Comparison of the Bioactivity of Two Glucoraphanin Hydrolysis Products Found in Broccoli, Sulforaphane and Sulforaphane Nitrile

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Epidemiological and laboratory studies suggest that dietary broccoli may prevent or delay a variety of cancers. Broccoli and other crucifers contain a relatively unique family of secondary metabolites called glucosinolates. Glucoraphanin, the major glucosinolate in broccoli, is hydrolyzed by an endogenous plant myrosinase to form either the potent anticarcinogen sulforaphane (SF) or sulforaphane nitrile (SF nitrile). The bioactivities of SF and SF nitrile were compared in rats and in mouse hepatoma cells. Male, 4-week-old, Fischer 344 rats were administered SF or SF nitrile (200, 500, or 1000 μmol/kg) by gavage daily for 5 days. Hepatic, colonic mucosal, and pancreatic quinone reductase and glutathione *S*-transferase activities were induced by high doses of SF, but not by SF nitrile. When Hepa 1c1c7 cells were exposed to increasing levels of each compound for 24 h, quinone reductase showed a 3-fold maximal induction over control at 2.5 μM SF and a 3.5-fold maximal induction over control at 2000 μM SF nitrile, the highest concentration tested. These results demonstrate that SF nitrile is substantially less potent than SF as an inducing agent of phase II detoxification enzymes. Therefore, glucoraphanin hydrolysis directed toward the production of SF rather than SF nitrile could increase the potential chemoprotective effects of broccoli.

Keywords: *Broccoli; glucosinolates; sulforaphane; sulforaphane nitrile; isothiocyanate; quinone reductase; glutathione S-transferase; Brassica oleracea*

INTRODUCTION

Increased consumption of cruciferous vegetables such as broccoli, Brussels sprouts, and cabbage correlates with a decreased risk for developing cancers of the pancreas (*1*), lung (*2*), colon (*3*), and prostate (*4*). It is hypothesized that much of this chemoprotective effect can be attributed to the physiological effects of isothiocyanates. These isothiocyanates are derived from a family of compounds called glucosinolates, which are secondary plant metabolites relatively unique to cruciferous vegetables.

When the tissue of cruciferous vegetables is disrupted, sequestered glucosinolates are released and come into contact with myrosinase enzyme (thioglucoside glucohydrolase, EC 3.2.3.1). Myrosinase breaks the β-thioglucoside bond of the glucosinolate molecule, producing glucose, sulfate, and an unstable aglucon intermediate. This aglucon spontaneously rearranges to form a diverse group of aglucon products. Depending upon the structure of the specific glucosinolate and the existing reaction conditions, isothiocyanates or nitriles usually constitute the majority of these aglucons (*5*, *6*). In broccoli, the primary glucosinolate is glucoraphanin [4-(methylsulfinyl)butyl glucosinolate] (*7*), which yields sulforaphane (SF) [4-(methylsulfinyl)butyl isothiocyanate] and sulforaphane nitrile (SF nitrile) [5-(methylsulfinyl)pentane nitrile] as its primary aglucon products following myrosinase-dependent hydrolysis (Figure 1). Several isothiocyanates, including SF, have been shown to be relatively potent monofunctional inducers of phase II detoxification enzymes (*8*−*10*). It is hypothesized that the induction of these enzymes may increase the clearance of chemical carcinogens, protecting cellular DNA from highly reactive electrophilic compounds (*11*).

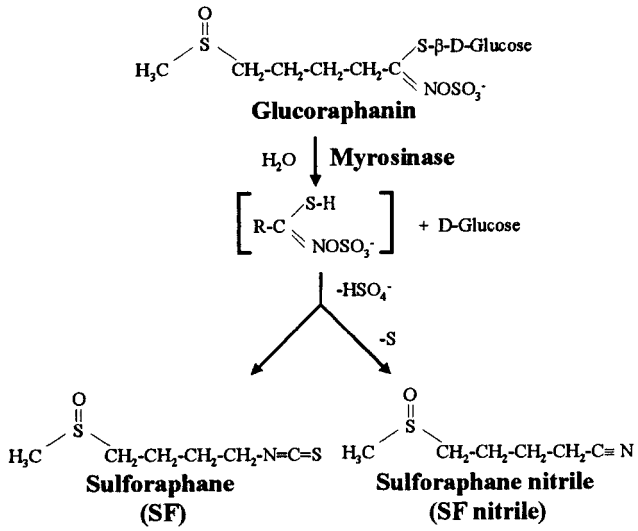

Figure 1. Enzymatic conversion of glucoraphanin to SF and SF nitrile.

Quinone reductase is a phase II enzyme responsible for the two-electron reduction of quinones to form hydroquinones, which are easily metabolized to form stable, water soluble conjugates that can be excreted in the urine. If there is insufficient reduction of quinones by quinone reductase, quinones undergo one-electron reduction catalyzed by the phase I enzyme NAD(P)H-cytochrome P450 reductase. The specific up-regulation of quinone reductase has been shown to inhibit the formation of quinone−DNA adducts in vitro (*12*, *13*).

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Using induction of quinone reductase as an end-point, a rapid screening assay has been developed for the detection of potential chemoprotective compounds in dietary constituents (*14*).

Glutathione *S*-transferases are another example of phase II detoxification enzymes that have been shown to be up-regulated by glucosinolate hydrolysis products. Glutathione *S*-transferases are responsible for conjugating glutathione (GSH) to electrophilic compounds, making the reactive compound stable and water soluble, thus protecting vital cellular components such as DNA. Total glutathione *S*-transferase activity can be used as another indicator of phase II enzyme induction and is easily measured by estimation of 1-chloro-2,4-dinitrobenzene (CDNB) conjugation with GSH (*15*).

Sulforaphane has been reported to induce both quinone reductase and glutathione *S*-transferase in vitro and in several organs in vivo (*9*, *16*, *17*). The induction of phase II enzymes has been correlated with protection of DNA from reactive carcinogenic intermediates and with a reduction in tumor formation in vivo (*18*, *19*). Indeed, SF has been shown to inhibit cancer initiation (*20*) and tumor growth (*16*, *21*) in vivo.

It has recently been reported that complete conversion of glucoraphanin to SF can occur when an exogenous myrosinase is used to hydrolyze the broccoli glucosinolates (*22*, *23*). Upon crushing of the plant tissue and subsequent hydrolysis by the endogenous plant myrosinase, nitrile compounds may be formed in similar or even greater concentrations than isothiocyanates. This has been reported for several cruciferous vegetables and seeds, including raw cabbage (*24*), garden cress (*25*, *26*), and turnip seeds (*27*). A recent study has shown that glucoraphanin levels in broccoli may not correlate with the potential to induce quinone reductase activity in vitro when exogenous myrosinase is not used (*28*). Most recently, several commercial broccoli cultivars were found to produce predominantly SF nitrile upon hydrolysis with the endogenous myrosinase (*29*). Sulforaphane nitrile has not been evaluated previously for its chemoprotective properties, and little work has been done to determine positive or negative health effects derived from this compound.

If the physiological effects of SF and SF nitrile differ substantially, then the health benefit of broccoli may depend on the ratio of SF to SF nitrile formed during the hydrolysis of glucoraphanin by myrosinase. The objective of this research was to compare the ability of the two glucoraphanin hydrolysis products, SF and SF nitrile, to induce the activity of the mammalian phase II detoxification enzymes quinone reductase and glutathione *S*-transferase.

MATERIALS AND METHODS

Materials. Both SF and SF nitrile were purified from broccoli seed using the extraction and purification method previously described (*29*). Briefly, broccoli seed (*Brassica oleracea* cv. Brigadier) was ground and defatted with hexane. Water was then added to the seed meal, and the resulting slurry was incubated at room temperature for 8 h and then extracted with methylene chloride. The dried methylene chloride extract was redissolved in water, filtered, and separated by HPLC using a Waters Prep-NovaPak C_{18} reversed-phase column. Fractions corresponding to SF and SF nitrile were collected and dried to obtain purified compounds. Compounds were tentatively identified using GC and confirmed using EI mass spectrometry (Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois, Urbana, IL). The purity of both compounds was >99% as determined by GC. All other chemical reagents were purchased from Sigma (St. Louis, MO). Hepa 1c1c7 cells were from American Type Culture Collection (ATCC).

Animals and Husbandry. Four-week-old male Fischer 344 rats weighing 55−75 g were purchased from Harlan, Inc. (Indianapolis, IN). Rats were housed individually in plastic shoebox cages in an environment of controlled temperature and humidity and were acclimated 5 days prior to the start of the experiment. During the acclimation period and throughout the experiment, rats were fed AIN 93G semipurified diet without *tert*-butylhydroquinone (TBHQ) (Harlan Teklad, Madison, WI). Water was provided ad libitum. Food was replaced daily and was available for 4 h at the same time each day. Food intake was calculated daily for the treatment groups, and pair-fed groups were fed the mean food intake of the previous day from their respective paired treatment group.

Experimental Design. SF and SF nitrile were dissolved in isotonic saline and administered by gavage daily at 9:00 a.m. for 5 days. In experiment 1, three treatment groups ($n = 5$) were administered 200, 500, or 1000 μmol of SF/kg. Three additional groups were pair-fed to the three SF treatment groups and received daily doses of saline. In experiment 2, three treatment groups were administered 200, 500, or 1000 μmol of SF nitrile/kg, and one group was administered 500 μmol of SF/kg (positive control). Three additional groups were pair-fed to the three SF nitrile treatment groups and received daily doses of saline.

Tissue Preparation. Rats were asphyxiated with carbon dioxide and killed by cervical dislocation 24 h after the final treatment. Livers were immediately perfused with ice-cold 1.15% KCl, and portions of liver and pancreas were snap frozen in liquid nitrogen. The proximal 5 cm of the colon was removed and flushed with ice-cold 1.15% KCl. The colon was cut lengthwise along the mesenteric artery and laid flat on an iced Petri dish. The mucosal cell layer was scraped with a glass slide, and the scrapings were collected and snap frozen in liquid nitrogen. Hepatic, pancreatic, and colonic mucosal samples were later thawed and homogenized in 0.15 M KCl−0.25 M potassium phosphate buffer and centrifuged at 11000*g* for 20 min at 4 °C. Supernatant from the colonic mucosa was collected and snap frozen in liquid nitrogen. Hepatic and pancreatic supernatants were centrifuged at 105000*g* for 60 min and the supernatants collected as cytosolic fractions and snap frozen in liquid nitrogen. Hepatic and pancreatic microsomal pellets were resuspended in 0.1 M sodium phosphate buffer (pH 7.4) containing 0.25 M sucrose and snap frozen in liquid nitrogen.

Enzyme Analyses. Quinone reductase activity was determined in cytosolic fractions from liver, pancreas, and colonic mucosa according to the method of Ernster (*30*) as modified by Benson and co-workers (*31*) measuring reduction of 2,6-dichlorophenol−indophenol (DPIP). Total glutathione *S*-transferase activity was measured in cytosolic fractions of liver, pancreas, and colonic mucosa according to the method of Habig and co-workers (*15*) using 1-chloro-2,6-dinitrobenzene (CDNB) as a second substrate. Hepatic microsomal ethoxyresorufin *O*-deethylase (EROD) activity was measured by quantification of resorufin formation using the method of Pohl and Fouts (*32*).

Determination of Quinone Reductase Activity in Hepa 1c1c7 Cell Culture. Hepa 1c1c7 cells were plated into 96 well plates (Costar 3595, Corning, Inc., Corning, NY) at 10000 cells/well using α-minimum essential medium (without ribonucleosides or deoxyribonucleosides) supplemented with 10% heat and charcoal-inactivated fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin (final concentrations). Cells were grown for 24 h, and then the medium was replaced with medium containing the test compound dissolved in dimethyl sulfoxide (DMSO) (0.1% v/v final concentration) or DMSO alone (vehicle control) and incubated for an additional 24 h. Cells grown with 1 μM β-naphthoflavone (BNF) were used in each experiment as a positive control. Quinone reductase activity was determined as previously described (*14*), except that protein was quantified in duplicate

Table 1. Quinone Reductase and Glutathione *S*-Transferase Specific Activities Following Five Daily Doses of Sulforaphane in Fischer 344 Rat Liver, Colon, and Pancreas (Experiment 1)

	quinone reductase activity[a] (nmol of DPIP/min/mg of protein)		glutathione *S*-transferase activity[a] (nmol of CDNB/min/mg of protein)	
organ	pair fed	treatment	pair fed	treatment
liver				
SF, 200 μmol/kg	63.3 ± 3.5	76.1 ± 5.9	372 ± 8.8	419 ± 26
SF, 500 μmol/kg	65.6 ± 5.7	92.6 ± 5.7*	391 ± 25	447 ± 24
SF, 1000 μmol/kg	63.8 ± 4.2	141 ± 9.8*	377 ± 16	478 ± 18*
pancreas				
SF, 200 μmol/kg	5.5 ± 0.8	8.3 ± 0.8*	63.9 ± 5.1	69.2 ± 6.7
SF, 500 μmol/kg	5.3 ± 0.8	16.5 ± 2.0*	67.0 ± 5.9	66.5 ± 5.1
SF, 1000 μmol/kg	6.5 ± 0.4	30.2 ± 5.4*	61.3 ± 2.6	78.4 ± 4.2*
colon				
SF, 200 μmol/kg	81.2 ± 5.4	137 ± 38	71.4 ± 5.0	77.8 ± 6.8
SF, 500 μmol/kg	86.6 ± 7.9	270 ± 29*	75.0 ± 3.2	95.8 ± 5.0*
SF, 1000 μmol/kg	69.9 ± 11	308 ± 38*	73.2 ± 3.2	118 ± 13*

[a] Mean ± SE. An asterisk (*) indicates the value is significantly greater than that for the pair-fed control (*t* test, $p < 0.05$).

Table 2. Quinone Reductase and Glutathione *S*-Transferase Specific Activities Following Five Daily Doses of Sulforaphane Nitrile in Fischer 344 Rat Liver, Colon, and Pancreas (Experiment 2)

	quinone reductase activity[a] (nmol of DPIP/min/mg of protein)		glutathione *S*-transferase activity[a] (nmol of CDNB/min/mg of protein)	
organ	pair fed	treatment	pair fed	treatment
liver				
SF, 500 μmol/kg		75.6 ± 6.9*		696 ± 19*
SF nitrile, 200 μmol/kg	40.8 ± 3.6	41.5 ± 2.0	562 ± 34	563 ± 17
SF nitrile, 500 μmol/kg	37.8 ± 2.0	35.3 ± 2.6	509 ± 13	516 ± 14
SF nitrile, 1000 μmol/kg	32.8 ± 3.1	42.6 ± 3.1	545 ± 12	546 ± 26
pancreas				
SF, 500 μmol/kg		20.5 ± 5.1*		54.1 ± 3.6
SF nitrile, 200 μmol/kg	4.8 ± 0.7	6.1 ± 0.7	51.7 ± 4.4	50.1 ± 3.2
SF nitrile, 500 μmol/kg	5.9 ± 0.9	6.1 ± 1.1	50.3 ± 2.0	52.6 ± 2.3
SF nitrile, 1000 μmol/kg	7.8 ± 1.2	8.2 ± 2.4	49.3 ± 2.5	43.4 ± 2.1
colon				
SF, 500 μmol/kg		241 ± 20*		89.1 ± 3.9*
SF nitrile, 200 μmol/kg	66.5 ± 5.9	63.5 ± 3.3	60.0 ± 3.3	55.2 ± 3.8
SF nitrile, 500 μmol/kg	68.5 ± 3.7	62.6 ± 4.2	59.4 ± 1.7	51.8 ± 2.8
SF nitrile, 1000 μmol/kg	65.1 ± 3.1	62.9 ± 7.3	62.8 ± 2.4	53.1 ± 2.8

[a] Mean ± SE. An asterisk (*) indicates that the value is significantly greater than the 200 μmol of SF nitrile/kg found for the pair-fed control (*t* test, $p < 0.05$).

plates and used to estimate specific activity. Protein was measured according to a modification of the method of Bradford (*33*) using a Bio-Rad kit (Bio-Rad Laboratories, Hercules, CA).

Statistical Analysis. Data were analyzed using SAS software (SAS, Inc., Cary, NC). *t* Tests were used to compare treatment to pair-fed groups for animal experiments 1 and 2. Protected ANOVA ($p < 0.05$) with LSD was used to compare effects of SF and SF nitrile in vitro.

RESULTS

Animal Experiments. *Experiment 1, SF Treatment.* In rats, hepatic quinone reductase activity was significantly induced at doses of 500 and 1000 μmol of SF/kg, with 2.2-fold induction at 1000 μmol of SF/kg. The lowest dose tested, 200 μmol of SF/kg, had no effect on hepatic quinone reductase activity. Pancreatic quinone reductase activity was significantly induced at all SF levels tested; the highest dose, 1000 μmol of SF/kg produced a 4.6-fold induction. As in the liver, colonic mucosal quinone reductase activity was significantly induced at 500 and 1000 μmol of SF/kg, with 4.4-fold induction at 1000 μmol of SF/kg (Table 1). In the colonic mucosa, glutathione *S*-transferase activity was significantly increased by 500 μmol of SF/kg, whereas this dose of SF had no effect on hepatic or pancreatic glutathione *S*-transferase. However, when the dose was increased to 1000 μmol/kg, hepatic, pancreatic, and colonic mucosal glutathione *S*-transferase activities were all significantly induced (1.3-, 1.3-, and 1.6-fold, respectively; Table 1).

Experiment 2, SF Nitrile Treatment. Rats receiving 500 μmol of SF/kg exhibited induction of quinone reductase activity in liver, pancreas, and colonic mucosa (1.8-, 4.2-, and 3.6-fold, respectively). These results were similar to those observed in experiment 1. No significant induction of quinone reductase activity was seen in rats receiving any of the doses of SF nitrile tested (Table 2). The positive control (500 μmol/kg dose of SF) gave a 1.2-fold induction of glutathione *S*-transferase activity in liver and a 1.5-fold induction of glutathione *S*-transferase activity in colonic mucosa. Pancreatic glutathione *S*-transferase was not significantly induced. No dose of SF nitrile tested significantly induced glutathione *S*-transferase activity (Table 2).

Hepatic EROD Activity. Neither SF nor SF nitrile altered hepatic EROD activity compared to pair-fed groups at any of the doses tested in either experiment. This indicates that, under the experimental conditions employed here, cytochromes P450 1A were not induced or suppressed by either compound (data not shown).

Quinone Reductase Activity in Hepa 1c1c7 Cells. When incubated with mouse Hepa 1c1c7 cells, SF induced quinone reductase significantly at all concentrations tested. A dose-dependent induction was observed from 0.078 to 2.5 μM SF, reaching a maximum

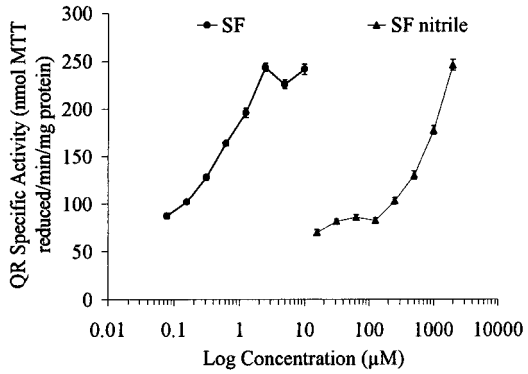

Figure 2. Effect of increasing concentrations of SF or SF nitrile on quinone reductase activity in Hepa 1c1c7 cells treated for 24 h. SF experiment: negative control = 78.1 ± 3.2 nmol of MTT reduced/min/mg of protein; positive control (1 μM BNF) = 398.3 ± 10.6 nmol of MTT reduced/min/mg of protein. SF nitrile experiment: negative control = 72.8 ± 2.1 nmol of MTT reduced/min/mg of protein; positive control (1 μM BNF) = 377.3 ± 11.4 nmol of MTT reduced/min/mg of protein. Mean ± SE (n = 8).

induction of 3.1-fold over control at a concentration of 2.5 μM (Figure 2). Concentrations of 5 and 10 μM did not cause any greater induction than did 2.5 μM. Protein content per well was significantly decreased compared to control ($p < 0.001$) at the highest concentration of SF tested (10 μM) (data not shown). SF nitrile significantly induced quinone reductase activity at concentrations of 31.25 μM and higher. A dose-dependent induction was observed, reaching an induction of 3.4-fold over control at 2000 μM, the highest concentration tested (Figure 2). Protein content per well was not significantly altered by SF nitrile treatment, even at 2000 μM, the highest concentration tested (data not shown).

Because SF and SF nitrile occur together in the diet, three concentrations of SF (0.1, 0.5, and 2.5 μM) were evaluated for their ability to induce quinone reductase activity when mixed with increasing concentrations of SF nitrile (7.8−2000 μM). At levels of 0 and 0.1 μM SF, SF nitrile caused a dose-dependent induction that could be accounted for by adding together data from SF alone and SF nitrile alone. However, at levels of 2.5 and 5 μM SF, SF nitrile caused a significant increase in quinone reductase induction over SF alone only at the highest concentration tested.

DISCUSSION

The observed induction of quinone reductase and glutathione *S*-transferase activity in vivo by SF is in agreement with previously published literature. In female CD-1 mice given 15 μmol of SF by gavage daily for 5 days, which is ~750 μmol of SF/kg mouse, a 2.5-fold increase in hepatic quinone reductase activity and a 1.9-fold increase in hepatic glutathione *S*-transferase activity were observed (*9*). In female BALB/c mice given 17 μmol of SF by gavage daily for 4 days, a 2.5-fold increase in mammary quinone reductase activity and a 2.3-fold increase in mammary glutathione *S*-transferase activity were found (*16*). In this second study, hepatic quinone reductase and glutathione *S*-transferase activities were unaffected. This discrepancy may result from the use of different mouse strains in the two experiments. Our results display an induction of hepatic, pancreatic, and colonic mucosal quinone reductase and glutathione *S*-transferase activities in response to SF in the rat and show this response to be dose-dependent.

Until now, little research has been conducted on the physiological effects of SF nitrile. A study performed by Ringenberg and Wallig found no induction of hepatic glutathione *S*-transferase activity in Fischer 344 rats gavaged with SF nitrile doses as high as 2000 μmol/kg for 3 days (*34*). In agreement with these data, doses of SF nitrile equivalent to effective doses of SF did not significantly up-regulate quinone reductase or glutathione *S*-transferase activity in rat liver, colonic mucosa, or pancreas. On the basis of these results, the substitution of the electrophilic isothiocyanate group with a nonelectrophilic cyano group may be responsible for a decreased reactivity and therefore a decreased potency for induction of quinone reductase and glutathione *S*-transferase activity in pancreas, liver, and colonic mucosa. It is also possible that the difference in molecular structure between SF and SF nitrile results in differences in the fraction absorbed from the gut and/or extent of cellular uptake, resulting in the observed decreased potency of SF nitrile compared to SF.

Glutathione *S*-transferase activity was notably less sensitive than quinone reductase to induction by SF. This is likely due to the fact that several subclasses of glutathione *S*-transferases exist, and the induction pattern of these subclasses has been reported to vary with exposure to different compounds from *Brassica* vegetables (*8*, *35*). Strong induction of a particular subunit may be partially masked by background activity of other subunits when total glutathione *S*-transferase activity is measured.

Hepatic EROD activity (cytochrome P450 1A family) was not significantly up-regulated by SF or SF nitrile. SF and other isothiocyanates have been previously reported to be monofunctional inducers, up-regulating synthesis of phase II detoxification enzymes without up-regulating synthesis of cytochrome P450 enzymes (*9*, *36*). Our work supports this suggestion.

The physiological effects of SF nitrile were further analyzed in a more sensitive cell culture system, using quinone reductase activity as an end-point (*14*). This assay has been commonly used as a screening assay for the detection of possible dietary chemoprotective agents (*9*). The observed induction of quinone reductase activity in vitro by SF is in agreement with previously published research (*9*, *16*). SF nitrile, however, has not been previously evaluated for its ability to induce quinone reductase activity in vitro. Here we report that SF nitrile is able to cause induction of quinone reductase activity in vitro, but only at levels that are ~3 orders of magnitude greater than those required for SF to produce the same effect. In addition, SF nitrile appears to compete with SF, causing SF to produce significantly less induction of quinone reductase in the presence of SF nitrile (Figure 3).

The results of this study indicate that SF nitrile is a less potent inducer of phase II detoxification enzymes than SF. This is an important finding because the ratio of isothiocyanate to nitrile formed in cruciferous vegetables may vary. It is well documented that different species and varieties of cruciferous vegetables express differing glucosinolate contents (*7*, *37*, *38*). Additionally, the ratio of isothiocyanate to nitrile produced from a parent glucosinolate has been found to vary among species and even among different varieties of the same species (*6*, *27*, *39*). The conditions under which hydrolysis takes place can also affect the ratio of isothiocyanates to nitriles formed. The pH during hydrolysis has been

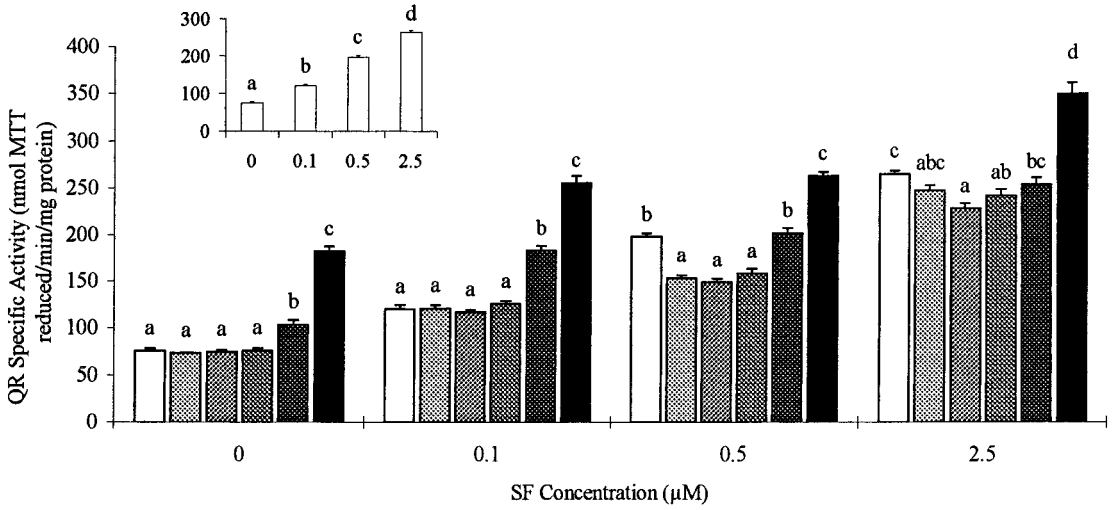

Figure 3. Effect of increasing concentrations of SF nitrile mixed with increasing concentrations of SF on quinone reductase activity in Hepa 1c1c7 cells treated for 24 h. (Inset) SF dose response without SF nitrile. Negative control = 75.5 ± 3.2 nmol of MTT reduced/min/mg of protein; positive control (1 μM BNF) = 327.2 ± 7.0 nmol of MTT reduced/min/mg of protein. Within each grouping, bars represent from left to right, 0 μM SF nitrile, 7.8 μM SF nitrile, 31.3 μM SF nitrile, 125 μM SF nitrile, 500 μM SF nitrile, and 2000 μM SF nitrile. Different letters within treatment groups indicate significant differences. Mean ± SE (n = 8).

found to be an important factor affecting both the activity of myrosinase (*40*) and the preferential formation of nitriles in place of isothiocyanates. Low pH has been reported to enhance the formation of nitriles, whereas neutral and high pH have been found to enhance the production of isothiocyanates (*41*). The presence of iron and thiol ions has been found to favor nitrile formation (*41*−*43*), whereas higher temperature and increased hydration have been found to favor isothiocyanate formation (*5*). Additionally, different crucifer species express differing isoforms of myrosinase (*44*), and the degree to which these isoforms may influence the aglucon products formed during natural glucosinolate hydrolysis is not well understood.

Relatively little is known about the effects of postharvest storage and processing on the conversion of glucoraphanin to SF or SF nitrile in commercially produced broccoli. Howard and co-workers (*45*) have reported that fresh broccoli (cv. Arcadia) stored for 21 days after harvest produced only 55% of the SF found in broccoli stored for 1 day. In addition, the ratio of SF to SF nitrile shifted during the storage period to favor isothiocyanate production. A detailed study of the effects of storage and processing upon glucosinolate hydrolysis could add greatly to our understanding of the factors involved in SF production.

In a recent study characterizing the relative potency of several broccoli cultivars to induce quinone reductase in a cell culture system, broccoli glucosinolates were extracted whole and then hydrolyzed using purified myrosinase from daikon (*Raphanus sativus*) prior to their evaluation in cell culture (*23*). Differences in hydrolysis conditions and species may influence the products derived from glucosinolate hydrolysis, so treatment of broccoli glucosinolates with exogenous daikon myrosinase may not accurately reflect how much of the final active component is formed from dietary broccoli. Faulkner and co-workers (*28*) have crossed a double-haploid broccoli breeding line derived from the cultivar Green Duke with wild *Brassica* species (*B. villosa* and *B. drepanensis*). In both crosses, glucoraphanin content was increased ~10-fold, but the potential for quinone reductase induction in culture was increased >100-fold. They hypothesized that the resulting hybrids provided an increased ratio of SF to SF nitrile upon hydrolysis of glucoraphanin. Recently we have shown that several commercial broccoli varieties are capable of producing primarily SF nitrile from glucoraphanin when a broccoli preparation is allowed to autolyze at room temperature without added exogenous myrosinase (*29*). It is therefore likely that upon consumption of broccoli, delivery of active SF may depend not only upon a high content of glucoraphanin present in the broccoli but also on the source of myrosinase and the conditions of hydrolysis that exist.

Here we report that two products of the hydrolysis of glucoraphanin by myrosinase in broccoli tissue, SF and SF nitrile, differ in potency for the induction of phase II detoxification enzymes. We hypothesize that this difference is due to the lack of an isothiocyanate functional group on SF nitrile. These findings also suggest a similar difference in anticarcinogenic potency between SF and SF nitrile. Therefore, to achieve maximum health benefit from broccoli, not only must glucoraphanin levels be high but preferential conversion of glucoraphanin to SF is necessary.

ABBREVIATIONS USED

SF, sulforaphane; SF nitrile, sulforaphane nitrile; EROD, ethoxyresorufin *O*-deethylase; BNF, β-naphthoflavone.

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