Inhibition of Rat Liver Microsomal Desaturases by Curcumin and Related Compounds

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Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) inhibited noncompetitively rat liver microsomal Δ5 desaturase (Kᵢ = 36 μM) and Δ6 desaturase (Kᵢ = 28 μM). Although curcumin has a symmetrical structure with a methylene group as the center, only half the structure is essential for the desaturase inhibition. The structure necessary for the inhibition is similar to that of alkyl gallate (H. Kawashima et al., Biochim. Biophys. Acta, in press), i.e., a 3-hydroxy group of the aromatic ring is essential for the inhibition and a free carboxyl group at the end opposite to the aromatic ring interferes with the inhibitory effect. The following structural features of curcumin are necessary for the desaturase inhibition: (i) the aromatic ring conjugated with the double bond between the 1 and 2 (or 6 and 7) positions; (ii) both 4-hydroxy and 3-methoxy groups (for both desaturase inhibitions); and (iii) only a 4-hydroxy group (for Δ6 desaturase inhibition).

Key words: curcumin; desaturase; Mortierella

During a study on the biosynthesis of C₂₀ polyunsaturated fatty acids by Mortierella fungi,¹⁰ we found three types of inhibitors of fatty acid desaturases. These desaturase inhibitors show unique effects on polyunsaturated fatty acid metabolism. For example, they can be used for the microbial production of dihomo-γ-linolenic acid (20 : 3), preventing the conversion of 20 : 3 to arachidonic acid (20 : 4), and the fatty acid compositions of animal tissues, especially the ratio of 20 : 3 and 20 : 4, change on their intake.¹⁰

The first type is lignan compounds in sesame seeds or oil, i.e., (+)-sesamin, (+)-episesamin, (+)-sesaminol, and sesamolin, which are specific inhibitors of Δ5 desaturase, which catalyzes the conversion of 20 : 3 to 20 : 4 in both fungal and rat liver microsomes.³⁵,⁵⁰ This is the first demonstration that specific inhibitors of Δ5 desaturase occur in nature. It has been found that sesamin and related compounds inhibit Δ5 desaturase noncompetitively, the Kᵢ values being in the range of 1.6–7.1 × 10⁻⁴ M.³⁵,⁵⁰ The second type is alkyl gallate, which is known as an antioxidant. Alkyl gallate is different from sesamin-like compounds in inhibiting not only Δ5 desaturase but also Δ6 desaturase.¹¹ We found that propyl gallate is a noncompetitive inhibitor with the Kᵢ values of which for Δ5 and Δ6 desaturases are 2.6 × 10⁻³ and 1.7 × 10⁻⁴ M, respectively, and that alkyl gallate requires the following structural features for the inhibition: (i) the aromatic ring carries a hydroxyl group at the metha-position and (ii) the carboxyl group is esterified with an alcohol of an appropriate carbon chain length. The third type is curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), a major component of the yellow spice, turmeric. It shows an inhibitory effect on Δ5 desaturase and a weak one on Δ6 desaturase.¹² It also shows antioxidant activity,¹³ inflammatory activity,¹⁴,¹⁵ and inhibitory effects on mammalian 5-lipoxygenase and cytochrome P450.¹⁶ Investigation of the inhibitory mechanism for the desaturase reaction may provide useful information on the relationship between the various interesting biological activities of curcumin. In this paper, we report kinetic analyses of the desaturase inhibition by curcumin, and show the necessary structures of curcumin and related compounds for the desaturase inhibition.

Materials and Methods

Chemicals. Curcumin was purchased from Wako Pure Chemicals (Osaka, Japan). [2⁻¹³C]Dihomo-γ-linolenic acid (20 : 3) (2.13 GBq mmol) and [1⁻¹³C]linoleic acid (18 : 2) (1.89 GBq mmol) were purchased from Amersham (Buckinghamshire, U.K.). All other reagents were of analytical grade.

Preparation of rat liver microsomes and assay of desaturase activity. Microsomes were prepared from male Wistar rats maintained on a standard pellet diet for 8 weeks as described previously.⁵¹ Protein was measured by the method of Bradford.¹⁷ The activity of the microsomal desaturase was measured using a labelled fatty acid (20:3 for Δ5 desaturase and 18:2 for Δ6 desaturase) as a substrate.⁴¹ Detection of the formed and resultant fatty acids was described previously.²⁻³⁻⁵⁻⁶⁻⁷⁻¹¹

Results and Discussion

Inhibition of Δ5 and Δ6 desaturases by curcumin

Double reciprocal plots of reaction velocity against 20 : 3 concentration gave convergent lines that intersected at the abscissa for various curcumin concentrations, indicating that the inhibition of rat liver microsomal Δ5 desaturase by curcumin is noncompetitive (Fig.), like those by sesamin-like compounds or alkyl gallate.⁵⁻⁵⁻¹¹ The calculated Kᵢ value for Δ5 desaturase was 36 μM, which is almost as much as that of propyl gallate (26 μM)¹¹ but smaller than that of sesamin (155 μM).³⁻⁵⁻⁷⁻¹¹ Δ6 desaturase was also inhibited noncompetitively: the Kᵢ for Δ6 desaturase was 28 μM, which is smaller than that of propyl gallate (170 μM).¹¹

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Abbreviations: 20:3, dihomo-γ-linolenic acid; 20:4, arachidonic acid; 18:2, linoleic acid.
**Table I. Inhibitory Effects of Curcumin and Related Compounds on Rat Liver Microsomal 15 and 16 Desaturases**

<table>
<thead>
<tr>
<th>R</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH=CH C CH₂ O CH=CH-OCH₃</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>CH=CH C CH₃ O</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>CH=CH C O CH₃ O</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>CH=CH C CH₂ OH</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>CH=CH C OH</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>C O CH₃</td>
<td>0</td>
</tr>
</tbody>
</table>

\*15 and 16 desaturase activities were measured in the presence of 0.1 mM of a compound as indicated. The 15 and 16 desaturase activities without inhibitors were 0.371 and 0.188 nmol min mg of microsomal protein, respectively.

**Table II. Inhibitory Effects of Ferulic Acid Methyl Ester and Related Compounds on Rat Liver Microsomal 15 and 16 Desaturases**

<table>
<thead>
<tr>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>Desaturase inhibition (*%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>OH</td>
<td>OCH₃</td>
<td></td>
<td>36 85</td>
</tr>
<tr>
<td>7</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td>90 &gt;98</td>
</tr>
<tr>
<td>8</td>
<td>OCH₃</td>
<td>OH</td>
<td></td>
<td>0 0</td>
</tr>
<tr>
<td>9</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td></td>
<td>0 0</td>
</tr>
<tr>
<td>10</td>
<td>H</td>
<td>OH</td>
<td></td>
<td>20 41</td>
</tr>
<tr>
<td>11</td>
<td>OH</td>
<td>H</td>
<td></td>
<td>0 &gt;98</td>
</tr>
<tr>
<td>12</td>
<td>H</td>
<td>H</td>
<td></td>
<td>0 0</td>
</tr>
<tr>
<td>13</td>
<td>O CH₃</td>
<td>O</td>
<td></td>
<td>0 0</td>
</tr>
</tbody>
</table>

\*15 and 16 desaturase activities were measured in the presence of 0.1 mM of a compound as indicated. The 15 and 16 desaturase activities without inhibitors are given in Table I.

We showed a sufficient inhibitory effect, we used methyl ferulate (3) and its derivatives for analysis of the effects of hydroxy and methoxy groups. As for alkyl gallate, we showed previously that the 3-hydroxy group of the aromatic ring is essential for the inhibition of both 15 and 16 desaturases, and that the presence of a methoxy group interferes with the inhibitory effects. Although 3 has an aromatic ring with hydroxy group(s) and a tail with a carboxyl group esterified with a methyl group, like alkyl gallate, 3 and the other curcumin-like inhibitors, having a methoxy group and no m-hydroxy group, do not fit the rule described above. Therefore, curcumin-like inhibitors seem quite different from alkyl gallate-like inhibitors. Among the tested compounds, methyl cinnamate (12) had no inhibitory effect on either desaturase, showing that the hydroxy and methoxy groups on the aromatic ring are necessary for the desaturase inhibition (Table II). Methyl 3,4-dihydroxycinnamate (7) showed great inhibitory effects (>90% inhibition) on both desaturases. Methyl 3-hydroxycinnamate (m-coumarate) (10) also inhibited both desaturases.
Interestingly, methyl 4-hydroxycinnamate (methyl p-coumarate) (11) showed an inhibitory effect on only $\Delta 6$ desaturase (>$98\%$ inhibition), i.e., not on $\Delta 5$ desaturase. This is the first demonstration of a specific inhibitor of $\Delta 6$ desaturase. On the other hand, methyl 3-hydroxy-4-methoxyxycinnamate (8), methyl 3,4-dimethoxyxycinnamate (9), and methyl 3,4-methylenedioxyxycinnamate (13) showed no inhibitory effects. 13 did not inhibit them, though it has a methylenedioxyphenyl group, which is a part of sesamin. This is similar to the case that 3,4-methylenedioxybenzoic acid methyl ester did not inhibit them. 11

As for $\Delta 5$ desaturase inhibition, 3, 7, and 10 showed inhibitory effects. Of the three compounds, 7 and 10 have a 3-hydroxy group and no methoxy group. On the other hand, 8, 9, 11, 12, and 13, which have methoxy group(s) or do not have a 3-hydroxy group, showed no inhibition. These suggest that the same rule for $\Delta 5$ desaturase inhibition found with alkyl gallate can be adopted for 7–13. Only 3 does not fit the rule and has no common feature in its structure, suggesting that the combination of the 4-hydroxy group, the 3-methoxy group, and the aromatic ring conjugated with the next double bond is essential for $\Delta 5$ desaturase inhibition.

3, 7, and 10 inhibited $\Delta 6$ desaturase as well as $\Delta 5$ desaturase. 11 is unique and does not fit the rule described above, because it has no 3-hydroxy group but shows a specific inhibitory effect on $\Delta 6$ desaturase. 11 has a 4-hydroxy group, like 3, suggesting that a 4-hydroxy group is necessary for $\Delta 6$ desaturase inhibition. As for alkyl gallate-type compounds, however, methyl 4-hydroxybenzoate showed no inhibitory effect on $\Delta 6$ desaturase. 11 Therefore, the combination of a 4-hydroxy group and some characteristic structure of curcumin-like compounds, for example, the presence of the aromatic ring conjugated with the next double bond, seems essential for $\Delta 6$ desaturase inhibition.

In conclusion, the desaturase inhibition by methyl ferulate derivatives as well as alkyl gallate almost always obeys this rule, i.e., a 3-hydroxy group is essential and a free carboxyl group rules out the inhibitory effect. In the case of curcumin-like compounds, the aromatic ring conjugated with the next double bond is essential for the inhibition. It was also demonstrated that the introduction of both 4-hydroxy and 3-methoxy groups leads to $\Delta 5$ desaturase inhibition and that only a 4-hydroxy group is necessary for $\Delta 6$ desaturase inhibition. These findings may be useful for discovering the mechanisms of $\Delta 5$ and $\Delta 6$ desaturation and the relationship between the desaturase inhibition and the other biological activities of curcumin.

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References