Aqueous extracts of selenium-fertilized broccoli increase selenoprotein activity and inhibit DNA single-strand breaks, but decrease the activity of quinone reductase in Hepa 1c1c7 cells.

Anna-Sigrid Keck a, John W. Finley b, *

a Department of Food Sciences and Human Nutrition, University of Illinois at Champaign-Urbana, 905 S Goodwin #84A, Urbana, IL 61801, United States
b United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, P.O. Box 9034, Grand Forks, ND 58202-9034, United States

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Abstract

Depending on growth conditions, broccoli may be enriched in the isothiocyanate sulforaphane and/or the mineral selenium (Se); both compounds may play an important role in the reduction of intracellular oxidative stress and chronic disease prevention. Sulforaphane up-regulates transcription of Phase II detoxification proteins (e.g. quinone reductase [QR]), whereas Se is needed for the production of thioredoxin reductase (TR) and glutathione peroxidase-1 (GPx1), both of which exhibit antioxidant activity. The objective of the present study was to determine whether the fertilization of broccoli with Se increases the antioxidant ability of broccoli. Hydrogen peroxide-induced DNA single-strand breaks (measured by single cell electrophoresis, Comet assay) and activity of antioxidant enzymes (GPx, TR and QR) were measured in mouse hepatoma cells (Hepa 1c1c7 cells) treated with purified sulforaphane, sodium selenite or extracts of selenized broccoli. When supplied separately as chemically pure substances, sodium selenite was more effective than sulforaphane for reduction of single-strand breaks. Se-fertilized broccoli extracts were the most effective for reduction of DNA single-strand breaks, and extracts that contained 0.71 μM Se and 0.08 μM sulforaphane inhibited 94% of DNA single-strand breaks. A significant positive association (r = 0.81, p = 0.009) between GPx1 activity and inhibition of DNA single-strand breaks as well as a 24 h lag time between addition of Se, sulforaphane or broccoli extract and inhibition of single-strand breaks suggests that some of the antioxidant protection is mediated through selenoproteins. Conversely, fertilization of broccoli with Se decreased the ability of broccoli extract to induce QR activity. These results demonstrate that Se and sulforaphane, alone or as a component of broccoli, may help decrease oxidative stress. They further suggest that Se is the most important for decreasing oxidative stress, but maximizing the Se content of broccoli also may compromise its ability to induce Phase II detoxification proteins.

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Abbreviations: GPx, glutathione peroxidase; Se, selenium; TR, thioredoxin reductase; QR, quinone reductase.

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* Corresponding author. Present address: A.M. Todd, 150 Domorah Drive, Montgomeryville, PA 19836, United States. Tel.: +1 215 469 1972; fax: +1 215 628 8651.

E-mail address: FinleyJ2@amtodd.com (J.W. Finley).

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1. Introduction

Epidemiologic studies suggest that a diet rich in broccoli (*Brassica oleracea*) protects against many common cancers (Verhoeven et al., 1996). Chemoprotection by broccoli is hypothesized to be from ingestion of bioactive isothiocyanates derived from glucosinolates. Broccoli contains high concentrations of the aliphatic glucosinolate glucoraphanin (0.8–21.7 μmol/g dry weight) (Kushad et al., 1999). The enzyme myrosinase or bacteria in the lower gut may hydrolyze glucoraphanin and form the bioactive isothiocyanate sulforaphane (Getahun and Chung, 1999; Shapiro et al., 1999).

One mechanism by which isothiocyanates may prevent cancer is by inducing Phase II detoxification enzymes. Recent studies have demonstrated that an element in the promoter of many of these enzymes, the antioxidant response element (ARE), may allow for transcriptional control of multiple enzymes in response to dietary activation factors (for review see Finley, 2003). Sulforaphane has been demonstrated to be the most powerful diet-derived inducer of the ARE (Fahey et al., 1997). In addition to detoxification reactions, Phase II proteins may also eliminate reactive oxygen species that potentially cause DNA damage (Ames, 1989; Lofthouse and Poulsen, 1996).

Selenium (Se) is an essential nutrient, and consumption of Se in amounts greater than the recommended intake has been demonstrated to reduce cancer (Clark et al., 1996). Broccoli can accumulate large amounts of Se if it is available from the soil or fertilizer, and Se-enriched broccoli protects against colon and mammary cancer in laboratory animals better than broccoli alone (Finley et al., 2000; Davis et al., 2002; Finley et al., 2001). Although the precise mechanism(s) by which Se inhibits cancer still are unclear, Se is essential for the activity of several important antioxidant enzymes including five glutathione peroxidase (GPx) isozymes and thioredoxin reductase (TR) (Brown and Arthur, 2001). Thioredoxin reductase may function as an isozymes and thioredoxin reductase (TR) (Brown and Arthur, 2001). Thioredoxin reductase may function as an antioxidant by reducing thioredoxin (McKenzie et al., 2002), or as a direct antioxidant enzyme by reducing lipoic acid, small thiols, lipid hydroperoxidases and oxidized ascorbic acid (Mustacich and Powis, 2000). The activity of TR is controlled in part through a translational mechanism that is responsive to Se availability. We have demonstrated that sulforaphane activates TR transcription through an ARE in the promoter region (Hintze et al., 2003a).

We recently reported that Se and sulforaphane concentrations in broccoli are inversely related; i.e. fertilization with Se results in dramatic reduction of sulforaphane content (Robbins et al., 2005). Thus by varying the amount of Se available to broccoli we are able to produce plants with high concentrations of one compound and low concentrations of the other, or we can produce broccoli that contains a moderate amount of both compounds. The objective of this study was to test, in cell culture, the antioxidant capacity of extracts of broccoli grown under three different Se concentrations. We exposed cultured mouse hepatoma cells to broccoli extracts, as well as pure sodium selenite and sulforaphane and we report that the activity of selenoproteins, as well as the resistance of the cells to oxidative stress, is modulated by Se and sulforaphane concentrations alone or the relative concentrations within broccoli extracts.

2. Materials and methods

2.1. Materials

Unless otherwise noted, all other materials used in this study were obtained from Sigma (St. Louis, MO). Purified sulforaphane was kindly provided by Dr. Elizabeth H. Jeffery at University of Illinois, Urbana-Champaign, IL.

2.2. Cell culture

Stock Hepa 1c1c7 cells were grown in 75 cm² polystyrene flasks (Corning, NY) by using Eagle’s minimum essential medium with s-modification and supplemented with 10% fetal bovine serum (Se content of basal media of 0.01 μmol/L). All cells were cultured at 37 °C in a humidified incubator with 5% CO₂. Media were changed every other day and cells were sub-cultured weekly. For Comet assay experiments, cells (40,000 cells/well) were grown in 24 well plates (Corning, NY) for 24 h, then exposed to aqueous broccoli extracts, pure sulforaphane or sodium selenite for 4 or 24 h. At the end of the treatment period cells were incubated with hydrogen peroxide for 30 min. All DNA single-strand break experiments contained negative and positive controls; negative controls were cells grown in cell culture medium without added sodium selenite, sulforaphane or broccoli extracts and not subjected to oxidative stress (no hydrogen peroxide treatment) and treated with cell culture medium only. Positive controls were cells grown with culture medium only without added sodium selenite, sulforaphane or broccoli extracts but oxidative stress was induced by exposure to 50 or 100 μM hydrogen peroxide. Cells were detached with 0.04% trypsin and 0.02% EDTA in 0.15 M phosphate buffer, pH 7.3, washed, and held on ice (~30 min) until used in the Comet assay. Cells used for enzyme activity were grown in 75 cm² culture flasks (1 × 10⁶ cells/flask, Corning, NY) for 24 h prior to exposure to the broccoli extracts or pure sulforaphane or sodium selenite for 4 or 24 h. After the treatments cells were detached with trypsin/EDTA buffer, washed with PBS, and lysed by sonication. Cell lysates were stored at −80 °C until analyzed for TR, GPx1 and QR activities. Experimental data are expressed as mean ± std error (n for each treatment given in tables) from one representative experiment. All experiments were replicated at least twice.

2.3. Broccoli extracts

Broccoli (*Brassica oleracea* var. Majestic) used in this study was grown and handled as previously described, except that the concentration of sodium selenate solution added varied depending on the desired concentration of Se in the broccoli florets (Finley et al., 2000). Broccoli used for this study contained 0.005 ± 0.001, 1.250 ± 0.007 or 11.143 ± 0.166 μmol Se/g dry weight (for low, medium and high-Se, respectively). Aqueous
extracts were prepared by mixing one part of finely ground lyophilized broccoli powder with nine parts of deionized water; this was wrapped in foil, and left for 8 h at room temperature. After centrifugation at 10,000 rpm for 10 min the supernatant was collected and the pellet re-extracted twice with deionized water. All supernatants were pooled, filtered and stored at −80 °C until analysis or used in the in vitro experiments. Stock solutions (50 mg dry weight broccoli/ml water) of low-Se broccoli extract contained 239 ± 2 μM sulforaphane and 1.3 ± 0.2 μM Se, medium-Se broccoli contained 157 ± 3 μM sulforaphane and 35.4 ± 1.3 μM Se and high-Se broccoli extract contained 41 ± 3 μM sulforaphane and 354 ± 56 μM Se.

2.4. Experimental design experiment 1. Effect of incubation time on DNA single-strand breaks

Extracts of low or high-Se broccoli equal to 10 or 100 μg dry weight broccoli/ml medium (final concentrations of sulforaphane and Se per well ranged from 0.01 to 0.48 μM and <0.001 to 0.7 μM, respectively) were incubated in duplicate with Hepa 1c1c7 cells for 4 or 24 h; cells were exposed to 50 μM hydrogen peroxide for 30 min and DNA single-strand breaks were determined by the Comet assay.

2.5. Experiment 2. Hydrogen peroxide concentration and DNA single-strand breaks

High-Se broccoli extracts equal to 5, 10, 50 and 100 μg dry weight broccoli/ml medium (final concentrations of 0.01–0.08 μM sulforaphane and 0.07–0.7 μM Se) were incubated in duplicate with Hepa 1c1c7 cells for 24 h. Cells were then exposed to hydrogen peroxide (50 or 100 μM) for 30 min and DNA single-strand breaks determined.

2.6. Experiment 3. Selenium concentration of broccoli and inhibition of DNA single-strand breaks

Extracts of low, medium, and high-Se broccoli in amounts equal to 10, 50, 100 and 500 μg dry weight broccoli/ml medium (final concentrations of 0.01–2.40 μM sulforaphane and <0.001–3.55 μM Se) were incubated with Hepa 1c1c7 cells for 24 h (n = 4 wells/treatment); cells were exposed to 50 μM hydrogen peroxide for 30 min and DNA single-strand breaks determined.

2.7. Experiment 4. Effect of chemically pure sulforaphane and sodium selenite on DNA single-strand breaks

Pure sulforaphane and sodium selenite (final concentrations of 0.5–2 μM for both) were incubated (n = 4 wells/treatment) with Hepa 1c1c7 cells for 24 h; cells were exposed to 50 μM hydrogen peroxide for 30 min and DNA single-strand breaks determined.

2.8. Experiment 5. Effect of broccoli extracts and pure sulforaphane and sodium selenite on antioxidant enzyme activities

Extracts of low-Se, medium-Se, and high-Se broccoli equal to 10, 50, 100 and 500 μg dry weight broccoli/ml medium (final concentrations of sulforaphane and Se per well ranged from 0.04 to 0.96 μM and 0.001 to 1.4 μM, respectively, Table 3) or pure sulforaphane and sodium selenite (0.5–2 μM) were incubated in triplicate with Hepa 1c1c7 cells for 4 or 24 h (n = 3 wells/treatment); before determining GPx, TR, and QR activities.

2.9. Alkali single cell gel electrophoresis (Comet) assay

DNA single-single-strand breaks were determined by single-cell electrophoresis, or the Comet assay, following the procedure of Burdette et al., 2002. The procedure used reagents and CometSlides™ from Trevigen (Gaithersburg, MD). In brief, 50 μL cell suspension was gently mixed with 500 μL LMAgarose (+42 °C) and a 75 μL aliquot was transferred onto the CometSlide. After the gel had solidified at 4 °C for 10 min, the slide was transferred to a pre-chilled lysis buffer for 30 min before it was submerged in pre-chilled electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH > 13) for 20 min. The slide was then electrophoresed on ice for 30 min at 300 A and 25 V. After the electrophoresis the slide was placed in 80% ethanol for 5 min and dried for 24 h. The DNA damage was evaluated using SYBR green staining solution and a fluorescent microscope with a FITC filter. Two hundred comets on each slide were scored visually according to the relative intensity of fluorescence in the tail. The intensity score of the DNA damage was ranked on a scale from 0 to 4 (n0, n1, n2, n3 and n4) where n0 is no damage (only an intact nucleus is observed) and n4 is the maximum damage (no nucleus is present and all DNA is in a diffuse ‘comet shaped’ form) was assigned to each cell. This type of non-parametric procedure (see equation below) is the most commonly used procedure to display DNA damage data that is visually collected by scoring fluorescent tail sizes (Burdette et al., 2002). The DNA damage was generated by the following formula: DNA damage = (1 ast n1 + 2 * n2 + 3 * n3 + 4 * n4)/(1/100) where 1 is the total number of nuclei counted (at least 150 and optimally 200). If there is no DNA damage the total score is zero and if all the nuclei have maximum damage the score is 400. The percent inhibition of DNA damage (%IDD) was then generated by the following formula %IDD = (100 – (mean of sample – mean of negative control) / (mean of positive control – mean of negative control) * 100). To insure that there was no bias in the counting, slides were assigned to and identified by a random number during the scoring process; the same person counted all slides for all experiments.

2.10. Selenium and enzyme activity analysis

Selenium was analyzed by hydride generation atomic absorption spectrometry following digestion in nitric acid and ashing in a muffle oven (with a matrix modifier to prevent volatilization) (Finley et al., 1996). Activity of QR was measured in microtiter plates using 3-(4,5-dimethylthiazolo-2-yl)-2,5-diphenyltetrazolium bromide as the substrate, following the method of Prochaska and Santamaria, 1988. Activity of TR was measured by the method of Holmgren and Bjornstedt, 1995 as modified by Hill et al. (1997) and activity of GPx1 was measured by method of Paglia and Valentine (1967) using 1.5 μM hydrogen peroxide as substrate. Protein concentrations were measured by the BioRad assay (Hercules, CA) based on the Bradford method (1976), using bovine serum albumin as standard.

2.11. Statistical analysis

Treatment effects were determined using analysis of variance (one-way ANOVA). Where a significant effect (p < 0.05) was found, Tukey’s studentized range test was used to determine differences between means. Pearson correlation coefficients were generated between enzyme activities and DNA single-strand breaks.

3. Results

3.1. DNA Single-strand break measured by the comet assay

3.1.1. Experiment 1

DNA single-single-strand breaks were inhibited by both low and high-Se broccoli extracts when they were included in incubation media for 24 h, however no inhibition was observed when extracts were incubated with cells for only 4 h (Table 1). For the high-Se broccoli at the 24 h incubation time point, the percent inhibition depended on the amount of extract added to the media (which affected the molar concentrations of Se and sulforaphane) while for the low-Se broccoli the protective effect was maximal.
already with 10 μg dry weight/ml. Relative to positive controls, DNA single-strand breaks were inhibited by as much as 93% when cells were incubated with high-Se broccoli extracts for 24 h.

### 3.1.2. Experiment 2

Broccoli extracts together with hydrogen peroxide concentrations of 100 μM resulted in as much as a 21-fold more DNA single-strand breaks than comparable treatments with 50 μM hydrogen peroxide (%IDD of 6.8 and 142.1 for the highest concentration of extract on cells treated with 50 and 100 μM hydrogen peroxide, respectively) (Table 2). Broccoli extracts containing less than 0.07 μM Se did not inhibit single-single-strand breaks induced by 100 μM hydrogen peroxide, whereas the same treatment inhibited 39% of the single-single-strand breaks in cells treated with 50 μM hydrogen peroxide. Selenium concentrations of 0.71 μM almost completely inhibited 50 μM hydrogen peroxide-induced single-single-strand breaks but only inhibited 44% of single-strand breaks in cells treated with 100 μM hydrogen peroxide.

### 3.1.3. Experiment 3

The inhibition of DNA single-strand breaks by broccoli extracts was dependent on the concentration of Se in the broccoli (Table 3). Cell culture medium with 0.71 μM Se and 0.08 μM sulforaphane resulted in 94% inhibition of DNA single-strand breaks (inhibition was not significantly different from the negative control). Means with different letters are significantly different from each other and from positive controls.
increased by medium concentrations of 3.55 l M and 0.4 l M sulforaphane), whereas extracts prepared from broccoli with lower Se concentrations resulted in only 51–66% inhibition. The highest concentration of low-Se broccoli extract resulted in media with the highest sulforaphane concentration (2.4 l M sulforaphane, 0.15 l M Se), but maximum inhibition (by this medium) was only 60%.

3.1.4. Experiment 4

Pure sulforaphane and sodium selenite inhibited DNA single-strand breaks in a dose dependent manner (Table 4). Similar to broccoli extracts, sodium selenite provided more protection than sulforaphane; 1 l M sodium selenite completely inhibited single-strand breaks, whereas the maximum concentration of purified sulforaphane (2 l M) only inhibited 62% of DNA damage (Table 4).

3.1.5. Experiment 5. Enzyme activities

Compared to control media, 0.5 l M Se increased TR and GPx activity ~100%; concentrations of Se > 0.5 l M did not cause a further increase in activity (Fig. 1). Overall, TR and GPx activities were significantly higher in cells incubated with extracts of high-Se broccoli, as compared to low-Se broccoli extracts. Activity of QR relative to control media was increased by addition of 0.5 l M sulforaphane; increased concentrations of sulforaphane did not cause a further increase in QR. Increasing amounts of broccoli extracts up-regulated QR activity, but Se fertilization of broccoli did not affect overall QR activity. Enzyme activities were not upregulated after 4 h incubation (data not shown). There was a significant correlation (p = 0.009) between DNA single-strand break protection and GPx activity (Table 5), a trend towards a correlation between TR activity and DNA single-strand breaks (p = 0.08), and no correlation between QR activity and DNA single-strand breaks (p = 0.6).

4. Discussion

It has been reported previously that TR, a selenoprotein with antioxidant activity, is regulated in part by both

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Table 3

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Extract (μg DW/ml)</th>
<th>SF (μM)</th>
<th>Se (μM)</th>
<th>DNA damage</th>
<th>% Inhibition of DNA damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg. control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Pos. control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>224.8 ± 9.7</td>
<td></td>
</tr>
<tr>
<td>Low-Se broccoli</td>
<td>10</td>
<td>0.05</td>
<td>&lt;0.001</td>
<td>110.9 ± 13.8</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.24</td>
<td>0.001</td>
<td>68.6 ± 12.6</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.48</td>
<td>0.003</td>
<td>115.2 ± 12.6</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2.40</td>
<td>0.015</td>
<td>90.4 ± 9.9</td>
<td>60</td>
</tr>
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<td>Medium-Se broccoli</td>
<td>10</td>
<td>0.03</td>
<td>0.01</td>
<td>152.4 ± 10.1</td>
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<tr>
<td></td>
<td>50</td>
<td>0.16</td>
<td>0.04</td>
<td>137.4 ± 12.6</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.31</td>
<td>0.07</td>
<td>79.7 ± 12.6</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>1.55</td>
<td>0.35</td>
<td>77.7 ± 8.3</td>
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</tr>
<tr>
<td>High-Se broccoli</td>
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<td>0.01</td>
<td>0.07</td>
<td>123.2 ± 16.6</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.04</td>
<td>0.35</td>
<td>49.8 ± 6.9</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.08</td>
<td>0.71</td>
<td>14.6 ± 5.8</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.40</td>
<td>3.55</td>
<td>3.6 ± 1.6</td>
<td>99</td>
</tr>
</tbody>
</table>

1. Amount of broccoli extract used per incubation expressed as μg dry weight (DW) broccoli/ml medium.
2. Final concentrations (μM) of sulforaphane (SF) and selenium (Se) incubated with broccoli extracts.
3. Negative controls represent basal DNA damage in cells incubated only in culture medium and not exposed to hydrogen peroxide; positive controls are cells exposed to 50 l M hydrogen peroxide for 30 min and incubated only in cell culture medium.
4. Data are means ± SE (n = 4) where 200 nuclei/sample were scored from one representative experiment; means with different superscripts are significantly different.

Table 4

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Concentration (μM)</th>
<th>DNA damage</th>
<th>% Inhibition of DNA damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg. control</td>
<td>0</td>
<td>24.9 ± 4.5</td>
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</tr>
<tr>
<td>Pos. control</td>
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<td>147.5 ± 5.7</td>
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<tr>
<td>Sulforaphane</td>
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<td>115.2 ± 2.6</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>95.2 ± 13.2</td>
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<tr>
<td></td>
<td>2.0</td>
<td>71.0 ± 9.2</td>
<td>62</td>
</tr>
<tr>
<td>Sodium selenite</td>
<td>0.5</td>
<td>72.3 ± 14.7</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>21.4 ± 5.3</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>24.2 ± 2.9</td>
<td>101</td>
</tr>
</tbody>
</table>

1. Concentration in incubation medium.
2. Negative controls represent basal DNA damage in cells incubated only in culture medium and not exposed to hydrogen peroxide; positive controls are cells exposed to 50 l M hydrogen peroxide for 30 min and incubated only in cell culture medium.
3. Data are means ± SE (n = 4) where 200 nuclei in each sample where scored from one representative experiment. Each experiment was repeated at least twice. Means with different superscripts are significantly different.
sulforaphane and Se (Hintze et al., 2003b) as well as by extracts of broccoli (Hintze et al., 2005). We recently reported that Se fertilization of broccoli decreased the accumulation of sulforaphane as unfertilized broccoli extracts contained 239 ± 2 μM sulforaphane and 1.3 ± 0.2 μM Se whereas fertilization with supre-nutritional levels of Se resulted in a broccoli-Se content of 354 ± 5.6 μM resulted in a total sulforaphane concentration of only 41 ± 3 μM (Robbins et al., 2005). These findings suggest that compounds in broccoli protect against oxidative stress, and the degree of protection may be affected by Se fertilization. The present study presents evidence that
Table 5
Association between protection against hydrogen peroxide-induced DNA damage, as measured by Comet assay, and antioxidant enzyme activities in Hepa 1c1c7 cells incubated with varying concentrations of Se and sulforaphane as pure chemicals or as broccoli extracts for 24 h prior to addition of 50 μM hydrogen peroxide.

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>DNA damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinone reductase</td>
<td>$r = 0.183$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.637$</td>
</tr>
<tr>
<td>Thioredoxin reductase</td>
<td>$r = -0.607$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.083$</td>
</tr>
<tr>
<td>Glutathione reroxidase</td>
<td>$r = -0.806$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.009$</td>
</tr>
</tbody>
</table>

Data are Pearson Correlation coefficients between means ($n = 9$).

components of broccoli protect against oxidative stress-induced DNA strand breaks as measured by the Comet assay, but while sulforaphane does provide some protection, the primary protective effect is from Se.

Many in vitro assays have been developed to determine the ‘antioxidant quality’ of foods or chemical substances, but most are of limited value because they do not measure a functional outcome (Prior and Cao, 1999). The single cell electrophoresis, or Comet assay, measures oxidative stress-induced DNA single-strand breaks (Bonniesen et al., 2001; Verhagen et al., 1995; Deng et al., 1998), and thus measures a functional outcome related to cancer resistance. DNA single-strand breaks as measured by the Comet assay have been reported to be inhibited by extracts of vegetables, especially tomatoes (Porrini and Riso, 2002), and by selenium compounds (Waleh et al., 1998). Brassica spp. reported to inhibit damage include mustard sprouts (Uhl et al., 2003) which inhibited benzo(a)pyrene-induced DNA single-strand breaks in human hepatoma cells in culture, and Brussels sprouts extracts which reduced hydrogen peroxide-induced DNA single-strand breaks in Hepa 1c1c7 cells (Zhu and Loft, 2003). Selenomethionine and selenocysteine were reported to inhibit peroxynitrite-induced DNA single-strand breaks in pBluescript II KS plasmids (Roussyn et al., 1996) and dietary Se protected against DNA single-strand breaks in canine prostate and lymphocytes (Waleh et al., 1998).

In the present study, when chemically pure compounds were compared on an equimolar basis, sodium selenite was more effective than sulforaphane for inhibition of DNA strand breaks. Likewise, when broccoli extracts were used, high-Se extracts provided more protection than did high-sulforaphane extracts. Although Se seemed to be the compound most associated with protection against strand breaks, synergism of Se with other factors in broccoli was suggested. Incubation of cells with high Se broccoli extracts that contained 0.35 μM Se and 0.04 μM sulforaphane (Table 3) resulted in 78% protection against single-strand breaks, whereas 0.5 μM sodium selenite or 0.5 μM pure sulforaphane provided only 61% or 26% protection, respectively.

That Se was the most protective compound in broccoli was demonstrated first by the observation that inhibition of DNA single-strand breaks by sodium selenite plateaued at 1.0 μM Se, but 2.0 μM pure sulforaphane only inhibited 62% of the single-strand breaks (Table 4). Also, despite possibly reduced phenolic acid content, and a sulforaphane content of only 17% of the low-Se broccoli extract, extracts of high-Se broccoli resulted in much greater protection against DNA damage than did low-Se broccoli extracts. The exact mechanism for the protective effect cannot be determined from the present study, however the lack of protection after 4 h of incubation argues against a direct antioxidant function. Further, that DNA strand break protection corresponded to an increase in TR and GPx activity (as well as a significant association between DNA strand break protection and GPx activity) suggests a role for selenoproteins, and warrants further investigation.

However, if selenoprotein activity is involved, it cannot account for all of the Se-mediated reduction of oxidative stress; and other antioxidant functions induced by Se may depend on the chemical form of Se. When cells where treated with sodium selenite, 0.5 μM saturated TR and GPx activity (Fig. 1), but 1.0 μM was required to maximally inhibit DNA single-strand breaks (Table 4). Conversely, when broccoli extracts were the source of Se, protection against DNA single-strand breaks was maximized at Se concentrations of 0.71 μM Se, whereas 3.55 μM Se was required to maximize GPx1 activities (Fig. 1). Some Se in broccoli may be in the form of selenite, but broccoli also contains many other species, including methylated forms such as Se-methyl selenocysteine (Roberge et al., 2003).

The present data show that in addition to altering the chemical profile (especially sulforaphane and phenolic acids) (Robbins et al., 2005), Se fertilization of broccoli also changes its potential nutritional benefits. Fig. 1 shows that compared to unfertilized broccoli, extracts of broccoli fertilized with Se were more effective inducers of selenoprotein activity and provided more protection against DNA strand breaks. The apparent synergism of Se and other compounds in broccoli (most likely sulforaphane, although several compounds in broccoli activate the TR ARE) (Hintze et al., 2005; Finley, 2003) makes it tempting to speculate that the health benefits of broccoli could be Improved by Se fertilization. However, QR activity, a Phase II detoxification protein responsive to sulforaphane (Fahey et al., 1997), was highest in medium and low-Se broccoli extracts, suggesting that the ability of broccoli to induce Phase II detoxification proteins may actually be lowered by Se fertilization. This suggests broccoli may function either as Se accumulator or as a source of glucosinolates, but it is presently not possible to combine high levels of both compounds in a single plant. Combining both will require delineating and then manipulating the biochemical reactions responsible for the antagonistic relationship between Se and sulforaphane. The antagonistic relationship between these compounds certainly provides
an example, and potential warning, that maximizing a single compound in a plant may come at the expense of other beneficial components of a plant.

In summary, the present data demonstrate that broccoli extracts, as well as purified compounds found in broccoli, have antioxidant function and inhibit DNA single-strand breaks in cultured cells. Although both Se and sulforaphane were able to inhibit strand breaks, Se was much more effective on an equimolar basis. The relative efficacy of broccoli extracts depended on the ratio of Se:sulforaphane—extracts of plants with a high ratio were quite effective antioxidants whereas extracts with a low ratio were much less effective. However Se fertilization also inhibits the accumulation of other compounds in broccoli, and Se-fertilized broccoli does not activate some Phase II detoxification proteins as well as unfertilized broccoli.

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