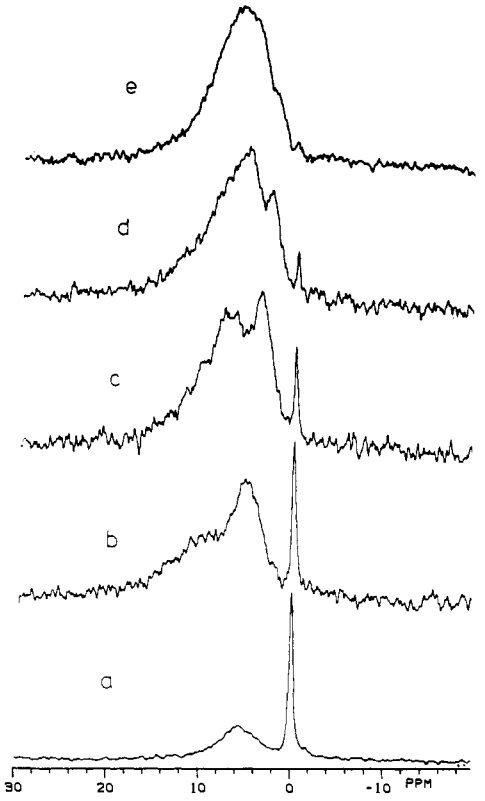


Figure 2. ^{31}P NMR spectra of egg lecithin (ELPC) (1.625×10^{-2} M) and $Pr(NO_3)_3$ in H_2O/benzene: (a) ELPC/Pr^{3+} = 52.4, H_2O/ELPC = 6.84; (b) ELPC/Pr^{3+} = 34.9, H_2O/ELPC = 8.55; (c) ELPC/Pr^{3+} = 34.9, H_2O/ELPC = 12.0; (d) ELPC/Pr^{3+} = 34.9, H_2O/ELPC = 15.4; (e) ELPC/Pr^{3+} = 34.9, H_2O/ELPC = 18.8.

any micelles would be populated with at least one Pr^{3+}. This procedure should detect *unmicellized* PPC's (if any). The results are reported in Table II in terms of the percent of the ^{31}P signal shifted downfield. In addition, experiments were carried out on ELPC in benzene where *both* the water content and the Pr^{3+} concentration were varied. Typical spectra are shown in Figure 2. As the Pr^{3+} concentration was increased from [PC]/[Pr^{3+}] = 52.4 to 34.9 where [H_2O]/[PC] = 8.55, the sharp upfield peak was still present. The latter diminished as shown with higher water content.

In the protic solvent *tert*-butyl alcohol, only one ^{31}P signal was obtained which simply broadened at higher Pr^{3+} and higher water content (Table II, last two entries). This result is interpreted in terms of lack of formation of reverse micelles, the line broadening being due to an averaging of the shift effect by the Pr^{3+} on homogeneously distributed DLPC.

A few experiments were carried out with an organic shift reagent, $Pr(DPM)_3$, as another probe to detect unmicellized PPC's. In this case, it was thought that only those PPC molecules in the bulk solvent free to interact with the shift reagent could experience the shift effect. This effect was indeed observed (Figure 3). Although the overlap of signals makes quantitative analysis difficult, qualitatively the results confirm what is suggested from the results with varying water content, namely, that the unshifted signal (high Pr^{3+}) is due to *unmicellized* PPC's. Thus with equimolar concentrations of $Pr(DPM)_3$ and DLPC in *dry* chlorobenzene, most of the signal is shifted (less than 30% not affected) indicative of a high proportion of monomers (or small aggregates). On the addition of water (H_2O/lipid = 8.13) to such a sample (mole ratio $Pr(DPM)_3$/DLPC = 0.50), it is estimated that 80% of the DLPC becomes micellized. This is in good agreement with the percent signal shifted under these conditions but with the Pr^{3+} shift reagent (Table II).

Finally, more definitive results were obtained when combined Pr shift reagnets were used: Pr^{3+} in the water phase and $Pr(DPM)_3$ in the organic phase. With Pr^{3+} alone in the system containing DLPC, the usual signals were observed (Figure 3).

Table II. Effect of Water Content and [Pr^{3+}] on the ^{31}P Spectra of Phospholipids in Organic Solvents

lipid	solvent	[lipid], mM	[lipid]/[Pr^{3+}]	[H_2O]/[lipid]	% signal shifted[a]
ELPC	benzene	16.2	46.8	4.1	
ELPC		16.2	46.8	5.82	64.9
ELPC		16.2	46.8	8.03	78.4
ELPC		16.2	46.8	12.3	90.5
DLPC	benzene	14.4	46.5	1.93	20.4
DLPC		14.4	46.5	4.24	49.8
DLPC		14.4	46.5	8.58	71.6
DLPC		14.4	46.5	11.5	81.8
DLPC		14.4	46.5	14.4	90.4
DLPC	chlorobenzene	16.4	52.9	1.70	32.0
DLPC		16.4	52.9	4.06	54.7
DLPC		16.4	52.9	8.13	79.8
DLPC		16.4	52.9	11.9	91.3
DLPC		16.4	52.9	14.1	93.7
DLPC	*o*-dichlorobenzene	13.9	44.8	4.0	47.9
DLPC		13.9	44.8	8.0	72.5
DLPC		13.9	44.8	12.0	86.6
DLPC		13.9	44.8	14.0	90.4
DPPC	benzene	74	109	0.82	
DPPC		74	109	4.10	16.1
DPPC		74	109	8.20	28.2
DPPC		74	109	12.3	41.0
DPPC		74	109	16.6	46.3[b]
DLPC	*tert*-butyl alcohol	16.3	354	7.6	
DLPC		16.3	32	51.0	

[a] The percent of the total signal shifted downfield from the sharp ^{31}P signal set = 0. [b] The aggregation number calculated under these conditions was 92.

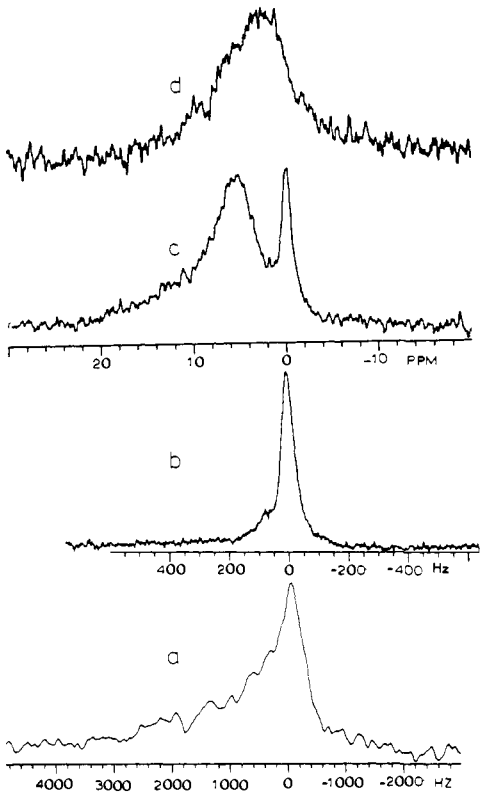


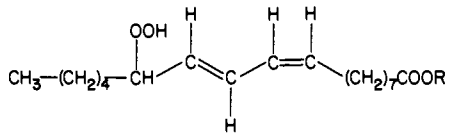
Figure 3. ^{31}P NMR spectra of dilinoleoylphosphatidylcholine (DLPC) in organic solvents containing shift reagents: (a) DLPC (1.64×10^{-2} M) and organic shift reagent, $Pr(DPM)_3$ (1.64×10^{-2} M) in dry chlorobenzene; (b) DLPC (1.64×10^{-2} M) and $Pr(DPM)_3$ (8.20×10^{-3} M) in chlorobenzene containing H_2O (H_2O/DLPC = 8.47). Using a deconvolution program, approximately 20% of the signal is estimated to be under shoulder downfield from the main signal: (c) DLPC (1.39×10^{-2} M), DLPC/Pr^{3+} = 44.8, H_2O/DLPC = 12.0; (d) the solution from part c to which was added $Pr(DPM)_3$(2.40×10^{-2} M).

Now when the organic solvent-soluble $Pr(DPM)_3$ is added, all of the sharp upfield peak collapses into the rest of the broad signal.

(2) Aggregation Studies by Sedimentation. It was desirable to have an independent method of measuring aggregation to compare with the ^{31}P studies. Therefore we applied the ultracentrifugation method to a few samples. ELPC, 15.6 mM in *o*-dichlorobenzene at 30 °C *without* added water, gave a molecular weight of 16000 ± 4000. On addition of water to $[H_2O]/[lipid] = 10$, the molecular weight increased to 36000 ± 4000. This would correspond to an average aggregation number of 45 ± 5. These results demonstrate that aggregates exist, at least in this solvent, even in the absence of added water and that as expected the amount of aggregation increases with the water content.

(3) Product Studies. Linoleic acid ((*Z*),(*Z*)-9,12-octadecadienoic acid) (Lin-H), methyl linoleate (Me-Lin), 1-palmitoyl-2-linoleoylglycerophosphatidylcholine (1P-2-LPC), and 1,2-dilinoleoylglycerophosphatidylcholine (DLPC) were oxidized in organic solvents. In one oxidation experiment, 1,2-dipalmitoylglycerophosphatidylcholine (DPPC) was mixed with 1P-2-LPC. Autoxidations were initiated by di-*tert*-butyl peroxyoxalate[13] or di-*tert*-butyl hyponitrite[14] at 30 °C and were carried out under air or pure oxygen.

Major products of the autoxidation of linoleic acid and methyl linoleate were the four major hydroperoxides described earlier.[9] These hydroperoxide products, **1–4**, were reduced with triphenylphosphine and analyzed by normal-phase high-pressure liquid chromatography (HPLC).



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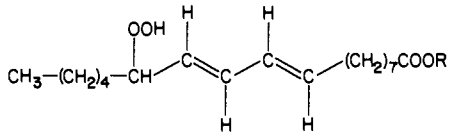

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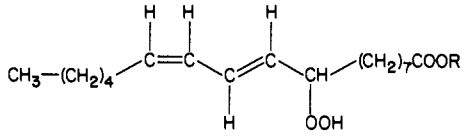

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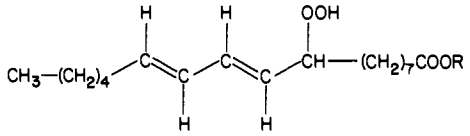

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Analogous hydroperoxide products were formed from 1P-2LPC and DLPC. For the phospholipid products, hydroperoxides were reduced with triphenylphosphine, and the glyceroesters were converted into methyl esters **1–4** by treatment with methanol/KOH. The compounds **1–4**, derived in this way from the phospholipids, were analyzed by HPLC.

Product distributions were monitored as a function of time of reaction and extent of oxidation. For autoxidations of free acid or methyl esters, the distribution of products (as measured by the ratio trans,cis/trans,trans products (**1** + **3**/**2** + **4**) changed very little in the early stages of oxidation (<10%). The ratio of trans,cis/trans,trans products decreased slightly with time in oxidations of phospholipids, and the value given was obtained at 5% oxidation or less. In Table III is given the product distribution for autoxidation of linoleic acid and methyl linoleate at 30 °C in benzene or chlorobenzene. Table IV shows product distributions obtained from autoxidation of linoleoyl phospholipids in benzene, chlorobenzene, and *o*-dichlorobenzene.

Table III. Products vs. Substrate Concentration for Autoxidation of Methyl Linoleate and Linoleic Acid at 30 °C

substrate	concn	solvent	t,c/t,t[a,b]
Me-Lin	0.002	Ph-Cl	0.18
Me-Lin	0.105	Ph-Cl	0.22 (0.02)
Me-Lin	0.098	Ph-H	0.21
Lin-H	0.24	Ph-H	0.24
Me-Lin	0.342	Ph-Cl	0.27 (0.02)
Me-Lin	0.34	Ph-H	0.33
Me-Lin	0.50	Ph-H	0.30 (0.01)
Lin-H	0.50	Ph-H	0.34 (0.01)
Me-Lin	0.50	Ph-Cl	0.31 (0.01)
Lin-H	0.50	Ph-Cl	0.38 (0.02)
Me-Lin	1.0	Ph-Cl	0.59 (0.03)
Me-Lin	1.0	Ph-H	0.54 (0.02)
Me-Lin	1.5	Ph-H	0.71 (0.01)
Me-Lin	1.5	Ph-Cl	0.77 (0.01)

[a] Ratio of products (**1** + **3**)/(**2** + **4**). [b] Values given in parentheses are standard errors. Over 80 separate analyses were made.

Table IV. Products vs. Substrate Concentration for Autoxidation of Linoleate Phospholipids in Organic Solvents at 30 °C

substrate	concn[a]	solvent	t,c/t,t[b,c]
DLPC	0.28	Ph-H	0.54 (0.02)
DLPC	0.26	Ph-Cl	0.55 (0.01)
DLPC	0.13	Ph-H	0.48 (0.03)
DLPC	0.13	Ph-H	0.45 (0.01)
DLPC	0.005	Ph-H	0.48 (0.04)
DLPC	0.0002	Ph-H	0.51 (0.04)
DLPC	0.001	Ph-Cl	0.48 (0.01)
1P-2LPC	0.002	Ph-H	0.32 (0.01)
1P-2LPC	0.04	Ph-Cl	0.33 (0.02)
1P-2LPC	0.11	Ph-Cl	0.34 (0.03)
1P-2LPC	0.15	Ph-Cl	0.40 (0.02)
1P-2LPC	0.20	Ph-Cl	0.40 (0.02)
1P-2LPC	0.00025	*o*-dichlorobenzene	0.30 (0.01)
1P-2LPC	0.0025	*o*-dichlorobenzene	0.31 (0.01)
1P-2LPC	0.0125	*o*-dichlorobenzene	0.31 (0.01)
1P-2LPC	0.025	*o*-dichlorobenzene	0.31 (0.01)
1P-2LPC	0.05	*o*-dichlorobenzene	0.33 (0.01)
1P-2LPC	0.1	*o*-dichlorobenzene	0.34 (0.01)
1P-2LPC	0.2	*o*-dichlorobenzene	0.40 (0.02)
1P-2LPC/DPPC[d]	0.002	Ph-H	0.21 (0.02)
1P-2LPC/DPPC[d]	0.0002	Ph-H	0.22 (0.02)

[a] Concentration of linoleate. For DLPC, twice the concentration of phospholipid. [b] Ratio of products (**1** + **3**)/(**2** + **4**). [c] Values given in parentheses are standard errors. [d] 1:10 ratio of 1P-2LPC:DPPC, total lipid concentration as given.

(4) Kinetics. The results of both the ^{31}P NMR and the product studies indicate that DLPC and ELPC aggregate in the nonprotic solvents used when water is present. Furthermore, the sedimentation analysis shows that ELPC will aggregate at least in *o*-dichlorobenzene without added water.

In order to carefully follow the effect of water-promoted aggregation on the kinetics, the synthetic phospholipid DLPC was selected for a quantitative study since the linoleate chain has a known oxidizability (vide infra). A series of measurements were carried out on predried DLPC in *initially dry* solvents to which known amounts of water were then added. The initiator was decomposed photolytically so that the rate of initiation (R_i) could be followed after each addition of water by measuring the dependence of the rate on the light intensity. The results are summarized in Table V along with some related thermally initiated rates and calculated values for aggregation and rates (see Discussion).

Discussion

(1) Aggregation Studies. Several methods have been presented in the literature showing that phospholipids aggregate into inverted micelles in organic solvents.[10,12,15] We studied aggregation in a

(13) Bartlett, P. D.; Benzing, E. P.; Pincock, R. E. *J. Am. Chem. Soc.* **1960**, *82*, 1762.
(14) Mendenhall, G. D. *J. Am. Chem. Soc.* **1974**, *96*, 5000.

number of solvents with various water content in order to relate aggregation under various conditions to our other studies on these systems, i.e., product and kinetic studies.

Our ^{31}P NMR aggregation studies in the presence of a water/phospholipid molar ratio of 20/1 (Table I) show that both unsaturated and saturated phospholipids aggregate in inert solvents commonly used for oxidation studies. The analysis by the method of Chen and Springer[12] gives aggregation numbers generally in the range 80–100. Aggregation is also maintained in a highly oxidized substrate.

Sedimentation analysis on a sample of ELPC in *o*-dichlorobenzene *without* added water, conditions where the ^{31}P method is not applicable, also indicates that a phospholipid will readily undergo aggregation in the absence of water. On the other hand, there was no evidence for aggregation into reverse micelles in *tert*-butyl alcohol by the ^{31}P NMR method.[16] Such a hydrophilic solvent apparently removes the tendency for a phospholipid to aggregate around added water. Such a solvent is therefore a useful isotropic medium for comparative studies on monomeric phospholipids.

The ^{31}P NMR method was used in several ways to show that monomers and micelles can be detected under appropriate conditions in organic solvents

(1) A series of experiments with higher $[PPC]/[Pr^{3+}] \simeq 45-53$ and varying water content (H_2O/PPC ca. 2–16) (Figure 1, Table II) illustrate that the percent ^{31}P signal shifted follows the amount of water.

(2) When the Pr^{3+} was increased to $[PPC]/[Pr^{3+}] \simeq 35$, which is high enough to ensure that *all micelles present* are populated with Pr^{3+} ions[12] and would have their ^{31}P signals shifted, the sharp upfield peak was still observed until additional water was added (Figure 2). This result is exactly what would be expected if the sharp upfield peak is due to unmicellized material and we are observing additional aggregation as more water is added until essentially all of the substrate is micellized (Figure 2e).

(3) The above conclusions were confirmed by using the organic shift reagent $Pr(DPM)_3$ to detect unmicellized PPC's in the organic phase. With this reagent *alone*, the ^{31}P signals for monomers and micelles are not well resolved. However, *qualitatively* the results confirm our findings using the resolved signals and Pr^{3+}. Namely, with $Pr(DPM)_3$ and DLPC in *dry* chlorobenzene, one observes a very broad shifted signal estimated to be ca. 70% of the absorption (Figure 3a). When water is added to this sample (H_2O/PC = 8.47) the shifted peak is now only a small shoulder on the main peak (Figure 3b) and the shifted signal, assigned now to monomers, is estimated to be ca. 20% of the total PPC.

Results were more definitive when *both* Pr^{3+} and $Pr(DPM)_3$ shift reagents were used. One starts by using conditions to generate both signals under conditions where the sharp unshifted peak is assigned to monomers (H_2O/DLPC = 12, PPC/Pr^{3+} = 44.8, Figure 3c) and then add $Pr(DPM)_3$. The narrow upfield peak now completely collapses into the remainder of the signal (Figure 3d). The narrow peak in Figure 3c can now be assigned entirely to monomeric PPC's. This provides convincing evidence that reverse micelles and monomers can co-exist and can be detected under appropriate conditions of water content.

These findings indicate that a water content in the range H_2O/PPC $\simeq$ 16–20 is required to micellize all of the phospholipids in benzene, chlorobenzene, and *o*-dichlorobenzene. Furthermore, below this water content we can assume that the unshifted peak in the presence of Pr^{3+} represents unmicellized PPC. A plot of percent signal shifted vs. H_2O/PPC (Figure 4) illustrates that the trend is very similar for the three solvents. The percent signal shifted can be taken as the micellized fraction of PPC. This information is important to the analysis of our kinetic data.

(2) Product Studies. The product studies reported here (Tables III and IV) provide independent evidence for aggregation of

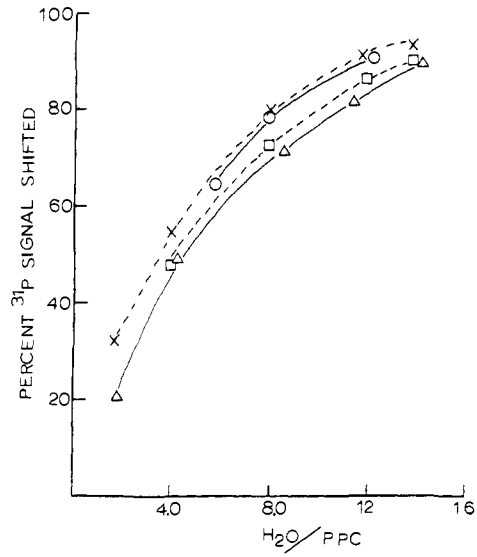

Figure 4. Effect of water content on ^{31}P NMR of phospholipids in organic solvents containing Pr^{3+}. Data are taken from Table II. (×) DLPC (16.4 mM) in chlorobenzene, DLPC/Pr^{3+} = 52.9; (□) DLPC (13.9 mM) in *o*-dichlorobenzene, DLPC/Pr^{3+} = 44.8; (△) DLPC (14.4 mM) in benzene, DLPC/Pr^{3+} = 46.5; and (○) ELPC (16.2 mM) in benzene, ELPC/Pr^{3+} = 46.8.

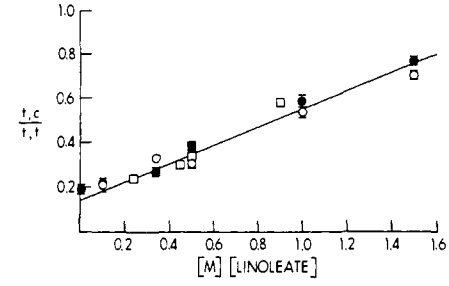

Figure 5. Ratio of linoleate oxidation products from autoxidation of linoleic acid or methyl linoleate in benzene or chlorobenzene: (□) linoleic acid in benzene, (■) linoleic acid in chlorobenzene, (○) methyl linoleate in benzene, and (●) methyl linoleate in chlorobenzene.

phospholipids in organic solvents and indicate that such aggregation affects the autoxidation. We have earlier shown[8] a direct relationship between the ratio of hydroperoxides formed (trans,cis/trans,trans) and the concentration of the oxidizable substrate. Kinetic analysis[8] suggests that the ratio of products is related to substrate concentration [R-H] directly as shown below, where *a* and *b* are constants:

$$\frac{\text{trans,cis}}{\text{trans,trans}} = \frac{\mathbf{1}+\mathbf{3}}{\mathbf{2}+\mathbf{4}} = a + b[\text{R-H}]$$

This relationship is demonstrated for linoleic acid or methyl linoleate in benzene or chlorobenzene in Table III and Figure 5. The ratio of products is independent of the nature of the substrate (free acid or methyl ester) in either chlorobenzene or benzene solvent. These experiments thus give an isotropic reference point where aggregation is not a factor and local concentration and bulk concentration are equal.

The data for phosphatidylcholine oxidation in organic solvents are revealing (Table IV and Figure 6). Of particular interest is the fact that, with a given concentration and solvent, the product ratio (trans,cis/trans,trans) increases in the order linoleate, 1P-2-LPC, DLPC. At 0.25 M, for example, the product ratio increases as follows: linoleate or linoleic acid = 0.22, 1P-2-LPC = 0.40, DLPC = 0.52. We suggest that this product difference is due to variations of the local linoleate concentration in these separate experiments.

With methyl linoleate and linoleic acid, the bulk concentration and local concentration are equivalent, while in the phospholipid systems with inverted micelles and local linoleate concentration

(15) Braedley, R. J.; Grant, D. H.; Reinsborough, V. C.; Ross, P. A. *Can. J. Chem.* **1976**, *54*, 3070.

(16) The reported molecular weight of egg lecithin in methanol corresponds to a trimer while larger aggregates form in longer chain alcohols. Elworthy, P. H.; McIntosh, D. S. *J. Pharm. Pharmacol.* **1961**, *13*, 633.

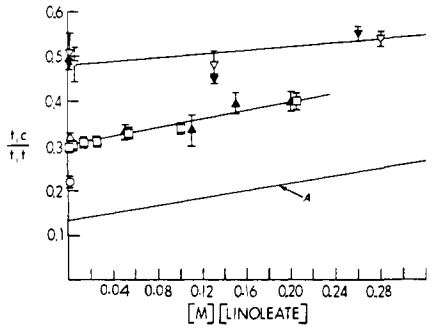

Figure 6. Ratio of linoleate oxidation products from autoxidation of 1-palmitoyl-2-linoleoylphosphatidylcholine (1P-2LPC) and dilinoleoylphosphatidylcholine (DLPC) in benzene, chlorobenzene, and *o*-dichlorobenzene; (▽) DLPC in benzene, (▼) DLPC in chlorobenzene, (□) 1P-2LPC in *o*-dichlorobenzene, (▲) 1P-2LPC in chlorobenzene, (△) 1P-2LPC in benzene, and (○) as in footnote *d*, Table IV. Line A is the least-squares fit from Figure 5.

in the micelle is significantly higher than the bulk linoleate concentration. This high local concentration is reflected in the high (trans,cis/trans,trans) product studies. For DLPC the local linoleate concentration is even higher than is the case of 1P-2-LPC. The former has two linoleate acyl chains, and this assures that at least one other linoleate will be nearby when the first linoleate of a phospholipid is undergoing oxidation.

A "local concentration" can be estimated by using the data presented in Figures 5 and 6. For example, in the concentration range for oxidation of egg lecithin and DLPC ((0.625–2.50) × 10^{-2} M), DLPC gives a trans,cis/trans,trans product ratio in the range 0.48–0.49 (Figure 6). If we now translate this product ratio to the plot of isotropic linoleates (Figure 5), we find that a product ratio of 0.48–0.49 corresponds to a bulk concentration of linoleate of approximately[9] 0.87 M. This indicates that the local inverted micelle concentration can be 35 to 140 times higher than the bulk concentration. It is interesting to note that the aggregation numbers found by the ^{31}P NMR method were also within this range (Table I).

One other observation supports the proposal that the trans,-cis/trans,trans ratio for oxidation in organic solvents is unusually high because of aggregation. While oxidation of a 2 × 10^{-3} M solution of 1P-2LPC in benzene gives a product ratio of 0.32, addition of 20 × 10^{-3} M DPPC to this mixture results in a product ratio (0.21) substantially lower than the value obtained with 1P-2LPC alone. This is consistent with a proposal that 1P-2LPC is diluted in the reverse micelles by addition of DPPC, a phospholipid that has no readily abstractable hydrogens. Thus, DPPC acts as a diluent and the local micelle linoleate concentration drops because of this dilution factor.

These observations on the aggregation and trans,cis/trans,trans product ratios have very important implications on kinetic studies of autoxidation in any solvents where reverse micelles form.

(3) Autoxidation Kinetics and Aggregation. The peroxidation of linoleic acid and other polyunsaturated fatty acids in homogeneous solution, like a wide variety of organic substrates, occurs via a radical chain reaction consisting of initiation, propagation, and termination steps:

Initiation

$$\text{formation of R}\cdot \text{ (or ROO}\cdot\text{) rate} = R_i$$

Propagation

$$\text{R}\cdot + \text{O}_2 \xrightarrow{\text{fast}} \text{ROO}\cdot$$

$$\text{ROO}\cdot + \text{R-H} \xrightarrow{k_p} \text{ROOH} + \text{R}\cdot$$

Termination

$$2\text{ROO}\cdot \xrightarrow{2k_t} \text{nonradical products} + \text{O}_2$$

The rate of oxygen consumption for this process follows the kinetic expression

$$-\frac{d[O_2]}{dt} = \frac{k_p[\text{R-H}]R_i^{1/2}}{(2k_t)^{1/2}}$$

The rate of chain initiation, R_i, can be controlled by using an initiator with a known rate constant for decomposition, k_i. Because not all of the radicals escape the solvent cage where they are produced, the actual R_i has to be measured, for example, by the inhibitor method with use of a phenolic antioxidant,[7] where

$$R_i = 2[\text{ArOH}]/\tau$$

The efficiency of chain initiation can then be expressed as $e = R_i/2k_i[\text{In}]$ and the chain length ν as $\nu = d[O_2]/dt/R_i$. Under these controlled conditions, the "oxidizability" of an organic substrate, $k_p/(2k_t)^{1/2}$, can be determined since

$$\frac{k_p}{(2k_t)^{1/2}} = \frac{-d[O_2]/dt}{[\text{R-H}]R_i^{1/2}}$$

The question which we wish to address here is—*In what manner does aggregation in reverse micelles affect the rates of autoxidation (and presumably the oxidizability) of phospholipids in organic solvents*?

The effect of aggregation on the rate of autoxidation is observed in a qualitative way by comparing the thermally initiated (DBHN) rate in benzene with that in benzene–water where complete micellization is observed (Table V, last two entries). The rate is more than doubled when complete aggregation occurs. Obviously one cannot expect to quantitatively study the dependence of rate on substrate concentration under these conditions where the solutions are not homogeneous. In fact we do find that the rates do not follow the substrate concentration for ELPC or DLPC in benzene, chlorobenzene, or *o*-dichlorobenzene, required for the classical autoxidation equation to be followed for a homogeneous solution. The effect of aggregation on the rates was not considered when an unusually high oxidizability for ELPC in chlorobenzene was reported earlier.[7] Aggregates form in *o*-dichlorobenzene and presumably in chlorobenzene under ordinary conditions (i.e., without additional water). This means that the reported oxidizability is not significant since we do not know if the substrate concentration is to be taken on the basis of the solvent volume (2.00 mL in the case of monomers) or the "micelle volume" if the substrate were completely micellized.

The experiments designed to determine the effect of water content on the rates of autoxidation (Table V) more explicitly indicate the effect of the fraction micellized on the measured rates. It is interesting to note that the measured rates of autoxidation of DLPC in initially anhydrous benzene, chlorobenzene, and *o*-dichlorobenzene all increase markedly on initial addition of water (e.g., the rates approximately double for a H_2O/PPC mole ratio in the range 5–8) and then the rate accelerations level off as the water content is increased to nearly H_2O/PPC ≃ 18. This is also the same kind of trend observed for the effect of water on the aggregation fraction as measured by the ^{31}P NMR method. One can extrapolate the micelle fraction from Figure 4 for the water content used in each solvent for the kinetics and these fractions are used in column 2 of Table V. Obviously the measured rates are increasing with the estimated increases in the micelle fraction. On the other hand, the rate in *tert*-butyl alcohol is not significantly increased on the addition of water, and this solvent does not lend itself to micelle formation according to the ^{31}P results.

In order to deal with the kinetics in these rather complex heterogeneous systems, we postulate that autoxidation is occurring in the bulk solvent as well as in the micellized phase and the measured rate thus depends on the fraction micellized. If it is assumed that the reactions are proceeding essentially independently in these two phases, we can calculate the rates from a linear combination of the rate typical of a homogeneous solution of DLPC plus the rate observed in an essentially micellized system if we know the appropriate fractions of homogeneous material and micellized material. For purposes of the calculation, we take the rate in *tert*-butyl alcohol (1.59 × 10^{-6} M s^{-1} at R_i = 2.63 × 10^{-8} M s^{-1}) as representing the rate in homogeneous solution. For

a micellized system we take the observed rate in water–benzene (Table V) (17.4×10^{-7} M s^{-1} at $R_i = 0.828 \times 10^{-8}$ M s^{-1}). In order to compare these, they must be placed on the same basis (i.e., the same R_i) so the rate in *tert*-butyl alcohol is corrected as follows:

$$\text{rate monomer} = (1.59 \times 10^{-7}\ \text{M s}^{-1})\left(\frac{0.828 \times 10^{-8}}{2.63 \times 10^{-8}}\right)^{1/2} = 0.892 \times 10^{-7}\ \text{M s}^{-1}$$

Now one can calculate the rate for a mixed monomer plus micelle system for any R_i by using the relationship:

$$\frac{-dO_2/dt}{R_i^{1/2}} = ((0.892 \times 10^{-7}\ \text{M s}^{-1})\ F_{\text{monomer}}) + ((17.4 \times 10^{-7}\ \text{M s}^{-1})F_{\text{micelle}})$$

These calculated rates are shown in the last column of Table V. There is excellent agreement with the measured rates (Table V). The average deviation from the measured rates is only 14% for the whole set, with the maximum single deviation being 28%.

Part of our question on the effect of aggregation on kinetics is answered. The rates in aggregates are higher, and indeed for mixtures of monomers and micelles one observes an average of the rates for the dispersed monomers and for the reverse micelles. The question of the oxidizability of the phospholipids in reverse micelles is more difficult to answer because of certain basic unknown features such as the reaction volume to use for calculations. Nevertheless, the oxidizabilities can be *estimated* by combining our various aggregation and kinetic studies in two independent ways.

(i) Oxidizabilities from ^{31}P NMR Aggregation and Rates of Autoxidation. If one assumes that the reaction volume is the same as the "dry" micelle volume,[17] the oxidizabilities where aggregation is essentially complete (95%) can be calculated from the measured rates in Table V where H_2O/PPC = 17.8. The oxidizability values for DLPC in benzene, chlorobenzene, and *o*-dichlorobenzene are then 0.121, 0.135, and 0.113 $M^{-1/2}$ $s^{-1/2}$, respectively.

(ii) Oxidizabilities from Product Studies and Rates of Autoxidation. Product studies have shown that, in the range of phospholipid bulk concentration of $0.625–2.50 \times 10^{-2}$ M used for kinetic studies, the result of aggregation is to increase the local linoleate concentration to an estimated 0.87 M. An oxidizability can be calculated from the equation $-d[O_2]/dt/[R\text{-}H]R_i^{1/2}$ by correcting each of the concentration terms to 0.87 M. If this is done for each of the rates measured for H_2O/PC = 17.8 in benzene, chlorobenzene, and *o*-dichlorobenzene, the oxidizability values are 0.130, 0.144, and 0.121 $M^{-1/2}$ $s^{-1/2}$, respectively, which are in good agreement with calculations based on the ^{31}P NMR method.

Summary and Conclusions

1. The ^{31}P NMR studies show that phosphatidylcholines aggregate in nonprotic solvents, benzene, chlorobenzene, and *o*-dichlorobenzene containing water to give aggregation numbers in the range 80–100 for a H_2O/PPC ratio of 20/1. In the presence of less water (H_2O/PPC $\simeq$ 4 to 12), the ^{31}P NMR method, employing the organic shift reagent, $Pr(DPM)_3$, as well as Pr^{3+}, gives evidence for both micelles and monomers in these solvents, the micellized fraction increasing as the water content increases.

2. Product studies of the trans,cis/trans,trans ratios of hydroperoxides formed in autoxidation of unsaturated phospholipids, 1P-2LPC and DLPC, show that these ratios are substantially higher than the product ratios from methyl linoleate or linoleic acid in these solvents. This provides independent evidence for aggregation of these phospholipids in such solvents.

3. The rates of autoxidation of DLPC in benzene, chlorobenzene, and *o*-dichlorobenzene increase with an increase in water content in the solvents, the measured rates being an average of the calculated rates for micellized phospholipid plus that calculated for homogeneously dispersed substrate.

The oxidizability of an unsaturated phospholipid *homogeneously dispersed* in an organic solvent is not so unusually high as reported earlier.[7] The value per single linoleate chain for DLPC in *tert*-butyl alcohol = 0.0315 $M^{-1/2}$ $s^{-1/2}$, which is similar to that for methyl linoleate in chlorobenzene (0.021 $M^{-1/2}$ $s^{-1/2}$)[18]. We have recently found the oxidizability of DLPC in multilamellar liposomes, thermally initiated by di-*tert*-butyl hyponitrite, to be 0.049 $M^{-1/2}$ $s^{-1/2}$ or 0.0245 per linoleate chain.[19] This means that the oxidizability of a linoleate chain organized in a bilayer is very similar to that in *homogeneous* solution. There is little or no reduction of the value in the bilayer. The oxidizability of unsaturated phospholipids (e.g., DLPC) organized in reverse micelles appears higher, by a factor of 2 to 3 times, than that of homogeneously dispersed material. If we assume that the linoleate chains within each phospholipid monomer and between adjacent monomers are all "lined up" adjacent to each other in the reverse micelle, it is reasonable to postulate that such a specific arrangement could cause a higher propagation rate constant, k_p, compared to randomly dispersed material. This would result in a higher overall oxidizability.

Experimental Section

Fatty acids and esters (99%+) were obtained from Nu Chek Prep (Elysian, MN) and used without further purification. Phospholipids were either purchased from Avanti Polar Lipids (Birmingham, AL) or prepared by known methods.[20–22] Di-*tert*-butyl peroxyoxalate and di-*tert*-butyl hyponitrite were prepared by known methods.[13,14] Bisdicyclohexylazonitrile from Polysciences, Inc., was recrystallized from cold methanol before use.

Aggregate Studies. (a) ^{31}P NMR spectra for determination of aggregation numbers were obtained at 52 °C for DPPC samples and at 30 °C for DLPC samples on a JEOL JNM FX 90 Q FT NMR spectrometer operating at 36.2 MHz.

Inverted micelles comprised of either DPPC or DLPC were prepared according to procedures similar to those of Chen and Springer.[12] Twenty microliters of 10 mM $Pr(NO_3)_3$ in D_2O were added to 40 mg of DPPC. One milliliter of deuteriobenzene was added to the DPPC/$Pr(NO_3)_3$/D_2O mixture. The solution was agitated for several minutes and then warmed above the phase transition temperature of DPPC (42 °C). The solution was then sonicated for 5 min on power level 2 with a Heat Systems W-375 Sonicator equipped with a standard microtip. Initially a turbid solution was formed, which disappeared after about 2 min of sonication. After the sonication was completed, the sample was maintained above the phase transition temperature of DPPC until a ^{31}P NMR spectrum could be obtained.

Other DPPC inverted micelle samples were prepared with lipid concentrations ranging from 20 to 80 mg/mL in deuteriobenzene, chlorobenzene, and *o*-dichlorobenzene by the same procedure outlined above. DLPC inverted micelle samples were prepared in a similar fashion at 30 °C. The D_2O/lipid molar ratio was maintained constant for all preparations. For DPPC inverted micelle samples, the water to lipid molar ratio was approximately 20.3/1, and for DLPC inverted micelles a ratio of 21.6/1 was used. Inverted micelle samples were also prepared with $La(NO_3)_3$ substituted for $Pr(NO_3)_3$. Other samples were prepared without water and shift reagent present or with water only.

(b) ^{31}P NMR spectra for determining the effect of varying water and shift reagents were obtained at 30 °C on a Nicolet 360NB FT NMR spectrometer. T_1 was found to be $^1/_2$ s for the broad shifted peak and

(17) For purposes of this calculation, the volume used for the expression $-(d[O_2]/dt)/[R\text{-}H]R_i^{1/2}$ was estimated as the volume of "neat" phospholipid using a density = 0.8,[7] and this volume was used in the units for $-d[O_2]/dt$, [R–H], and $R_i^{1/2}$, respectively. The actual micelle volume will probably be larger due to the containment of water and solvent. A doubling of the micelle volume used in the calculations increases the resulting oxidizability by approximately 40%.

(18) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1967**, *45*, 793. We recently determined the oxidizability of methyl linoleate (2.26×10^{-2} to 9.04×10^{-2} M) in chlorobenzene initiated by azobisisobutyronitrite to be 0.023 ± 0.003 $M^{-1/2}$ $s^{-1/2}$, in agreement with the literature.

(19) Barclay, L. R. C.; Locke, S. J.; MacNeil, J. M.; Van Kessel, J.; Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1984**, *106*, 2479.

(20) Gupta, C. M.; Radhakrishan, R.; Khorana, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4315.

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(22) Mason, T.; Broccoli, A. V.; Huang, C. H. *Anal. Biochem.* **1981**, *113*, 96.

Table V. Aggregation and Rates of Autoxidation of Dilinoleoylphosphatidylcholine (3.13×10^{-5} mol) in Organic Solvents (2.00 mL) Photoinitiated[a] with the Bisbicyclohexylazonitrile (4.10×10^{-6} mol) or Thermally with Di-*tert*-butyl Hyponitrite (6.08×10^{-6} mol)

solvent	H_2O/PC	micelle fraction[b] from ^{31}P NMR	measured rate $\times 10^{-6}$, M s^{-1}	10^{-5}[α-Toc], M	$10^{2}\tau$, s	$10^{-8}R_i$,[c] M s^{-1}	calcd rates $\times 10^{-6}$,[d] M s^{-1}
benzene			1.53				
	8.88	0.76	3.30				3.48
	17.80	0.94	3.59	1.28	4.60	5.57	4.25
chlorobenzene		0.25[e]	2.08				1.58
	2.66	0.43	3.42				2.49
	6.20	0.70	3.81				3.93
	11.53	0.91	4.41				5.01
	17.80	0.96	4.81	1.28	3.12	8.21	5.27
o-dichlorobenzene			1.63				
	5.34	0.59	3.59				3.59
	10.64	0.82	4.25				4.88
	17.80	0.94	4.35	1.25	2.70	9.48	5.55
tert-butyl alcohol[f]			0.159	1.28	9.72	2.63	
benzene[g]			0.743	1.44	3.78	0.770	
benzene[g]	20.0	1.00	1.74	1.44	3.48	0.828	

[a] Solutions were irradiated through Pyrex with a focused 250-W lamp. [b] Estimated from a plot of percent ^{31}P shifted signal vs. the ratio H_2O/PPC, using Figure 4. [c] R_i throughout a single photoinitiated run was assumed to be constant, since the order with respect to light intensity was 0.50 ± 0.05 after each H_2O addition. [d] Calculated from a linear combination of the rate of monomers in *tert*-butyl alcohol (0.892×10^{-7} at $R_i = 8.28 \times 10^{-9}$ M s^{-1}) and reverse micelles in benzene–water (17.4×10^{-7}, at $R_i = 8.28 \times 10^{-9}$ M s^{-1}) using the following relationship: calcd rate = $[(0.892 \times 10^{-7})F_{monomer} + (17.4 \times 10^{-7})F_{micelle}]\,(R_i/8.28 \times 10^{-9})^{1/2}$. [e] Estimated from fraction shifted with $Pr(DPM)_3$. [f] Oxidizability, $-dO_2/dt/[R\text{-}H]R_i^{1/2} = 0.063$ $M^{-1/2}$ $s^{-1/2}$. [g] Thermally initiated runs.

1 s for the narrow peak; therefore, a delay time of at least 5 s was used between pulses to avoid selective saturation of the peaks. The phospholipids were dried under reduced pressure (<0.1 mm) over P_2O_5 for at least 2 h. The solvents were dried over anhydrous calcium chloride and distilled immediately before use. Aqueous $Pr(NO_3)_3$, 0.62 m or 0.031 M, and water were added as required from a precision syringe, and the samples were sonicated for 5 min between additions with a Bransonic 220 bath sonicator. A repetition of runs indicated that the integrations (percent shifted of the total signal) are reproducible to approximately ±5%. Some difficulty was encountered in dissolving water in the phospholipid–*o*-dichlorobenzene samples. The water was observed to volatilize rapidly on sonication, rather than disperse in the solvent. This can be avoided by rapid vortex stirring immediately before sonication.

(c) The molecular weights on ELPC were obtained on a Beckman-Spinco Model E ultracentrifuge operating at 40 000 rpm. Samples were inserted in an aluminum double-sector cell.

Product Studies. (a) Fatty Acid or Methyl Ester Oxidations. Extreme care was taken to ensure that the fatty acid was not significantly oxidized before the experiment was begun. Storage of the fatty acids in methanol stock solutions under an argon atmosphere and at low temperatures prevented unwanted oxidation. The extent of oxidation was monitored by titrating hydroperoxides formed with triphenylphosphine. Addition of a known quantity of phosphine to an aliquot of the oxidation mixture was followed by TLC analysis to determine if all phosphine was oxidized to phosphine oxide. One-half percent increments of phosphine, based on starting oxidizable substrates, were generally added until some phosphine remained unoxidized. When the system was sufficiently oxidized (~ 1–2% conversion), the hydroperoxy fatty acids were reduced with excess Ph_3P at 0 °C for 30 min. After removal of solvent in vacuo, the hydroxy fatty acid oxidation products were analyzed on a Whatman 5 μ Partisil silica column. The integrated peak areas of the products were divided by the absorbances of each component to obtain the actual distribution of products.[8]

(b) Phospholipid Autoxidation in Organic Solvents. The phosphatidylcholine was purified by RP HPLC prior to each experiment.[9,21] After oxidation an aliquot was cooled to 0 °C and the hydroperoxides were reduced with excess Ph_3P. The phospholipid esters were transmethylated with 0.5 M methanolic KOH (room temperature, 10 min), the solution neutralized with 1.0 M NH_4Cl, and the hydroxy methyl linoleates extracted with hexane–ether (1:1, twice). The solvent was removed in vacuo and the oxidation products analyzed by NP HPLC on the 10 μ Porasil column.

Kinetic Studies. The autoxidations were carried out at 30 °C under 760 torr of oxygen on an automatic recording gas absorption apparatus similar to that described.[7] Studies on the effect of water on the rates (Table V) were carried out with use of solvents and phospholipids dried as described above. Autoxidations with bisdicyclohexylazonitrile (Table V) were photoinitiated with use of a focused 200V super pressure mercury light source filtered through Pyrex.

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