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Antioxidant and Prooxidant Effects of Tocopherol

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Abstract—A computer simulation of the kinetics of free-radical oxidation of lipids in the presence of tocopherol was performed. The induction periods and initial rates of oxidation as functions of initial tocopherol concentrations were found to exhibit extremums. The extended kinetic scheme used in this study describes all types of observed relationships and eliminates illusory contradictions between two groups of experiments in which the antioxidant and prooxidant effects of tocopherol were found. The model can form a basis for the prediction of the effects of tocopherol and its analogs under real conditions.

INTRODUCTION

Current concepts of the effect of tocopherol on the free-radical oxidation of lipids contain serious contradictions. A great body of publications is known in which the antioxidant role of tocopherol was demonstrated; however, on the other hand, a number of publications illustrated the prooxidant effect of this substance [1, 2, 4–7]. In a number of cases, the dependence of the induction period on the initial concentration of tocopherol exhibited an extremal character (an increase and then a decrease with increasing initial concentrations of tocopherol) [1, 2, 4]. The initial rates of oxidation increased with the initial concentration of tocopherol; in some cases, they were higher than those in the absence of this compound [1, 2, 5–7]. Hypotheses on the effects of the reactions of oxidation chain propagation by tocopheroxyl radicals were proposed for the qualitative explanation of these phenomena [1, 2]. However, the problem cannot be considered as solved until the development of a mathematical model that describes all observed effects based on a single kinetic scheme.

Moreover, the role of reactions of the intermediate product methylenequinone [2, 8, 9] was not studied; the role of reaction of tocopherol with a hydroperoxide, which is possible for a number of other phenols [10], is not clearly understood either.

The aim of this work was to simulate experimentally observed phenomena with the use of an extended kinetic scheme of free-radical oxidation, which includes the above reactions, to find the reasons for the above contradictions, and to compare different performance criteria for the antioxidant activities of preparations.

CALCULATION PROCEDURE

The studies were performed using mathematical computer simulation [11–13]. The rate constants of elementary reactions, which were used in the calculations, were taken from [1, 2, 14–17] and converted to 50°C. In the absence of published data, estimated values, which were based on an analogy with other processes and on the reactivities of reacting species, were used [10, 14, 18]. These values were corrected in the course of solving the problems formulated in this work.

The kinetic scheme, which initially consisted of ten elementary reactions that were commonly used previously [1, 2], was extended by a number of cross reactions, elementary steps specific to tocopherol with the participation of methylenequinone (TMQ) [2, 8, 9], and the reaction of tocopherol with a hydroperoxide. In this case, for the optimization and evaluation of the effects of particular elementary steps on the reaction kinetics, the rate constants were varied within one or two orders of magnitude. As a result, a base version of the conceivable reaction mechanism was obtained, which is presented in the kinetic scheme (see the table). The following initial conditions, which are typical of systems containing the esters of unsaturated fatty acids, were taken [16]: [RH] = 3 and [ROOH] = 10^{-4} mol/l. The calculations were performed at initiation rates (w_i) of 5×10^{-11} (simulation of autoxidation) and 5×10^{-9} mol l^{-1} s^{-1} (simulation of an initiated oxidation regime).

In special computer experiments, it was found that the kinetics of oxygen absorption, substrate consumption, and diene conjugate or hydroperoxide buildup exhibited the same character. This was also supported by published data [19]. Therefore, to illustrate the considerations of this study, we chose peroxide concentration as a typical parameter. In the subsequent text, the kinetic curve implies the kinetic curve of change in the total hydroperoxide concentration. Changes in the initial hydroperoxide concentration within a range from 0 to 10^{-4} mol/l had no effect on the shape of kinetic curves.

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Kinetic scheme of the oxidation of lipids (RH) in the presence of tocopherol (TOH)

No.	Reaction	k, l mol^{-1} s^{-1}
1	$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$	1×10^8
2	$ROO^{\bullet} + RH \longrightarrow R^{\bullet} + ROOH$	1×10^2
3	$R^{\bullet} + ROOH \longrightarrow ROO^{\bullet} + RH$	1×10^5
4	$R^{\bullet} + R^{\bullet} \longrightarrow NRP$	1×10^8
5	$R^{\bullet} + ROO^{\bullet} \longrightarrow NRP$	5×10^7
6	$ROO^{\bullet} + ROO^{\bullet} \longrightarrow O_2 + NRP$	1×10^7
7	$TOH + ROO^{\bullet} \longrightarrow TO^{\bullet} + ROOH$	2×10^6
8	$TO^{\bullet} + ROOH \longrightarrow TOH + ROO^{\bullet}$	10
9	$TOH + R^{\bullet} \longrightarrow TO^{\bullet} + RH$	1×10^7
10	$TO^{\bullet} + RH \longrightarrow TOH + R^{\bullet}$	0.5
11	$TO^{\bullet} + O_2 \xrightarrow{RH} TMQ + HOOH + R^{\bullet}$	1.0
12	$TO^{\bullet} + O_2 \longrightarrow OTOO^{\bullet}\ (\equiv ROO^{\bullet})$	1.0
13	$TO^{\bullet} + R^{\bullet} \longrightarrow TMQ + RH$	1×10^7
14	$TO^{\bullet} + ROO^{\bullet} \longrightarrow ROOH + TMQ$	1×10^7
15	$TO^{\bullet} + ROO^{\bullet} \longrightarrow QP$	1×10^7
16	$TO^{\bullet} + TO^{\bullet} \longrightarrow TOH + TMQ$	3×10^3
17	$TO^{\bullet} + TO^{\bullet} \longrightarrow TD$	1×10^3
18	$TMQ + R^{\bullet} \longrightarrow TMQR^{\bullet}$	1×10^5
19	$TMQR^{\bullet} + R^{\bullet} \longrightarrow NRP$	1×10^7
20	$TMQR^{\bullet} + ROO^{\bullet} \longrightarrow NRP$	1×10^7
21	$TMQ + ROO^{\bullet} \longrightarrow TMQROO^{\bullet}$	1×10^2
22	$TMQROO^{\bullet} + R^{\bullet} \longrightarrow P$	1×10^8
23	$TMQROO^{\bullet} + ROO^{\bullet} \longrightarrow NRP$	5×10^7
24	$TMQROO^{\bullet} + TO^{\bullet} \longrightarrow NRP$	5×10^7
25	$ROOH + ROOH \longrightarrow ROO^{\bullet} + RO^{\bullet}(R^{\bullet}) + H_2O$	1×10^{-8}
26	$QP \longrightarrow TO^{\bullet} + ROO^{\bullet}$	1×10^{-5} (s^{-1})
27	$ROOH + TOH \longrightarrow TO^{\bullet} + RO^{\bullet}(R^{\bullet}) + H_2O$	4×10^{-6}
28	$HOOH + TOH \longrightarrow TO^{\bullet} + HO^{\bullet}(R^{\bullet}) + H_2O$	4×10^{-6}

Note: NRP is a nonradical product; TMQ is methylenequinone; and QP is a quinolide peroxide.

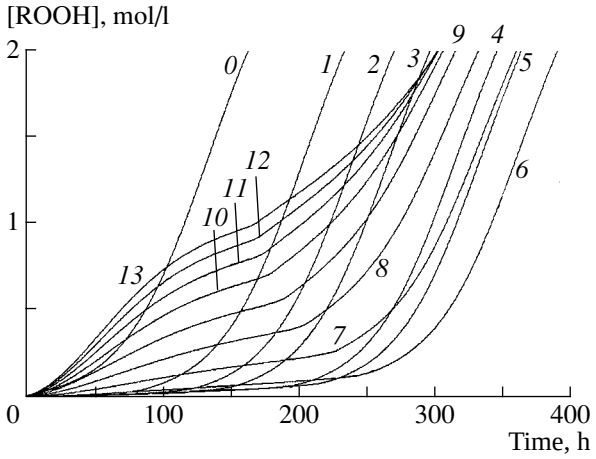

Fig. 1. Kinetic curves of the total hydroperoxide concentration at the initial concentrations of tocopherol (mmol/l): (*0*) 0, (*1*) 0.05, (*2*) 0.10, (*3*) 0.15, (*4*) 0.3, (*5*) 0.4, (*6*) 1.0, (*7*) 3.0, (*8*) 6.0, (*9*) 10, (*10*) 15, (*11*) 20, (*12*) 25, and (*13*) 30. Rate of initiation: 5×10^{-11} mol l^{-1} s^{-1}.

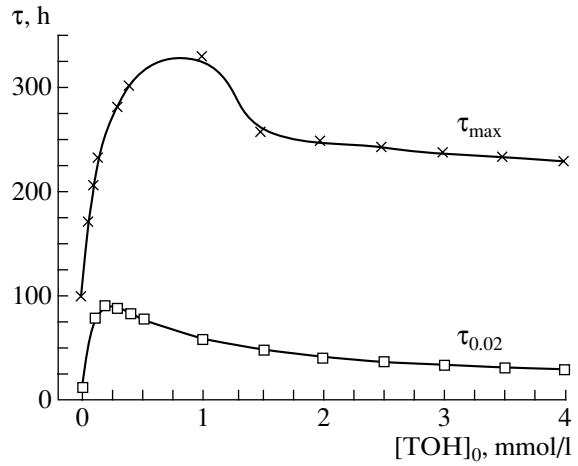

Fig. 2. Dependence of the induction period τ, determined by two techniques, on the initial concentration of tocopherol at a rate of initiation equal to 5×10^{-11} mol l^{-1} s^{-1}.

RESULTS AND DISCUSSION

Figure 1 demonstrates the kinetic curves of hydroperoxide buildup under autoxidation conditions. As the concentration of tocopherol was increased from 0 to 1 mmol/l, the curves shifted to the right (induction periods increased); however, their shape changed insignificantly. The curves ended with portions parallel to each other, which correspond to a developed oxidation process. However, as the concentration was further increased, the shape of the curves dramatically changed: the slopes of the initial portions increased, whereas the slopes of the end portions decreased. As the initial concentrations of tocopherol were increased, the curves completely shifted to the left; this fact is indicative of a decrease in the inhibiting effect. The initial tocopherol concentration $[TOH]_0 = 1$ mmol/l can be considered as critical.

Note that, at $[TOH]_0$ higher than the critical value, the kinetic curves exhibited a sharp bend, which corresponds to the most rapid increase in the rate of oxidation (Fig. 1).

The technique used for determining induction periods is of special importance because of significant differences in the shape of kinetic curves. The induction period was determined by the following two techniques: (1) as the time (τ_{max}) that corresponds to the point of the most rapid increase in the rate of oxidation and (2) as the time ($\tau_{0.02}$) it takes for the total hydroperoxide concentration to reach a value of [ROOH] = 0.02 mol/l [2]. Figure 2 demonstrates each of these times as functions of the initial concentration of tocopherol. Both of these functions passed through a maximum; however, the position of this maximum essentially depends on the technique used for evaluating the induction period.

Moreover, at tocopherol concentrations higher than the critical value, τ_{max} gradually loses its meaning as the time over which the oxidation process is practically suppressed, because the degree of oxidation considerably increases in this interval. Because the concentration of oxidation products is an extensive factor of the process, it was of interest to examine the kinetics of changes in an intensive factor, the concentration of peroxide radicals. In the chemiluminescence method, which is commonly used for this purpose [18–21], the intensity of luminescence (I) is proportional to the rate of disproportionation of these radicals ($I \sim [ROO^{\bullet}]^2$). Figures 3 and 4 illustrate the kinetics of changes in

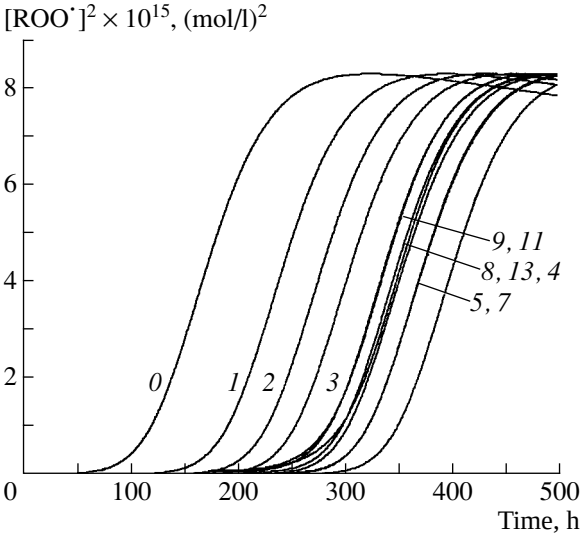

Fig. 3. Curves of changes in the peroxide radical concentration squared during autoxidation. The rate of initiation and the numbering of curves are specified in Fig. 1.

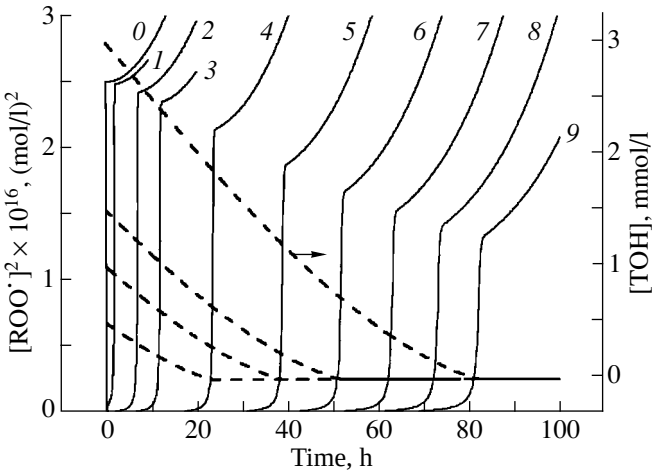

Fig. 4. Time dependence of $[ROO^{\bullet}]^2$ and [TOH] in initiated oxidation ($w_i = 5 \times 10^{-9}$ mol l^{-1} s^{-1}) at the initial concentrations of tocopherol (mmol/l): (*0*) 0, (*1*) 0.02, (*2*) 0.10, (*3*) 0.2, (*4*) 0.5, (*5*) 1.0, (*6*) 1.5, (*7*) 2.0, (*8*) 2.5, and (*9*) 3.0.

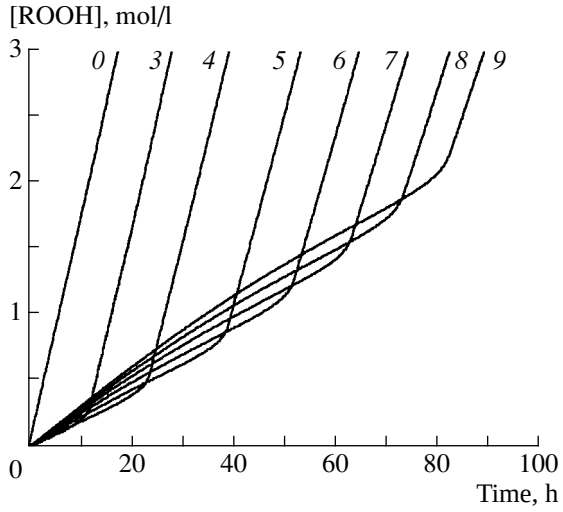

Fig. 5. Kinetic curves of the total hydroperoxide concentration. The rate of initiation and the numbering of curves are specified in Fig. 4.

$[ROO^{\bullet}]^2$. In this case, all kinetic curves of $[ROO^{\bullet}]^2$ are uniform in shape and exhibit induction periods, which are most clearly defined in the kinetic curves under conditions of initiated oxidation (Fig. 4). This fact is indicative of certain advantages of the chemiluminescence method over studying the kinetics of hydroperoxide buildup. As can be seen in Fig. 5, the latter method exhibits contradictions that are described below.

A researcher who measures induction periods by observing their increase draws the conclusion that the antioxidant effect of tocopherol increases with an increase in its initial concentration. However, another researcher, measuring the initial rate of oxidation and observing its increase, draws the opposite conclusion: a decrease in the antioxidant efficiency. In this case, it is of crucial importance to choose a criterion of efficiency because the efficiency factors change in opposite directions.

As mentioned above, a procedure for measuring the kinetics of chemiluminescence ($I \sim [ROO^{\bullet}]^2$) is free of such contradictions. The character of changes in the concentration of peroxide radicals is always the same: the introduction of tocopherol causes a decrease in this concentration, and the recovery of luminescence intensity after the induction period corresponds to the complete disappearance of the antioxidant (Fig. 4). Therefore, the monitoring of the concentration of peroxide radicals by chemiluminescence seems to be a more direct technique for evaluating the efficiency of antioxidants and their kinetic parameters than the examination of the kinetics of accumulation of the molecular products of oxidation.

Burlakova *et al.* [1, 2] called attention to the extremal character of the dependence of the initial rate of oxidation on the initial concentration of tocopherol. As can be seen in Fig. 6, the mathematical model used in this work also reflects these characteristics of the kinetics of tocopherol action.

Thus, is tocopherol an antioxidant or prooxidant? The answer: a combination of both. Under conditions of autoxidation, high concentrations (>10 mmol/l) of tocopherol produce higher rates of generation of free radicals than those in the absence of this compound; thus, it is responsible for a prooxidant effect. This is likely due to reactions with hydroperoxides (reaction nos. 27 and 28 in the table) because the introduction of reaction nos. 10–12 (chain propagation by radicals formed from tocopherol) without considering reaction nos. 27 and 28 decreased the efficiency of the antioxidant action; however, it did not increase the rate of oxidation as compared with the uninhibited process.

Is it appropriate to apply the term *antioxidant* to substances similar to tocopherol in action? It is likely that the use of this term is allowable in the sense that this substance can decrease the rate of oxidation over a certain concentration range, as compared with an uninhibited process, and shift the step of developed uninhibited oxidation in time. Nevertheless, while on the subject of antioxidant action, the degree of conversion should be specified.

In comparative studies of the efficiency of tocopherol and related antioxidants under different conditions, the authors should use a sufficiently wide concentration range in order to have a grasp of the shape of the entire kinetic curve (to sufficiently high degrees of con-

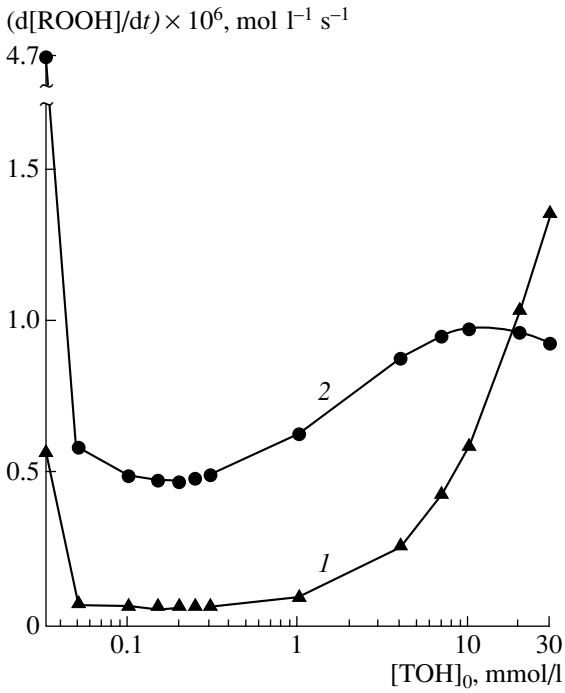

Fig. 6. Dependence of the rate of oxidation on the initial concentration of tocopherol (*1*) at the rate of initiation equal to 5×10^{-11} mol l^{-1} s^{-1} 20 h after the addition of tocopherol and (*2*) at the rate of initiation equal to 5×10^{-9} mol l^{-1} s^{-1} 30 min after the addition of tocopherol.

version) and perform a comparison within similar portions of the kinetic curves.

CONCLUSIONS

In this work, using a direct computer simulation, we found that the kinetic scheme used is sufficient to describe all types of the experimentally observed relationships and to resolve all apparent contradictions. The antioxidant and prooxidant effects of tocopherol are not contradictory, but they are the sides of a single set of properties of this compound. The kinetic model studied can form a basis for predicting the effects of tocopherol and its analogs under real conditions and for developing models of the combined effects of substances on free-radical oxidation in both homogeneous and heterogeneous systems.

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