

Cruciferous Vegetables: Cancer Protective Mechanisms of Glucosinolate Hydrolysis Products and Selenium

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Dietetic professionals urge Americans to increase fruit and vegetable intakes. The American Institute of Cancer Research estimates that if the only dietary change made was to increase the daily intake of fruits and vegetables to 5 servings per day, cancer rates could decline by as much as 20%. Among the reasons cited for this health benefit are that fruits and vegetables are excellent sources of fiber, vitamins, and minerals. They also contain nonnutritive components that may provide substantial health benefits beyond basic nutrition. Examples of the latter are the glucosinolate hydrolysis products, sulforaphane, and indole-3-carbinol. Epidemiological studies provide evidence that the consumption of cruciferous vegetables protects against cancer more effectively than the total intake of fruits and vegetables. This review describes the anticarcinogenic bioactivities of glucosinolate hydrolysis products, the mineral selenium derived from crucifers, and the mechanisms by which they protect against cancer. These mechanisms include altered estrogen metabolism, protection against reactive oxygen species, altered detoxification by induction of phase II enzymes, decreased carcinogen activation by inhibition of phase I enzymes, and slowed tumor growth and induction of apoptosis.

Keywords: *cruciferous vegetables; glucosinolates; cancer protection; isothiocyanates sulforaphane; indole-3-carbinol; selenium*

Cancer is the second-leading cause of death in the United States, and the American Cancer Society estimates that one third of all cancer deaths are related to dietary factors. Lack of anticarcinogenic compounds in our diet (eg, tocopherols, ascorbic acid, selenium, polyphenolics, and isothiocyanates) may contribute more to our risk of developing cancer than the presence of dietary carcinogenic compounds (eg, heterocyclic amines and aflatoxin). This hypothesis is supported by studies reporting a reduction in cancer risk with increased intake of fruits and vegetables.² The primary cancer protective benefit of such a dietary change may not be associated with decreased

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fat or increased fiber intake as much as it is with the increased intake of nonnutritive dietary components such as lycopene, isothiocyanates, and isoflavones.

Cancer Prevention by Crucifer-Rich Diets

Many studies report a strong inverse relationship between the intake of crucifers and the risk for many cancers.^{3,5} This association has been found to be stronger than the association between cancer risk and fruit and vegetable intake in general.⁶ Epidemiologic studies have demonstrated inverse associations between crucifer intake and the incidence of lung, pancreas, bladder, prostate, thyroid, skin, stomach, and colon cancer.³ Prospective dietary assessment of 628 men diagnosed with prostate cancer found that increasing crucifer intake from 1 to 3 or more servings per week resulted in a 41% decreased apparent risk.⁷ A 10-year cohort study of 47,909 men reported that increased crucifer intake, but not fruits and other vegetables, was associated with decreased risk for bladder cancer (relative risk = 0.49, 95% confidence interval = 0.32-0.75, $P = .008$).⁶ Verhoeven and coworkers reviewed the results of 7 cohort studies and 87 case control studies and reported that 67% of the case control studies found inverse associations between total crucifer intake and cancer risk.³ Inverse associations between cancer risk and intakes of cabbage, broccoli, cauliflower, or brussels sprouts were noted in 70%, 56%, 67%, and 29% of the control studies, respectively. The cohort studies showed inverse associations between intakes of cabbage, cauliflower, or broccoli and risks

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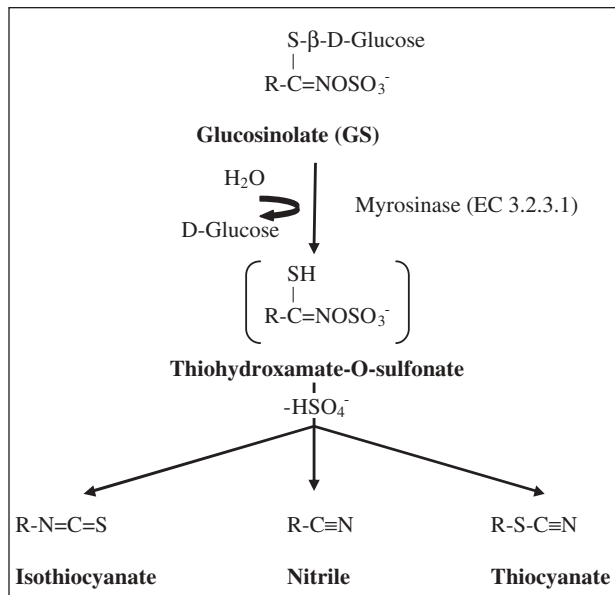


Figure 1 Bioactivation of glucosinolates. Hydrolysis of glucosinolates by the endogenous enzyme myrosinase, to form glucosinolate hydrolysis products: nitriles, isothiocyanates, and thiocyanates.

for lung cancer; between total crucifer intake and risk for stomach cancer; and between broccoli intake and risk for all cancers. As a result of many studies showing inverse associations between dietary intake of crucifers and cancer, the National Research Council, Committee on Diet, Nutrition and Cancer specifically recommended in 1982 that Americans increase consumption of cruciferous vegetables.³

Crucifers contain a group of secondary metabolites called glucosinolates (GS),⁹ as well as numerous other bioactive compounds, that play a role in cancer protection. The plant family Cruciferae (also called the mustard family or Brassicaceae) includes broccoli, parsnip, brussels sprouts, Chinese cabbage, radish, horseradish, wasabi, white mustard, watercress, and cauliflower. Crucifers also contain many other bioactive components including flavonoids such as quercetin,¹⁰ minerals such as selenium (Se),¹¹ S-methyl cysteine sulfoxide, and 1,2-dithiole-3-thione.¹² This communication summarizes research conducted on bioactive hydrolysis products of GS and the mineral Se and the mechanisms by which these compounds protect against cancer.

GS and Their Bioactive Hydrolysis Products

The chemical structures of GS are similar in all the plants in which they are present (> 3000 crucifer species). Their basic structure consists of a β -D-thioglucose group, a sulfonated oxime group, and a side chain derived from methionine, phenylalanine, tryptophane, or branched-chain amino acids (desig-

nated as R in Figure 1).⁹ The sulfate group of a GS molecule is strongly acidic, and plants accumulate GS by sequestering them as potassium salts in plant vacuoles.⁹ More than 120 GS have been characterized, but no essential role for GS in plant metabolism has been found. The potent odor and taste of GS has resulted in a proposed role of GS in herbivore and microbial defense.⁹

Dietary Sources of GS

The source and amount of GS in human diets varies among different countries. The primary dietary sources in North America include vegetables of the genus *Brassica* such as cabbage, kale, brussels sprouts, cauliflower, broccoli, turnip, and rutabaga.¹³ However, in Japan, radishes are a primary source of GS, as they make up more than 1% of all food sold.⁹ Per capita consumption of crucifers by the Japanese is ~5-fold greater than that of Americans.^{9,13}

GS Profile of Crucifers

The chemical form and total amount of GS differ more than 10-fold within and between crucifer species. Glucobrassicin and glucoraphanin are generally found in high concentrations in broccoli (0.1-2.8 and 0.8-21.7 mmol/g DW, respectively) and constitute as much as 95% of the total amount of GS.¹⁴ 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.⁹

GS concentrations also vary between plant tissues and are affected by environmental conditions.¹⁵ Seeds often contain several-fold greater GS concentrations than the edible portions of the plant.⁹ Environmental factors that affect GS accumulation include cultivation, climate, and soil conditions (eg, soils rich in sulfate produce plants with increased concentrations of GS).¹⁵ Indolyl GS, such as glucobrassicin, are affected primarily by environmental conditions, whereas aliphatic GS, such as glucoraphanin, are primarily under genetic control.¹⁶

Hydrolysis of GS to Bioactive Products

GS are not bioactive in the animal that consumes them until they have been enzymatically hydrolyzed.¹⁷ This occurs when endogenous myrosinase is released during the disruption of the plant cell by harvesting, processing, or mastication (Figure 1). Hydrolysis produces nitriles, isothiocyanates, and thiocyanates (Table 1), some of which are known to be bioactive. Hydrolysis of glucoraphanin results in sulforaphane (SF) and sulforaphane nitrile (SFN), hydrolysis of sinigrin results in allyl isothiocyanate (AITC),

gluconasturtiin produces phenethyl isothiocyanate (PEITC), and glucobrassicin produces indole-3-carbinol (I3C).

The relative proportion of GS hydrolysis products depends on many factors including the plant species and cultivar, the site of hydrolysis (eg, inside the plant or in the gut), the tissue, the presence of cofactors (eg, vitamin C), and the environmental conditions (eg, temperature, pH, moisture). The specific activity of myrosinase varies between plant species^{9,15}; broccoli converts 80% to 90% of glucoraphanin to SFN and 10% to 20% to SF,¹⁸ but daikon myrosinase converts almost all glucoraphanin to SF.¹⁹ Rabot and coworkers discovered that the gut microflora has the capacity to hydrolyze GS,¹⁷ thus accounting for the bioactivity of GS from cooked crucifers.²⁰ Therefore, the total amount of bioactive GS hydrolysis products in a meal depends on the specific crucifer used, food preparation conditions, and the composition of the host gut microflora.

Mechanisms of Cancer Protection by Isothiocyanates

In vitro and in vivo isothiocyanates are potent anticarcinogenic compounds.²¹ The most studied bioactive isothiocyanates are SF, PEITC, AITC, and I3C,²¹⁻²³ but many other isothiocyanates present in lower quantities may contribute to the anticarcinogenic properties of crucifers.

Detoxification

The anticarcinogenic properties of isothiocyanates have been attributed to their ability to alter detoxification pathways,^{21,22} leading to decreased activation of procarcinogens and increased excretion of carcinogens. Some isothiocyanates appear to increase both phase I and phase II enzymes; that is, they act as bifunctional inducers that activate both the antioxidant response element (ARE) and the xenobiotic response element (XRE) in the gene promoter region. Other isothiocyanates may upregulate only phase II enzymes, thus functioning as monofunctional inducers through the ARE. Some researchers believe that phase II detoxification pathways are not rate limiting and therefore cannot play a role in blocking carcinogenesis.²⁴ This may be true for low levels of carcinogens, but at higher levels, the phase II enzymes probably do become rate limiting.²⁵ This hypothesis is supported by the observation that drugs such as acetaminophen are activated more rapidly than they are conjugated, resulting in an accumulation of toxic reactive intermediates.²⁵

There is in vitro and in vivo evidence that dietary isothiocyanates regulate phase I and II enzyme activities. Isothiocyanates such as PEITC and SF inhibit

Table 1. Major Glucosinolates and Their Hydrolysis Products in Cruciferous Vegetables

<i>Glucosinolate</i>	<i>Natural Abundance</i>	<i>Isothiocyanate</i>	<i>Nitrile</i>
Glucoraphanin	Broccoli	Sulforaphane	Sulforaphane nitrile
Gluconasturtiin	Chinese cabbage, radishes, and watercress	Phenethyl isothiocyanate	
Sinigrin	Brussels sprouts, cabbage, and cauliflower	Allyl isothiocyanate	
Glucobrassicin	All crucifers	Indole-3-carbinol	
Progoitrin	Crambe (oil seed)	Crambene	

phase I enzyme isozymes, including cytochrome P450 1A1, 2B1/2, 3A4, and 2E1 in cell culture.^{26,27} In cell culture and animals, SF increases the activity of phase II enzymes such as quinone reductase (QR) and glutathione-S-transferases (GST).^{21,28,29} Preliminary QR activity data using rodents suggest that SF present in whole broccoli is more beneficial than purified SF.³⁰ In rodents, PEITC increases hepatic glutathione (GSH) concentrations, QR, and GST activity.³¹ Rats given the parent compound of AITC (24 mg/d) for 11 days had significantly increased total GST activity,³² and brussels sprouts high in the parent GS of AITC increased hepatic and intestinal GST.³³ I3C is a unique isothiocyanate because it can increase phase I isozymes including cytochrome P450 1A,³⁴ increase phase II enzymes, and act as a phytoestrogen.³⁵ The in vitro and in vivo findings with isothiocyanates are supported by human studies; daily intake of 300 g brussels sprouts^{20,36} or 300 g red cabbage³⁶ induced plasma GST, and daily intake of 500 g broccoli induced blood cytochrome P450 enzymes involved in 2-hydroxylation of estrogen.³⁷

Protection Against Oxidative Stress

Oxidative stress resulting from excessive exposure to environmental pollutants, ultraviolet light, or ionizing radiation may overwhelm the body's antioxidant system and result in oxidative damage to proteins and nuclear acids. This may lead to initiation of cancer and other degenerative diseases. Extracts of crucifers have direct free radical-scavenging properties ex vivo,³⁸ but the direct antioxidant properties do not correlate with GS content, suggesting that GS play a minor role in direct oxidant protection.

Tumor Growth Inhibition and Apoptosis

Isothiocyanates may slow proliferation and increase apoptosis of cancer cells, resulting in a retardation of tumor growth. I3C arrests human breast cancer cells²³

and prostate cancer cells³⁹ in the G1-phase of the cell cycle. Cell cycle arrest is accompanied by abolished expression of cyclin-dependent kinase-6 and increased apoptosis.²³ Sulforaphane arrests human colon cancer cells in G2/M-phase and increases expression of cyclin A and B, bax, and cell death by apoptosis.⁴⁰

Altered Estrogen Metabolism

I3C may lower the risk of hormone-dependent cancers by altering estrogen metabolism. Plant estrogen agonists (phytoestrogens) lower the growth-promoting activities of estrogens by increasing 2-hydroxylation of estrogen. Hydroxylation on the 2-position lowers the concentration of 16-hydroxylation estrogen products, which are stronger estrogen receptor agonists.³⁵ In vitro, I3C inhibits estradiol-induced cell proliferation in estrogen-dependent cells.⁴¹ Intake of 300 mg I3C/day increased the 2/16-alpha-hydroxyestrone ratio in women at risk for breast cancer.⁴² I3C was given to women with cervical intraepithelial neoplasia (CIN) (a risk factor for uterine cervix cancer). After 12 weeks, more than 40% of the women taking 200 mg or 400 mg I3C/day experienced complete regression of CIN, but no women in the placebo group showed regression.⁴³ In addition, women taking I3C had a dose-dependent increase in 2/16 alpha-hydroxyestrone ratio. Oral I3C provided to patients with recurrent respiratory papilloma resulted in reduced or cessation of papilloma growth in 66% of the subjects.⁴⁴ These results suggest that intake of I3C and crucifers rich in I3C may protect against hormone-dependent cancers.

Mechanisms of Cancer Protection by Nitriles

Relative little research has been conducted with GS-derived nitriles, perhaps because only a few bioactive nitriles have been discovered. The nitrile crambene is a hydrolysis product of progoitrin and is abundant in brussels sprouts, cabbage, and crambe (an oil seed), and SFN is a hydrolysis product of glucoraphanin.

Detoxification

Nitriles may increase phase II detoxification enzymes in vitro and in vivo. Rats given crambene had significantly increased QR, GST, and GSH.⁴⁵ The amount of crambene needed to induce hepatic QR activity in rats was similar to that reported for SF,⁴⁶ but in cell culture, crambene was only 1% as effective as SF.⁴⁷ Sulforaphane nitrile bioactivity was similar to crambene in cell culture, but SFN (1 mmol/kg rat) did not increase phase II enzyme activities in rats.⁴⁶ This suggests that some nitriles may play a role in the ability of crucifers to protect against carcinogenesis.

Tumor Growth Inhibition

Nitriles also may prevent tumor growth. Crambene induced cell cycle arrest without affecting cell viability (95%) in the G₂/M phase in mouse Hepa 1c1c7 cells, rat H4IIEC3 cells, and human HepG2 cells.⁴⁷ The mechanism of cell cycle arrest is not clear, but crambene arrested the cells in the same phase as cells treated with SF.⁴⁰

Selenium and Cancer Protection

Human and animal studies have demonstrated that the micronutrient selenium (Se) protects against several common cancers. Compared to placebo controls, supplementation of humans with 200 µg Se/day as Se-enriched-yeast reduced overall cancer mortality by 41%, prostate cancer by 52%, lung cancer by 26%, and colorectal cancer by 54%.⁴⁸ The specific bioactivity of Se depends on its chemical form, and some studies suggest that methylated seleno compounds (eg, Se methyl selenocysteine) may be especially cancer preventive.⁴⁹ The specific mechanism by which Se inhibits cancer is unclear. Potential mechanisms may include pathways mediated through selenoproteins, altered detoxification enzyme activities, antioxidant protection, cell cycle arrest, apoptosis, and production of specific Se metabolites.^{50,51}

Selenium Content of Plants

Vegetables, including crucifers, typically contain low amounts of Se (0.1-0.3 µg Se/g DW). However, certain plants, when grown on soils with high Se content, have the unique ability to accumulate concentrations of Se many orders of magnitude above normal. For example, broccoli or canola plants grown on the west side of the central valley of California may contain up to 7 µg Se/g DW,⁵² and when grown under experimental conditions, they may contain more than 950 µg Se/g DW.⁵³ Other crucifers that accumulate Se include radish, brussels sprouts, Indian mustard, and cabbage⁵⁴⁻⁵⁶ (Table 2). Noncrucifer Se-accumulator plants include garlic, onion, ramps, and milk vetch (*Astragalus* spp, Fabaceae), and these plants may accumulate up to 3000 µg Se/g DW.^{55,57}

Selenium is taken up and metabolized by plants similar to sulfur; the preferred chemical form is selenate. Selenate is metabolized by the sulfur assimilation pathway to adenosine 5'-phosphoselenate (APSe)⁵⁸ (Figure 2); this enzymatic step is probably rate limiting. Activated selenate is reduced to selenite and selenide before being incorporated into selenocysteine (SeCys) by cysteine synthase.⁵⁸ Selenocysteine can be converted to other seleno amino acids, incorporated into selenoproteins, or methylated and sequestered in vacuoles.⁵⁸

Table 2. Highest Selenium Concentrations ($\mu\text{g/g DW}$) Measured in Accumulator Plants When Grown in High Selenium Areas or Under High Selenium Experimental Conditions

<i>Selenium Accumulator Plant</i>	<i>Selenium ($\mu\text{g/g DW}$)</i>	<i>Reference</i>
Cruciferous vegetables		
Indian mustard (<i>Brassica juncea</i>)	140-1000	54,57,67,68
Ethiopian mustard (<i>Brassica carinata</i>)	40-1000	54
Brussels sprouts (<i>Brassica oleracea</i>)	34	69
Radish (<i>Raphanus sativum</i>)	1400-2000	56
Canola (<i>Brassica napus</i>)	3-7 ^a	52,68
Broccoli (<i>Brassica oleracea</i>)	150-950 3-5 ^a	11,53,61,63,70 52
Other plants		
Milk vetch (<i>Astragalus praleongus</i> or <i>A. bisulcatus</i>)	1660-3180 ^b 520	71 57
Garlic (<i>Allium sativum</i>)	70-1350	49,55,57,70,72,73
Onion (<i>Allium cepa</i>)	100-140	55,57,70
Ramp (<i>Allium tricoccum</i>)	50-520	57

- a. Naturally grown in high selenium area of California.
- b. Naturally grown in high selenium area of South Dakota.

Accumulator plants convert the majority of Se into analogues of sulfur amino acids⁵⁹ and methylated seleno amino acids.⁵⁷ Methylation is catalyzed by methyl transferases that transfer methyl groups from S-adenosyl methionine to the seleno amino acid (Figure 2). Common methylated seleno compounds include Se-methyl selenocysteine (SeMSC), Se-methyl selenocysteine selenoxide, Se-methyl methionine, methyl selenol, dimethyl selenide, and trimethyl selenonium.⁵⁵ Some laboratories have reported that SeMSC may account for more than half of the Se in high-Se garlic and broccoli.^{49,55}

Dietary Se may be utilized to synthesize essential selenoproteins, incorporated randomly as selenomethionine (SeMet) into proteins or excreted (Figure 3). Beta-lyases may catalyze the conversion of SeMSC and SeCys to hydrogen selenide. Hydrogen selenide may be sequentially methylated to mono-, di-, and trimethylated metabolites that are ultimately excreted in the human urine and/or breath.

Accumulation of Se in broccoli enhances the anticarcinogenic potential of broccoli in the chemically induced rat aberrant crypt model. Rats fed 2 mg Se/kg diet as high-Se broccoli had 60% fewer aberrant crypts (preneoplastic markers of colon cancer) compared to rats fed selenite or broccoli without Se.⁶⁰ High Se broccoli also reduced total intestinal tumor burden in a strain of mice with spontaneous intestinal tumors⁵³ and mammary tumors in DMBA-treated mice.⁶¹ High-Se broccoli was less effective in restoring Se tissue

concentrations and glutathione peroxidase activities in Se-depleted rats as compared to selenite, selenate, or selenomethionine.⁶² This supports the hypothesis that high-Se broccoli contains forms of Se that are not utilized for selenoprotein synthesis to the same extent as other dietary seleno compounds.⁶³ Development of varieties of broccoli that spontaneously accumulate Se under normal soil conditions may enhance its cancer protective ability.

Mechanisms of Cancer Protection by Se-Enriched Crucifers

Although the mechanism of the cancer protective effects of Se-enriched crucifers is unknown, studies with pure SeMSC may give some insight. In rodents, SeMSC was more effective than selenomethionine, selenite, and selenocysteine in suppressing DMBA-induced mammary tumorigenesis.^{64,65} The addition of arsenic, which inhibits the methylation of Se compounds, decreases the cancer protection of SeMSC.⁶⁴ These data suggest that the cancer protective effect of Se may be related to production of methylated seleno compounds, especially methylselenol.⁶⁴ The specific biological action of methylated seleno compounds is still unknown.

Isothiocyanates in crucifers may upregulate the selenoprotein thioredoxin reductase (TR). It is well known that Se is essential for the activity of TR, a selenoprotein that reduces thioredoxin and may thus regulate cell growth by providing reducing power for p53 and redox cycling of endogenous antioxidants such as vitamin C and lipoic acid.⁵¹ Recent evidence suggests that broccoli-derived SF may upregulate TR activity in a dose-dependent manner in rats.³⁰ Reporter gene constructs show that TR is regulated by SF through an ARE in the promoter region.⁶⁶ It is not known if upregulation of TR is associated with the anticarcinogenic properties of high-Se broccoli or SF.

Summary

Americans have a 30% to 50% probability of developing cancer in their lifetime, and 1 in 4 deaths in the United States are from cancer. Furthermore, one third of all cancer deaths are estimated to be related to dietary factors. A diet rich in crucifers, such as brussels sprouts and broccoli, is inversely associated with the risk of many common cancers. Furthermore, epidemiological studies have shown that crucifers provide an even greater protection against cancer than a diet high in a general mixture of fruits and vegetables. GS and their hydrolysis products may be the mechanism by which crucifers provide this protection. The concentration of GS in crucifers can vary more than 10-fold depending on the species, cultivar, and environ-

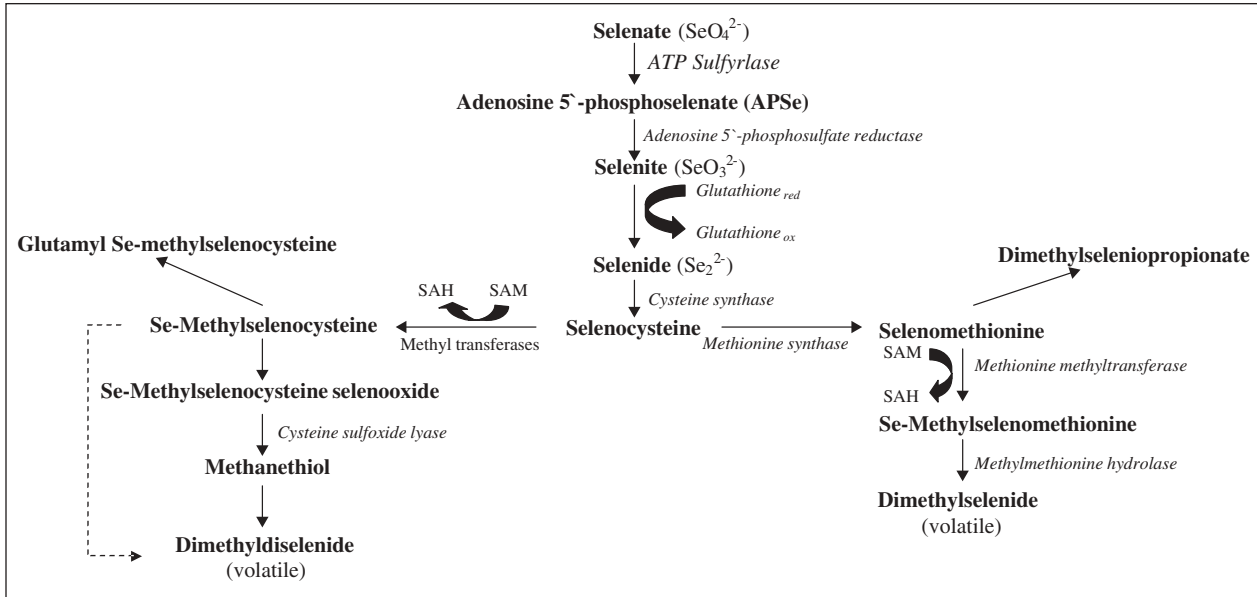


Figure 2 Proposed pathway of selenium metabolism in plants is similar to sulfate metabolism.⁵⁸ Production of Se-methylselenocysteine selenoxide has been detected only in Brassica and Raaphanus plants. Cysteine sulfoxide lyase has only been detected in Allium and Brassica plants. SAM = S-adenosyl methionine; SAH = S-adenosyl homocysteine.

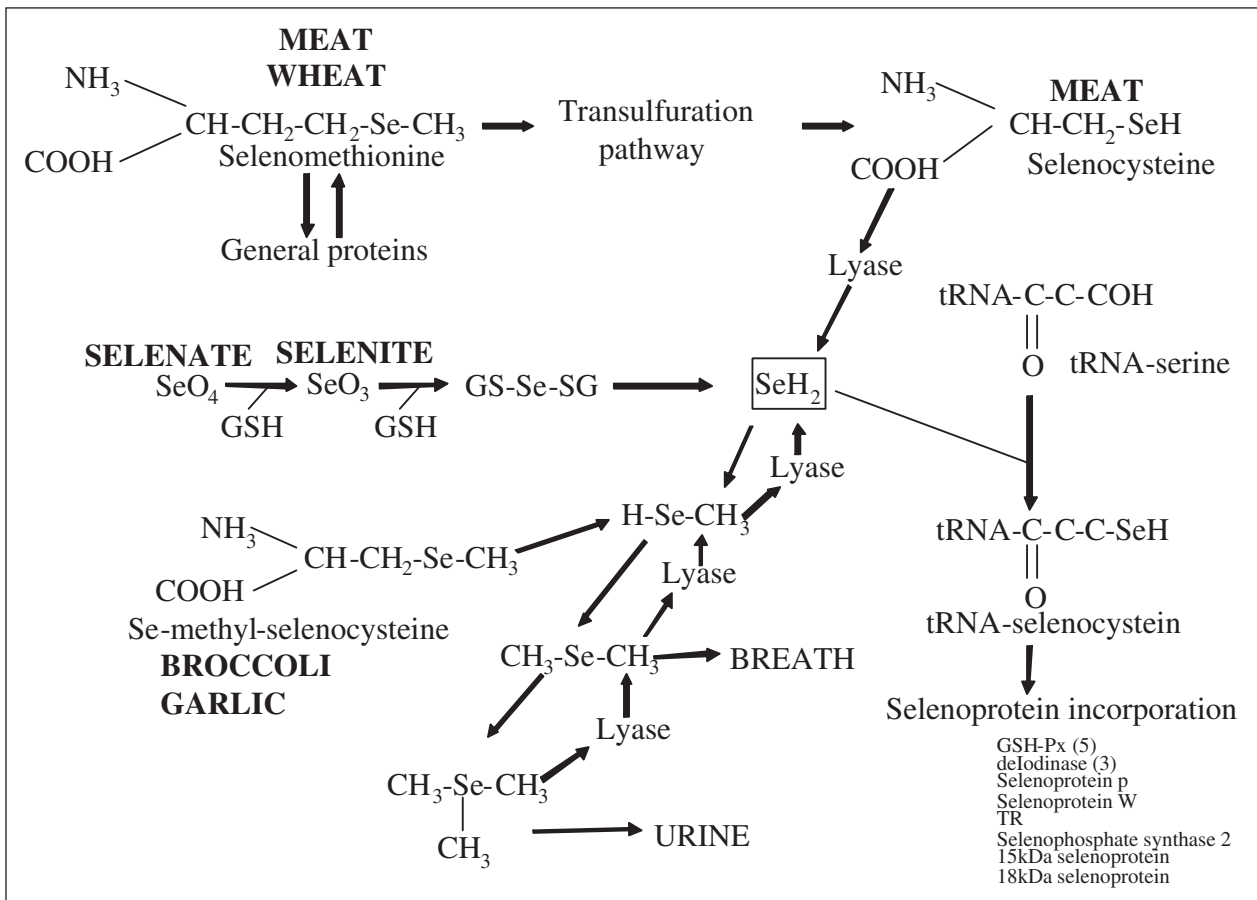


Figure 3 Metabolic fate of the chemical forms of selenium when consumed by animals. Each form of selenium enters the metabolic pathway at a different point and has a unique metabolic fate.

mental conditions. The total amount of bioactive GS hydrolysis products formed depends on food preparation as well as the site of hydrolysis. GS hydrolysis products, particularly isothiocyanates such as SF, PEITC, AITC, and I3C, and perhaps nitriles such as crambene, may act as anticarcinogens by blocking initiation of cancer through altering phase I and phase II detoxification enzymes. They also may arrest the cell cycle and induce apoptosis, resulting in slowed tumor growth. Enhancement of the Se content of broccoli further increases its anticarcinogenic properties. Development of crucifers high in both Se and GS may result in crucifers with greater cancer protective benefits than the crucifers currently available on the American market.

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