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The effect of selenium deficiency on peroxidative injury in the house fly, *Musca domestica*

A role for glutathione peroxidase

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Selenium-dependent glutathione peroxidase activity is documented for the first time in insects. Reduction in glutathione peroxidase activity in the cytosol of adult house flies by lowering selenium in the diet results in significant increases in peroxidative injury. Catalase activity, while higher in low-selenium flies than in selenium-supplemented flies, does not prevent lipid peroxidation. The discovery of glutathione peroxidase activity in insects eliminates an anomaly which partially limited the usefulness of these animals as models for the study of the antioxidant defense system.

Selenium; Glutathione peroxidase; Catalase; Peroxidative injury; Cytosol; *(Musca domestica)*

1. INTRODUCTION

The selenoenzyme glutathione peroxidase (GSH-PX) and catalase (CAT) are the two enzymes responsible for the detoxification of hydrogen peroxide (H_2O_2) in biological systems [1]. Based primarily on studies with insects as models for the free radical theory of ageing [2], CAT is believed to be the primary enzyme for metabolism of H_2O_2 in these invertebrates. The importance of GSH-PX has not been examined because earlier reports [3,4] indicated that this enzyme is absent in insects. The absence of GSH-PX in insects has partially limited the usefulness of these animals as models for the study of the antioxidant defense system.

Reduced GSH-PX activity as a result of low dietary selenium (Se) has been shown to result in cardiomyopathy in both rodents [5] and humans [6]. In in vitro studies with isolated perfused hearts, CAT has been shown to be important in metabolism of H_2O_2 [7] and in the prevention of myocardial damage which has been attributed to oxygen-derived free radicals in ischemic-reperfused hearts [8,9]. Nevertheless, recent studies in our laboratory [10,11], as well as in others [12–14], question the relative importance of CAT. These in vivo studies [10,11,14] indicate that, in mammals at least, GSH-PX plays a more important role than CAT in the detoxification of endogenous H_2O_2 and subsequent peroxidative injury. The importance of GSH-PX in mammals provided the impetus to re-examine whether or not GSH-PX activity is present in insects.

We report here for the first time that selenium-dependent GSH-PX activity is present in the cytosol of insects. The effect of low dietary Se on GSH-PX activity, CAT activity, and peroxidative injury in the adult house fly, *Musca domestica* is also examined.

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2. MATERIALS AND METHODS

2.1. *Animal treatment*

Larvae were reared in batches on a diet composed of 240 g alphacel (<0.02 ppm Se) (ICN Biochemicals Inc., Cleveland, OH), 100 g of low-Se torula yeast (<0.02 ppm Se) (Dyets, Pennsylvania), 60 g alfalfa meal (0.07 ± 0.02 ppm Se) (Bio Serv Inc., Frenchtown, NJ), 15 g grade I sucrose (Sigma, St. Louis, MO), 600 mg methylparaben (Sigma) and 600 ml deionized water. The final concentration of Se in the larval diet was less than 0.02 ppm. Newly emerged adult males were separated into populations of 200 flies. Each group was housed in a 5400 cm^3 cage kept at 23°C, relative humidity of 40% and 12 h light/12 h dark cycle. Adult flies were fed a dry diet of low-Se yeast and sucrose (1 : 1) (<0.02 ppm Se). Selenium, as sodium selenite, was administered in deionized drinking water at concentrations of either 0, 1.0 or 2.0 ppm Se (w/v) for 10 days.

Within each treatment group, flies were pooled for tissue homogenization and biochemical analyses.

2.2. *Tissue preparation*

10% (w/v) homogenates were made in a 0.25 M sucrose solution using a teflon-glass homogenizer. Homogenates were centrifuged at 15000 × *g* for 15 min at 4°C and the supernatant was recentrifuged at 100000 × *g* for 60 min at 4°C. The final supernatant (cytosolic fraction) was used for enzyme assays.

Thiobarbituric acid (TBA)-reactive substances in homogenates were measured to assess peroxidative injury according to [10].

GSH-PX activity was measured spectrophotometrically by following the oxidation of NADPH by H_2O_2 (0.25 M) at 340 nm at ambient temperature (23 ± 2°C) [15].

CAT activity was determined as described by [16].

Protein was measured by the dye-binding method described in [17]. Bovine serum albumin was used to construct the standard curve.

Total Se in the diet and tissues was assayed fluorimetrically according to [18].

3. RESULTS

Addition of 1.0 ppm Se to the drinking water of flies resulted in an 8-fold increase in total Se compared to unsupplemented flies (table 1). A 15-fold increase over the unsupplemented flies was observed in populations given 2.0 ppm Se.

Compared to animals maintained on drinking water supplemented with 1 and 2 ppm Se, unsupplemented flies displayed a 27% decrease in GSH-PX activity, 43% higher levels of TBA-reactive substances, and a 44% increase in catalase activity (table 1).

Pupae from larvae reared on a stock diet, consisting predominantly of wheat bran (0.16 ppm

Table 1

Effects of various levels of selenium treatment for 10 days on lipid peroxidation and cytosolic catalase and GSH-PX activities in the adult housefly

	Dietary selenium (ppm)		
	0.0	1.0	2.0
GSH-PX (nmol NADPH oxid./ min per mg protein)	27.8 ± 2.7	37.8 ± 4.3**	38.1 ± 2.3**
TBA-RX Substs. (Abs./mg tissue × 10^3)	7.3 ± 1.3	4.8 ± 0.9*	4.9 ± 0.8*
Catalase (k/s per mg protein × 10^{-3})	42.3 ± 3.9	28.9 ± 8.6*	30.0 ± 5.0**
Selenium (μg/g tissue wet wt)	0.092 ± 0.011	0.690 ± 0.087**	1.375 ± 0.109**

All values reported as mean ± SD of 4 populations of flies (200 flies per population). Statistical significance was calculated by the one-way analysis of variance (ANOVA), compared to the 0.0 ppm Se group. * $P < 0.05$; ** $P < 0.01$

Se), were also tested for GSH-PX activity. The mean GSH-PX activity of these stock pupae from day of pupation to day of adult ecdysis was 9.3 ± 2.0 nm NADPH oxid./min per mg protein. The activity in pupae is substantially lower than that in the adult. Here it was 67% lower than the unsupplemented adult flies and 75% lower than the supplemented adults (table 1) obtained from larvae reared on the low-Se experimental diet.

4. DISCUSSION

The results presented here indicate for the first time that (i) GSH-PX activity is present in insects, (ii) this activity is modulated by dietary Se, (iii) reduction in this activity is associated with increased peroxidative injury, and (iv) this injury is not prevented by an increase in CAT activity.

Contrary to previous observations [3,4], we found that Se-dependent GSH-PX activity is present in the cytosol of house flies. In one paper [3], the assertion that GSH-PX activity is absent in insects is based on 'personal observation' for which no details are elaborated. In the other paper [4], GSH-PX activity was not detected in homogenates of fleshfly (*Sarcophaga* sp.) and silk moth (*Antheraea pernyi*) pupae. The pupal stage of endopterygotic insects is marked by an overall reduction in metabolic rate as indicated by low oxygen consumption and reduced levels of enzymes involved with energy release [19]. Most likely, the activities of antioxidant enzymes such as GSH-PX are reduced in response to lower metabolic rates and generation of fewer oxygen radicals. For example, our data show that GSH-PX activity in house fly pupae is 67–75% lower than that found in adults. It is therefore plausible that the dipteran and lepidopteran pupae surveyed in this earlier study possessed levels of GSH-PX activity that could not be detected by the assay employed.

As in other animals [10,11], reduction in GSH-PX activity in house flies was associated with increased quantities of TBA-reactive substances. Our data indicate that not only do flies possess GSH-PX activity, but that marked reductions in this activity can result in peroxidative injury. Thus, Se appears to be an essential trace element for insects.

Interestingly, cytosolic CAT activity was higher in the low-Se flies. A similar phenomenon has been documented in our laboratory for rats [11]. There are at least two possible explanations for this trend. Catalase activity may be higher in order to compensate for decreased GSH-PX activity and greater H_2O_2 concentrations, or lower GSH-PX activity may lead to increased damage of peroxisomal membranes and leakage of CAT into the cytosol. Regardless of the validity of these explanations, our results clearly show that increased CAT activity does not prevent peroxidative injury that occurs as a consequence of Se deficiency. As discussed elsewhere [11,13], it is relevant to point out that (i) in mammals the cytosolic selenoenzyme GSH-PX has a considerably greater affinity for H_2O_2 than does catalase (a K_m of 1 μM vs 1 mM), (ii) catalase is localized in peroxisomes while GSH-PX is distributed throughout the cytosol, and (iii) GSH-PX can react with lipid peroxides as well as H_2O_2, while catalase is limited to the latter substrate. It should be noted that the importance of lipid peroxide metabolism by GSH-PX in vivo has been questioned [20].

Because whole fly preparations were used, changes in GSH-PX activity, CAT activity and TBA-reactive substances in the flies may be underestimated by our data. In rats the primary site of Se-deficient peroxidative injury is the heart [11], where signs of peroxidative damage are manifested prior to manifestation in the kidney and liver [10]. Our use of whole flies may thus have masked greater differences in individual organs. For instance, we have shown that in adult house flies up to 84.5% of the total Se is associated with the midgut which can possess Se concentrations 15-fold greater than those found in the remainder of the carcass [21].

The discovery of GSH-PX in house flies removes a limitation on the use of insects as models for research on free radicals. The previous failure of pro-oxidants to provoke lipid peroxidation in insects has been attributed to the compensatory action of reduced metabolic rates and increased glutathione levels [2]. However, glutathione itself has not conclusively been shown to exhibit antioxidant properties in vivo [22]. Its efficacy in mammals is generally attributed to the activity of the selenoenzyme GSH-PX [1]. Hereafter, the significance of glutathione levels in insects can also be explained in terms of Se-dependent GSH-PX activity.

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