Increased malic enzyme activity in selenium-deficient rat liver

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The activity of cytosolic malic enzyme was significantly higher (61%) in the liver of rats fed a selenium-deficient diet compared with the controls. Activities of two other NADPH-generating enzymes, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, were also increased but to a lesser extent (13%). Glutathione reductase, an NADPH-consuming enzyme involved in the metabolism of glutathione, was higher (26%) in the liver of selenium-deficient rats, possibly as a result of an increase in glutathione synthesis and enzymic processes that maintain glutathione in the reduced state. It is concluded that an increased demand for NADPH in selenium deficiency, possibly resulting from increased glutathione metabolism, may induce NADPH-generating enzymes, especially malic enzyme. (J. Nutr. Biochem. 5:314–316, 1994.)

Keywords: malic enzyme; selenium deficiency; glutathione; glutathione reductase; rats

Introduction

Malic enzyme (ME, EC 1.1.1.40) provides NADPH needed for synthesis of fatty acids and other compounds. Its activity is regulated by nutritional and hormonal status.[1] The activity of malic enzyme also is increased by the activation of detoxification processes that consume NADPH,[2] and the diminution of glutathione in the liver.[3] Maintenance of glutathione in the reduced state requires NADPH and glutathione reductase.

The trace element selenium is an integral part of the enzymes glutathione peroxidase (GSH-Px, EC 1.11.1.9)[4] and Type I iodothyronine deiodinase (DI, EC 3.8.1.4).[5] GSH-Px catalyzes the reduction of H_2O_2 and other hydroperoxides by glutathione, while DI catalyzes the deiodination of iodothyronines, notably the conversion of thyroxine to triiodothyronine. Selenium deficiency causes a marked depression in the activity of these two enzymes[6,7] and affects xenobiotic metabolism.[8–10] Hill and Burk[11] found that the synthesis of glutathione increased in selenium-deficient hepatocytes. Using hemoglobin-free perfused livers, Hill and Burk[11] demonstrated that selenium-deficient livers released four times as much GSH into the caval perfusate as control liver, and plasma GSH + GSSG in selenium-deficient rats was twofold higher than in control rats.

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In a recent preliminary study, we reported a 77% increase in malic enzyme activity in selenium-deficient rat livers.[12] This led us to study further the metabolic alterations caused by selenium deficiency that might have an effect on the activity of malic enzyme (ME). Because the function of malic enzyme is to provide NADPH, the increase in malic enzyme could result from increased demand for NADPH in selenium deficiency. We assayed the specific activity of malic enzyme in the liver cytosol along with two other NADPH-generating enzymes, glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44). Glutathione reductase (GSSG-R, EC 1.6.4.2), an important enzyme using NADPH for glutathione reduction, also was assayed.

Methods and materials

Animals and treatment

Weanling male Sprague-Dawley rats were fed a torula yeast-based diet for 12 wks. One group of six rats was fed the diet deficient in selenium (−Se) (0.01 ppm Se by analysis)[13] and the control group of six rats was fed the diet supplemented with 0.1 ppm Se as sodium selenite (+Se). The basal diet consisted of (%): torula yeast (30), sucrose (60), corn oil (5), mineral mix AIN-76 (3.5), vitamin mix AIN-76A (1), DL-methionine (0.3), and choline bitartrate (0.2). Rats were kept individually in wire bottom stainless-steel cages with free access to diets and drinking water.

Enzyme preparation and assays

For preparation of tissue extracts, rats were anesthetized by diethyl ether and were killed by cardiectomy. Livers were perfused with

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cold 0.15 M KCl, rapidly removed, and homogenized in 3 vol ice-cold 0.05 M Tris-HCl buffer, pH 7.4, containing 0.25 M sucrose, 4 mM EDTA, and 3 mM DTT. Homogenates were centrifuged for 15 minutes at 25,000*g*, and the supernatants were centrifuged again for 1 hr at 105,000*g*. All procedures were performed at about 4° C. Supernatants were collected and stored at − 60° C for subsequent enzyme assays.

ME was assayed according to the procedure of Ochoa et al.[14] G6PD and 6PGD were assayed according to the methods published by Bergmeyer.[15] GSSG-R was assayed by the method of Worthington and Rosemeyer.[16] GSH-Px was assayed according to Prohaska et al.[17] Protein content in the supernatants was assayed according to Lowry et al.[18] using bovine serum albumin as a standard.

Statistical methods

Comparisons between −Se and +Se group were done using the Student's *t* test.

Results

The final average weights (means ± SD) were 319 ± 14 and 340 ± 31 g for −Se and +Se rats, respectively (the difference was not statistically significant). *Table 1* shows the specific activities of the three NADPH-generating enzymes in selenium-deficient rat livers and the controls. Specific activity of ME increased 61% in selenium-deficient livers cytosol compared with controls. The specific activities of G6PD and 6PGD increased slightly but significantly (13%). GSSG-R activity also significantly increased (27%) in selenium-deficient livers. GSH-Px activity was, as expected, very low in selenium-deficient livers *(Table 1)*. Protein content of cytosolic fractions (105,000*g*) was not significantly different between selenium deficient livers and the controls (25.9 ± 3.2 and 23.8 ± 2.5 mg/mL, respectively).

Discussion

Our results indicate that NADPH-linked glutathione metabolism may play an important role in the induction of liver ME in selenium-deficient rats. Earlier studies showed that the concentration of GSH in the livers is maintained relatively constant regardless of selenium status.[19] Plasma GSH increased in selenium-deficient rats and was accompanied by a substantial increase in GSH synthesis in the liver.[11] Because glutathione is released to the circulation almost entirely in the reduced form,[11] it is likely that enzymic processes needed to maintain glutathione in reduced form would be increased in selenium deficiency. We found in a preliminary study (data not shown) and in this study that the specific activity of GSSG-R was significantly increased in selenium-deficient livers over the controls. Studies by Reiter and Wendel[9,10] showed 60% or more increase in the specific activity of GSSG-R in selenium-deficient mice. However, Burk et al.[20] found no change in the activity of GSSG-R (activity per gram of liver) in perfused selenium-deficient rat livers compared with the controls.

The activity of GSH-Px in selenium-deficient rats was reduced to less than 4% of the controls *(Table 1)*. GSH-Px needs GSH and NADPH for peroxide destruction,[4] thus it would be expected that the consumption of NADPH would be less. However, other NADPH consumption reactions may be dominant in total NADPH use.

Ayala et al.[2] found that administration to rats of either t-butyl hydroperoxide or phenobarbital produced an increase in specific activity of GSSG-R and NADPH cytochrome c reductase accompanied by a significant increase in the specific activity of ME. When rats were fed a diet containing a very low level of methionine, it was found that the specific activity of GSSG-R was increased along with the activity of NADPH cytochrome c reductase and thioredoxin reductase. ME activity was increased significantly.[3] The results from these two studies and our study demonstrate a responsive relationship between an NADPH-consuming enzyme, GSSG-R, and ME, an NADPH-generating enzyme.

The specific activities of two other NADPH-generating enzymes, G6PD, and 6PGD, also were increased in selenium-deficient livers in our study. Although the increases in the activity of these enzymes were small compared with the increase in ME, all the changes pointed in the same direction; toward an increase in NADPH synthesis. In a study by Wendel and Otter,[21] selenium deficiency was reported to cause an increase in the specific activity of 6PGD by 60% in the perfused livers of mice, whereas the activity of G6PD was unchanged. In contrast, Burk et al.[20] found a decrease in the activity (per gram of perfused liver) of G6PD in selenium-deficient rats. The difference between their results and ours is unexplained.

Hitomi et al.[22] recently reported a liver-specific induction of ME, G6PD, and 6PGD in rats that were fed a diet containing polychlorinated biphenyls. They concluded that the induction of these NADPH-generating enzymes was a result of an increased demand for NADPH needed for lipogenesis in the liver.

Thyroid hormone T_3 (3,3′,5-triiodothyronine) stimulates activity of ME.[23] However, plasma T_3 concentrations in selenium-deficient rats were found to be either lower or about the same as the controls, although concentrations of the prehormone thyroxine (T_4) were slightly higher in selenium-deficient rats.[6,7]

Beckett et al.[24] reported higher ME activity in kidney

Table 1 Specific activity of malic enzyme, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase, and glutathione peroxidase in rat liver cytosol

Enzyme activity	Dietary treatment	
	−Se	+Se
Malic enzyme (mU/mg protein)[a]	15.0 ± 1.9**	9.3 ± 2.1
Glucose-6-phosphate dehydrogenase (mU/mg protein)[a]	16.5 ± 1.8*	14.5 ± 1.1
6-phosphogluconate dehydrogenase (mU/mg protein)[a]	39.6 ± 4.4*	34.8 ± 2.7
Glutathione reductase (mU/mg protein)[b]	62.5 ± 5.9**	49.3 ± 3.1
Glutathione peroxidase (U/mg protein)[c]	0.04 ± 0.06**	1.07 ± 0.23

[a]1 U equals 1 micromol NADPH formed/min.
[b]1 U equals 1 micromol NADPH oxidized/min.
[c]1 U equals 1 micromol GSH oxidized/min.
*$P < 0.05$, **$P < 0.001$, compared with +Se group by Student's *t* test. Values are means ± SD for six rats per group.

cytosol of rats fed a selenium deficient diet for 5 weeks, whereas a statistically significant increase in liver cytosol of − Se rats was not observed. The diets used in their study were based on amino acids. Our results demonstrate that when rats were fed a torula yeast-based, selenium-deficient diet for 12 wks, the activity of hepatic ME can be stimulated. The increase in ME activity may result from an alteration in xenobiotic metabolism involving glutathione, with an accompanying increased requirement for NADPH.

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