Expression analysis of mammalian selenocysteine lyase

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Abstract Selenium (Se) is an essential trace element of mammals and plays important roles in the form of selenocysteine residues in selenoproteins. Selenocysteine lyase (SCL) specifically catalyzes the decomposition of L-selenocysteine into Se and L-alanine and is proposed to function as a Se delivery protein to selenophosphate synthetase in selenoprotein biosynthesis. However, the physiological role of SCL has not been established. In this study, the expression levels of SCL in various tissues of Se-deficient/supplemented mice were studied. We also examined effect of oxidative stress or excess sodium selenite on the expression of SCL by western blot analysis. Expression levels of SCL were not significantly changed by Se status in many tissues. However, the levels of SCL in the stomach of 7 out of 8 mice fed a Se-supplemented diet were markedly lower than those of mice fed a Se-deficient diet. By the administration of 1 mg/kg sodium selenite, the expression levels of SCL were elevated in stomach of the mice fed a Se-supplemented diet. This is the first report demonstrating the possible existence of a Se-dependent regulation system for SCL.

Key words : Selenium, Deficiency, Mouse, Selenocysteine lyase, Expression, Western blot.

Introduction

Selenium (Se) is an essential trace element of many organisms, including humans. Deficiency of Se is related to cancer [1], cardiovascular diseases [2], neural diseases [3], rheumatoid arthritis and immune disorders [4]. Se is incorporated into proteins in the form of selenocysteine residue [5-7]. Selenocysteine lyase (SCL) specifically catalyzes the decomposition of L-selenocysteine into Se and L-alanine and is proposed to function as a selenium delivery protein to selenophosphate synthetase in selenoprotein biosynthesis [8-11]. Previously, we reported cDNA cloning and characterization of SCL from mouse liver [9]. The enzyme shows high substrate specificity towards L-selenocysteine and mainly localizes to liver, kidney, testis, spleen, and brain. The distribution pattern of the enzyme is similar to that of selenophosphate synthetase, supporting the idea that SCL and selenophosphate synthetase may cooperate with each other to function in selenoprotein biosynthesis. However, *in vivo* relevance of SCL to Se metabolism has not been established. Expression of many enzymes is regulated by their substrates, products or metabolically related compounds. Thus, experiments were conducted to test whether expression of SCL is affected by Se deficiency or administration of sodium selenite. Several selenoproteins function as anti-oxidant proteins, and metabolism of Se is thought to be regulated by the oxidative status. To investigate whether changes in the oxidative status affect the function of SCL, the effect of free-radical generating lipopolysaccharide (LPS) on SCL expression was also monitored.

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Accepted: Sep 13, 2004

Materials and Methods

Weaning female C57BL/6 mice (3weeks old) were purchased from Shimizu Laboratory Supplies Co. Ltd. One group (n = 12) was fed a Se-deficient diet, and another (n = 12) was fed a Se-supplemented diet containing 0.4 ppm sodium selenite as a Se source. After 6 months, 1 mg/kg sodium selenite in saline was administered orally to Se-deficient mice (n = 3) and Se-supplemented mice (n = 3). Another group of Se-deficient mice (n = 3) and Se-supplemented mice (n= 3) was injected intraperitoneally with 1 mg/kg *Escherichia coli* LPS. Twenty-four hours after administration, mice were killed by vertebral dislocation and decapitation. The liver, brain, stomach and spleen were immediately excised, and the brain tissues (cerebrum, midbrain and cerebellum) were dissected. The tissues were immediately frozen in liquid nitrogen and stored at -80°C until use. The tissues were homogenized with a glass-Teflon homogenizer in 50 mM KPB (pH 7.0) containing a protease inhibitors cocktail (Roche), and the homogenates were centrifuged at 10,000 rpm for 30 min at

4°C to obtain the supernatants. Protein was determined with a dye-binding assay kit (Nacalai Tesque) by following a manufacturer's instruction with bovine serum albumin as a standard. An antiserum against SCL was prepared as described previously [9]. Western blot analysis was performed by established procedures [12]. Briefly, equal amounts of total protein were loaded and separated on sodium dodecyl sulfate (SDS)-12.5% polyacrylamide gels and then transferred to PVDF membranes. Membranes were dried and washed in 1x Tween (0.1%)-Tris-buffered saline three times for 5 min each. Primary antibodies diluted 1:10000 in 5% skim milk were allowed to incubate overnight at 4°C. After washing, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Cell Signaling Technology) diluted 1:50000 in 5% skim milk. Subsequently, membranes were washed and incubated with a chemiluminescence substrate (ECL plus, Amersham) at room temperature for 5 min. The blots were then visualized with a chemiluminescent detection system (ECL plus, Amersham) as described by the manufacturer.

Results and Discussion

Expression level of SCL in the tissues of the normal and the Se-deficient mice—Expression levels of SCL in the cerebrum of the Se-deficient mice were similar to those of the Se-supplemented mice. Our previous study indicated that mRNA expression levels of cellular glutathione peroxidase (Gpx1), gastrointestinal glutathione peroxidase (Gpx2), cytosolic thioredoxin reductase (TR1), type 2 triiodothyronine deiodinase (DI2) and selenophosphate synthetase 2 (SPS2) were increased by Se deficiency [12]. Therefore, it is likely that the regulation of SCL expression may be different from those selenoproteins in cerebrum. Also in midbrain, cerebellum and spleen, SCL expression levels were not significantly different between the Se-deficient mice and the Se-supplemented mice. In contrast, the expression level of SCL in the liver of the Se-supplemented mice were slightly lower than those of the Se-deficient mice. To our surprise, remarkable difference in the SCL expression levels was observed in the stomach. The levels of SCL in 5 out of 6 Se-deficient were very high. However, the expressions of SCL in 4 out of 5 Se-supplemented mice were almost completely repressed. This is consistent with our previous observation that the expression level of SCL in stomach is markedly lower than liver and spleen in mice fed a Se-adequate diet [9]. These results suggest that SCL expression may be induced under Se deficiency in stomach. The gastrointestinal tract is particularly susceptible to reactive oxygen species which lead to carcinogenesis. An important role in defense strategy against reactive oxygen species is played by antioxidants including glutathione. Gpx2 is exclusively expressed in the gastrointestinal tract in rodents [13]. Interestingly, Gpx2 mRNA levels increase during selenium deficiency, whereas Gpx1 mRNA levels markedly decrease [13]. Thus, the metabolism of Se in stomach may be different from other tissues. A specialized Se metabolism in stomach may be related to the stomach-specific regulation of the expression of SCL in response to Se status.

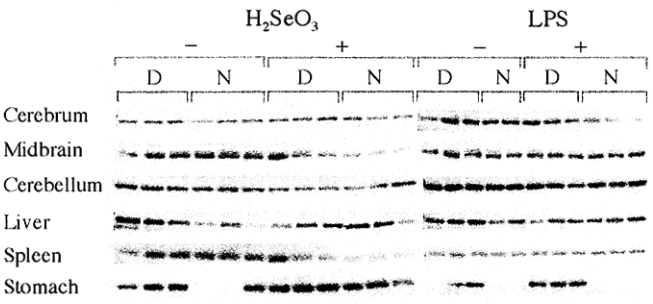

Fig. 1. Western blot analysis of SCL in mouse tissues.
Tissue extracts from mouse (cerebrum, midbrain, cerebellum, liver, spleen, stomach) were subjected to 12.5% SDS-PAGE, blotted onto a polyvinylidene difluoride membrane, and analyzed with a polyclonal anti-SCL antibody. Each lane was loaded with 25 μg of protein. 'N' indicates the mice fed a Se-supplemented diet, 'D' indicates the mice fed a Se-deficient diet. Samples indicated by '+' were obtained from mice administrated 1 mg/kg sodium selenite (left) or 1 mg/kg LPS (right).

Expression level of SCL in the tissues of the normal and the Se-deficient mice administrated excess sodium selenite—An excess amount of sodium selenite (1 mg/kg) was administrated to mice which were fed a Se-deficient diet or a Se-supplemented diet for 6 months. The SCL expression levels of midbrain and spleen of the Se-supplemented/selenite-administrated mice were slightly lower than those of the Se-supplemented mice. However, the excess selenite did not affect the SCL levels of midbrain and spleen of Se-deficient mice. The levels of SCL in the liver of 2 out of 3 Se-supplemented/selenite-administrated mice was higher than the levels of the Se-supplemented mice, although some individual differences were observed. Interestingly, we observed the expression of SCL in the stomach of the Se-supplemented/selenite-administrated mice. This result indicates that excess selenite may strongly induce the expression of SCL in stomach. The result that both the long-term Se deficiency and the refeeding of excess sodium selenite induced the SCL expression may indicate that the expression of SCL is indirectly regulated by Se status or

the expression of SCL is repressed in a certain range of Se concentration.

Expression level of SCL in the tissues of the normal and the Se-deficient mice administrated LPS—Macrophages activated by microbial LPS produce bursts of nitric oxide and reactive oxygen species [14]. Redox protection systems are essential for the survival of the macrophages since the nitric oxide and reactive oxygen species can be toxic. We investigated the effect of oxidative stress induced by LPS [15] on the expression of SCL with respect to Se status. At sixteen hours after administration of LPS, mice produced shaking behavior. One Se supplemented mouse died 2 hours later. SCL expression levels in cerebrum and cerebellum of the LPS-administrated mice were lower than those of the mice without administration of LPS. In contrast, the level of SCL in the liver and the spleen of the Se-supplemented/LPS-administrated mice were slightly higher than those of the Se-supplemented mice. In contrast to that the Se-supplemented/selenite-administrated mice contain high level of SCL, the Se-supplemented/ LPS-administrated mice did not produce SCL. The result indicates that oxidative stress induced by LPS does not affect the expression of SCL in stomach, and therefore, the induction of SCL levels by the administration of excess sodium selenite may be due to a specific response rather than the general stress response which can be produced by reactive oxygen species.

Although the physiological relevance of the expression level of SCL to Se status remains to be solved, this study demonstrated for the first time the possible existence of a Se-dependent regulation system for SCL.

References

1) Gey KF : Vitamins E plus C and interacting conutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. Biofactors 7 : 113-174, 1998.
2) Sinatra ST, DeMarco J : Free radicals, oxidative stress, oxidized low density lipoprotein (LDL), and the heart: Antioxidants and other strategies to limit cardiovascular damage. Conn Med 59 : 579-588, 1995.
3) Wasantwisut E : Nutrition and development : Other micronutrients' effect on growth and cognition. Southeast Asian J Trop Med Public Health 28 : 78-82, 1997.
4) Finch JM. Turner RJ : Effects of selenium and vitamin E on the immune responses of domestic animals. Res Vet Sci 60 : 97-106, 1996.
5) Low SC, Berry MJ. : Knowing when not to stop : Selenocysteine incorporation in eukaryotes. Trends Biochem Sci 21 : 203-208, 1996.
6) Stadtman TC : Selenocysteine. Annu Rec Biochem 65 : 83-100, 1996.
7) Behne D, Hilmert H Scheid S, Gessner H, Kyliakopoullos A, Elger W : Selenium in biology and medicine. Springer-Verlag, Berlin, 1988.
8) Esaki N, Nakamura T, Tanaka H, Soda K : Selenocysteine lyase, a novel enzyme that specifically acts on selenocysteine. Mammalian distribution and purification and properties of pig liver enzyme. J Biol Chem 257 : 4386-4391, 1982.
9) Mihara H, Kurihara T, Watanabe T, Yoshimura T, Esaki N : cDNA cloning, purification, and characterization of mouse liver selenocysteine lyase. Candidate for selenium delivery protein in selenoprotein synthesis. J Biol Chem 275 : 6195-200, 2000.
10) Lacourciere GM, Mihara H, Kurihara T, Esaki N, Stadtman TC : *Escherichia coli* NifS-like proteins provide selenium in the pathway for the biosynthesis of selenophosphate. J Biol Chem 275 : 23769-23773, 2000.
11) Mihara H, Esaki N : Selenocysteine lyase from mouse liver. Methods Enzymol 347 : 198-203, 2002.
12) Kuwana E, Kurokawa S, Mihara H, Kurihara T, Yoshimura T, Esaki N : Effects of selenium deficiency on the expression of selenoprotein mRNAs in mouse brain. Biomed Res Trace Elements, 14 : 293-296, 2003.
13) Wingler K, Brigelius-Flohe R : Gastrointestinal glutathione peroxidase. Biofactors, 10 : 245-249, 1999.
14) Muller JM, Ziegler-Heitbrock HW, Baeuerle PA : Nuclear factor kB, a mediator of lipopolysaccharide effects. Immunobiology 187 : 233-256, 1993.
15) Sato H, Kuriyama-Matsumura K, Hashimoto T, Sasaki H, Wang H, Ishii T, Mann GE, Bannai S : Effect of oxygen on induction of the cystine transporter by bacterial lipopolysaccharide in mouse peritoneal macrophages. J Biol Chem 276 : 10407-10412, 2001.