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SELENIUM MODULATES PEROXIDATION IN THE ABSENCE OF GLUTATHIONE PEROXIDASE IN *MUSCA DOMESTICA*

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SUMMARY: Adult houseflies fed a low-selenium diet showed a 73% decrease in total Se compared to those given 1.0 ppm Se in their drinking water. This decrease was associated with a 84.4% increase in thiobarbituric acid reactants and a 16.3% increase in conjugated dienes. These increases were unrelated to activities of glutathione S-transferases, superoxide dismutases and catalase and to levels of reduced and oxidized glutathione, all of which were unaltered by Se deficiency. Since houseflies lack glutathione peroxidase, Se apparently modulates peroxidation in these animals independent of the antioxidant enzymes and glutathione.

The discovery by Rotruck *et al.* (1) that selenium is an integral component of the selenoenzyme, glutathione peroxidase (EC 1.11.1.9) provided an explanation for the antioxidant property of this element (2). There is also some evidence to suggest that Se affects peroxidation apart from its role in the selenoenzyme (3). However, in mammals it is difficult to modulate tissue Se levels without simultaneously affecting glutathione peroxidase activity.

There is considerable evidence indicating that insects lack glutathione peroxidase (4-8). Consequently, these animals are ideal *in vivo* models for studying the biochemistry of Se independent of its function in this enzyme. In the present study, the antioxidant property of Se is dissociated from the antioxidant enzymes and glutathione in the housefly (*Musca domestica*). Thus, Se can alter peroxidation in *M. domestica* by a mechanism other than its well-documented role in glutathione peroxidases of vertebrates.

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MATERIALS AND METHODS

Animal treatment

A stock colony of houseflies (*M. domestica*) was maintained as described (9). Eggs were collected from the stock colony and larvae were reared on a low-Se diet (<0.02 ppm Se) as described (10). Low-Se animals were separated as pupae into 10 groups of 200 flies. Each group was housed in a 5400 cm^3 cage kept at 23°C, approximately 40% relative humidity, and a 12 h light/12 h dark photoperiod. Newly emerged flies were fed deionized drinking water and a dry diet consisting of low-Se torula yeast and sucrose (1:1) (<0.02 ppm Se). After all the flies had emerged, sodium selenite was administered in drinking water to 5 of the groups at a concentration of 1.0 ppm Se (w/v). After 10 days of treatment, sodium selenite was omitted from drinking water of all groups for 24 h to clear the gut of dietary Se prior to homogenization.

Flies were anesthetized with carbon dioxide and homogenized in chilled 0.25 M sucrose containing 1 mM EDTA, pH 7.4, to give 10% (w/v) homogenates. Cell debris were sedimented by centrifugation at 700 g for 10 min to yield a crude extract which was sonicated on ice with 15 s bursts for a total of 2 min.

Analyses

The Se-independent glutathione peroxidase activity of glutathione S-transferases was measured by the coupled assay procedure of Lawrence and Burk (11) as modified by Simmons *et al.* (8). A unit of activity is defined as the amount of enzyme required to oxidize 1 nmole of NADPH/min.

Glutathione S-transferase activity was measured by conjugation of glutathione to 1-chloro-2,4-dinitrobenzene according to the method of Habig *et al.* (12). A unit of activity corresponds to the formation of 1 nmole conjugate/min.

Superoxide dismutase activity was assayed in crude extracts by a modification of the method of Winterbourn *et al.* (13). Total (Mn + Cu/Zn-containing) and cyanide insensitive (Mn-containing only) superoxide dismutase activity was determined by addition of potassium cyanide to the reaction mixture to give a final concentration of 0.02 mM and 2.0 mM, respectively. Cyanide-sensitive activity (Cu/Zn-containing only) was calculated by subtracting the cyanide-insensitive activity from the total. A unit of activity is defined as the amount of enzyme which inhibits by 50% the reduction of nitroblue tetrazolium by riboflavin-generated superoxide radical.

Catalase activity was measured in crude extracts by the method of Cohen *et al.* (14). Activity is expressed in terms of the first-order reaction rate constant.

Protein was quantified according to the dye-binding method of Bradford (15) using Coomassie Brilliant Blue G-250. Bovine serum albumin was used as the standard.

Total Se was determined by the method of Watkinson (16) as modified by Gasiewicz and Smith (17).

Glutathione was measured by the method of Hissin and Hilf (18). The assay was modified in that approximately 0.125 g of tissue was homogenized in a total of 4 ml buffer containing metaphosphoric acid. Also, N-ethylmaleimide was immediately added to aliquots of homogenate for glutathione disulfide determination prior to centrifugation.

Thiobarbituric acid reactants were quantified in 10% (w/v) homogenates in 0.25 M sucrose according to the method of Ohkawa *et al.* (19) as modified by Jamall and Smith (20). The absorption maximum of thiobarbituric acid reactants from tissues homogenized in sucrose was 517 nm, in agreement with Schlafer and Shepard (21).

Conjugated dienes were measured by the procedure of Buege and Aust (22). Their procedure was modified in that lipids were extracted from 300 ml aliquots of 10% (w/v) homogenates. Absorbance of conjugated dienes at 234 nm was normalized against the protein moiety of extracted proteolipids, which was calculated from absorbance at 260 nm and 280 nm using the Warburg and Christian equation (23).

RESULTS

Total Se levels in tissue from flies fed a low-Se diet were 27% of those of Se-supplemented flies (Table 1). A 84.4% increase in thiobarbituric acid reactants was associated with tissue Se depletion. Because the thiobarbituric acid assay has been criticized by some (24,25) as being more a measure of *in vitro* rather than *in vivo* peroxidation, conjugated dienes were measured as a second index of peroxidation. A 16.3% increase in levels of conjugated dienes in Se deficient flies (Table 1) substantiated the results obtained using the thiobarbituric acid assay.

Selenium deprivation did not affect the Se-independent glutathione peroxidase or glutathione conjugation activities of glutathione S-transferases; nor were the activities of catalase, cytosolic (Cu/Zn) and mitochondrial (Mn) superoxide dismutase, or levels of reduced and oxidized glutathione affected by Se deficiency (Table 1). The reduced glutathione to glutathione disulfide ratio of approximately 5 observed here using a fluorometric assay is low compared to that in mammals. This observation is in agreement with Sohal (7) who reported a ratio of 4 for houseflies using an enzymatic method.

DISCUSSION

Since glutathione peroxidase is absent in the housefly (7,8), Se modulated peroxidation by a mechanism other than detoxification of H_2O_2 by this selenoenzyme. Changes in peroxidation could not by explained by the activities of the other antioxidant defense enzymes or levels of reduced and oxidized glutathione, all of which were unaltered during Se deficiency. The ability of Se to affect peroxidation apart from its role in the selenoenzyme, has been previously suggested in rats by Burk *et al.* (3). They used time course experiments to demonstrate that injection of Se-deficient rats with sodium selenite protected against diquat-induced peroxidation, without a concurrent increase in glutathione peroxidase activity.

The biochemical function of Se which affects peroxidation in houseflies and rats independent of glutathione peroxidase is unkown. It is plausible that a

Table 1. Effects of dietary Se on activities of glutathione S-transferase and antioxidant defense enzymes, and on levels of Se, peroxidation and glutathione in houseflies supplemented with sodium selenite in the drinking water for 10 days

	Dietary selenium (ppm)	
	<0.02	1.0
Selenium ($\mu g \cdot g$ tissue wet wt^{-1})	0.23 ± 0.08	0.87 ± 0.34*
Thiobarbituric Acid Reactants (absorbance units•g tissue wet wt^{-1})	55.9 ± 4.3	30.3 ± 8.7*
Conjugated Dienes (absorbance units•μg $protein^{-1}$)	1.43 ± 0.06	1.23 ± 0.11*
Se-independent Glutathione Peroxidase (units•mg $protein^{-1}$)	11.1 ± 2.8	7.9 ± 3.0
Glutathione S-transferase (units•mg $protein^{-1}$)	107.0 ± 12.1	115.6 ± 4.2
Cu/Zn-Superoxide Dismutase (units•mg $protein^{-1}$)	31.8 ± 5.7	27.1 ± 8.1
Mn-Superoxide Dismutase (units•mg $protein^{-1}$)	39.6 ± 7.4	39.6 ± 7.4
Catalase ($k \cdot sec^{-1} \cdot g$ $protein^{-1}$)	12.7 ± 6.0	12.0 ± 4.4
Reduced Glutathione (nmoles•mg tissue wet $wt.^{-1}$)	3.71 ± 0.31	3.79 ± 0.66
Glutathione Disulfide (nmoles•mg tissue wet wt^{-1})	0.71 ± 0.09	0.75 ± 0.10

All values are reported as mean ± S.D. of 5 populations of flies (200 flies per population). Statistical significance calculated by the one-way ANOVA, *$P<0.05$.

seleno-compound other than glutathione peroxidase is involved. For example, seleno-amino acids such as selenocysteine, selenocystine, selenocystamine and selenomethione can catalyze the reduction of of peroxides *in vitro*, and they may be capable of functioning as antioxidants *in vivo* (26-29). Alternatively, a metabolic effect of Se may be involved. Selenium deficiency alters a number of metabolic processes in mammals and some of these alterations may promote peroxidation (30).

In conclusion, although Se appears to protect against diquat-induced peroxidation in mammals apart from its role in glutathione peroxidase (3), further investigation is limited by the difficulty of dissociating changes in Se status from alterations in glutathione peroxidase activity in vertebrates. In the present study, this limitation was overcome by using the adult housefly which lacks the selenoenzyme (7,8). Our findings agree with those of Burk *et al.* (3) in that Se affects peroxidation independent of glutathione peroxidase. Moreover, we have demonstrated a relationship between Se deficiency and peroxidation in the absence of a dietary pro-oxidant.

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