Note

Kinetics for the Autoxidation of Conjugated Linoleic Acid

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The oxidation rates for conjugated linoleic acid (CLA), linoleic acid (LA), and their methyl and ethyl esters were measured in aqueous and solvent systems by the induction period method. In an aqueous system, the initiation rate was almost the same for all the samples, except for ethyl conjugated linoleate (ECL). The propagation rate, oxidizability, and kinetic chain length were different for the ester and free types of CLA and LA. CLA was the most stable, its oxidizability being half that of LA. However, ECL was very susceptible to free radical oxidation. In a solvent system, the CLA derivatives had higher or almost same oxidizability as the LA derivatives, although the propagation rates of CLA and LA were lower than those of their esters.

Key words: autoxidation; conjugated linoleic acid; kinetics; linoleic acid

Conjugated linoleic acid (CLA) is the general name for a mixture of isomers of linoleic acid (LA) with conjugated double bonds at positions 9 and 11 or 10 and 12. CLA is mainly in animal foods such as milk fat, natural and processed cheeses, and meat products.1,2 It has also been detected in human serum and bile.3 CLA is potentially valuable as a functional food and for its medicinal properties because it exhibits important physiological characteristics including anticarcinogenic, hypcholesterolemic and antiatherogenic effects.4-6 It is thus important to know the stability of CLA in order to use it in foods and medicines. In this present work, we investigate the oxidative rate of CLA in solvent and aqueous systems.

CLA and LA, and their methyl esters (MCL and ML) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A). CLA and MCL were mixtures of the following isomers: 9,11-isomer, 49%; 10,12-isomer, 51%. Ethyl esters of CLA and LA (ECL and EL) were prepared from the corresponding methyl esters by reacting with sodium ethylate in ethanol.

The oxidation rates of the CLA and LA derivatives were measured by the induction period method.7 The aqueous oxidation system used a reaction mixture consisting of 20 mM lipids, 4 mM 2,2'-azobis(2-aminopropan) dihydrochloride (AAPH) as a radical initiator, 0.4 μM α-tocopherol as a radical inhibitor, and a 0.24 M sodium deoxycholate-25 mM phosphate buffer (pH 7.5). The reaction mixture was incubated at 37°C, and the oxygen uptake was monitored by a YSI model 5300 biological oxygen monitor.

The solvent oxidation system used 5 ml of benzene containing 36 mM lipids, 25 mM 2,2'-azobis(2,4-dimethyl-valeronitrile) (AMVN) as a radical initiator and 0.4 μM α-tocopherol in a 10-ml test tube with a Teflon cap. The mixture was incubated at 37°C, and the oxygen uptake was measured as previously reported by using TCD-gas chromatography.8 Each experiment was performed in six parallel runs, and we discuss here only the kinetic data with significant differences (p<0.005).

Table 1 shows the kinetics for the oxidation of CLA and LA and their esters in the aqueous system. The initiation rate (Ri) expressed by Eq. 1 was determined by the concentration [IH] of α-tocopherol and the induction period (t_{ind}):

\[ R_i = \frac{2[\text{IH}]}{t_{\text{ind}}} \]  (1)

The measured t_{ind} values were 7200 s for CL and ML, 8400 s for CLA and EL, 7800 s for MCL, and 13200 s for ECL. Although the Ri values were almost the same for all samples, ECL had the lowest Ri value.

On the other hand, the propagation rate (R_p) expressed by Eq. 2 was evaluated by the slope of the oxygen uptake curve:

\[ R_p = k_p [\text{I}]R_i^{1/2}/(2k_i) \]  (2)

where k_p is the propagation rate constant, k_i is the termination rate constant, and [I] is the substrate concentration. Each sample gave a different R_p value. The R_p values of the CLA derivatives were in this order: ethyl ester (ECL) > methyl ester (MCL) > free (CLA), while those of the LA derivatives were in the opposite order (LA > ML > EL). CLA showed a lower R_p value than LA, while ECL showed a higher R_p value than EL. A similar pattern was also obtained when trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) was used as the radical inhibitor instead of α-tocopherol (data not shown). Acyl groups were one of the factors determining the propagation rate of CLA.

The oxidizability of CLA could be obtained by modifying Eq. 2 as follows:

\[ \text{oxidizability} = \frac{k_i}{(2k_i)^{1/2}R_i^{1/2}} = R_p/[\text{LA}]R_i^{1/2} \]  (3)

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Abbreviations: AAPH, 2,2'-azobis(2-aminopropan) dihydrochloride; AMVN, 2,2'-azobis(2,4-dimethyl-valeronitrile); CLA, conjugated linoleic acid; ECL, ethyl conjugated linoleate; EL, ethyl linoleate; LA, linoleic acid; MCL, methyl conjugated linoleate; ML, methyl linoleate; R_i, initiation rate; R_p, propagation rate; t_{ind}, induction period
Table 1. Kinetics for the Oxidation of Linoleic Acid (LA, ML and EL) and Conjugated Linoleic Acid Derivatives (CLA, MCL and ECL) in an Aqueous System

<table>
<thead>
<tr>
<th></th>
<th>( R_p ) (M/s)</th>
<th>( R_s ) (M/s)</th>
<th>Oxidizability (M/s(^{-1/2}))</th>
<th>( R_p/R_s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA</td>
<td>9.5 x 10(^{-11})</td>
<td>4.3 x 10(^{-9})</td>
<td>22.3 x 10(^{-3})</td>
<td>45.7</td>
</tr>
<tr>
<td>MCL</td>
<td>10.5 x 10(^{-11})</td>
<td>8.7 x 10(^{-9})</td>
<td>43.4 x 10(^{-3})</td>
<td>84.8</td>
</tr>
<tr>
<td>ECL</td>
<td>6.1 x 10(^{-11})</td>
<td>1.1 x 10(^{-9})</td>
<td>74.8 x 10(^{-3})</td>
<td>190.9</td>
</tr>
<tr>
<td>LA</td>
<td>11.1 x 10(^{-11})</td>
<td>10.1 x 10(^{-9})</td>
<td>48.3 x 10(^{-3})</td>
<td>92.1</td>
</tr>
<tr>
<td>ML</td>
<td>11.1 x 10(^{-11})</td>
<td>7.9 x 10(^{-9})</td>
<td>37.9 x 10(^{-3})</td>
<td>72.3</td>
</tr>
<tr>
<td>EL</td>
<td>9.5 x 10(^{-11})</td>
<td>6.5 x 10(^{-9})</td>
<td>33.5 x 10(^{-3})</td>
<td>68.5</td>
</tr>
</tbody>
</table>

\( R_p \), initiation rate; \( R_s \), propagation rate.

Table 2. Kinetics for the Oxidation of Linoleic Acid (LA, ML and EL) and Conjugated Linoleic Acid Derivatives (CLA, MCL and ECL) in a Solvent System

<table>
<thead>
<tr>
<th></th>
<th>( R_p ) (M/s)</th>
<th>( R_s ) (M/s)</th>
<th>Oxidizability (M/s(^{-1/2}))</th>
<th>( R_p/R_s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA</td>
<td>4.4 x 10(^{-11})</td>
<td>41.9 x 10(^{-11})</td>
<td>17.6 x 10(^{-4})</td>
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<tr>
<td>MCL</td>
<td>7.4 x 10(^{-11})</td>
<td>63.8 x 10(^{-11})</td>
<td>20.7 x 10(^{-4})</td>
<td>8.6</td>
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<tr>
<td>ECL</td>
<td>5.5 x 10(^{-11})</td>
<td>68.5 x 10(^{-11})</td>
<td>25.8 x 10(^{-4})</td>
<td>12.3</td>
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<tr>
<td>LA</td>
<td>7.4 x 10(^{-11})</td>
<td>31.3 x 10(^{-11})</td>
<td>10.4 x 10(^{-4})</td>
<td>4.2</td>
</tr>
<tr>
<td>ML</td>
<td>4.4 x 10(^{-11})</td>
<td>56.3 x 10(^{-11})</td>
<td>23.6 x 10(^{-4})</td>
<td>12.8</td>
</tr>
<tr>
<td>EL</td>
<td>4.4 x 10(^{-11})</td>
<td>53.7 x 10(^{-11})</td>
<td>22.6 x 10(^{-4})</td>
<td>12.1</td>
</tr>
</tbody>
</table>

\( R_p \), initiation rate; \( R_s \), propagation rate.

As shown in Table 1, CLA showed lower oxidizability than LA, while ECL showed higher oxidizability than EL. The kinetic chain length expressed by \( R_p/R_s \) means the number of times that the chain reaction was repeated by a free radical. The \( R_p/R_s \) values were 46 (CLA) to 191 (ECL), and showed similar patterns to the propagation rate and oxidizability. These results show that CLA was more stable than LA, while ECL was the most susceptible to free radical oxidation in the aqueous system. These differences in the oxidative rate should be important factors in the quality of lipid foods.

Table 2 shows the kinetics for the oxidation of CLA and LA in benzene. The \( R_s \) value differed according to the type of lipid. The \( R_s \) value of CLA was lower than that of LA, while MCL had a higher \( R_s \) value than ML. In general, the ester derivatives showed higher \( R_s \) values than the free compounds similar to aqueous system results. The \( R_p \) value of LA was lower than that of CLA. The oxidizability of LA was lower than CLA, although MCL and ECL showed almost the same values as those of ML and EL. The \( R_p/R_s \) values were 4 (LA) to 13 (ML), with LA showing the lowest \( R_p/R_s \) value.

The oxidation rates of the CLA and LA derivatives were measured in the aqueous and solvent systems by the induction period method. In the aqueous system, the initiation rate was almost the same for all the samples, except for ECL. The propagation rate, oxidizability, and kinetic chain length were different between the ester and free types of CLA and LA. CLA was the most stable, its oxidizability being half that of LA. However, acyl groups accelerated the oxidation rate, especially the propagation rate of CLA, although they reduced the oxidation rate of LA. The oxidizability values show that ECL was the most susceptible to free radical oxidation among the tested lipids in the aqueous system. On the other hand, the CLA derivatives had higher or almost the same oxidizability as the LA derivatives in benzene.

It is interesting that the propagation rate of both CLA and LA was lower than that of their respective esters in benzene. The carboxyl group may have trapped the free radicals in the solvent system. Zhang and Chen,\(^9\) and van den Berg et al.\(^{10}\) have measured the decomposition rates of CLA and LA during autoxidation in a bulk system, and reported that CLA was oxidized faster than LA. Our results are different from theirs. CLA was not always oxidized faster than LA. In the aqueous system, CLA was more stable to free radical oxidation than LA. The propagation rates were different according to the types of CLA and LA derivatives, although the initiation rates were almost same in the aqueous system. These observations suggested that the intermolecular distance for fatty acids associated with the micellar conformation might be different between the CLA and LA derivatives: i.e. the longer intermolecular distance of CLA might result in lower propagation rate and oxidizability than those of LA in the aqueous system. On the contrary, ECL seemed to be susceptible to free radical oxidation due to its short intermolecular distance in aqueous system. We have demonstrated in this study that the oxidation rate of CLA could be affected by acyl groups and the type of oxidation system. Our observations suggest that the oxidative stability of CLA could be modified by the lipid structure and storage conditions.

References