Desmutagenic Activities of Vegetables on Broiled Fish

Kentaro YOSHIKAWA*, Kayo MUI**, Ryuichiro ISHII*, Takao TERASHITA*, Jiko SHISHIYAMA* and Matashi KÔNO*

Synopsis

The extracts of certain vegetables (eggplant, broccoli etc.) reduced the mutagenicity of pyrolysate mutagens by inhibiting metabolic activation with rat liver preparation S-9. Juices prepared from 90 kinds of vegetables and fruit were almost all capable of irreversibly suppressing the mutagenicity of broiled fish by direct enzymatic inactivation of the mutagenic principle. Some pyrolysated fish products contained components resistant to vegetable desmutagenic action.

Introduction

Roasted meats and broiled fish contain highly mutagenic substances produced when amino acids and proteins are heated. Crude extracts of many vegetables counteract the in vitro mutagenicity of the pyrolysates derived from certain amino acids. Inoue et al. isolated a hemoprotein from cabbages that has peroxidase and NADPH-oxidase activity, and that acts desmutagenically on tryptophan pyrolysate (Trp-P-2) both by on direct enzymatic inactivation and by suppression of metabolic activation. A similar substance has been isolated from broccoli.

Here, we did an extensive survey of the desmutagenic activities of vegetables and other foodstuffs to estimate their direct capability of inactivating the mutagenicity of broiled fish. Most vegetable extracts strongly inhibited metabolic activation or irreversibly inactivated mutagenicity. However, pyrolysated fish products contained substances that resisted vegetable desmutagenic capabilities.

Materials and Methods

To prepare a crude mutagenic extract, 100 g of raw aji fish (Trachurus japonicus) was broiled on a gas burner. About 20 g of the burned surface was extracted with a 1:1 mixture of chloroform and methanol. The solvent was evaporated at 45°C under reduced pressure and 5 g of the residue was dissolved in 5 ml of dimethyl-sulfoxide (Me2SO).

Vegetable samples were purchased at a supermarket in Higashi-Osaka city. Each vegetable was purred in juicer and filtered through gauze. The juice obtained was centrifuged at 35,000 x g (18,000 rpm) for 15 min at 0°C. The supernatant was separated and stored at -20°C until use.

In the experiment with vegetable juice, 1 g of the extract in Me2SO was mixed with 5 ml of juice and kept at 37°C for 60 min. Then the mixture was extracted with 16 ml of ethyl acetate at
pH 12 with vigorous shaking for 15 min. Eight milliliters of the supernatant was dried under reduced pressure at 0°C and the dry material was dissolved in 0.5 ml of Me₂SO. As a control, 5 ml of distilled water was used instead of the vegetable juice.

The rat preparation S-9 was prepared from rats treated with polychlorobiphenyl by the method by Ames et al. The Ames assay was done with 0.1 ml of the solution in Me₂SO by the pouring of a 0.1 ml suspension of salmonella typhimurium TA98 containing 0.3 ml of the S-9 mixture onto a selective agar plate. The plates were incubated at 37°C for 48 hr and the number of His⁺ colonies was counted.

Separately, mutagenicity assays were also done with the Me₂SO extract of broiled fish without vegetable juice or with a mixture of broiled fish extract and a vegetable juice without ethyl acetate extraction. All experiments were done at least twice with triplicates each time, and results show the mean of two experiments.

Results

The addition of the S-9 preparation to the extract prepared from broiled aji fish conferred mutagenicity on the extract (Fig. 1). About 200 His⁺ revertants were found per plate when 1 mg of dry pyrolysate was added. The effects of three vegetable juices on the mutagenicity of the aji fish extract are shown in Fig. 2. The mutagenicity of the aji extract was reduced by the addition of burdock, eggplant, or broccoli juice.

![Fig. 1. Mutagenicity of the extract from broiled aji fish (Trachurus japonicus). See text for preparation of the sample. Salmonella typhimurium TA98 was used.](image-url)

- □, with S-9 preparation
- ○, without S-9 preparation
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Fig. 2. Effects of vegetable juices on the mutagenicity of broiled aji fish. Juice was mixed with the fish extract, bacteria, and S9 preparation in melted soft agar solution (3 ml, 47°C) and poured onto a solid agar plate.

- Burdock
- Eggplant
- Broccoli

Fig. 3. Heat sensitivity of the desmutagenic activity of eggplant. See text for details of experiment.

- Broiled fish extract was mixed with eggplant juice, and the mutagenicity of the mixture was assayed.
- Broiled fish extract mixed with eggplant juice was extracted with ethyl acetate, and the mutagenicity of the extract was assayed.

The suppression of mutagenicity might be caused by direct inactivation of mutagenic substances or by inhibition of metabolic activation by the S9 preparation. Next, the mutagenic principle in the mixture of vegetable juice and fish extract was extracted with an organic solvent, ethyl acetate. The desmutagenic activity of crude unheated eggplant extract was higher than that of heat-treated juice or the fraction extracted with ethyl acetate, and about half of the mutagenicity that had seemed to be lost was recovered in the extracted fraction (Fig. 3). Some of the desmutagenic capacity of juices resisted heating; about 50% of the mutagenicity remained after heat treatment. A similar inactivation curve was obtained with heated juice (100°C for 30 min).

We wanted to identify vegetables that when made into juice would act directly on the mutagens of broiled fish before digestion. A survey was done, and results are shown in Table 1, which lists 90 vegetables, fruit, and other foodstuffs. Some foodstuffs (golden apple, pomegranate, chrysanthemum, etc.) had strong desmutagenic effects on aji fish pyrolysis products. More than half of the foods had moderate desmutagenic activity. However, the pyrolysate fish products included factors resistant to vegetable desmutagenic capabilities.

Inhibitors of metabolic activation and desmutagens are quite different. One example will be given. Enokidake mushrooms (Flammulina velutipes), which had a desmutagenic activity of 47%
Table 1. Desmutagenic effects of foodstuffs on the broiled Aji extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golden apple (Malus pumila  ather Mill)</td>
<td>84</td>
</tr>
<tr>
<td>Pomegranate (Panica granatum L.)</td>
<td>81</td>
</tr>
<tr>
<td>Garland chrysanthemum (Chrysanthemum coronarium)</td>
<td>78</td>
</tr>
<tr>
<td>Ponkan orange (Citrus reticulate)</td>
<td>76</td>
</tr>
<tr>
<td>Lily bulb (Lilium auratum Lindl.)</td>
<td>74</td>
</tr>
<tr>
<td>Grape (Vitis spp.)</td>
<td>73</td>
</tr>
<tr>
<td>Kidney bean (Pisum sativum L.)</td>
<td>71</td>
</tr>
<tr>
<td>Plantain (Musa acuminata)</td>
<td>71</td>
</tr>
<tr>
<td>Royal fern (Osmoda japonica Thunb.)</td>
<td>70</td>
</tr>
<tr>
<td>Corn (Zea mays L.)</td>
<td>69</td>
</tr>
<tr>
<td>Hime apple (Malus pumila)</td>
<td>69</td>
</tr>
<tr>
<td>Ginger (Zingiber officinale Rosc.)</td>
<td>67</td>
</tr>
<tr>
<td>Kaioke (Raphanus sativus L.)</td>
<td>65</td>
</tr>
<tr>
<td>Sweet pepper (Piper nigrum L.)</td>
<td>64</td>
</tr>
<tr>
<td>Red onion (Allium cecio L.)</td>
<td>63</td>
</tr>
<tr>
<td>Garlic (Allium sativum)</td>
<td>63</td>
</tr>
<tr>
<td>Shallot (Alliumfistulatum)</td>
<td>63</td>
</tr>
<tr>
<td>Small onion (Allium cecio L.)</td>
<td>61</td>
</tr>
<tr>
<td>Papaya (Carica papaya L.)</td>
<td>61</td>
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<tr>
<td>Ginkgo nut (Ginkgo biloba L.)</td>
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<tr>
<td>Radish (Raphanus sativus L.)</td>
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</tr>
<tr>
<td>Mitsuba water dropwort (Cryptolaenia japonica)</td>
<td>56</td>
</tr>
<tr>
<td>Chinese quince (Chaenomeles sinensis)</td>
<td>54</td>
</tr>
<tr>
<td>Mugwort (Artemisia vulgaris L.)</td>
<td>53</td>
</tr>
<tr>
<td>Sunny lettuce (Lactuca sativa L.)</td>
<td>53</td>
</tr>
<tr>
<td>Sweet potato (Ipomoea batatas)</td>
<td>53</td>
</tr>
<tr>
<td>Onion (Allium cecio L.)</td>
<td>53</td>
</tr>
<tr>
<td>Japanese butterbur (Petasites japonicus)</td>
<td>51</td>
</tr>
<tr>
<td>Spinach (Spinacia oleracea L.)</td>
<td>51</td>
</tr>
<tr>
<td>Kiwifruit (Actinidia chinensis)</td>
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</tr>
<tr>
<td>Fuji apple (Malus pumila)</td>
<td>51</td>
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<tr>
<td>Parsley (Petroselinum sativum)</td>
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</tr>
<tr>
<td>Banana (Musa sapientum L.)</td>
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</tr>
<tr>
<td>Kabosa citrus fruit (Citrus sphaeroarpa)</td>
<td>50</td>
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<tr>
<td>Japanese persimmon (Diospyros Kaki L.)</td>
<td>47</td>
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<tr>
<td>Enokidake mushroom (Flammulina velutipes)</td>
<td>47</td>
</tr>
<tr>
<td>Saiakadoki pumpkin (Cucurbita sp.)</td>
<td>45</td>
</tr>
<tr>
<td>Pine apple (Ananas camusus)</td>
<td>12</td>
</tr>
<tr>
<td>Toukoku bean (Faba spp.)</td>
<td>42</td>
</tr>
</tbody>
</table>
Violet cabbage (Brassica oleracea L.) 42
Soybean sprout (Glycine max.) 41
Rape (Brassica campestris L.) 41
Eggplant (Solanum melongena L.) 40
Lemon (Citrus limon) 40
Soybean (Glycine max.) 40
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Navel orange (Citrus sinensis) 34
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Pumpkin (Cucurbita moschata) 25
Cherry (Prunus armeniaca) 23
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Kintaki bean (Phaseolus vulgaris L.) 20
Celery (Apium graveolens L.) 20
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Mustard (Brassica juncea) 16
Broccoli (Brassica oleracea L.) 16
Budo bean (Glycine max) 14
Sweet pepper (Piper nigrum L.) 13
Azuki bean (Vigna angularis) 13
Head lettuce, butterhead type (Lactuca sativa L.) 9
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Buckwheat (Fagopyrum esculentum Moench) 7
Mung bean (Vigna radiata Wilcz.) 7
Head lettuce (Lactuca sativa L.) 5
Mushroom (Agrarius hisporus) 2
Yamanohimo (Dioscorea japonica) 2
Indian lotus root (Nelumbo nucifera) 2
Kinosaya pea pods, immature (Vigna sinensis) 1
Fern bracken (Wasabi japonica) 1
Namae mushroom (Pholiota nameko)
Grapefruit (Citrus paradisi)
Orange (Citrus sinensis Osbeck)
Strawberry (Fragaria grandiflora)
aji fish (Trachurus japonicus) pyrolysate (control)

*Less than 0 (activation of mutagenicity)
The results are shown as the activity relative to the control as % . Details of the procedure are in the text.

Fig. 4. Heat sensitivity of the desmutagenic activity of cuokidake mushrooms (Flammulina velutipes)
(a) Extract of broiled fish was mixed with fresh or heated juice, and the mutagenicity of the mixture was assayed.
- mixed with fresh juice
■ mixed with heated juice (100°C for 10 min)
△ mixed with heated juice (100°C for 30 min)
(b) Extract of broiled fish was mixed with fresh or heated juice, and ethyl acetate extraction was done. The mutagenicity of the extract was assayed.
○, mixed with fresh juice
□, mixed with heated juice (100°C for 10 min)
△, mixed with heated juice (100°C for 30 min)

was studied to identify the mechanism of the desmutagenic activity. The juice of these mushroom had a heat-resistant inhibitor of metabolic activation (Fig. 4a), but its desmutagen was heat-labile (Fig. 4b).

Discussion

Our results showed that most kinds of vegetables were capable of some degree of direct inactivation of the mutagenicity of broiled fish pyrolysates. With some vegetable, metabolic activation by the S-9 preparation was inhibited, and mutagenic substances were recovered by
Desmutagenic activities that affect tryptophan pyrolysate are involved in the direct enzymatic inactivation of mutagens and also suppression of metabolic activation. In our examination using broiled aji fish as the mutagen, desmutagenicity was found in some vegetable juices. The mutagenicity of this broiled fish arises from several highly mutagenic products of pyrolysis, such as Trp-P1, Trp-P2, Glu-P1, Glu-P2, 2-Amino-3 methylimidazo[4,5-f]quinoline, 2-Amino-3 dimethylimidazo[4,5-f]quinoline. The results obtained here did not show what kind of action the vegetable juices had. It is not known whether the components of the pyrolysate from broiled fish unaffected by vegetable juice are carcinogenic or not.

References