Antimutagenicity of Sweetpotato (Ipomoea batatas) Roots

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Received October 1, 1998; Accepted November 4, 1998

Antimutagenicity of the water extracts prepared from the storage roots of four varieties of sweetpotato with different flesh colors was investigated using Salmonella typhimurium TA 98. The extract from the whole roots of the purple-colored Ayamurasaki variety effectively decreased the reverse mutation induced not only by Trp-P-1, Trp-P-2, IQ, B[a]P, and 4-NQO but also by dimethyl sulfoxide extracts of grilled beef. Comparison of the inhibitory activity of the extracts from the normal Ayamurasaki and its anthocyanin-deficient mutant one suggested that the anthocyanin pigment in the flesh decreases the mutagenic activity of the mutants as heterocyclic amines. Two anthocyanin pigments purified from purple-colored sweetpotato, 3-(6,6'-caffeylferulohydroxysophoroside)-5-glucoside of cyanidin (YGM-3) and peonidin (YGM-6) effectively inhibited the reverse mutation induced by heterocyclic amines, Trp-P-1, Trp-P-2, and IQ in the presence of rat liver microsomal activation systems.

Key words: antimutagenicity; sweetpotato storage root; anthocyanin; phenolics

Sweetpotato represents the sixth most important food crop in the world. Kozai et al. reported that sweetpotato will be important in solving the global issues of food, energy, and natural resources and the environment in the 21st century. Sweetpotato will especially be an important food crop in Asian and African countries where the populations are expected to increase significantly in the future. Several varieties of sweetpotato contain higher contents of various vitamins, minerals, and protein than other vegetables. Despite its agronomic and nutritional advantages, consumption of sweetpotato has declined in recent decades. To circumvent this problem, interest has been focused on the development of new uses. Therefore, further understanding of the physiological functions of sweetpotato is considered to be one of the important factors for developing new uses. Several investigators reported the suppression of melanogenesis of mouse melanoma B16 and antioxidiant activity by sweetpotato extract as well as the reducing effects of purple-colored sweetpotato juice against carbon tetrachloride-induced liver injury. Recently, new varieties of sweetpotato with different flesh color have been released for the new uses by the Kyushu National Agricultural Experiment Station. Estimation of general nutritive value such as vitamin or mineral contents of these varieties has been investigated but too little is known about their physiological function.

Recent development of screening methods for environmental carcinogens by measuring their mutagenicity has allowed various types of mutagens and carcinogens to be detected and identified in daily foods. Some of these substances have been found to be generated during storage, cooking, and digestion of foods. Trp-P-1 and Trp-P-2 are mutagenic pyrolysates of tryptophan, which were first isolated from broiled beef and demonstrated to be carcinogenic in rats. IQ was isolated from baked fish and also demonstrated to be carcinogenic in the mouse. On the other hand, it is now known that various types of inhibitors that act against mutagens and carcinogens exist in our daily food, and they play an important role in reducing the risks of mutagenesis and carcinogenesis. However, antimutagenic activity of sweetpotato varieties has not been investigated.

In this paper, we describe the effects of the water extracts of the storage roots of several varieties of sweetpotato with different flesh colors and two anthocyanin pigments purified from purple-colored sweetpotato on the mutagenicity of some mutagens.

Materials and Methods

Sweetpotato materials. Four varieties of sweetpotato with different flesh color (Table 1), Koganesen, Kyushu-114, Joy White, and Ayamurasaki and its anthocyanin-deficient mutant were cultivated in 1996 under the same conditions in an experimental field at Miyakonojo (Japan). An anthocyanin-deficient mutant of Ayamurasaki variety with yellow flesh was generated spontaneously in the experimental field of Kyushu.
Agricultural Experiment Station. Harvested roots were cut into two portions and one half was used as the whole root. The remaining one half was separated into the peeled outer layer (about 0.5 cm thick) as the outer tissue and the inner portion without its outer layer as the inner tissue. The peeled outer layer comprised all of the cortical region, which included the periderm, laticifer, and cambium. All sections in each case were diced, lyophilized, and ground to flour. The flour samples were kept at -20°C until use.

Chemicals and bacteria. Trp-P-1, Trp-P-2, IQ, 4-NQO, and BlaIP were obtained from Wako Pure Chemical Industries Ltd. Chlorogenic acid (ChA) was the product of Sigma Chemical Co. S-9 fraction prepared from rat liver treated with phenobarbital and 5,6-benzoflavone and cofactors were the products of Oriental Yeast Co., Ltd. Folin-Ciocalteau phenol reagent was from Nacalai tesque, Inc. Other chemicals used were of special grade. Strain TA 98 of Salmonella typhimurium was supplied by the Institute for Fermentation, Osaka, Japan (IFO). The bacteria was cultured in nutrient broth for 16 h at 37°C before the mutagenicity assay.

Preparation of sweetpotato-water extract and anthocyanin pigments. The extract was made from the lyophilized flour (1 g), using 20 ml of ice-cold water for 1 h. The suspension was centrifuged at 18,000×g for 20 min, and the resultant precipitate was re-extracted under the same conditions. The collected supernatant was lyophilized.

Two anthocyanin pigments, 3-(6,6'-caffeylferuloyll-sophoroside)-5-glucoside of cyanidin and peonidin, which correspond to the third and sixth peaks (Fig. 1), respectively, seen in an earlier HPLC analysis of the crude anthocyanin extract from the purple-colored flesh of the Yamagawamura sakari variety, were purified and designated as YGM-3 and YGM-6, since anthocyanin pigments of purple-colored sweetpotato have been closely identified in this variety. 11-13

Assay of antimitogenicity. The mutagenicity assay was done by a modification of the method of Yahagi et al. 14 The antimitogenic activity was evaluated for TA 98 using several mutants, Trp-P-1, Trp-P-2, IQ, BlaIP, and 4-NQO, and a dimethyl sulfoxide (DMSO) extract of grilled beef (DEGB). These mutagens other than 4-NQO require metabolic activation to cause mutations in TA98. S-9 mix contained 50 μmol of sodium phosphate buffer (pH 7.4), 4 μmol of MgCl2, 16.5 μmol of KCl, 2.5 μmol of glucose-6-phosphate, 2 μmol of NADH, 2 μmol of NADPH, and 50 μl of S-9 fraction in a total volume of 0.5 ml. For the inhibition test, 0.1 ml of each mutagen, 0.1 ml sweetpotato-water extract or DMSO-dissolved pigment solution, and 0.5 ml S-9 mix or phosphate buffer were simultaneously incubated with 0.1 ml of bacterial suspension at 37°C for 20 min, and then poured on minimal-glucose-agar plates with 2 ml of soft agar.

Preparation of N-OH-Trp-P-1 and DEGB. Preparation of N-OH-Trp-P-1 and DEGB was done by the method of Yamada and Tomita. 15 Trp-P-1 (0.3 μg/plate) was incubated with 0.5 ml of S-9 mix for 7 min at 37°C, and then the enzyme was completely inactivated in boiling water for 2 min.

For the preparation of DEGB, sliced beef (fillet, purchased from a market) was grilled to well-done, lyophilized, and ground to flour. Five grams of the lyophilized flour were extracted with 10 ml of DMSO for 60 min at room temperature. The solution was sterilized by filtration (Minisart NML 0.45 μm, Sartorius). DEGB was added to the reaction mixture at a dose of 100 μl/plate without dilution.

Extraction and measurement of phenolics. The lyophilized sweetpotato flour was vigorously mixed with 10 times its equivalent volume of 80% ethanol. The mixture was boiled for 5 min under a hood and centrifuged at 5000×g for 10 min. The residue was mixed with additional 80% ethanol and boiled for 10 min to re-extract the phenolics and centrifuged under the same conditions. The extracts were combined and made up to 10 ml. This extract was used for the measurement of total phenolics. Total phenolics were measured by the procedure described by Cosentino and Lee. 16 The alcohol extract was diluted to obtain an absorbance reading within the range of the standards (800-40 μg ChA/ml). The absorbance in the microplate wells was measured at 600 nm with a dual wavelength flying spot scanning densitometer (Shimadzu Co.), with a microplate system. The results were expressed as mg ChA/100 g flour.

HPLC analysis of anthocyanin pigments from Ayamurasaki variety and its mutant. Individual anthocyanin pigments were analyzed using a HPLC (Model LC-9A, Shimadzu Co.) with a variable wavelength spectrum flow monitor. The samples for HPLC analysis of anthocyanin pigments were prepared by extraction with 50% acetic acid. Separation of the pigments was done on a 4.6 mm×25 cm Intersil ODS-2 column (Shimadzu Co.) and detected at 530 nm. The column was conditioned with Solvent A (1.5% phosphoric acid), and the pigments were eluted using a linear solvent gradient of 0-100% Solvent B (1.5% phosphoric acid, 20% acetic acid, 25% acetonitrile) for 30 min at a flow rate of 1 ml/min.

Results and Discussion

Effects of water extracts from whole storage roots on the mutagenicity of Trp-P-1

The antimutagenic effect of the water extracts from whole storage roots of four varieties with different flesh colors and the anthocyanin-deficient Ayamurasaki mutant was examined using Trp-P-1 at a dose of 0.075 μg/plate. The extract was used at doses of 1, 5, and 10 mg/plate. Table 1 shows the results at a dose of 1 mg/plate. The inhibitory activity was 41% at a dose of 1.0 mg/plate of the extract from Ayamurasaki and the extract showed a dose-dependent antimutagenicity (data not shown). The extracts from Koganesengan, Joy White, and Kyushu-114 variety showed the inhibitory ac-
The HPLC analysis indicated that anthocyanin pigments are rarely contained in the flesh of the anthocyanin-deficient mutant of Ayamurasaki variety. On the comparison with the inhibitory activities between the water extracts from Ayamurasaki normal and its mutant, the inhibitory activities of the extract from the Ayamurasaki’s roots were 37% at doses of 1.0 mg/g of plate of the extract. However, the inhibitory activity of the extract from Ayamurasaki mutant was 1% at a dose of 1.0 mg/g of plate of the extract (Table 1). Thus, the antimutagenicity of the extract from Ayamurasaki mutant was at the same level as the ones from Koganesengan, Joy White, and Kyushu-114 with yellow, white, and orange flesh. These results suggests that the effective inhibition of reverse mutation by Ayamurasaki extract may be attributed to the anthocyanin pigments in the purple flesh.

Phenolics are known to inhibit the reverse mutations induced by various mutagens. We measured the phenolic content of sweetpotato roots to discover the relationship between the phenolic contents and the antimutagenicity. The contents of total phenolics in the whole storage roots of the varieties tested were expressed as mg/g of flour. The average contents in Ayamurasaki root were 1800 mg/g of flour, while in the other three varieties and Ayamurasaki mutant the contents were about 200 mg/g to 300 mg/g of flour. The high phenolic content of Ayamurasaki probably increases the reaction of anthocyanin pigments with Folin-Ciocalteu phenol reagent. Odake et al. and Goda et al. reported that anthocyanins of the purple-colored sweetpotato have a caffeoyl or ferulyl group in their structural formula. These data suggested a relationship between the phenolic contents and the antimutagenicity. However, the main phenolic component in the Ayamurasaki extract to inhibit the reverse mutation seems to be anthocyanin pigments.

Effects of water extract from Ayamurasaki root on the mutagenicity of various mutagens

The antimutagenic activity of the extract was evaluated using several mutagens, such as Trp-P-2, IQ, B[a]P, 4-NQO, N-OH-Trp-P-1, and DEGB (Fig. 2). Trp-P-2, IQ, B[a]P, 4-NQO, and N-OH-Trp-P-1 were added at doses of 0.02, 0.02, 10, 0.8, and 0.3 μg/plate, respectively. DEGB was used at a dose of 100 μl/plate without dilution. Of these mutagens, S-9 mix was added for the assay using Trp-P-2, IQ, B[a]P, and DEGB to cause mutations in TA 98, but not for 4-NQO and N-OH-Trp-P-1. The extract was used at doses of 1, 5, and 10 mg/plate. The extract inhibited Trp-P-2-induced mutation by 76%, IQ by 77%, B[a]P by 60%, and 4-NQO by 53% respectively at the concentration of 10 mg/g. Thus, the Ayamurasaki-water extract effectively decreased the reverse mutations induced by all purified mutagens tested. We went on to confirm whether the Ayamurasaki-water extract inhibits the reverse mutation induced by mutagenic substances in daily foods such as DEGB. As shown in Fig. 2, the Ayamurasaki-water extract also showed the dose-dependent antimutagenicity against the reverse mutation induced by DEGB, as well as by Trp-
P-1, Trp-P-2, and IQ.

Trp-P-1 is known to be converted into the N-hydroxy form, N-OH-Trp-P-1, by metabolic enzymes to develop mutagenic activity.17 Yamada and Tomita18 indicated that the water extract from black tea or oolong tea inhibited the mutagenic activity of Trp-P-1 by direct reactions with activated mutagen rather than by the inactivation of metabolic enzymes. The effect of the Ayamurasaki-water extract on the mutagenicity of N-OH-Trp-P-1 prepared by incubation of Trp-P-1 with S-9 just before the mutagenicity assay was investigated for elucidation of the mechanism of its antimutagenicity. The Ayamurasaki-water extract effectively decreased the mutagenicity of N-OH-Trp-P-1 (Fig. 2). These results suggest that the Ayamurasaki-water extract inhibited the mutagenic activity of the mutagen partly by direct reactions with activated mutagen rather than by the inactivation of metabolic enzymes.

The water extract from Ayamurasaki with purple-colored flesh effectively inhibited the reverse mutation induced not only by various kinds of purified mutagens but also by DEGB. The strong inhibition of the reverse mutation by the Ayamurasaki-water extract reveal that the antimutagenic activity of Ayamurasaki might be due to the anthocyanin pigment in the flesh. However, the antimutagenic activity of the Ayamurasaki-water extract may be due to water-soluble components such as amino acids,19 vitamins20,21 and phenolics other than the anthocyanin pigments. Yamada and Tomita18 have suggested that many kinds of extracts containing compounds analogous to caffeic acid or CHA effectively decrease the mutagenic activity of the mutagens as heterocyclic amines. We also confirmed that CHA notably inhibits Trp-P-1- or Trp-P-2-induced mutation on Salmonella typhimurium TA 98 (data not shown). Hayase and Kato9 proposed that the effective antioxidant activity of the sweetpotato extract was mainly based on the synergistic effect of phenolic compounds with amino acids. Therefore, it is necessary to confirm the antimutagenicity of anthocyanins purified from sweetpotato with purple flesh color.

![Graph](image_url)

**Fig. 2.** Effects of Ayamurasaki-Water Extract on the Mutagenicity of Trp-P-2, IQ, B[a]P, 4-NQO, DEGB, and N-OH-Trp-P-1 against Salmonella typhimurium TA 98.

- Trp-P-2 (0.02 μg/plate, in the presence of S-9 mix); ○, IQ (0.02 μg/plate, in the presence of S-9 mix); △, B[a]P (10 μg/plate, in the presence of S-9 mix); ▲, 4-NQO (0.8 μg/plate, in the presence of S-9 mix); ◯, N-OH-Trp-P-1 (0.3 μg/plate, in the absence of S-9 mix); ●, DEGB (100 μl/plate without dilution, in the presence of S-9 mix). The His+ revertant value of control on Trp-P-2, IQ, B[a]P, 4-NQO, N-OH-Trp-P-1, and DEGB was 866 ± 57, 921 ± 22, 252 ± 24, 305 ± 9, 393 ± 28, and 229 ± 20/plate, respectively.

![Chemical Structure](image_url)

**Fig. 3.** Chemical Structures of YGM-3 and YGM-6.
### Table 2. Effects of YGM-3 and YGM-6 on the Mutagenicity of Trp-P-1, Trp-P-2, IQ, and DEGB against Salmonella typhimurium TA 98

<table>
<thead>
<tr>
<th>Mutagena (μg or μl / plate)</th>
<th>Inhibition (%)b</th>
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<tbody>
<tr>
<td></td>
<td>YGM-3</td>
</tr>
<tr>
<td>Trp-P-1 (0.075 μg)</td>
<td>51</td>
</tr>
<tr>
<td>Trp-P-2 (0.020 μg)</td>
<td>77</td>
</tr>
<tr>
<td>IQ (0.020 μg)</td>
<td>42</td>
</tr>
<tr>
<td>DEGB (100 μl)</td>
<td>57</td>
</tr>
</tbody>
</table>

a Mutagenicity was tested with S-9 mix.  
b YGM-3 and YGM-6 was used at a concentration of 0.5 mg/plate. Each value represents the mean ± S.D. of triplicate plates. The values shown have had the spontaneous mutation frequency subtracted. The His+ revertant value of control on Trp-P-1, Trp-P-2, IQ, and DEGB was 693 ± 43, 825 ± 9, 884 ± 20, and 288 ± 11/plate, respectively.

### Effects of anthocyanin pigments on the mutagenicity of various mutagens

Two pigments purified from the Yamagawamurasaki variety were used to confirm the hypothesis that anthocyanin pigments of purple-colored sweetpotato inhibit the reverse mutation induced by several mutagens. Chemical structures of YGM-3 and YGM-6 are shown in the Table. 3 Table 3 shows the antagonism of YGM-3 and YGM-6 at a dose of 0.5 mg/plate against the reverse mutations induced by Trp-P-1, Trp-P-2, IQ, and DEGB. YGM-3 appears to have stronger antimutagenic activity against the mutagens tested than YGM-6. This reflects the structural difference between cyanidin and peonidin (Fig. 3). Odake et al.9) and Goda et al.10) reported that anthocyanins of the purple-colored sweetpotato have a caffeoyl or feruloyl group in their structural formula. Those results agree with the report by Yamada and Tomita,11) that compounds analogous to caffeic acid or chlorogenic acid effectively decrease the mutagenic activity of the mutagens as heterocyclic amines. The strong inhibition of reverse mutation by anthocyanins of purple-colored sweetpotato may be due to the caffeoyl or feruloyl group in their chemical structure. Further work on the relationship between the chemical structure and antimutagenic activity is required to support the release of high quality varieties of purple-colored sweetpotato.

At present, the flour from Ayamurasaki variety has been developed as a material for noodles, breads, confectioneries, and a new type of alcohol drink. Furthermore, the pigment extract from Ayamurasaki variety has been used as a food colorant because the anthocyanin pigment from purple-colored sweetpotato is more stable than other anthocyanins from red cabbage, elderberry and purple corn.12) On the other hand, the reports related to physiological functions of purple-colored sweetpotato are few. Suda et al.13) has only reported a protective effect of purple-colored sweetpotato juice against carbon tetrachloride-induced liver injury. Therefore, an in vivo study on anticarcinogenesis of the anthocyanins is required to increase the demand for purple-colored sweetpotato in the future.

### References