Benzophenone-photosensitized autoxidation of linoleate in solution and sodium dodecyl sulfate micelles

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Diffusion studies show that benzophenone (BP), linoleic acid, and methyl linoleate partition completely into the micelles of phosphate buffer/0.10 *M* SDS. Water-soluble compounds 4-sulphomethylbenzophenone, sodium salt (BP^-), azobis(2-amidinopropane·HCl) (ABAP) and 2,5,7,8-tetramethyl-6-hydroxychroman-2-carboxylate (Trolox) show partial partitioning into the micelles. BP- and BP^--photosensitized oxidation of linoleic acid in 0.50 *M* SDS exhibited characteristics of free radical chain reactions including: (*1*) inhibition by phenolic antioxidants, (*2*) no retardation by singlet oxygen quenchers, and (*3*) the formation of conjugated hydroperoxides with *cis,trans* to *trans,trans* ratios of geometrical isomers typical of autoxidation. Quantitative kinetic studies of the order in substrate, RH, and the rate of chain initiation, R_i, show that the classical rate law, $-d[O_2]/dt = k_p/2k_t^{1/2}[RH]R_i^{1/2}$ applies to BP-photoinitiated autoxidation of linoleic acid in SDS and the oxidizability ($k_p/2k_t^{1/2} = 4.42 \times 10^{-2}\ M^{-1/2}\ s^{-1}1/2$) is the same as that found with a thermal initiator. The rotating sector method gave absolute rate constants for linoleic acid autoxidation in 0.50 *M* SDS for propagation ($k_p = 36.2\ M^{-1}\ s^{-1}$) and termination ($2k_t = 3.52 \times 10^5\ M^{-1}\ s^{-1}$), significantly lower than values in polar organic solvents; attributed to solvation of polar peroxyls in aqueous SDS. Depressed inhibition rate constants (k_{inh}) for α-tocopherol, Trolox, and pentamethylhydroxychroman (PMHC) in 0.50 *M* SDS compared to k_{inh} in *tert*-butyl alcohol are attributed to hydrogen bonding effects on the peroxyl radicals and on the inhibitors.

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Des études de diffusion démontrent que la benzophénone (BP), l'acide linoléique et le linoléate de méthyle se repartissement complètement dans les micelles du tampon de phosphate/0,10 *M* SDS. Les composés qui sont solubles dans l'eau, comme le sel de sodium de la sulfo-4 méthylbenzophénone (BP^-), l'azobis (amidino-2 propane·HCl) (ABAP) et le tétramethyl-2,5,7,8 hydroxy-6 chromanecarboxylate-2 (Trolox), ne se répartissent que partiellement dans les micelles. L'oxydation photosensibilisée par le BP ou le BP^- de l'acide linoléique dans du SDS à 0,50 *M* présente les caractéristiques des réactions radicalaires en chaînes, y compris : (*1*) l'inhibition par les antioxydants phénoliques, (*2*) le fait que les pièges à oxygène singulet ne retardent pas la réaction et (*3*) la formation d'hydroperoxydes conjugués contenant des rapports des isomères géométriques *cis,trans* à *trans,trans* qui sont typiques d'auto-oxydations. Des études cinétiques quantitatives de l'ordre en substrat, RH, et en vitesse d'initiation de chaîne, R_i, démontrent que l'équation de vitesse classique, $-d[O_2]/dt = k_p/2k_t^{1/2}[RH]R_i^{1/2}$ s'applique à l'auto-oxydation photo-initiée par le BP de l'acide linoléique dans le SDS et que la facilité d'oxyder ($k_p/2k_t^{1/2} = 4{,}42 \times 10^{-2}\ M^{-1/2}\ s^{-1/2}$) est la même que celle qui a été observée avec un initiateur thermique. Utilisant la méthode du secteur tournant, on a déterminé les vitesses absolues pour la propagation ($k_p = 36{,}2\ M^{-1}\ s^{-1}$) et pour la terminaison ($2k_t = 3{,}52 \times 10^5\ M^{-1}\ s^{-1}$) de la réaction d'oxydation de l'acide linoléique dans du SDS à 0,50 *M*; ces valeurs sont beaucoup plus basses que celles observées dans des solvants organiques polaires et on attribue ces différences à la solvatation des radicaux peroxyles polaires par le SDS aqueux. Les constantes d'inhibition de vitesse (k_{inh}) que l'on observe pour l'α-tocophérol, le Trolox et le pentaméthylhydroxychromane (PMHC), dans le SDS à 0,50 *M*, sont faibles par rapport à la valeur de k_{inh} que l'on observe dans l'alcool *tert*-butylique; on attribue ces différences à des effets de liaisons hydrogènes sur les radicaux peroxyles et sur les inhibiteurs.

[Traduit par la revue]

Introduction

The photosensitized action of oxygen on biological systems (the photodynamic effect) results in a number of known detrimental effects including membrane damage, protein damage, mutagenesis, and skin cancer (1–3). The possible involvement of singlet oxygen in the photoxidation of biological systems has attracted great interest (1–3) and even some application; as in photodynamic therapy (1).

The variety of examples in the literature where singlet oxygen is reported to be involved in photosensitization processes in model membrane environments such as phospholipid bilayers (4–9) and various types of micelles (10–22) usually involve a dye or porphyrin as sensitizer. These reports were most concerned with the detection of singlet oxygen but little attention was focused on the fate of an oxidizable substrate in these studies in microenvironments.

This investigation examines in detail the photo-oxidation of the linoleate chain in an aqueous sodium dodecyl sulfate (SDS) micellar system containing a simple aromatic ketone (benzophenone) as sensitizer. The results reported herein will show that (*1*) the benzophenone-sensitized oxidation of linoleate in 0.50 *M* SDS proceeds by a free-radical chain autoxidation reaction (Type I) rather than a reaction involving singlet oxygen (Type II); (*2*) the classical rate law

$$[1] \quad \frac{-d[O_2]}{dt} \quad \frac{k_p[RH]R_i^{1/2}}{(2k_t)^{1/2}}$$

well known for autoxidation in homogeneous solution (23–25), is applicable to this system where RH is the linoleate concentration in the micellar phase, k_p and $2k_t$ are the chain propagation and bimolecular termination rate constants, respectively, and R_i is the rate of free radical chain initiation measured by the inhibitor method (26) using phenolic antioxidants; (*3*) the products of autoxidation of linoleate show the same trend with linoleate micellar concentration for photoinitiation with

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benzophenone as that found with lipid-soluble and water-soluble thermal azo initiators, and (*4*) this ketone-sensitized micellar system provides a very useful model microenvironment for *quantitative* kinetic studies of the substrate oxidizability ($k_p/2k_t^{1/2}$) and this system has important advantages for the precise determination of the absolute rate constants for chain propagation, termination, and inhibition for autoxidation of the linoleate chain.

Results

1. Partition coefficients between buffer and 0.10 M *SDS*

Whereas a previous study on thermally initiated autoxidation of linoleic acid in SDS micelles *assumed* that the substrate partitioned completely into the micellar phase (27), it was important for this quantitative study to actually determine the partition coefficients of this and other pertinent compounds between SDS micellar and phosphate buffer phases. For this purpose, we used a variation of the simple method to measure diffusion coefficients (28). The procedure and results of this study are outlined in Table 1.

TABLE 1. Distribution coefficients of organic compounds between phosphate buffer (0.01 *M*, pH 7.0) and SDS (0.10 *M*) at 25°C[a]

Compound[b]	$D \times 10^5$ cm^2 s^{-1}		
	Buffer	SDS	f[c]
Linoleic acid	0.556 ± 0.032	0.158 ± 0.013	0.952
Methyl linoleate	[d]	0.192 ± 0.012	1.0
Benzophenone	0.833 ± 0.014	0.166 ± 0.004	0.960
BP^-	0.666 ± 0.013	0.473 ± 0.044	0.366
ABAP	0.592 ± 0.013	0.180 ± 0.010	0.907
DBHN	1.013 ± 0.020	0.224 ± 0.016	0.902
Trolox	0.621 ± 0.063	0.531 ± 0.031	0.186
PMHC	0.685 ± 0.019	0.185 ± 0.001	0.914
α-Tocopherol	[d]	[e]	1.0[d]
Anthracene	[d]	0.140 ± 0.025	1.0[d]
Phenanthrene	[d]	0.138 ± 0.005	1.0[d]

[a]Diffusion coefficients were calculated from the equation (58): $D = 0.2310r^2t_r/(W_{1/2})^2$ where r is the radius of the tube, t_r is the retention time of the solute, and $W_{1/2}$ is the width of the dispersion curve at half height. The distribution coefficient, f, is then calculated from the equation $D = fD_m + (1 - f)D_w$ (28) where D and D_w are the diffusion coefficients of a solute in 0.10 *M* SDS and buffer, respectively, and D_m is the diffusion coefficient of the micelles (see footnote *d*).

[b]Concentrations of the injected samples were less than 10^{-3} *M* and the detector wavelength was such that the absorbance of the stock solution was at least 0.5.

[c]The deviation is estimated to be 5–10%.

[d]Solubility was too low for measurement. These were taken as "tracer" compounds and their average $D = D_m$ (28).

[e]Eluted peaks were not Gaussian.

2. Inhibition studies with phenolic antioxidants

Experiments were conducted on the effects of the known efficient phenolic antioxidants (29), α-tocopherol (E), 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC), and 2,5,7,8-tetramethyl-6-hydroxychroman-2-carboxylate (Trolox) on the ketone-sensitized oxidation of linoleic acid in SDS micelles. Typical oxygen uptake traces showing the inhibition periods for these antioxidants in reactions initiated by benzophenone and by the sodium salt of 4-sulphomethylbenzophenone (BP^-) are illustrated in Fig. 1 A and B, respectively.

The relative effectiveness of the three antioxidants, E, PMHC, and Trolox, was also measured in a qualitative manner for the photoinitiated oxidation of methyl linoleate in SDS micelles. Typical traces illustrated in Fig. 2 show the relatively poor antioxidant activity of vitamin E *in this system*. Similar results (not shown) were obtained when the water-soluble thermal initiator azobis(2-amidinopropane) hydrochloride (ABAP) was used to initiate autoxidation of methyl linoleate in SDS micelles. This latter system appears to be inhomogeneous (see Discussion); therefore, subsequent quantitative studies were conducted with linoleic acid in SDS.

3. Singlet oxygen quenchers

The inhibition studies with phenolic antioxidants indicate that linoleate undergoes reaction by a free-radical chain autoxidation under our conditions. Nevertheless, a few experiments were carried out with known singlet oxygen quenchers in the aqueous and micellar phases to determine whether they had any effect on the autoxidation. Sodium azide (5×10^{-3} *M*) had no effect on the rate of oxygen uptake and this concentration of azide added with α-tocopherol did not affect the typical induction period observed for this inhibitor (Fig. 1*A*). Similarly, diphenylisobenzofuran (DPBF) did not affect the rate of oxygen uptake in concentrations of 8.2×10^{-4} *M* in the micellar phase and did not affect the length of the induction period of α-tocopherol (1.17×10^{-4} *M*).

Irradiation of aqueous SDS micellar solutions containing methylene blue is a known method to generate singlet O_2 in the aqueous phase (14, 20, 22). The effect of the free-radical trap, Trolox, on an irradiated micellar solution containing methylene blue showed a very interesting contrast with its inhibiting action in the BP-sensitized experiments. In the presence of aqueous $^1O_2$, Trolox caused a rapid upsurge in the rate of oxygen consumption which is more pronounced with increased Trolox. Similarly, injection of α-tocopherol (8.5×10^{-4} *M*) did not cause an induction period. HPLC analysis indicated consumption of E with time, presumably due to attack by singlet oxygen in the micellar phase. These results are attributed to a rapid chemical reaction between singlet oxygen and the inhibitors (30, 31). On the other hand, ground state O_2 shows a low reactivity toward the tocopheroxyl radical (32).

4. Product studies

The products of autoxidation of linoleate are known to be conjugated diene 9- and 13-hydroperoxide positional isomers, having the *trans,trans* and *cis,trans* configuration (33). Reaction of linoleate with singlet oxygen yields a different mixture with approximately equal amounts of two conjugated (9- and 13-) and two non-conjugated (10- and 12-) hydroperoxides (34). In other words, the products from the $^1O_2$ reaction are not controlled by abstraction of the reactive allylic hydrogens as is the case for the autoxidation procedure. Porter and co-workers (35–39) have conducted a detailed study of the products formed in autoxidation of linoleic acid in solution and from phospholipid emulsions using a lipid-soluble initiator (e.g. di-*tert*-butylhyponitrite, DBHN). As a result, they developed a mechanism for lipid peroxidation which involves a competition between H-atom abstraction and β-scission of peroxyl radicals to account for the dependence of the relative amounts of the kinetic (*cis,trans*) and thermodynamic (*trans,trans*) products on the hydrogen atom donating ability, i.e. linoleate concentration, of the medium.

In order to determine if the Porter mechanism for linoleate autoxidation is applicable to oxidations conducted in aqueous SDS micelles, we investigated the dependence of the ratio of the various oxidation products as a function of micellar linoleate

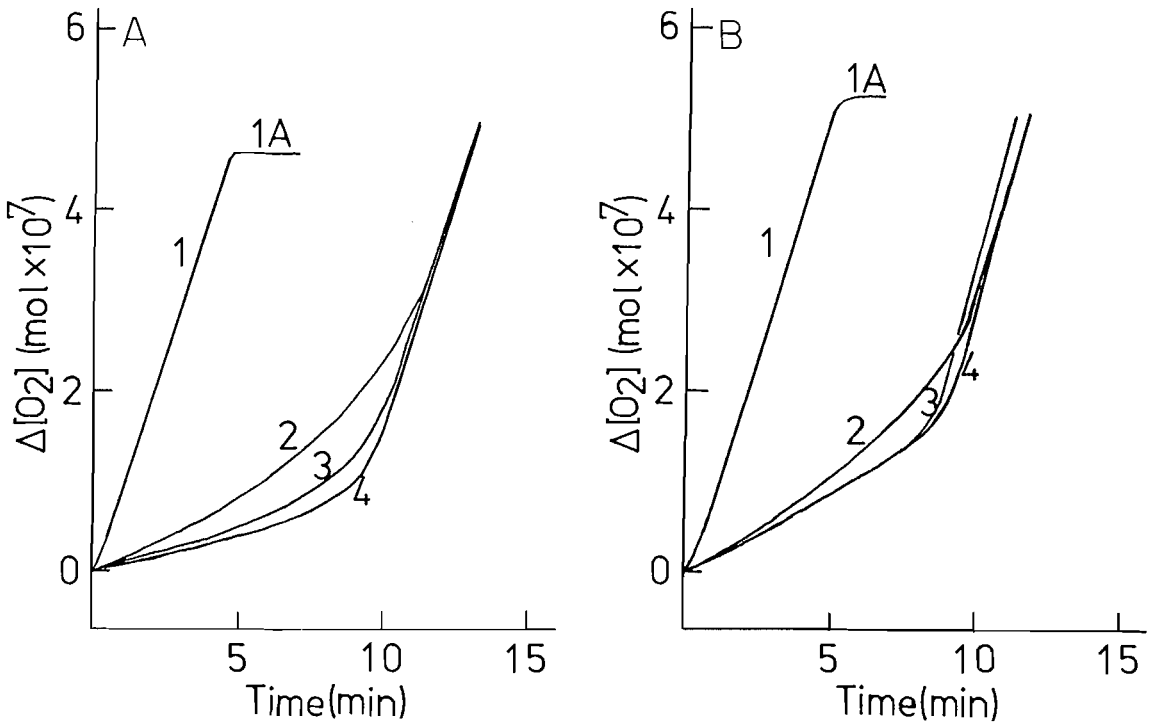


FIG. 1. (*A*) Autoxidation of linoleic acid (6.43×10^{-5} mol) in 2.00 mL of 0.50 *M* SDS (pH 7.0) at 30°C, photoinitiated by benzophenone (2.82×10^{-6} mol): *1*, uninhibited reaction (1A: lamp off); *2*, inhibited with α-tocopherol (1.67×10^{-8} mol); *3*, inhibited with Trolox (1.66×10^{-8} mol); *4*, inhibited with PMHC (1.62×10^{-8} mol). (*B*) Autoxidation of linoleic acid (4.83×10^{-5} mol) in 1.50 mL of 0.50 *M* SDS (pH 7.0) at 30°C, photoinitiated by BP^- (2.07×10^{-6} mol): *1*, uninhibited reaction (1A: lamp off); *2*, inhibited with α-tocopherol (3.75×10^{-8} mol); *3*, inhibited with Trolox (3.83×10^{-8} mol); *4*, inhibited with PMHC (3.88×10^{-8} mol).

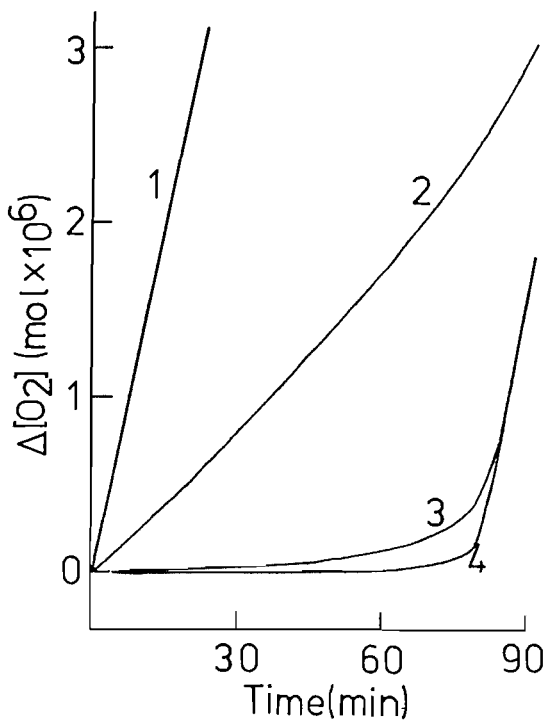


FIG. 2. Autoxidation of methyl linoleate (1.21×10^{-4} mol) in 2.00 mL of 0.50 *M* SDS (pH 7.0) at 30°C, photoinitiated by benzophenone (1.10×10^{-6} mol): *1*, uninhibited reaction; *2*, inhibited with α-tocopherol (3.70×10^{-8} mol); *3*, inhibited with Trolox (3.65×10^{-8} mol); *4*, inhibited with PMHC (3.60×10^{-8} mol).

concentration. Oxidations initiated by both water-soluble and lipid-soluble azo compounds (ABAP and DBHN, respectively) and water- and lipid-soluble benzophenones were studied. In all four cases, the products separated by HPLC (following reduction and methylation) were the alcohols known to form by free-radical autoxidation; namely **1**–**4** (Scheme 1).

The analytical data for the products of micellar linoleate autoxidations when thermally initiated by the azo compounds, ABAP and DBHN, are outlined in Table 2 while those for reactions photoinitiated by the benzophenones (BP and BP^-) are given in Table 3.

HPLC analyses were also undertaken to determine the fate of benzophenone during prolonged irradiation. A typical sample containing 1.54×10^{-4} *M* BP in 0.50 *M* SDS, saturated with O_2 *but without linoleic acid*, gave >95% recovery of benzophenone with only traces of benzhydrol and benzopinacol after $2\frac{1}{4}$ h of irradiation at full lamp intensity. Also, there was no observed uptake of oxygen during the experiment. Similar results were obtained when the experiment was carried out in the presence of linoleic acid (0.46 *M*). After $2\frac{1}{4}$ h of irradiation, greater than 90% of the BP remained while approximately 2.5% of the initial amount was accounted for by benzhydrol and a trace of benzopinacol. During the course of a typical kinetic autoxidation experiment (the equivalent of 2–3 h of continuous irradiation at full lamp intensity) essentially all of the benzophenone is recovered.

5. Kinetic results

In order to determine if the classical rate law (eq. [1]) is applicable to these photo-oxidations in SDS micelles, the

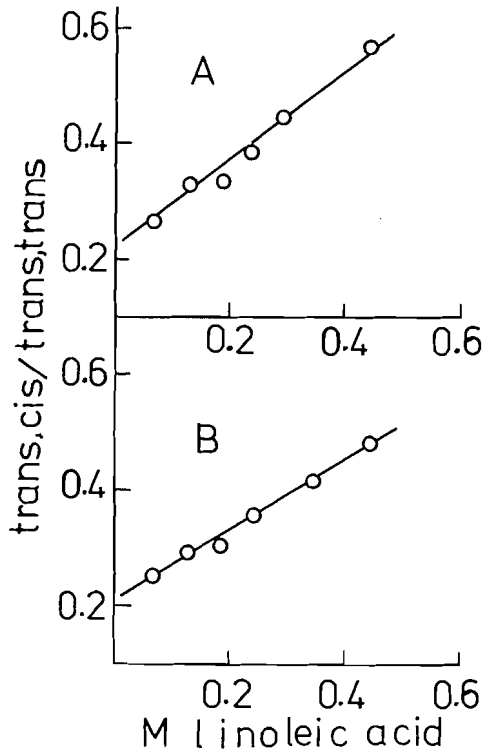


FIG. 3. Concentration dependence of product ratios (*trans,cis/trans,trans*) of hydroxy fatty esters derived from autoxidation of linoleic acid in 0.50 *M* SDS (pH 7.0) at 30°C. Values taken from Table 2. *A*, Initiation by DBHN (1.03×10^{-5} mol); *B*, initiation by ABAP (2.67×10^{-5} mol).

dependence of the rate of oxygen uptake on micellar linoleic acid concentration was measured at five concentrations over the range 0.0630 *M* to 0.459 *M* and the dependence of the rate of O_2 consumption with variation in R_i was measured by the application of five different lamp intensities at each linoleic acid concentration. The results of these experiments are summarized in Table 4.

The absolute rate constants for chain propagation, k_p, and termination, $2k_t$, were measured by the rotating sector method (40, 41).

The separation of the absolute rate constants for chain propagation and termination involves measurement of the lifetime, τ, of the reaction chain. The chain lifetime is defined by the equation

$$[2] \quad \tau = (2k_tR_i)^{1/2}$$

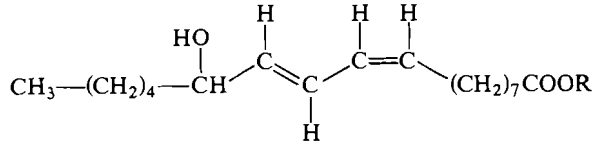
where R_i is the rate of photoinitiation. A sectored disc is placed in the light path between the lamp and the reaction vessel and the rate of oxygen consumption is measured at various rotation speeds of the disc. If the ratio of dark to light periods is r, the rate at fast speeds is

$$[3] \quad R_f = K[I/(r + 1)]^{1/2}$$

where $KI^{1/2}$ is the oxidation rate at a steady light intensity, I. At slow rotation speeds, the rate is

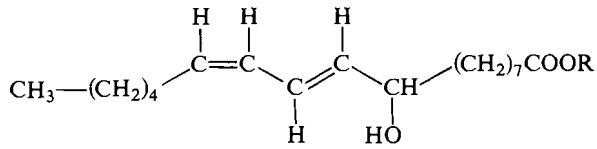
$$[4] \quad R_s = KI^{1/2}/(r + 1)$$

At intermediate sector rotation speeds, the reaction rate has a complicated dependence on the duration of the light flash, λ, and the lifetime of the reaction chain. The value of λ is



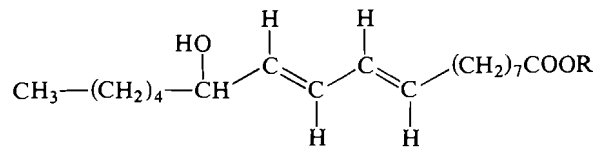
1

Methyl 13-hydroxy-*cis*-9, *trans*-11-octadecadienoate



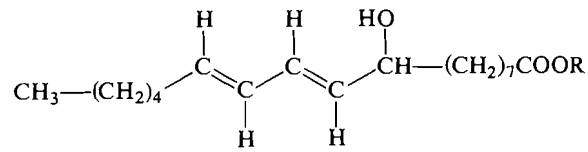
2

Methyl 9-hydroxy-*trans*-10, *cis*-12-octadecadienoate



3

Methyl 13-hydroxy-*trans*-9, *trans*-11-octadecadienoate



4

Methyl 9-hydroxy-*trans*-10, *trans*-12-octadecadienoate

SCHEME 1

determined by the rotation speed of the sector

$$[5] \quad \lambda = \frac{r + 1}{\text{slow speed (rpm)}}$$

Under steady illumination, the rate of oxygen consumption, R_0, can be represented by the classical rate law:

$$[1a] \quad R_0 = k_p[\text{RH}]\left(\frac{R_i}{2k_t}\right)^{1/2}$$

Burnett and Melville (40) have given values of the ratio R_λ/R_0 in terms of λ/τ for various values of r. As a result, $2k_t$ can be readily obtained from eq. [2] once the R_i has been measured. Substitution of R_i and $2k_t$ into eq. [1*a*] yields k_p. To improve the accuracy of the measurements, we used the modification (41) of finding a particular λ by varying the sector speed to obtain a rate midway between R_f and R_s and then repeatedly measured R_0 and R_λ.

In the above discussion it has been assumed that chain termination is a bimolecular process. First order chain termination, which can be represented as

$$[6] \quad \text{ROO}\cdot \rightarrow \text{inactive products}$$

causes a reduction in the value of R_f/R_0. The measured ratio enables determination of a quantity, γ, by means of the following expression:

$$[7] \quad \frac{R_f}{R_0} = \frac{\{(\gamma^2 r + 1)/(r + 1)\}^{1/2}\gamma}{1 - \gamma}$$

TABLE 2. Product ratio distribution for autoxidation of linoleic acid in 0.50 *M* SDS (pH 7.0) at 30°C; initiated by azo intiators

Initiator	Concentration (mol × 10^5)	[Linoleic acid][a] (mol L^{-1})	*t,c* products[b] / *t,t* products
DBHN	1.03	0.0631	0.264
		0.124	0.333
		0.182	0.332
		0.238	0.386
		0.292	0.450
		0.444	0.571
ABAP	2.67	0.0631	0.252
		0.124	0.296
		0.182	0.305
		0.238	0.357
		0.345	0.417
		0.444	0.483

[a]A micellar reaction volume of 2.5×10^{-4} liters for 2.00 mL of 0.50 *M* SDS was used in concentration calculations (27) in micelles throughout (Tables 2, 3, 4, 6).
[b]**(1 + 2)/(3 + 4)**. Products were analyzed as the methyl hydroxylinoleates (see text) by three HPLC analyses on each experiment. The reproducibility was approximately 5%.

TABLE 3. Product ratio distribution for autoxidation of linoleic acid in 0.50 *M* SDS (pH 7.0) at 30°C; photoinitiated by benzophenones

Initiator	Concentration (mol × 10^7)	[Linoleic acid][a] (*M*)	*t,c* products[b] / *t,t* products
BP	5.48	0.124	0.255 ± 0.007
		0.238	0.284 ± 0.019
		0.445	0.429 ± 0.032
		0.536	0.443[c]
		0.742	0.602 ± 0.034
BP^-	5.49	0.0631	0.182 ± 0.011
		0.124	0.264 ± 0.014
		0.238	0.283 ± 0.039
		0.445	0.454 ± 0.045
		0.742	0.630 ± 0.025

[a]See footnote *a*, Table 2.
[b]Results from two different experiments, also see footnote *b*, Table 2.
[c]One experiment.

where γ is defined as $k_x/(k_x^2 + 8k_tR_i)^{1/2}$. For a particular γ, the value of R_λ/R_0 yields a second quantity m, defined as

$$[8] \quad m = \frac{\lambda(k_x^2 + 8k_tR_i)^{1/2}}{2}$$

The rate constant for first order termination, k_x is readily calculated from γ and m:

$$[9] \quad k_x = \frac{2\gamma m}{\lambda}$$

The rate constants for bimolecular termination and chain propagation are then obtained from the following expressions:

$$[10] \quad 2k_t = \frac{m^2}{\lambda^2 R_i} - \frac{k_x^2}{4R_i}$$

and

TABLE 4. Kinetics of autoxidation of linoleic acid in 0.50 *M* SDS (pH 7.0) at 30°C; photoinitiated by benzophenone (1.13×10^{-2} *M*)[a]

[Linoleic acid][a]	$-d[O_2]/dt$ ($M\ s^{-1} \times 10^6$)	Transmittance[b]
0.0631	1.57	1.00
	1.43	0.794
	1.00	0.501
	0.708	0.251
	0.341	0.100
0.124	3.26	1.00
	2.82	0.794
	2.10	0.501
	1.50	0.251
	0.869	0.100
0.238	6.69	1.00
	5.99	0.794
	4.38	0.501
	3.18	0.251
	1.89	0.100
0.344	9.55	1.00
	8.39	0.794
	6.30	0.501
	4.62	0.251
	2.67	0.100
0.459	12.5	1.00
	10.8	0.794
	8.86	0.631
	8.23	0.501
	5.81	0.251
	4.42	0.159
	3.52	0.100

[a]See footnote *a*, Table 2.
[b]The transmittance was controlled by inserting neutral density filters into the light path.

$$[11] \quad k_p = \frac{R_0 2(2k_t)\gamma}{[RH]k_x(1 - \gamma)}$$

The absolute rate constants were determined in two organic solvents, acetonitrile and *tert*-butyl alcohol, by photoinitiation with benzophenone as well as the more conventional azobiscyclohexylnitrile (ACHN) (41). For each solvent, reasonably good agreement was obtained by the two methods, as illustrated in Table 5.

The rotating sector method was applied to a series of autoxidations of linoleic acid in aqueous SDS micelles with benzophenone as sensitizer. The absolute rate constants so determined are reported in Table 6.

Equation [12] is the rate equation for inhibited autoxidations,

$$[12] \quad \frac{-d[O_2]}{dt} = \frac{k_p[RH]R_i}{nk_{inh}[ArOH]}$$

where the stoichiometric factor $n = 2$ for the phenolic antioxidant (ArOH) used. Since the k_p is now known for our conditions we were able to determine inhibitor rate constants, k_{inh}, for the termination of peroxyls via the process,

$$ROO\cdot + ArOH \rightarrow ROOH + ArO\cdot$$

in homogeneous solution and in SDS micelles. Some typical value are shown in Table 7.

TABLE 5. Absolute rate constants for the autoxidation of linoleate (0.420 M) in homogeneous solution at 30°C[a]

No.[b]	Initiator ($M \times 10^3$)	Solvent	R_i[c] ($M\,s^{-1} \times 10^7$)	$\frac{-d[O_2]/dt}{[RH]R_i^{1/2}}$ ($M^{-1/2}s^{-1/2} \times 10^2$)	ν[e]	γ	m	k_x[d]	$2k_t$[d] ($M^{-1}s^{-1} \times 10^{-6}$)	k_p[d] ($M^{-1}s^{-1}$)
1	ACHN (6.76)	CH_3CN[f]	2.26	3.59	38	0.070	3.49	0.133	4.42	94.9
2(*a*)	BP (1.63)	CH_3CN	1.85	4.03	39	0.072	4.07	0.132	4.33	96.5
(*b*)			1.91	3.98	37	0.070	3.08	0.118	3.74	90.5
3	ACHN (6.85)	TBA[f]	6.95	1.72	30	0.075	3.75	0.153	15.1	75.6
4(*a*)	BP (1.66)	TBA	1.80	1.95	19	0.092	4.36	0.308	15.5	78.5
(*b*)	(1.68)		1.17	1.45	17	0.203		0.650	21.0	90.4
5	BP (1.63)	CH_3CN	1.71	4.37	44	0.040	4.53	0.051	2.43	70.3

[a]Under such conditions, the order with respect to the R_i (i.e. light intensity) was 0.457–0.522.
[b]Nos. 1–4 used linoleic acid, no. 5 methyl linoleate.
[c]The R_i was measured by the induction period method. The value reported is the average of at least two such measurements with Trolox.
[d]The variation in measurements of the rate constants within an experiment (by repeated measurements of R_0 and R_λ) was between 5 and 18% and generally less than 10%.
[e]The kinetic chain length; $\nu = -d[O_2]/dt//R_i$.
[f]The dielectric constants for CH_3CN = 38, for *tert*-butyl alcohol (TBA) = 11.

TABLE 6. Absolute rate constants for the benzophenone-sensitized autoxidation of linoleic acid (0.238 M)[a] in 0.50 M SDS (pH 7.0) at 30°C

No.	BP ($M \times 10^2$)	R_i[b] ($M\,s^{-1} \times 10^7$)	$\frac{-d[O_2]/dt}{[RH]R_i^{1/2}}$ ($M^{-1/2}s^{-1/2} \times 10^2$)	ν[d]	γ	m	k_x[c] (s^{-1})	$2k_t$[c] ($M^{-1}s^{-1} \times 10^{-5}$)	k_p[c] ($M^{-1}s^{-1}$)
1	1.01	5.20	4.94	16	0.140	2.62	0.143	4.98	44.5
2	1.01	5.05	4.78	16	0.136	3.95	0.107	3.05	35.8
3	1.01	4.22	4.46	16	0.164	4.50	0.106	2.44	30.9
4	0.203	0.914	3.95	31	0.250	2.43	0.088	3.08	32.4
5	0.203	0.803	3.95	33	0.250	2.58	0.093	4.06	37.4

[a]See footnote *a*, Table 2.
[b]See footnote *c*, Table 5.
[c]The variation in rate constants within an experiment (by repeated measurement of R_0 and R_λ) was between 8 and 30%.
[d]See footnote *e*, Table 5.

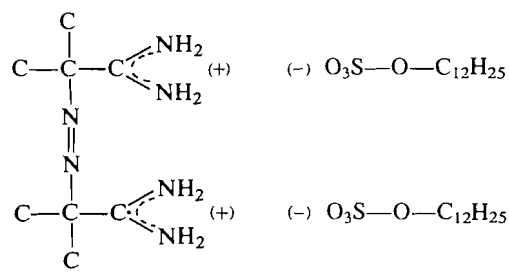
Discussion

1. Partitioning of substrates, initiators and antioxidants between the aqueous and micellar phases

The partition coefficient determinations (Table 1) reveal that the diffusion coefficients for anthracene and phenanthrene approach a limiting value, indicative of localization within micelles. As such, these highly hydrophobic compounds are said to act as "tracers" of the micellar diffusion and the average value of their diffusion coefficients is assigned to D_m (28). From the table it can also be seen that linoleic acid is completely (within experimental error) in the micellar phase. The diffusion coefficient for methyl linoleate in 0.10 M SDS is similar to that for the "tracer compounds". This and the fact that the ester has such poor solubility in the buffer used (precluding measurement of D) lead to the conclusion that both linoleates partition entirely into the micellar phase. Subsequent calculations assume this to be the case.

The partitioning of the initiators employed in this study provides some very interesting results. Benzophenone resides in the micellar phase ($f = 0.96$) while its water-soluble derivative BP^- partitions considerably ($f = 0.37$) into the micelles of 0.10 M SDS. It has been suggested earlier that BP^- was "essentially" in an aqueous environment in the presence of 0.2 M SDS from spectroscopic properties (42). It is our conclusion that the determination of diffusion coefficients provides a more sensitive quantitative measurement of partitioning than do spectroscopic methods.

The behaviour of the azo initiator, ABAP, appears to be very surprising. It is readily soluble in water (aqueous solutions of 1 M or more are easily prepared), but it partitions to the extent of 91% into 0.1 M SDS. We speculate that this may be due to electrostatic interactions between the positive amidino groups of ABAP and the anionic surface of the micelle, such as shown below



This model implies that ABAP *may* be associated with the surface of the micelles. Such electrostatic interactions could account for the unusual observation that ABAP decomposes three times *less rapidly* in 0.50 M SDS than in water or in liposome dispersions (43). We suggest that these dipolar

TABLE 7. Inhibition rate constants for phenolic antioxidants in the photo-initiated autoxidation of linoleic acid[a] in solution and in 0.50 *M* SDS (pH 7.0) at 30°C

Medium	Initiator ($M \times 10^3$)	R_i ($M\,s^{-1} \times 10^7$)	Inhibitor ($M \times 10^5$)	k_p ($M^{-1}s^{-1}$)	k_{inh}[b] ($M^{-1}\,s^{-1} \times 10^{-4}$)
TBA	BP (1.69)	1.30	α-T (7.37)	85.1	21.8 ±4.3
		1.22	(7.12)		24.2 ±4.2
		1.25	PMHC (7.67)		15.3 ±2.0
		1.12	(7.43)		26.8 ±1.8
		1.04	Trolox (7.72)		13.4 ±2.5
		1.02	(7.46)		15.9 ±2.2
CH_3CN	BP (1.63)	1.85	Trolox (10.3)	93.8	8.55±0.99
		1.74	(10.1)		11.2 ±0.9
	BP (1.63)	1.91	(10.4)		10.6 ±0.8
		1.84	(10.3)		9.27±0.99
	ACHN (6.76)	2.12	(11.1)	94.9	11.7 ±1.0
		1.93	(11.0)		12.4 ±0.8
SDS	BP (10.4)	2.04	α-T (6.19)	36.2	1.79±0.19[c]
	(11.5)	1.08	(11.7)		1.59±0.20
	(10.4)	2.12	PMHC (6.00)		4.43±0.58[c]
	(11.5)	1.74	(12.2)		2.99±0.64
	(10.4)	2.19	Trolox (6.15)		3.51±0.29[c]
	(11.5)	1.44	(16.4)		2.10±0.37

[a]Initial linoleic acid concentration was 0.420 *M* in both TBA and CH_3CN and 0.238 *M* (in the micellar phase) for 0.50 *M* SDS.

[b]Inhibition rate constants were determined for at least four points along each induction period where the chain length was in the range 2–9. The variation is shown for each induction period. The method of calculation of ref. 29 was used.

[c]The calculation for k_{inh} assumes that α-T, PMHC, and Trolox partition completely into the micellar phase in 0.50 *M* SDS (see text).

interactions act to inhibit cleavage of the molecule and reduce the rate of nitrogen liberation.

Trolox is an effective antioxidant in either organic solvents (29) or aqueous systems (43, 44). As shown in Table 1, it partitions significantly (19%)[2] into the micellar phase and its versatility as an antioxidant is undoubtedly due to this partitioning action between the two phases.

2. Product studies with azo and benzophenone initiators. The mechanism for benzophenone-initiated oxidations

The photosensitized reaction of oxygen with an organic substrate may be initiated by hydrogen abstraction from the substrate by the sensitizer triplet followed by free radical autoxidation (Type I mechanism). Alternately, the Type II process involves initial energy transfer from the sensitizer to form singlet oxygen which can react with the hydrogen donor in a variety of ways (such as the ene reaction with olefins), usually distinct from the free radical route. These two reaction types have been studied extensively and reviewed (2, 3). Product studies provide one method of distinguishing Type I from Type II (*vide supra*).

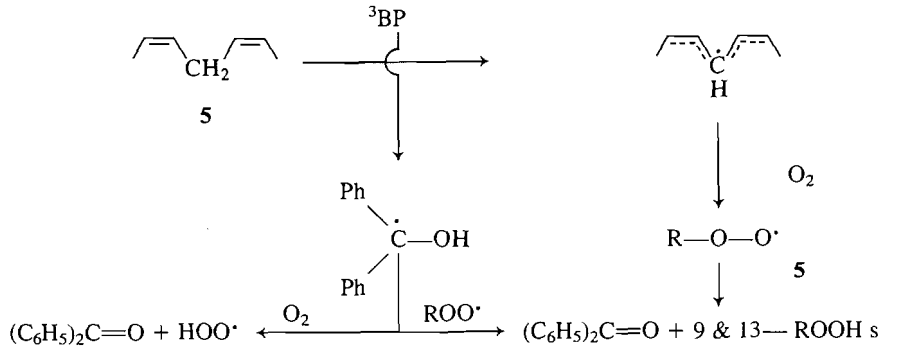
In Fig. 3 *A* and *B* are shown plots of the dependence of the ratio of *cis,trans*-substituted hydroperoxides (**1** + **2**) to *trans,trans* products (**3** + **4**) as a function of micellar linoleic acid concentration for thermal initiation by DBHN and ABAP, respectively. Good linear correlations were obtained with both lipid-soluble DBHN and water-soluble ABAP. Since ABAP is associated with the micellar phase, it most likely forms initiating peroxyls in (or near) the same region as DBHN.

Plots of *cis,trans/trans,trans* product ratios for oxidations photoinitiated by benzophenone and BP^- are shown in Fig. 4 *A* and *B*, respectively. These plots show that the ratio of kinetic (*c,t*) to thermodynamic (*t,t*) products shows a linear dependence on linoleate concentration remarkably similar to that found for the thermal azo initiators. These results are entirely consistent with the free radical autoxidation (Type I) mechanism for linoleate oxidations in micellar SDS whether thermally initiated (by DBHN or ABAP) or photoinitiated (by BP or BP^-).

At least two important factors operate to account for the occurrence of Type I mechanism in our system. (*1*) One important competition which controls whether initiation occurs by Type I (radical chain) or Type II (singlet O_2) is between substrate and oxygen for the triplet sensitizer (2). Allylic hydrogens are known to be effective quenchers of benzophenone triplets by hydrogen abstraction (45) and the doubly allylic hydrogens in linoleate are readily abstracted. (2) While singlet oxygen is known to form in micelles by various photosensitization methods (10–22) and its lifetime is "longer" (53 μs, ref. 17) in SDS, peroxyl radicals have lifetimes orders of magnitude longer. Furthermore, termination of linoleate peroxyls is very much retarded in the presence of SDS micelles (*vide infra*). As a result of these factors, $^1O_2$ quenchers such as azide and DPBF had no effect on our reactions and the peroxyl radicals carried a chain reaction as proposed in Scheme 2.

The recycling of benzophenone as initiator is an interesting characteristic of our system. This could occur via some chain

[2]This higher partition fraction over that reported (2%) is probably related to the higher SDS concentration used here compared to only 0.0150 *M* used earlier (44).



SCHEME 2

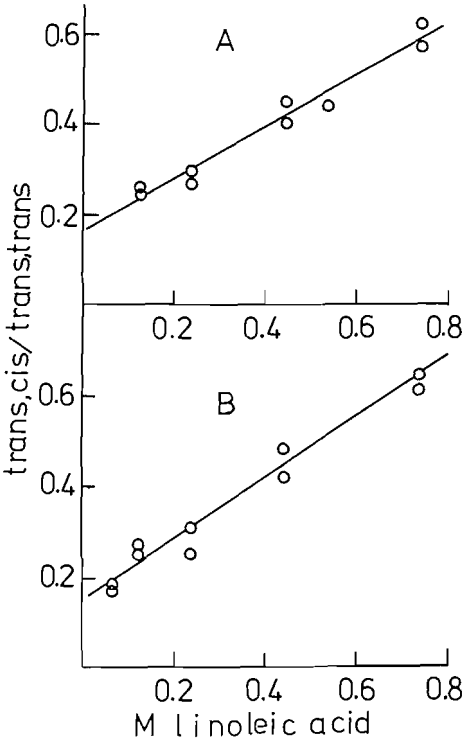


FIG. 4. Concentration dependence of product ratios (*trans,cis/trans,trans*) of hydroxy fatty esters derived from autoxidation of linoleic acid in 0.50 *M* SDS (pH 7.0) at 30°C. Values taken from Table 3. *A*, Photoinitiated by benzophenone (5.48×10^{-7} mol); *B*, photoinitiated by BP$^-$ (5.49×10^{-7} mol).

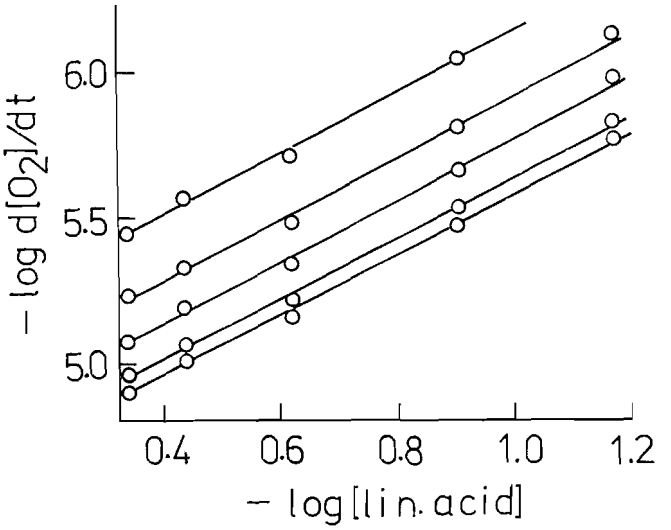


FIG. 5. Kinetic order plots for autoxidation of linoleic acid in 0.50 *M* SDS (pH 7.0) at 30°C; photoinitiated by benzophenone (1.13×10^{-2} *M*). Rate of oxygen consumption as a function of substrate concentration at five different light intensities. Values taken from Table 4.

termination by H-atom transfer from the ketyl radical to peroxyl or possibly to oxygen, a known reaction in homogeneous solution (46).

3. Kinetics for benzophenone-initiated autoxidation. Medium effects on rate constants of autoxidation and inhibition

The benzophenone-sensitized autoxidation has very significant advantages over the usual azo initiators for these quantitative measurements. Evolution of nitrogen and consumption of oxygen from the latter require large corrections to the measured rates in microenvironments (47), and such corrections are difficult to make for photo-decomposition of azo initiators since the necessary rate constants are unknown. In addition, azo initiators give thermal (dark) rates of autoxidation which can cause large errors in determinations of rate constants. These limitations do not occur in benzophenone-sensitized autoxidations; there is no dark rate and no gas is absorbed/produced when BP is irradiated in oxygenated 0.50 *M* SDS.

Log plots of the oxygen uptake for benzophenone-initiated reactions in SDS micelles are shown in Fig. 5 over a 7.3-fold change in linoleate concentration and for the five different light intensities used. Excellent linear correlations were obtained in all cases and the order in linoleate is essentially unity (1.02–1.06). Plots of the kinetic order in light transmittance shown in Fig. 6 for these conditions also exhibit linear correlations. The reaction order with respect to the rate of free radical chain initiation (R_i) obtained from the slopes vary somewhat, 0.627 to 0.540 with linoleate concentration. This is attributed to some first order chain termination[3] of peroxyls accompanying the usual bimolecular termination at low concentration. Subsequent kinetic measurements of rate constants were made at intermediate concentration (0.238 *M*) where the classical rate law for autoxidation (eq. [1]) is followed.[4]

The absolute rate constants for chain propagation, k_p, and termination, $2k_t$, for autoxidation of linoleate in two polar solvents, *tert*-butyl alcohol and acetonitrile (Table 5), provide

[3]Others (41) have postulated first order termination of peroxyls but the detailed mechanism is not known.

[4]The probability of simultaneously generating two radicals in the micelle is nil. In these and other related free radical reactions in SDS micelles (27, 44, 51), the fact that bimolecular termination occurs also implies that the radicals in question (e.g. linoleoylperoxyl) diffuse rapidly between micelles to allow radical recombination.

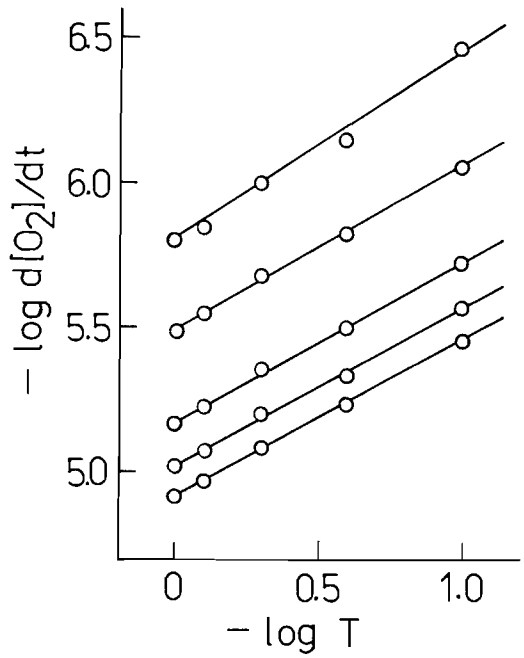


FIG. 6. Kinetic order plots for autoxidation of linoleic acid in 0.50 *M* SDS (pH 7.0) at 30°C; photoinitiated by benzophenone (1.13×10^{-2} *M*). Rate of oxygen consumption as a function of light intensity at five substrate concentrations. Values taken from Table 4.

basic data for comparison with the values obtained in micelles. As observed earlier by Howard and Ingold (48), the effect of an increase in solvent polarity is mainly on the rate of chain *termination*, which is decreased because the solvent stabilized polar peroxyl radicals (48) are destroyed in this step. Our finding that the $2k_t$ is lower in CH_3CN ($4.16 \pm 0.30 \times 10^6$ M^{-1} s^{-1}) than in *tert*-butyl alcohol ($17.2 \pm 2.7 \times 10^6$ M^{-1} s^{-1}) is consistent with the effect of the more polar solvent to decrease the termination rate.

Kinetic data for benzophenone-initiated autoxidation of linoleate (Table 6) indicate that the substrate oxidizability obtained by this method ($4.42 \pm 0.46 \times 10^{-2}$ $M^{-1/2}$ $s^{-1/2}$) is the same as that obtained earlier (4.48×10^{-2} $M^{-1/2}$ $s^{-1/2}$) using a thermal azo initiator in 0.50 *M* SDS (27).

Significant differences are shown in the absolute rate constants in micelles compared to those in organic solvents. The chain propagating rate constant in SDS (36.2 ± 4.8 M^{-1} s^{-1}) is one-half that found in acetonitrile. The effect on the $2k_t$ is more dramatic; the value in 0.50 *M* SDS ($2k_t = 3.52 \pm 0.90 \times 10^5$ M^{-1} s^{-1}) being reduced by at least an order of magnitude. We suggest that these effects on the rate constants are due, at least in part, to the polarity of peroxyl radicals.[5] Once formed, the radical centre would rapidly move to the micelle-aqueous interface where the polar environment of the aqueous buffer would substantially lower the $2k_t$ by strongly solvating the radicals by polar and hydrogen bonding interactions (50),[6] in this case of the resonance-stabilized peroxyls:

$$R{-}\ddot{O}{-}\ddot{O}\cdot \Longleftrightarrow R{-}\overset{(+)}{\dot{O}}{-}\overset{(-)}{\ddot{O}}\!: \quad \cdots \quad H{-}OH$$

Such solvation effects would also account for the lower k_p observed since the reactivity of the peroxyls would thereby be suppressed.

[5]The dipole moment of a peroxyl radical was found to be 2.4 D (49).

[6]In addition, the fact that a radical must exit a micelle in order to terminate with one in another micelle can be a controlling factor (51).

Values for inhibitor rate constants (k_{inh}) are of interest because they are an indication of the antioxidant activity. Accurate determinations are reported for various phenolic antioxidants in chlorobenzene solution (29). However, other reported values for k_{inh} in different solvents (52) and in micelles (44) can be quite unreliable because they are based on values of k_p measured in solution under different conditions. Evaluation of k_{inh} in microenvironments such as micelles and bilayers are of particular interest because they have implications for the effectiveness of antioxidants as biomembrane protectors. It is reported that the antioxidant properties of α-tocopherol and PMHC are similar within micelles and liposomes but that the phytyl side chain of α-tocopherol suppresses its transfer between liposomes (53).

Our values of k_{inh} reported in Table 7 are corrected for the k_p applicable for the medium in which the determination was made. Rather large errors are associated with these numbers (see Table 7), but they are sufficiently reliable to determine the effect of a micellar environment compared to a polar organic solvent, which is our main objective. To a certain extent, it is also possible to compare the activities of the three antioxidants in the same medium. The k_{inh} for α-tocopherol is retarded by a factor of 14 on changing from solution ($k_{inh} = 23 \pm 1.2 \times 10^4$ M^{-1} s^{-1} in *tert*-butyl alcohol) to micelles ($k_{inh} = 1.69 \pm 1.0 \times 10^4$ M^{-1} s^{-1}). This supports the proposal that diffusion of this lipophilic phenol between micelles is a limiting factor in antioxidant activity (44). The overall result of the micelles is a change in the relative activities of the three antioxidants. In 0.50 *M* SDS, PMHC and Trolox are now approximately twice as active as E; whereas in homogeneous alcohol solution, E and PMHC have the same activity, within experimental error, followed by Trolox. The absence of a definite induction period when α-tocopherol is used with methyl linoleate dispersed in SDS micelles (Fig. 2) can now be understood in terms of the reduced mobility of this inhibitor between micelles combined with a lack of homogeneity in such a system. It was suggested that methyl linoleate may "pool" (54) or form a heterogeneous microemulsion (55) in detergents (56) so that exchange of E between droplets is limiting. This demonstrates the importance of selecting the model system for quantitative study with considerable care.

Our actual values of k_{inh} are significantly different from values known in less polar solvents (e.g. α-tocopherol, $k_{inh} = 320 \times 10^4$ M^{-1} s^{-1} in chlorobenzene (29)). We suggest that a polar protic solvent (TBA) reduces the k_{inh} substantially by solvation of the polar peroxyl radicals. Trolox shows a further reduction in k_{inh} on changing from TBA ($k_{inh} = 14.7 \pm 1.3 \times 10^4$ M^{-1} s^{-1}) to more polar acetonitrile ($k_{inh} = 10.6 \pm 1.3 \times 10^4$ M^{-1} s^{-1}) most probably due to solvent effects on the peroxyl radical reactivity. The overall drop in activity of the three antioxidants observed in aqueous SDS micelles is attributed to a combination of strong hydrogen bonding aqueous solvent effects on the polar peroxyl radicals, and the effect water may have of tying up the electron lone pairs on the oxygen *para* to the phenolic hydroxyl group of the antioxidants. This latter hydrogen bonding would reduce the reactivity of the phenolic hydrogen by damping the stereoelectronic effect (29) exerted by the lone pairs on the para oxygen.

Experimental

1. Distribution coefficients

(1) Materials and preparation

The buffer used for diffusion coefficient measurements was 0.005 *M*

each of NaH_2PO_4 and Na_2HPO_4 (pH 7.0) in glass-distilled water containing 1×10^{-5} *M* EDTA. Stock 0.10 *M* SDS was prepared in this buffer. Anthracene (Eastman-Kodak) and phenanthrene (Fisher) were each recrystallized from ethanol.

Dilute solutions (generally less than 10^{-3} *M*) of the various compounds were prepared in both buffer and 0.10 *M* SDS by vortex-stirring and sonication (except DBHN and ABAP solutions). The solution concentration was such that it gave an absorbance between 0.5 and 1.5 at λ_{max} as measured in a 1.0 cm cell with a Varian Cary 219 spectrophotometer. Because of poor solubility, methyl linoleate was dissolved in 0.30 *M* SDS prior to determination of its diffusion coefficient in 0.10 *M* SDS. Also, the poor water solubility of linoleic acid necessitated the addition of sufficient aqueous NaOH to increase the pH of the injected buffer sample to 8.5.

(2) Apparatus

The Taylor diffusion apparatus used in this study is based on the design of Burkey *et al.* (28). Using a Spectra Physics SP8000 HPLC pump, the solvent was pumped at constant flow (0.2 mL/min) through 10 m of stainless steel tubing (0.03 in. i.d., Chromatographic Specialities) which was enclosed in the HPLC oven and thermostatted at 25 ± 0.1°C. A Nupro CA relief valve was placed in-line between the pressure transducer and injector of the HPLC to provide adequate back pressure (800–1200 psi) for effective flow referencing while not interfering with solute diffusion. The sample was loaded into a 10 μL loop and injected into the diffusion apparatus. Solute dispersion was detected at (or near) λ_{max} with a Schoeffel SF 770 variable wavelength detector and recorded on a Varian CDS 401 data system.

2. Autoxidations

(1) Materials and preparations

(a) Phosphate buffer

Solutions were prepared in 0.05 *M* of each of NaH_2PO_4 and Na_2HPO_4 (pH 7.0) in glass distilled water containing 1×10^{-4} *M* EDTA. Prior to use, the buffer was passed through a column of Chelex 100 (50–100 mesh) (BioRad) to remove traces of heavy metal ions (57).

(b) Solvents

Hexane, methanol, and acetonitrile were of HPLC grade and purchased from Caledon or Anachemia. *tert*-Butyl alcohol was purified by distillation.

(c) Sodium dodecyl sulfate

Electrophoresis purity SDS was purchased from BioRad. Stock solutions of 0.50 *M* SDS were prepared in the 0.10 *M* phosphate buffer (pH 7.0) described above.

(d) Linoleic acid, methyl linoleate

Linoleic acid and methyl linoleate were purchased from Nu-Chek Prep, Inc. (>99%). The lipids were found to be free of hydroperoxide by periodic TLC analysis on silica gel with *N,N*-dimethyl-*p*-phenylenediamine spray.

(e) Initiators

Di-*tert*-butylhyponitrite was synthesized and stock solutions were prepared in 0.50 *M* SDS as reported earlier (54). Azobis(2-amidinopropane·HCl) and azobiscyclohexylnitrile were obtained from Polysciences, Inc. and stored at −30°C. Benzophenone (Fisher) was recrystallized from pentane, while 4-sulphomethylbenzophenone, sodium salt (a gift from Dr. J. C. Scaiano) was used as received. Stock solutions of ABAP were prepared in phosphate buffer. BP and BP^- were dissolved in 0.50 *M* SDS by vortex-stirring and sonication. For autoxidations carried out in homogeneous solution, benzophenone and ACHN were readily dissolved in either *t*-butanol or CH_3CN. All initiator solutions were stored at 4°C.

(f) Inhibitors

For micellar autoxidations, the following procedure was used: Stock solutions of α-tocopherol (Eastman-Kodak) were prepared in 0.50 *M* SDS as previously described (27) while solid PMHC (a gift from Dr. G. W. Burton) was readily dissolved in SDS. Standard solutions of Trolox, a gift from Hoffman-LaRoche, were prepared in argon-degassed phosphate buffer. Stock solutions of PMHC, α-T and Trolox were also prepared in *t*-butanol or acetonitrile, as required. All inhibitor solutions were stored under argon and at 4°C.

(g) Quenchers

Solutions of 1,3-diphenylisobenzofuran (Aldrich) were prepared in 0.5 *M* SDS by vortex-stirring and sonication. Sodium azide and 1,4-diazabicyclo[2.2.2]octane (DABCO, Aldrich, 97%) were dissolved in argon-degassed buffer. All quencher solutions were stored refrigerated and under argon.

(2) Autoxidation procedure

Autoxidations were carried out at 30°C and 760 mm Hg of O_2 using a sensitive automatic recording gas absorption apparatus similar to that described (47). For DBHN-initiated autoxidations, a 2.00 mL aliquot of DBHN in 0.50 *M* SDS was added to a calibrated reaction cell, followed by the appropriate amount of linoleic acid and isomolar NaOH. The cell and contents were then attached to the gas absorption apparatus and the rate of O_2 consumption measured. For the ABAP-initiated autoxidations, typically 100 μL of 0.4 *M* ABAP solution in buffer was added to 2.00 mL of 0.50 *M* SDS in a calibrated cell. The cell and contents were attached to the apparatus and allowed to shake for up to 1 h (before the addition of linoleic acid and NaOH) to permit solubilization of the ABAP.

In a typical photoinitiated run, a 2.00 mL aliquot of benzophenone in the appropriate solvent (or BP^- in 0.50 *M* SDS) was added to a calibrated reaction cell followed by the desired amount of linoleic acid (and NaOH for micellar autoxidations). The cell and contents were then attached to the apparatus and shaken to thermally equilibrate. The reaction was initiated by irradiation through Pyrex with a 200 W super pressure mercury lamp, the intensity of which was monitored using fiber optics sampling of the light beam and a phototube detector. Various additives were added to the reaction mixtures from precision syringes. The extent of oxidation was generally less than 10%.

(3) Absolute rate constants

Two 45° sectors were cut from each of two discs of blackened metal with dimensions such that $r = 3$. The discs were placed back-to-back between the light source and the gas absorption apparatus equipped with a shutter. The disc nearest the lamp was driven by an electric motor through a series of gears which could be changed to provide nine different rotation speeds from 0.286 to 4.20 rpm. The disc nearer the oxidation apparatus was driven through gears which enabled rotation speeds of 700 to 2400 rpm.

The reaction order with respect to the rate of photoinitiation was determined by measuring the rate of oxygen consumption when the reaction mixture was exposed to irradiation of different intensities. A series of neutral density filters of known transmittance (Ealing Scientific) was placed in the light path, the shutter opened and the rate of oxidation measured on a strip chart recorder.

The parameter γ was determined by measuring R_f/R_0. This ratio is measured three times using the rates obtained at 2400 rpm (R_f) and steady illumination (R_0).

The next step involved a preliminary measurement of R_λ/R_0 for several of the possible slow sector speeds, λ. From ref. 40, it can be shown that for a sector with $r = 3$, the rotating sector method is most accurate when $m = 3.70$. Using the sector rotation speed which produces m nearest to 3.70, the ratio R_λ/R_0 was determined twice by alternately measuring R_λ and R_0. The R_i was then measured by the addition of an aliquot of Trolox solution (in the appropriate solvent). This process was repeated until at least four values of R_λ/R_0 (i.e. m) and two values of R_i have been determined. From this the absolute rate constants are then calculated according to eqs. [9]–[11].

HPLC analyses

(1) Linoleate autoxidation products

After 5% oxidation (as measured by the consumption of O_2), 5 mL of 10 m*M* PPh_3 was added to a 500 μL aliquot of the reaction mixture followed by the addition of 50 mL of methanol/water (50/50). The aqueous solution was extracted twice with diethyl ether; 35 and 15 mL. The combined extracts were dried over Na_2SO_4 and then treated with excess CH_2N_2 in ether at 0°C. The solution was gently warmed to room

temperature to remove excess diazomethane. Samples were then stored at −30°C until required for analysis.

Immediately prior to analysis by HPLC, the solvent was removed at reduced pressure, followed by pumping at <0.25 mm Hg for at least 30 min. The residue was brought up in 2 mL of HPLC solvent: hexane/2-propanol/acetone (992/4/4). Brief centrifugation was usually necessary to remove excess solid PPh_3. Separation of the four methyl hydroxylinoleates was achieved using a Waters 5100 HPLC pump fitted with a 30 cm × 10 μm Apex silica column (Jones Chromatography). With a flow rate of 1.6 mL/min, the products were detected by monitoring eluent absorption at 234 nm with a Schoeffel SF 770 variable wavelength detector. Identification of the four compounds was achieved by cross-comparison of an autoxidation product mixture from our lab with a reference mixture obtained and analyzed (39) under similar conditions in the laboratories of Dr. N. A. Porter of Duke University.

Relative amounts of the four hydroxylinoleates were determined by correcting the peak areas, as reported by a Varian CDS 401 data system, for the appropriate molar absorptivity (33): 13-*c*,*t* (λ_{max} 236, ϵ = 27 200); 13-*t*,*t* (λ_{max} 233 nm, ϵ = 30 500); 9-*c*,*t* (λ_{max} 236 nm, ϵ = 28 300); 9-*t*,*t* (λ_{max} 233 nm, ϵ = 31 600).

(2) Benzophenone product analysis

A 100 μL aliquot was removed from the reaction mixture and added to 1 mL of methanol/water (50/50). After vortex-stirring for 15 s, the aqueous solution was extracted twice with 1 mL of HPLC solvent: hexane/2-propanol/acetone (992/4/4). Following brief centrifugation, the organic layers were removed. To 500 μL of the combined extracts was added 25 μL of 2.82×10^{-3} *M* *p*-methoxyacetophenone (the internal standard) in hexane. The sample was eluted using the Waters 5100 pump and the Apex silica column. To aid in separation, the solvent was pumped at 0.8 mL/min for 10 min, then increased to 1.6 mL/min for the remainder of the analysis. The eluent was monitored at 225 nm with the Schoeffel SF 770 detector.

(3) α-Tocopherol analysis

A 250 μL aliquot of the reaction mixture (initially 3.94×10^{-5} *M* α-T) was removed from the reaction vessel and added to 500 μL of absolute ethanol in a small test tube. After vortex-stirring for 15 s, the solution was extracted twice with 250 μL of hexane. Brief centrifugation was necessary to aid separation of the two layers. To 250 μL of the combined hexane extracts was added 10 μL of the internal standard solution, 9.28×10^{-4} *M* PMHC in hexane. Analysis was performed by injecting 50 μL of the resulting solution onto the Apex silica column with the solvent; hexane/2-propanol (99/1) flowing at 0.9 mL/min. The column effluents were monitored at 220 nm with the Schoeffel SF 770.

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