Kinetic Modeling of Lipoprotein Peroxidation Initiated by Copper and Azo Compounds

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ABSTRACT: Oxidation of low density lipoprotein (LDL) has been postulated as the main cause of atherogenesis, resulting in formation of foam cells, triggering of various pathways, and leading to the development of the disease. Therefore, antioxidants would naturally be expected to attenuate the progress of atherosclerosis. α-Tocopherol (α-TocH) is the most abundant form of vitamin E in nature and the major antioxidant of biological membranes. α-TocH is also present at a much higher concentration than other antioxidants in plasma lipoproteins. The large amount of α-TocH present in LDL leads to the expectation that lipid peroxidation would be strongly inhibited by α-TocH. However, advanced atherosclerotic plaques are not deficient in the presence of antioxidants such as vitamins C and E, despite the occurrence of massive lipid oxidation. Thus conventional mechanism associated with the antioxidant properties of α-TocH cannot explain why lipid peroxidation occurs in atherosclerotic lesions in the presence of compounds that are usually considered to be antioxidants. A kinetic model was developed and applied to explore the mechanism of lipid peroxidation process under various conditions and to examine the possible occurrence of lipid peroxidation in the presence of α-tocopherol. The model incorporated many factors, that were not included in previous models but we believe to play important roles in the different outcome of the process. As a result, the numerical simulation illustrated that lipid peroxidation in the lipoprotein particle could occur in the presence of vitamins E (α-TocH) and C under certain conditions, including high initiation rate, high initial α-TocH level, low ratio of [vitamin C]/[α-TocH], and small lipoprotein particles. The kinetic scheme developed in this study defined the type of relationship that α-TocH in an environment exhibits either pro- or anti-oxidative property. Thus, antioxidant and pro-oxidant effects of tocopherol merely depend on the condition in which its properties are exhibited.

KEYWORDS: Lipid Peroxidation, Tocopherol, Atherosclerosis, Kinetic Modeling, Simulation.

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease, which involves a build up of cholesterol and fatty deposits within the arterial wall. “Fatty streaks” formed on the interior walls of arteries are the first visible signs of atherosclerosis. These fatty streaks contain oxidized low density lipoprotein particles (LDL), which are strongly implicated in the initiation and development of atherosclerosis. Antioxidants would therefore be expected to attenuate atherosclerosis, and a number of human and animal studies has indicated that this is the case. Vitamin E is by far the major free radical antioxidant in LDL lipid, and α-tocopherol (α-TocH) is biologically and chemically the most active form. The very high reactivity of α-TocH toward peroxy radicals (LOO·) and the relatively large amount of α-TocH present in LDL lead one to expect that LDL peroxidation would be very strongly inhibited by α-TocH. However, recent studies of the kinetics of LDL peroxidation showed that α-TocH could act as a pro-oxidant under certain experimental conditions. Hypotheses regarding the effects of tocopheroxyl radicals (Toc·) on the reactions of oxidation chain propagation have been proposed for the qualitative explanation of these phenomena. In addition, other studies have reported that the content of α-TocH in advanced human atherosclerotic lesion and LDL-like particles derived from them were at least comparable to that of healthy arteries and circulating LDL. The conventional mechanism associated with the antioxidant properties of vitamin E can not explain why LDL peroxidation occurs in atherosclerotic lesions in the presence of compounds that are usually considered to be antioxidants. Moreover, recent randomized human clinical trials indicated that vitamin antioxidants failed to retard atherosclerosis and may have caused adverse effects.

The susceptibility of low density lipoprotein (LDL) to radical peroxidation is believed to be an important
factor in the process responsible for foam-cell formation and atherosclerosis. Appropriate methods for determination of the susceptibility of LDL to oxidation \textit{in vivo} are not available. The common methods of evaluation of LDL susceptibility to oxidation \textit{in vitro} are based on LDL peroxidation induced by transition metals (usually copper ions)\textsuperscript{7, 13, 14}. However, the copper-mediated oxidation mechanism that could explain oxidative attack of LDL is not well defined, and the involvement of copper ions in the first steps of LDL oxidation is still a subject of discussion.

The lipids contained in high density lipoprotein (HDL) can also be oxidized. This process has received less attention than LDL oxidation, in spite of the possibility that it may have several important pathophysiological implications. HDL oxidation may affect the oxidation of LDL. Addressing this possibility requires data on the relative oxidizability of HDL and LDL. The published results regarding this issue are quite confusing. Several authors found that HDL is more susceptible than LDL to copper induced oxidation\textsuperscript{15-17}. On the other hand, several other studies indicated that the susceptibility of HDL to oxidation is either similar or smaller than that of LDL\textsuperscript{21, 18}.

\textit{In vivo} lipid peroxidation occurs in a heterogeneous open system with permanent exchange of lipids, oxidants, antioxidants and peroxidation products between membranes and their environments. It is also under the influence of a multitude of enzymatic activities. These factors usually interact in complicated ways. In view of this complexity, several authors have attempted to gain understanding of the mechanisms responsible for oxidation in simple model systems, made by dispersing oxidizable lipids in the form of liposomes, emulsions, and micellar systems\textsuperscript{19, 20}. In such systems it is possible to monitor peroxidation under varying conditions while changing the factors that govern the reaction in a controllable fashion, one at a time. Although these studies yielded interesting results concerning the influence of various factors on the kinetics of lipoprotein peroxidation, the extrapolation of \textit{in vitro} results on to the biological environment is not straightforward. These \textit{in vitro} quantitative determinations of kinetic parameters of individual reactions in the simple systems such as homogeneous solution and micelle, though less informative about integrative aspects, seem more robust than results from \textit{in vitro} studies of the responses of more complete systems. However, the observed experimental responses arising from those known individual reactions are difficult to explain due to the complexity of the biological system. Mathematical techniques can be useful in revealing gaps and inconsistencies in the knowledge about the reactional mechanisms of such responses.

Several workers have applied mathematical models\textsuperscript{13, 22} and theoretical analysis\textsuperscript{19, 24} to the study of lipid peroxidation and associated processes. For example, valuable insights were gained from the kinetic modeling of radical initiated lipid peroxidation in low density lipoproteins carried out by Waldeck and Stocker in 1996\textsuperscript{23}. Later in 1998, Pinchuk \textit{et al}.\textsuperscript{24} did a kinetic analysis of copper-induced peroxidation of LDL. Their efforts clearly illustrate how mathematical modeling can be the appropriate methodology to study mechanism of lipid peroxidation process since it is possible to simulate the overall lipid peroxidation process mathematically using a set of those elementary reactions. Besides reproducing the overall process of lipid peroxidation in lipoprotein particles, such simulations provide insight into the elementary reactions and their interaction. Such an approach can also offer solutions to controversial issues such as the relative importance of antioxidants, the role of initiating radicals, and potential pro-oxidantive effects of antioxidant within a lipoprotein particle.

The purposes of this study were firstly to set up a basic model for lipid peroxidation that describes the overall process as it has usually been observed in the experiments. The kinetic model was based upon the hierarchy of known radical reactions\textsuperscript{1, 5, 13, 19, 21-35}. The theoretical model was developed as a Personal Computer based system programmed in SAAM II ver 1.1 (RFKA, University of Washington, Seattle, WA). The second goal was to examine the influence of the system parameters, such as the rate of initiation, the concentration of the major antioxidant on the kinetics of the overall process and the elementary reactions. Of particular interest was the response of lipid soluble antioxidant (vitamin E, \(\alpha\)-TocH) to various rates of initiation. Thirdly, we examined the possibility of lipid peroxidation occurring in the presence of compounds that are usually considered to be antioxidants. The role of transition metals such as copper ion as a promoter of lipoprotein peroxidation was employed in the model construction and investigation. Generally, transition metals remain tightly bound to binding protein in \textit{vivo} and are not available to participate in the oxidative biochemistry. However, it is our hypothesis that under certain conditions, such as low pH which may prevail in micro-environments within the arterial wall, metals can become displaced from their binding proteins and may then promote lipoprotein oxidation in the presence of antioxidants.

\textbf{METHODS}

\textbf{Mathematical Model}

To investigate the kinetic reactions responsible for oxidizability of lipid in the microenvironment of a
lipoprotein particle, a kinetic model was developed to obtain an overall picture of lipid peroxidation process and to illuminate the relative importance of individual processes in the lipid peroxidation system. The kinetic model incorporated the following features: the classic reaction scheme of lipid peroxidation process; antioxidant and pro-oxidant effects of vitamins C and E; peroxyl radical- or copper-initiated lipid peroxidation process; and the compartmental nature of the system.

The theoretical model for lipid peroxidation process was developed as a personal computer based system. The computer model was programmed in SAAM II ver. 1.1 (RFKA, University of Washington, Seattle, WA). This software can solve systems of ordinary (linear or nonlinear) differential equations, estimate parameters and simulate the behavior of the model under various conditions. A general diagram of the processes that are considered in our model is presented in Scheme 1. The reaction equations identified to be involved in the process are given in Table 1.

In the model, the flux is defined by rates of reactions (v_i), calculated by the following equation:

\[ v_i = k_i [A][B] \]  

where \( k_i \) is the apparent rate constant of a bimolecular reaction (M\(^{-1}\)s\(^{-1}\)) and [A] and [B] are the concentrations of the substrates A and B, respectively.

The rate expressions of the individual reactions in Table 1 are as follows.

\[
\begin{align*}
v_1 &= 2v_{12} = k_{14} [\alpha\text{-TocH}] [\text{LH}] \\
v_2 &= 2v_{13} = k_{15} [\alpha\text{-TocH}] [\text{LOOH}] \\
v_3 &= k_{16} [\text{L}] [\text{L}] \\
v_4 &= k_{17} [\text{L}] [\text{LOO}'] \\
v_5 &= k_{18} [\text{LOO}] [\text{LOO}] \\
v_6 &= k_{19} [\text{LOO}] [\alpha\text{-TocH}] \\
v_7 &= k_{20} [\text{LOO}] [\text{Asc}^-] \\
v_8 &= k_{21} [\text{ROO}] [\text{LOOH}] \\
v_9 &= k_{22} [\text{ROO}] [\alpha\text{-TocH}] \\
v_{10} &= k_{23} [\text{ROO}] [\alpha\text{-TocH}] \\
v_{11} &= k_{24} [\text{ROO}] [\alpha\text{-TocH}] \\
v_{12} &= k_{25} [\text{LOOH}] [\alpha\text{-TocH}] \\
v_{13} &= k_{26} [\text{ROO}] [\text{Asc}^-] \\
v_{14} &= k_{27} [\text{ROO}] [\text{Asc}^-] \\
v_{15} &= k_{28} [\text{ROO}] [\text{Asc}^-] \\
v_{16} &= k_{29} [\text{ROO}] [\text{Asc}^-] \\
v_{17} &= k_{30} [\text{ROO}] [\text{Asc}^-] \\
v_{18} &= k_{31} [\text{ROO}] [\text{Asc}^-] \\
v_{19} &= k_{32} [\text{ROO}] [\text{Asc}^-] \\
v_{20} &= k_{33} [\text{ROO}] [\text{Asc}^-] \\
v_{21} &= k_{34} [\text{ROO}] [\text{Asc}^-] \\
v_{22} &= k_{35} [\text{ROO}] [\text{Asc}^-] \\
v_{23} &= k_{36} [\text{ROO}] [\text{Asc}^-] \\
v_{24} &= k_{37} [\text{ROO}] [\text{Asc}^-] \\
v_{25} &= k_{38} [\text{ROO}] [\text{Asc}^-] \\
v_{26} &= k_{39} [\text{ROO}] [\text{Asc}^-] \\
v_{27} &= k_{40} [\text{ROO}] [\text{Asc}^-] \\
v_{28} &= k_{41} [\text{ROO}] [\text{Asc}^-] \\
v_{29} &= k_{42} [\text{ROO}] [\text{Asc}^-] \\
v_{30} &= k_{43} [\text{ROO}] [\text{Asc}^-] \\
v_{31} &= k_{44} [\text{ROO}] [\text{Asc}^-] \\
v_{32} &= k_{45} [\text{ROO}] [\text{Asc}^-] \\
v_{33} &= k_{46} [\text{ROO}] [\text{Asc}^-]
\end{align*}
\]

The above rate constants \( k_i, i = 1, 2, \ldots, 33 \), have been found experimentally and their literature sources are indicated in Table 1.

**Model of Azo Compound-Induced Lipid Peroxidation**

Azo compound was used in order to obtain a constant and known rate of chain initiation, which is essential to the kinetic study.

The oxidation of polyunsaturated fatty acids (LH) initiated with azo compound (R-N=N-R) and inhibited by antioxidant (IH) can be represented by the following scheme.

**Initiation:**

\[
\text{R-N=N-R} \quad \rightarrow \quad (1-e)\text{R-R} + 2e + \text{N}_2
\]

**Propagation:**

\[
\text{R} + \text{O}_2 \quad \rightarrow \quad \text{ROO}^+ \quad \rightarrow \quad \text{ROO}^+ + \text{LO}^+ \rightarrow \text{LOO}^+ + \text{L}^+ \quad \rightarrow \quad \text{LOOH} + \text{L}^+
\]

**Inhibition:**

\[
\text{LOO}^+ + \text{IH} \quad \rightarrow \quad \text{LOOH} + \text{I}^-
\]

**Termination:**

\[
\text{LOO}^+ + \text{I}^- \quad \rightarrow \quad \text{NRPs}
\]

**NRPs**

From the nature of species involved in the model, an assumption was made that most species are present in only one phase. Of all the reaction equations in Table 1, reactions 1-3, 11-15, 18-24, 26-27, and 29-30 were used in order to model the basic scheme of azo compound-induced lipid peroxidation. The concentration-time profile for each species in the azo
Table 1. Chemical reactions considered in the lipid peroxidation model.

<table>
<thead>
<tr>
<th>No.</th>
<th>Reaction</th>
<th>Rate constant (unit)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initiation : Azo compound</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>AAPH + Heat + O₂ → ROO⁻&lt;sub&gt;v&lt;/sub&gt;</td>
<td>1.36 x 10&lt;sup&gt;-6&lt;/sup&gt; (s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>AMVN + Heat + O₂ → ROO⁻&lt;sub&gt;v&lt;/sub&gt;</td>
<td>2.5 x 10&lt;sup&gt;-6&lt;/sup&gt; (s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>ROO⁻&lt;sub&gt;v&lt;/sub&gt; + LH → ROOH + L⁻</td>
<td>3.1 x 10&lt;sup&gt;3&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>21</td>
</tr>
<tr>
<td><strong>Initiation : Copper ion</strong></td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td>Cu²⁺ + LOOH → Cu⁺ + LOO⁻ + H⁺</td>
<td>3 x 10&lt;sup&gt;-3&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>Cu⁺ + LOOH → Cu²⁺ + LO⁻ + HO⁻</td>
<td>1 x 10&lt;sup&gt;2&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>13</td>
</tr>
<tr>
<td><strong>Cu²⁺/Cu⁺ redox cycling</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Cu²⁺ + α-TocH → Cu⁺ + α-Toc⁻ + H⁺</td>
<td>2.9 (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>Cu⁺ + AscH⁻ → Cu⁺ + Asc⁻ + H⁺</td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>Cu⁺ + reducing agent → Cu⁺</td>
<td></td>
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</tr>
<tr>
<td>9</td>
<td>Cu⁺</td>
<td></td>
<td></td>
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<tr>
<td><strong>Initiation : Autoxidation</strong></td>
<td></td>
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<tr>
<td>10</td>
<td>LH → LOO⁻</td>
<td>3 x 10&lt;sup&gt;8&lt;/sup&gt; (s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>21</td>
</tr>
<tr>
<td><strong>Oxygenation</strong></td>
<td></td>
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<tr>
<td>11</td>
<td>L⁻ + O₂ → LOO⁻</td>
<td>3 x 10&lt;sup&gt;8&lt;/sup&gt; (s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>21</td>
</tr>
<tr>
<td><strong>Propagation</strong></td>
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<tr>
<td>12</td>
<td>LOO⁻ + LH → LOOH + L⁻</td>
<td>3.1 x 10&lt;sup&gt;3&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>21</td>
</tr>
<tr>
<td>13</td>
<td>LOH + L⁻ → LO⁻ + L⁻</td>
<td>4 x 10&lt;sup&gt;7&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>22</td>
</tr>
<tr>
<td>14</td>
<td>α-Toc⁻ + LH → α-Toc⁻ + L⁻</td>
<td>1 x 10&lt;sup&gt;3&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>19</td>
</tr>
<tr>
<td>15</td>
<td>α-Toc⁻ + LOOH → α-Toc⁻ + LOO⁻</td>
<td>1 (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>19</td>
</tr>
<tr>
<td><strong>Termination</strong></td>
<td></td>
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<tr>
<td>16</td>
<td>L⁻ + L⁻ → NRPs</td>
<td>1 x 10&lt;sup&gt;9&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>27</td>
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<tr>
<td>17</td>
<td>L⁻ + LOO⁻ → NRPs</td>
<td>5 x 10&lt;sup&gt;7&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>27</td>
</tr>
<tr>
<td>18</td>
<td>LOO⁻ + LO⁻ → NRPs</td>
<td>1 x 10&lt;sup&gt;3&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>13</td>
</tr>
<tr>
<td>19</td>
<td>LOO⁻ + α-Toc⁻ → NRPs</td>
<td>2.5 x 10&lt;sup&gt;9&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>28</td>
</tr>
<tr>
<td>20</td>
<td>LOO⁻ + Asc⁻ → LOO⁻ + Asc⁻</td>
<td>1 x 10&lt;sup&gt;9&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>29</td>
</tr>
<tr>
<td>21</td>
<td>ROO⁻ + LOO⁻ → NRPs</td>
<td>1 x 10&lt;sup&gt;3&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>13</td>
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<tr>
<td><strong>Inhibition by vitamin E</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>22</td>
<td>ROO⁻ + α-TocH → ROOH + α-Toc⁻</td>
<td>5.9 x 10&lt;sup&gt;9&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>30</td>
</tr>
<tr>
<td>23</td>
<td>ROO⁻ + α-Toc⁻ → NRPs</td>
<td>2.5 x 10&lt;sup&gt;9&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>28</td>
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<tr>
<td>24</td>
<td>LOO⁻ + α-Toc⁻ → LOOH + α-Toc⁻</td>
<td>5.9 x 10&lt;sup&gt;9&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>30</td>
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<tr>
<td>25</td>
<td>LO⁻ + α-Toc⁻ → LOH + α-Toc⁻</td>
<td>1 x 10&lt;sup&gt;8&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>31</td>
</tr>
<tr>
<td><strong>Inhibition by vitamin C</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>26</td>
<td>ROO⁻ + AscH⁻ → ROOH + Asc⁻</td>
<td>1 x 10&lt;sup&gt;3&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>29</td>
</tr>
<tr>
<td>27</td>
<td>LOO⁻ + AscH⁻ → LOOH + Asc⁻</td>
<td>1 x 10&lt;sup&gt;3&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>29</td>
</tr>
<tr>
<td>28</td>
<td>LO⁻ + AscH⁻ → LOH + Asc⁻</td>
<td>1.6 x 10&lt;sup&gt;9&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>30</td>
</tr>
<tr>
<td><strong>Reactions involving vitamin E and/or vitamin C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>α-Toc⁻ + α-Toc⁻ → NRPs</td>
<td>1 x 10&lt;sup&gt;4&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>33</td>
</tr>
<tr>
<td>30</td>
<td>α-Toc⁻ + Asc⁻ → α-Toc⁻ + Asc⁻</td>
<td>5 x 10&lt;sup&gt;9&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>34</td>
</tr>
<tr>
<td>31</td>
<td>Asc⁻ + Asc⁻ + H⁺ → Asc⁻ + DHA</td>
<td>0.2 x 10&lt;sup&gt;9&lt;/sup&gt; (M&lt;sup&gt;-1&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>35</td>
</tr>
<tr>
<td>32</td>
<td>Asc⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Asc⁻</td>
<td></td>
<td></td>
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</tbody>
</table>

Compound-induced lipid peroxidation can therefore be described by the following sets of differential equations:

\[
\frac{d[\text{LH}]}{dt} = -v_3 - v_{12} - v_{14} \tag{6}
\]

\[
\frac{d[L^+]}{dt} = v_3 - v_{11} + v_{12} + v_{14} \tag{7}
\]

\[
\frac{d[LOO^\cdot]}{dt} = v_{11} - v_{12} + v_{15} - 2v_{18} - v_{19} - v_{20} - v_{21} - v_{24} - v_{27} \tag{8}
\]


\[
\frac{d[\text{LOOH}]}{dt} = v_{12} - v_{13} + v_{24} + v_{27} \tag{9}
\]

\[
\frac{d[\alpha-\text{TocH}]}{dt} = v_{14} + v_{15} - v_{22} - v_{24} + v_{30} \tag{10}
\]

\[
\frac{d[\alpha-\text{Toc}^{*}]}{dt} = -v_{14} - v_{15} - v_{19} + v_{22} - v_{23} + v_{24} - 2v_{29} - v_{30} \tag{11}
\]

\[
\frac{d[\text{AscH}^{-}]}{dt} = -v_{26} - v_{27} - v_{30} \tag{12}
\]

\[
\frac{d[\text{Asc}^{*}]}{dt} = -v_{20} + v_{26} + v_{27} + v_{30} \tag{13}
\]

\[
\frac{d[\text{DHA}]}{dt} = v_{20} \tag{14}
\]

\[
\frac{d[\text{ROO}^{*}]}{dt} = v_{5} - v_{21} - v_{22} - v_{23} - v_{26} \tag{15}
\]

where the rate expressions of the individual reactions are described in (5).

Model of Copper Ion-Mediated Lipid Peroxidation

Copper ion-mediated peroxidation of lipoproteins involves the continuous production of free radicals via a redox cycle between Cu^{2+} and Cu^{+}. From the nature of the species involved in the model, the assumption was made that most species are present in only one phase. From the reaction equations in Table 1, reactions 4-7, 11-15, 18-20, 24-25, and 27-30 were used in order to model the basic scheme of copper ion-mediated lipid peroxidation. The concentration-time profile for each species in the copper mediated lipid peroxidation could therefore be described by the following set of differential equations:

\[
\frac{d[\text{Cu}^{2+}]}{dt} = -v_{4} + v_{5} - v_{6} - v_{7} \tag{16}
\]

\[
\frac{d[\text{Cu}^{+}]}{dt} = v_{4} - v_{5} + v_{6} + v_{7} \tag{17}
\]

\[
\frac{d[\text{LH}]}{dt} = -v_{12} - v_{13} - v_{14} \tag{18}
\]

\[
\frac{d[\text{L}^{*}]}{dt} = -v_{11} + v_{12} + v_{13} + v_{14} \tag{19}
\]

\[
\frac{d[\text{LOO}^{*}]}{dt} = v_{4} + v_{11} + v_{12} - v_{15} - 2v_{18} - v_{19} - v_{20} - v_{24} - v_{27} \tag{20}
\]

\[
\frac{d[\text{LOOH}]}{dt} = -v_{4} - v_{5} + v_{12} - v_{15} + v_{24} + v_{27} \tag{21}
\]

\[
\frac{d[\text{LO}^{*}]}{dt} = v_{5} - v_{13} - v_{25} - v_{28} \tag{22}
\]

\[
\frac{d[\text{LOH}]}{dt} = v_{13} + v_{25} + v_{28} \tag{23}
\]

\[
\frac{d[\alpha-\text{TocH}]}{dt} = -v_{6} + v_{14} + v_{15} - v_{24} - v_{25} + v_{30} \tag{24}
\]

\[
\frac{d[\alpha-\text{Toc}^{*}]}{dt} = v_{6} - v_{14} - v_{15} - v_{19} + v_{24} + v_{25} - 2v_{29} - v_{30} \tag{25}
\]

\[
\frac{d[\text{AscH}^{-}]}{dt} = -v_{7} - v_{27} - v_{28} - v_{30} \tag{26}
\]

\[
\frac{d[\text{Asc}^{*}]}{dt} = v_{7} - v_{20} + v_{27} + v_{28} + v_{30} \tag{27}
\]

\[
\frac{d[\text{DHA}]}{dt} = v_{20} \tag{28}
\]

where the rate expressions of the individual reactions are described in (5).

Results

Accuracy of the Constructed Model

In order to check for the degree of accuracy in our model derived above, the simulated LOOH profiles for different α-TocH concentrations were plotted against experimental data obtained from the literature. We see that although the simulated curve fits the experimental data well for low α-TocH there was an observable discrepancy between the simulated curve and experimental results at 5 µM α-TocH. The greatest discrepancy occurs in the late lag phase and at the point where a sharp increase of LOOH begins. Since the lipid peroxidation was modeled here as a homogeneous system, we think that this discrepancy is due to the fact that we have not taken into consideration the following reaction.

\[
\text{TocH}_{\text{reactive}} + S \rightleftharpoons \text{TocH—S}_{\text{unreactive}} \tag{I}
\]

where \( S \) is the polar solvent and \( K_s \) is the equilibrium constant, taking into account the possibility that the reactive TocH may react with the polar solvent and transform into an unreactive TocH—S. Then, one is led to the reaction

\[
\text{TocH}_{\text{reactive}} + \text{LOO}^{*} \rightarrow \text{Toc}^{*} + \text{LOOH} \tag{II}
\]

with \( k_{24}^{s} = \frac{k_{24}^{s}}{(1 + K_s[S])} \tag{29} \)

where \( k_{24}^{s} \) is the rate constant of reaction 24 (in Table 1) in apolar medium.
With equation (29) incorporated into our model, the simulated curve exhibited a more smoothly rising LOOH level during the late lag phase and beginning of the propagation phase (Figure 1(b)), which fits the experimental data quite well.

**Lipid Peroxidation in Homogeneous System**

As shown in Figure 2, the simulated time course of 2,2'-azobis(2,4-dimethylvaleronitrile)-induced lipid peroxidation process in a homogeneous system consisted of two stages: the first stage is readily identified as the lag phase and lasts as long as antioxidants are present. In this figure, the simulation of our model, consisting of equations (6)–(15), was carried out with the system parametric values utilized in a previous experimental study by Shi *et al.*\(^3\) in order to compare our simulated profile with theirs. Just like in the experiments, the lag phase was accompanied by a depletion of \(\alpha\)-TocH. Only when the antioxidants were used up completely could chain propagation run efficiently, leading to a sharp increase in the rate of lipid hydroperoxide (LOOH) formation.

By variation of some selected parameters, it is possible to find out more about the relative importance and the interactions of the elementary reactions of the lipid peroxidation process. Of particular interest is the response of lipid soluble antioxidant (vitamin E, TocH) to various rates of initiation. Figure 3 shows the effect of increased rate of radical generation from 0.5 nM/s to 8 nM/s on kinetic profile of lipid hydroperoxide.

Fig 1. Comparison of simulation curves and experimental data of oxidation of methyl linoleate (453 mM) in acetonitrile induced by AMVN (0.2 mM) in the absence or presence of \(\alpha\)-TocH (5 \(\mu\)M). □ corresponds to experimental data where \(\alpha\)-TocH is present, ○ corresponds to that without \(\alpha\)-TocH, while the solid curve and dashed curves correspond to simulated results in the two cases, respectively. (Experimental data taken from Figure 2 in the study of Watson *et al.*\(^4\)).
formation, residual $\alpha$-TocH (%TocH) and concentration of tocopheroxyl radicals (Toc·). The results obtained from the model simulation showed that the rate of $\alpha$-TocH depletion depends on the rate of radical generation, and propagation phase starts after complete depletion of $\alpha$-TocH.

In order to demonstrate the influence of $\alpha$-TocH on the lag time and the rate of LOOH formation during the lag phase, a series of simulations with different initial concentrations of $\alpha$-TocH were performed under conditions of absence or presence of tocopheroxyl radicals (Toc·) participating in chain propagation reactions (TMP reaction). Figure 4 shows the influence of $\alpha$-TocH on the lag phase. The length of the lag phase increases as the initial concentration of $\alpha$-TocH increases. The simulated result shows that the rate of LOOH formation increases slightly during the lag phase when TMP reaction is included. Figure 5 shows the influence of TMP reaction on LOOH formation and $\alpha$-TocH consumption. Here again, slightly greater LOOH formation was observed during lag phase when TMP reaction was present.

**Lipid Peroxidation Process in Lipoprotein Particle**

For a complete kinetic picture, it is necessary to consider the lipoprotein dispersion to consist of compartments (lipoprotein particles) and to discriminate the processes within the compartments.
from between the compartments.

The schematic model of compartmental lipoprotein particle is shown in Figure 6. A lipoprotein particle is considered as a spherical particle with a diameter of 21 nm for LDL and 8 nm for HDL. As the lipid volume of a lipoprotein particle (e.g. \( V_{\text{LDL}} = 5.575 \times 10^{-24} \text{ m}^3 \)) is very small, it may be assumed that a lipoprotein particle can hold only one radical inside a particle. Then reactions 18, 19 and 29 (in Table 1) should not be occurring in a lipoprotein particle. In addition, for each species in a lipoprotein particle that is considered in the lipid peroxidation model, one should use “local concentration” for simulating the kinetic data, because it localizes reactants within a very small compartment, thereby increasing effective concentrations. Hence, the rate and extent of their reactions are increased \( \approx 400 \) fold for \([\text{LDL}]_{\text{system}} = 1 \mu \text{M}, \) compared with the same reaction occurring in a homogeneous solution. We can write the following equations for the system volume \( V_{\text{system}} \), the volume of the aqueous compartment \( V_{\text{aq}} \), and the volume of the lipid compartment \( V_{\text{lp}} \).

\[
V_{\text{system}} = V_{\text{aq}} + V_{\text{lp}} \quad (30)
\]

\[
V_{\text{lp}} = \frac{4}{3} \pi r^3 \cdot N_A \cdot [L]_{\text{system}} \cdot V_{\text{system}} \quad (31)
\]

\[
V_{\text{aq}} = \left(1 - \frac{4}{3} \pi r^3 \cdot N_A \cdot [L]_{\text{system}} \right) \cdot V_{\text{system}} \quad (32)
\]

where \( \frac{4}{3} \pi r^3 \) is the lipid volume of an LDL particle and \( N_A \) is the Avogadro’s number.

Thus, equation (31) yields the local concentration

**Abbreviations**

- PL : Phospholipid
- FC : Free Cholesterol
- CE : Cholesteryl ester
- TG : Triglyceride
- \( \alpha\)-TOH : Vitamin E
of substrate in a lipoprotein particle as

$$[X]_p = \left(\frac{4}{3}\pi r^3 \cdot N_A \cdot [\text{Lipoprotein}]_{\text{system}}\right)$$ (33)

**Azo Compound Induced Lipid Peroxidation**

This study used kinetic models of azo compound-induced lipid peroxidation to investigate the effect of location and rate of peroxyl radical (ROO•) generation (intra- or extra-particle). The setup of the kinetic model for simulated water-soluble 2,2′-azobis (2-amidinopropane) dihydrochloride and lipid-soluble 2,2′-azobis (2,4-dimethyl-valeronitrile or AMVN)-induced lipid peroxidation is based on a network of chemical reactions and location of substance in each reaction. The rate of reaction located in lipid environment will be multiplied by a factor:

$$\text{Factor} = \frac{4}{3}\pi r^3 \cdot N_A \cdot [\text{Lipoprotein}]_{\text{system}}$$ (34)

which is deduced from equation (33) above.

Figure 7 shows the influence of TMP reaction on LOOH formation and α-ToCH consumption induced by AMVN. The simulation results indicated that tocopheroxyl radical (Toc•) can drive lipid peroxidation process. Thus, the kinetic model in this study included TMP reactions in the network of lipid peroxidation reactions.

As observed in Figure 7, the level of tocopheroxyl radicals (Toc•) depended on the initial condition of α-ToCH in a lipoprotein particle. The relationship between the concentration of α-ToCH in a lipoprotein particle and kinetic profile of LOOH formation were investigated. A series of simulation results indicated that the degree of TMP depended on the initial concentrations of α-ToCH and rates of radical generation. High concentration of α-ToCH and low flux rate of radical enhance the participation of TMP reactions in lipid peroxidation process. As the tocopheroxyl radical (Toc•) can be recycled back to α-ToCH by water-soluble antioxidant, model simulation also illuminated the effect of vitamin C on the kinetic profile of LOOH formation and α-ToCH concentration. A previous study showed that vitamin C can trap only peroxyl radicals in aqueous phase. Then, vitamin C can slow down lipid peroxidation process via the decrease of the rate of initiation and recycle of tocopheroxyl radicals when peroxyl radicals are generated in aqueous phase. Vitamin C can prolong lag phase of lipid peroxidation and stop TMP reactions when vitamin C can have access to all tocopheroxyl radicals at that time.

**Copper mediated lipid peroxidation**

Initiation of lipid peroxidation by copper requires reduction of Cu²⁺ to Cu⁺ as a first step. It is unclear, however, whether this reaction occurs in the course of lipoprotein oxidation. It is also unknown which reductant, if any, can drive the reduction of Cu²⁺. Lipid hydroperoxide (LOOH) and α-ToCH can reduce copper via reactions 4 and 6 in Table 1, respectively. Increased preexisting lipid hydroperoxide concentrations enhances copper mediated lipid peroxidation. Under condition of very low preexisting concentration of lipid hydroperoxides, α-ToCH could trigger copper mediated lipid peroxidation (Figure 8). More specifically, if we compare the solid curve corresponding to an absence of α-ToCH with the other curves with α-ToCH present, we see that the level of LOOH rises more sharply in the absence of any α-ToCH once the oxidation process progresses. However, in the same Figure, we can also observe that the level of LOOH begins to rise sooner and more steeply with increasing levels of α-ToCH present. This is because, in this process initiated by copper, Cu²⁺ needs to be converted to Cu⁺ before the propagation process can proceed. This conversion or
reduction step can be made with α-TocH (equation 6 in Table 1), in the very early stages of the lag phase when no LOOH is present initially. Therefore, LOOH level rises faster with higher levels of α-TocH present. Without any α-TocH present (solid curve), the conversion of Cu^{2+} cannot go through equation 6 of Table 1 and thus takes a lot longer to initiate the peroxidation process. This is what sets apart the copper-initiated peroxidation process from that initiated by the azo compounds, which does not need the first reduction step. It clearly shows that the amount of copper and the initial level of α-TocH are the crucial factors which delineate the role of α-TocH as a pro-oxidative or anti-oxidative agent. Moreover, under conditions of non-removal of tocopheroxyl radicals by water-soluble antioxidant, α-TocH can be regenerated via reactions 14 and 15 in Table 1, which further perpetuate the process.

**DISCUSSION**

Our model yielded simulated curves, which compare well with experimental data, after taking into account of the solvent effect (reaction (II)) in the homogeneous system model, and using the correction factor in equation (34) in the compartmental system model.

We must point out here, however, that there are certain limitations to our models. In particular, our compartmental model assumes that all lipid compartments (or particles) in the system have the same composition. Moreover, initial concentrations of lipid particles have not to be too high, otherwise the overall structure of the system will be different and the compartments cannot avoid overlapping, and thus our compartmentalizing assumption is no longer valid.
Despite these confounding factors, our models still are capable of yielding insightful discoveries of a complex biochemical process. From our model construction and simulations, we found that the discriminating factors which differentiate the apparent role of vitamin E as a pro-oxidant or an antioxidant are the rate of initiation of the process, the initial concentration of vitamin E, and the pH within the microenvironment, although there has been only a few reports in the literature regarding the latter factor. Our model for copper-mediated lipid peroxidation has been able to illustrate clearly the important role that copper ions in the process play in the lipid peroxidation even in the presence of \( \alpha \)-TocH during the lag phase. Since transition metals usually are tightly bound to the binding proteins in vivo, they are not generally available to participate in the oxidative biochemistry. However, conditions of low pH reported in the study of Lamb and Leake can lead to transition metals being dislodged from the binding protein to become available for oxidative activity. Since our model clearly shows that higher levels of transition metals, specifically copper, give rise to greater lipid peroxidation in the presence of \( \alpha \)-TocH, it is therefore reasonable to deduce that in vivo, the determining condition for lipid peroxidation to occur even with \( \alpha \)-TocH present would be a low pH level in the microenvironment within the arterial wall.

In order to provide a more solid support of the above conclusion, in vivo data are necessary from clinical experiments. However, from measurements reported in the literature, clinical data collected in vivo appear to be extremely contradictory, varying drastically from one report to another, and extremely sensitive to changes in the microenvironments. In such a scenario, the important role of computer and mathematical models becomes evident as a viable tool to assist in our feasible interpolation from in vitro observations to conditions in vivo.

Earlier studies, such as in the studies of Abuja et al. and Mosinger, only included reactions 22 and 23 in Table 1, which assumed \( \alpha \)-TocH is converted into \( \alpha \)-Toc that is very inert and does not react any further. Our model, however, includes reactions 14, 15 and 30 (Table 1) as well, which allow \( \alpha \)-Toc to react with LH, LOOH or AscH and regenerate into the reduced form of vitamin E, \( \alpha \)-TocH. \( \alpha \)-TocH in turns can bind with available free radicals, continuing to act as an antioxidant.

From a total of 33 reactions in Table 1, only 10 of them were taken into account in modeling azo compound-induced lipid peroxidation, and 13 were taken into account in modeling copper ion-mediated lipid peroxidation. In deciding which reactions should be excluded, the values of the rate constants \( k \), reported in various literatures, given in Table 1, were considered. Relatively small values of \( k \) imply that the corresponding reactants are less significant and may be neglected. We left out the factors whose magnitudes are of the order \( 10^{-8} \) or less, which are significantly smaller than the other coefficients. We were thus reduced to 10 differential equations in the first model and 13 in the latter. Including more equations might improve the accuracy only slightly, but leaves the model too mathematically intractable so that analysis of the model becomes practically impossible and insightful interpretations too difficult. It should be noted that several parameters are involved. Altogether 23 reaction equations have been taken into account, requiring 23 rate constants to be specified. Since the values of these parameters have been measured by many groups of researchers, some deviations in the reported values of a parameter have been observed. The computer programme, SAAM II ver. 1.1 (RFKA, University of Washington, Seattle, WA) used in the model simulations is capable of overcoming such reservations about some of the parametric values to a certain extent. It allows us to specify the values of some of the parameters needed to simulate the equations, leaving some of them unfixed. The programme would then proceed to generate the appropriate values of the remaining parameters from given initial estimates so that the simulated curve best fits the given experimental data taken from the literature. We have experimented with different sets of reported parametric values and observed no discernable difference in the resulting simulated curves.

In fact, the rate limiting step in our model system is reaction 12 (Table 1). However, if the rate of initiation of oxidation becomes very low, reactions 14 and 15 will become more significant and become the rate limiting steps instead. This is why our model has been able to demonstrate different outcomes in the lipid peroxidation process for different initiation rates. Without the inclusion of reactions 14 and 15, as in previous studies, the models would only exhibit the same profile for all rates of initiation, as reactions 14 and 15 only come into play at very low initiation rates.

Moreover, reaction (I), which was included in our modeling of the homogeneous system, led to a better fit of the simulated curves with experimental profiles. The reactive TocH may be oxidised to its unreactive form which allows more oxidation to occur during the lag phase, The resulting simulated curve exhibits the rise in LOOH when \( \alpha \)-TocH has not completely vanished as experimentally observed.

Therefore, it is our contention that our model of
azo compound-induced lipid peroxidation consisting of the system of differential equations (6)–(15) with (II), and that of copper ion-mediated lipid peroxidation consisting of the system of differential equations (16)–(28) with (33), provide an improvement on earlier studies. Specifically, in the work of Waldeck and Stocker\textsuperscript{2}, peroxy radicals were allowed to react only with $\alpha$-TocH, while our model includes the reaction with LH also. Their model therefore cannot illustrate, as our model is able to, how the role of $\alpha$-TocH may change from being an anti-oxidant agent to a prooxidant one at different initial levels. The results reported by Abuja and Eserbauer\textsuperscript{13} are similar to those of Waldeck and Stocker\textsuperscript{2} and bear the same limitations. Finally, the studies done by Pinchuk \textit{et al.} concentrated only on the analysis of the system at equilibrium, in order to compute the rate of accumulation of LOOH. Our effort reveals deeper insights about the dynamics of the reacting components at different stages of the entire process. The possible lipid peroxidation in the presence of agents such as $\alpha$-TocH, formally thought to be an antioxidant, and the conditions under which this event can be expected, provide valuable information for clinicians in their prevention or treatment of atherosclerosis. Traditionally, $\alpha$-TocH, the most active form of vitamin E, has been considered as the major free radical antioxidant. In view of our study, prescription of $\alpha$-TocH may not invariably lead to inhibition of peroxidation. Other precautioning conditions or factors, indicated in this study, are also worthy of note. In agreement with our modeling results, recent randomized human clinical trials indicated that vitamin antioxidants may fail to retard atherosclerosis and may even have caused adverse effects\textsuperscript{4, 11, 12}.

**References**

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