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Bioactivities of Sulfur Compounds in Cruciferous Vegetables

Mee Ree Kim

Department of Food Science and Nutrition, Chungnam National University,
Daejeon 305-764, Korea. E-mail: mrkim@cnu.ac.kr

ABSTRACT

Cruciferous vegetables are rich in organosulfur compounds such as isothiocyanates and sulfides. While the isothiocyanates, corresponding to pungent principle, are generated from myrosinase-catalyzed hydrolysis of glucosinolates, the sulfides can be generated non-enzymatically. Recent studies provide evidences that some sulfur compounds in these vegetables show a chemopreventive action against carcinogenesis; while isothiocyanates such as sulforaphane induce phase 2 enzymes (glutathione S-transferase/quinone reductase), disulfides tends to elevate the level of phase 1 and 2 enzymes. Especially, sulforaphane rich in Cruciferae vegetables has been reported to express anticarcinogenic effect in some organs such as liver, kidney or intestine. When the level of sulfur compounds in Cruciferous and Alliaceous vegetables was determined by GC/MS (SIM), the richest in sulforaphane is broccoli followed by turnip, cabbage, radish, kale, cauliflower and Chinese cabbage. Meanwhile, the sulfides are predominant in Alliaceous vegetables such as onion. In related study, the administration of vegetable extract elevated the GST level by 1.5 fold for broccoli, 1.4 fold for radish, and 1.3 for onion. Thus, the vegetables frequently used in Korean dish contain relatively high amount of anticarcinogenic sulfur compounds. Moreover, the combination of broccoli and radish extracts elevated the GST induction up to 1.84 folds of control. In addition, the Kakdugi, fermented radish Kimchi was observed to show a comparable GST induction despite the decomposition of methylthio-3-butenylisothiocyanate (MTBI). Therefore, the combination of vegetables including broccoli, and fermented radish Kimchi would be useful as a functional food for chemoprevention.

Key words: bioactivity, sulfur compounds, cruciferous vegetables

INTRODUCTION

Cruciferae vegetables, such as broccoli, cabbage, Brussels sprouts, cauliflower, bok choy, kale, mustard seed and radishes have characteristic flavor, which are derived from isothiocyanates, nitriles or sulfides. Most of these organo sulfur compounds are derived from glucosinolate (Fenwick et al., 1983).

Glucosinolates

Glucosinolate is named trivially sinigrin, consisiting of β-D-thioglucose group, a sulfonated oxime group and a side chain derived from amino acid. Glucosinolates, sequestered within the intact vegetable tissue, are released by physical damage such as chopping or chewing, and then exposed to the enzyme myrosinase, and finally hydrolyzed to isothiocyanates (R-N=C=S), nitriles and thiocyanate ions. This reaction is responsible for the development of the sharp taste of horseradish, mustard and radish (MacLeod, 1976). The Cruciferous vegetables contain different kinds of R groups in glucosinolates (Fenwick et al., 1983; Fahey et al., 2001). The major glucosinolates in the cruciferae are as shown in Table 1. The amount of glucosinolates varies greatly within and between crucifer

Table 1. Major glucosinolates and their hydrolysis products in Cruciferous vegetables

Glucosinolate	Natural abundance	Isothiocyanate	Nitrile
Glucoraphanin	Broccoli	Sulforaphane	Sulforaphane nitrile
Gluconasturtiin	Chinese cabbage, radishes, watercress	Phenethyl isothiocyanate	
Sinigrin	Brussels sprouts, cabbage, cauliflower	Allyl isothiocyanate	
Glucobrassicin	All crucifers	Indole-3-carbinol (3-Indolymethylglucosinolate)	
Progoitrin	Cramble (oil seed)	Crambene	

species. Glucobrassicin and glucoraphanin, generally found in high concentrations in broccoli, constitute as much as 30~65% of the total amount of glucosinolates (Kushhad et al., 1999). In contrast, Brussels sprouts, cabbage, and cauliflower contain little or no glucoraphanin. Crucifers, other than broccoli, generally contain high concentrations of sinigrin. Gluconasturtiin is abundant in Chinese cabbage, radishes, and watercress (Fenwick et al., 1983). Within the same species, plant tissue and environmental condition also affect glucosinolate concentration. Seeds often contain greater glucosinolate concentrations than the edible portion of the plant. Skin of radish contains higher amount glucosinolate than the inner part (Kim, 1988). Glucosinolate content is influenced by environmental factors such as cultivation, climate, and soil conditions (Josefsson, 1967). Indolyl glucosinolate, such as glucobrassicin, are affected primarily by environmental condition, whereas aliphatic glucosinolate, such as glucoraphanin, are primarily under genetic control (Brown et al., 2001). Non-enzymatic degradation may be caused by treatments such as cooking of cruciferous vegetables, which reduces the levels of glucosinolates remarkably.

Generation of isothiocyanates from glucosinolates

Isothiocyanates are one of hydrolysis product of glucosinolates, and some of isothiocyanates are known to be bioactive; sulforaphane (SF), allyl isothiocyanate (AI), phenylether isothiocyanate (PEI), indole-3-carbinol (I3C) and 4-methylthio-3-butenyl isothiocyanate (MTBI).

Myrosinase Myrosinase (thioglucoside glucohydrolase, E.C. 3.2.3.1.) is a group of enzymes which utilize various kinds of glucosinolates as substrates. The enzyme requires optimum condition for maximal activity. Myrosinase purified from radish harvested in Korea, showed an optimum pH of 6.5 and was stable at pH 6 to 7 at room temperature, but unstable below pH 4. The enzyme possessed an optimum temperature of 37°C, and gave a Vmax value of 40moles/mg min and a Km value of 0.12 mM for sinigrin. The purified myrosinase was activated at low conc. (<1 mM) of ascorbic acid with maximum activation at 0.6 mM, but somewhat inhibited by more than 2 mM ascorbic acid (Kim et al., 1989). The specific activity of myrosinase differs between plant tissues or species (Josefsson, 1967; Fenwick et al., 1983). Radish skin shows higher specific activities than inner part. Broccoli converts 80% to 90% of glucoraphanin to sulforaphane and 20% to SF (Matusheski et al., 2001) but daikon radish myrosinase converts almost all of glucoraphanin to sulforaphane (Zhang et al., 1992).

Factors affecting enzymatic hydrolysis of glucosinolates The hydrolysis of glucosinolate depends on many factors including the plant species and cultivar, the site of hydrolysis (inside the plant or in the gut), the tissue (skin vs. central part) of plant, ascorbate level and the environmental conditions (temperature, pH, moisture). Total glucosinolate in radish was estimated to range from 200~450 μmoles/100 g by coupled enzymes assay (Kim and Rhee, 1992). Quantitative determination of MTB-glucosinolate by RP-HPLC assay combined with myrosinase pretreatment showed that the peeled radishes contained about 200 μmoles/100 g and skin around 275 μmoles/100 g (Kim, 1988).

Isothiocyanates

Physiological and flavor properties Pungent or biting taste and flavor of cruciferous vegetables are derived from isothiocyanates (MacLeod, 1976). The pungent flavor of mustard seeds is largely due to allyl isothiocyanate. Aromatic isothiocyanates may provide much of the taste of horseradish. Broccoli flavor is from suforaphane. Phenylethyisothiocyanate (PEI) is the main isothiocyanate of Chinese cabbage. Pungent and biting taste of radish is mainly due to 4-methylthio-3-butenyl isothiocyanate (MTBI), constituting about 85% of the total isothiocyanates (Kim and Rhee, 1985)

Amount and stability of isothiocyanates in vegetable extracts The amount of isothiocyanate produced from inherent glucosinolates in vegetable homogenates depends on mainly the activity of myrosinase. Cooking process such as heating inactivates the enzyme myrosinase activity or decomposes glucosinolates. In an attempt to see the change of MTBI in radish, we developed RP-HPLC method to quantitate (Kim and Rhee, 1986). RP-HPLC assay (65% CH3CN) was proved to be convenient, precise (<3% error) and reproducible, showing a good linearity between 10 nmoles/mL and 120 nmoles/mL. MTBI was stable in the organic solvent, but was hydrolyzed slowly in the basic medium and decomposed rapidly in the acidic aqueous medium. RP-HPLC analysis and the DTNB color reaction indicate that the decomposition of MTBI in the aqueous medium leads to the formation of methanethiol and a polar product (λ max=269 nm). The amount of MTBI formed in the radish homogenate under optimum condition (pH 8.5, 1 min) corresponds to 210~420 μmoles/100 g, which differs between cultivars and harvest season. MTBI in radish was higher in small cultivars than large ones (Kim et al., 1994). Meanwhile, in cooked radish, 4-methylthiobutyl isothiocyanate, the stable isothiocyanates is the most predominant. In Kimchi, the amount of MTBI decreased to 68%, 21%, 5% and zero after 1 day, 2 days, 3 days, and 4 days of fermentation at 20°C, in accordance with the gradual decrease of pungency during fermentation (Kim and Rhee, 1993). This was ascribed to the decomposition of glucosinolate and the reduction of myrosinase activity, which was caused by the lowered pH during fermentation (Kim and Rhee, 1993).

Nutrakinetics: Absorption and metabolism

In cruciferous plants, the content of glucosinolates is relatively high, approaching 1% or more of their dry weight (Rosa et al., 1997). Glucosinolate consumption is estimated to be as high as 300 mg/d (~660 μmol/d) (ILSI, 1999). The average daily consumption of allyl isothiocyanate from a typical diet is in the range 2~7 mg (Fenwick et al., 1983). Isothiocyanates are known to be absorbed through GI tract of humans. Consumption of 10 g and 20 g mustard led to the urinary excretion of 5.4 mg and 12.8 mg, respectively, of allyl isothiocyanate during the first 12 hours after consumption (Jiao et al., 1994). *In vivo*, isothiocyanates are conjugated with glutathione and then sequentially metabolized to mercapturic acids according to glutathione detoxification pathway (Fig. 1). These metabolites are collectively designated dithiocarbamates. In human urine after oral consumption, about 50 percent of benzyl isothiocyanate was excreted as a N-acetylcysteine conjugate. The N-acetylcysteine conjugate of phenethyl isothiocyanate in the urine is known to be a good marker (Chung, 1992). Thorough chewing of fresh vegetables exposes the glucosinolates to plant myrosinase and significantly increases dithiocarbamate excretion. Recent study indicates that humans can convert glucosinolates to isothiocyanates through the action of the microflora of the gastrointestinal tract (Shapiro et al., 2001; Getahun et al., 1999), suggesting that glucosinolates in cooked vegetables, lacking in myrosinase activity, can be converted to isothiocyanate in gut.

Bioactivities

Many studies report a strong inverse relationship between the intake of crucifers and the risk for many cancers.

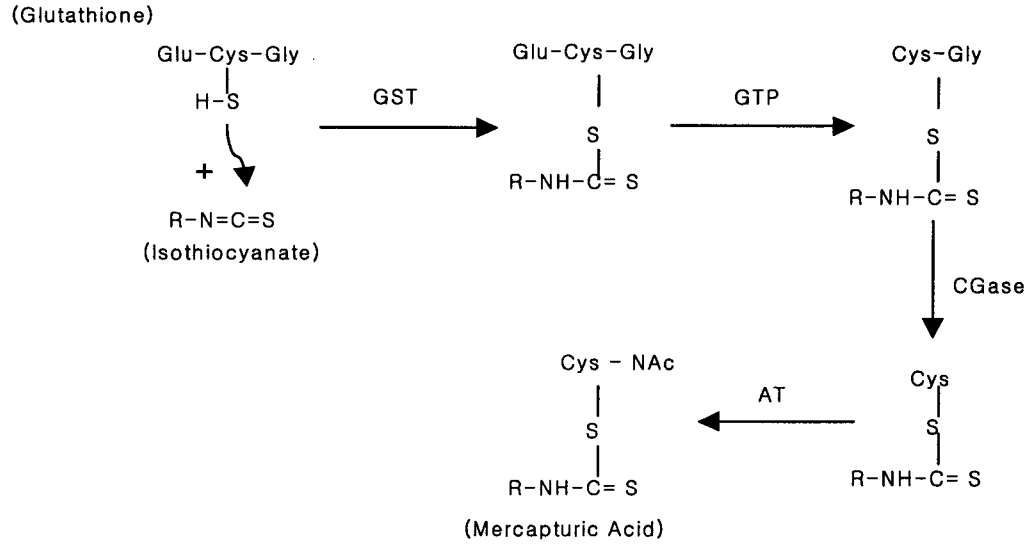

Fig. 1. Isothiocyanates are conjugated to glutathione by glutathione S-transferase (GST), then metabolized sequentially by γ-lutamyltranspeptidase (GTP), cysteinylglycinase (CGase), and acetyltransferase (AT) ultimately to form mercapturic acids (Shapiro et al., 2001).

Besides that, cruciferae vegetables show bioactivities as goitrogenic, antibacterial, antifungal, and antiprotozoal activities as well as insect repellants. Consumption of cruciferous vegetables has been associated with a reduction in the incidence of cancer at several sites in human body (Graham et al., 1978; Verhoeven et al., 1996). Feeding of crucifers is known to induce enzymes responsible for xenobiotic metabolism, and thereby accelerate the metabolic disposal of xenobiotics (Wattenberg, 1985; Beecher, 1994; Hecht, 1995). Especially, induction of phase 2 detoxification enzymes, such as glutathione S-transferase (EC 2.5.1.18) and quione reductase [NAD(P)H : (quionone : acceptor) oxidoreductase, EC 1.6.99.2] in rodent tissue affords protection against carcinogens and other toxic electrophiles (Wattenberg, 1985; Habig, 1974). It is well known that isothiocyanates are produced from myrosinase-catalyzed hydrolysis of glucosinolate in the extract (Fenwick et al., 1983). The induction of GST activity was attributed mainly to isothiocyanates which are mostly monofunctional inducers of phase 2 enzymes (Prochaska & Talalay, 1988; Talalay et al., 1995; Zhang et al., 1992) or in part to sulfides, inducers of phase 1 and 2 enzymes (Brady et al., 1988; Gudi and Singh, 1991).

Mechanism of anticarcinogenic action of isothiocyanates

There are two types of anticarcinogenic enzyme inducers: (a) bifunctional inducers that elevate both phase 2 enzymes (e.g., glutathione S-transferase, UDP-glucuronosyltransferases and quionone reductase) and phase 1 enzymes (e.g., cytochrome P450); and (b) monofunctional inducers that others selectively induce only phase 2 enzymes (monofunctional inducers) (Prochaska and Talalay, 1988). Phase 1 enzymes (cytochromes P450) convert procarcinogens to reactive electrophilic ultimate carcinogens that can damage susceptible centers of DNA bases and initiate carcinogenesis. However, Phase 2 enzymes prevent the damage of DNA and other macromolecules by reactive electrophiles by forming the conjugates with endogenous ligands (e.g., glutathione, glucuronic acid), and by inactivating electrophiles and by promoting their excretion. In addition, glutathione, the principal cellular antioxidant, which is similarly regulated by phase 2 enzymes, plays a major role in protection against electrophiles and reactive oxygen species. One possible mechanism for anticarcinogenic effects of isothiocyanates is to induce the activity of phase 2 enzymes and/or inhibit phase 1 enzymes (Prochaska and Talalay, 1988; Talalay et al., 1995;

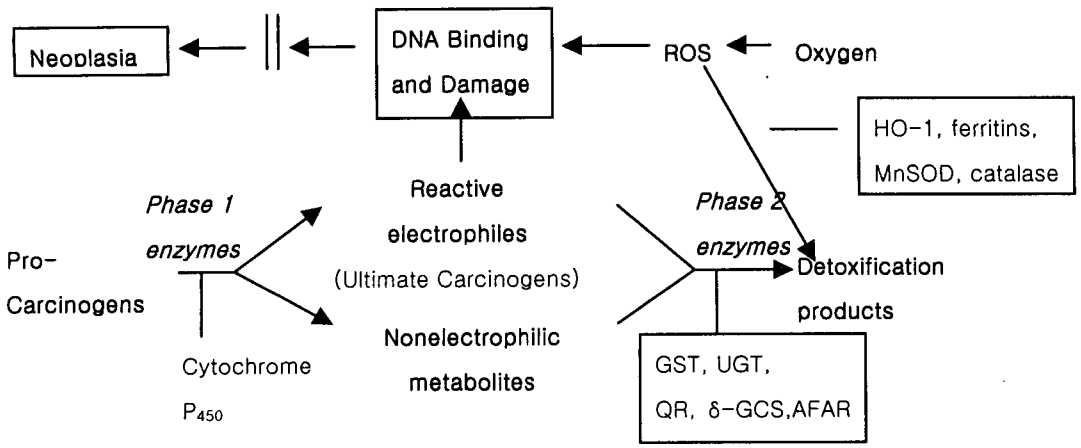

Fig. 2. Role of metabolism in chemical carcinogenesis. Susceptibility to carcinogen damage is controlled by the balance between phase 1 activation and phase 2 detoxication enzymes. GST, glutathione *S*-transferases; γ-GCS, γ-glutamylcysteine synthase; HO-1, heme oxygenase 1; MnSOD, manganese superoxide dismutase; QR, quinone reductase (NQO1); ROS, reactive oxygen species; UGT, UDP-glucuronosyl transferases; AFAR, aflatoxin B1 aldehyde reductase (Talalay and Fahey, 2001).

Talalay and Fahey, 2001). Recent studies in humans also revealed that a high consumption of Cruciferous led to an increase in glutathione *S*-transferase activity, supporting the potential role of isothiocyanates in the prevention of human cancer (Verhoeven et al., 1996). The second putative mechanism involves the suppression of tumor development by deleting the initiated cells from damaged tissue by apoptosis (Adesida et al., 1996).

Anticarcinogenic activity

The isothiocyanates are the most potent anticarcinogenic agents known (Wattenberg, 1977; Wattenberg, 1985). Consumption of broccoli, Brussels sprouts and cauliflower enhanced the activity of biotransforming enzymes and exhibited anticarcinogenic activity in both animals and humans (Aspry and Bjeldanes, 1983; McDanell et al., 1988; Stoner et al., 1991). Cabbage and broccoli enhanced colon mucosal glutathione levels (Chen et al., 1995). Consumption of 300 g of cooked Brussels sprouts per day by healthy human volunteers significantly elevated glutathione-S-transferase levels in blood plasma (Bogaards et al., 1994).

Sulforaphane Sulforaphane is one of the representative monofunctional inducers, which induces phase 2 enzymes selectively without the induction of aryl hydrocarbon receptor-dependent cytochromes P-450 phase 1 enzymes. Among synthetic sulforaphane analogues (CH_3-SO_m-$(CH_2)_n$-NCS), sulforaphane (n=1, m=4) is the most potent inducer, and the presence of oxygen on sulfur enhances potency (Zhang et al., 1992).

Phenethylisothiocyanate Another effective isothiocyanate may be phenethylisothiocyanate, which inhibited iso-enzyme P4502E1 in stimulated rat liver microsome, and increased tissue level of glutathione. Synthetic analogs of phenethyl isothiocyanate with a longer alkyl chain length possessed greater inhibitory activity against NNK-induced lung tumors in mice (Morse et al., 1990). Phenhexyl isothiocyanate inhibited chemically induced lung tumors in strain A mice by >80 percent when administered at a dose that was 50-fold lower than the dose of NNK (Morse et al., 1992). Phenhexyl isothiocyanate, the most potent anticarcinogen tested, was 50～100 times more potent than phenethyl isothiocyanate (Chung, 1992), due possibly to the increased lipophilicity and stability of the phenhexyl derivative (Chung, 1992).

GST induction by fresh cruciferae vegetables commonly consumed vegetables Because sulforaphane acts as a monofunctional inducer in chemoprevention, at first, the quantitative determination of sulforaphane in 20 cruciferous

vegetables of Korean origin was performed. Homogenate of vegetable was extracted with dichloromethane, and the extract, after drying, was subjected to GC/MS analysis, which was based on single ion monitoring (SIM) at m/z 72, 160, 55, 114 and 177. The content of sulforaphane was found to be the highest in broccoli followed by turnip, red cabbage, radish and kale. Also, the content of sulforaphane differed according to the cultivars and the portion of the vegetables: highest inthe stem of '1243' and the floret of Pilgrim the amount of sulforaphane was the highest (>700 ppm). Processing or cooking conditions affect sulforaphane content. Sulforaphane was maximally produced from the homogenate in 0.1 M phosphate buffer containing 1 mM of vitamin C and storing for 1 hr at room temperature. In cooked broccoli, the amount of sulforaphane decreased according to the boiling time, and after 30 min to 10% of control (Kim et al., 1997).

The anticarcinogenic effect of commonly consumed cruciferous and lilaceae vegetables, which were harvested in Korea, was compared by assessing the induction of phase 2 enzymes. In addition, the combinational effects of two types of vegetables were assessed. The solvent extract of vegetable in propylene glycol (5 mL/kg body wt.) was administered to ICR mice 6 to 8 weeks old via gavage during 5 days. The induction of GST activity in liver cytosol of mice was greatest with broccoli, followed by radish, wild green onion, turnip and green onion. The induction of GST activity in liver cytosol increased up to 1.5 to 1.8-folds at a dose of 24 g fresh vegetable/ mouse. The induction of combination between vegetables was the highest with the combination of broccoli and radish,1.83-fold, followed by that of broccoli and green onion, 1.72-fold and that of broccoli and turnip, 1.50-fold (Kim et al., 1999). Crucufer vegetables containing higher sulforaphane amount showed higher GST induction, while onion family vegetables with higher sulfides induced higher GST activity, compared to control.

CONCLUSION

Cruciferae vegetables contain several kinds of bioactive sulfur compounds derived from myrosinase hydrolysis from glucosinolates such as isothiocyanates. This paper dealt with the sulfur compounds and their related compounds concerning chemical properties, nutrakinetic, bioability, and anticarcinogenesis.

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