Note

Anti Influenza Virus Activity of a Red-Fleshed Potato Anthocyanin

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We have bred red-fleshed potato from hybrid seedlings between cultivars of Solanum tuberosum ssp. tuberosum and S. tuberosum ssp. andigena. The anthocyanin of red-fleshed potato inactivated both influenza viruses A (IVA) and B (IVB). The IC50 of red-fleshed potato anthocyanin was 48 µg/ml (IVA) and 54 µg/ml (IVB). The IC50 of pelanin was 107 µg/ml (IVA) and 83 µg/ml (IVB). The antiviral activities of pelanin against influenza viruses were lower than the red-fleshed potato anthocyanin. In our previous reports black currant anthocyanins showed an additive antiviral effect. Therefore, we believe that the antiviral activity of the potato anthocyanin comes from the additive or synergistic effect of each anthocyanin pigments and other coexisting pigments.

Keywords: potato anthocyanin, anti-influenza activity, pelanin, pelargonidin

Several plant polyphenols have antiviral activity against influenza viruses (Nagai et al., 1990, 1995a, 1995b; Zakay-Rones et al., 1995). Anthocyanins obtained from plants have been used as a coloring material and are known to have antioxidant activities.

In our previous report, we showed that the mixture of delphinidin 3-glucoside, delphinidin 3-rhamnoglucoside, cyanidin 3-glucoside, cyanidin 3-rhamnoglucoside and other anthocyanins from black currants (Ribes nigrum L.) extract had anti-influenza virus activity against influenza virus types A (IVA) and B (IVB). (Knox et al., 2001).

We have bred anthocyanin-rich tetraploid potatoes from hybrid seedlings between cultivars of Solanum tuberosum ssp. tuberosum and S. tuberosum ssp. andigena (Hayashi et al., 1997; Naito et al., 1998; Mori et al., 2000), and red-fleshed potato ‘Inca Red’ was selected. This paper reports the antiviral activity of red-fleshed potato anthocyanin against IVA and IVB.

Materials and Methods

Red-fleshed potato ‘Inca Red’ was cultivated in the fields of the Potato Breeding Laboratory, Hokkaido National Agricultural Experiment Station (Memuro, Hokkaido).

Pelargonidin, pelargonidin 3-p-coumaroylglucose, 5-glucose and pelargonidin 3-p-coumaroylglucose, 5-malonylgucose were purified from red flowers of Hyacinthus orientalis (Hosokawa et al., 1995).

Red-fleshed potatoes were sliced to the thickness of 1 mm with a slicer and extracted with 3% trifluoroacetic acid (TFA) aqueous solution overnight at 4°C. After filtration through a paper filter No. 3 (Advantec Toyo, Tokyo), the pigment extract was passed through an Amberlite XAD-7 (Organo, Tokyo) column (30 mm I.D. × 450 mm). After washing the column with water, the adsorbed pigments containing anthocyanins were eluted with 0.3% TFA-methanol. The pigment eluate was concentrated under vacuum at 40°C and then dissolved in 0.1% TFA-methanol. The pigment eluate was filtered with a paper filter No. 5c (Advantec Toyo, Tokyo) and dried under vacuum at 40°C. After dissolving in a small amount of 0.1% TFA-methanol, the anthocyanin was precipitated by diethyl ether. The precipitate was collected by centrifugation and dried under vacuum at 40°C. The diethyl ether precipitation was repeated several times, and then the purified anthocyanin powder was obtained. (Hayashi et al., 1997)

Analytical high performance liquid chromatography (HPLC) was carried out on an Inertsil ODS-3 column (4.6 mm I.D. × 250 mm, GL Science Inc., Kyoto) using an isocratic elution in 0.1% TFA-acetonitrile (82.5:17.5, V/V), at a flow rate of 0.8 ml/min at 45°C. Anthocyanin composition was determined from the HPLC peak area recorded at 525 nm. (Hayashi et al., 1996)

Preparative HPLC was carried out with an Inertsil ODS-3 column under the same conditions as analytical HPLC. Pelanin was purified from the crude anthocyanin by preparative HPLC, then dried in a vacuum at 40°C, and powdered pelanin was obtained.

Mardin-Darby canine kidney (MDCK) cells were obtained from RIKEN Cell Bank (Tsukuba, Japan), and cultivated with Eagle’s minimum essential medium (MEM) supplemented with 10% newborn calf serum (MEM-NCS10).

The A/PR/8/34 (H1 N1) strain of influenza virus type A (IVA) and the B/Gifu/2/73 strain of influenza virus type B (IVB), passaged in our laboratory, were propagated in 11-day-old embryonated eggs. All viruses were titrated by a plaque-forming assay.
and stored in small aliquots at −80°C.

Test samples were serially diluted with distilled water before or after adjusting the pH of samples to pH 7.4. The assay of antiviral activities of the test samples against influenza viruses was carried out by the 50% plaque reduction method as described previously (Hisaki et al., 1999). Briefly, confluent monolayers of MDCK cells grown in a 24-well microplate were infected with about 50 plaque-forming units of the A/PR/8/34 strain or the B/Gifu/2/73 strain per well. After 1.5 h of incubation at 37°C, the cell sheets were washed three times with MEM and overlaid with MEM containing 1 μg/ml trypsin (TPCK-treated, Type XIII; Sigma, St Louis, MO), a three-fold concentration of MEM amino acids and vitamins (Nissui Seiyaku Co. Ltd., Tokyo.), 1 mg/ml of glucose, 0.1 mg/ml of DEAE-dextran, 0.8% Agar Noble (Difco, Detroit, MI) and serially diluted test samples. The cells were incubated at 37°C for 2 days, fixed with formalin and stained with crystal violet after removal of the overlay agar media by suction. The plaque counts were expressed as a percentage of the number obtained in control wells and were plotted to give dose-response lines, from which the inhibitory percentage of the number obtained in control wells and were plotted to give dose-response lines, from which the inhibitory concentration of samples required to inhibit the virus plaque number by 50% (IC50, μg/ml) was determined.

Fig. 1. Fractionation of red-fleshed potato anthocyanin by HPLC.

Fig. 2. Structure of pelanin from the red-fleshed potato anthocyanin. Pelanin: 3-O-[6-O-(4-O-p-coumaroyl-α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-5-O-[β-D-glucopyranosyl-pelargonidin.

Table 1. Anti-influenza virus activities of the purified red-fleshed potato anthocyanin and pelain.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>IC50 (μg/ml)</th>
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<tbody>
<tr>
<td>IVA</td>
<td>IVB</td>
</tr>
<tr>
<td>Purified red potato anthocyanin</td>
<td>48</td>
</tr>
<tr>
<td>Pelanin</td>
<td>107</td>
</tr>
<tr>
<td>Pg</td>
<td>22</td>
</tr>
<tr>
<td>Pg 3-pC-glc, 5-glc</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Pg 3-pC-glc, 5-Ma-glc</td>
<td>1,550</td>
</tr>
</tbody>
</table>

*Value was not able to settle because sample quantity was sufficient.

IVA: influenza virus type A, IVB: influenza virus type B
Pg 3-pC-glc, 5-glc: pelargonidin 3-p-coumaroylglucose, 5-glucose
Pg 3-pC-glc, 5-Ma-glc: pelargonidin 3-p-coumaroylglucose, 5-malonylglucose

Test samples were serially diluted with distilled water before or after adjusting the pH of samples to pH 7.4.

Results and Discussion

The purified anthocyanin powder (148 mg) was obtained from 100 g of fresh red-tubers. Ten types of pigments were detected from red-fleshed potato ‘Inca Red’ anthocyanin (Fig. 1). The main pigment was 3-O-[6-O-(4-O-p-coumaroyl-α-L-rhamnopyranosyl)-β-D-glucopyranosyl]-5-O-[β-D-glucopyranosyl-pelargonidin (pelanin) (Ishii et al., 1996; Hayashi et al., 1997; Naito et al., 1998) (Fig. 2). Pelanin (640 mg) was isolated from 1 g of purified red-fleshed potato anthocyanin by preparative HPLC.

Antiviral activities of the purified anthocyanin fraction from red-fleshed potato and pelargonidin type anthocyanin pigments are shown in Table 1. The anthocyanin of red-fleshed potato and pelanin inactivated both IVA and IVB. The IC50 of red-fleshed potato anthocyanin was 48 μg/ml (IVA) and 54 μg/ml (IVB), and the IC50 of pelanin was 107 μg/ml (IVA) and 83 μg/ml (IVB).

Pelargonidin, an aglycone of pelanin, showed higher antiviral activity against IVA and IVB compared to the red-fleshed potato anthocyanin. However, the antiviral activities of pelargonidin 3-p-coumaroylglucose, 5-glucose and pelargonidin 3-p-coumaroylglucose, 5-malonylglucose were slightly weak. From this result, it was conceivable that the inhibition activity against influenza virus originated from the structure of the anthocyanin pigment. Pelanin was the main pigment of red-fleshed potato anthocyanin. The antiviral activities of pelanin against influenza viruses were lower than those of the red-fleshed potato anthocyanin. Therefore, strong activity substances different from those the anthocyanin, might be included.

In our previous reports (Knox et al., 2001), black currant anthocyanins showed an additive antiviral effect. Amoros et al. (1992) have reported a synergistic antiviral effect of flavones and flavonoids against herpes simplex virus 1. Therefore, we believe that the antiviral activity of the potato anthocyanin is shown the result of the additive or synergistic effect of other constituents including anthocyanin.

Black currant anthocyanins have showed antiviral activity against both IVA and IVB (Knox et al., 2001). The same effect was shown to the red-fleshed potato anthocyanin. The mechanism of the interaction is not yet understood. Thus, further studies are needed.

The anthocyanin from potatoes is a natural pigment, so that the mechanism of anti-influenza virus activity is thought be of serious significance to this study.
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References