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Desmutagenicity of Vegetables and Fruits

Kazuki SHINOHARA

Division of Nutrition and Physiology, National Food Research Institute
(Tsukuba, Ibaraki, 305 Japan)

Abstract

Aqueous dialyzates from various kinds of vegetables and fruits such as spinach, broccoli, eggplant and apple inhibited the mutagenicity of Trp-P-2 toward *Salmonella typhimurium* TA 100 and TA 98, and the desmutagenicity was retained even after heating at 100°C for 20 min. Dialyzates of burdock, eggplant, spinach and apple also inhibited the mutagenicity of Trp-Ps, benzo-[a]pyrene, sterigmatocystin, aflatoxin B1, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide and *N*-methyl-N'-nitro-*N*-nitrosoguanidine. It was suggested that desmutagenic principles of apple and spinach be polymerized polyphenol fractions and relatively high molecular weight components, respectively.

Discipline: Food
Additional key words: Ames test, carcinogens, mutagens

Introduction

Some epidemiological studies indicate that green- and yellow-colored vegetables reduce the occurrence of cancers. This suggests that vegetables and fruits have some antitumoric functions. One of the functions of constituents of vegetables and fruits is an inactivating function on the mutagenicity of mutagens and carcinogens (desmutagenic function). Many mutagens and carcinogens have been detected in natural environments. Especially, findings of powerful mutagens and carcinogens in the pyrolyzates of amino acids and proteins, broiled fish and roasted meat have received much attention. Recent studies revealed that some desmutagenic substances existed in vegetables and fruits. It has already been identified that vitamin C, vitamin A, cysteine, polyphenols, peroxidase, fibers, and lignin-like compounds were desmutagenic[2–9,13]. Most of them have relatively low molecular weight of constituents, except peroxidase and fibers. Desmutagenicity of the high molecular weight of constituents in vegetables and fruits is to be clearly identified yet. Vegetables and fruits comprise a number of constituents, and mutual interactions among those constituents take place during processing, storage and cooking to produce many types of output. It is plausible to expect that unknown desmutagenic substances may exist among these constituents or products.

This report describes the desmutagenic effect of nondialyzable extracts of fresh and freeze-dried vegetables and fruits on some mutagens and carcinogens by use of Ames test[1].

Desmutagenic assay

Ames method[1] with *Salmonella typhimurium* TA 100 and TA 98 was used to examine desmutagenicity of dialyzates on several mutagens, including 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyridol[4,3-*b*]indole (Trp-P-2), benzo[*a*]-pyrene (B[*a*]P), sterigmatocystin (ST), aflatoxin B1 (AB1), 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) and *N*-methyl-*N*-nitroso-*N'*-nitrosoguanidine (MNNG). Among these mutagens, Trp-P-1, Trp-P-2, B[*a*]P, ST and AB1 are indirect mutagens and require S9 rat liver fraction and its cofactors (S9 mixture: S9) for their activation, while MNNG and AF-2 are direct mutagens and do not require S9. Trp-P-1, Trp-P-2, B[*a*]P, AF-2 and MNNG were dissolved in dimethylsulfoxide to give concentrations of 10, 10, 20, 0.1 and 10 μg/m*l*, respectively. ST was dissolved in acetone to give 20 μg/m*l* , and AB1 in benzene-acetonitrile mixture to give 1 μg/m*l*. An aliquot of each dialyzate was mixed with a mixture of 0.1 m*l* of TA 100 or TA 98 suspension, either 0.1 m*l* of S9 or 0.1 m*l* of 0.2 M phosphate buffer (pH 6.7), 0.1 m*l* of mutagen or carcinogen and 0.1 m*l* of phosphate buffer. The total volume was adjusted to 1 m*l* with sterile distilled water. After the mixture had been pre-incubated at 37°C for 20 min, 2 m*l* of top agar was added, and the entire mixture was poured on to a histidine-deficient Vogel-Bonner agar plate (15 × 100 mm petri dish). The plate was incubated at 37°C for 2 days, and the number of occurring revertants was counted.

Preparation of aqueous dialyzates from vegetables and fruits[10)]

The following kinds of fresh or freeze-dried vegetables and fruits were used in this study: broccoli, burdock, cabbage, carrot, Chinese chive, Chinese cabbage, chingentsuai, cucumber, eggplant, enokitake, green pepper, Japanese radish, kinusayaendo, komatsuna, matsutake, nigauri, onion, potato, pumpkin, spinach, sweet potato, tomato, welsh onion, amanatsu, apple, and natsudaidai. One hundred grams of each vegetable and fruit were homogenized in 40% ethanol. The homogenized samples were kept standing for 2 days at room temperature to extract their constituents as much as possible and then centrifuged or filtered, the supernatant of each filtrate being evaporated to remove the ethanol. The concentrated extracts were then dialyzed against distilled water at 4°C for 4 days to remove the low molecular weight constituents. The prepared dialyzates were applied to a desmutagenic assay after sterilizing by filtration through a millipore filter (0.45 μm).

Desmutagenic effect of dialyzates of vegetables and fruits[10,11)]

All of aqueous dialyzates of vegetables used inhibited the mutagenicity of Trp-P-2 on TA 100 and TA 98. Table 1 shows some results of the selected vegetables. Among the vegetables, the dialyzates of broccoli, burdock, cucumber, eggplant, komatsuna, green pepper and spinach showed a high desmutagenic effect. The desmutagenic activity of these dialyzates on Trp-P-2 was still retained even after heating them at 100°C for 20 min, indicating that heat-stable desmutagens exist in these dialyzates. Heated dialyzates of eggplant, spinach and burdock also inhibited the mutagenicity of MNNG, AF-2, B[*a*]P, ST, AB1 and Trp-P-1 (Table 2).

Table 1. Desmutagenic effect of the dialyzates of vegetables on Trp-P-2 toward *Salmonella typhimurium* TA 100

Vegetable and fruit	Inhibition percentage	
	Unheated	Heated
Eggplant	82.5	82.3
Broccoli	79.5	74.0
Komatsuna	77.6	75.7
Spinach	76.7	74.2
Green pepper	73.0	52.0
Cucumber	75.5	58.3
Burdock	67.8	64.6
Radish	48.3	39.3
Tomato	46.1	26.8
Onion	35.8	12.1
Cabbage	35.3	21.7
Carrot	24.5	26.3
Potato	25.3	12.3
Amanatsu	20.0	14.4
Apple	58.0	35.4
Natsudaidai	50.4	53.2
Natsudaidai pericarp	61.2	61.9
Natsudaidai envelope	41.8	37.2

Table 2. Desmutagenic effect of heated dialyzates of eggplant, spinach and burdock on several mutagens toward *Salmonella typhimurium* TA 100

Vegetable	Inhibition percentage					
	MNNG	AF-2	B[*a*]P	ST	AB1	Trp-P-1
Eggplant	29.5	59.4	76.4	66.4	89.1	86.2
Spinach	21.4	8.0	40.8	24.8	55.5	55.2
Burdock	26.9	14.7	40.8	34.5	55.5	75.2

The mutagenicity of the indirect mutagens was more strongly inhibited by those dialyzates than that of the direct mutagens. It was confirmed that the dialyzates of vegetables were not mutagenic toward TA 100. In addition to the dialyzate of edible part of eggplant and cucumber, the dialyzates prepared from their leaves also inhibited the mutagenicity of Trp-P-2. Similar high desmutagenic effects on Trp-P-2 toward TA 98 were observed in the dialyzates of leaves of sweet potato and Japanese radish and roots of spinach.

Dialyzates from the sarcocarp, pericarp or envelope of amanatsu, apple and natsudaidai also inhibited the mutagenicity of Trp-P-2 on TA 100 (Table 1). The desmutagenic activity of these dialyzates was retained after heating at 100°C for 20 min.

Partial isolation of desmutagenic principles of dialyzates spinach and apple[10,12]

The dialyzate of spinach was separated into two major fractions of SPW1 and SPW2 by a Sephadex G-100 gel-filtration (Fig. 1). Both fractions showed a desmutagenic activity on Trp-P-2 dose-dependently toward TA 98 (Fig. 1). However, SPW1 fraction had a marked killing effect on both strains. The desmutagenic SPW2 fraction was separated into four major fractions of SPW2-1, SPW2-2, SPW2-3 and SPW2-4 with a Sephadex G-25 gel-filtration (Fig. 2). Each fraction showed a desmutagenic activity on Trp-P-2 toward TA 98 (Fig. 2). The activity of SPW2-3 and SPW2-4 fractions was especially prominent. The desmutagenic activity of these fractions on Trp-P-2

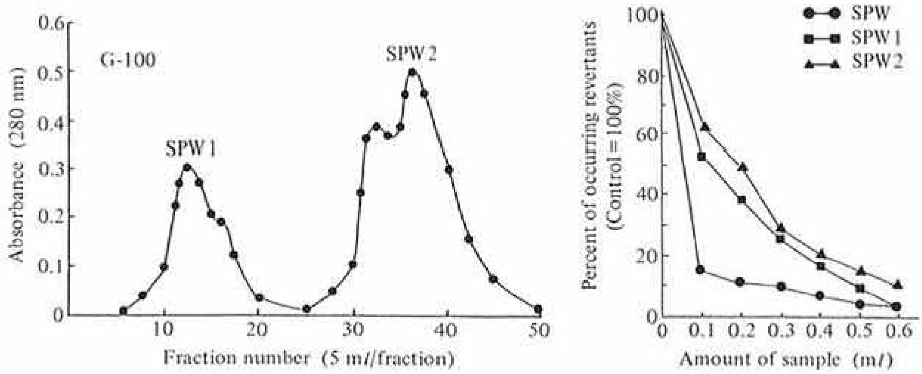

Fig. 1. Sephadex G-100 gel-filtration of dialyzate of spinach and desmutagenic effect of the fractions on Trp-P-2 toward TA 98 and TA 100

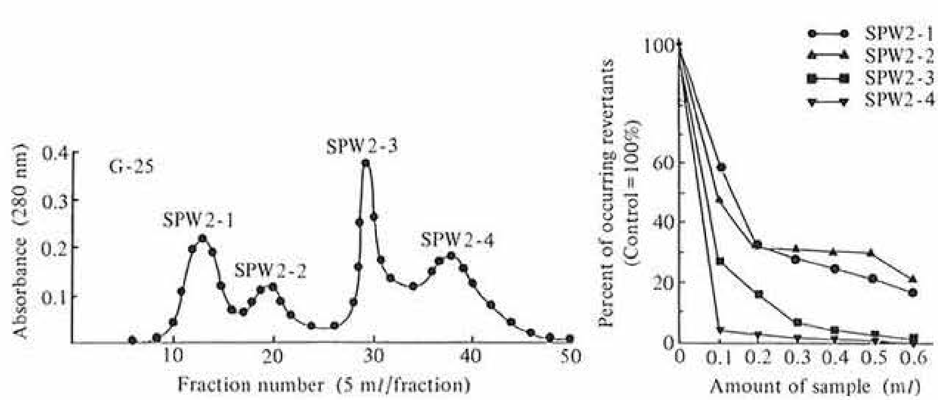

Fig. 2. Sephadex G-25 gel-filtration of SPW2 fraction and desmutagenic effect of the fractions obtained on Trp-P-2 toward TA 98

was maintained even after heating them at 100°C for 20 min. No killing and mutagenic effects on TA 98 and TA 100 were found in these fractions. All of these fractions inhibited the mutagenicity of B[*a*]P and MNNG (Table 3).

On the other hand, the heated dialyzates prepared from apple sarcocarp with 40% ethanol containing 0.1% sodium sulphite were separated into two main fractions with a Sepharose CL 6B gel-filtration column. The desmutagenic activity on Trp-P-2 and AF-2 was found in the polyphenol-rich fractions (Fig. 3). The dialyzate prepared from apple pericarp with 0.1 N HCl was separated into three peaks. Their desmutagenic effect on Trp-P-2 and AF-2 was also found in the polyphenol-rich fractions. From these results, it was concluded that the desmutagenic principles existing in aqueous dialyzates of apple were the fractions containing polyphenols with relatively high molecular weights, probably polymerized polyphenols.

Table 3. Effect of Sephadex G-25 gel-filtrated fractions of dialyzates of spinach on the mutagenicity of B[*a*]P and MNNG toward *Salmonella typhimurium* TA 100

Fraction	Percentage of occurrence His$^+$ revertants[c]	
	B[*a*]P[a]	MNNG[b]
SPW2-1	57.5	28.1
SPW2-2	73.0	37.8
SPW2-3	36.3	19.9
SPW2-4	33.1	31.3

a): In the presence of S9 mix.
b): In the absence of S9 mix.
c): The number of His$^+$ revertants in the control was 1,252 for B[*a*]P (10 μg/m*l*) and 825 for MNNG (10 μg/m*l*).

Discussion

Our present study has revealed that new des-

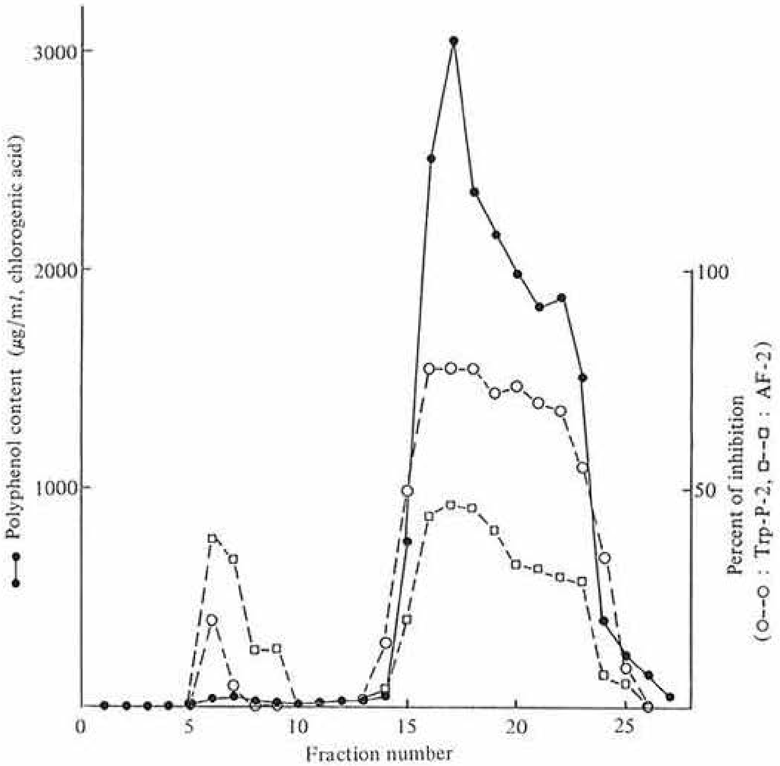

Fig. 3. Sepharose CL 6B gel-filtration of dialyzates of apple sarcocarp and desmutagenic effect of fractions on Trp-P-2 and AF-2 toward *Salmonella typhimurium* TA 100

mutagenic components on Trp-P-1, Trp-P-2, AF-2, B[*a*]P, ST, AB1 and MNNG existed in aqueous dialyzates of fresh and freeze-dried vegetables and fruits. The highest desmutagenic activity was observed in the dialyzates of spinach, cabbage and eggplant among the vegetables used in this study. It is quite noticeable that the dialyzates prepared from apple and natsudaidai pericarp, leaves of eggplant, cucumber, sweet potato and Japanese radish, and root of spinach also showed a marked desmutagenic activity on Trp-P-2. The desmutagenic activity of these dialyzates was likely to be higher than the activity of dialyzates of edible portion of those vegetables. The desmutagenic activity of these dialyzates was still retained after heating at 100°C for 20 min, although some dialyzates lost some of their desmutagenic activity after heating. This result suggests that even if vegetables and fruits are cooked by boiling, they still possess their desmutagenic effects. An aqueous dialyzate of spinach was partially separated by the gel-filtration with Sephadex G-100 and G-25 column chromatographies. Among the fractions obtained, the P2-3 and P2-4 fractions which were lately eluted on a Sephadex G-25 gel-filtration were found to have the highest desmutagenic effect on Trp-P-2 toward TA 98 and TA 100. These fractions also inhibited the mutagenicity of B[*a*]P and MNNG, suggesting that the fractions of dialyzate of spinach inhibit the direct and indirect mutagens. It was also suggested that the possible desmutagenic substances having heat-stability in the dialyzates of apple be polymerized polyphenols. It is thus clear that vegetables and fruits contain many kinds of desmutagenic constituents that can reduce the mutagenic activity of mutagens and carcinogens.

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