Note

Thermal Isomerization of All-trans-Lutein in a Benzene Solution

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Four major cis-lutein isomers could be identified after thermal isomerization of all-trans-lutein in a benzene solution system; namely, 13′- , 13-, 9′- and 9-cis-luteins. Using both all-trans-lutein and a mixture of these purified cis-luteins as starting materials, the quantitative changes determined by HPLC response show that this thermal isomerization was a reversible reaction. The equilibrium constants (Kc) of 13′- and 13-cis-luteins were higher than those of 9′-, and 9-cis-luteins, suggesting that the amount formed of the former isomers was higher than that of the latter.

Key words: carotenoid; isomerization; lutein

Lutein, a carotenoid, is an important substance to filter out the visible blue light which can cause free-radical damage to the eye.10 Our previous study has shown that lutein and its fatty acid esters both played a role in the color change of banana peel during ripening.2,3 An extract of marigold flowers, in which lutein is the major pigment, is commercially used as a food colorant.9 We have reported in a previous paper the method for preparing lutein from this flower, in which lutein exists mostly as the all-trans isomer.9 Structurally, carotenoids have the capacity for geometrical isomerism, and in nature, they can exist as the cis isomers and the more normal all-trans isomer.11 The characteristics of carotenoids are different for the all-trans and cis isomers. On the other hand, the cis isomers are also well known as thermal and photo degradation products of the all-trans isomer.11 This study deals with the thermal isomerization of all-trans-lutein, including identification of the lutein isomers formed, and the rate constant of the reaction in a benzene solution.

All-trans-lutein prepared from an extract of marigold flower3 was dissolved in benzene (0.47 ± 0.05 μmol/ml), put into capped test tubes (3 ml), and incubated in the dark at a moderate temperature (30, 45, or 60°C) for 4 days to achieve thermal isomerization. At specific times, the samples were analyzed by HPLC (Fig. 1). The sample before incubation contained more than 95% of all-trans-lutein (peak 5), and some small peaks with a retention time of around 22 min. These small peaks increased during incubation at 60°C for 24 hours, while the all-trans-lutein decreased. Some very small and broad peaks also appeared during early elution. The 30°C and 40°C incubation also gave a similar pattern in the HPLC analysis, but the change of all-trans-lutein was slower than that at 60°C (data not shown).

To identify the cis isomers, the lutein isomers were transformed from all-trans-lutein by incubating at 60°C for 24 hours, and were prepared by Capcell C-18 3G-120 column chromatography (310 mm i.d. × 250 mm; Shinseido Co., Tokyo, Japan; eluent, water:ethyl acetate:MeOH=10:10:80, v/v/v). This separation gave similar chromatographic pattern to that by HPLC, and 4 major peaks (peaks 5, 6, 7 and 8) could be collected.

UV-VIS spectrophotometry (Shimadzu UV-160A) showed λmax for peak 5 in benzene of (340), (433), (457), 486 nm, whereas λmax values for samples from peaks 6, 7 and 8 were somewhat lower than that of peak 5. As an indicator of the cis isomer,11 the percentage absorbance of the cis peak (Dc) to that of each maximum (Dm), namely %Dc/Dm, for peaks 6, 7 and 8 was 30.0%, 30.4%, and 41.2%, respectively. However, these values were much higher than that of peak 5 (5.1%). FAB-MS data show that peak 5 had a parent peak at 568 (M+) which coincided with lutein.12 Moreover, an epoxy test on samples from peaks 5, 6, 7 and 8 gave negative results, suggesting that these peaks did not have an epoxy functional group.

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Fig. 1. Chemical Structure of All-trans-lutein and Some cis-Luteins, and HPLC Pattern of Lutein Extracted from Marigold Flowers. HPLC was done in a YMC-Pack ODS-A column (4.6 mm i.d. × 250 mm); solvent, hexane:methylene chloride:MeOH:acetonitrile (2:2:10:86); flow rate, 0.4 ml/min; detection, 447 nm.

Solid line, all-trans-lutein before incubation; dotted line, all-trans-lutein incubated at 60°C for 24 hours. See the text and Table 1 for peak numbering.
Table 1. Vinyl Proton Chemical Shifts by $^1$H-NMR of Lutein Isomers in CDCl$_3$ (δ ppm)

<table>
<thead>
<tr>
<th>Lutein isomer</th>
<th>HPLC peak</th>
<th>Vinyl proton chemical shifts*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6'</td>
<td>7'</td>
</tr>
<tr>
<td>9'-Cis</td>
<td>2.36</td>
<td>6.12</td>
</tr>
</tbody>
</table>

* A chemical shift with an isomerization shift of more than 0.02 is shown in **bold italic.**

The $^1$H-NMR (Jeol JNM-GX500) data in CDCl$_3$ show that the vinyl proton chemical shifts of peak 5 were close to those of all-trans-lutein, in which the β- and ε-ionone rings have different chemical shifts (Table 1). Compared to peak 5, the vinyl proton chemical shift of peak 6 at positions 7', 8', 10', 11' and 12' was shifted +0.02, +0.54, −0.10, +0.10 and −0.10 ppm, respectively. This difference (isomerization shift) would be useful for elucidating the structure of cis isomers of the carotenoids. Two chemical shifts were obtained from the methyl protons of peak 8, whereas those of all-trans-lutein resulted in only one chemical shift, suggesting that there were two isomers in this peak (data not shown). On the basis of the results from the spectrophotometric analysis, TLC, HPLC, epoxy test, and isomerization shift, and by comparing with the reference data, we conclude that peak 5 was all-trans-lutein, peaks 6 and 7 were 9'- and 9-cis-luteins, respectively, whereas peak 8 was a mixture of 13'- and 13-cis-luteins.

Using purified all-trans-lutein and a mixture of 13- and 13'-cis-luteins as external standards, the change in concentration of the lutein isomer during incubation was monitored by HPLC. The total luteins (all-trans and the four major cis isomers) did not change until about 18 hours, after which there was a decrease, like the case of all-trans-lutein (Fig. 2A). This phenomenon suggests that only isomerization of all-trans-lutein to 9', 9-, 13'- and 13-cis-luteins occurred until about 18 hours had elapsed. Further evidence is shown in Fig. 2B, in which the 13'- and 13-cis-luteins increased promptly during early incubation, but decreased slowly after about 18 hours. Similar phenomena were also observed in the case of 9'- and 9-cis-luteins, but the time when lutein began to decrease (the turning point) was somewhat earlier than that of the 13'- and 13-cis-luteins. This result sug-

Fig. 2. Changes in Lutein Isomer Concentration during Incubation at 60°C after Starting with All-trans-lutein.

(A) □ all-trans-lutein; ● total lutein (all-trans, 9'-, 9-, 13'- and 13-cis-luteins). (B) ● 13'- and 13-cis-luteins; ○ 9-cis-lutein; * 9'-cis-lutein.

Fig. 3. Changes in Lutein Isomer Concentration (A), and Ratio of cis and All-trans Isomer Concentration (B) during Incubation at 60°C after Starting with a Mixture of Purified cis-Luteins.

(A) □ total lutein; ■ 13'- and 13-cis-luteins; ● all-trans-lutein; ○ 9-cis-lutein; * 9'-cis-lutein. (B) ● [13'- and 13-cis-luteins]/[all-trans-lutein]; ○ [9-cis-lutein]/[all-trans-lutein]; * [9'-cis-lutein]/[all-trans-lutein].
gests that, after the turning point, the 9'-, 9-, 13'- and 13-cis-luteins changed to other lutein derivatives, or were degraded to peroxo and/or smaller components. Moreover, the ratio of cis/trans for each cis isomer also promptly increased during early incubation, reached a specific ratio, and then remained constant at this specific ratio (data not shown). The point at which the value reached this specific ratio was close to the turning point. These data indicate that the isomerization of carotenoids might be a reversible reaction as previously reported.\(^\text{12}\)

For that reason, a mixture of all-trans:9'-cis:9-cis:13'- and 13-cis-luteins (1:1:2:13) was also isomerized by using the same method as that for all-trans-lutein as already described. Incubation at 60°C caused the amount of the cis isomers to promptly decrease, while the all-trans isomer increased (Fig. 3A). The ratio of cis/all-trans dropped after a few hours of incubation, becoming specific and constant after about 18 hours, depending on the type of cis isomer (Fig. 3B). This evidence proves that the thermal isomerization of carotenoids is a reversible reaction.

To investigate the rate constant of this thermal isomerization, eight series of all-trans-lutein samples (5 replications) were incubated at 60°C for 50 hours, and at specific times, the concentration of each lutein isomer was analyzed by HPLC. The data were analyzed by a complete rate equation procedure with the differential method as described elsewhere.\(^\text{13}\) The mechanism for the thermal isomerization is hypothesized as follows:

\[
\begin{align*}
\text{T} & \xrightarrow{k_1} \text{A} \\
& \xrightarrow{k_2} \text{B} \\
& \xrightarrow{k_3} \text{C} \\
& \xrightarrow{k_4} \text{D} \\
& \xrightarrow{k_5} \text{E}
\end{align*}
\]

Where T is all-trans-lutein, A is 9'-cis-lutein, B is 9-cis-lutein, and C is 13'- and 13-cis-luteins. It is assumed that this mechanism only occurred before the turning point for the cis isomers. Referring to this mechanism, the rate equations can be derived as follows:

\[
\begin{align*}
-r_T &= -\frac{dC_T}{dt} = r_A + r_B + r_C \\
r_A &= \frac{dC_A}{dt} = k_1 f(C_T) - k_2 f(C_A) \\
r_B &= \frac{dC_B}{dt} = k_3 f(C_T) - k_4 f(C_B) \\
r_C &= \frac{dC_C}{dt} = k_5 f(C_T) - k_6 f(C_C)
\end{align*}
\]

Table 2 shows the rate constants of isomerization calculated by using this method. The rate constant of isomerization was different for each cis isomer, and in the order of \( k_1 < k_3 < k_5 \) and \( k_2 < k_4 < k_6 \). Furthermore, the ratio of the \( \text{trans} \rightarrow \text{cis} \) rate constant to the \( \text{cis} \rightarrow \text{trans} \) rate constant (equilibrium constant, \( k_{C/T} \))\(^\text{13}\) was also different, for 9'-, 9-, and 13'- and 13-cis being 0.06, 0.07 and 0.83, respectively, suggesting that the amount of 13'- and 13-cis-luteins formed was higher than that of 9'- and 9-cis-luteins.

When a mixture of all-trans and cis-lutein is stored at a specific temperature, reversible isomerization will occur until the ratio of all-trans and cis-luteins reaches a specific value, after which they will gradually degrade to other components. This specific ratio depends on the temperature and type of the cis isomer. Therefore, these phenomena should be taken into consideration when lutein is being used as an additive in food, or is being stored over a long period. The differences between all-trans and cis-luteins, especially their antioxidative activity, can be a subject for further study.

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