Note

Comparison of Acylated Plant Pigments: Light-resistance and Radical-scavenging Ability

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The acylated plant pigments synthesized by lipase-catalyzed transesterification with aromatic acids were compared in respect of their light-resistance and radical-scavenging ability. With both the flavonols and anthocyanins, their acylated derivatives were more stable against illumination with fluorescent light than their non-acylated glucosides. Their radical-scavenging ability partially decreased or was retained by acylation to the glucoside molecules.

Key words: plant pigment; flavonoid; anthocyanin; light-resistance; radical-scavenging ability

Flavonoids are important natural products which are used as food ingredients and cosmetic additives, as well as in a variety of commodities. These plant pigments have recently been reported to possess immunomodulatory, antioxidative, and antiviral activities. Flower colors, a type of naturally occurring pigment consisting of sugar-containing flavonoids and anthocyanins, are often present in the acylated form with several aromatic acids such as p-coumaric acid at a specific hydroxy group of their sugar moieties. These pigments have been reported to be stabilized in plant tissues because of their intramolecular hydrophobic interactions caused by acylation with aromatic acids. As a result, it has been recognized that such physiological functions as the light-resistance, thermostability, and radical-scavenging ability of the flavonoids in higher plants are due to the presence of these acylated derivatives in their tissues.

However, little information on the physiological functions is known about the effects of the acyl moiety in the acylated flavonoid glucosides. To investigate the structure-stability relationship of the flavonoid aglycon and aromatic acid moiety, we have developed a new method for the regioselective and direct acylation of flavonoid β-D-glucosides via acylation by lipase-catalyzed transesterification with aromatic acid vinyl esters as the acyl donors, and by an enzymatic reaction system from plant cultured cells with aromatic acids. We describe here the effects of acylation of the flavonoid glucosides on the light-resistance and radical-scavenging ability. We also discuss the contribution of the acyl moiety in the flavonoid glucoside molecules.

Quercetin, cyanidin, and isoquercitrin (quercetin 3-O-β-D-glucopyranoside) were purchased from Extrasynthese (Genay, France). Chrysanthemin (cyanidin 3-O-β-D-glucopyranoside) was kindly provided by Dr. T. Ichi, San-Ei Gen. F. F. I., Inc. (Osaka, Japan). The acylated flavonoid glucosides were enzymatically synthesized from their non-acylated forms with vinyl cinnamate or vinyl p-coumarate in dry acetone by the lipase, Chirazyme L-2, c.-f., c-2, lyo (Roche Diagnostics, lipase B from Candida antarctica), at the C6-hydroxy group of the glucose moieties according to our methods described previously.

To interpret the light-resistance of the acylated flavonoid glucosides, we measured the decrease in their maximum absorbance under illumination by white fluorescent light as shown in Fig. 1. With both the flavonol glucoside (isoquercitrin) and the anthocyanin (chrysanthemin), p-coumarate derivatives of the glucosides were the most stable under illumination by light than their glucosides (non-acylated forms). The cinnamate esters also had more light-resistance than their glucosides. The polyphenol aglycons (quercetin and cyanidin) were the most liable in each of the four kinds of compound. Similar results were apparent in thermostability (heated for few days at 50°C under dark conditions) for both the flavonols...
The acylated flavonoid glucosides were synthesized from their non-acylated forms with vinyl cinnamate or vinyl \( p \)-coumarate in dry acetone by Chirazyme L-2, and their structures were elucidated by NMR (\(^1\)H- and \(^{13}\)C-) and MS analyses as described previously.\(^7\)\(^\text{-}^{11}\) The residual amounts of the plant pigments without incubation are expressed as 100 \( z \): anthocyanins (450 mM) were incubated at room temperature in 1 ml of a 100 mM McIlvain buffer (pH 6.0) under illumination by white fluorescent light (one side, 5,000 lx) for various times; chrysanthemin \( p \)-coumarate (500 nm, \( \bullet \)), chrysanthemin cinnamate (500 nm, \( \triangle \)); chrysanthemin (530 nm, \( \# \)), cyanidin (580 nm, \( \circ \)).

B: flavonols (40 mM) were incubated under illumination by light (one side, 20,000 lx) in 1 ml of the buffer (pH 7.0); isoquercitrin \( p \)-coumarate (350 nm, \( \bullet \)); isoquercitrin cinnamate (350 nm, \( \triangle \)); isoquercitrin (350 nm, \( \# \)); quercetin (350 nm, \( \circ \)).

An analysis by electron spin resonance spectrometry (Jeol JES-FR30, Japan)\(^{13}\) indicated no significant decrease in the radical-scavenging ability of the four tested anthocyanins and flavonols against a superoxide anion radical due to glycosylation and acylation, although the radical-scavenging ability of the flavonols against the radical was stronger than that of the anthocyanins, and the scavenging ability of the each polyphenol was slightly higher than that of its glucoside and acylated glucoside forms. Thus, the \textit{in vitro} radical-scavenging ability of the anthocyanins and the flavonols against the two radicals was not essentially changed by acylation with the aromatic acids.

These results were verified by measuring the protective effect on malondialdehyde (MDA) formation in Chinese hamster ovary cultured cells (CHO-cells) when the anthocyanins and flavonols were added to a culture medium with Fe\(^{2+}\)/ascorbic acid, which produced a hydroxy radical.\(^{14}\) The protective effect on MDA formation was quite evident by the addition of cyanidin and chrysanthemin, although the cinnamate and \( p \)-coumarate decreased the effect (Fig. 2). No significant protective effect on MDA formation was found in any of the four flavonol compounds. According to the results of experiments on the cultured cells, it seems that the decreased radical-scavenging ability of the acylated anthocyanins against the hydroxy radical was caused by the introduction of the aromatic acids into the glucoside molecules, resulting in increased water-insolubility in the cells.\(^4\)

The occurrence of highly acylated anthocyanins has recently been reported in some plant tissues.\(^{15}\) Moreover, quercetin, cyanidin, and their glucosides
Table 1. Radical-scavenging Abilities of Plant Pigments and Their Derivatives

<table>
<thead>
<tr>
<th>Antioxidative Substrate (30 nmol)</th>
<th>TAS$^{11}$ (mmol/l)</th>
<th>Antioxidative Substrate (30 nmol)</th>
<th>TAS$^{11}$ (mmol/l)</th>
</tr>
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<tbody>
<tr>
<td>Anthocyanins</td>
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<td>Flavonols</td>
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<td>3.49</td>
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$^{11}$ TAS = Total antioxidant status.

Fig. 2. Protective Effect on Malondialdehyde (MDA) Formation in CHO-Cells.

After pre-incubating CHO-cells for 1 h in 3 ml of the medium with 150 mM anthocyanins, 500 mM of Fe(NH$_4$)$_2$SO$_4$)/5 mM ascorbic acid was added, and then the mixture was incubated for 3 h. The protective effect on MDA formation was measured as described in the text. Control (CHO-cells with Fe$^{2+}$/ascorbic acid) (1), cyanidin (2), chrysanthemin (3), chrysanthemin cinnamate (4), chrysanthemin p-coumarate (5).

were considered to take part in protecting against intracellular oxidative stress resulting from the radical attack. We have demonstrated that the structural stabilization of the flavonoid glucosides was due to the introduction of the acyl moiety into their glucoside molecules. Acylation of the flavonoid glucosides with aromatic acids might partially decrease their radical-scavenging ability, however, the decreasing scavenging ability was not essential. The prolonged and sustained radical-scavenging ability of the flavonoid glucosides are expected as the results of the acylation. A detailed structural elucidation of the acylated plant pigments synthesized by lipase-catalyzed transesterification$^{7-10}$ will be reported in our forthcoming paper.

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