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Glutathione-related enzymic activities in rats receiving high cholesterol or standard diets supplemented with two forms of selenium

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Abstract

Selenium deficiency was produced in rats fed a high cholesterol diet for 57 days (Group 1). It was characterized by an increase in malondialdehyde (MDA) an end product of lipid peroxidation and by the dramatic collapse of selenium-dependent glutathione peroxidase activity (GSHPx) in plasma, erythrocytes and in homogenate supernatant fraction of liver, kidney and heart compared with rats fed a standard diet containing sodium selenite (Group 3). A compensatory rise in the activity of liver glutathione S-transferase (GST) activity and also in glutathione reductase (GSSGR) activity was accompanied by an increase in NADPH-generating enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Adequate dietary selenium supplementation by Se-rich Spirulina corrected all the selenium deficiency effects (Group 2), then, GSHPx and NADPH-consuming enzymes activities were of the same magnitude as those exhibited by rats fed a standard diet containing adequate selenium in the form of sodium selenite. Based on this study, it is concluded that Se-enriched Spirulina behave as an excellent selenium carrier.

1. Introduction

The nutritional need for trace amounts of selenium has been recognized for many years (Schwarz & Foltz, 1957). Its antioxidant function was unknown until Rotruck et al. (1972) discovered that selenium is an integral component of the active site of the mammalian enzyme glutathione peroxidase (GSH-Px). This enzyme catalyzes the reduction of hydroperoxides and thus protects the cells from oxidative damage. A low activity of this enzyme is related to selenium deficiency (Rotruck et al., 1972) and, thus, low selenium status may increase the risk of oxidative damage. There is interest in supplementing human subjects with selenium for prevention of diseases such as cancer (Clark & Combs, 1986) and cardiovascular disease (Salonen, Alfthan, Huttunen, Pikkarainen, & Puska, 1982). Cholesterol feeding was shown to result in an increase in the rate of lipid peroxidation and a decrease in GSH-Px activity in rat liver (Tsai, 1975) and the damaging process of lipid peroxidation may be an important factor in the etiology of atherosclerosis; therefore, a diet rich in cholesterol and deficient in both selenium and vitamin E (shown to quench peroxy radicals during lipid peroxidation) was used here.

The cyanobacteria *Spirulina platensis* (blue-green algae) is commercially available for human consumption. Spirulina represent one of the richest protein sources of plant origin (60–70%) and are a good source of vitamins and minerals (Bourges, Sotomayor, Mendoza, & Chavez, 1971; Dillon, Phuc, & Dubacq, 1995) although a low amount of selenium (Se) is found (from 0.01 to 0.04 mg/kg). These microalgae are now used as a health food source for humans (Kay, 1991). The simple cultivation technology and the good quality of its protein, as well as the absence of any toxic side effects (Chamarro, Herrera, Salazar, Salazar, & Ulloa, 1988;

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Yoshino, Hirai, Takahashi, Yamamoto, & Yamazaki, 1980), favor their large scale production. Moreover, the ability to control chemical composition by varying cultivation conditions makes Spirulina the most easily Se-supplementable vegetable by way of the aquatic environment.

The purpose of this study was to assess the ability of Se-enriched Spirulina to influence the enzymic antioxidant defence in tissues of rats fed a Se-deficient atherogenic diet, so that a potential pharmaceutical application becomes of interest compared to a classical supplementation with sodium selenite.

2. Materials and methods

2.1. Experimental design

Male Sprague Dawley rats (Iffa Credo, L'Arbresle, France) ($n=21$) weighing 110 ± 9 g were divided into three equal groups of seven each, according to average body weight. They were randomly assigned to experimental torula yeast-based diets (Table 1), yielding three dietary treatment groups, either low in vitamin E (4.5 mg α-tocopherol/kg diet), deficient in selenium (0.006 mg/kg diet) and rich in cholesterol (1%) supplemented (Group 2) or not (Group 1) with Se-improved Spirulina (0.200 mg Se/kg diet), or adequate in vitamin E (346 mg α-tocopherol/kg diet) and selenium (0.200 mg Se as Na_2SeO_3/kg diet) (Group 3). The animals were individually housed in stainless steel metabolic cages in a temperature-controlled room (22°C) with constant humidity and with 12 h light:dark cycles (lights on, 0700 to 1900 h). All dietary groups received daily the same quantity of food and had free access to distilled water; body weight was measured twice a week throughout the 57-day experimental period.

Table 1
Composition of experimental diets (g/100 g)[a]

Diet component	Group 1	Group 2	Group 3
Torula yeast[b] ($N \times 6.25$)	32.30	32.30	32.30
DL-methionine	0.10	0.10	0.10
Corn starch	29.19	29.20	34.40
Sucrose	14.40	14.40	17.20
Cellulose	–	–	5.00
Corn oil	2.00	2.00	5.00
Lard	15.00	15.00	–
Cholesterol	1.00	1.00	–
Mineral mix[c]	–	–	4.00
Mineral mix[d]	4.00	4.00	–
Vitamin mix[e]	–	–	2.00
Vitamin mix[f]	2.00	2.00	–
Spirulina[g] (as mg/kg diet)	–	92.50	–
Total vitamin E (mg/100 g)	0.45	0.45	34.56
Total selenium (μg/100 g)	0.60	20.00	20.00
Metabolizable energy (kcal/g)	475.00	475.00	412.00

[a] All diets were formulated to contain 15% protein.
[b] Torula yeast contained 7.42% nitrogen and 0.02 ppm Se.
[c] Mineral mixture contained (mg/kg diet): $CaHPO_4$, 17,200; KCl, 4000; NaCl, 4000; MgO, 420; $MgSO_4$, 2000; Fe_2O_3, 120; $FeSO_4.7H_2O$, 200; trace elements, 400 (Na_2SeO_3, 0.44; $MnSO_4.H_2O$, 98; $CuSO_4.5H_2O$, 20; $ZnSO_4.7H_2O$, 80; $CoSO_4.7H_2O$, 0.16; KI, 0.32; sufficient starch to bring to 40 g (per kg diet).
[d] Mineral mix as described above and free from Na_2SeO_3.
[e] Vitamin mixture containing (mg/kg diet): retinol, 12; cholecalciferol, 0.125; thiamin, 40; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyanocobalamin, 0.1; all-*rac*-α-tocopherol, 340; menadione, 80; nicotinic acid, 200; choline, 2720; folic acid, 10; p-aminobenzoic acid, 100; biotin, 0.6; sufficient starch to bring to 20 g (per kg diet).
[f] Vitamin mixture as described above without all-*rac*-α-tocopherol.
[g] Weight of Spirulina supplying the same quantity of selenium than mineral mixture given to Group 3 (i.e. 0.2 mg Se per kg diet).

2.2. Analytical procedures

Animals were anesthetized with pentobarbital (Pentobarbital 6%, 60 mg/kg body wt) and killed by exsanguination. The liver was perfused with 1.15% KCl to remove residual blood, rapidly excised, rinsed in ice cold saline, blotted dry, weighed and stored in liquid nitrogen. The subsequent steps were performed at 4°C; tissue was homogenized in 5 vol ice cold 0.1 M potassium phosphate buffer (pH 7.4). An aliquot of the homogenate was removed for the determination of lipid peroxidation. The remaining homogenate was spun at $13{,}000\times\boldsymbol{g}$ for 15 min and the supernatant was re-centrifuged at $105{,}000\times\boldsymbol{g}$ for 60 min. Aliquots of the resulting supernatant (cytosol) were stored at −80°C for subsequent assay of glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST), glutathione reductase (GSSGR) and NADPH-producing enzymes: glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD) and malic enzyme (ME).

Blood was collected by heart puncture in heparinized tubes. The erythrocytes were separated by centrifugation of blood at $1000\times\boldsymbol{g}$ for 15 min, the cells were washed with cold 0.9% saline and were lysed with distilled water. Aliquots of plasma and hemolysate were stored at −80°C for subsequent assay of GSHPx.

Free radical damage was estimated by measuring malondialdehyde (MDA) using the thiobarbituric (TBA) test according to Esterbauer and Cheeseman (1990) in the presence of butylated hydroxytoluene. TBA-reacting compounds were expressed as nmoles of MDA per gram of protein. Glutathione peroxidase activity was assessed by the method of Paglia and Valentine (1967) as modified by Lawrence and Burk (1976) using hydrogen peroxide as the substrate and including 1.0 mM azide to inhibit catalase, so that only Se-dependent GSHPx was measured. GST activity was determined by the increment of absorbance at 340 nm due to 2,4-dinitrophenyl S-glutathione formation from 1-chloro-2,4-dinitrobenzene and GSH measured as

reported by Habig, Pabst, and Jakoby (1974). Glutathione reductase acivity was monitored by following the rate of reduction of $NADP^+$ to NADPH in the presence of oxidized glutathione (Cohen & Duvel, 1988). G6PD and 6PGD were assayed according to the method of Glock and McLean (1953) and ME according to the procedure of Ochoa, Mehler, and Kornberg (1948). For analysis of glutathione, 100 mg samples of tissue were homogenized in 15 vols perchloric acid (2 g/liter). After centrifugation, the supernatant fractions (200 µl) were assayed for total glutathione according to the procedure of Anderson (1985) where GSH content was determined by its reaction with 5-5′-dithiobis-(2-nitrobenzoic acid) to yield the yellow chromophore, 5-thio-2-nitrobenzoic acid at 412 nm. Protein determinations were performed according to Smith et al. (1985) using bovine serum albumin as standard.

2.3. Statistical analysis

Data are shown as the mean ± SEM of seven observations per group. The statistical significance of differences between treatments was established by ANOVA using a Stat View 512^+ microcomputer program (Brain Power, Calabasas, CA) incorporating a calculation of Fisher's least significant difference between groups. Differences were considered significant when $p < 0.05$.

3. Results

The results are presented in Tables 2 and 3. Dietary cholesterol supplementation and vitamin E depletion affected body weight gain since diets 1 and 2 supplied more metabolizable energy than diet 3. It significantly increased the liver size. Plasma cholesterol concentration was lower in the presence of Se (Group 2) than in its absence (Group 1), when the diets were inadequate in vitamin E. Plasma HDL-C concentration was low in rats fed the high-cholesterol inadequate vitamin E diets (Groups 1 and 2) in comparison with that from Group 3 fed low cholesterol adequate Se and vitamin E diet. In the absence of dietary Se (Group 1), rats exhibited high LDL-C levels compared to Group 2, when the animals were fed the same diet supplemented with Spirulina; this value was brought to the same level seen in animals fed sufficient vitamin E and Se diets. The liver, kidney and heart lipid peroxidation, i.e. MDA concentration, was significantly increased by high cholesterol diets (Groups 1 and 2) compared with the standard diet (Group 3); nevertheless rats subjected to an altered vitamin E diet and receiving an adequate dose of selenium in the form of Spirulina (Group 2) showed a marked decrease in hepatic (about 30%), renal (48%) and cardiac (49%) levels of MDA with respect to those of Group 1. As expected, Se deficiency caused a significant decrease in the GSHPx activity in all the tissues studied (Table 3) and this decrease was independent of and unaffected by the vitamin E status of the rats. The percentage losses of GSHPx activity due to selenium deficiency were 82, 46, 84, 52 and 41%, respectively, in plasma, erythrocytes, liver, kidney and heart. Vitamin E deficiency was without effect on the GST and GSSGR (Table 3) activity since no significant difference appeared between Groups 2

Table 2
Body weight, food intake, liver weight and plasma cholesterol in rats fed the experimental diets[a]

	Group 1	Group 2	Group 3
Initial body wt (g)	110 ± 6a	110 ± 2a	110 ± 2a
Final body wt (g)	371 ± 5a	371 ± 6a	352 ± 5a
Growth (g/d)	4.56 ± 0.08a	4.57 ± 0.10a	4.23 ± 0.08b
Food intake (g/d)	16.50 ± 0.13a	16.61 ± 0.15a	16.83 ± 0.18a
Liver wt (g/100 g BW)	3.49 ± 0.08a	3.46 ± 0.06a	3.18 ± 0.15b
Total cholesterol (mg/100 ml)	67.3 ± 5.0a	51.3 ± 4.0b	61.0 ± 3.7a
HDL-cholesterol (mg/100 ml)	30.8 ± 2.6a	31.9 ± 4.3a	47.3 ± 3.7b
LDL-cholesterol (mg/100 ml)	12.1 ± 1.8a	6.71.3b	5.9 ± 0.9b

[a] Values are means ± SEM of seven observations per group; values in a row with different letters are significantly different ($p < 0.05$).

Table 3
Whole blood glutathione, tissue malondialdehyde (MDA) and tissue activitiy of glutathione peroxidase (GSHPx), glutathione reductase (GSSGR), glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate deshydrogenase (6PGD) and malic enzyme (ME) in rats fed the experimental diets[a]

	Group 1	Group 2	Group 3
Plasma			
GSHPx (mU/mg protein)	1.38 ± 0.06a	7.88 ± 0.40b	7.54 ± 0.80b
Red blood cells			
GSHPx (mU/mg protein)	28.8 ± 1.7a	53.9 ± 0.6b	51.5 ± 1.1b
Whole blood			
total GSH (nmol/mg protein)	587 ± 5a	493 ± 7b	535 ± 12b
Liver			
MDA (nmol/g protein)			
bound	19.4 ± 1.5a	13.8 ± 1.6b	3.4 ± 0.6c
free	11.1 ± 1.1a	7.4 ± 0.8b	3.2 ± 0.5c
GSHPx (mU/mg protein)	93.36 ± 5.7a	594.4 ± 23.8b	671.0 ± 42.0b
GST (mU/mg protein)	69.2 ± 13.3a	23.0 ± 3.0b	29.3 ± 2.9b
GSSGR (mU/mg protein)	127.8 ± 9.6a	98.0 ± 5.6b	84.2 ± 8.5b
G6PD (mU/mg protein)	19.7 ± 1.5a	14.4 ± 0.9b	10.8 ± 1.1b
6PGD (mU/mg protein)	39 ± 1a	28 ± 1b	28 ± 1b
ME (mU/mg protein)	71.3 ± 1.9a	73.3 ± 5.1a	94.5 ± 6.4b
Kidney			
MDA (pmol/g protein)			
bound	315.1 ± 33.3a	215.0 ± 24.2b	129.9 ± 11.2c
free	268.0 ± 34.4a	88.2 ± 9.4b	18.3 ± 7.8c
GSHPx (mU/mg protein)	591.4 ± 31.1a	1227.8 ± 55.8b	1518.0 ± 77.4a
Heart			
MDA (pmol/g protein)			
bound	148.0 ± 18.8a	72.0 ± 8.5b	29.5 ± 4.6c
free	87.7 ± 13.2a	38.3 ± 7.2b	2.8 ± 0.3c
GSHPx (mU/mg protein)	187.4 ± 16.5a	317.4 ± 18.1b	305.2 ± 19.9b

[a] Values are means ± SEM of seven observations per group; values in a row with different letters are significantly different ($p < 0.05$).

and 3 whatever may be the enzyme regarded. However, selenium deficiency triggered off a significant rise in liver GST (66%) and GSSGR (23%) activity in comparison with rats from Group 2 which were supplied selenium by Spirulina; the activities exhibitd by these animals were not significantly different from those seen in Group 3. The specific activities of the three NADPH-producing enzymes are also shown in Table 3. The activities of G6PD and 6PGD increased significantly in Group 1 (about 27–28%) with regard to Groups 2 and 3, whereas that of ME was unchanged in Groups 1 and 2 and significantly lower than that of Group 3.

4. Discussion

This experiment investigated the ability of dietary Spirulina to behave like a carrier of selenium as measured by liver GSHPx activity and glutathione-related enzyme activities (GSSGR, GST), the activities of NADPH-generating enzymes (G6PD, 6PGD and ME) were also monitored. Rats submitted to an oxidative stress were used in order to test any beneficial effect of Se-rich microalgae. Additionally, rats fed standard diets were also utilized. In this work we found that a 57-day dietary deficiency of antioxidant nutrients at high-cholesterol level significantly affected rat plasma LDL-C; rats fed a diet deficient in both vitamin E and Se had an increased LDL-C of 79% compared either with rats fed an identical diet but supplemented with Se-enriched Spirulina or to rats fed a standard diet adequate in both antioxidants. Therefore, selenium deficiency alone was responsible for the elevated LDL-C level. This is in agreement with the work reported by others (Stone, Stewart, Nicholas, & Pavuluri, 1986; Mazur et al., 1996). Stone et al. (1984) suggested that the hypercholesterolemic effect of Se deficiency was amplified by addition of 1% dietary cholesterol known to decrease the plasma clearance rate of LDL (Slater, Shepherd, & Packerd, 1982), thereby allowing LDL to undergo increased in vivo lipid peroxidation. Dietary deficiencies of both vitamin E and Se promote in vivo lipid peroxidation (Hafeman & Hoekstra, 1977) and this was emphasized here by low MDA levels (either free or bound) seen in all the studied tissues of rats from Groups 3 fed an adequate vitamin E and Se diet. Moreover, the selenium supply by Spirulina (Group 2) resulted in significantly lower MDA level in liver, kidney and heart when compared with animals from Group 1. Although the same amount of selenium was fed to Groups 2 and 3, they differ significantly from one another in MDA, free and bound, in liver, kidney and heart: these higher tissue levels of MDA in rats from Group 2 can be attributed to the diet which is naturally rich in lard and cholesterol and low in vitamin E; this is not the case for rats from Group 3. Selenium deficiency resulted in a general and dramatic fall of Se-GSHPx activity. It must be pointed out that dietary Spirulina supplementation (Group 2) allowed rats to restore Se-GSHPx activities at an identical level to that observed in antioxidant-adequate-fed rats (Group 3). GST, which is present in large amounts in liver, catalyses the conjugation by glutathione of a wide range of xenobiotics. It has been reported by Lawrence and Burk (1978) and Lawrence, Parkhill, and Burk (1978) that, in rats, selenium deficiency is accompanied by a compensatory rise in GST activity. The 84% decreased hepatic Se-GSHPx activity was accompanied here by a 66% increase in GST activity. Studies have shown that GST activity increases as much as 200% in Se-deficient rat liver (Burk, Lawrence, & Correia, 1980); that level of increase was not achieved in the rats from Group 1. Regarding the specific activity of GSSGR, which was significantly increased in rats from Group 1 over those from Group 2, some authors (Reiter & Wendel, 1984, 1985) reported an increase of this enzymic activity in Se-deficient mice, whereas others (Burk, Nishiki, Lawrence, & Chance, 1978) found no change in the liver of Se-deficient rats. The specific activities of NADPH-generating enzymes behaved differently in Se-deficient rats: although G6PD and 6PGD activities were increased, no change was observed in that of ME compared to rats from Group 2. Our results clearly demonstrate a responsive relationship between an NADPH-consuming enzyme (GSSGR) and G6PD and 6PGD, NADPH-generating enzymes. Nevertheless, during selenium deficiency, malic enzyme activity did not change toward an increased NADPH production as did G6PD and 6PGD, possibly to maintain glutathione in a reduced state. Ayala, Lobato, and Machado (1986) reported such a relationship between GSSGR and ME in rats receiving t-butyl hydroperoxide. Vadhanavikit and Ganther (1993, 1994) found increased cytosolic activity of NADPH-generating enzymes (G6PD, 6PGD and ME) and GSSGR in the liver of rats fed a Se-deficient diet compared with controls, whereas studies by Burk et al. (1978) reported a decreased G6PD activity in Se-deficient rats. The slight discrepancy which appeared between these results and ours is unexplained but could be attributed to the high fat diets used here. Nevertheless, dietary Se-supplementation by Spirulina in rats from Group 2 triggered off a reduced demand for NADPH in comparison with Se-deficiency, thereby supplying enough glutathione for the reduction of hydroperoxides by GSHPx, as seen in standard rats from Group 3.

The high recovery of Se-GSHPx activity and the gross restoration of the studied parameters demonstrated that Spirulina behave as an efficient vehicle for selenium after dietary Se-rich algae supplementation in rats submitted to peroxidative damage. Nevertheless, although one of us showed the algal cytoplasmic localization of the element and any contamination from extracellular source in the final dry powdered Spirulina (unpublished

data), the possible organic nature of selenium remains to be assessed.

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