Structure-Dependent Photodegradation of Carotenoids Accelerated by Dimethyl Tetrasulfide under UVA Irradiation

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Carotenoids are used in wide-ranging food applications, but they are susceptible to degradation by many factors including light. We examined the photodegradation of five kinds of carotenoids and three kinds of anthocyanins to clarify which structures of pigments were favorable to accelerated degradation by sulfides under UVA irradiation. Under UVA irradiation, crocetin and crocin were decomposed more rapidly in the presence of dimethyl tetrasulfide than in the absence of the sulfide, but not as rapidly as β-carotene, zeaxanthin and β-cryptoxanthin were. However, cyanidin was decomposed more slowly in the presence of sulfide than in the absence of sulfide. Moreover, the photodegradation of kuromanin and keracyanin was not affected by the addition of a sulfide. We also examined the mechanism for this accelerated degradation. Normal hexane was more favorable to the photodegradation of β-carotene than methanol and ethanol. The accelerated degradation was inhibited by free radical scavengers, but enhanced by the addition of deuterium oxide. These results suggest that conjugated double bonds were favorable to the accelerated photodegradation by sulfide and that this reaction was mediated by free radicals.

Key words: carotenoid; anthocyanin; dimethyl tetrasulfide; photodegradation; UVA

Carotenoids are highly colored groups of fat-soluble plant pigments, and notable for their wide distribution, structural diversity, and various functions. The use of carotenoids requires a detailed knowledge of their stability with respect to possible degradation processes in order to optimize food packaging and storage. It has been widely reported that carotenoids are susceptible to light, heat and oxygen.1,2 However, the influence of the presence of other functional food additives on the direct or sensitized photobleaching of carotenoids has not been fully investigated.

Sulfides are characteristic flavor components abundant in plants of the Allium family. The major organosulfur breakdown products contain monosulfides, disulfides, trisulfides, and tetrasulfides.3) Epidemiological evidence has indicated these organosulfur compounds to possess many health-related biological effects such as antioxidative, antithrombotic, antihypertensive, and chemopreventive properties.4,5) Most of these studies have been focused on the functional effects of alkenyl sulfides, but little is known about the effects of dimethyl sulfides, but little is known about the effects of dimethyl tetrasulfide (Me₄S₄). Our previous report indicated Me₄S₄ to be one of the most potent accelerators for the photodegradation of carotenoids.6) Therefore, we used Me₄S₄ to investigate the effects of the pigment structure and free radical scavengers on the acceleration of photodegradation in this study.

Carotenoids have accessory functions by which they serve as light harvesting antennae in photosynthetic systems and play a protective role from photodamage by dissipating excess light.7) Carotenoid degradation in processed foods has been attributed to oxidation and subsequent fragmentation of the carotenoid molecule8) which are often linked to oxidative degradation of co-existing components such as unsaturated lipids.9) The common mechanism and kinetics in the oxidation of carotenoids have been described as free-radical reactions.10) The structure of carotenoids breaks down under attack by free radicals such as singlet molecular oxygen and other reactive species.

Our previous report has indicated the significant effect of irradiation by γ rays or UVA on the stability of functional food components, including carotenoids and their derivatives, in liposome model systems.11,12) Our previous study found that sulfides had an accelerative effect on the degradation of carotenoids under UVA irradiation depending on the number of sulfur atoms.5) In this study, we used dimethyl tetrasulfide (Me₄S₄) as a photodegradation accelerant to further investigate the photodegradation of carotenoids. Anthocyanin pig-

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Abbreviations: D₂O, deuterium oxide; DTT, dithiothreitol; Me₄S₄, dimethyl tetrasulfide
ments were selected for structural comparison in order to elucidate the importance of the carotenoid structure in its photodegradation accelerated by Me$_2$S$_4$ under UVA irradiation. In addition, the effects of solvent, free radical scavenger and deuterium oxide (D$_2$O), an enhancer of the half-life of $^{1}$O$_2$, on the accelerated photodegradation of carotenoids were studied to clarify the mechanism. The structures of the carotenoids and anthocyanins used in this study are shown in Fig. 1.

**Materials and Methods**

*Materials.* Beta-carotene, NaN$_3$ and thiourea were obtained from Nacalai Tesque (Kyoto, Japan). Beta-
cryptoxanthin and zeaxanthin were purchased from Extrasynthese (Genay, France), and crocetin, crocin, cyanidin chloride, kuromanin chloride, keracyanin chloride and dithiothreitol were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dimethyl tetrasulfide and dithiothreitol were purchased from Wako Pure Chemical Industries (Osaka, Japan). The solvents used in this study were all of analytical grade.

Photodegradation of carotenoids and anthocyanins. Each carotenoid was dissolved in methanol, ethanol or n-hexane, and dimethyl tetrasulfide in ethanol was added to the carotenoid solution. The solution was then irradiated in a screw-capped quartz cell at 25 °C by a UVA lamp (FL20SBLB, National, Tokyo, Japan) with a light intensity of 5.91 W/m² as previously described. Each carotenoid was irradiated at an initial concentration of 3 μM for β-carotene, β-cryptoxanthin, zeaxanthin and crocetin, and at 1.2 μM for crocin in the presence or absence of dimethyl tetrasulfide (Me₂S₄, 10 μM).

—The photodegradation did not fit the first-order kinetic model when the residual concentration of a carotenoid was higher than 95% after 30 min of irradiation, and the relative coefficient was less than 0.90.

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Solvent</th>
<th>Me₂S₄ (+)</th>
<th>Me₂S₄ (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>k (min⁻¹)</td>
<td>Relative coefficient</td>
</tr>
<tr>
<td>β-carotene</td>
<td>Ethanol</td>
<td>0.0117</td>
<td>R² = 0.9929</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.0491</td>
<td>R² = 0.9963</td>
</tr>
<tr>
<td></td>
<td>n-Hexane</td>
<td>0.0804</td>
<td>R² = 0.9999</td>
</tr>
<tr>
<td>β-cryptoxanthin</td>
<td>Ethanol</td>
<td>0.0156</td>
<td>R² = 0.9720</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.0531</td>
<td>R² = 0.9975</td>
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<tr>
<td></td>
<td>n-Hexane</td>
<td>0.1184</td>
<td>R² = 0.9953</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>Ethanol</td>
<td>0.0167</td>
<td>R² = 0.9732</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.0605</td>
<td>R² = 0.9998</td>
</tr>
<tr>
<td></td>
<td>n-Hexane</td>
<td>0.1207</td>
<td>R² = 0.9904</td>
</tr>
<tr>
<td>Crocin</td>
<td>Ethanol</td>
<td>0.0068</td>
<td>R² = 0.9988</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.0273</td>
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</tr>
<tr>
<td>Crocetin</td>
<td>Ethanol</td>
<td>0.0081</td>
<td>R² = 0.9988</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.0361</td>
<td>R² = 0.9991</td>
</tr>
</tbody>
</table>

Each value was calculated from six experiments.

In order to examine whether free radicals were involved in the accelerated photodegradation of carotenoids, 10 mM of Na₂S, a singlet oxygen and hydroxyl radical scavenger, 10% D₂O, a chemical to extend the lifetime of singlet oxygen, 0.2 mM of thiourea, a hydroxyl radical (·OH) scavenger, or 10 mM of DTT, a free radical scavenger, was added to a solution containing 3 μM of β-carotene in the presence of 5 μM or 10 μM of dimethyl tetrasulfide. The absorbance of the solution at 450 nm was measured after 20, 40 and 60 min of UVA irradiation.

Differences were examined by means of Student’s t-test, and probability values of less than 1% were considered significant.

Results

Photodegradation of carotenoids accelerated by dimethyl tetrasulfide under UVA irradiation

We demonstrated that sulfides accelerated the photodegradation of β-carotene, β-cryptoxanthin and zeaxanthin by UVA irradiation and that a larger number of sulfur atoms in the coexisting sulfides was more favorable to the accelerative effect of the sulfide. We investigated the effect of dimethyl tetrasulfide (Me₂S₄) on the photodegradation of other carotenoids by UVA irradiation and the effect of the solvent on the accelerated photodegradation of carotenoids by Me₂S₄. The time course of the relative value of the residual concentration to the initial concentration of pigment was fitted to the first-order kinetic model, R(t) = R(0) exp(−kt), to calculate the rate constant, k, where R(t) is the relative concentration of pigment at irradiation time t as previously described. The rate constants of photodegradation are summarized in Table 1. Carotenoids in
Accelerated Photodegradation of Carotenoids by Me$_2$S$_4$

In ethanol, methanol or n-hexane were irradiated with UVA in the presence or absence of Me$_2$S$_4$. Note that β-cryptoxanthin, zeaxanthin, crocetin and crocin in ethanol or methanol were not decomposed by UVA irradiation in the absence of Me$_2$S$_4$.

In ethanol, the photodegradation of β-carotene, β-cryptoxanthin and zeaxanthin in the absence of Me$_2$S$_4$ was much slower than that in the presence of Me$_2$S$_4$ under UVA irradiation (Table 1), this being consistent with our previous result. In methanol, a representative spectrum for β-carotene photodegradation accelerated by Me$_2$S$_4$ under UVA irradiation is shown in Fig. 2A. The spectral patterns for β-carotene solutions with or without Me$_2$S$_4$ were very similar, but with an obvious difference in the absorbance at 450 nm after 60 min of UVA irradiation. Dimethyl tetrasulfide significantly decreased the relative residual concentration of β-carotene by 51.8% under UVA irradiation for 60 min (P < 0.001) (Fig. 2B).

The rates of photodegradation of two other carotenoids, crocetin and crocin, were also investigated (Table 1). Under UVA irradiation, crocetin and crocin in ethanol were decomposed more quickly in the presence of Me$_2$S$_4$ than in its absence. These results suggest that the conjugated double bond was favorable to accelerating the photodegradation of carotenoids by sulfides.

The addition of Me$_2$S$_4$ accelerated the UVA-induced photodegradation of these carotenoids in methanol and n-hexane, as well as in ethanol. These results show that the photodegradation of carotenoids in the absence of Me$_2$S$_4$ was slower than that in the presence of Me$_2$S$_4$ under UVA irradiation, regardless of the type of solvent. In addition, irrespective of the type of carotenoid, the effect of solvent on accelerating the photodegradation was in the following order: n-hexane > methanol > ethanol.

Furthermore, the photodegradation of crocetin and crocin was accelerated by Me$_2$S$_4$ in ethanol and methanol, but was slower than that of β-carotene, β-cryptoxanthin and zeaxanthin. These results suggest that a larger number of conjugated double bonds was favorable to accelerating the photodegradation of carotenoids by sulfides. The difference in photostability among the carotenoids with a large number of conjugated double bonds was in the order of zeaxanthin > β-cryptoxanthin > β-carotene, suggesting that the substitution of a hydroxyl group in their structures was favorable to accelerating the photodegradation of carotenoids by sulfides.

There was no significant difference between the rate constants for crocetin and those of crocin (Table 1). These results suggest that the glycosidation of carotenoids was not favorable to accelerating the photodegradation by Me$_2$S$_4$ and the photodegradation by UVA. Note that neither crocetin nor crocin can be dissolved in n-hexane and that up to 1.2 μM crocin can be dissolved in ethanol and methanol. In this study, the time course for the relative value of the residual concentration to the initial concentration of pigment was fitted to the first-order kinetic model. However, our previous study has shown that the degradation rate constant was increased with increasing β-carotene concentration. These results suggest that crocetin could have greater photostability than crocin in ethanol or methanol.

Photodegradation of anthocyanins in the presence or absence of dimethyl tetrasulfide under UVA irradiation

In order to confirm that a conjugated double bond was favorable to accelerating the photodegradation of car-
otenoids by dimethyl tetrasulfide, we investigated the decomposition of anthocyanins, cyanidin, kuromanin and keracyanin by UVA in the presence or absence of Me₃S₄ (Fig. 3). Anthocyanins are flavylium-derived compounds which have an aromatic conjugated double bond, but not an aliphatic one.
Anthocyanin pigments are red with a stable structure of flavylium cations in an acidic aqueous solution (pH < 1). We determined the residual concentration of each anthocyanin after vaporizing the solvent of the irradiated solution and re-dissolving the residue in 0.1 N of an HCl solution. The relative residual concentration for each anthocyanin was determined by the absorbance at the maximum absorption wavelength ($\lambda_{\text{max}}$).

Cyanidin was significantly decomposed by UVA irradiation in the absence of Me$_2$S$_4$, but neither kuromanin nor keracyanin was substantially decomposed by UVA irradiation. Dimethyl tetrasulfide accelerated the carotenoid decomposition induced by UVA irradiation, whereas it significantly suppressed the decomposition of cyanidin induced by UVA irradiation (P < 0.001) (Table 1, Figs. 2 and 3). Kuromanin (P < 0.001) and keracyanin (P < 0.01) were significantly degraded by UVA irradiation in the absence of sulfide, but dimethyl tetrasulfide had no effect on the photodegradation of kuromanin chloride (P = 0.063) or keracyanin chloride (P = 0.56).

Mechanistic study on the photodegradation of carotenoids accelerated by dimethyl tetrasulfide under UVA irradiation

Taken together, it is suggested that conjugated double bonds were involved in the photodegradation of carotenoids and in the accelerated photodegradation by sulfide. In order to clarify the mechanism for this acceleration by Me$_2$S$_4$ of the photodegradation of carotenoids, we employed the free radical scavengers, NaN$_3$, thiourea and DTT, and D$_2$O, a reagent to extend the half-life of singlet oxygen. Each chemical was added to a methanol solution containing $\beta$-carotene and Me$_2$S$_4$, and the solution was irradiated with UVA for 20, 40 and 60 min. The initial absorbance of a $\beta$-carotene methanol solution in the absence of the sulfide before UVA irradiation was 100%. Each value is the mean ± S.D. from four experiments.

Fig. 4. Effect of NaN$_3$, Thiourea, DTT and D$_2$O on the Degradation of $\beta$-Carotene in the Presence of Dimethyl Tetrasulfide under UVA Irradiation. 3 µM of $\beta$-carotene in methanol was added with NaN$_3$ (10 mM) or D$_2$O (10%) in the presence of 5 µM of dimethyl tetrasulfide, or added with thiourea (0.2 mM) or DTT (10 mM) in the presence of 10 µM of dimethyl tetrasulfide, before being irradiated with UVA for 20, 40 and 60 min. The initial absorbance of a $\beta$-carotene methanol solution in the absence of the sulfide before UVA irradiation was 100%. Each value is the mean ± S.D. from four experiments.

Accelerated Photodegradation of Carotenoids by Me$_2$S$_4$ in order to investigate which structure of a carotenoid was most favorable to accelerated photodegradation by Me$_2$S$_4$, we investigated the decomposition of carotenoids and anthocyanins under UVA in the presence of Me$_2$S$_4$. Me$_2$S$_4$ accelerated the photodegradation of carotenoids under UVA irradiation, but not the photodegradation of anthocyanins. These results suggest that an aliphatic conjugated double bond was more favorable
to accelerating by Me$_2$S$_4$ the UVA-induced photodegradation than an aromatic conjugated double bond.

It has been reported that anthocyanins were relatively stable to glycosidation as compared with those in the form of an aglycone.$^{18,19}$ This is consistent with our results that cyanidin was significantly more degraded by UVA irradiation than kuromanin and keracyanin, suggesting that the glycosidation of anthocyanins was favorable for their photostability.

In the presence of Me$_2$S$_4$, the rate constants for the photodegradation of carotenoids in $n$-hexane were larger than those in ethanol and methanol, and the rate constants for the photodegradation of carotenoids in methanol were larger than those in ethanol. These results suggest that the polarity of the solvent could have been involved in the rate of photodegradation. Liu et al.$^{20}$ have found that the apparent photodegradation rate constant for phenylureas in various solvents increased with decreasing polarity. However, our results show that the rate constant for the photodegradation of carotenoids in ethanol was smaller than that in methanol. This difference may be attributed to the capability of ethanol to scavenge free radicals,$^{21}$ which raises the possibility that free radicals were involved in the accelerated photodegradation of carotenoids by sulfides.

The choice of solvent for food processing and preservation is based on effect of the solvent on the food material. Methanol, ethanol and $n$-hexane are employed to extract carotenoids from several kinds of food material.$^{22,23}$ We investigated here the effect of solvent on the accelerated photodegradation of carotenoids by Me$_2$S$_4$. These results suggest that ethanol and methanol could be more useful than $n$-hexane for food processing and preservation in terms of photodegradation.

In addition, this study shows that the difference in photostability among $\beta$-carotene, $\beta$-cryptoxanthin and zeaxanthin was attributable to the number of hydroxyl groups. The radical cation of zeaxanthin is less stable toward phenoxyl radicals than that of $\beta$-carotene because of two hydroxyl groups.$^{24}$ These results suggest that radical cations may be involved in the accelerated photodegradation by dimethyl tetrasulfide under UVA irradiation.

Sulfides did not induce decomposition of carotenoids without UVA irradiation,$^9$ but dimethyl tetrasulfide was also decomposed by UVA irradiation (data not shown). The accelerated decomposition was inhibited by free radical scavengers, but enhanced by the addition of deuterium oxide (D$_2$O) (Fig. 4). Taken together, we hypothesize that a complex free radical chain reaction could be attributed to the sulfide-accelerated photodegradation of carotenoids. Under UVA irradiation, the excited singlet state of a carotenoid was produced by direct light absorption by the carotenoid, and then the carotenoid in the excited state could be easily oxidized by sulfide via adduct formation or losing an electron from the polyene chain. Therefore, it is suggested that the addition of an antioxidative agent to food would be effective for protecting against photodegradation during food processing and preservation.

In conclusion, our results suggest that reactions mediated by free radicals may be involved in the accelerated photodegradation of carotenoids by sulfides, that sulfides can enhance the production of free radicals and that solvents can change the lifetime of the free radicals. These free radicals may subsequently attack the aliphatic conjugated double bonds, since the bleaching process of carotenoids involves disruption of the aliphatic conjugated double bonds.$^{25}$ Nevertheless, the mechanism for the sulfide-accelerated degradation of carotenoids under UVA irradiation remains to be clarified.

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References


