Probabilistic Kinetic Model of Slow Oxidation of Low-Density Lipoprotein. 3. Hydroperoxide-Free Initiation

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A theoretical model is presented that explains slow copper-induced oxidation of low-density lipoprotein in the systems free of seeded hydroperoxydes. The model is based on the probabilistic kinetic theory, modified to take into account different radical generation rates by oxidized and reduced forms of the metal ions. It is shown that the initiation and progression of the LDL oxidation can take place in any LDL dispersion by metal-induced oxidation of α-tocopherol, one of the constituents of LDL, and formation of slowly reacting α-tocopheroxyl radical. Selected values of several adjustable parameters define actual temporal profiles of the quantities defining the oxidation process.

1. INTRODUCTION

In numerous mechanistic and kinetic studies of oxidation of plasma low-density lipoprotein (LDL), mediated by the transition-metal ions, notably copper, the presence of lipid hydroperoxide in the LDL system is simply assumed. In copper-driven oxidation the break-down of LOOH is associated with the oxidation of copper, Cu(I) → Cu(II). In order for copper to act as a catalyst, reduction of Cu(II) by some of the LDL constituents is needed. There is now overwhelming evidence that α-tocopherol, imbedded in the lipid domain of the LDL particles, is capable of reducing copper, taking part in that process.[1−6] At the same time α-tocopheroxyl radical, T•, is introduced into the system. That radical reacts with lipids. Although the rate constant for its reaction with unsaturated lipids is low,[4] $k = 0.12\ M^{-1}\ s^{-1}$, the lifetime of T• is long enough to make that process efficient in the absence of other, more competitive reactions. That is the basis for the pro-oxidant action of α-tocopherol and the *tocopherol-mediated peroxidation* (TMP), the process introduced by Bowry and co-workers[1] and further elaborated in a number of publications by the same authors and their research groups.

To visualize the complete picture of the LDL oxidation, often the absolute requirement of the seeded or preformed hydroperoxides was emphasized.[7−10] In some other studies, based mostly on the experiments in which essentially no hydroperoxide was detected,[5,11] it was claimed that no preformed peroxides were required for the LDL oxidation. The question arises how reliable are the methods for the hydroperoxide evaluation. Whereas in the LDL samples prepared by ultracentrifugation, the iodometric[12] and FOX[13] methods usually indicate several hydroperoxide molecules per LDL particle, the luminescence-based methods[14,15] detect about 2 orders of magnitude lower concentration. It is probable that the former two methods overestimate and the latter methods underestimate lipid peroxide groups.[16] Thus, relying only on the measured amount of hydroperoxides in the LDL preparation is not quite safe to conclude on the existence of and requirement for the seeded peroxides for the lipoprotein oxidation.

In the present study the essentials of the probabilistic kinetic theory of LDL oxidation[17] have been adopted to show in a quantitative way that the LDL oxidation might be initiated and progressed by transition-metal ions, notably copper, in any LDL system containing α-tocopherol, regardless of the presence of seeded hydroperoxides.

2. THEORETICAL FOUNDATIONS

The microscopic probabilistic kinetic model of LDL oxidation[17] by the transition-metal ions explains the processes under quasi-stationary conditions in closed systems in a satisfactory manner.[18] The rates of the metal-ion oxidation and reduction are assumed to be equal during the time of observation. In that time other parameters might change significantly. For such a quasi-stationary process the presence of lipid hydroperoxides is required. The model may be extended to the nonstationary processes, such as prevailing in the systems that initially do not contain any hydroperoxides.

To account for the LDL systems lacking seeded hydroperoxides it is assumed that at any instant of time the rate of radical generation in reducing the metal ions by α-tocopherol, G_T, generally differs from the radical generation rate in oxidizing metal ions by hydroperoxides, G_P.

The generation rates related to a single LDL particle could be expressed as

$$G_T^* = k_T^*\, C_{M(n+1)}^* C_T^*$$

$$G_P^* = k_P^*\, C_{M(n)}^* C_P^* \qquad (1)$$

where $C_{M(n+1)}^*$ and $C_{M(n)}^*$ refer to the concentration of the metal ions in the oxidized and reduced states, respectively

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(in moles per particle). The generation rates expressed per liter of the LDL dispersion of concentration C_{LDL} are

$$G_T = G_T^* \cdot C_{LDL} \cdot N_A$$

$$G_P = G_P^* \cdot C_{LDL} \cdot N_A \quad (2)$$

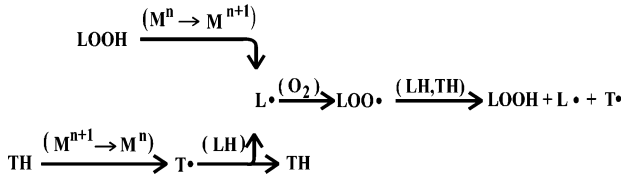
where N_A is the Avogadro constant. If the starred quantities referring to a single particle are transformed into standard forms[19] (per liter of the LDL dispersion), eqs 2 turn into

$$G_T = k_T n_T n_M r \cdot C_{LDL}^0 \cdot C_{LDL}$$

$$G_P = k_P n_P n_M \cdot (1 - r) \cdot C_{LDL}^0 \cdot C_{LDL} \quad (3)$$

Here n_T, n_P, and n_M mean the number of α-tocopherol molecules, peroxides, and metal ions, respectively, per LDL particle, r is the fraction of the metal ions in the oxidized state, $r = C_{M(n+1)}/C_M$, and C_{LDL}^0 is the molar concentration of "pure" LDL, $C_{LDL}^0 = 4 \cdot 10^{-4}$mol L^{-1}. It comes into the expressions in transformation of k_T^* to k_T and k_P^* to k_P, having in mind that the values of k's that could possibly be found in the literature would refer to a pure lipid system and not to the dispersed "lipid" particles in water. In eq 3 n_P, r and consequently $G = G_T + G_P$ depend on time.

To find out the time dependence of the quantities that define the oxidation process, first the chemical pathway is to be defined and then appropriate calculations performed. As described in more details,[17] the L• radicals generated by oxidation of α-tocopherol (generation rate G_T) and by reduction of lipid peroxides (generation rate G_P) subsequently react with oxygen (probability $(1 - P_\lambda(0))$) or escape that reaction (probability $P_\lambda(0)$; $P_\lambda(0)$ is the Poisson probability of finding an LDL particle without any oxygen molecule in it at the time of L• production). The formed peroxyl radical, LOO•, is either permanently trapped at protein (probability γ_2) or participates in further oxidizing process (probability $(1\text{-}\gamma_2)$) by reacting with the lipid (probability γ_3) or α-tocopherol molecules (probability $(1\text{-}\gamma_3)$). Thus, in the entire process new L• and hydroperoxides are formed, and α-tocopherol oscillates between T• and TH. The metal ions, by reacting with peroxide and α-tocopherol and consequently flipping between the reduced and oxidized forms, maintain the circle of tocopherol-mediated peroxidation. The entire process is presented in a simplified form in the scheme below:



As it is seen, L• might circulate in an "auxiliary" circle by reacting with oxygen, without reacting with the metal ions (propagation of oxidation).

G_T and G_P, defined by eqs 3, could be numerically calculated in the finite difference approach, in a step-by-step basis. For a sufficiently small increment of time, δt, given the state of any variable X_i at time t, its value at time $t + \delta t$ is given by

$$X_i(t + \delta t) = X_i(t) + f_i(X_1(t), X_2(t), \ldots)\delta t \quad (4)$$

For an actual calculation, the initial values of all variables have to be known, and the adjustable parameters have to be selected. In contrast to the procedure extensively elaborated,[17] in the present treatise we will solve just eqs 3 and show that any other quantities of interest are related to G_T and G_P.

The change of G ($G = G_P + G_T$) in the ith time increment is

$$\delta G(i) = G(i) \cdot \delta t \quad (5)$$

Changes of other quantities in the ith time increment are as follows.

Change of r:

$$\delta r(i) = \frac{1}{C_M} \cdot [\delta G_P(i) - \delta G_T(i)] \quad (6)$$

Change of concentration of oxygen in the solution:

$$\delta C_{O_2}(i) = [\delta G_P(i) + \delta G_T(i)] \cdot [(1 - P_\lambda(i)) \cdot (Z(i) + 1)] \quad (7)$$

Change of concentration of peroxide:

$$\delta C_{LOOH}(i) = [\delta G_P(i) + \delta G_T(i)] \cdot Z(i) - \delta G_P(i) \quad (8)$$

The term $Z(i)$ in the above relations means the probability for the L• radicals to stay in the "auxiliary" circle in the propagation phase before leaving it

$$Z(i) = \sum_{j=1}^{\infty} [(1 - P_\lambda(i)) \cdot (1 - \gamma_2) \cdot \gamma_3]^j$$

The sum of the above geometrical progression is

$$Z(i) = \frac{(1 - P_\lambda(i)) \cdot (1 - \gamma_2) \cdot \gamma_3}{1 - (1 - P_\lambda(i)) \cdot (1 - \gamma_2) \cdot \gamma_3} \quad (9)$$

The change of the value of any quantity between two consecutive steps is

$$X(i + 1) = X(i) + \delta X(i) \quad (10)$$

3. RESULTS

For the calculations of the oxidation process in the LDL systems lacking seeded hydroperoxide the following initial values were used: $C_{O_2}(0) = 0.22$ mmol L^{-1}, $C_{LDL}(0) = 5-20$ μmol L^{-1}, $n_P(0) = 0$, $n_T(0) = 5$, and $C_M = 0.1 \cdot C_{LDL}$, and for simplicity, the presence of possible co-oxidants was neglected. As for the rate constants, we adopted[2] $k_T = 0.5$ mol^{-1} L^{-1} and explored the influence of k_P on the oxidation process by introducing the parameter q, $q = k_P/k_T$, tested in the range of 50−200. Parameter r was varied from 0.001 to 0.999. Other parameters used in the calculations were the same as for the quasi-stationary model.[17] The system is assumed to be closed (no supply of additional oxygen).

Figure 1 shows the time dependence of concentration of oxygen in the aqueous phase of LDL dispersion, lipid hydroperoxide (expressed in the mole to mole of LDL ratio, n_P) (panel A), ratio r, and the radical generation rate, $G = G_T + G_P$ (panel B), for four different concentrations of LDL. For the calculation $r = 0.9$ and $q = 100$ were used. As it is

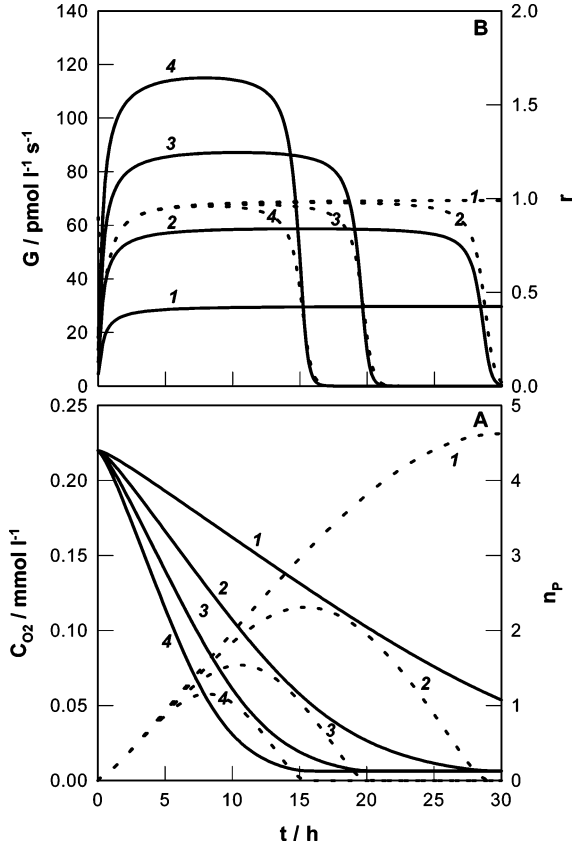


Figure 1. Calculated time dependence of oxygen consumption (full lines) and hydroperoxide production (dotted lines) (panel A) and radical generation rate (full lines) and parameter r (dotted lines) in panel B, in a closed LDL system, for four different values of C_{LDL}: 5 μmol L^{-1} (curves 1), 10 μmol L^{-1} (curves 2), 15 μmol L^{-1} (curves 3), and 20 μmol L^{-1} (curves 4). For the calculation the following initial values of the parameters were used: $C_{O_2}(0) = 0.22$ mmol L^{-1}, $n_T(0) = 5$, $n_P(0) = 0$, $r = 0.9$, and $q = 100$. The values of other parameters were the same as in our earlier study.[17]

seen, the oxidation process is initiated and progresses although at the beginning there is no hydroperoxide in the system.

The reaction starts by oxidation of α-tocopherol and consequent production of the initial amount of hydroperoxide needed for maintaining the oxidation process. The hydroperoxide level gradually increases, reaches maximum, and then decreases. The C_{LOOH} maxima are higher for the lower LDL concentration: $n_P = 4.62$, 2.31, 1.54, and 1.15 for C_{LDL} values 5 μmol L^{-1}, 10 μmol L^{-1}, 15 μmol L^{-1}, and 20 μmol L^{-1}, respectively. After the initial sharp increase, the total radical generation rate reaches its plateau and then sharply drops to zero. At the plateau G_T and G_P are essentially equal, defining the quasi-stationary state.[17] Ratio r initially sharply drops (not clearly noticed in the time scale of Figure 1) and then behaves in a manner similar to that of G. Concentration of oxygen gradually decreases and under the assumed conditions approaches certain nonzero value.

Essentially the same behavior is predicted for other selections of the initial and adjustable parameters. Figure 2 illustrates the influence of the initial value of r on the temporal profiles of the quantities studied. For all used initial values (0.1, 0.3, 0.6, and 0.9), r initially sharply drops (poorly seen in the illustration), then gradually increases to the level of the quasi-stationary state, and finally sharply drops to zero, together with vanishing of G and C_{LOOH}. The temporal

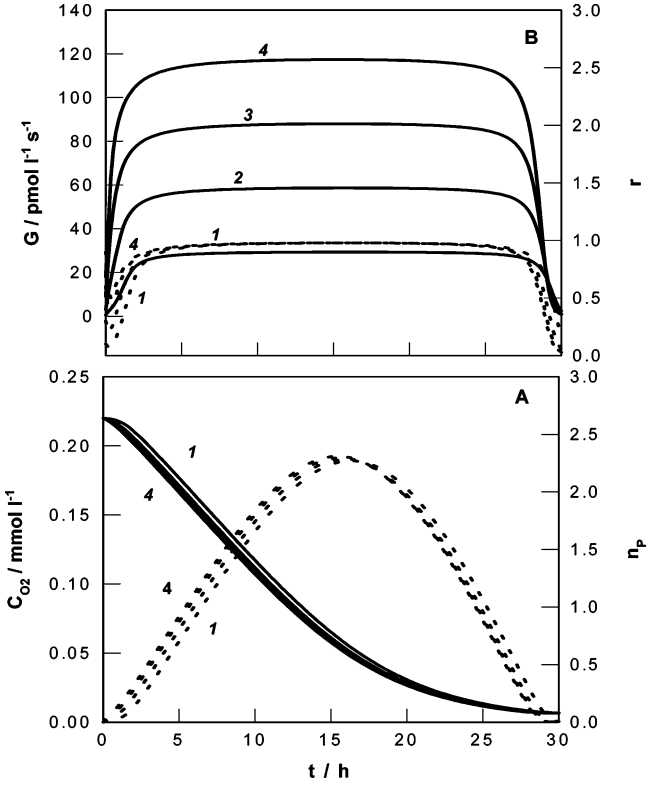


Figure 2. Influence of the selected values of r on the temporal behavior of oxidation markers C_{O_2}, n_P, G, and r: $r(0) = 0.1$ (curves 1), $r(0) = 0.3$ (curves 2), $r(0) = 0.6$ (curves 3), and $r(0))$ 0.9, for $C_{LDL} = 10$ μmol L^{-1}. Other conditions are the same as for Figure 1.

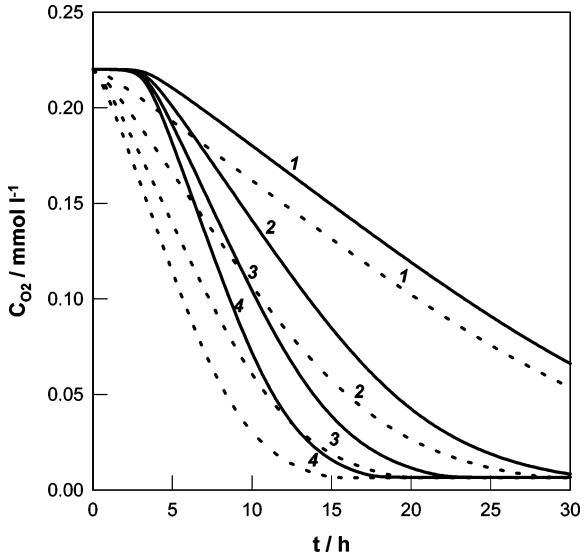


Figure 3. Temporal behavior of C_{O_2} for two extreme values of r, $r = 0.001$ (full lines) and $r = 0.999$ (dotted lines) for different values of C_{LDL}: 5 μmol L^{-1} (curves 1), 10 μmol L^{-1} (curves 2), 15 μmol L^{-1} (curves 3), and 20 μmol L^{-1} (curves 4).

variation of oxygen in the LDL systems of four different concentrations for two extreme values of r (0.001 and 0.999) are shown in Figure 3. It turns out that r is not a very influential parameter, except at the initiation in the approach to the quasi-stationary state and in the descent of the process.

Parameter q is also not very influential. Temporal plots of G, n_P, r, and C_{O_2} with various initial values of q are essentially the same as those presented in Figure 2 for variable r (not shown).

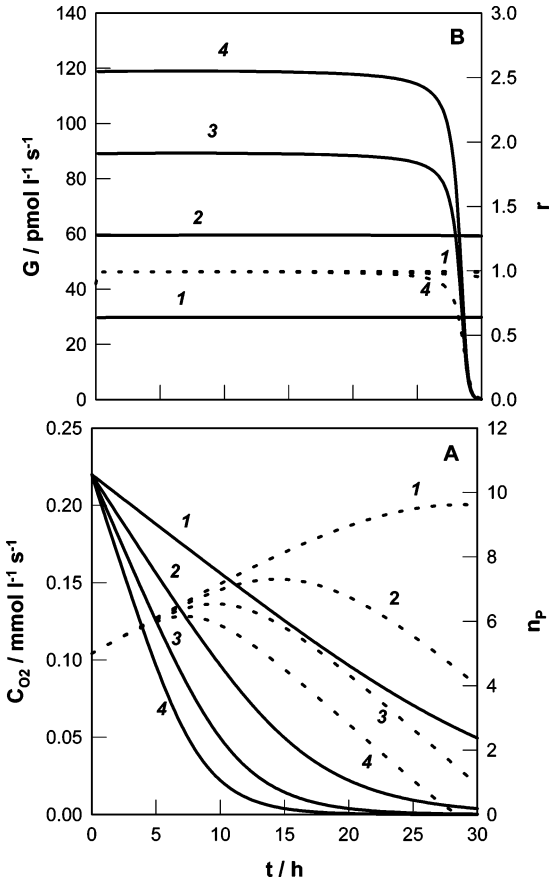


Figure 4. The time dependence of the oxidation markers for four different values of C_{LDL}, for $n_P(0) = 5$. Other conditions are the same as for Figure 1.

The present approach could also be used to calculate the time dependence of the oxidation processes in the LDL dispersions containing preformed hydroperoxide as well as in the open LDL systems (allowed continuous influx of oxygen). The curves for the former systems (Figure 4) and for the latter systems (not shown) are very similar to those derived from the quasi-stationary model.[17]

4. DISCUSSION

In the present report we have demonstrated that the foundations of the microscopic probabilistic model of LDL oxidation[17] by low concentrations of the transition-metal ions ($C_M/C_{LDL} < 1$) could explain initiation and progression of the oxidation process regardless of the presence of seeded hydroperoxides. The original model has to be modified in the way that the generation of radicals by the metal ion oxidation and the metal ion reduction were separately treated, allowing for unequal rates of these two processes. The LDL oxidation is initiated by reduction of the metal ions by α-tocopherol and simultaneous formation of the α-tocopheroxyl radical. Subsequently some hydroperoxides and the metal ions in the reduced state are formed. These two components and oxygen are necessary for propagation of the tocopherol-mediated peroxidation. The entire process is operative for the entire range of r values, $0 \leq r < 1$.

It is quite certain that besides the two emphasized processes in the catalytic action of copper ions, some other reactions might also contribute to the overall process, in particular, reduction of Cu(II) by lipid hydroperoxide[16] and direct oxidation of Cu(I) by oxygen.[20,21] Although the former reaction has been experimentally demonstrated in rather simple lipid systems, in the more complex systems, containing protein and α-tocopherol, such a reaction is less important in competition with other thermodynamically and kinetically more favorable reactions.[8,16,22] Besides, the fit of the experiments with varying α-tocopherol content[18] with the general probabilistic kinetic model clearly demonstrates the dominant role of α-tocopherol in reduction of Cu(II) (tocopherol-mediated peroxidation).

The contribution of the direct oxidation of Cu(I) by oxygen in such a complex system like ours is not quite clear. It is probable that at the beginning and the descent of the oxidation, when lipid peroxides are low or even nonexistent, this process might be important or even dominant. The support for that possibility is the ceasing of the oxidation process predicted by the present model at the stage at which a certain amount of oxygen is still present in the system, the behavior that is not expected in reality. However, the inclusion of such a term in a theoretical model would require more knowledge of the respective kinetic data.

For the process to be initiated, at least a fraction of copper has to be in the cupric state, to act on α-tocopherol. The increase of copper concentration would increase the rate of oxidation processes. However, more than one copper ion in a single LDL particle could provide conditions for more complex reactions, not just for tocopherol-mediated peroxidation.

In any case, regular physiological conditions and processes are much more complex, and the tocopherol-mediated peroxidation, as presented here, might not be of general importance.[23] However, favorable conditions for TMP might prevail at a particular time at some local places. Also, the developed model nicely explains vulnerability of LDL to oxidation, independent of the existence of seeded hydroperoxides. The model also takes into account the processes in open systems, the systems with a permanent supply of oxygen. Actually, the approach based on unequal rates of the metal oxidation and reduction might better explain any process of slow LDL oxidation than the quasi-stationary model.[17]

A larger number of adjustable parameters would certainly result in a better fit of the theory to the experimental data, but often the actual choice lacks solid justification.

The peroxide-free initiation of oxidation is not easily experimentally verified. It is practically impossible to prepare LDL samples without at least a minute amount of peroxides.

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