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DIFFERENTIAL CHANGES OF GLUTATHIONE *S*-TRANSFERASE ACTIVITY BY DIETARY SELENIUM

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Abstract—Dietary selenium deficiency produced increased activity of the glutathione *S*-transferases in the liver, kidney and duodenal mucosa. In these tissues, the residual activity of total glutathione peroxidase that included selenium-independent activity was considerably higher than that of selenium-dependent glutathione peroxidase. The enhanced activity of glutathione *S*-transferases was restored to control level 48 hr after an injection of selenite equivalent to the amount of daily selenium intake. Under the same conditions, selenium-dependent glutathione peroxidase activity increased with time and reached 11.9, 11.6 and 46.2% of the activity in the liver, kidney and duodenal mucosa of selenium-supplemented rats, respectively, 48 hr after selenite injection, whereas total glutathione peroxidase activity was not altered except in the kidney. These differential changes of glutathione *S*-transferase activity were intimately related to those of selenium-dependent glutathione peroxidase activity produced by selenium depletion and repletion, suggesting that the glutathione *S*-transferase activity was regulated by dietary selenium. Present findings support the idea that glutathione *S*-transferases having selenium-independent glutathione peroxidase activity function as a substitute for selenium-dependent glutathione peroxidase in selenium-deficient rats.

Glutathione *S*-transferases are important enzymes that participate in the detoxication of various xenobiotics [1]. Recently, it was demonstrated that these enzymes have an additional ability to reduce lipid peroxides as selenium-independent glutathione peroxidase [2–4]. Lawrence *et al.* [5] have reported that the increase of glutathione *S*-transferase activity contributes to selenium-independent glutathione peroxidase activity in the liver of selenium-deficient rats. To clarify the role of glutathione *S*-transferases, we need to investigate whether the enhanced activity of glutathione *S*-transferases in selenium-deficient rats is restored by selenium supplementation. Further, comparative studies of the relative activity and distribution of selenium-dependent and selenium-independent glutathione peroxidases and glutathione *S*-transferases are needed in various tissues of selenium-deficient and sufficient rats.

In this paper, we present evidence that dietary selenium-deficiency produced marked stimulation of glutathione *S*-transferase activity in the liver, kidney and duodenal mucosa of rats and that the enhanced activity was decreased to that of selenium-sufficient rats by the injection of a trace amount of selenite.

MATERIALS AND METHODS

1-Chloro-2,4-dinitrobenzene (CDNB) was purchased from the J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A. Reduced glutathione (GSH) was from the Sigma Chemical Co., St. Louis, MO, U.S.A. Cumene hydroperoxide was purchased from the Nakarai Chemical Co., Kyoto, Japan. All other chemicals used were of analytical grade.

Male Sprague–Dawley rats (3-weeks-old) were divided into two groups designated selenium-deficient (selenium content: 0.012 ppm) and selenium-sufficient diet (added 0.5 ppm selenium as sodium selenite) and were maintained for 6 weeks on a diet consisting of torula yeast as described previously [6]. Diet and water were provided *ad lib.*

Animals were killed by decapitation, and tissues were homogenized in 0.02 M potassium phosphate buffer (pH 7.0) containing 0.1 M KCl, 1 mM EDTA, and 0.5% Triton X-100. The homogenate was centrifuged at 15,000 *g* for 15 min, and the resulting supernatant fraction was used for the enzyme assay.

Assay of glutathione peroxidase activity was carried out by the method of Prohaska and Ganther [7]. Selenium-dependent glutathione peroxidase activity was measured with 1 mM H_2O_2 as a substrate. Total glutathione peroxidase activity, that is the sum of selenium-dependent and -independent activities, was assayed with 1 mM cumene hydroperoxide in place of H_2O_2. Glutathione *S*-transferase activity was measured with 1 mM CDNB as a substrate using the method of Habig *et al.* [8]. The concentration of GSH used was 1 mM for both enzyme assayes. Kinetic parameters of glutathione *S*-transferases for CDNB were determined by varying the CDNB concentration (0.1 to 1.0 mM) in the presence of 1.0 mM GSH and those for GSH by varying the GSH concentration (0.1 to 1.0 mM) in the presence of 1.0 mM CDNB. Selenium content was measured by the method of Watkinson [9]. Protein was determined by the method of Lowry *et al.* [10].

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RESULTS

Selenium content varied considerably among tissues (Table 1). Of the tissues examined, the selenium level was the highest in the kidney, followed by the testis and liver. The amounts in the heart, lung and brain were considerably lower. In the gastrointestinal tract, selenium content in the stomach mucosa was found to be higher than that in the duodenal, jejunal or ileal mucosa.

The tissue distribution of glutathione peroxidase activity was found to be similar to that of selenium content (Table 2). After 6 weeks of feeding the selenium-deficient diet, marked decreases in selenium content and glutathione peroxidase activity were observed in almost all the tissues examined. In the brain and testis, however, the decreases in selenium content and enzyme activity were much less than those of other tissues. The decrease of tissue selenium was proportional to that of selenium-dependent glutathione peroxidase activity assayed with H_2O_2 as a substrate rather than to that of total glutathione peroxidase activity with cumene hydroperoxide as a substrate. A possible cause may have been the considerable amount of total glutathione peroxidase activity that was in the liver, kidney and duodenal mucosa even in selenium deficiency; residual ratios of total glutathione peroxidase activity in these tissues were higher than those of selenium-dependent activity. This suggests that glutathione *S*-transferase with selenium-independent glutathione peroxidase activity may contribute to the high residual activity of total glutathione peroxidase in selenium-deficient rats.

Glutathione *S*-transferase activity in selenium-deficient and -sufficient rats is shown in Table 3. In selenium-sufficient rats, the testis and the liver exhibited the highest activities of glutathione *S*-transferases; the activities in heart, lung and brain were ten times lower than that of the testis. In the gastrointestinal tract, the duodenum and jejunum contained high activity that was equivalent to, or somewhat higher than, the activity in the kidney, whereas the activity in the stomach and ileum was rather low.

Selenium deficiency was followed by stimulation of glutathione *S*-transferase activity in the liver, kidney and duodenal mucosa. In particular, the activity in the liver increased to about 2-fold that of the selenium-sufficient group. In other tissues, however, there was no difference between the dietary groups. Apparent K_m and V_{max} values were determined to evaluate the effects of dietary selenium deficiency on the kinetics of glutathione *S*-transferases in the liver, kidney and duodenal mucosa (Table 4). Selenium deficiency markedly increased the V_{max} value for CDNB without influencing the K_m value. The V_{max} for GSH, as determined by varying the GSH concentration, was increased, but the K_m for GSH was not altered.

To clarify the relationship between the glutathione peroxidase and glutathione *S*-transferase activities, the influence of the injection of a small amount of selenite on both enzyme activities was investigated in selenium-deficient rats (Fig. 1). The enhanced activity of glutathione *S*-transferase in the selenium-deficient rats was gradually decreased to the levels of selenium-sufficient rats by the injection of sodium

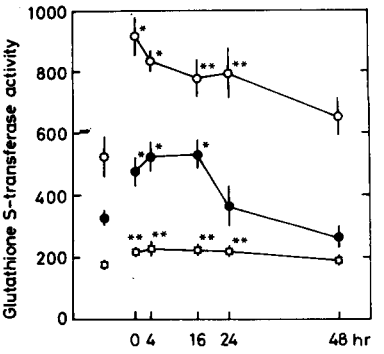

Fig. 1. Decrease after selenite injection of glutathione *S*-transferase activity in the tissues of selenium-deficient rats. Sodium selenite was injected at a dose of 1.0 μmole/kg (s.c). Enzyme activity was expressed as nmoles CDNB conjugated per min per mg protein. Points and bars represent means ± S.E. of four to six rats. Left points and bars are means ± S.E. of the values of selenium-sufficient rats. Significantly different from selenium-sufficient rats (*$P < 0.01$ and **$P < 0.05$). Key: (○) liver, (□) kidney, and (●) duodenal mucosa.

Table 1. Effect of dietary selenium-deficiency on selenium content in rat tissues*

Tissue	(ng selenium/g tissue) Se(+)	Se(−)	$\frac{Se(-)}{Se(+)}$ 100(%)
Liver	409.2 ± 17.4	13.7 ± 1.7†	3.3
Kidney	1263.3 ± 86.2	113.5 ± 13.8†	9.0
Brain	99.2 ± 1.6	73.7 ± 4.7†	74.3
Heart	218.5 ± 6.3	34.8 ± 2.6†	15.9
Lung	227.8 ± 10.4	38.8 ± 5.2†	17.0
Testis	710.0 ± 26.9	343.5 ± 31.8†	48.4
Gastrointestinal mucosa			
Stomach	378.7 ± 12.5	34.4 ± 4.2†	9.1
Duodenum	253.0 ± 6.5	34.2 ± 6.3†	13.5
Jejunum	186.2 ± 10.0	29.0 ± 6.3†	15.6

*Each value is the mean ± S.E. of six rats.
†Significantly different from selenium-sufficient rats ($P < 0.01$).

Table 2. Effect of dietary selenium deficiency on glutathione peroxidase activity in rat tissues*

Tissue	Enzyme activity (nmoles NADPH oxidized/min/mg protein) Selenium-dependent activity Se(+)	Se(−)	Se(−)/Se(+) 100(%)	Total activity Se(+)	Se(−)	Se(−)/Se(+) 100(%)
Liver	117.21 ± 13.27	0.46 ± 0.10†	0.4	120.81 ± 10.92	23.62 ± 1.44†	19.6
Kidney	122.18 ± 11.05	3.79 ± 0.58†	3.1	120.76 ± 8.48	8.42 ± 0.72†	7.0
Brain	9.98 ± 0.46	7.11 ± 0.43†	71.2	10.21 ± 0.42	7.27 ± 0.41†	71.2
Heart	113.57 ± 10.24	10.05 ± 1.08†	8.8	98.21 ± 9.22	8.62 ± 0.75+	8.8
Lung	71.98 ± 5.95	9.08 ± 1.01†	12.6	66.22 ± 3.68	8.56 ± 0.57†	12.9
Testis	38.84 ± 3.63	18.60 ± 1.83†	47.9	42.64 ± 3.40	22.71 ± 0.41†	53.3
Gastrointestinal mucosa						
Stomach	156.38 ± 13.27	4.14 ± 0.67†	2.6	136.11 ± 13.85	7.76 ± 0.55†	5.7
Duodenum	22.09 ± 2.65	3.11 ± 0.49†	14.1	24.93 ± 2.72	11.91 ± 1.01†	47.8
Jejunum	24.03 ± 2.40	2.35 ± 0.34†	9.8	22.51 ± 2.45	4.81 ± 0.57†	21.4

*Selenium-dependent and total glutathione peroxidase activities were assayed with H_2O_2 and cumene hydroperoxide, respectively, as substrate. Each value is the mean ± S.E. of six to seven rats.
†Significantly different from selenium-sufficient rats ($P < 0.01$).

selenite (1.0 μmole/kg, s.c.). The dose of selenite used was thought to be roughly equivalent to daily selenium intake. However, the selenite injection did not influence the glutathione S-transferase activity in the tissues where its activity was not altered by selenium deficiency (data not shown).

With regard to glutathione peroxidase, selenium-dependent activity was increased with time and reached 11.9, 11.6 and 46.2% of the activities in the liver, kidney and duodenal mucosa of selenium-supplemented rats, respectively, 48 hr after selenite injection (Fig. 2A). However, total glutathione peroxidase activity assayed with cumene hydroperoxide as a substrate did not increase in the liver and the duodenal mucosa in spite of selenite injection (Fig. 2B). In other tissues, both selenium-dependent and total glutathione peroxidase activities were found to increase (data not shown).

DISCUSSION

It has been demonstrated that glutathione S-transferases exhibit selenium-independent glutathione peroxidase activity [2–4]. Recently, Lawrence *et al.* [5] have reported that selenium deficiency causes an increase in glutathione S-transferase activity of rat liver. However, the regulation of glutathione S-transferase activity by dietary selenium and the relationship between glutathione peroxidase and glutathione S-transferases in the various tissues are not known yet. In the present experiment, by feeding the tolura yeast-based diet for 6 weeks, the weight gain of selenium-deficient rats was suppressed moderately (by about 10%) as compared to that of selenium-supplemented rats, but there was no apparent difference in either food or water consumption between the dietary groups. Under the experimental conditions, glutathione S-transferase activity markedly increased in the liver, kidney and duodenal mucosa of selenium-deficient rats. The enhanced activity of glutathione S-transferases in selenium-deficient rats was restored to the control

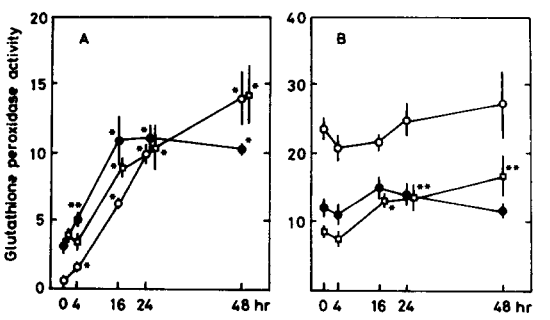

Fig. 2. Increase of glutathione peroxidase activity after selenite injection in the tissues of selenium-deficient rats. Sodium selenite was injected at a dose of 1.0 μmole/kg (s.c.). Enzyme activity was assayed with H_2O_2 (A) and cumene hydroperoxide (B) as substrate, respectively, and is expressed as nmoles NADPH oxidized per min per mg protein. Points and bars represent means ± S.E. of four to seven rats. Significantly different from selenium-deficient rats (*$P < 0.01$ and **$P < 0.05$). Key: (○) liver, (□) kidney, and (●) duodenal mucosa.

Table 3. Effect of dietary selenium deficiency on glutathione *S*-transferase activity in rat tissues*

Tissue	Enzyme activity (nmoles CDNB conjugated/min/mg protein) Se(+)	Se(−)	$\frac{Se(-)}{Se(+)}$ 100(%)
Liver	528.7 ± 63.1	918.4 ± 62.9†	173.7
Kidney	174.2 ± 5.9	212.2 ± 11.0‡	121.8
Brain	79.5 ± 5.4	83.7 ± 5.6	105.3
Heart	39.9 ± 3.2	40.3 ± 3.3	101.0
Lung	77.1 ± 6.7	89.4 ± 6.4	116.0
Testis	956.1 ± 87.3	948.2 ± 85.0	99.2
Gastrointestinal mucosa			
Stomach	118.0 ± 8.2	114.3 ± 7.9	96.9
Duodenum	324.4 ± 23.5	473.3 ± 21.2†	145.9
Jejunum	182.6 ± 14.5	190.7 ± 14.1	104.4

*Each value is the mean ± S.E. of six rats.
†,‡Significantly different from selenium-sufficient rats (†$P < 0.01$ and ‡$P < 0.05$).

level by the injection of selenite equivalent to the daily selenium intake. These results indicate the possiblity that glutathione *S*-transferase activity is regulated by dietary selenium status.

Under the same conditions, glutathione peroxidase activity assayed with H_2O_2 as a substrate gradually increased with time up to 48 hr after selenite injection. The activity assayed with H_2O_2 represents selenium-dependent glutathione peroxidase activity. Considering previous information on glutathione peroxidase synthesis [11, 12], stimulated activity of selenium-dependent glutathione peroxidase after selenite treatment is thought to be due to the biosynthesis of glutathione peroxidase. However, total glutathione peroxidase activity assayed with cumene hydroperoxide in place of H_2O_2 was not altered. This seems to reflect a balance between increased synthesis of glutathione peroxidase and decreased activity of glutathione *S*-transferases, since the activity assayed with cumene hydroperoxide is the sum of selenium-dependent and -independent activities. This supports the idea that the high residual ratio of total glutathione peroxidase activity as compared to that of selenium-dependent activity in selenium-deficient rats may be explained by the increased activity of glutathione *S*-transferases in liver, kidney and duodenal mucosa. These findings suggest that glutathione *S*-transferases in these tissues may function as one of the compensatory mechanisms against severe depletion of glutathione peroxidase in selenium-deficient rats.

In the case of other tissues, there were no relative alterations between selenium-dependent glutathione peroxidase and glutathione *S*-transferase activities. Considering recent findings that glutathione peroxidase activity in the bovine retina, which is lacking a seleno-enzyme, is associated with glutathione *S*-transferases [13], it is conceivable that glutathione *S*-transferases may partly exist as selenium-independent glutathione peroxidase in tissues deficient in glutathione peroxidase in selenium deficient rats. However, it is not clear whether glutathione *S*-transferases may actually function as selenium-independent glutathione peroxidase in the tissues where considerable amounts of glutathione peroxidase exist, because of the low catalytic activity of glutathione *S*-transferase for organic hydroperoxides [4]. In fact, Awasthi *et al.* [14] have reported that the administration of butylated hydroxytoluene induces glutathione *S*-transferases without a corresponding increase of selenium-independent glutathione peroxidase activity.

According to Stone and Dratz [15], rats fed a selenium and vitamin E deficient diet have increased

Table 4. Kinetic parameters of glutathione *S*- transferases for CDNB and GSH*

Tissue	K_m (mM) Se(+)	Se(−)	V_{max} (nmoles/min/mg protein) Se(+)	Se(−)
(A) CDNB				
Liver	0.168 ± 0.011	0.158 ± 0.005	466.0 ± 29.2	793.9 ± 34.0†
Kidney	0.547 ± 0.054	0.511 ± 0.031	169.4 ± 13.1	301.0 ± 16.3†
Duodenum	0.566 ± 0.037	0.609 ± 0.035	469.8 ± 42.9	824.7 ± 69.1†
(B) GSH				
Liver	0.107 ± 0.006	0.102 ± 0.003	422.8 ± 60.9	793.9 ± 34.0†
Kidney	0.121 ± 0.002	0.118 ± 0.014	168.0 ± 26.9	248.7 ± 14.0‡
Duodenum	0.155 ± 0.011	0.159 ± 0.013	341.1 ± 14.9	574.5 ± 64.9‡

*Each value is the mean ± S.E. of four rats.
†,‡Significantly different from selenium-sufficient rats (†$P < 0.01$ and ‡$P < 0.05$).

activity of glutathione S-transferases in the liver, kidney, testis, brain and retinal pigment epithelium. The increase of the enzyme activity of the testis and brain seems to depend on either vitamin E deficiency or vitamin E plus selenium deficiency, because in the present study the enzyme activity in these tissues did not increase in the rats deficient in selenium alone. However, it has been reported recently that liver glutathione S-transferase activity is not affected by vitamin E deficiency in rats [16]. Thus, it is likely that the increase of glutathione S-transferase activity is primarily specific to selenium deficiency and that the phenomenon is probably intensified by a concomitant deficiency of vitamin E. Such alterations of glutathione S-transferase activity, as well as the degree of glutathione peroxidase depletion, may possibly contribute to the differences in the susceptibility of different organs to oxidative injury in antioxidant deficient rats.

With regard to the roles of gastrointestinal glutathione peroxidase and glutathione S-transferases, there has been little investigation [17, 18]. Vilas *et al.* [19], based on findings that dietary peroxides increased glutathione peroxidase activity in the mucosa, have suggested that glutathione peroxidase may be involved in protecting the gastric mucosa from damage caused by dietary peroxides. They have also emphasized that the decrease of glutathione peroxidase activity in the intestinal mucosa resulting from selenium-deficiency was less than that in any other tissue, but their results represented only the changes of total glutathione peroxidase activity. In the present study, although considerable activity of total glutathione peroxidase remained owing to the contribution of glutathione S-transferases even in selenium-deficiency, selenium-dependent glutathione peroxidase activity was observed to decrease in the intestinal mucosa to an extent similar to that of other tissues. On the other hand, glutathione S-transferases are known to play an important role in the metabolism of xenobiotics in the gastrointestinal tract as well as in the liver [20, 21]. Further, significant amounts of GSH, substrate for both enzymes, are present in the gastrointestinal mucosa [22]. Both glutathione peroxidase and glutathione S-transferases exhibited characteristic localization; the former activity was high in the gastric mucosa, whereas the latter was high in the duodenal and jejunal mucosa where absorption of dietary constituents and xenobiotics mainly occurs. Thus, such characteristic localization of both enzymes and differential changes of glutathione S-transferase activity by selenium depletion and repletion are interesting when attempting to assess the possible roles and relative contributions of the enzymes in the different gastrointestinal tracts to the metabolism of dietary peroxides as well as peroxides formed *in vivo*.

It has been reported that selenium has an anticarcinogenic activity [23]. Although the role of glutathione peroxidase in carcinogenesis is not understood well, an inverse correlation between susceptibility to chemical carcinogens and glutathione S-transferase activity has been proposed [24]. Thus, the possibility that the increase of glutathione S-transferase activity in the liver, kidney and duodenal mucosa is one of the mechanisms to protect selenium-deficient rats from carcinogens cannot be ruled out. Further studies will be required to investigate this possibility.

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