Table VI. Rate Constants for Bond Homolysis of *dl* Dimer **2** as a Function of Temperature and Solvent

solvent	temp, °C	k, s^{-1}	temp, °C	k, s^{-1}
ethanol	24.9	$(1.12 \pm 0.01) \times 10^{-3}$	45.9	$(1.18 \pm 0.2) \times 10^{-2}$
	33.4	$(3.65 \pm 0.03) \times 10^{-3}$		
1,2-dimethoxy-ethane	38.9	$(4.71 \pm 0.05) \times 10^{-5}$	60.9	$(6.43 \pm 0.03) \times 10^{-4}$
	49.2	$(1.97 \pm 0.01) \times 10^{-4}$		
benzene	50.6	$(1.65 \pm 0.03) \times 10^{-5}$	70.8	$(2.29 \pm 0.01) \times 10^{-4}$
	60.7	$(5.35 \pm 0.06) \times 10^{-5}$		

wrapped with aluminum foil to assist heat transfer, and the cell was placed in the thermostated cell holder. The cell was temperature equilibrated for 7 min, the DPPH solution was vigorously mixed with the TM-3 dimer in the cuvette compartment, and the spectrometer program was started. The procedure was performed at three temperatures approximately 10 °C apart for each of the three solvents. The absorbance at infinity was obtained by immersing the cell in a constant-temperature bath at 70 °C until no further reaction was discernible by visible spectroscopy.

Data Collection and Reduction. The HP-8450 spectrometer was preprogrammed prior to analysis. Initially a balance measurement was performed on a sample of pure solvent and stored in memory. The λ_{max} of DPPH was determined to be 516 nm; consequently, the average absorbance from 514 to 518 nm was measured. A data point was collected every 10 s, and after 400 data points, the data were transferred to memory. The data were later transferred to the HP 85 and stored on magnetic tape. The data were analyzed by least-squares fitting of the data to first-order kinetics plotting, $-\ln (A_t - A_\infty)$ vs. time with the HP 85. The slope was the rate constant for bond homolysis, Table VI. The free energy of activation was calculated from k, and the other activation parameters were obtained from standard least-squares analysis of $\ln k$ and $\ln (k/T)$ vs. $1/T$ plots. All values are reported at 25 °C. Errors expressed are standard deviations from the least-squares analysis.

Acknowledgment. We are indebted to many people for their assistance with this work including Gareth Eaton for his advice on quantitative EPR spectroscopy, Gary Bishop and Stan Gill for assistance with the computer programing, Carl Koval, Ed King, and David Griller for helpful discussions, and Don Kleyer for some preliminary experiments.

Registry No. 1, 53153-52-9; **2**, 53153-53-0; **3**, 57765-64-7.

Phospholipid Inverted Micelles. Autoxidation Kinetics and ^{31}P NMR Exchange Rates

L. Ross C. Barclay,*† Bruce J. Balcom,† and Bruce J. Forrest‡

Contribution from the Department of Chemistry, Mount Allison University, Sackville, N.B., Canada E0A 3C0, and Department of Chemistry, Dalhousie University, Halifax, N.S., Canada B3H 4J3. *Received August 2, 1985*

Abstract: Autoxidation in phospholipid reverse micelles in benzene and chlorobenzene is initiated by a water-soluble initiator azobis(2-amidinopropane) (ABAP) hydrochloride inserted into the aqueous core and inhibited when micelles containing the water-soluble antioxidant 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate (Trolox) are added. Autoxidation of egg phosphatidyl choline (EPC) is studied quantitatively in water–benzene by using ABAP and Trolox in the aqueous cores. The oxidizability value ($k_p/2k_t^{1/2} = 0.023$ $M^{-1/2}$ $s^{1/2}$) obtained is similar to that of EPC in bilayers. Autoxidation initiation and inhibition were also observed between saturated, dipalmitoylphosphatidyl choline (DPPC) reverse micelles containing ABAP and unsaturated, dilinoleoylphosphatidyl choline (DLPC) reverse micelles containing Trolox in the aqueous cores in benzene. Similar results were obtained when the locations of the ABAP and Trolox were switched. Kinetic studies indicate the oxidizability of DLPC in these reverse micelles to be 0.092 $M^{-1/2}$ $s^{1/2}$. Absolute rate constants for propagation, k_p, and termination, $2k_t$, determined for DLPC in reverse micelles in benzene gave values of k_p similar to that in homogeneous solution while the $2k_t$ is an order of magnitude smaller. The exchange rates and lifetimes of phospholipids in DLPC reverse micelles, containing Pr^{3+} shift reagent in the aqueous cores, were measured by ^{31}P NMR magnetization transfer techniques. The micelle to micelle phospholipid exchange lifetime for DLPC (27 ms) is similar to its monomer to micelle lifetime (34 ms), whereas for DPPC the latter lifetime is twice as long. Certain preparations of reverse micelles in nonprotic organic solvents exhibit an instability and they break up after several hours.

Phospholipids are known to form inverted (reverse) micelles especially in nonprotic organic solvents in the presence of water.[1] Interest in such inverted micelles has developed because of the polar core's catalytic effects and the idea that they provide a model for processes in biomembranes[2] including trans bilayer transport[3] and fusion.[4] Various reviews reflect the attention given to ionic reactions in inverted micelles.[2,5,6] Comparatively little is known of the effect of inverted micelles on *free radical* reactions. Free radical lipid peroxidation is related to important pathological events[7-10] resulting in current interest in the free radical autoxidation in such microenvironments as micelles and bilayers[11-14] as models for biomembranes. There is also related interest in the

† Mount Allison University.
‡ Dalhousie University.

(1) Elworthy, P. H.; McIntosh, D. S. *J. Phys. Chem.* **1964**, *68*, 3448.
(2) Fendler, J. H. *Acc. Chem. Res.* **1976**, *9*, 153.
(3) Cullis, P. R.; Dekruijff, B. *Biochim. Biophys. Acta* **1979**, *559*, 399; **1980**, *602*, 474.
(4) Verklelj, A.; Van Echteld, C. J. A.; Gerritsen, W. J.; Cullis, P. R.; Dekruijff, B. *Biochim. Biophys. Acta* **1980**, *600*, 620.
(5) Kitahara, A. *Adv. Colloid Inferface Sci.* **1980**, *12*, 109.
(6) Eicke, H.-F. *Top. Curr. Chem.* **1980**, *87*.
(7) Pyror, W. A. In "Free Radicals in Biology"; Pryor, W. A., Ed.; Academic Press: New York, 1976; Vol. I, Chapter 1, pp 1-49.
(8) Mead, J. F. In "Free Radicals in Biology"; Pryor, W. A., Ed.; Academic Press: New York, 1976; Vol. I, Chapter 1, pp 51-68.

0002-7863/86/1508-0761$01.50/0

Table I. Autoxidation and Inhibition between EPC Reverse Micelles and between Micelles and Bulk Phase

run	EPC,[c] mol × 10^5	ABAP,[d] mol × 10^6	inhibitor,[d] mol × 10^8	τ, s × 10^{-3}	R_i,[e] Ms^{-1} × 10^7	$-d[O_2]/dt$,[e] Ms^{-1} × 10^5	chain length	$k_p^e/2kt^{1/2}$, $M^{-1/2}$ $s^{-1/2}$
1[a]	3.25	3.66	α-T 2.71	0.905	18.5	2.96	16	0.022
2[a]	3.12	2.56	α-T 4.65	3.50	8.49[f]	2.46[f]	29	0.027
	3.91		Trolox 0.75	0.705	5.44	1.99	37	0.027
	5.47		Trolox 1.50	0.936	5.84	1.39	24	0.018
3[b]	4.20	3.66	Trolox 1.01	0.936	5.13	1.43	28	0.019

[a] In chlorobenzene solvent. [b] In benzene solvent. [c] The H_2O/EPC mole ratio is 18/1. [d] Reverse micelles containing ABAP (aqueous) and Trolox (aqueous) were prepared separately before initiation by irradiation with 200-W Pyrex-filtered Hg source. [e] The reaction volume is approximated by the lipid volume. [f] The R_i and the rate during a run decreases due to photodecomposition of the initiator.

mechanism of autoxidation inhibition by antioxidants.[11,15-21]

We have recently shown that the rate of photochemically initiated free radical autoxidation of 1,2-dilinoleoyl-*sn*-glycero-3-phosphatidylcholine (DLPC) in benzene, chlorobenzene, and *o*-dichlorobenzene is accelerated by added water.[22] This increase in rate is related to the fraction of phospholipid which is aggregated into reverse micelles in these solvents, the susceptibility to autoxidation (*oxidizability*) being 2–3 times greater than that of either homogeneously dispersed substrate or substrate in a bilayer.[22] In order to better understand the effect of aggregation into reverse micelles on the autoxidation of phospholipids, we now address two fundamental questions concerning these systems: (*1*) *Can autoxidation reactions initiated in the aqueous cores of one type of reverse micelle (saturated or unsaturated) be inhibited by antioxidants in other reverse micelles or in the bulk phase*? (*2*) *If the answer to (1) is yes, what are the dynamics of these reverse micelles which permit such interactions*? In order to address the first question, we will report on some autoxidation kinetics and inhibition results obtained by sequestering free radical initiators and inhibitor in separate aqueous cores of phospholipid reverse micelles. The second question on phospholipid dynamics in reverse micelles is examined by the use of ^{31}P NMR magnetization transfer experiments on reverse micelles containing a praseodymium shift reagent.

Results

(1) Autoxidation: Initiation and Inhibition between Reverse Micelles. The peroxidation of polyunsaturated fatty acids in homogeneous solutions and in microenvironments such as micelles[11] follows the classical rate law so that the uptake of oxygen is given by eq 1, where [R–H] is the substrate concentration, R_i

$$-\frac{dO_2}{dt} = \frac{k_p}{2k_t^{1/2}}[R\text{–}H]R_i^{1/2} \quad (1)$$

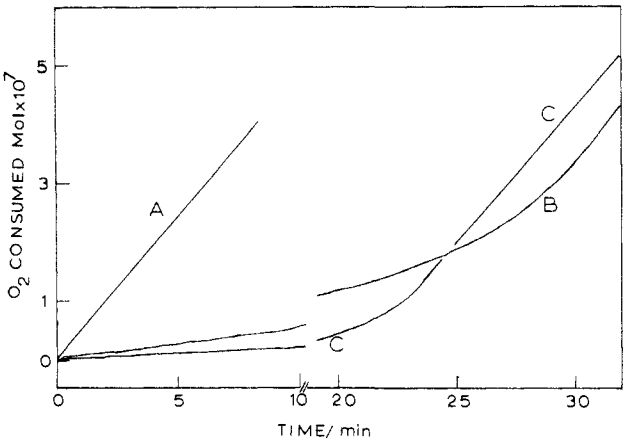

Figure 1. Autoxidation of egg phosphatidylcholine (EPC) (5.11 × 10^{-5} mol) in reverse micelles in benzene–water. H_2O/EPC mole ratio = 18/1. Initiated with ABAP (3.66 × 10^{-6} mol) by irradiation (see text). (A) Uninhibited reaction; (B) inhibited with α-tocopherol (3.88 × 10^{-8} mol); (C) inhibited with Trolox (2.02 × 10^{-8} mol).

is the rate of free radical chain initiation, and k_p and $2k_t$ are the rate constants for the rate-controlling chain propagation step (here hydrogen abstraction from substrate by initiating peroxyl radicals) and for the termination step, respectively. The rate constant ratio, $k_p/2k_t^{1/2}$, is generally referred to as the *oxidizability* of the substrate. The rate of chain initiation (R_i) is generally determined by the inhibitor method[23] using a chain-breaking phenolic antioxidant, ArOH, which has been shown to react with two peroxyls, so that R_i is given by eq 2, where τ is the time during which

$$R_i = 2[ArOH]/\tau \quad (2)$$

oxidation is suppressed. The chain length is given by the rate ratios

$$\nu = \frac{-dO_2/dt}{R_i} \quad (3)$$

In order to test for interaction between aqueous cores of reverse micelles, we located a water-soluble azo initiator, azobis(amidinopropane) (ABAP) hydrochloride, in the water phase of phospholipid reverse micelles while the water-soluble phenolic inhibitor 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate (Trolox) was located in the water phase of other phospholipid reverse micelles. In addition, the organic solvent-soluble inhibitor, α-tocopherol (α-T) was used in the bulk phase so that a direct comparison could be made of inhibition from the different phases. Experiments were conducted with sufficient water content (water/phospholipid mole ratios ≥18) to aggregate essentially all of the phospholipids, known to give aggregation numbers of 80–100 for DPPC and DLPC.[22]

Our initial results were obtained with reverse micelles prepared in benzene and chlorobenzene from the oxidizable substrate egg phosphatidylcholine (EPC). A typical trace for the uninhibited autoxidation of EPC initiated by photochemical decomposition of ABAP in the aqueous phase is shown in Figure 1A and the inhibition by α-tocopherol added to the bulk phase is shown in Figure 1B. For experiments employing the water-soluble initiator

(9) Porter, N. A. In "Free Radicals in Biology"; Pryor, W. A., Ed.; Academic Press: New York, 1980; Vol. IV, Chapter 8, pp 261–294.
(10) "Oxygen Radicals in Chemistry and Biology", 3rd International Conference, Neuherberg, Federal Republic of Germany; Bors, W., Saran, M., Tait, D., Eds.; W. de Gruyter: Berlin, New York, 1984.
(11) Barclay, L. R. C.; Locke, S. J.; MacNeil, J. M. *Can. J. Chem.* **1985**, *63*, 366.
(12) 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.
(13) Yamamoto, Y.; Haga, S.; Niki, E.; Kamiya, Y. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 1260.
(14) Yamamoto, Y.; Niki, E.; Kamiya, Y.; Shimasaki, H. *Biochim. Biophys. Acta* **1984**, *795*, 332.
(15) Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 6472.
(16) Burton, G. W.; Hughes, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1983**, *105*, 5950.
(17) Doba, T.; Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1983**, *105*, 6505.
(18) Doba, T.; Burton, G. W.; Ingold, K. U.; Matsuo, M. *J. Chem. Soc., Chem. Commun.* **1984**, 461.
(19) Winterle, J.; Dulin, D.; Mill, T. *J. Org. Chem.* **1984**, *49*, 491.
(20) Niki, E.; Saito, T.; Kawakami, A.; Kamiya, Y. *J. Biol. Chem.* **1984**, *259*, 4177.
(21) Niki, E.; Kawakami, A.; Saito, M.; Yamamoto, Y.; Tsuchiya, J.; Kamiya, Y. *J. Biol. Chem.* **1985**, *260*, 2191.
(22) Barclay, L. R. C.; MacNeil, J. M.; VanKessel, J.; Forrest, B. J.; Porter, N. A.; Lehman, L. S.; Smith, K. J.; Ellington, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 6740.
(23) Boozer, C. E.; Hammond, G. S.; Hamilton, C. E.; Sen, J. N. *J. Am. Chem. Soc.* **1955**, *77*, 3233.

Table II. Autoxidation and Inhibition between DLPC and DPPC Reverse Micelles

run[a]	DLPC,[b,c] mol × 10^5	DPPC,[b,c] mol × 10^5	ABAP, mol × 10^6	inhibitor,[c] mol × 10^8	τ, s × 10^{-3}	R_i,[d] Ms^{-1} × 10^5	$-d[O_2]/dt$,[d] Ms^{-1} × 10^5	chain length	$k_p/2k_t^{1/2}$,[d] $M^{-1/2}$ $s^{-1/2}$
1	3.12	0.83	3.66	Trolox 1.50	1.41	5.44	5.38	99	0.091
2	3.12		3.66	α-T 1.55	1.07	9.25[e]	10.80[e]	117	0.112
	3.12	1.66		Trolox 2.40	3.81	2.69	3.27	121	0.095
3	1.25	1.33	2.93	Trolox 2.01	1.19	13.5[e]	4.00	30	0.069
				α-T 0.194	0.222	6.99			
				α-T 0.490	0.698	5.55	4.00[e]	72	0.107
4	3.12	1.66	2.93	Trolox 5.27	5.68	2.89	2.40	83	0.092
				α-T 0.12	0.366	0.989	1.45	147	0.095

[a] In benzene solvent. [b] The H_2O/phospholipid mole ratio is ≃18/1. [c] See footnote d, Table I. Runs 1–3 contained ABAP in the DLPC micelles and Trolox in the DPPC micelles; in Run 4 the locations of initiator and inhibitor are switched. [d] The same as footnote e, Table I. [e] The R_i and the rate during a run decrease due to photodecomposition of the initiator.

Table III. Absolute Rate Constants for Propagation, k_p, and Termination, $2k_t$, for Autoxidation of DLPC Reverse Micelles

run[a]	ACHN, mol × 10^6	order in[b] light intensity	inhibitor, mol × 10^8	τ, s × 10^{-3}	R_i,[c] Ms^{-1} × 10^6	$-d[O_2]/dt$,[c] Ms^{-1} × 10^4	chain length	$k_p/2k^{1/2}$,[c] $M^{-1/2}$ $s^{-1/2}$	k_p M^{-1} s^{-1}	$2k_t$, M^{-1} s^{-1} × 10^{-5}
1	4.51	0.51	α-T 7.75	0.408	12.2	2.42	40	0.149	[d]	[d]
2	5.38	0.63	α-T 12.6	2.93	2.75	3.60	131	0.217	118	2.05
3	3.28	0.50	α-T 6.30	1.48	2.72	3.61	132	0.218	132	8.34
4	4.51	0.56	α-T 6.30	1.01	3.98	4.15	163	0.208	163	3.88
5	DACMP 2.67	0.61	α-T 3.10	0.448	6.10	4.14	68	0.167	[d]	[d]

[a] In benzene solvent. The H_2O/DLPC mole ratio = $^{18}/_1$. [b] Determined by using neutral density filters between the light source and the sample. [c] The same as footnote e, Table I. [d] Not measured.

(ABAP) and the water-soluble inhibitor (Trolox), separate samples of EPC micelles containing these chemicals were mixed in the autoxidation apparatus. A trace of a typical inhibition period obtained is shown in Figure 1C. A summary of several kinetic runs carried out on EPC reverse micelles is given in Table I. In calculations of the rates of chain initiation (R_i) and the rates of oxygen uptake, the reaction volume used was that of the total "neat" phospholipid volume as before.[22,24] Using this reaction volume, we calculate the oxidizability, $k_p/2k_t^{1/2}$, of EPC in reverse micelles to average 0.023 ± 0.004 $M^{-1/2}$ $s^{1/2}$.

Experiments were also carried out with the initiator in the aqueous core of unsaturated oxidizable (DLPC) reverse micelles and the inhibitor (Trolox) in the aqueous core of saturated, nonoxidizable, dipalmitoylphosphatidylcholine (DPPC) reverse micelles or in the bulk phase (α-tocopherol). Typical traces of such experiments are illustrated in Figure 2. A summary of kinetic runs on these combined systems is given in Table II, including an example of a reaction when the positions of the Trolox and the ABAP were switched (No.4).[24] The oxidizability, calculated by using the combined lipid volume as the reaction volume, of the DLPC is 0.092 ± 0.012 $M^{-1/2}$ $s^{-1/2}$.

We recently applied the rotating sector technique[25,26] to determine the absolute rate constants of propagation, k_p, and termination, $2k_t$, for autoxidation in bilayers and micelles.[27] We now extend this method in an exploratory way to estimate k_p and $2k_t$ for autoxidations of DLPC reverse micelles. The method is based upon measurement of the lifetime of the reaction chains. The reaction is initiated by photochemical decomposition of the initiator with a rotating sectored disk of known dimensions between the light source and the reaction flask. Rates of oxygen uptake are measured at various sector speeds, and the $2k_t$ is calculated as described.[28] The k_p is then calculated from the rate at steady

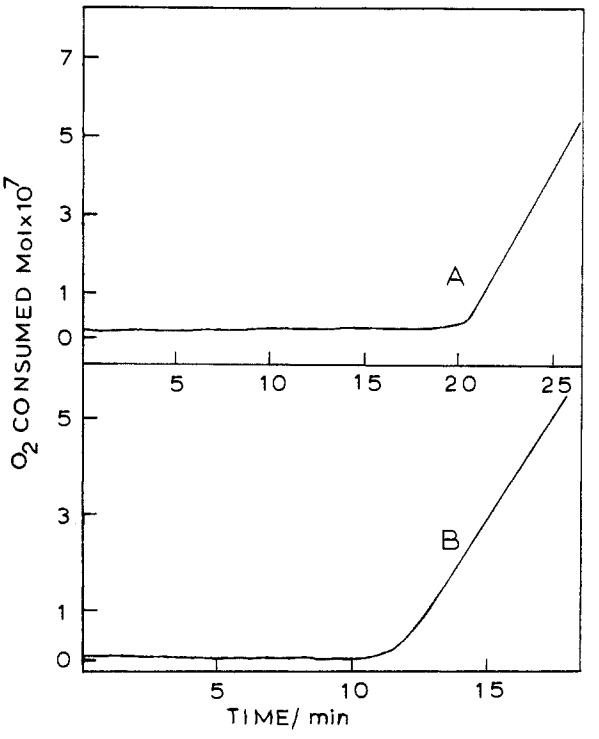

Figure 2. Autoxidation of dilinoleoylphosphatidylcholine (DLPC, 1.25 × 10^{-5} mol) initiated with ABAP (2.93 × 10^{-6} mol) in DLPC reverse micelles in benzene. H_2O/PPC ratio = 18/1. (A) Inhibited with Trolox (4.85 × 10^{-8} mol) in dipalmitoylphosphatidylcholine (DPPC) reverse micelles. (B) Inhibited with α-tocopherol (0.49 × 10^{-8} mol) in the bulk solvent (see Table II, footnote e).

illumination from eq 1 by substituting for the measured $2k_t$ and the R_i. For eq 1 to be followed exactly, chain termination must be bimolecular and the oxidation rate proportional to $R_i^{1/2}$. This is tested by using an initiator of relatively high thermal stability (e.g., azobis(cyclohexanenitrile), ACHN) and measuring the reaction rate order, which should be half order, with respect to the light intensity.

Some results for determinations of k_p and $2k_t$ for autoxidation of reverse micelles of DLPC are given in Table III. The thermal dark rate was generally less than 5% of the photochemical rate in the reactions initiated by ACHN. Reactions with orders in

(24) We postulate that the water-soluble initiator/inhibitor combinations ABAP/Trolox do not undergo rapid intermicellar transfer on a laboratory time scale and that efficient interaction between peroxyl radicals and Trolox occurs by rapid lipid intermicellar exchange (vide infra). This exchange will mix saturated and unsaturated lipids so that the effective reaction volume is estimated by the total lipid volume. As a referee points out, our results might also be accounted for by intermiceller exchange of ABAP and Trolox.

(25) Burnett, G. M.; Melville, H.; W. "Technique of Organic Chemistry"; Friess, S. L., Lewis, E. S., Weissberger, A., Eds.; Interscience: New York, 1963; Vol. III, Part II, Chapter 20.

(26) Howard, J. A. *Adv. Free Radical Chem.* **1972**, *4*, 49.

(27) Barclay, L. R. C.; Locke, S. J.; MacNeil, J. M.; VanKessel, J. *Can. J. Chem.* **1985**, *63*, 2633.

(28) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1965**, *43*, 2729, 2737.

Table IV. Lifetimes of Phospholipids in Reverse Micelles and in Bulk Phase from ^{31}P NMR Magnetization Transfer Experiments

phospholipid[a]	H_2O/PPC, mol ratio	PPC/Pr^{3+}, mol ratio	PPC exchange measured	λ_2,[b] s^{-1}	β[c]	k_A,[b] s^{-1}	τ,[d] ms
DLPC	18.0	101	micelle/micelle	−37.50	0.50	25	27
DLPC	9.0	80	monomer/micelle	−29.80	0.52	19.6	34
DPPC	9.5	84	monomer/micelle	−13.71	0.55	7.1	73

[a] DLPC (3.13×10^{-5} mol) and DPPC (3.37×10^{-5} mol) in 2.00 mL of benzene. [b] $\lambda_2 = -k_A(1 + \beta)$ where k_A is the rate constant for the faster exchange out of the upfield NMR peak. [c] β is obtained from the relative ^{31}P NMR peak areas. [d] τ is the reduced lifetime $= \tau_0\tau_1/(\tau_0 + \tau_1)$ where τ_0 = time in micelle with no Pr^{3+} and τ_1 = time in micelle with Pr^{3+}.

light intensity significantly higher than the "theoretical" 0.50 appear to have some first-order termination in addition to bimolecular chain termination and corrections are made for this by using the described method.[25] In addition a reaction was carried out with the structurally similar (to ACHN) water-soluble initiator, *N,N'*-dimethyl-4,4'-azobis(4-cyano-1-methylpiperidinium) dinitrate (DACMP), in the aqueous core of DLPC reverse micelles to determine the oxidizability by initiation inside the aqueous core (Table III, run 5).

(2) Exchange Rates of Phospholipids by ^{31}P NMR Magnetization Transfer Techniques. In order to understand the dynamics of phospholipids in reverse micelles that permit the observed initiation and inhibition between micelles in autoxidation, we used the ^{31}P NMR magnetization transfer techniques to study the exchange of phospholipids between reverse micellar and monomeric forms as well as intermicellar exchange. The feasibility of such experiments relies on the ability to produce samples that are composed either entirely of reverse micelles or of mixtures of micelles and monomers.[22] This is accomplished by varying the amount of water present. As water is added, increasing amounts of phospholipid form reverse micelles, and at water:phosphatidylcholine mole ratios of 18:1 micellization is essentially complete.[22] In addition to preparing the necessary populations of micelle and monomer species, saturation transfer requires that the NMR signal of the two populations be resolved. This is accomplished by the use of a water-soluble shift reagent, such as praseodymium nitrate, which shifts downfield and broadens the resonance of those ^{31}P nuclei with which it comes in contact (e.g. in the reverse micelles). In the case of complete micellization, a low ratio of Pr^{3+}/PPC results in a situation where some of the reverse micelles are populated by a Pr^{3+} ion, while others are not. In the case of the coexistence of micelles and monomers known to be produced by very small amounts of added water,[22] higher concentrations of Pr^{3+} are used to ensure that virtually all reverse micelles formed contain at least one Pr^{3+} ion.

The McConnell equations[29] describe the transfer of magnetization for exchange between two sites, such as is the case here. Specifically[30,31]

$$M_A(t) = c_1e^{\lambda_1 t} + c_2e^{\lambda 2 t} + M_A^{\infty} \quad (4)$$

$$M_B(t) = c_3e^{\lambda_1 t} + c_4e^{\lambda 2 t} + M_B^{\infty} \quad (5)$$

$M_A(t)$ and $M_B(t)$ are the time-dependent magnetizations, while M_A^{∞} and M_B^{∞} are the equilibrium magnetizations for sites A and B, and c_1–c_4 are constants. In the case of fast exchange, which is the case here (vide infra),

$$\lambda_1 = -R_{1A}\frac{\beta}{1+\beta} - R_{1B}\frac{1}{1+\beta} \quad (6)$$

$$\lambda_2 = -k_A(1 + \beta) \quad (7)$$

where $k_{1A} = R_{1A} + k_A$, $k_{1B} = R_{1B} + k_B$, k_A and k_B are the exchange rate constants out of site A and B, R_{1A} and R_{1B} are the spin lattice relaxation rates of the two sites, and β is defined as

$$\beta = \alpha\frac{M_A^{\infty}}{M_B^{\infty}} = \frac{k_B}{k_A} \quad (8)$$

(29) McConnell, H. M. *J. Chem. Phys.* **1958**, *28*, 430.
(30) Forsen, S.; Hofmann, R. A. *J. Chem. Phys.* **1963**, *39*, 2892.
(31) Led, J. J.; Gesmar, H. *J. Magn. Reson.* **1982**, *49*, 444.

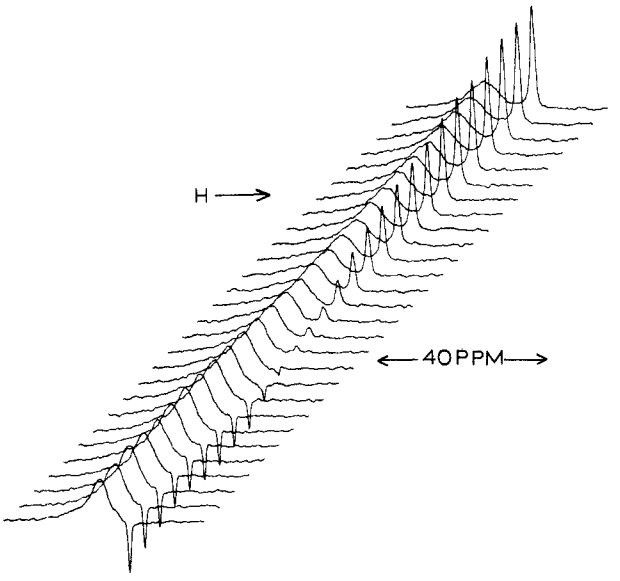

Figure 3. ^{31}P NMR magnetization transfer experiment (monomer/micelle) on DLPC (3.13×10^{-5} mol) in benzene (2.00 mL). H_2O/DLPC mole ratio = 18/1. DLPC/Pr^{3+} mole ratio = 101/1. Sampling NMR pulse (90°) 31 μs, pulse width 2μ s, delay in DANTE sequence 100 μs, intersequence delay 7 s.

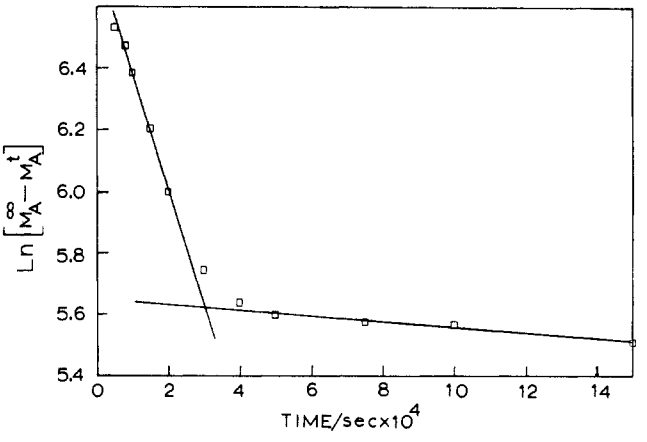

Figure 4. Plot of ^{31}P magnetization transfer (monomer/micelle) for DPPC in benzene (2.00 mL). DPPC = 3.37×10^{-5} mol, H_2O/DPPC mol ratio = 9.5/1, DPPC/Pr^{3+} = 84/1. M_A = equilibrium magnetization for site A (upfield peak), M_A' = magnetization at time t.

In eq 8, α is the ratio of the relative line widths of the two exchanging signals which allow signal heights to be measured instead of signal areas. In the present case signal A, the upfield peak, is selectively inverted and the effect of this inversion on both signals A and B can be monitored. In the case of fast exchange, $k_A >> R_{1A} + R_{1B}$ and the two time constants λ_1 and λ_2 will dominate different parts of the recovery of signal A from inversion. At short times, λ_2 will dominate with the term involving λ_1 offering little pertubation. Thus for fast exchange, the exchange rate out of site A, k_A, can be measured from the initial time dependence of signal A alone.

Figure 3 is typical of the ^{31}P time lapse recovery NMR spectra, in this case from a spin inversion of the monomer component

micelle/monomer dipalmitoylphosphatidylcholine (DPPC) system. Figure 4 gives a typical plot of ln ($M_A^\infty - M_A^t$) vs. t, in this case for the DLPC micelle/micelle exchange, showing how the two time constants, λ_1 and λ_2, dominate different parts of the recovery of a signal A from inversion. Exchange rate data are obtained from the initial slope and results are summarized in Table IV.

Discussion

(1) The Question of Initiation and Inhibition of Autoxidation between Reverse Micelles and between Micelles and Bulk Phase. Our initial results (illustrated in Figure 1) with oxidizable (EPC) reverse micelles clearly show that reactions initiated in the aqueous core of micelles by photochemical decomposition of the initiator (ABAP) can be effectively inhibited with an antioxidant (α-tocopherol) in the bulk phase or by a water-soluble antioxidant (Trolox) sequestered in other EPC reverse micelles.[24] Quantitative free radical kinetic studies, requiring the measurements of rates of oxygen uptake and the rates of free radical chain initiation (R_i), are readily carried out in these heterogeneous systems. The overall oxidizability of EPC obtained by both techniques of determining the R_i (α-tocopherol and Trolox) averages 0.023 ± 0.004 $M^{-1/2}$ $s^{-1/2}$. This value is only 1.4 times that found for EPC aggregated into bilayers.[32] Thus we now conclude that EPC is *not* much more highly oxidizable in an organic solvent than in a bilayer. The earlier conclusion on its much higher oxidizability in chlorobenzene[32] failed to take into account the tendency for aggregation into reverse micelles in organic solvents.

Free radical chain initiation and inhibition can also be observed for reverse micellar systems constituted from mixtures of inert saturated (DPPC) and unsaturated, oxidizable (DLPC) phospholipids. Characteristically sharp breaks in the inhibition periods are observed whether the water-soluble initiator (ABAP) and water-soluble inhibitor (Trolox) were located in the aqueous cores of saturated (inert) DPPC and in the core of unsaturated (oxidizable) DLPC micelles, respectively, or if the location of initiator and inhibitor were initially switched.[24] Reactions could also be inhibited with α-tocopherol added to the bulk solvent phase (see Figure 2B). The oxidizability, from quantitative treatment of the kinetic data (Table II) for the DLPC in mixed DLPC + DPPC reverse micelles, is 0.092 ± 0.012 $M^{-1/2}$ $s^{-1/2}$. This value is 1.5–2 times lower than that observed for reverse micelles of DLPC alone (ref 22, $k_p/2k_t^{1/2}$ = 0.130 $M^{-1/2}$ $s^{-1/2}$ and, Table III, $k_p/2k_t^{1/2}$ = 0.192 ± 0.032 $M^{-1/2}$ $s^{-1/2}$. It was postulated earlier[22] that the oxidizability of DLPC in reverse micelles is higher than in *homogeneous* solution due to close alignment of the unsaturated monomers of DLPC in the micelles resulting in a higher k_p compared to randomly dispersed material. Our exploratory data on absolute rate constants (Table III) reveal that the propagation rate constant, k_p, in reverse micelles (118–163 M^{-1} s^{-1}) is not significantly different from that in homogeneous solution in *tert*-butyl alcohol (150 M^{-1} s^{-1}).[27] The main difference appears in the termination rate constant, $2k_t$, found to be 6.8 × 10^6 M^{-1} s^{-1} in *tert*-butyl alcohol[27] compared to a value that is an order of magnitude lower in reverse micelles (the average $2k_t$ = 4.8 × 10^5 M^{-1} s^{-1}, Table III). Peroxyl radicals are known to be highly polarized.[32,33]

$$\text{R-}\ddot{\text{O}}\text{-}\ddot{\text{O}}\cdot \leftrightarrow \text{R-}\dot{\ddot{\text{O}}}^{+}\text{-}\ddot{\text{O}}:^{-}$$

We spectulate that such polarized peroxyl radicals, aggregated in close contact with water, could be stabilized by the polar aqueous phase compared to the termination products (peroxides). The effect of water would thus be to stabilize the peroxyl radical relative to the termination products with a resulting lowering of the observed $2k_t$. In this respect, such a medium effect on the termination rate is similar to that observed on peroxyl radicals in *homogeneous* solution.[34,35]

The observed peroxyl free radical initiations and inhibitions between reverse micelles with various locations of initiators and inhibitors resulting in autoxidations with substantial chain lengths clearly demonstrates that there is dynamic interaction between phospholipid reverse micelles during reaction. It is most desirable that the time frame of this interaction be quantified so that its nature may be better understood.

(2) The Question of the Dynamics of Phospholipids between Reverse Micelles and between Monomers and Micelles. The dynamics of molecules between reverse micelles remains a very intriguing physical chemical problem. Most of the research has focused on the exchange of solubilizates, such as ions, between the aqueous pools of reverse micelles.[36-41] Our interest is in the exchange of the phospholipids which constitute our reverse micelles (*micelle/micelle exchange*) and exchange of monomers from the bulk solvent phase with the micellar phospholipids (*monomer/micelle exchange*). An important study by Chen and Springer[42] on ^{31}P NMR spectra of reverse DPPC micelles in benzene established that the average lifetime of a lipid molecule in a micellar aggregate at 50–52 °C is of the order of milliseconds. Their NMR method did not determine the separate exchange rates for solubilized Pr^{3+} ion and the PPC components of the reverse micelles. They were only able to determine that one or both exchange rapidly on the laboratory time scale while each exchanged slowly on the NMR time scale.

Our ^{31}P NMR magnetization transfer results resolve this problem and place definite values on the lifetimes of phospholipids in micellar and in monomer states. The results in Table IV show that the average lifetime of a DLPC molecule in a micelle (27 ms) is similar to the lifetime of a monomer in bulk solution (34 ms). This may be due to the mechanisms of the exchange processes (vide infra). On the other hand, the monomer/micelle exchange rate in the DPPC system is considerably slower, with a resulting lifetime (73 ms) twice that of DLPC monomers. The different lifetime of DPPC monomer/micelle compared to DLPC may be due to the difference in the nature of aggregation (or packing) of the individual phospholipid chains, saturated compared to unsaturated, in the reverse micelles.

Other "lifetime" experiments on these reverse micelles were unsuccessful due to an unusual and unexpected instability encountered. On several samples prepared in the usual way (DPPC in benzene at 52 °C, DLPC in chlorobenzene or *o*-dichlorobenzene), the ^{31}P NMR signal "collapsed" after several hours of data acquisition. Droplets of water settled out and, in certain cases with DLPC, an insoluble "glass" separated that could not be dispersed again. This may be a different "pseudophase" of the phospholipid but its exact nature is not yet determined. This instability of phospholipid reverse micelles has important implications for physical studies over longer times; greater than approximately 4 h. The ^{31}P spectra provide a convenient check on the stability of such aggregates used in any experiments. The DLPC reverse micelles in benzene appeared to maintain their aggregate structures for at least 8 h. Autoxidation experiments on this system were completed within 2 h.

The mechanisms considered[42] for phospholipid transfer between reverse micelles include: (A) *direct diffusion*, where a lipid molecule leaves its micelle, diffuses through the solvent, and enters a new micelle; (B) *nonfusing collision*, involving transfer induced by collisions of micelles; (C) *fusing collisions*, of micelles forming larger aggregates, which could lead to transfer of lipids. The fact that micelle/micelle and monomer/micelle exchange rates are very similar for DLPC indicates that process A is a definite possibility

(32) Barclay, L. R. C.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 6478.
(33) Fessenden, R. W.; Hitachi, A.; Nagarajan, V. *J. Phys. Chem.* **1984**, *88*, 107.
(34) Hendry, D. G.; Russell, G. A. *J. Am. Chem. Soc.* **1964**, *86*, 2368.
(35) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1966**, *44*, 1119.
(36) Schelly, Z. A. In "Studies in Physical and Theoretical Chemistry. Aggregation Processes in Solution"; Wyn-Jones, E., Gormally, J., Eds.; Elsevier: (1983). Amsterdam, 1983; Vol. 26, Chapter 6, p 140.
(37) Boicelli, C. A.; Conti, F.; Giomini, M.; Giuliani, A. M. *Chem. Phys. Lett.* **1982**, *89*, 490.
(38) Atik, S. S.; Thomas, J. K. *J. Am. Chem. Soc.* **1981**, *103*, 3543.
(39) Tamura, K.; Shelly, Z. A. *J. Am. Chem. Soc.* **1981**, *103*, 1018.
(40) Robinson, B. H.; Steytler, D. C.; Tack, R. D. *J. Chem. Soc., Faraday Trans.* **1979**, *75*, 481.
(41) Chen, S.-T.; Springer, C. S. *Biophys. Chem.* **1981**, *14*, 375.
(42) Chen, S.-T.; Springer, C. S. *Chem. Phys. Lipids* **1979**, *23*, 23.

as a mechanism for lipid transfer. A collision-mediated mechanism (i.e., B) is also possible. The time, t, between collision may be quantified. Eq 9,[43] where η is the viscosity of the medium, r the

$$t = \pi\eta r^3/kTB \quad (9)$$

radius of the particle, k Boltzmann's constant, and B the volume fraction occupied by the micelles, gives an estimated time between collisions of 1 μs. Collisions are thus estimated to be several orders of magnitude faster than the lifetime of a phospholipid in a micelle.

Summary and Conclusions

1. Free radical chain autoxidations were initiated by a water-soluble initiator, ABAP, sequestered in the aqueous core of EPC reverse micelles in organic solvents and inhibited by the water-soluble inhibitor, Trolox, in other EPC reverse micelles or by α-tocopherol in the bulk phase. The *oxidizability* of ELPC in such reverse micelles (0.023 $M^{-1/2}$ $s^{-1/2}$) is similar to that in EPC bilayers.

2. Free radical initiation and inhibition interactions are also readily observed between saturated, DPPC, reverse micelles, and unsaturated, DLPC, reverse micelles in benzene: water with the initiator and inhibitor located in the aqueous cores of either type of phospholipid. The oxidizability of reverse DLPC micelles when mixed with saturated DPPC micelles (0.092 $m^{-1/2}$ $s^{-1/2}$) is lower than that of DLPC reverse micelles alone (0.192 $M^{-1/2}$ $s^{-1/2}$). This is probably due to fast and efficient exchange of phospholipids between micelles (vide infra). Absolute rate constants were determined for propagation, k_p (118–162 M^{-1} s^{-1}), and termination, $2k_t$ ($\simeq 4.8 \times 10^5$ M^{-1} s^{-1}), for autoxidation of DLPC reverse micelles. The $2k_t$ term appears to dominate differences in oxidizability of DLPC in homogeneous solution compared to that in reverse micelles.

3. The dynamics of exchange between reverse DLPC micelles and between monomers in solution and micelles were measured using the ^{31}P NMR magnetization transfer method[30] and Pr^{3+} shift reagent in the aqueous cores. Exchange rates are fast on a laboratory time scale. The lifetime of a DLPC molecule in a micelle (27 ms) is similar to that of a monomer in bulk solution (34 ms) of a system containing micelles. This indicates that direct diffusion through the solvent between micelles is a most probable mechanism for efficient intermicellar exchange. Monomer/micellar exchange in DPPC is slower (73 ms) probably due to differences in packing aggregation between saturated and unsaturated micelles.

Experimental Section

Phospholipids were purchased from Avanti Polar Lipids (Birmingham, AL). Samples for ^{31}P NMR studies 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 before use. Azobis(2-amidinopropane) (ABAP) hydrochloride, and azobis(cyclohexanenitrile) (ACHN) were obtained from Polysciences, Inc. *N,N'*-Dimethyl-4,4'-azobis(4-cyano-1-methylpiperidinium) dinitrate (DACMP) was synthesized by a known method.[44] α-Tocopherol was obtained from Eastman Kodak Co. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylate (Trolox) was a gift from Hoffmann-LaRoche, Nutley, NJ. The phospholipids, initiators, and inhibitors were all stored at −30 °C in the dark.

Autoxidation Kinetics. The autoxidations were carried out at 30 °C under 760 torr of oxygen on an automatic gas absorption apparatus similar to that described.[22,32] To prepare a sample of reverse micelle for autoxidation, the phospholipid was dissolved in benzene (i.e., 25 mg of DLPC in 2.00 mL of benzene) followed by injection of sufficient water (i.e., 10 μL) to bring the H_2O:PPC ratio to approximately 18:1. This mixture was vortexed stirred, then sonicated in a Bransonic 220 bath sonicator until it was clear, approximately 10 min. When water-soluble initiators (ABAP, DACMP) and inhibitor (Trolox) were used, they were dissolved in the water used, the micellar solutions with initiators and inhibitor were prepared separately, and these were mixed in the glass autoxidation cell immediately before initiation of the reaction. The cell was attached to the gas absorption apparatus in a constant temperature bath and the reaction initiated by irradiation with a Pyrex-filtered 200-W super pressure mercury arc light. The light intensity was monitored by a sampling fiber optics light guide. In the rotating sector experiments, to determine k_p and $2k_t$, the order in light intensity was first measured by measuring the rate with a series of six neutral density filters (ranging from 10% to 80% of full light) between the light source and the sample cell. The rates were measured at various rotating sector speeds and fast speed (1800 rpm). Then the R_i was measured by injection of a known amount of an α-tocopherol solution.

^{31}P NMR Studies. Samples of reverse micelles were prepared by the same procedure as used in autoxidation experiments, except that aqueous $Pr(NO_2)_3$, 0.62 or 0.031 M, and water were added as required from a precision syringe before mixing and sonication as before. ^{31}P NMR spectra were recorded on a Nicolet 360NB spectrometer operating at 146.16 MHz at a temperature of 303 K. In a typical experiment the narrow upfield signal was selectively inverted using a DANTE pulse sequence of Morris and Freeman.[45] Specifically, the sequence was $[D5-(P3-D3)_n-D1-P_2-DE-Acquire]_{NA}$ where D5 is the intersequence delay to allow relaxation to equilibrium, D1 is a variable exchange delay, and P3 and D3 are the pulse width and delay associated with the DANTE selective inversion pulse which is accomplished by repeating P3–D3 n times. P2 is a nonselective 90° sampling pulse (31 μs) and DE is a short delay to prevent pulse feedthrough during the data acquisition. The sequence was repeated NA times (usually 200) to increase the signal to noise ratio. Typically P3 and D3 were set to 2 and 100 μs, respectively, with $n = 31$. The transmitter was placed directly on the frequency of the narrow upfield peak and 4 K data points were used. Under these conditions the DANTE pulse train excites a bandwidth of ±80 Hz about the carrier. Excitation is also produced at intervals of 10 kHz from the carrier. However, these frequencies are well away from the ^{31}P phosphorus resonances and offer no interference to the study of the magnetization transfer.

Acknowledgment. This research was supported by the Natural Sciences and Engineering Research Council of Canada. ^{31}P NMR spectra were obtained at the Atlantic Magnetic Resonance Centre, Halifax, N.S., Canada.

Registry No. DPPC, 2644-64-6; DLPC, 998-06-1; ACHN, 2094-98-6; DACMP, 32256-09-0; ABAP, 30393-74-9; α-T, 59-02-9; trolox, 56305-04-5.

(43) Mysels, K. J. "Introduction to Colloid Chemistry"; InterScience: New York, 1959.

(44) Neuman, R. C.; Pankratz, R. P. *J. Org. Chem* **1971**, *36*, 4046.

(45) Morris, G. A.; Freeman, R. *J. Magn. Reson.* **1978**, *29*, 433.