JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Phytochemicals in Broccoli Transcriptionally Induce Thioredoxin Reductase

KORRY J. HINTZE,† KARL WALD,‡ AND JOHN W. FINLEY*,‡

Department of Animal and Range Sciences, North Dakota State University, Fargo, North Dakota 58105, and Grand Forks Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Grand Forks, North Dakota 58202

Previous studies have demonstrated transcriptional induction of thioredoxin reductase (TR) by sulforaphane (SF) purified from broccoli; the mechanism of induction is via an antioxidant response element (ARE) in the promoter region of the gene. The purpose of the present study was to further characterize the induction of TR by compounds in broccoli and to determine if SF is the primary compound responsible for this induction. Aqueous extracts were made from broccoli with low or high concentrations of selenium (Se) and/or SF and tested in a TR/luciferase reporter gene system in cultured cells. Phenolic acids commonly found in broccoli (sinapic, caffeic, ferulic, and protocatechuic) and ascorbic acid were also tested. At SF concentrations of ≤ 2 μM, broccoli extracts and purified SF activated transcription equally well, but 4 μM SF in broccoli extracts resulted in almost twice as much induction as 4 μM purified SF ($P < 0.05$). All broccoli extracts significantly increased TR and quinone reductase activity relative to controls ($P < 0.05$), but only extracts highest in Se increased glutathione peroxidase activity ($P < 0.05$). No phenolic acids tested induced transcription, but ascorbic acid resulted in modest dose-dependent induction between 0 and 120 μM ($P < 0.001$). These data suggest that SF accounts for most of the ARE-activated transcriptional induction of antioxidant genes by broccoli.

KEYWORDS: Quinone; reductase; antioxidant response element; selenium; sulforaphane

INTRODUCTION

Oxidative stress may initiate molecular events in the cancer process, and reduction of oxidative stress may protect against carcinogenesis (*1*). Numerous foods contain bioactive compounds with putative antioxidant properties, and a number of in vitro tests have been developed that assess the ability of a food extract to reduce an oxidized substrate (*2*). However, the problem with many of these tests is a lack of evidence of a correlation between in vitro reduction of an oxidized substrate and positive in vivo changes. A variety of enzymes, including many in the phase II detoxification system, eliminate oxidative stressors; thus, compounds that up-regulate these enzymes may be considered antioxidants with in vivo function (*3*). The antioxidant responsive element (ARE) is a cis-acting element in the promoter of numerous phase II and antioxidant genes that acts to turn on transcription in response to certain chemical signals (*4*). Activation of the ARE begins with a bioactive chemical that interfaces with a redox sensitive tethering protein (keap1) causing the release of the transcription factor Nrf2, which translocates to the nucleus and binds to a promoter-embedded ARE (*5*). Because many genes up-regulated by the ARE catalyze the destruction of reactive oxygen species, activation of an ARE may be a measure of in vivo antioxidant potential (*6*).

Mammalian thioredoxin reductase (TR) is a selenium (Se)-dependent enzyme with in vitro antioxidant ability; Se availability partially regulates TR production through a translational mechanism (*7*). TR reduces a variety of molecules including lipoic acid, small thiols, lipid hydroperoxides, NK-lysin, vitamin K_3, dehydroascorbic acid, ascorbyl free radical, and the tumor suppressor d protein p53 (*7*) and indirectly regenerates oxidized vitamin E semiquinone through reduced ascorbate (*8*). We have previously reported that a TR/luciferase reporter gene construct is transcriptionally regulated by broccoli-derived sulforaphane (SF) through an ARE sequence (*9*). This observation has led to the hypothesis of the duality of regulation of TR; it responds to Se availability similar to a classic selenoprotein, but, similar to many phase II proteins, it also responds to transcriptional activation through the ARE.

The consumption of cruciferous vegetables such as broccoli is associated with protection against cancer at several sites including the prostate (*10*), bladder (*11*), and breast (*12*). The protective mechanism effect is thought to be activation of phase II antioxidant and detoxification enzymes, perhaps mediated through an ARE (*13*). Mice with a disrupted Nrf2 gene were

* Address correspondence to this author at the USDA, ARS, GFHNRC, P.O. Box 9034, Grand Forks, ND 58202-9034 [telephone (701) 795-8416; fax (701) 795-8220; e-mail jfinley@gfhnrc.ars.usda.gov].
† North Dakota State University.
‡ U.S. Department of Agriculture.

10.1021/jf0580059 CCC: $30.25
Published on Web 06/18/2005

Table 1. Selenium and SF Concentrations of Broccoli Extracts[a]

Se content (mg/kg)	extract Se (μM)	extract SF (μM)	extract name
1000	279	41.3	low SF
100	27	156	moderate SF
0	0.1	240	high SF

[a] Freeze-dried broccoli differing in Se content (0, 100, or 1000 mg/kg) was hydrolyzed in deionized water for 24 h at room temperature, centrifuged, and filtered.

more sensitive to benzopyrene-induced tumors than wild-type animals, and wild-type mice were partially protected from carcinogenesis by treatment with oltipraz (a well-characterized phase II enzyme inducer), whereas Nrf2 knockout mice were not protected (*14*). Sulforaphane is the most potent dietary inducer of phase II enzymes (*15*). Purified SF induces TR transcription through an ARE (*9*), but it is unknown whether whole extracts of broccoli also induce TR.

Broccoli also contains numerous antioxidant substances that could potentially induce antioxidant enzymes (*16*, *17*), and combinations of these may synergistically increase phase II enzyme expression (*18*, *19*). Phenolic acids have antioxidant (*20*, *21*) and anticarcinogenic activities (*22*−*24*), and multiple phenolic acids are found in broccoli (*25*). Apart from sweet red peppers, broccoli also contains the most vitamin C per serving of commonly consumed vegetables [∼100 mg/serving (*26*)], and vitamin C has been extensively studied for its antioxidant properties (*27*). Hepatic TR reduces dehyroascorbic acid (DHA) to the active antioxidant form of ascorbic acid (AA) (*8*). Consequently, cells exposed to increased concentrations of AA may require a concomitant increase in TR expression.

The primary objective of the present study was to characterize the ability of aqueous extracts of broccoli, as well as substances in broccoli other than SF, to induce TR. Broccoli accumulates Se, and Se-enriched broccoli suppresses colon (*28*) and mammary cancers (*29*). However, Se fertilization decreases the SF content (*25*) of broccoli; thus, Se fertilization makes it possible to produce broccoli with widely different ratios of SF and Se. A secondary objective of this study was to use extracts of these broccoli plants to characterize the differential effects of SF and Se on activity of TR, quinone reductase (QR), and glutathione peroxidase (GSH-Px). Finally, this paper provides supporting evidence that dietary constituents help to regulate TR in a manner similar to other phase II proteins, providing additional evidence for multiple controls on TR production and activity.

MATERIALS AND METHODS

Broccoli Extracts. Broccoli plants with different concentrations of Se and SF were produced as described elsewhere (*25*). The concentrations of Se and SF were inversely related (**Table 1**), resulting in three types of broccoli: broccoli with high SF and low Se, broccoli with intermediate SF and Se, and broccoli with low SF and high Se. Cell culture experiments used extracts of the various broccolis; extracts were generated by hydrolyzing 3 g of freeze-dried broccoli in 60 mL of deionized water for 24 h at room temperature. Extracts were then centrifuged at 3000 rpm for 10 min, and the supernatant was passed through a Büchner funnel using no. 1 Whatman filter paper. The Se content of extracts was determined by hydride generated atomic absorption spectroscopy (*30*) and SF by HPLC (*31*).

Cell Culture. Enzyme activity studies were conducted in Hepa1c1c7 mouse hepatoma cells (ATTC) seeded at 1×10^6 cells/flask in 75 cm^2 culture flasks. Cells were grown for 24 h in control medium (α-modified MEM with 26.2 mM sodium bicarbonate and 10% fetal bovine serum). Experimental media contained 1.79% broccoli extracts (v/v) resulting in the following treatments: (1) low-SF broccoli extract, (2) moderate-SF broccoli extract, and (3) high-SF broccoli extract. Treatments were left on cells for 24 h, and then cells were trypsinized, centrifuged at 5000 rpm for 5 min, washed with PBS, recentrifuged, and lysed by sonication.

Reporter gene experiments used human hepatoma HepG2 cells obtained from ATCC and seeded at 8.0×10^5 cells/dish grown for 24 h on 60 × 15 mm collagen-coated culture dishes (Corning) in MEM medium (Sigma) with 2.2 M sodium bicarbonate, 1 mM sodium pyruvate, and 10% fetal bovine serum. After 24 h, cells were transfected with pGL3 luciferase reporter constructs (Promega) containing the entire TR promoter (*9*) along with pRL-SV40, an expression vector for renilla luciferase (Promega). Transfections were performed using FuGene6 according to the manufacturer's instructions (Roche Biochemicals). Twenty-four hours after transfection, media were changed and experimental treatments added.

Experimental Treatments. The effect of SF on ARE activation was studied by adding different amounts of high-SF broccoli extract to control cell culture media, resulting in SF contents of 0.5, 1, 2, and 4 μM. Likewise, low-SF broccoli extracts were used resulting in SF concentrations of 0.1, 0.2, 0.4, and 0.8 μM. Broccoli extracts were compared to media that used purified SF in concentrations of 0.5, 1.0, 2.0, and 4.0 μM. Sulforaphane was HPLC-purified from broccoli seed according to the method of Matusheski et al. (*31*). Enzyme activity in Hepa1c1c7 cells was determined with treatments of 1.79% of the three extracts, resulting in Se/SF concentrations of 2 nM and 4.3 μM, 500 nM and 2.8 μM, and 5 μM and 0.7 μM (concentrations of Se and SF, respectively); these treatments were compared to control medium with 0 Se and 0 SF.

Phenolic acid (ferulic, sinapic, caffeic, and protocatechuic; Sigma) activation of reporter constructs used media that was formulated with increasing concentrations of test chemicals compared to control media and media containing 2 μM SF. Phenolic acids were dissolved in dimethyl sulfoxide (DMSO), and all media contained 0.1% DMSO. Vitamin C studies used ascorbic acid (Sigma) dissolved directly in the basal media. After 24 h of exposure to experimental treatments, cells were washed with PBS, lysed in 1.8 mL of passive lysis buffer (Promega) for 15 min at room temperature, transferred to 2 mL tubes, and then frozen at −80 °C.

Biochemical Analysis. *Enzyme Assays.* Quinone reductase activity was determined according to the method of Benson et al. (*32*), and specific activity is reported as nanomoles of DPIP reduced per minute per milligram of protein. Thioredoxin reductase activity was determined by following the reduction of 5,5-dithiobis[2-nitrobenzoic acid] (DTNB) at 412 nm in the presence of NADPH (*33*). Enzymatic TR activity was determined by subtracting the time-dependent increase in absorbance at 412 nm in the presence of the TR activity inhibitor aurothioglucose (20 mM) from total TR activity. One unit of activity is described as 1 mmol of TNB formed per minute per milligram of protein. Glutathione peroxidase activity was determined using the coupled enzyme method of Paglia and Valentine (*34*) using H_2O_2 as the substrate and NADPH as the source of reducing equivalents. Protein concentrations were measured by the Bio-Rad assay (Hercules, CA) on a SpectraMax 190 plate reader (Molecular Devices, Sunnyvale, CA).

Luciferase Assay. Cell lysates were analyzed for firefly and renilla luciferase activities by using the dual-luciferase reporter assay system (Promega), following the manufacturer's protocol. Luminescence was measured on a Turner Designs TD 20/20 luminometer (Sunnyvale, CA). To normalize data, all values were reported as the ratio of firefly luminescence (experimental plasmid) to renilla luminescence (pRL-SV40, noninducible plasmid).

Statistical Analysis. Treatment effects were determined by one-way ANOVA. When a significant effect ($P < 0.05$) was found, Tukey's Studentized range was used to determine differences between means (*35*).

RESULTS

Broccoli Extracts and Enzyme Activities. All broccoli extracts significantly increased TR and QR activities relative to controls ($P < 0.05$; see **Figure 1A**,**B**). Thioredoxin reductase

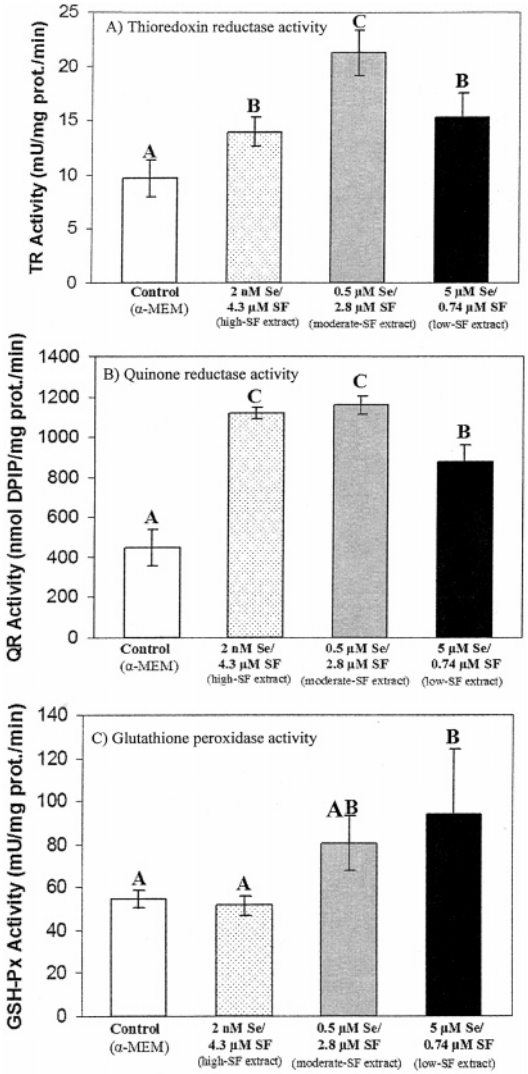

Figure 1. Effect of equal amounts of water-extracted broccoli (high-SF, moderate-SF, and low-SF extract; 1.79% volume extract/volume α-MEM) containing different concentrations of Se and SF on Hepa1c1c7 cell (**A**) TR activity, (**B**) QR activity, and (**C**) GSH-Px activity. Values are means ($n = 4$) ± SD. Differently labeled columns are significantly different ($P < 0.05$).

activity was highest in the intermediate Se/SF, whereas QR was significantly higher in the intermediate and high-SF extract (**Figure 1C**), as compared to control and low-SF extract ($P < 0.05$). Relative to control media, high-SF and intermediate-SF extract had no effect on GSH-Px activity ($P = 0.35$; **Figure 1C**), but the low extracts (which were also the highest in Se) significantly increased GSH-Px activity ($P < 0.05$).

Broccoli Extracts and TR Reporter Construct Activity. Increasing amounts of SF from broccoli or as the pure chemical caused a dose-dependent increase in construct activity ($P < 0.05$; see **Figure 2**), but only SF concentrations of ≥2 μM significantly increased reporter gene activity relative to controls. Purified SF and SF from broccoli resulted in similar reporter gene activity for all treatments except 4 μM SF, in which SF from broccoli doubled reporter gene activity relative to purified SF.

Effect of Phenolic Acids on TR Reporter Construct Activity. Thioredoxin reductase reporter gene activity was not induced by any of the phenolic acids at any of the concentrations tested (**Figure 3**).

Effect of Vitamin C on TR Reporter Construct Activity. Ascorbic acid at concentrations of ≥60 μM resulted in a modest but significant increase in reporter activity ($P < 0.05$; **Figure 4**).

DISCUSSION

We and others have previously reported that SF up-regulates TR activity (*36*, *37*) and TR transcription via an ARE found in the promoter region of the gene (*9*). This paper demonstrates that unpurified aqueous extracts of broccoli also induce TR transcription; however, SF appears to be the primary compound responsible for induction. Se-enriched broccoli is effective for the suppression of colon and mammary cancers (*28*, *29*), but Se fertilization also changes its chemical composition and, specifically, causes a great decrease in SF concentrations (*28*). This paper shows that changing the chemical composition of broccoli by fertilization with Se also changes the ability of broccoli extracts to induce transcription of TR and activity of QR and GSH-Px.

Reporter genes are activated only by transcriptional regulators (e.g., ARE inducers), whereas enzyme activity is the sum of transcriptional, translational, and post-translational controls. Sulforaphane activates the ARE, and, as expected, extracts with the highest concentration of SF but lowest amount of Se caused the greatest transcriptional induction of TR reporter constructs. However, total TR activity in Hepa 1c1c7 cells [enzyme activity was determined in Hepa1c1c7 cells rather than Hep-G2 cells because QR response to phytochemicals has been extensively studied in this model (*38*, *39*) and because our initial report of induction of TR by SF used Hepa1c1c7 cells (*36*)] was not maximized by extracts with the highest SF, but rather by extracts with moderately high concentrations of both SF and Se. This is explained by the up-regulation of TR transcription by SF and the simultaneous increase in TR translation by Se; thus, both compounds work synergistically to increase TR activity.

The differential activation of transcriptional and translational controls by SF and Se, respectively, was further demonstrated by their effects on QR and GSH-Px activity. Quinone reductase and GSH-Px both have antioxidant functions; QR is a phase II enzyme that catalyzes the reduction of quinones to hydroquinones (*40*) and eliminates redox cycling by a semiquinone intermediate. Individuals homozygous for a QR polymorphism that eliminates QR activity (*41*) are vulnerable to urothelial tumors, leukemia, cutaneous basal cell carcinoma, and benzene-induced hematotoxicity (*42*). Glutathione peroxidase is a selenoprotein that responds rapidly to changes in Se availability (*43*) and exhibits in vivo antioxidant activity during times of acute oxidative stress (*44*). Quinone reductase is a phase II protein with an ARE and does not contain Se; as expected, QR activity was maximized by broccoli extracts that contained the highest concentrations of SF, but was unaffected by Se. Alternatively, the selenoprotein GSH-PX has not been reported to be regulated like a phase II enzyme; GSH-PX activity was maximized by extracts highest in Se but was unaffected by SF concentration.

This paper also demonstrates that SF is the primary component in broccoli extracts responsible for TR transcriptional induction (presumably through the ARE). There were no differences in reporter gene activity between pure SF and broccoli extracts when treatments of ≤2 μM SF were compared, suggesting that within this range of concentrations other components of broccoli have little effect on ARE-mediated transcription. However, incubation of cells with broccoli extracts that contained 4 μM SF resulted in more than twice as much

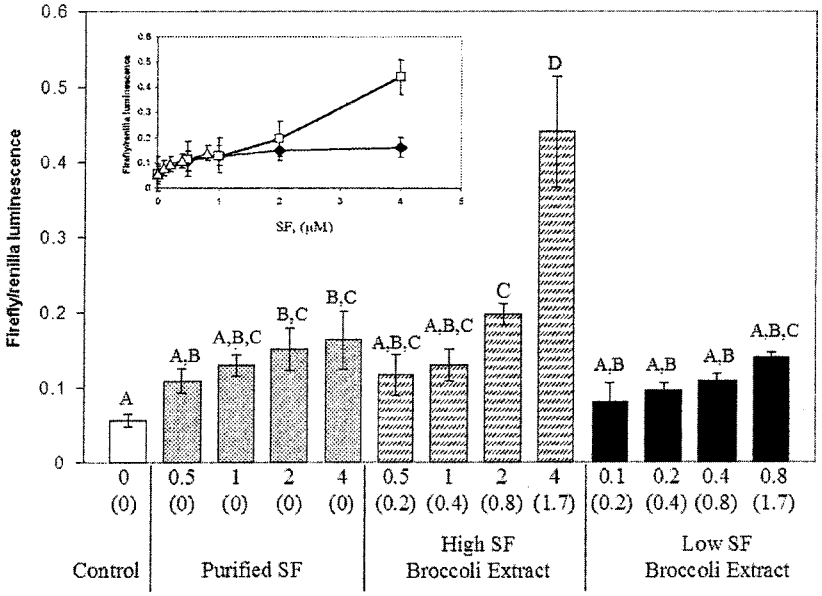

Figure 2. Effect of equal amounts of water-extracted broccoli extract containing different concentrations SF or purified SF added to basal media on TR reporter construct activity. Numbers in parentheses on the *X*-axis are the percentages of the medium that is broccoli extract. Values not in parentheses are the SF (μM) concentrations of the media. Data are the mean ratio of firefly luminescence to renilla luminescence. Values are means ± SD, $n = 3$. Differently lettered columns are significantly different ($P < 0.05$). Contrasts were used to test for linear trend of concentration within each type of extract or purified SF. (Inset) Comparison of different micromolar concentrations of only purified SF (◆) and high-SF broccoli extracts (□).

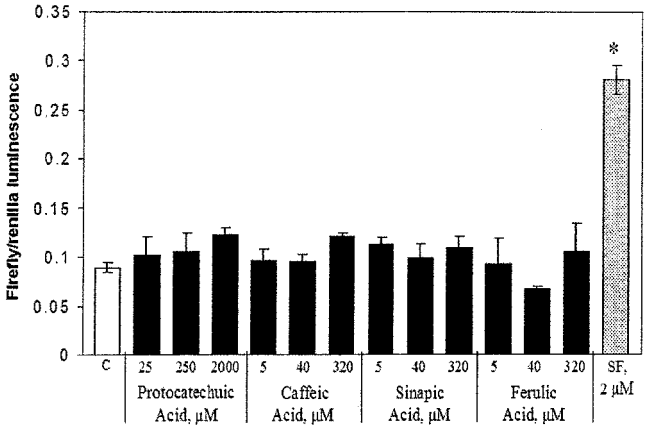

Figure 3. Effect of phenolic acids (protocatechuic, caffeic, sinapic, and ferulic acid) commonly found in broccoli, 2 μM SF treated cells, and untreated cells on TR reporter activity. Data are the mean ratio of firefly luminescence to renilla luminescence. Values are means ± SD, $n = 3$. Columns marked with an asterisk are significantly different from control ($P < 0.05$).

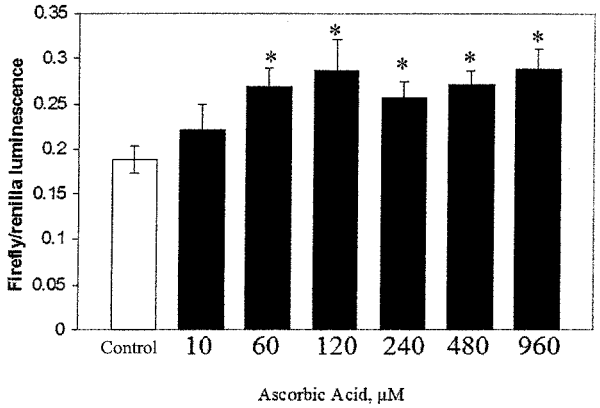

Figure 4. Effect of increasing concentrations of ascorbic acid on TR reporter activity. Values are means ± SD, $n = 3$. Columns marked with an asterisk are significantly different from control ($P < 0.05$).

reporter activity as 4 μM purified SF. This result may represent a threshold response triggered by supraphysiologic concentrations of multiple bioactive inducers in addition to SF. Other than SF, cruciferous vegetables contain many other mostly uncharacterized compounds with the potential to activate genes (*13*, *17*). Sulforaphane is a member of a general class of bioactive compounds termed isothiocyanates, and there is limited evidence that isothiocyanates other than SF also may activate genes (*13*). Ye et al. (*45*) reported that the total isothiocyanate concentration in human plasma was 2.3 μM following ingestion of 200 μmol of total broccoli sprout isothiocyanate. Consequently, medium containing broccoli extracts with 4 μM SF most likely contained very high concentrations of SF as well as many other bioactive compounds. Thus, a synergistic interaction of multiple bioactive inducers provided at supraphysiologic concentrations may have triggered a TR response much greater than an equal concentration of SF alone.

We recently characterized phenolic acids in broccoli and reported that Se fertilization affects the relative abundance of various specific phenolic acids (*46*). The structures of many phenolic acids and their metabolites are similar to those of chemicals known to induce the ARE; thus, it is hypothesized that phenolic acids might also induce the ARE, but none of the phenolic acids tested had any effect on TR reporter activity. This is somewhat surprising because curcuminoids (which can be derived from phenolic acids) have been noted to be especially potent inducers of the ARE (*47*), although the same authors also noted that the most effective ARE-inducing phenolic compounds contain ortho-hydroxyl groups not found in compounds tested in the current study. It also is possible that phase I activation may be needed to convert phenolic acids to compounds more likely to bind to the ARE.

Ascorbic acid concentrations used in this study represent the range found in human plasma; concentrations below 11 μM are considered to be deficient, whereas people consuming the recommended daily allowance of vitamin C have concentrations between 35 and 45 μM. Intakes of 100 mg or more result in plasma AA concentrations of 60 μM (*48*), and such intakes have

been hypothesized to decrease cancer risk (*49*). In the current study, 60 μM AA significantly induced TR reporter activity, although compared to SF, induction was relatively modest. It is unclear whether the mechanism for increased TR expression by AA is mediated through the ARE or by other unknown sequences in the TR promoter.

This study provides additional evidence for the duality of regulation of TR. Relative to low-Se media, TR activity was, as expected for a selenoprotein, increased by extracts with higher concentrations of Se. However, similar to other phase II proteins, activity also was increased by extracts low in Se but high in SF. The duality of regulation of TR may relate to a duality of function; oxidative stress may induce the phase II antioxidant functions of TR through an ARE-mediated mechanism, whereas the other functions of TR may be regulated by completely different mechanisms.

There is a wealth of evidence suggesting increased broccoli consumption can protect against cancer (*13*). There is also strong evidence that suggests Se-enriched broccoli protects against colon and breast cancers (*28*). However, it appears these unique modes of chemoprevention by broccoli are mutually exclusive. Sulforaphane seems to be the greatest single contributor to phase II enzyme activation (*15*), yet enriching broccoli with Se depresses SF concentrations by as much as 80% (*25*). This has implications for the development of "functional foods"; if a plant is altered in a way to maximize a single bioactive component, there may be other unintended changes that reduce the overall health benefits of the food.

LITERATURE CITED

(1) Sun, Y. Free radicals, antioxidant enzymes and carcinogenesis. *Free Radical Biol. Med.* **1990**, *8*, 583−599.

(2) Brawley, O. W.; Barnes, S.; Parnes, H. The future of prostate cancer prevention. *Ann. N. Y. Acad. Sci*. **2001**, *952*, 145−152.

(3) Kong, A. N.; Yu, R.; Hebbar, V.; Chen, C.; Owuor, E.; Hu, R.; Ee, R.; Mandlekar, S. Signal transduction events elicited by cancer prevention compounds. *Mutat. Res.* **2001**, *480*−*481*, 231−241.

(4) Rushmore, T. H.; Kong, A. N. Pharmacogenomics, regulation and signaling pathways of phase I and II drug metabolizing enzymes. *Curr. Drug Metab*. **2002**, *3*, 481−490.

(5) Hayes, J. D.; McMahon, M. Molecular basis for the contribution of antioxidant reponsive element to cancer chemoprevention. *Cancer Lett.* **2001**, *174*, 103−113.

(6) Prestera, T.; Zhang, Y.; Spencer, S. R.; Wilczak, C. A.; Talalay, P. The electrophile counterattack response: protection against neoplasia and toxicity. *Adv. Enzyme Regul.* **1993**, *33*, 281−296.

(7) Mustacich, D.; Powis, G. Thioredoxin reductase. *Biochem. J.* **2000**, *346* (Part 1), 1−8.

(8) May, J. M.; Cobb, C. E.; Mendiratta, S.; Hill, K. E.; Burk, R. F. Reduction of the ascorbyl free radical to ascorbate by thioredoxin reductase. *J. Biol. Chem.* **1998**, *273*, 23039−23045.

(9) Hintze, K. J.; Wald, K. A.; Zeng, H.; Jeffery, E. H.; Finley, J. W. Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles via an antioxidant response element. *J. Nutr.* **2003**, *133*, 2721−2727.

(10) Jain, M. G.; Hislop, G. T.; Howe, G. R.; Ghadirian, P. Plant foods, antioxidants, and prostate cancer risk: findings from case-control studies in Canada. *Nutr. Cancer* **1999**, *34*, 173−184.

(11) Michaud, D. S.; Spiegelman, D.; Clinton, S. K.; Rimm, E. B.; Willett, W. C.; Giovannucci, E. L. Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. *J Natl. Cancer Inst.* **1999**, *91*, 605−613.

(12) Terry, P.; Wolk, A.; Persson, I.; Magnusson, C. Brassica vegetables and breast cancer risk. *JAMA*−*J. Am. Med. Assoc.* **2001**, *285*, 2975−2977.

(13) Talalay, P.; Fahey, J. W. Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *J. Nutr.* **2001**, *131*, 3027S−3033S.

(14) Ramos-Gomez, M.; Kwak, M. K.; Dolan, P. M.; Itoh, K.; Yamamoto, M.; Talalay, P.; Kensler, T. W. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. *Proc. Natl. Acad. Sci U.S.A* **2001**, *98*, 3410−3415.

(15) Prestera, T.; Holtzclaw, W. D.; Zhang, Y.; Talalay, P. Chemical and molecular regulation of enzymes that detoxify carcinogens. *Proc. Natl. Acad. Sci. U.S.A* **1993**, *90*, 2965−2969.

(16) Jeffery, E. H.; Jarrell, V. Cruciferous vegetables and cancer prevention. In *Nutraceuticals and Functional Foods*; Wildman, R., Ed.; CRC Press: Boca Raton, FL, 2001.

(17) Keck, A. S.; Staack, R.; Jeffery, E. H. The cruciferous nitrile crambene has bioactivity similar to sulforaphane when administered to Fischer 344 rats but is far less potent in cell culture. *Nutr. Cancer* **2002**, *42*, 233−240.

(18) Wallig, M. A.; Kingston, S.; Staack, R.; Jeffery, E. Induction of rat pancreatic glutathione-S-transferase and quinone reductase activities by a mixture of glucosinolate breakdown derivatives found in brussels sprouts. *Food Chem. Toxicol.* **1998**, *36*, 365−373.

(19) Nho, C. W.; Jeffery, E. The synergistic upregulation of phase II detoxification enzymes by glucosinolate breakdown products in cruciferous vegetables. *Toxicol. Appl. Pharmacol.* **2001**, *174*, 146−152.

(20) Kanski, J.; Aksenova, M.; Stoyanova, A.; Butterfield, D. A. Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure−activity studies. *J. Nutr. Biochem.* **2002**, *13*, 273−281.

(21) Ogiwara, T.; Satoh, K.; Kadoma, Y.; Murakami, Y.; Unten, S.; Atsumi, T.; Sakagami, H.; Fujisawa, S. Radical scavenging activity and cytotoxicity of ferulic acid. *Anticancer Res.* **2002**, *22*, 2711−2717.

(22) Hudson, E. A.; Dinh, P. A.; Kokubun, T.; Simmonds, M. S.; Gescher, A. Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. *Cancer Epidemiol. Biomarkers Prev.* **2000**, *9*, 1163−1170.

(23) Yang, C. S.; Landau, J. M.; Huang, M. T.; Newmark, H. L. Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu. Rev. Nutr.* **2001**, *21*, 381−406.

(24) Tseng, T. H.; Wang, C. J.; Kao, E. S.; Chu, H. Y. Hibiscus protocatechuic acid protects against oxidative damage induced by *tert*-butylhydroperoxide in rat primary hepatocytes. *Chem.*−*Biol. Interact.* **1996**, *101*, 137−148.

(25) Robbins, R.; Keck, A. S.; Banuelos, G.; Finley, J. Cultivation conditions and selenium fertilization alter the phenolic profile glucosinolate and sulforaphane content of broccoli. *J. Med. Foods* **2004**. In press.

(26) U.S. Department of Agriculture. ARS USDA nutrient database for standard reference, release 14; Nutrient Data Laboratory homepage http://www.nal.usda.gov/fnic/foodcomp, 2004.

(27) Johnston, C. Vitamin C. In *Present Knowledge in Nutrition*; Bowman, B., Russell, R., Eds.; ILSI Press: Washington, DC, 2004.

(28) Finley, J. W.; Davis, C.; Feng, Y. Selenium from high-selenium broccoli protects rats from colon cancer. *J. Nutr.* **2000**, *130*, 2384−2389.

(29) Finley, J. W.; Ip, C.; Lisk, D.; Davis, C.; Hintze, K. J.; Whanger, P. D. Cancer-protective properties of high-selenium broccoli. *J. Agric. Food Chem.* **2001**, *49*, 2679−2683.

(30) Finley, J.; Matthys, L.; Shuler, T.; Korynta, E. Selenium content of foods purchased in North Dakota. *Nutr. Res.* **1996**, *16*, 723−728.

(31) Matusheski, N. V.; Wallig, M. A.; Juvik, J. A.; Klein, B. P.; Kushad, M. M.; Jeffery, E. H. Preparative HPLC method for the purification of sulforaphane and sulforaphane nitrile from *Brassica oleracea*. *J. Agric. Food Chem.* **2001**, *49*, 1867−1872.

(32) Benson, A. M.; Hunkeler, M. J.; Talalay, P. Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5216−5220.

(33) Hill, K.; McCollum, G.; Boeglin, M. E.; Burk, R. Thioredoxin reductase activity is decreased by selenium deficiency. *Biochem. Biophys. Res. Commun*. **1997**, *234*, 293−295.

(34) Paglia, D.; Valentine, W. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **1967**, *70*, 158−169.

(35) SAS Institute Inc. *SAS OnlineDoc 9.1.2*; Cary, NC, 2004.

(36) Hintze, K. J.; Keck, A. S.; Finley, J. W.; Jeffery, E. H. Induction of hepatic thioredoxin reductase activity by sulforaphane, both in Hepa1c1c7 cells and in male Fisher 344 rats. *J. Nutr. Biochem.* **2003**, *14*, 173−179.

(37) Zhang, J.; Svehlikova, V.; Bao, Y.; Howie, A. F.; Beckett, G. J.; Williamson, G. Synergy between sulforaphane and selenium in the induction of thioredoxin reductase 1 requires both transcriptional and translational modulation. *Carcinogenesis* **2003**, *3*, 497−503.

(38) Jang, D. S.; Park, E. J.; Hawthorne, M. E.; Vigo, J. S.; Graham, J. G.; Cabieses, F.; Santarsiero, B. D.; Mesecar, A. D.; Fong, H. H.; Mehta, R. G.; Pezzuto, J. M.; Kinghorn, A. D. Constituents of *Musa* × *paradisiaca* cultivar with the potential to induce the phase II enzyme, quinone reductase. *J. Agric. Food Chem.* **2002**, *50*, 6330−6334.

(39) Ryoyama, K.; Mori, N.; Nara, M.; Kidachi, Y.; Yamaguchi, H.; Umetsu, H.; Fuke, Y. Augmented gene expression of quinone reductase by 6-(methylsulfinyl)hexyl isothiocyanate through avoiding its cytotoxicity. *Anticancer Res.* **2003**, *23*, 3741−3748.

(40) Merk, H. F.; Jugert, F. K. Cutaneous NAD(P)H:quinone reductase: a xenobiotica-metabolizing enzyme with potential cancer and oxidation stress-protecting properties. *Skin Pharmacol.* **1991**, *4* (Suppl. 1), 95−100.

(41) Wiencke, J. K.; Spitz, M. R.; McMillan, A.; Kelsey, K. T. Lung cancer in Mexican-Americans and African-Americans is associated with the wild-type genotype of the NAD(P)H:quinone oxidoreductase polymorphism. *Cancer Epidemiol. Biomarkers Prev.* **1997**, *6*, 87−92.

(42) Ross, D.; Kepa, J. K.; Winski, S. L.; Beall, H. D.; Anwar, A.; Siegel, D. NAD(P)H:quinone oxidoreductase 1 (NQO1): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. *Chem.−Biol. Interact.* **2000**, *129*, 77−97.

(43) Arthur, J. R. The glutathione peroxidases. *Cell Mol. Life Sci.* **2000**, *57*, 1825−1835.

(44) Fu, Y.; Cheng, W. H.; Ross, D. A.; Lei, X. Cellular glutathione peroxidase protects mice against lethal oxidative stress induced by various doses of diquat. *Proc. Soc. Exp. Biol. Med.* **1999**, *222*, 164−169.

(45) Ye, L.; Dinkova-Kostova, A. T.; Wade, K. L.; Zhang, Y.; Shapiro, T. A.; Talalay, P. Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans. *Clin. Chim. Acta* **2002**, *316*, 43−53.

(46) Shapiro, T. A.; Fahey, J. W.; Wade, K. L.; Stephenson, K. K.; Talalay, P. Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans. *Cancer Epidemiol. Biomarkers Prev.* **2001**, *10*, 501−508.

(47) Dinkova-Kostova, A. T.; Massiah, M. A.; Bozak, R. E.; Hicks, R. J.; Talalay, P. Potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis depends on their reactivity with sulfhydryl groups. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3404−3409.

(48) Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R. W.; Washko, P. W.; Dhariwal, K. R.; Park, J. B.; Lazarev, A.; Graumlich, J. F.; King, J.; Cantilena, L. R. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3704−3709.

(49) Cameron, E.; Pauling, L.; Leibovitz, B. Ascorbic acid and cancer: a review. *Cancer Res.* **1992**, *39*, 663−681.

Received for review January 3, 2005. Revised manuscript received April 22, 2005. Accepted April 28, 2005.

JF0580059