Action of Curcumin as an Antioxidant against Lipid Peroxidation

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The antioxidant activity of curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, a natural antioxidant obtained from rhizome of Curcuma longa L., and related compounds was measured against lipid peroxidation in various media. Curcumin reacted with stable radicals such as galvinoxyl and N,N-diphenyl-1-picrylhydrazyl, indicating it to possibly serve as a potent hydrogen donor, but no curcumin radical could be observed directly by electron spin resonance spectroscopy. Neither could the radical be spin trapped with α-(4-pyridyl-1-oxide)-N-t-butylnitrone. Curcumin suppressed the oxidation of methyl linoleate in organic homogeneous solution and aqueous emulsions, soybean phosphatidylcholine liposomal membranes, and rat liver homogenate induced by free radicals. Curcumin was a stronger antioxidant than 2,6-di-t-butyl-4-methylphenol, Comparable to isoeugenol, but weaker than 3,4-dihydroxybenzoic acid and 2-t-butyl-4-methoxyphenol.

1 Introduction

As the experimental and epidemiological evidence suggesting the involvement of free radical-mediated oxidations of lipids in a variety of diseases, cancer and aging has been accumulated1,2, the role of antioxidants has received increasing attention. Numerous types of antioxidants with different functions are known3,4. Among others, the antioxidants in foods have received much attention in connection with disease prevention and novel, natural antioxidative substances have been explored and found in the plant materials. Curcumin 1, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, the main coloring constituent of the rhizomes of the perennial plant Curcuma longa L. (turmeric), has been found to act as an antioxidant5,6,7,8). It has also been reported to exhibit anti-inflammatory9) and anti-thrombotic properties10). It has also been found to inhibit the mutagenicity of benz[a]pyrene and 7,12-dimethylbenz[a]anthracene11) and also to inhibit tumor promotion12).

From its structure, it is not surprising that curcumin acts as an antioxidant. However, it has been also observed that, under certain conditions, curcumin accelerates oxygen radical formation by reducing ferric ion to give more active iron(II) ion13,14. The antioxidant activity of curcumin, especially the chemical reactivity toward peroxyl radical, has not been evaluated quantitatively, and the present study has been undertaken to assess the activity of curcumin as a radical-scavenging antioxidant against lipid peroxidation in a model system which is suitable for kinetic study.

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Abbreviations used : AAPH : 2,2′-azobis(2-amidinopropane) dihydrochloride ; AMVN : 2,2′-azobis (2,4-dimethylvaleronitrile) ; ESR : electron spin resonance ; DPPH : N,N-diphenyl-1-picrylhydrazyl ; POBN : α-(4-pyridyl-1-oxide)-N-t-butylnitrone ; PC : phosphatidylcholine ; HPLC : high performance liquid chromatography ; PMC : 2,2,5,7,8-pentamethyl-6-chromanol ; EDTA : ethylenediaminetetraacetic acid ; TBARS : thiobarbituric acid reactive substances
2 Materials and Methods

Figure-1 shows the antioxidants used in this study. Curcumin was purchased from Sigma Chemical (St. Louis, MO). (2R, 4'R, 8'R)-α-Tocopherol and 2,2,5,7,8-pentamethyl-6-chromanol (PMC) were kindly supplied from Eisai Co. Ltd. (Tokyo, Japan). Other antioxidants were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Methyl linoleate and soybean phosphatidylcholine (PC) were chosen as substrates. These compounds are most suitable as oxidizable lipids for kinetic study since they give conjugated diene hydroperoxides quantitatively\(^{19-21}\), which makes it possible and easy to follow the extent of oxidation and evaluate the antioxidant activity accurately. Commercial methyl linoleate and soybean PC obtained from Sigma were used after purification with column chromatography as reported previously\(^{22}\). 2,2'-Azo-bis(2,4-dimethylvaleronitrile) (AMVN) and 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) used as lipophilic and hydrophilic radical initiators, respectively, were supplied from Wako Pure Chemical (Osaka, Japan). It has been shown previously that the use of these azo radical initiators enables us to measure the antioxidant activities of various compounds quantitatively\(^{23}\).

The oxidation of methyl linoleate in homogeneous solution was performed in acetonitrile at 37°C in air. The free radical-mediated oxidation of methyl linoleate gives four kinds of isomeric, conjugated diene hydroperoxides\(^{19-21}\). They were analyzed by a high performance liquid chromatography (HPLC) from their absorption at 234 nm. The LC-18 column (Supelco, 25 cm) was used. A mixture of methanol/t-butyl alcohol/40 mM phosphate buffer (60:30:10 by volume) was used as an eluent at a flow rate of 1.0 mL per min. The rate of oxidation of methyl linoleate micelles in aqueous dispersions was measured by following the consumption of oxygen with an oxygen electrode. The oxidation of soybean PC liposomes was measured similarly by following the formation of PC hydroperoxides at 234 nm with an HPLC using LC-Si column (Supelco, 25 cm) and methanol/40 mM phosphate buffer (90:10 by volume) as an eluent at a flow rate of 1.0 mL per min.

The oxidation of rat liver homogenate was performed in 0.1 M sodium phosphate buffer
(pH 7.4) at 37°C under air in the absence or presence of 1 mM EDTA. The stock methanol solution of curcumin (4 mM) was added to 2.5% homogenate suspension (final curcumin concentration was 40 μM) before starting the oxidation with the addition of (final concentration 10 mM) AAPH. The extent of the oxidation was measured by the formation of the thiobarbituric acid reactive substances (TBARS) spectrophotometrically as described in the literature24). Freshly diluted tetraethoxypropane was used as a standard and results were expressed as μM of malonaldehyde equivalents.

The consumption of curcumin and PMC was followed with an HPLC equipped with an electrochemical detector using LC-18 column (Supelco, 25 cm and methanol/water (80:20 by volume) containing 50 mM NaClO₄ as an eluent at a flow rate of 1.0 mL/min. The consumption of curcumin was also followed spectrophotometrically at 417 nm.

The ESR spectra were recorded on an X-band JEOL-FE 1 X spectrometer with the following conditions: magnetic field, 329 ±5 mT; sweep time, 8 mT per min; microwave power, 1 mW; modulation frequency, 100 kHz; and modulation amplitude, 0.02 mT.

3 Results

3.1 Curcumin as a hydrogen donor as studied by ESR

It is known that the potent radical-scavenging antioxidants are known to react rapidly with the stable radicals, galvinoxyl and N,N-diphenyl-1-pirclyhydrazyl (DPPH)25). The interaction of curcumin with these radicals can be followed with ESR. When a methanol solution of curcumin (5 mM) was mixed with an equal amount of a methanol solution containing 4.5 mM galvinoxyl, the ESR spectrum of galvinoxyl was reduced considerably. When the concentrations of curcumin and galvinoxyl was 5 mM and 0.45 mM respectively, the ESR spectrum of galvinoxyl totally disappeared in approximately 30 min. No new ESR spectrum was observed. When solution of curcumin was mixed with an equal concentration of DPPH, the ESR spectrum of DPPH disappeared rapidly. Again, no new ESR spectrum was observed. Similarly, no ESR signal was observed when curcumin reacted with DPPH in methanol in the presence of a spin trap, POBN. These results suggest that curcumin has an active hydrogen atom and that the curcumin radical is too unstable to be observed directly and does not readily form a stable spin adduct. The hindered phenols having substituents at both ortho-positions, such as α-tocopherol and 2,6-di-t-butyl-4-methylphenol give stable phenoxyl radicals which have been observed directly by ESR26). The apparent instability of the phenoxyl radical derived from curcumin may be because one of the ortho-positions is not substituted.

3.2 Inhibition of oxidation of methyl linoleate in acetonitrile solution by curcumin

The results of oxidations of methyl linoleate in acetonitrile induced by AMVN in the absence and presence of curcumin are shown in Fig.-2. This system is the most suitable for measuring chemical reactivity of the antioxidant toward free radicals. The rate of oxidation was measured by following the accumulation of methyl linoleate hydroperoxide. Curcumin retarded the oxidation in a dose-dependent manner, but it did not produce a clear induction period.

Curcumin was consumed by the radicals generated from AMVN (Fig.-3). The rate
of consumption of curcumin increased with an increasing concentration of AMVN, corresponding to an increasing rate of radical flux. In the absence of radical initiator, curcumin was not consumed appreciably. The consumption of curcumin and 2,2,5,7,8-pentamethyl-6-chromanol (PMC) induced by free radicals when they were present together is shown in Fig.-4. PMC was consumed predominantly at first while curcumin was spared and then consumed after PMC was substantially depleted.

In Figure 5 and Table 1, the antioxidant activities of curcumin, its related compounds and α-tocopherol (vitamin E) against the oxidation of methyl linoleate in acetonitrile are compared. Curcumin was a stronger antioxidant than 2,6-di-t-butyl-4-methylphenol, as potent as isoeugenol, but weaker than 3,4-dihydroxycinnamic acid and 2-t-butyl-4-methoxyphenol.

These results shown in Figs.-2～5 and Table 1 show that curcumin acts as an antioxidant by scavenging chain-initiating radicals derived from AMVN and/or chain-

Table 1 Relative antioxidant activities of curcumin and related compounds against peroxidation of methyl linoleate in acetonitrile induced by AMVN at 37°C in air.

<table>
<thead>
<tr>
<th>Antioxidant [IH], μM</th>
<th>5</th>
<th>10</th>
<th>50</th>
</tr>
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<tbody>
<tr>
<td>Curcumin</td>
<td>0.556</td>
<td>0.371</td>
<td>0.104</td>
</tr>
<tr>
<td>4-Hydroxy-3-methoxycinnamic acid</td>
<td>0.784</td>
<td>0.666</td>
<td>0.207</td>
</tr>
<tr>
<td>3,4-Dihydroxycinnamic acid</td>
<td>0.490</td>
<td>0.281</td>
<td>0.059</td>
</tr>
<tr>
<td>4-Hydroxyxycinnamic acid</td>
<td>0.968</td>
<td>0.877</td>
<td>0.689</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>0.571</td>
<td>0.357</td>
<td>0.091</td>
</tr>
<tr>
<td>2-t-Butyl-4-methoxyphenol</td>
<td>0.249</td>
<td>0.144</td>
<td>0.034</td>
</tr>
<tr>
<td>2,6-Di-t-butyl-4-methylphenol</td>
<td>0.622</td>
<td>0.440</td>
<td>0.115</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>0.023</td>
<td>0.011</td>
<td></td>
</tr>
</tbody>
</table>

Note: Methyl linoleate (453 mM) was oxidized in acetonitrile at 37°C in air in the presence of 0.20 mM AMVN and antioxidant and the accumulation of methyl linoleate hydroperoxides was followed. The rates of formation of hydroperoxides were measured in the presence and absence of antioxidant, \( R_{\text{inh}} \) and \( R_{o} \), respectively, and their ratio was calculated.
carrying lipid peroxyl radicals, but that its antioxidant activity is much smaller than that of \( \alpha \)-tocopherol.

### 3.3 Inhibition of oxidation of methyl linoleate micelles by curcumin

The results of oxidations of methyl linoleate micelles in aqueous dispersions induced by AMVN in the absence and presence of curcumin are shown in Fig.-6. The oxidation inhibited by \( \alpha \)-tocopherol is also included for comparison. Curcumin retarded the oxidation dose-dependently, but its antioxidant activity was much smaller than \( \alpha \)-tocopherol and it did not produce clear induction period.

### 3.4 Inhibition of oxidation of soybean PC liposomes by curcumin

The results of oxidations of soybean PC multilamellar liposomal membranes induced by AMVN in the absence and presence of curcumin are shown (Fig.-7). Curcumin retarded the oxidation dose-dependently, but it did not give clear induction period.

### 3.5 Inhibition of oxidation of rat liver homogenate by curcumin

AAPH induced the oxidation of rat liver homogenate in phosphate buffer suspensions. As shown in Fig.-8, a faster rate of oxidation was observed in the absence of EDTA than in its presence, probably because of the sequestration of metal ions by EDTA. Curcumin suppressed the oxidations of liver homogenate in the presence and absence of EDTA.

### 4 Discussion

The above results clearly show that curcumin acts as a hydrogen donor and suppresses the oxidations of lipids in various systems, although it is not clear at present
whether the initial step is a direct hydrogen atom transfer or an electron transfer, followed by proton transfer. The direct ESR analysis and spin trapping study show that the radical derived from curcumin is short-lived and unstable. Similar observations have been reported recently by Scharich\textsuperscript{12).}

The phenoxyl radical and carbon-centered radical at keto-enol double bond are postulated\textsuperscript{12).}

The results in Fig.-2, 6 and 7 show that curcumin retarded the oxidations of lipids but that, unlike \( \alpha \)-tocopherol, it did not produce clear induction period. This is apparently because curcumin is not reactive enough toward peroxyl radical to scavenge peroxyl radicals efficiently and inhibit the oxidation. Figures-3 and 4 also support this conclusion. The rates of consumption of curcumin induced by 1 mM and 5 mM AMVN were calculated from the data in Fig.-3 as 1.36 \( \times 10^{-10} \) M/s and 3.92 \( \times 10^{-10} \) M/s, respectively, showing that, although the rate of consumption of curcumin increased with increasing AMVN, it was not directly proportional to AMVN concentration. This suggests that some peroxyl radicals derived from AMVN were not scavenged by curcumin but terminated by their mutual interactions. Figure-4 shows that PMC was consumed at a rate 3 times faster than that for curcumin. This is explained by incomplete scavenging of radicals by curcumin and/or larger stoichiometric number of radicals trapped by each curcumin molecule than that by PMC.

The above results and discussion show that curcumin is not active enough to scavenge radicals efficiently before the radicals attack lipids and/or react with other radicals. Under these circumstances, the absolute rate constant for scavenging of peroxy radical by curcumin can not be measured from kinetic analysis\textsuperscript{27\textendash}30).

On the other hand, the ratio of the rate of oxidation inhibited by antioxidant to that of uninhibited oxidation (\( R_{inh}/R_o \)) gives the relative antioxidant potency, that is, how much does the antioxidant reduce the rate of oxidation. It depends on reaction conditions such as antioxidant concentration and rate of chain initiation. Table-1 shows, although semiquantitatively, that the antioxidant activity decreases in the order of \( \alpha \)-tocopherol \( \approx \) 2-t-butyl-4-methoxyphenol \( > \) 3,4-dihydroxycinnamic acid \( > \) curcumin \( \approx \) isoeugenol \( > \) butylated 2,6-di-t-butyl-4-methylphenol \( > \) 4-hydroxy-3-methoxycinnamic acid \( > \) 4-hydroxycinnamic acid.

In conclusion, curcumin can act as a radical-scavenging antioxidant with modest reactivity. The antioxidant properties of curcumin may explain its reported medicinal activity. Furthermore, curcumin and related compounds may be an important component in food and play a vital role in vivo as natural, safe antioxidant.

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脂質酸化に対するクルクミンの酸化防止作用

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種々の脂質酸化系におけるクルクミンと関連化合物の酸化防止活性を測定した。クルクミンはガルビノケシル、DPPH に対反応し、水素供与体として作用することが確認されたが、クルクミン由来のラジカルは直接、あるいはスピントラップ剤存在下でも認められなかった。クルクミンは、リノール酸メチルの均一溶液中においては水懸濁液中での酸化。大豆ホスファチジルコレリンリポソーム膜、およびラット肝ホモジネートのラジカル酸化反応を抑制した。その抑制効果は 2,6-ジトールチル-4-メチルフェノールより大きく、3,4-ジヒドロキシケイ皮酸、2-トールチル-4-メチルフェノールより劣り、α-トコフェロールに比べると明らかに小さかった。

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