Analysis of Lipid Peroxidation Kinetics. 1. Role of Recombination of Alkyl and Peroxyl Radicals

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The kinetics of the lipid peroxidation reaction is only partly understood. Although the set of reactions constituting the overall reaction are believed to be known, it has not been possible to predict how the reaction will respond to a change of one or more of the parameters, e.g., initial concentrations of reactants or different ways of initiating the reaction, nor has it been possible to predict the time dependence of the products. The reason for these problems is the complicated structure of the kinetic scheme, which includes a chain reaction. In this work we perform an in-depth analysis of the importance of the individual reaction steps, and we introduce a new quasi-stationary concentration method based on the assumption that one or more concentrations vary much slower than the others. We show that it is justified to use a quasi-stationary concentration approximation for the alkyl radical $L^{\bullet}$, but not for the peroxyl radicals $LO_2^{\bullet}$ as assumed in previous works. The method allows us to derive manageable analytical expressions. On the basis of literature values of the rate constants, we are able to introduce specific simplifications that allow us to obtain simple analytical expressions for the time dependence of all concentrations involved in the process.

1. Introduction

Lipid peroxidation has attracted much attention because of its possible contributions to cancer and aging. It is an oxidative deterioration of polyunsaturated fatty acids, by which the polyunsaturated fatty acids are converted into lipid hydropeoxides. Lipid peroxidation is a free radical-related process. Oxidants can react with the double layer of polyunsaturated fatty acids of the cell and cellular organelle membranes to form toxic metabolites. For instance, brain tissue being rich in polyunsaturated fatty acids is very susceptible to lipid peroxidation. Lipid peroxidation is to a large extent responsible for the destruction of cellular membranes, and peroxidation of membrane lipids can have numerous effects, including increased membrane rigidity, decreased activity of membrane-bound enzymes (e.g., sodium pumps), altered activity of membrane receptors, and altered permeability.

Previous attempts to describe the kinetics[1–5] have used numerical simulations and approximate analytical descriptions of the lipid peroxidation kinetics to interpret experimental data in terms of rate constants of the individual stages of the process. However, the derived reaction rates and the concentrations of the reactants are rather scattered in the literature and differ, sometimes by orders of magnitude, from each other. Part of the discrepancies between the values of the parameters can be explained either by the different lipid compositions of the investigated systems or by the different experimental methods, which are not all equally accurate. A numerical simulation using a particular data set is sufficient neither to draw general conclusions about the kinetics of the process nor to predict how the kinetics will proceed for another parameter set. Only a detailed analytical, theoretical analysis will be able to answer these questions.

Our original interest in the lipid peroxidation reaction was caused by the presence of radical pair steps in the reaction scheme. It is well-known that radical pair recombination reactions are magnetosensitive and that this effect is explained by the radical pair mechanism (RPM), which requires an intermediate radical pair state in the reaction pathway; cf. some recent reviews.[6–11] There are a vast number of papers reporting experimental findings of magnetic field effects on chemical reactions in liquids and micelles, and they have been satisfactorily explained by the RPM. However, there are only a few observations of the magnetic field effect on biological reactions. The lipid peroxidation, an autocatalytic reaction, contains several radical recombination stages and therefore can be expected to be magnetosensitive. It contains a chain reaction with alkyl and peroxyl radicals that opens up an exciting possibility, the existence of a magnetically sensitive trigger effect where a small change of a parameter may cause a large change in the kinetics. So far, there appear to be only two experimental observations of the effect of a stationary magnetic field on lipid peroxidation. In one of the works,[12] the effect of a static magnetic field, ranging from 0 to 280 mT, on the kinetics of lipid peroxidation was studied. It was found that the kinetics of nonenzymatic lipid peroxidation was accelerated by a weak stationary magnetic field in a model system consisting of liposomes obtained from 1,2-dioleoylphosphatidylcholine. Another work[13] investigated the effect of a static magnetic field on lipid peroxidation in phospholipid membranes. Unfortunately, these results have not been reproduced by other groups. At present, it is therefore uncertain to what extent the magnetosensitive stages contribute to the kinetics and what magnitudes of magnetic field effects can be expected. Theoretical predictions would require a detailed knowledge of the importance of the individual steps of the kinetic scheme, but an exhaustive literature search revealed that

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such information is missing. All previous considerations either are very simplified or build on assumptions of quasi-stationary regimes for all radicals.

In the present work, we present a detailed analytical investigation of lipid peroxidation kinetics. We show that it is justified to use a quasi-stationary concentration method for the alkyl radical $L^\bullet$, but not for the peroxyl radicals $LO_2^\bullet$ as assumed in the previous works. This approach allows us to derive manageable analytical expressions. By incorporating the known information about the relative magnitudes of the kinetic parameters, these expressions can be simplified further and used to derive simple analytical expressions for the time dependence of all concentrations involved in the process.

2. Kinetic Scheme and Kinetic Equations

Lipid-containing substances, investigated in vitro, most often consist of suspensions of liposomes or mitochondrial inner membranes. Liposomes are aggregates consisting of self-assembled layers of lipid molecules, and the lipid system is thus essentially an open heterogeneous system. A complete description therefore should take into account any possible effect of the heterogeneity. This would require a complex description of the fluxes of reactants from the surrounding water solution to the liposome layer, the following diffusive motion and subsequent recombination of the lipid radicals within the liposome layer, etc. However, to make the system tractable, it is usual practice to treat the lipid peroxidation process within the framework of formal chemical kinetics in the homogeneous phase. We will use the same approximate description in the present work since it allows us to obtain analytical results. In the future, it will be interesting to investigate the importance of the heterogeneity and, if it is significant, to see whether it can be included by correction terms in the present results or whether a numerical treatment is needed.

The simplest case occurs when the suspension initially contains only the lipid LH (fat acid). The lipid peroxidation is then a simple chain reaction that consists of the following three stages: initiation, propagation, and termination.

2.1. Initiation. There are two kinds of initiation, instant and continuous.

Instant initiation occurs when the active radical (e.g., $OH^\bullet$) is produced by a short pulse, e.g., an ultraviolet (UV) laser pulse. The initial reaction step is then

$$OH^\bullet + LH \xrightarrow{k_1} L^\bullet + H_2O \tag{1}$$

which can be considered as a fast process that creates alkyl radicals $L^\bullet$ with initial concentration $[L^\bullet]_0$.

Continuous initiation, e.g., caused by continuous UV irradiation, produces alkyl radicals $L^\bullet$

$$LH \xrightarrow{I} L^\bullet \tag{2}$$

with a constant rate

$$\{\partial_t[L^\bullet]_t\}_{\text{init}} = I \tag{3}$$

The rate of creation of radicals $L^\bullet$ is, in principle, proportional to the instantaneous concentration $[LH]_t$ of LH, and thus, a more accurate expression would be

$$I = k_I[LH]_t \tag{4}$$

However, in the present work we neglect the change of the lipid concentration for the continuous case.

2.2. Propagation. Chain propagation proceeds according to the reactions

$$L^\bullet + O_2 \xrightarrow{k_2} LO_2^\bullet \tag{5}$$

$$LO_2^\bullet + LH \xrightarrow{k_3} LOOH + L^\bullet \tag{6}$$

The first reaction is an oxidation of the alkyl radical, which produces a peroxyl radical $LO_2^\bullet$. This radical reacts with a lipid molecule to give hydroperoxide LOOH and an alkyl radical, thus propagating the chain and accumulating the hydroperoxidation products.

2.3. Termination. The termination of the chain propagation is thought to be due to radical recombination:

$$L^\bullet + L^\bullet \xrightarrow{k_4} P_1 \tag{7}$$

$$L^\bullet + LO_2^\bullet \xrightarrow{k_5} P_2 \tag{8}$$

$$LO_2^\bullet + LO_2^\bullet \xrightarrow{k_6} P_3 \tag{9}$$

where P_1, P_2, and P_3 are various molecular products that are not involved in further chain reactions. The most remarkable feature of these reactions is their magnetosensitivity, which they share with any radical recombination reaction. Since there are three reactions, we have to investigate the importance of each of them. However, a more general question arises: Can or do these reactions terminate the chain propagation and thus stop the lipid peroxidation?

2.4. Kinetic Equations and Parameter Values. We shall answer these questions by using formal chemical kinetics corresponding to the reaction schemes introduced above. We write the kinetic scheme as

$$\partial_t[L^\bullet]_t = I - v_2 + v_3 - 2v_4 - v_5 \tag{10}$$

$$\partial_t[LO_2^\bullet]_t = v_2 - v_3 - v_5 - 2v_6 \tag{11}$$

$$\partial_t[LOOH]_t = v_3 \tag{12}$$

$$\partial_t[LH]_t = -v_3 - I \tag{13}$$

where the reaction rates v_2 and v_3 are defined as

$$v_2 = k_2[O_2][L^\bullet]_t \equiv k_2'[L^\bullet]_t, \quad v_3 = k_3[LH]_t[LO_2^\bullet]_t \equiv k_3'[LO_2^\bullet]_t \tag{14}$$

The pseudo-first-order rate constants are introduced as in ref 1

$$k_2' = k_2[O_2], \quad k_3' = k_3[LH]_t \tag{15}$$

and in the following, we will assume that the oxygen and lipid concentrations are in excess. The radical recombination rates, corresponding to eqs 7 and 8, are

$$v_4 = k_4[L^\bullet]_t^2, \quad v_5 = k_5[L^\bullet]_t[LO_2^\bullet]_t, \quad v_6 = k_6[LO_2^\bullet]_t^2 \tag{16}$$

Literature values of the rate constants, cf. Table 1, provide important information on the relative magnitudes of the rates, although the estimated values do not always agree.

The rate (v_2) of reaction 5 is recognized to be fast, and the estimated values of the rate constant k_2 are reasonably close to

conclude that it is on the order of 10^9 M^{-1} s^{-1}. The estimated values of k_3 vary tremendously. No variation of the recombination rate constants k_4 and k_5 were found. The estimates of the recombination rate constant of the peroxyl radicals k_6 vary between 10^3 and 10^7 M^{-1} s^{-1}.

The data presented in ref 2 imply that the "oxidizability"

$$Z_{LO_2^\bullet} = \frac{k_3}{\sqrt{2k_6}} \approx 0.04\ M^{-1/2}\ s^{-1} \tag{17}$$

which is within the range $0.02-0.08$ $M^{-1/2}$ s^{-1} given in ref 5, while refs 3 and 4 yield $Z_{LO_2^\bullet} \approx 0.7$ $M^{-1/2}$ s^{-1} and $Z_{LO_2^\bullet} \approx 4.5 \times 10^3$ $M^{-1/2}$ s^{-1}, respectively. The oxidizability determines the peroxidation rate under continuous initiation when only recombination between peroxyl radicals is important.[2,5]

Literature estimates of the concentrations are $[O_2] = 1.0 \times 10^{-4}$ M from ref 2 and [LH] = 25 mM from ref 2. The constant initiation rate can be estimated as $I \leq 5 \times 10^{-6}$ M/s from the estimated light absorption in 3 mL of solution, which equals 0.96×10^{16} and 1.11×10^{16} quanta/s at wavelengths of 254 and 300 nm, respectively.[1]

3. Role of Radical Recombination Processes on the Decay Kinetics of Peroxyl Radicals

In this section, we examine the importance of the various radical recombination reactions, eqs 7–9. This has never been completely established, and different arguments have been used to introduce different and rather strong simplifications of the reaction scheme.

In refs 2, 3, and 5 only the recombination reaction of peroxyl radicals, eq 9, was taken into account. The arguments were that the oxidation reaction 5 of alkyl radicals is fast and the quasi-stationary concentration of alkyl radical $L^\bullet$ must thus be rather low. Consequently, the recombination rate of alkyl radicals (v_4) and the reaction rate of alkyl radicals with peroxyl radicals (v_5) appear to be small compared with the recombination rate of peroxyl radicals (v_6).

Reference 1, on the other hand, only included the recombination of alkyl radicals, i.e., reaction 7. The argument was that the rate constant of the peroxyl recombination reaction (k_6) is 5 or 6 orders of magnitude smaller than the recombination rate constant of alkyl radicals[4] (k_4) and therefore only the latter recombination needs to be included. However, the rate constant k_5 of the recombination between alkyl and peroxyl radicals, eq 8, is large enough[4] to influence the recombination kinetics.

Our treatment is based on a fast attainment of a quasi-stationary state of the alkyl radicals. Due to the fast oxidation reaction of alkyl radicals, eq 5, the concentration of $L^\bullet$ achieves a quasi-stationary value for times satisfying $k_2't \geq 1$. Since the time derivative $\partial_t[L^\bullet] = 0$ at the quasi-stationary state, it follows from eqs 10 and 14–16 that

$$2k_4[L^\bullet]_{qs}^2 + (k_2' + k_5[LO_2^\bullet]_t)[L^\bullet]_{qs} - (k_3'[LO_2^\bullet]_t + I) = 0 \tag{18}$$

where the subscript t indicates the time variable. This equation determines the quasi-stationary concentration of the alkyl radicals:

$$[L^\bullet]_{qs} = \frac{1}{4k_4}\left[\sqrt{(k_2' + k_5[LO_2^\bullet]_t)^2 + 8k_4(k_3'[LO_2^\bullet]_t + I)} - (k_2' + k_5[LO_2^\bullet]_t)\right] \tag{19}$$

Notice that the right-hand side and thus $[L^\bullet]_{qs}$ depend on time. This time dependence, caused by the time dependence of the concentration of the peroxyl radicals, is, however, very slow. For all available data, the following inequalities are satisfied:

$$k_5[LO_2^\bullet]_t \ll k_2' \quad \text{and} \quad k_5[LO_2^\bullet]_{qs} \ll k_2' \tag{20}$$

The peroxyl radicals attain a time-independent stationary state on a longer time scale than that of the quasi-stationary state of the lipid radicals. This implies that both the lipid and the peroxyl radicals attain time-independent stationary concentrations on the longer time scale, but not on the shorter one. It is shown below that the stationary concentration of peroxyl radicals, $[LO_2^\bullet]_{qs}$, for continuous initiation is equal to

$$[LO_2^\bullet]_{qs} = \sqrt{\frac{I}{2k_6}} \tag{21}$$

Using this result, the second inequality of eq 20 can be rewritten as

$$k_5 \ll k_2'\sqrt{\frac{2k_6}{I}} = 2\eta\frac{k_6k_2'}{k_3'} \tag{22}$$

where we have introduced the dimensionless parameter

$$\eta = \frac{k_3'}{\sqrt{2k_6I}} = Z_{LO_2^\bullet}\frac{[LH]_t}{\sqrt{I}} \tag{23}$$

This parameter is on the order of unity for all reasonable values of [LH] and I and for the literature values of the rate constant displayed in Table 1 using the definitions in eq 15. The only exception is the value in ref 3, which is 4 orders of magnitude larger.

In the following we assume that

$$\frac{k_3'k_4}{k_2'} \ll k_5 \ll \frac{k_6k_2'}{k_3'} \tag{24}$$

which is readily seen to be true for all literature values of the rate constants. This is true even for the out of range k_3 value from ref 3. Actually, we cannot check these inequalities consistently for the data of ref 3 since it contains no data for k_4 and k_5, but these inequalities hold true even for k_3 taken from ref 3 and k_4 and k_5 from the other references.

For instant initiation ($I = 0$) we must use the initial value $[LO_2^\bullet]_0$ in the first inequality of eq 20. This initial value $[LO_2^\bullet]_0$ is the concentration of $LO_2^\bullet$ radicals immediately after the quasi-stationary concentration of $L^\bullet$ is reached ($t \geq 1/k_2'$). This instant of time will now be considered as the initial time. As a rule, $[LO_2^\bullet]_0 \leq [LO_2^\bullet]_{qs}$.

The oxidizability $Z_{LO_2^\bullet}$ for the reaction of $LO_2^\bullet$ was introduced in eq 17 as the ratio of the decay rate constant k_3 and the square root of 2 times the recombination rate constant k_6. By analogy, we introduce oxidizability for the reaction of alkyl radicals as

TABLE 1: Literature Values of the Rate Constants (M^{-1} s^{-1}) (Corresponding Reactants in Parentheses)

	k_2 ($L^\bullet + O_2$)	k_3 ($LO_2^\bullet$ + LH)	k_4 ($L^\bullet + L^\bullet$)	k_5 ($L^\bullet + LO_2^\bullet$)	k_6 ($LO_2^\bullet + LO_2^\bullet$)
ref 2	3.0×10^8	1.4×10^1			6.6×10^4
ref 3	(10^9, 10^{10})	3.0×10^5			2.2×10^3
ref 4	3.0×10^8	3.1×10^1	10^9	5.0×10^7	1.0×10^5
refs 14–16	9×10^6		2×10^8		3×10^7

$$Z_{L\bullet} = \frac{k_2}{\sqrt{2k_4}} > 10^3\ \mathrm{M}^{-1/2}\ \mathrm{s}^{-1} \tag{25}$$

for the published rate constants.[3,4] The corresponding dimensionless parameter, cf. eq 23, is

$$\xi = \frac{k_2'}{\sqrt{2k_4 I}} \equiv Z_{L\bullet}\frac{[O_2]}{\sqrt{I}} \gg 1 \tag{26}$$

From eqs 23 and 26 it follows that

$$\eta \ll \xi \quad \text{or} \quad Z_{LO_2\bullet}[LH]_t \ll Z_{L\bullet}[O_2] \tag{27}$$

and

$$4\eta/\xi^2 \ll 1 \quad \text{and} \quad 4/\xi^2 \ll 1 \tag{28}$$

The inequalities 27 and 28 are satisfied even for the data from ref 3. Note that inequality 27 agrees with inequality 24. Under the conditions in eq 28, the second term in the square root of eq 19 is much smaller than the first term, which allows us to expand the square root in eq 19 in a Taylor series. By introducing the small dimensionless parameters

$$x = \frac{8k_4(k_3'[LO_2^\bullet]_t + I)}{(k_2')^2} \quad \text{and} \quad y = \frac{k_5[LO_2^\bullet]_t}{k_2'} \tag{29}$$

and expanding eq 19 to second order with respect to the small parameters x and y, we obtain

$$[L^\bullet]_{qs} \approx \frac{k_2'}{8k_4}x\left(1 - \frac{1}{4}x - y\right) \tag{30}$$

A closed rate equation for peroxyl radicals can be derived with the same accuracy using eqs 11 and 14–16

$$\begin{aligned}\partial_t[LO_2^\bullet]_t &= k_2'(1-y)[L^\bullet]_{qs} - k_3'[LO_2^\bullet]_t - 2k_6[LO_2^\bullet]_t^2 \\ &\approx \frac{(k_2')^2}{8k_4}x\left(1 - \frac{1}{4}x - 2y\right) - k_3'[LO_2^\bullet]_t - 2k_6[LO_2^\bullet]_t^2 \\ &= -k_0\left(1 + \frac{k_3'k_4}{k_2'k_5}\right)[LO_2^\bullet]_t - \\ &\quad 2k_6\left(1 + \frac{k_3'k_5}{k_2'k_6} + \frac{\eta^2}{\xi^2}\right)[LO_2^\bullet]_t^2 + I\left(1 - \frac{1}{\xi^2}\right)\end{aligned} \tag{31}$$

Here we have introduced the "monomolecular rate"

$$k_0 = \frac{2k_5}{k_2'}I \tag{32}$$

which depends on the initiation rate I. Equation 31 can be simplified by use of the inequalities 24, 26, and 27 and retaining only the first nonvanishing terms in each set of parentheses:

$$\partial_t[LO_2^\bullet]_t \approx -k_0[LO_2^\bullet]_t - 2k_6[LO_2^\bullet]_t^2 + I \tag{33}$$

We have now obtained a simple, closed kinetic equation for the decay of peroxyl radicals due to recombination processes. It is seen that recombination between radicals with the smallest oxidizability (cf. eq 27) plays a dominant role in the oxidation process. The reaction rate k_0 is rather small, but it was included because it induces a first-order reaction. To investigate the role of this "monomolecular recombination", we first determine the quasi-stationary concentration $[LO_2^\bullet]_{qs}$. Substituting $\partial_t[LO_2^\bullet]_t = 0$ into eq 33, we obtain

$$[LO_2^\bullet]_{qs} = \frac{1}{4k_6}\left(\sqrt{k_0^2 + 8k_6 I} - k_0\right) \tag{34}$$

By using eq 32, we see that inequality 22 is equivalent to

$$k_0 \ll 2\sqrt{2k_6 I} \tag{35}$$

Thus, when calculating the quasi-stationary value of the peroxyl concentration, we can neglect k_0 and arrive at eq 21.

The general solution to eq 33 is

$$[LO_2^\bullet]_t = \frac{[LO_2^\bullet]_{qs} - \alpha([LO_2^\bullet]_{qs} + k_0/(2k_6))\exp[-(4k_6[LO_2^\bullet]_{qs} + k_0)t]}{1 + \alpha\exp[-(4k_6[LO_2^\bullet]_{qs} + k_0)t]} \tag{36}$$

where

$$\alpha = \frac{[LO_2^\bullet]_{qs} - [LO_2^\bullet]_0}{[LO_2^\bullet]_{qs} + [LO_2^\bullet]_0 + k_0/(2k_6)} \tag{37}$$

Strictly speaking, we can neglect the quantity k_0 in the exponents in eq 36 for $k_0 t \ll 1$, i.e., for $t \leq 10^4$ s, due to the condition in eq 35. For longer times, the kinetic decay is already so substantial that any additional decay is not noticeable.

For $I \to 0$, the recombination of $LO_2^\bullet$ radicals must lead to power law kinetics, while the "monomolecular reaction" gives an exponential decay. Nevertheless, we can neglect k_0 since it is proportional to the initiation rate I and thus vanishes for $I \to 0$. This leads to the kinetic equation

$$\partial_t[LO_2^\bullet]_t = -2k_6[LO_2^\bullet]_t^2 + I \tag{38}$$

which has the general solution

$$[LO_2^\bullet]_t = [LO_2^\bullet]_{qs}\frac{[LO_2^\bullet]_{qs}\sinh(kt) + [LO_2^\bullet]_0\cosh(kt)}{[LO_2^\bullet]_{qs}\cosh(kt) + [LO_2^\bullet]_0\sinh(kt)} \tag{39}$$

This result also follows from eq 36 by setting $k_0 = 0$; the quasi-stationary concentration $[LO_2^\bullet]_{qs}$ is given by eq 21. Here we have introduced k as an effective rate constant for attaining the quasi-stationary value of $[LO_2^\bullet]_t$:

$$k = 2k_6[LO_2^\bullet]_{qs} = \sqrt{2k_6 I} \tag{40}$$

For instant initiation, $t = 0$ means the time when a quasi-stationary concentration of alkyl radicals $L^\bullet$ is reached ($\approx 1/k_2' \approx 10^{-8}$ s); this concentration equals $[LO_2^\bullet]_0$. Note that the quasi-stationary concentration of peroxyl radicals $[LO_2^\bullet]_{qs}$ in the case of continuous production is attained much later, i.e., for $t \gg 1/(2k_6 I)^{1/2}$, since

$$\frac{1}{k_2'} \ll \frac{1}{\sqrt{2k_6 I}} = \frac{1}{k} \quad \text{or} \quad \frac{k_2'}{k_3'}\eta \gg 1 \tag{41}$$

4. Kinetics of Hydroperoxide Accumulation

The chain reaction of the lipid peroxidation leads to an accumulation of hydroperoxide LOOH determined by kinetic eqs 12 and 13, which, by insertion of eq 14, can be written as

$$\partial_t[LOOH]_t = k_3[LO_2^\bullet]_t[LH]_t \partial_t[LH]_t = -k_3[LO_2^\bullet]_t[LH]_t - I \tag{42}$$

These equations and eq 38 give a closed set of kinetic equations that are rather cumbersome due to the dependence of I on the

concentration $[LH]_t$; cf. eq 4. We will therefore divide the following investigation of the solution to these equations into the two initialization cases.

4.1. Instant Initialization. Let us first consider the instant initiation case, $I = 0$. Since $\partial_t([LOOH]_t + [LH]_t) = 0$, we immediately have

$$[LH]_t + [LOOH]_t = [LH]_0 + [LOOH]_0 \tag{43}$$

where for generality we have included the concentration $[LOOH]_0$ at $t = 0$. Inserting this relation into the first equation of eq 70 leads, together with eq 38, to a closed set of two kinetic equations:

$$\partial_t[LOOH]_t = k_3[LO_2^\bullet]_t([LH]_0 + [LOOH]_0 - [LOOH]_t)$$
$$\partial_t[LO_2^\bullet]_t = -2k_6[LO_2^\bullet]_t^2 \tag{44}$$

The solution to these equations is

$$[LOOH]_t = [LOOH]_0 + [LH]_0\left\{1 - \exp\left(-k_3\int_0^t [LO_2^\bullet]_t\, dt\right)\right\} \tag{45}$$

where

$$[LO_2^\bullet]_t = \frac{[LO_2^\bullet]_0}{1 + 2k_6[LO_2^\bullet]_0 t} \tag{46}$$

Equation 46 is a particular case of eq 39, obtained in the limit $I \to 0$.

The "initial" concentration of peroxyl radicals, $[LO_2^\bullet]_0$, i.e., the value when the quasi-stationary concentration of alkyl radicals, $[L^\bullet]_{qs}$, has been established, can easily be estimated from eqs 10 and 11 (with $I = 0$) if we neglect the recombination processes ($\upsilon_4 = \upsilon_5 = \upsilon_6 = 0$) and the change of the LH concentration during the short initial period. This gives $\partial_t([LO_2^\bullet]_t + [L^\bullet]_t) = 0$, and thus

$$[LO_2^\bullet]_t = [L^\bullet]_0 - [L^\bullet]_t \tag{47}$$

where $[L^\bullet]_0$ is the initial concentration of alkyl radicals that initiated the chain propagation.

The rate equation for the alkyl radical follows from eqs 14 and 15

$$\partial_t[L^\bullet]_t = -(k_2' + k_3')[L^\bullet]_t + k_3'[L^\bullet]_0 \tag{48}$$

and is easily solved to

$$[L^\bullet]_t = [L^\bullet]_{qs} - ([L^\bullet]_{qs} - [L^\bullet]_0)\exp[-(k_2' + k_3')t] \tag{49}$$

where

$$[L^\bullet]_{qs} = \frac{k_3'}{k_2' + k_3'}[L^\bullet]_0 \approx \frac{k_3'}{k_2'}[L^\bullet]_0 \tag{50}$$

is the quasi-stationary concentration of alkyl radicals; note that $k_3' \ll k_2'$. We see that the quasi-stationary value is obtained for $t \geq 1/(k_2' + k_3') \approx 1/k_2'$. Inserting eq 50 into eq 47 gives

$$[LO_2^\bullet]_0 = [L^\bullet]_0 - [L^\bullet]_{qs} = \frac{k_2'}{k_2' + k_3'}[L^\bullet]_0 \approx [L^\bullet]_0 \tag{51}$$

More details can be found in Appendix A.

The stationary concentration of peroxyl radicals, $[LO_2^\bullet]_{qs}$, is equal to zero, cf. eqs 34 and 46 for instant initiation ($I = 0$), and it is attained with the rate $2k_6[LO_2^\bullet]_0$. However, the peroxidation kinetics is determined by the time integral of eq 46, cf. eq 45, and this quantity

$$\int_0^t [LO_2^\bullet]_t\, dt = \frac{1}{2k_6}\ln(1 + 2k_6[LO_2^\bullet]_0 t) \tag{52}$$

diverges at $t \to \infty$. By substituting this result into eq 45, it is seen that the decay of lipid acid LH and the accumulation of hydroperoxide LOOH will proceed as

$$[LOOH]_t = [LOOH]_0 + [LH]_0\left\{1 - \frac{1}{(1 + 2k_6[LO_2^\bullet]_0 t)^{k_3/2k_6}}\right\} \tag{53}$$

until the fatty acid LH has completely disappeared. This result implies that the recombination of $LO_2^\bullet$ radicals cannot terminate the chain propagation. It can only change the kinetic law from exponential, in the absence of recombination

$$[LOOH]_t = [LOOH]_0 + [LH]_0\{1 - \exp(-k_3[LO_2^\bullet]_0 t)\} \tag{54}$$

to the slower algebraic form of eq 53. This is clearly seen for the situation described in refs 2 and 4, i.e., when

$$k_3 \ll 2k_6 \tag{55}$$

When this condition is satisfied, eq 53 can be expanded and rewritten as

$$[LOOH]_t = [LOOH]_0 + [LH]_0\frac{k_3}{2k_6}\ln(1 + 2k_6[LO_2^\bullet]_0 t) \tag{56}$$

For short times, specified as

$$t \ll 1/2k_6[LO_2^\bullet]_0 \ll 1/k_3[LO_2^\bullet]_0 \tag{57}$$

eq 56 gives a linear growth of the hydroperoxide concentration:

$$[LOOH]_t \approx [LOOH]_0 + [LH]_0 k_3[LO_2^\bullet]_0 t \tag{58}$$

Equation 54 gives an identical behavior both for short times and for intermediate times, defined as

$$1/2k_6[LO_2^\bullet]_0 \ll t \ll 1/k_3[LO_2^\bullet]_0 \tag{59}$$

However, for intermediate times, eq 56 predicts a logarithmic dependence of the lipid peroxidation, i.e., a very slow increase of the LOOH concentration with time. Finally, for long times, i.e., for

$$t \gg 1/k_3[LO_2^\bullet]_0 \tag{60}$$

the kinetic law in eq 54 is exponential, while eq 56 preserves a very slow time dependence up to very large values of the logarithmic function

$$\frac{k_3}{2k_6}\ln(1 + 2k_6[LO_2^\bullet]_0 t) \approx 1 \tag{61}$$

which corresponds to a very long time, never achieved in a real experiment. Thus, although radical recombination cannot terminate the chain propagation, it can decrease the accumulation rate of the peroxidation considerably.

4.2. Continuous Initiation. Let us now consider continuous initiation. Kinetic eqs 38 and 70 cannot be solved analytically if we include the dependence of the initiation rate I on the concentration of LH given by eq 4. Therefore, as in real experiments, we restrict ourselves to a slower decay of lipid LH, given by the condition in eq 55 and use the approximation $[LH]_t \approx [LH]_0$. The initiation rate (eq 4) can thus be considered

time independent. By substituting $[LO_2^\bullet]_0 = 0$ into eq 39, we then obtain (cf. Appendix B for more details)

$$[LO_2^\bullet]_t = [LO_2^\bullet]_{qs} \tanh(kt) \quad (62)$$

where the quasi-stationary concentration $[LO_2^\bullet]_{qs}$ and the rate k are determined by eqs 21 and 40, respectively. The time integral of this kinetics is found to be

$$\int_0^t [LO_2^\bullet]_t \, dt = \frac{1}{2k_6}\left\{kt + \ln\left(\frac{1+\exp(-2kt)}{2}\right)\right\} \quad (63)$$

To calculate the hydroperoxide accumulation kinetics, we also neglect the decay of lipids, i.e., $[LH]_t \approx [LH]_0$, which can be justified under the condition in eq 55 and the additional condition

$$\frac{k_3}{2k_6}kt = Z_{LO_2^\bullet}\sqrt{It} \approx \frac{\eta I}{[LH]_0}t \ll 1 \quad (64)$$

where the oxidizability $Z_{LO_2^\bullet}$ and the parameter η were determined in eqs 17 and 23, respectively. The condition in eq 64 can be rewritten as

$$It \ll [LH]_0\frac{1}{\eta} \quad \text{or} \quad It \ll [LH]_0 \quad (65)$$

where we have used $\eta \geq 1$. These conditions allow us to neglect the terms on the right-hand side of the second equation in eq 70, giving $[LH]_t \approx [LH]_0$. Substituting eq 63 into the first equation of eq 70 finally gives an expression for the peroxide accumulation kinetics (under the stated conditions):

$$[LOOH]_t \approx [LOOH]_0 + [LH]_0\frac{k_3}{2k_6}\left\{kt + \ln\left(\frac{1+\exp(-2kt)}{2}\right)\right\} \quad (66)$$

For short times ($kt \ll 1$ but $k_2't \gg 1$; see eq 41), i.e., before the quasi-stationary concentration of peroxyl radicals is attained but after the quasi-stationary concentration of alkyl radicals has been attained, eq 66 simplifies to

$$[LOOH]_t \approx [LOOH]_0 + \frac{1}{2}[LH]_0 I k_3 t^2 \quad (67)$$

This quadratic time dependence of the peroxide concentration corresponds to pure chain propagation reaction 6. This law is due to the linear time dependence of the concentration of $LO_2^\bullet$, $[LO_2^\bullet]_t = It$, at the initial stage of the continuous initiation, when recombination is not important.

For intermediate times

$$1 \ll kt \ll \frac{2k_6}{k_3} \quad (68)$$

i.e., when the quasi-stationary concentration of peroxyl radicals is attained, the peroxide concentration follows a linear kinetic law:

$$[LOOH]_t \approx [LOOH]_0 + \frac{1}{2}[LH]_0\frac{k_3}{k_6}kt \equiv [LOOH]_0 + [LH]_0 k_3\sqrt{\frac{I}{2k_6}}t \quad (69)$$

This kinetics is formally equivalent to the kinetic law for instant initiation and pure chain propagation, eq 58, when the influence of the recombination is negligible. However, for continuous initiation, eq 69 is obtained for all times satisfying eq 65 by replacing the initial concentration $[LO_2^\bullet]_0$ in eq 58 by the quasi-stationary value $[LO_2^\bullet]_{qs}$, given by eq 21. Note that the quasi-stationary value $[LO_2^\bullet]_{qs}$ is attained entirely due to recombination.

Thus, under continuous initiation, the recombination of radicals cannot terminate the chain reactions but only change a quadratic kinetic law to a linear one.

TABLE 2: Lipid Peroxidation Kinetics for Instant Initiation of the Reaction

species	concentration kinetics for instant initiation
$[LO_2^\bullet]$	$[LO_2^\bullet]_t = [LO_2^\bullet]_0/(1 + 2k_6[LO_2^\bullet]_0 t)$
LOOH	$[LOOH]_t = [LOOH]_0 + [LH]_0\{1 - 1/(1 + 2k_6[LO_2^\bullet]_0 t)^{k_3/2k_6}\}$
LH	$[LH]_t = [LH]_0 + [LOOH]_0 - [LOOH]_t$
$L^\bullet$	$[L^\bullet]_t = k_3[LH]_t[LO_2^\bullet]_t/k_2[O_2]$

TABLE 3: Lipid Peroxidation Kinetics for Continuous Initiation of the Reaction

species	concentration kinetics for continuous initiation
$LO_2^\bullet$	$[LO_2^\bullet]_t = (I/2k_6)^{1/2}\tanh[(2k_6 I)^{1/2}t]$
LOOH	$[LOOH]_t \approx [LOOH]_0 + [LH]_0(k_3/2k_6)\{(2k_6 I)^{1/2}t + \ln[[1 + \exp[-2(2k_6 I)^{1/2}t]]/2]\}$
LH	$[LH]_t = [LH]_0 + [LOOH]_0 - [LOOH]_t$
$L^\bullet$	$[L^\bullet]_t = (k_3[LH]_t[LO_2^\bullet]_t + I)/k_2[O_2]$

5. Summary

We have investigated the kinetic description of the lipid peroxidation chain reaction. Since a general analytical solution of the kinetic equations does not appear feasible, we have used a quasi-stationary approximation of the radical concentrations in the kinetic description of the lipid peroxidation chain reaction. When applicable, this is a very useful and accurate description. However, its validity depends on how the reaction is initiated. We considered two common initialization procedures, an instantaneous or pulsed initiation and a continuous initiation. We found that this quasi-stationary approximation can always be used for alkyl radicals. However, for peroxyl radicals, the approximation can only be used for continuous initiation in a restricted time interval, and for instant initiation, it cannot be applied at all since the time scale of the decay of the $LO_2^\bullet$ radical is on the same order as that of the hydroperoxide accumulation. Furthermore, we showed that radical recombination cannot terminate the chain propagation; it can only change the kinetics of the accumulation of peroxide.

The role of the different recombination processes of the radicals produced in the peroxidation reaction was investigated. We showed that only recombination between radicals with the least oxidizability needs to be taken into account, i.e., the recombination between peroxyl radicals. The complicated chain reaction scheme in eqs 10–13 could thus be reduced to a much simpler and more transparent reaction scheme consisting of the following three rate equations for the concentration of LOOH, LH, and $LO_2^\bullet$:

$$\partial_t[LOOH]_t = k_3[LO_2^\bullet]_t[LH]_t$$

$$\partial_t[LH]_t = -k_3[LO_2^\bullet]_t[LH]_t - I$$

$$\partial_t[LO_2^\bullet]_t = -2k_6[LO_2^\bullet]_t^2 + I \quad (70)$$

These simplifications are based on a set of conditions for rate constants and concentrations of reactants which are satisfied for all available literature values.

For convenience we summarize below the derived expressions for the kinetics of the species and the conditions of their applicability. Since these are different for instant and continuous initiation of the lipid peroxidation reaction, we consider the two cases separately.

The analytical expressions for the kinetic behavior are given in Table 2 for instant initiation. The conditions of applicability are

$$[O_2] = \text{constant}$$

$$\frac{k_3 k_4}{k_2}\frac{[LH]_t}{[O_2]} \ll k_5 \ll \frac{k_2 k_6}{k_3}\frac{[O_2]}{[LH]_t}$$

$$k_5[LO_2^\bullet]_t \ll k_2[O_2] \quad (71)$$

The analytical results for the kinetic behavior under continuous initiation are given in Table 3. The conditions of applicability are those for instant initiation plus the following two conditions for the initiation rate:

$$It \ll [LH]_0, \qquad \frac{k_2[O_2]}{\sqrt{2k_4 I}} \gg 1 \quad (72)$$

The former condition requires that the light initiation does not lead to any significant change of the LH concentration.

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Appendix A: Quasi-Stationary Radical Concentration at Instant Initiation

The kinetic equations for the radical concentrations, under the condition $I = 0$, are given by eqs 10 and 11 plus eqs 14–16, i.e.

$$\partial_t[L^\bullet]_t = -k_2'[L^\bullet]_t + k_3'[LO_2^\bullet]_t - 2k_4[L^\bullet]_t^2 - k_5[L^\bullet]_t[LO_2^\bullet]_t$$

$$\partial_t[LO_2^\bullet]_t = k_2'[L^\bullet]_t - k_3'[LO_2^\bullet]_t - 2k_6[LO_2^\bullet]_t^2 - k_5[L^\bullet]_t[LO_2^\bullet]_t \quad (73)$$

Under the derived conditions for the rate constants, eqs 22, 24, 27, and 28, both the recombination of $L^\bullet$ radicals and that of $L^\bullet$ with $LO_2^\bullet$ (processes 7 and 8) have negligible influence on the peroxidation kinetics. Only the recombination of $LO_2^\bullet$ radicals, i.e., process 9, is important and must be taken into account; cf. eq 38. Consequently, the peroxidation kinetics does not depend on the values of the rate constants k_4 and k_5, and thus, we are free to choose the following model values:

$$k_4 = k_6, \quad k_5 = 2k_6 \quad (74)$$

These values satisfy all the inequalities used for our derivations and simultaneously allow us to obtain an exact analytical solution. In addition, we assume that

$$k_3' \ll k_2' \quad (75)$$

which is necessary for applying the quasi-stationary approximation for the concentration of $L^\bullet$ and which is satisfied for all available data. Equation 73 can then be written as

$$\partial_t[L^\bullet]_t = -k_2'[L^\bullet]_t + k_3'[LO_2^\bullet]_t - 2k_6[L^\bullet]_t^2 - 2k_6[L^\bullet]_t[LO_2^\bullet]_t$$

$$\partial_t[LO_2^\bullet]_t = k_2'[L^\bullet]_t - k_3'[LO_2^\bullet]_t - 2k_6[LO_2^\bullet]_t^2 - 2k_6[L^\bullet]_t[LO_2^\bullet]_t \quad (76)$$

It is convenient to introduce the quantity

$$[R]_t = [L^\bullet]_t + [LO_2^\bullet]_t \quad (77)$$

with the initial condition

$$[R]_0 = [L^\bullet]_0 \quad (78)$$

This implies that $L^\bullet$ is the only radical that is initially present in the system and thus capable of initiating the chain reaction. The rate equation for $[R]_t$ is obtained by summing both parts of eq 76, yielding

$$\partial_t[R]_t = -2k_6[R]_t^2 \quad (79)$$

which is easily solved to

$$[R]_t = \frac{[L^\bullet]_0}{1 + 2k_6[L^\bullet]_0 t} \quad (80)$$

Equation 77 then immediately gives

$$[LO_2^\bullet]_t = [R]_t - [L^\bullet]_t \quad (81)$$

By inserting this relation into the first equation of eq 76, we obtain a linear inhomogenious differential equation of first order

$$\partial_t[L^\bullet]_t = -(k_2' + k_3' + 2k_6[R]_t)[L^\bullet]_t + k_3'[R]_t \quad (82)$$

which is solved to

$$[L^\bullet]_t = \frac{[L^\bullet]_0}{1 + 2k_6[L^\bullet]_0 t}\left\{\frac{k_3'}{k_2' + k_3'} + \frac{k_2'}{k_2' + k_3'}e^{-(k_2' + k_3')t}\right\} \quad (83)$$

and which, using eq 81, gives

$$[LO_2^\bullet]_t = \frac{k_2'}{k_2' + k_3'}\frac{[L^\bullet]_0}{1 + 2k_6[L^\bullet]_0 t}\{1 - e^{-(k_2' + k_3')t}\} \quad (84)$$

Thus, we see that, after the concentration of the $L^\bullet$ radicals has become quasi-stationary, i.e., when $(k_2' + k_3')t \approx k_2't \gg 1$, then

$$[L^\bullet]_{qs} = [L^\bullet]_t \approx \frac{k_3'}{k_2'}\frac{[L^\bullet]_0}{1 + 2k_6[L^\bullet]_0 t} \quad (85)$$

and

$$[LO_2^\bullet]_t \approx \frac{[L^\bullet]_0}{1 + 2k_6[L^\bullet]_0 t} \quad (86)$$

The time dependence of $[L^\bullet]_t$ in the quasi-stationary period is now due solely to the time dependence of $[LO_2^\bullet]_t$ since

$$[L^\bullet]_t \approx \frac{k_3'}{k_2'}[LO_2^\bullet]_t \quad (87)$$

This is in full accordance with eqs 29, for $I = 0$, and 30 when the second-order terms required for obtaining the second equation of eq 44 are neglected. When eq 51 is used, eq 86 is indeed a solution to the second equation of eq 44. Thus, a quasi-stationary value of the concentration of $L^\bullet$ radicals indeed does take place for $k_2't \gg 1$ followed by a slow decay due to the decrease of the $LO_2^\bullet$ concentration induced by the recombination of $LO_2^\bullet$ radicals; cf. eq 87.

Appendix B: Quasi-Stationary Radical Concentration under Continuous Initiation

The quasi-stationary approximation under continuous initiation can be investigated similarly to the procedure used in Appendix

A. The only difference is that the first kinetic equation in eq 76 of the model contains an additional term, I, responsible for the continuous production of radicals.

It is convenient again to use the quantity $[R]_t$, introduced in eq 77, but now with the initial condition

$$[R]_0 = 0 \tag{88}$$

The rate equation for $[R]_t$ is now

$$\partial_t[R]_t = -2k_6[R]_t^2 + I \tag{89}$$

which is formally identical with eq 38, and thus, the solution is (cf. eq 39 with $[LO_2^\bullet]_0 = 0$)

$$[R]_t = [R]_{qs}\tanh(kt) \equiv \sqrt{\frac{I}{2k_6}}\frac{1-\exp(-2kt)}{1+\exp(-2kt)} \tag{90}$$

By substituting eq 81 into the first equation of eq 76 (with a constant initiation rate I), we again obtain an inhomogeneous linear differential equation of first order:

$$\partial_t[L^\bullet]_t = -(k_2' + k_3' + 2k_6[R]_t)[L^\bullet]_t + k_3'[R]_t + I \tag{91}$$

where $[R]_t$ is given by eq 90. The solution to this equation, with initial condition $[L]_0 = 0$, is

$$[L^\bullet]_t = \frac{k_3'}{k_2'}[R]_t + \frac{I}{k_2'} + \frac{2I\exp(-k_2't)}{k_2'(1+\exp(-2kt))} \tag{92}$$

where we have used inequalities 41 and 75. It is seen that a quasi-stationary concentration of alkyl radicals is reached when

$$k_2't \gg 1 \tag{93}$$

with a value

$$[L^\bullet]_{qs} = [L^\bullet]_t = \frac{k_3'}{k_2'}[R]_t + \frac{I}{k_2'} \tag{94}$$

This quasi-stationary value obeys the inequality

$$[L^\bullet]_{qs} \ll [R]_t \tag{95}$$

which, by use of eqs 40, 75, and 90, is equivalent to

$$\frac{k_2'}{k}\frac{1-\exp(-2kt)}{1+\exp(-2kt)} \gg 1 \tag{96}$$

This condition is most difficult to satisfy for $kt \ll 1$, but for that case eq 96 becomes equivalent to eq 93, i.e., the condition required for obtaining the quasi-stationary regime. Thus, after the quasi-stationary regime is reached, the second term in eq 81 can be neglected, yielding

$$[LO_2^\bullet]_t \approx [R]_t \tag{97}$$

By use of eq 90, it is seen that this relation is identical to eq 62. Note that eqs 94 and 97 agree with eqs 29 and 30 when the second-order terms are neglected, which is required for obtaining eq 38. Thus, the method is self-consistent.

References and Notes

(1) Costanzo, L. L.; de Guidi, G.; Giuffuda, S.; Sortino, S.; Gondorelli, G. *J. Inorg. Biochem.* **1995**, *59*, 1.

(2) Antunes, F.; Salvador, A.; Marinko, H. S.; Alves, R.; Pinto, R. *Free Radical Biol. Med.* **1996**, *21*, 917.

(3) Tang, L.; Zhang, Y.; Qian, Z.; Shen, X. *Biochem. J.* **2000**, *352*, 27.

(4) Vanarojana, S.; Chantharaksi, U.; Wilairat, P.; Lenbury, Y. *Sci. Asia* **2005**, *31*, 369.

(5) Frankel, E. N. *Lipid Peroxidation*, 2nd ed.; The Orly Press: Bridgwater, England, 2005.

(6) Salikhov, K. M.; Molin, Yu. N.; Sagdeev, R. Z.; Buchachenko, A. L. *Spin Polarization and Magnetic Effects in Radical Reactions*; Elsevier: New York, 1984.

(7) Buchachenko, A. L. *J. Phys. Chem. A* **2001**, *A105*, 9995.

(8) Brocklehurst, B. *Chem. Soc. Rev.* **2002**, *31*, 301311.

(9) Steiner, U. E.; Ulrich, T. *Chem. Rev.* **1989**, *89* (1), 51.

(10) Grissom, C. B. *Chem. Rev.* **1995**, *95* (1), 3.

(11) Harkins, T. T.; Grissom, C. B. *Science* **1994**, *263* (5149), 958.

(12) Lalo, U. V.; Pankratov, Y. V.; Mikhailik, O. M. *Redox Rep.* **1994**, *1* (1), 71.

(13) Aristarkhov, V. M.; Klimenko, L. L.; Deev, A. I.; Ivanekha, E. V. *Biofizika* **1983**, *28* (5), 800–6.

(14) Uri, N. In *Autoxidation and Antioxidant*; Lundberg, W. O. Ed.; Wiley-Interscience: New York, 1961; pp 55−106.

(15) Chambers, D. E.; Parks, D. A.; patterson, G.; Roy, R.; McCord, J. H.; Yoshida, S.; Parmley, L. F.; Downey, J. M. *J. Mol. Cell. Cardiol.* **1985**, *17*, 145.

(16) Aaes-Jorgensen. In *Autoxidation and Antioxidant*; Lundberg, W. O. Ed.; Wiley-Interscience: New York, 1962; Vol. II, pp 1045−1094.

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