4-(Methylthio)-3-butenyl Isothiocyanate, a Principal Antimutagen in Daikon (*Raphanus sativus*; Japanese White Radish)

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The antimutagenic activity of *n*-hexane extracts from eight strains of daikon (*Raphanus sativus*; Japanese white radish) have been examined using the UV-induced mutation assay of *Escherichia coli* B/r WP2. A correlation was found between the potency of antimutagenicity and the amount of 4-(methylthio)-3-butenyl isothiocyanate (MTBITC) in their *n*-hexane extracts. Because the pure MTBITC also showed antimutagenicity, MTBITC is presumably the active antimutagen principle in *n*-hexane extracts of daikon. Among the eight strains of daikon studied, Aokubi, the improved common strain in Japan, contained 71.0 μmol of MTBITC in 100 g of fresh daikon. In contrast, Karami and Momoyama, which are original wild strains, contained much more MTBITC (363.5 and 168.0 μmol/100 g, respectively). In addition, phenethyl isothiocyanate was found in a lesser amount (5−33 nmol/100 g) in eight strains of daikon, and allyl isothiocyanate and benzyl isothiocyanate were not detectable in any strains (<3 nmol/100 g). The amount of total isothiocyanate in grated daikon was 7.0 times higher than that in cut daikon measured after 30 min of cooking. Through eating habits, humans might be able to consume substantial amounts of the antimutagen MTBITC from dishes using the grated form of wild strains of daikon. Therefore, it is possible to substantially increase the intake of the antimutagenic ingredient of daikon (i.e., MTBITC) by changing food preferences and preparation procedures (i.e., using the grated form of the wild strains).

Keywords: *Isothiocyanate; antimutagen; vegetable; radish; Raphanus*

INTRODUCTION

Organic isothiocyanates (R−N=C=S) are highly reactive compounds in which the central carbon atom is strongly electrophilic and is attacked by nucleophiles such as amino groups. Isothiocyanates are, therefore, capable of reacting with various cellular targets and inducing several biological responses: antimicrobial (*1*), antimutagenic (*2*), and anticarcinogenic (*3*) activities.

Although isothiocyanates are known to be present in many cruciferous vegetables and are believed to be conducive to health, the isothiocyanates are not readily found in row cruciferous vegetables but are rapidly formed from its precursor glucosinolate by myrosinase when the vegetables are wounded. The process of wounding is, therefore, very important in the formation of isothiocyanate in vegetables. Unfortunately, in general cooking practice, very few cruciferous vegetables are prepared with vigorous wounding.

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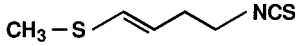

Figure 1. Chemical structure of 4-(methylthio)-3-butenyl isothiocyanate.

Daikon (Japanese white radish) has been widely used in Japanese cuisine for more than 1000 years. The Japanese people often eat grated daikon with soy source, boiled small fish, or *Nametake* (Japanese mushroom). In addition, the Japanese people usually garnish tempura (a traditional Japanese dish of deep-fried fishes and vegetables) and grilled fish with grated daikon. Daikon that has been vigorously wounded (i.e., grated) before cooking is therefore considered to be a properly prepared cruciferous vegetable.

4-(Methylthio)-3-butenyl isothiocyanate (MTBITC; the chemical structure is shown in Figure 1) has been mainly considered as a principal isothiocyanate having pungency in daikon for a long period. However, its biological effect was first identified as antimicrobial to *Escherichia coli*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, and *Aspergillus orizae* in 1982 (*4*). No other activity has been observed so far.

Recently, we found a antimutagenic activity of MTBITC in a UV-induced *E. coli* B/r WP2 mutation assay. In this study, we surveyed the antimutagenicity of four different extraction fractions (*n*-hexane, chloroform, ethyl acetate, and aqueous) of Aokubi daikon (common improved strain in Japan). We further compared the antimutagenic activities in *n*-hexane fractions between

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the common strain and seven traditional wild strains of daikon. The four isothiocyanates [MTBITC, allyl isothiocayanate (AITC), benzyl isothiocyanate (BITC), and phenethyl isothiocyanate (PEITC)] in their *n*-hexane extract were also quantitatively determined. Finally, we identified the best way to develop isothiocyanates during daikon food preparation.

MATERIALS AND METHODS

Chemicals. Allyl isothiocyanate, benzyl isothiocyanate and phenethyl isothiocyanate were purchased from Sigma Chemical Co. (St. Louis, MO). 1,2-Benzenedithiol was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 4-(Methylthio)-3-butenyl isothiocyanate was extracted and purified from the root of daikon (*Raphanus sativus*) according to the method described previously with a slight modification (*5*). The purity of the MTBITC was 94.3% as estimated by HPLC.

Vegetables. Daikon (*R. sativus*) were harvested in November or December 1999 in open-field culture at the Kyoto Prefectural Agricultural Research Institute, Japan. We used eight strains of daikon: Aokubi, Karami, Kuki, Momoyama, Nezumi, Sabaga, Shogoin, and Tokinashi.

Extraction and Preparation of Sample from Daikon. A whole root of daikon was washed with water, peeled, and then grated with a daikon grater or cut into 2 cm cubes. After 30 min of incubation, an aliquot of the grated daikon (100 g) was extracted with methanol (100 mL, three times). In the case of cubed daikon, it was homogenized in methanol and then extracted. The methanol extracts were combined and evaporated to 100 mL of crude aqueous extract. The extract was combined, and 3 mL of 1 M HCl was added to avoid degradation of isothiocyanates in alkaline pH; the mixture was partitioned three times with *n*-hexane, chloroform, and ethyl acetate, in that order, with 100 mL of solvent each time. The *n*-hexane, chloroform, and ethyl acetate layers were evaporated to dryness at <35 °C, and the aqueous layer was lyophilized.

Measurement of Total Isothiocyanate Content. The level of total isothiocyanate was measured by using the method of Zhang et al. with a slight modification (*6*). In brief, 50 μL of *n*-hexane extract from daikon was diluted with the mixture of 0.45 mL of methanol and 0.45 mL of 50 mM $Na_2B_4O_7$−HCl buffer (pH 8.5), and then 50 μL of 8 mM 1,2-benzenedithiol was added and mixed well in a 1.5 mL plastic tube. The tube was heated at 65 °C for 1 h, and then the isothiocyanate content was calculated by reading the sample absorption at 365 nm from a linear standard equation derived from the absorption readings of a serial dilution of known phenethyl isothiocyanate concentrations.

Measurement of AITC, BITC, MTBITC, and PEITC Contents. AITC, BITC, MTBITC, and PEITC in *n*-hexane extracts were measured by gas chromatography on a Shimadzu (Kyoto, Japan) model GC-12A instrument with a flame ionization detector (FID-GC). The operating conditions for gas chromatography were as follows: injection volume, 1.0 μL; injector and detector temperature, 250 °C; DB-5 (25 m × ∼0.2 mm, 0.33 μm film thickness; J&W Scientific, Folsom, CA); column temperature, 70 °C for 70 s increased to 170 °C at 3 °C/min; carrier gas, 180 kPa He; injection, splitless (closed for 70 s).

Assay for Antimutagenicity. The assay was carried out as described previously (*7*). In brief, UV-irradiated (254 nm; 20 J/m^2) cell suspensions (∼1.5 × 10^9 cells/mL) of *E. coli* B/r WP2 *trpE65* were diluted with PBS 10 and 1 × 10^6 times the original concentration and plated to detect revertants and survivors, respectively. Fifty microliters of extract sample solution dissolved in dimethyl disulfoxide (DMSO), 0.2 mL of UV-irradiated and diluted cells, and 2 mL of 0.7% molten top agar were mixed well in 0.5 mL of PBS and poured onto semi-enriched minimal agar medium (SEM). The numbers of revertants and survivors were counted as colony-forming units on the same organized SEM plates after incubation at 37 °C for 2 days. Antimutagenicity was expressed as relative mutagenic activity (RMA, percent of control) and calculated using the formula

$$[(m/M)/(s/S)] \times 100$$

where m is the number of revertant colonies in the presence of the test sample, M is the number of revertant colonies in the absence of the test sample, s is the number of surviving colonies in the presence of the test sample, and S is the number of surviving colonies in the absence of the test sample. IC_{50} (the concentration needed to achieve an RMA of 50%) values and 95% confidence intervals were calculated from a linear regression derived from at least 15 points taken over five doses.

Table 1. IC_{50} of Aokubi Daikon Extracts on UV-Induced Mutation in *E. coli* B/r WP2[a]

fraction	yield[b] (mg)	IC_{50} (μg/plate)	yeild (μg)/IC_{50} (μg/plate)
n-hexane	32	20.2 ± 7.7	1584
chloroform	66	72.0%; 100[c] μg/plate	—[d]
ethyl acetate	101	124.3 ± 9.4	813
aqueous	1389	55.3%; 1000[c] μg/plate	—[d]

[a] Each value represents average ± 95% confidence interval of three individual samples. [b] Dry weight of each fraction extracted from 100 g of fresh daikon. [c] Value of lowest RMA shown at the dose. [d] Unable to calculate. IC_{50} is not shown in the fraction.

RESULTS

Antimutagenicity of Daikon Extracts. First, we examined the antimutagenicity of the four different solvent fractions (*n*-hexane, chloroform, ethyl acetate, and aqueous) of Aokubi daikon, a common improved strain in Japan. The antimutagenicity is evaluated by determining the RMA, that is, the mutagenic activity adjusted by sample toxicity. The sample was considered as strongly antimutagenic when it showed <50% of RMA. On the basis of this criterion, extracts in *n*-hexane and ethyl acetate fractions were considered as strongly antimutagenic, but extracts in chloroform and aqueous fractions were not (Table 1). The IC_{50} values, that is, the concentration needed to achieve an RMA of 50%, are 20.2 μg/plate for the *n*-hexane extract and 124.3 μg/plate for the ethyl acetate extract. The yields, that is, the dry weight of each fraction extracted from 100 g of fresh body of daikon, are 32 mg for the *n*-hexane extract and 101 mg for the ethyl acetate extract. The fraction showing the higher value of yield/IC_{50} is supposed to be more significant. Therefore, we focused on the most significant fraction, the *n*-hexane extract of daikon in the present study. The antimutagenicity was significantly higher in the *n*-hexane extract from Aokubi daikon at 30 min than at 0 and 60 min of incubation after grating (data not shown). The antimutagenicity was therefore detected in the *n*-hexane extract of daikon at 30 min of incubation after grating in this study.

Next, we compared the activities of the *n*-hexane fraction among common and seven kinds of traditional wild strains of daikon in order to identify strains possesses high antimutagenic compounds. The morphology of typical strains of daikon is shown in Figure 2. Aokubi (A) and Sabaga (D) are cone-shaped, whereas Kuki (B) and Momoyama (C) show cylindrical shapes. In contrast, Karami (E) and Shogoin (F) are globular in shape. Tokinashi and Nezumi (not shown in Figure 2) are morphologically similar to Aokubi (A) and Momoyama (C), respectively. When the amount of *n*-hexane extract from 0.1 g of fresh daikon was added into a plate to detect antimutagenicity, Karami, Momoyama, and

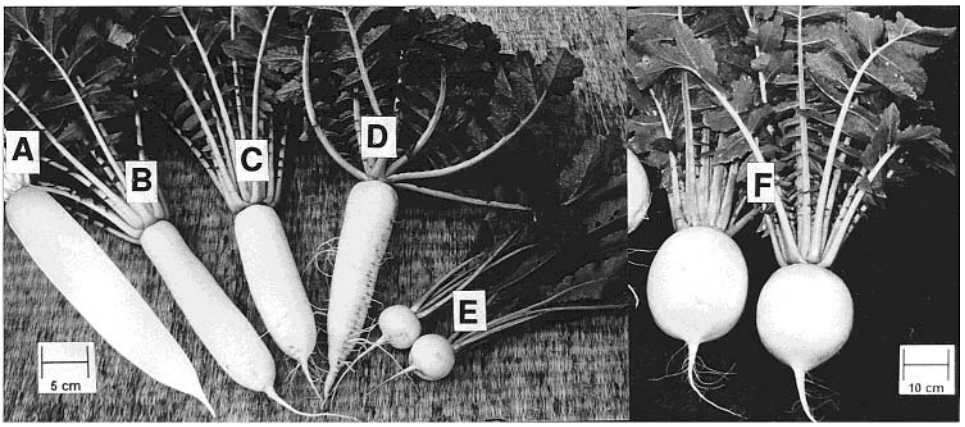

Figure 2. Morphological differences of six strains of daikon: (A) Aokubi; (B) Kuki; (C) Momoyama; (D) Sabaga; (E) Karami; (F) Shogoin.

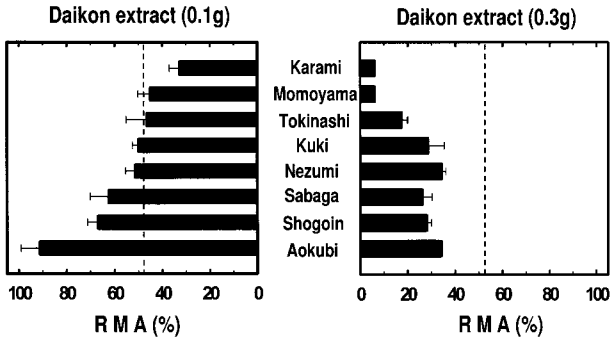

Figure 3. Antimutagenicity of *n*-hexane extract from eight strains of daikon. Cell suspensions of *E. coli* in a Petri dish were irradiated with a germicidal lamp at a dose of 20 J/m^2. The cell suspension (3×10^7 cells for mutant cell detection; 3×10^2 cells for viable cell detection) was poured onto an SEM plate with soft agar containing 0.1 or 0.3 g of daikon extract. The numbers of mutant cells and viable cells were determined by colony formation on SEM plates. Each value represents the mean of duplicate plates in three experiments. The numbers of mutant colonies and survival colonies were 264 ± 17 and 271 ± 9 in UV-irradiated cells.

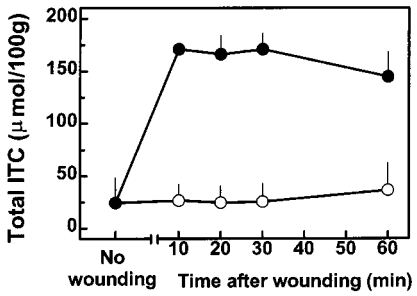

Figure 4. Change of total ITC in Shogoin daikon by wounding. For quantification of total ITC, 50 μL of the diluted *n*-hexane extract was reacted with 50 μL of 8 mM 1,2-benzenedithiol at 65 °C for 1 h, and then the absorption of the reactant was calculated at 365 nm. The amount of total ITC was measured at 10, 20, 30, and 60 min after Shogoin daikon had been grated (●) or cut into 2 cm cubes (○). Each point represents mean with range (vertical error bar) of two experiments.

Tokinashi strains showed strong antimutagenicity with RMA values of 32.8, 45.1, and 46.7%, respectively (Figure 3). However, Aokubi, Shogoin, Sabaga, Nezumi, and Kuki did not show striking antimutagenicity, as their RMA values were >50%. When the amount was increased to 0.3 g, all daikon strains showed strong antimutagenic activities, in particular, both Karami and Momoyama strains, which had RMA values of 6.1% (Figure 3). All samples tested were not cytotoxic at the dose used. From these results, Karami and Momoyama strains were found to have significantly higher antimutagenic activities.

Measurement of Isothiocyanates. First, we determined the preparation that provided the maximum level of total ITC in daikon. The amount of total ITC was quantified in daikon prepared according to different commonly used methods in Japanese cuisine, that is, grating and cutting. Shogoin daikon is well adapted to grating and cutting forms of dishes in Japanese cuisine; therefore, we used Shogoin daikon here. To see the change of the level of total ITC after daikon had been grated or cut into 2 cm cubes, total ITC was measured at 10−60 min after Shogoin daikon had been grated. Without wounding, 100 g of shogoin daikon contained only 24.4 μmol of total ITC, due to the lack of myrosinase activity (Figure 4). At 10 min after grating, the level increased to 170.3 μmol. The level of total ITC remains unchanged until 30 min after grating. It started to decrease slightly over the next 30 min. On the other hand, the level of total ITC did not significantly increase until 60 min after the daikon had been cut into 2 cm cubes. At 30 min after grating, the total ITC level was 7.0 times higher than that without wounding. In contrast, the amount of total ITC changed slightly in 60 min after cutting. The level was only 1.5 times higher than the level without wounding. Therefore, we measured isothiocyanates in daikon at 30 min after grating in the present study.

Next, we measured the amount of isothiocyanates in *n*-hexane extracts of grated daikon. MTBITC was found within the range of 71.0−363.5 μmol/100 g of fresh daikon at 30 min after grating (Table 2). Karami and Momoyama strains showed high contents at 363.5 and 168.0 μmol, respectively. In contrast, the amount of MTBITC was 71.0 μmol/100 g in the common improved strain, Aokubi. Karami and Momoyama strains contain MTBITC at levels 5.1 and 2.4 times higher than that of Aokubi, respectively. Total ITC was found within the range of 91.2−389.8 μmol/100 g. Karami and Momoyama strains also showed high total ITC levels at 389.8 and 205.3 μmol, respectively. Of the total ITC, 61.1% (in Sabaga) to 93.2% (in Karami) of isothiocyanate in the *n*-hexane extract was determined to be MTBITC. The Momoyama strain also contains a high level of MTBITC in total ITC (81.8%). On the other

Table 2. Levels of Isothiocyanate in *n*-Hexane Extract from Eight Strains of Daikon[a]

	μmol/100 g		nmol/100 g		
strain	total ITC	MTBITC	PEITC	AITC	BITC
Karami	389.8 ± 42.0	363.5 ± 75.5	20 ± 15	<3	<3
Momoyama	205.3 ± 21.9	168.0 ± 34.0	9 ± 4	<3	<3
Kuki	167.1 ± 6.2	111.3 ± 10.0	33 ± 8	<3	<3
Tokinashi	166.2 ± 20.2	128.3 ± 25.2	6 ± 2	<3	<3
Nezumi	156.9 ± 11.0	131.4 ± 17.0	9 ± 2	<3	<3
Shogoin	147.5 ± 13.1	124.6 ± 12.4	20 ± 2	<3	<3
Sabaga	123.6 ± 14.6	75.5 ± 18.2	7 ± 6	<3	<3
Aokubi	91.2 ± 7.9	71.0 ± 1.3	5 ± 2	<3	<3

[a] A whole root of daikon grated and incubated for 30 min was extracted with methanol and then partitioned with *n*-hexane. For total isothiocyanate (total ITC), 50 μL of the diluted *n*-hexane extract was reacted with 50 μL of 8 mM 1,2-benzenedithiol at 65 °C for 1 h, and then the absorption of the reactant was calculated at 365 nm. Each isothiocyante (MTBITC, PEITC, AITC, and BITC) was analyzed with FID-GC. Each value represents average ± SE of three to six individual samples.

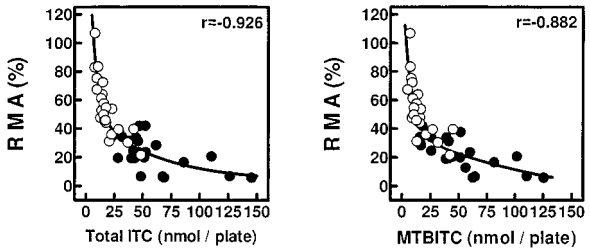

Figure 5. Correlation between antimutagenicity and isothiocyanate contents: (○) 0.1 g of daikon extract; (●) 0.3 g of daikon extract. For quantification of total ITC, 50 μL of the diluted *n*-hexane extract was reacted with 50 μL of 8 mM 1,2-benzenedithiol at 65 °C for 1 h, and then the absorption of the reactant was calculated at 365 nm. MTBITC was analyzed with FID-GC. The correlation plots were applied with two site binding hyperbola. The correlation was observed between RMA and total ITC content ($r = -0.926$) or MTBITC content ($r = -0.882$). Each point represents the mean of duplicate plates in one experiment.

hand, PEITC was a very minor isothiocyanate in daikon, ranging from 5 to 33 nmol/100 g. AITC and BITC were not detected in any of the strains tested (<3 nmol/100 g). From these results, the principal isothiocyanate in *n*-hexane extract of daikon was found to be MTBITC.

Correlation between Antimutagenicity and Isothiocyanate Contents. Experiments were carried out on the correlation between antimutagenicity and amounts of MTBITC. Figure 5 shows a correlation plot adapted with two site binding hyperbola between the amount of total ITC or MTBITC in eight strains of daikon and their RMAs. The RMA is shown as a function of the dose of *n*-hexane extract from 0.1 g (open circle) and 0.3 g (solid circle) of fresh daikon for one assay plate. A dose response was seen between the antimutagenicity and the amount of MTBITC ($r = -0.882$). Furthermore, a similar dose response curve was shown between the antimutagenicity and the amount of total ITC ($r = -0.926$). From these results, MTBITC appears to be the most likely antimutagenic principle in the *n*-hexane extracts of daikon.

Antimutagenicity of MTBITC and PEITC. The antimutagenicity of MTBITC was indeed confirmed by experiments shown in Figure 6. MTBITC purified from Aokubi daikon was antimutagenic at the dose range of 60−2000 nmol/plate without toxicity. The IC_{50} of MTBITC was 302.0 nmol/plate. PEITC also had an antimutagenic activity, and its IC_{50} was 60.3 nmol/plate, which is 5.0 times stronger than that of MTBITC.

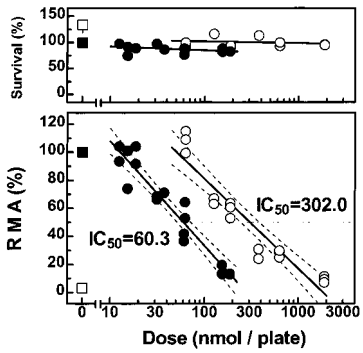

Figure 6. Antimutagenicity of MTBITC and PEITC on UV-induced mutation in *E. coli* B/r WP2. Cell suspensions of *E. coli* in a Petri dish were irradiated with a germicidal lamp at a dose of 20 J/m^2. The cell suspension (3×10^7 cells for mutant cell detection; 3×10^2 cells for viable cell detection) was poured onto an SEM plate with soft agar containing MTBITC or PEITC. The numbers of mutant cells and viable cells were determined by colony formation on SEM plates. Each value represents the mean of duplicate plates in one experiment. The numbers of mutant colonies and survival colonies were 245 ± 10 and 262 ± 12 in UV-irradiated cells. A linear regression and its 95% confidence interval were expressed with solid and dotted lines: (□) negative control (without UV irradiation); (■) positive control (with UV irradiation); (○) MTBITC; (●) PEITC.

DISCUSSION

We have found an antimutagenic activity of MTBITC in the *n*-hexane extraction fraction of daikon (improved strain Aokubi), a Japanese white radish, in a UV-induced mutation assay of *E. coli* B/r WP2. We first observed the antimutagenic activity in the *n*-hexane extraction fraction of daikon (Table 1). Then a correlation was found between the antimutagenicity and the amounts of MTBITC in the extraction fraction (Figure 5). Finally, we confirmed the antimutagenicity of pure MTBITC (Figure 6). There are more than 20 kinds of daikon strains in Japan. In particular, in Kyoto Prefecture, which is near the center of the Japan archipelago, various wild traditional strains of daikon are available, because Kyoto was the ancient capital of Japan for a long period and many novel strains of vegetables were gathered for an offering to the emperor. Some of them have been well preserved in Kyoto. Here we focused our study on seven wild traditional strains harvested in Kyoto and compared the antimutagenicities of their *n*-hexane extracts. Among eight strains of daikon (seven wild and one improved strains), there is a 5.1-fold difference in MTBITC content between Aokubi (lowest) and Karami (highest) (Table 2). The Momoyama strain also contains MTBITC at a level 2.4 times higher than that in Aokubi. Thereby, the *n*-hexane extracts of Karami and Momoyama strains show significantly higher antimutagenic activities than other strains (Figure 3), and their activities are correlated with the amount of MTBITC (Figure 5). In addition, all seven traditional wild strains contain higher amounts of MTBITC than the improved strain, Aokubi. These seven wild strains are called "*Kyo-yasai*", meaning traditional vegetables in Japanese. Recently some *Kyo-yasai* have been reported to be superior to their counterpart of common improved vegetables in Japan with regard to antimutagenicity (*7*). One of the reasons considered is

that *Kyo-yasai* have been carefully preserved without selection for breeding for more than 300 years, so they retain their original phenotypes including the stronger antimutagenicity (*7*). The same reason applies to daikon, as a preference for the milder taste and the avoidance of a strong pungent taste caused by MTBITC in daikons by the consumer might result in the selective breeding of the present improved strain.

Compounds that decrease gene mutation are generically called antimutagens. One of them may suppress the change of a premutagen to a mutagen. Another may degrade a mutagen chemically or enzymatically before the mutagen reaches DNA in cells. After DNA lesion is caused by a mutagen, some compounds (i.e., bioantimutagens) may increase the level of error-free DNA repair or increase the opportunity for DNA repair by delaying DNA replication and mutation fixation, so the cell can be repaired to become normal again (*8*). In the present assay, MTBITC showed antimutagenicity when it was exposed to cells right after UV irradiation. Therefore, MTBITC is considered to be a bioantimutagen. Bioantimutagens identified so far are <10% of antimutagens. However, a bioantimutagen food factor that can act even after a cell has a DNA lesion is as important as other antimutagens.

The *n*-hexane extract from cruciferous vegetables usually contains oleaginous compounds. Some isothiocyanates can be also extracted with *n*-hexane. Typical isothiocyanates in *n*-hexane extracts from cruciferous vegetables are allyl, benzyl, and phenethyl isothiocyanates, and they are well investigated for cancer chemopreventive and chemotherapeutic effects (*9*, *10*). In contrast, MTBITC has not been studied for any effects against cancer or antimicrobial effects (*4*) and should be investigated in the future. Comparison of the IC_{50} values of MTBITC and PEITC for antimutagenicity shows that the activity of PEITC is ~5 times stronger than that of MTBITC (Figure 6). However, the amount of MTBITC in daikon is ~3000−20000 more than that of PEITC (Table 2). If we focus only on PEITC, daikon might not be considered a valuable vegetable on the basis of antimutagenicity. It should be noted that (−)-(1*R**,3*S**,3′*R**)-1-(2′-pyrrolidinethione-3′-yl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, which formed from L-tryptophan and MTBITC in fermented daikon, showed mutagenicity within the range of 0.63−3.77 μmol/plate in *S. typhimurium* TA98 with S9 mix (*11*). However, the dose is greater than the IC_{50} of MTBITC (0.3 μmol/plate), and the mutagenic risk is therefore supposed to be lowered in a bacterial assay. In addition, the IC_{50} of the crude *n*-hexane fraction (20.2 μg/plate) was lower than that of MTBITC alone (48.0 μg; 302 μmol/plate) (Table 1 and Figure 6). It was supposed that a synergistic component was contained in the *n*-hexane fraction for MTBITC.

Grated daikon has been widely used in Japanese cuisine. For instance, grated daikon is usually served with tempura, a Japanese deep-fried food. Grated Karami daikon has been used for seasoning instead of Wasabi (Japanese horseradish) with "*Soba-tsuyu*", a fish broth for Japanese soba noodles to provide pungency. Despite a high content of MTBITC, Karami daikon is not popular due to its much stronger pungency compared with other daikons. In contrast, the pungency of the Momoyama daikon is not as severe as that of the Karami daikon, despite a high amount of MTBITC. Momoyama daikon, therefore, might be recommended to provide significant amounts of MTBITC in the diet. The pungency of these vegetables can be also alleviated by adding a couple of drops of rice vinegar. Therefore, a person can easily ingest 100 g or more of daikon in this manner. The level of total ITC was constant until 30 min after grating in this study (Figure 4). It has been reported that MTBITC started degrading at 15−30 min after incubation with L-ascorbic acid to form a yellow pigment, 2-thioxo-3-pyrrolidinecarbaldehyde (*12*, *13*). These findings suggest that daikon should be eaten within 30 min after grating to provide the maximum amount of MTBITC.

Finally, because this study is based on a bacterial antimutagenic assay, the results should be verified using mammalian and human cell mutation assays with different physical and chemical mutagens. This and the cancer chemopreventive effect of MTBITC will be studied in the future.

ABBREVIATIONS USED

AITC, allyl isothiocyanate; BITC, benzyl isothiocyanate; MTBITC, 4-(methylthio)-3-butenyl isothiocyanate; PEITC, phenethyl isothiocyanate; total ITC, total isothiocyanate in *n*-hexane extract; RMA, relative mutagenic activity.

LITERATURE CITED

(1) Hashem, F. A.; Saleh, M. M. Antimicrobial components of some Cruciferae plants. *Phytother. Res.* **1999**, *13*, 329−332.

(2) Hamilton, S. M.; Teel, R. W. Effects of isothiocyanates on cytochrome P-450 1A1 and 1A2 activity and on the mutagenicity of heterocyclic amines. *Anticancer Res.* **1996**, *16*, 3597−3602.

(3) Hecht, S. S. Chemoprevention of cancer by isothiocyanates, modifiers of carcinogen metabolism. *J. Nutr.* **1999**, *129*, 768S−774S.

(4) Esaki, H.; Onozaki, H. Antimicrobial action of pungent principles in radish root. *Eiyo To Shokuryo* **1982**, *35*, 207−211 (in Japanese).

(5) Esaki, H.; Onozaki, H. Colorimetric determination of pungent principles in radish root. *Eiyo To Shokuryo* **1980**, *33*, 161−167 (in Japanese).

(6) Zhang, Y.; Cho, C.-G.; Posner, G. H.; Talalay, P. Spectroscopic quantitation of organic isothiocyanates by cyclocondensation with vicinal dithiols. *Anal. Biochem.* **1992**, *205*, 100−107.

(7) Nakamura, Y.; Suganuma, E.; Kuyama, N.; Sato, K.; Ohtsuki, K. Comparative bio-antimutagenicity of common vegetables and traditional vegetables in Kyoto. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1161−1165.

(8) Kada, T.; Inoue, T.; Namiki, M. Environmental desmutagens and antimutagens. In *Environmental Mutagenesis, Carcinogenesis and Plant Biology*; Klekowski, E. J., Jr., Ed.; Praeger Scientific: New York, 1981; pp 132−151.

(9) Hirose, M.; Yamaguchi, T.; Kimoto, N.; Ogawa, K.; Futakuchi, M.; Sano, M.; Shirai, T. Strong promoting activity of phenylethyl isothiocyanate and benzyl isothiocyanate on urinary bladder carcinogenesis in F344 male rats. *Int. J. Cancer* **1998**, *77*, 773−777.

(10) Hecht, S. S.; Kenney, P. M.; Wang, M.; Trushin, N.; Upadhyaya, P. Effects of phenethyl isothiocyanate and benzyl isothiocyanate, individually and in combination, on lung tumorigenesis induced in A/J mice by benzo[*a*]-pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Lett.* **2000**, *150*, 49−56.

(11) Ozawa, Y.; Uda, Y.; Matsuoka, H.; Abe, M.; Kawakishi, S.; Osawa, T. Occurrence of stereoisomers of 1-(2′-

pyrrolidinethione-3′-yl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid in fermented radish roots and their different mutagenic properties. *Biosci., Biotechnol., Biochem*. **1999**, *63*, 216−219.

(12) Ozawa, Y.; Uda, Y.; Ohshima, T.; Saito, K.; Maeda, Y. Formation of yellow pigment by the reaction of 4-methylthio-3-butenyl isothiocyanate with L-ascorbic acid and some dihydroxyphenolic compounds. *Agric. Biol. Chem*. **1990**, *54*, 605−611.

(13) Uda, Y.; Ozawa, Y.; Ohshima, T.; Kawakishi, S. Identification of enolated 2-thioxo-3-pyrrolidinecarbaldehyde, a new degradation product of 4-methylthio-3-butenyl isothiocyanate. *Agric. Biol. Chem*. **1990**, *54*, 613−617.

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