Arm-to-Arm Autoxidation in a Triglyceride: Remote Group Reaction Kinetics[1]

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Autoxidation in glyceryl trilinoleate does not obey the usual (ideal) kinetic rate law; instead the oxygen is consumed more rapidly than would be expected in *dilute* solutions of the lipid.[3] Herein, this anomaly is shown to be caused by intramolecular (*arm-to-arm*) hydrogen atom abstraction by the peroxyl radical, a mode of autoxidation which generates di- and trihydroperoxides in the dilute solutions. It is also shown that the relative rates of intra- vs intermolecular hydrogen abstraction are accurately predicted by a simple reaction volume model for the *effective molarity* of the remote active group.

A paradigm of uninhibited autoxidation kinetics is that the rate of oxidation (R_p) should be *proportional* to the product of the substrate concentration [LH] and the square root of the rate of radical initiation R_i,[4]

$$R_p \propto [LH]R_i^{1/2} \quad \text{(I)}$$

The proportionality constant—the *oxidizability* of the lipid—is found from the kinetic rate law for an autoxidation chain reaction. That is, for an azo-initiated reaction,

initiation:

$$^1/_2RN{=}NR \xrightarrow{-N_2\ O_2} \rightarrow ROO^\bullet \text{ (rate, } R_i) \quad (1)$$

propagation:

$$LH + {-}OO^\bullet \xrightarrow{k_p\ O_2} \rightarrow LOO^\bullet + {-}OOH\ (R_p) \quad (2)$$

termination:

$$-OO^\bullet + -OO^\bullet \xrightarrow{2k_t} \text{nonradical products} \quad (3)$$

(where $-OO^\bullet$ represents $LOO^\bullet$ or $ROO^\bullet$). From this scheme the propagation rate[4]

$$R_p = \{k_p/(2k_t)^{1/2}\}[LH]R_i^{1/2} \quad \text{(II)}$$

so that the oxidizability of a lipid in regular solution (i.e., experimentally $R_p/[LH]R_i^{1/2}$) *should* be a constant, viz., $k_p/(2k_t)^{1/2}$ and, indeed, this quantity is almost invariably found to be a constant.

One glaring exception came, however, from a careful study by Cosgrove, Church, and Pryor (CCP)[3] of the autoxidation of glyceryl trilinoleate in chlorobenzene at 37 °C. These workers found that although eq I was satisfactory for solutions of glyceryl mono- and dilinoleate (also in chlorobenzene at 37 °C), there was a marked deviation with glyceryl trilinoleate. In particular, the oxidizability of the triglyceride rose sharply as its concentration was reduced to below ~7 mM (i.e., [LH] < 3 × 7 ≈ 20 mM in Figure 1). This deviation from ideal kinetics was attributed to aggregation of the lipid.[3] Intuitively, however, a triglyceride would be far *less* likely to form molecular aggregates in a nonpolar solvent than a mono- or diglyceride (which contain hydrogen-bonding hydroxy groups). Moreover, aggregation normally *decreases* upon dilution rather than rising sharply below a threshold concentration (cf. fatty acid micelle formation in water).

A potential solution to this long-standing "triglyceride problem" appeared to me to be that its origins did indeed lie in an "aggregation effect" but that the triglyceride *itself* was the "aggregate". That is, each molecule of glyceryl trilinoleate contains three active lipid moieties (LH groups) and therefore, as the bulk concentration of the triglyceride is decreased, there will come a point where the concentration of one LH group *relative to a second on a different "arm" of the molecule* will exceed that of LH groups (on other molecules) in the surrounding medium. Consequently, for dilute solutions the measured oxidizability (as defined above) will increase because propagation will begin to take place by *arm-to-arm* transfer of the peroxyl radical chain, i.e., by H-atom abstraction from one linoleate arm by a peroxyl radical on a different arm.

The CCP oxygen uptake data will now be reevaluated with this idea in mind. The total *effective* concentration of LH groups (from the view point of a $LOO^\bullet$ radical) will be the sum of the inter- and intramolecular concentrations

$$[LH]_{eff} = [LH] + [LH]_{intra} \quad \text{(III)}$$

The lipid's oxidizability ($k_p/(2k_t)^{1/2}$) should therefore be redefined in terms of $[LH]_{eff}$, i.e.

$$k_p/(2k_t)^{1/2} = R_p/(R_i^{1/2}[LH]_{eff}) = R_p/\{R_i^{1/2}([LH] + [LH]_{intra})\} \quad \text{(IV)}$$

or

$$R_p/R_i^{1/2} = (k_p/(2k_t)^{1/2})([LH] + [LH]_{intra}) \quad \text{(V)}$$

A plot of $R_p/R_i^{1/2}$ vs [LH] for CCP's glyceryl trilinoleate is indeed linear ($\langle r \rangle = 0.993$), see Figure 2, with

$$\text{slope} = \textit{intermolecular} \text{ oxidizability} = k_p/(2k_t)^{1/2} = 0.024_6\ M^{-1/2}\ s^{-1/2}$$

$$\text{intercept} = 5.2 \times 10^{-4}\ M^{1/2}\ s^{-12}$$

and hence,

$$[LH]_{intra} = \text{intercept/slope} = 21\ \text{mM}$$

In Figure 3 it is shown that a "correction" with $[LH]_{intra}$ = 21 mM brings the CCP oxidation data for this triglyceride into conformity with eq I. Furthermore, applying half of this correction to CCP's diglyceride data[3] reduces the mean oxidizability of the diglyceride from the rather high value of $0.029_5\ M^{-1/2}\ s^{-1/2}$ to a more reasonable *intermolecular* value of $0.025_5\ M^{-1/2}$. This value for the diglyceride is

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(3) Cosgrove, J. P.; Church, D. F.; Pryor, W. A. *Lipids* 1987, *22*, 299–303.

(4) Herein: PUFA ≡ polyunsaturated fatty acid; LH ≡ bis-allylic methylene group; propagation rate, $R_p \equiv -d[LH]/dt = -d[O_2]/dt$ (see ref 3).

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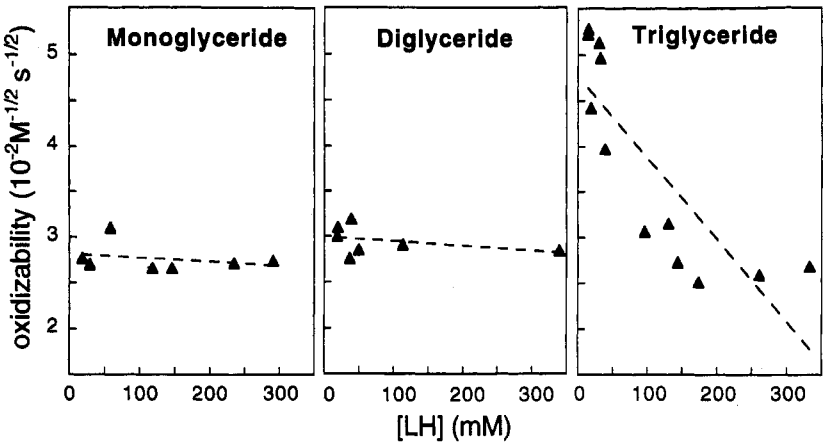


Figure 1. Oxidizability of glycerol linoleates as a function of their PUFA concentration (see ref 4). Data from ref 3 ("oxidizability" = $R_p/[LH]R_i^{1/2}$).

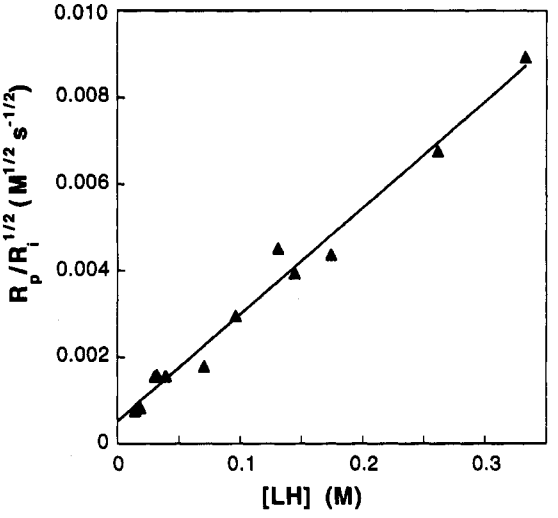


Figure 2. $R_p/R_i^{1/2}$ vs [LH] from oxygen uptake data for glyceryl trilinoleate.[3]

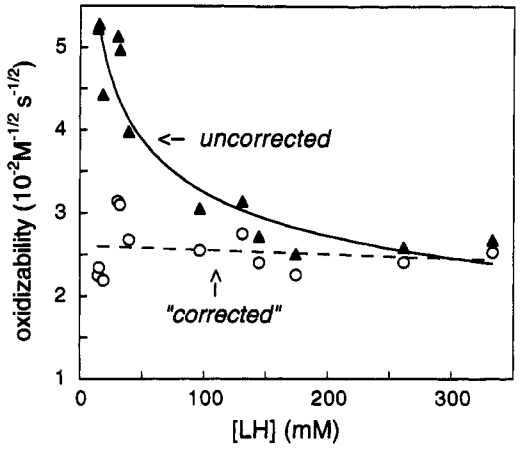


Figure 3. Triglyceride oxidizability corrected for *arm-to-arm* propagation. Data from ref 3 with corrected (intermolecular) *oxidizability* = $R_p/\{R_i^{1/2}([LH] + 21\ mM)\}$ (eq IV).

now in line with the (necessarily intermolecular) oxidizability of the monoglyceride, 0.026_8 $M^{-1/2}$ $s^{-1/2}$ and with the intermolecular oxidizability of the triglyceride, 0.024_6 $M^{-1/2}$ $s^{-1/2}$ (as calculated above).

The occurrence of arm-to-arm autoxidation of the triglyceride implies that in *dilute* solutions ([LH] < 20 mM) the products will be mainly di- and trihydroperoxides.

Table 1. Relative Yields of Glyceryl Trilinoleate Mono-, Di-, and Trihydroperoxide (MHP, DHP, and THP) from Azo-Initiated Autoxidation of Glyceryl Trilinoleate (TG)[a]

[TG], mM	[LH], mM	THP, %	DHP, %	MHP, %
110	330	–	8	92
5	15	9	46	45
1.0	3	64	21	15
1.0[b]	3	–	1	99

[a] HPLC yields (% total hydroperoxides) from 37 °C thermolysis of 2,2'-azobis(2,2-dimethylvaleronitrile) (0.8 mM) in chlorobenzene solutions of glycerol trilinoleate (TG). [b] Same as *a* with 2 mM α-tocopherol added and reaction time increased (from 1–2 h) to 25 h.

The azo-initiated autoxidation of glyceryl trilinoleate at 37 °C was therefore examined. Glyceryl trilinoleate mono-, di-, and trihydroperoxides (MHP, DHP, and THP, respectively) were readily resolved and quantified using Nef et al.'s HPLC method.[5] The initial[6] relative yields of DHP and THP increased as the solution was diluted with chlorobenzene until, for [LH] = 3 mM, the main product was the trihydroperoxide (Table 1). Analysis of the data according to the pseudo-first-order steady-state relation:

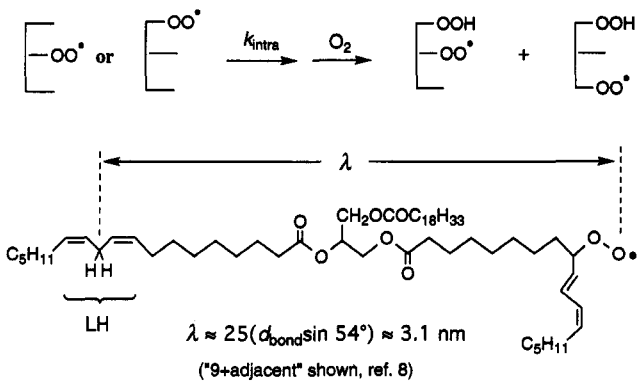
$$([THP] + [DHP])/[MHP] = [LH]_{intra}/[LH]$$

affords $[LH]_{intra} \approx 18$ mM, which is pleasantly close to the value calculated from CCP's oxygen absorption data. Addition to the most dilute reaction mixture of α-tocopherol (which is an outstanding hydrogen atom donor to peroxyl radicals) eliminated the THP and DHP products (Table 1). This is because the peroxyl radical first formed on a triglyceride molecule is "capped" before it can undergo an arm-to-arm H-transfer (cf. ref 7).

A simple model of calculating the effective concentration of an LH group relative to a peroxyl radical on a different arm of the same molecule is to envisage the OO• group "tethered" to the LH group. The *length* of this "tether" (λ) is the *maximum* distance between the OO• group and the LH group which is allowed by the "string" of intervening bonds (Scheme 1). Thus, placing the OO• group at the center of a sphere of radius λ, the effective concentration of the LH group at the other end of tether

(5) Nef, E. E.; Frankel, E. N.; Miyashita, K. *Lipids* **1990**, *25*, 33–39.
(6) Beyond ~10% conversion, di- and trihydroperoxides are also formed even in concentrated solutions via repeated H-abstraction from the hydroperoxy group (LOOH → LOO•) followed eventually by intramolecular H-abstraction (cf. ref 7).
(7) Porter, N. A. *Acc. Chem. Res.* **1981**, *14*, 95–102.

Scheme 1



will be

$$[LH]_{intra} = (1/N_A)\ \text{mole}/V \qquad \text{(VI)}$$

where N_A is Avogadro's number and V is the volume of the reaction sphere:

$$V = 4\pi\lambda^3/3 \qquad \text{(VII)}$$

The tether length for a triglyceride radical λ will be 3.1–3.7 nm (see Scheme 1 and ref 8) and hence the overall value for $[LH]_{intra}$ can be calculated to be *ca.* 21 mM.[8] This intramolecular concentration is in remarkably good agreement with the concentrations calculated from CCP's kinetic data (21 mM) and the present product data (18 mM). This agreement between a very simple theory and experiment proves that there is no special barrier (e.g., ring strain) to the intramolecular H-abstraction.

In conclusion, the effective molarity of remote LH-groups in a glyceryl trilinoleate peroxyl radical (Scheme 1) is simply equal to the total number of LH groups in the peroxyl radical divided by the volume within which they are constrained to react. This appears to be the first *quantitative* demonstration of such an intuitively obvious (*post facto*) relationship. The tether model should apply equally well to other reactions between two remote groups attached to a long *floppy* molecule. It would, of course, break down when the two groups are attached to a short or stiff molecule because there will be retardation by ring strain in the cyclic transition state.[9]

Experimental Section

Glyceryl trilinoleate (Sigma, 99%) was purified immediately before use by chromatography under nitrogen.[5] Freshly made solutions of 2,2′-azobis(2,2-dimethylvaleronitrile) (0.8 mM) and the lipid in spectral grade chlorobenzene were thermostated (37 ± 1 °C) in glass vials, and the oxidation products were quantified by HPLC[5] (assuming equal absorption at 234 nm for each conjugated double bond in the hydroperoxide products). Reaction mixtures were analyzed before 2% of the lipid had been oxidized (as estimated by HPLC via a standard of methyl linoleate hydroperoxide). Calculated radical chain lengths were greater than six (i.e., $(d[LOOH]/dt)/R_i > 6$) and no LOOH decomposition products were detected.

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(8) The OO• group can form at either the 9- or 13-positions on a linoleate arm ($\lambda_{adj} \approx 3.1$ or 3.6 nm) and can then react with either an adjacent or, for the triglyceride, nonadjacent arm ($\lambda_{nonadj} \approx \lambda_{adj} + 0.1$ nm); i.e., a remote LH group may therefore contribute 13 or 9 mM, or 12 or 8 mM, respectively, to the overall $[LH]_{intra}$. The value $[LH]_{intra} \approx 21$ mM for the latter *overall* concentration assumes a stochastic distribution between 9- and 13-peroxyls and between the three arms of the triglyceride.

(9) For a discussion of ring strain vs ring size: Porter, N. A.; Chang, V. H.-T.; Magnin, D. R.; Wright, B. T. *J. Am. Chem. Soc.* 1988, *110*, 3554–3560.