— **Minireview** —

New Aspects of Physiological and Pharmacological Roles of Selenium

Seiichiro Himeno* and Nobumasa Imura

Kitasato University, School of Pharmaceutical Sciences, Department of Public Health and Molecular Toxicology, 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan

(Received June 30, 2000)

The biological functions of selenium (Se) are mediated by the expression of selenoproteins such as glutathione peroxidase. Recent progress in biochemical characterization of selenoproteins has elucidated new functions of newly identified and classical selenoproteins. Most selenoproteins are involved in protection against oxidative injury and in redox regulation of cellular events. On the other hand, mechanisms of anticarcinogenic effects of Se compounds appear to include not only the expression of selenoproteins but also pharmacological actions of Se metabolites.

Key words —— selenium, selenoprotein, glutathione peroxidase, thioredoxin reductase, cancer chemoprevention

INTRODUCTION

Recent progress in selenium (Se) research has expanded the spectrum of physiological and pharmacological functions of Se. The logical basis for the role of Se as an essential micronutrient has been provided primarily by the two findings that Se comprises the active site of glutathione (GSH) peroxidase[1] and that the mortality and morbidity of endemic cardiomyopathy (Keshan disease) in China were dramatically reduced by supplementation of sodium selenite.[2] However, in addition to the first identified GSH peroxidase, which is now called cellular or classical GSH peroxidase (cGPx), a variety of new selenoproteins have been isolated in this decade, and some of them seem to be more important than cGPx. On the other hand, epidemiological and experimental studies have provided cumulative evidence on the protective roles of Se in the development of cancer. Trials of cancer chemoprevention by supplementation with Se compounds are now drawing increasing attention.[3] However, the use of Se compounds in humans expecting their pharmacological effects will require more intensive studies on the toxicity and metabolism of Se compounds. In this article, new aspects of physiological and pharmacological roles of Se are briefly reviewed.

*To whom correspondence should be addressed: Kitasato University, School of Pharmaceutical Sciences, Department of Public Health and Molecular Toxicology, 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan. Tel.: +81-3-5791-6266; Fax: +81-3-3442-4146; E-mail: himenos@pharm.kitasato-u.ac.jp

New Functions of Selenoproteins

Cellular Glutathione Peroxidase —— As shown in Table 1, 17 selenoproteins have so far been identified in mammals. The GPx family is comprised of cGPx (GPX1), extracellular GPx (eGPx or GPX2), gastrointestinal GPx (GPx-GI or GPX3), and phospholipid hydroperoxide GPx (PHGPx or GPX4). Cellular GPx is the most abundant selenoprotein in animals. Since the major part of cGPx is located in the cytosol, the primary role of cGPx seems to scavenge H_2O_2 in the cytosol, while H_2O_2 produced in peroxisomes is scavenged predominantly by catalase that is specifically localized in peroxisomes. We have shown that the activity of cGPx in the liver and kidney of guinea pigs is extremely low, but it is compensated by the high activity of catalase in the cytosol in this species.[4] This, in turn, suggests an important role for cGPx in removing cytosolic H_2O_2 in other species. However, as cGPx cannot use phospholipid hydroperoxide as a substrate,[5] cGPx may not play an important role in the protection of biomembranes against oxidative stress. Recently, cGPx null mice were developed, but these mice did not show any dysfunctions in reproduction or growth, suggestive of a less important role for cGPx in survival of animals.[6] On the other hand, when animals are subjected to Se-deficient diet, the amount

Table 1. Selenocysteine-Containing Proteins in Mammals

Selenoproteins	Functions	Location
Glutathione peroxidase		
Cellular glutathione peroxidase (cGPx or GPX1)	Removal of hydroperoxides	Ubiquitous
Gastrointestinal glutathione peroxidase (GPx-GI or GPX2)	Removal of hydroperoxides	Gastrointestinal tracts
Extracellular glutathione peroxidase (eGPx or GPX3)	Removal of hydroperoxides	Plasma
Phospholipid hydroperoxide glutathione peroxidase (PHGPx or GPX4)	Removal of phospholipid hydroperoxides	Ubiquitous, testis
5′ Triiodothyronine deiodinase (5′-DI)		
Type 1 5′-DI	Conversion of T4 to T3, T4 to rT3	Thyroid gland, liver, kidney
Type 2 5′-DI	Conversion of T4 to T3	Pituitary gland, CNS, brown adipose tissue
Type 3 5′-DI	Conversion of T4 to rT3	Placenta
Thioredoxin reductase (TrxR)		
TRxR1	Reduction of thioredoxin	Ubiquitous (cytosol)
TRxR2	Reduction of thioredoxin	Ubiquitous (mitochondria)
TRxR3	?	Testis
Selenoprotein P	Antioxidant ? Se carrier ?	Plasma
Selenoprotein W	?	Ubiquitous, muscle
Selenophosphate synthetase 2 (SPS2)	Synthesis of selenophosphate (?)	Ubiquitous
15-kDa selenoprotein	?	Ubiquitous
SelT	?	?
SelR/SelX	?	?
SelN	?	?

of cGPx decreases more rapidly than that of other selenoproteins, and the supplementation of Se to Se-deficient animals restores the amount of cGPx less rapidly than that of other selenoproteins such as selenoprotein P. Thus, cGPx may have dual roles in animal tissues, *i.e.*, as an antioxidant enzyme and as a pool of Se in the body.

Phospholipid Hydroperoxide Glutathione Peroxidase —— In contrast to cGPx, PHGPx has broad substrate specificity including phospholipid hydroperoxide and oxidized cholesterol, and is associated with membrane fractions. Therefore, PHGPx seems to have more important roles in the protection of biomembranes against lipid peroxidation. However, since the amount of PHGPx is very low except for the testis and the activity of PHGPx is hardly affected by the change in nutritional status of Se, physiological roles of PHGPx have not been fully elucidated. Recently, however, Imai *et al*. established two cell lines, in which either mitochondrial or non-mitochondrial PHGPx was overexpressed, and found that the mitochondrial PHGPx plays an important role as an antiapoptotic enzyme in the mitochondria,[7] while non-mitochondrial PHGPx is associated with nuclear membrane and is involved in the regulation of leucotriene production.[8] The detailed properties of the two types of PHGPx will be described by Nakagawa *et al*. in this issue.[9] On the other hand, it was shown that the PHPGx expressed in the testis has dual functions depending on the stage of spermatogenesis, *i.e.*, as an antioxidant enzyme in the spermatids and as a structure protein in the midpiece of matured sperm.[10]

Thioredoxin Reductase —— In 1996, Tamura *et al*. isolated a 57-kDa selenoprotein from human lung adenocarcinoma cells and identified it as thioredoxin reductase.[11] This enzyme reduces the oxidized form of thioredoxin (Trx), a low-molecular-weight protein responsible for the reduction of protein disulfides and other oxidized biomolecules. Mammalian Trx reductase (TrxR) is longer than bacterial TrxR in its size due to the additional 150 amino acids in the C-terminal domain. The selenocysteine (SeCys) moiety of mammalian TrxR is located penultimate to the C-terminal end of this protein, while bacterial TrxR does not contain SeCys. Several pieces of experimental evidence have indicated that the C-terminal SeCys residue, in addition to N-terminal cysteine residues, plays an important role in enzymatic activity of mammalian TrxR.[12,13] Recently, numerous studies reported that the Trx and TrxR system plays important roles in the redox regulation of various cellular events including activation of transcription factors as well as in the protection against oxidative injury.[14] Furthermore, it was reported that the induction of the TrxR gene occurs in response to oxidative stress, as has not been observed for other selenoproteins.[15] Elucidation of the mode of expression and cellular functions of TrxR will provide a new insight into the physiological roles of Se.

Orphan Selenoproteins —— Selenoprotein P and selenoprotein W have been called 'orphan selenoproteins' since the functions of these selenoproteins remain still unclear.[16] Selenoprotein P is the major selenoprotein in blood plasma, containing about a half of the Se in plasma. When Se is administered to animals, a substantial amount of newly synthesized selenoprotein P appears in blood plasma in a few hours, suggesting an important role of selenoprotein P. The structure of rat and human mRNAs of selenoprotein P containing 10 in-frame UGA codons suggests that this protein contains 10 SeCys residues.[17,18] However, peptide analyses of rat and human selenoprotein P revealed only 7–8 SeCys residues in mature proteins.[19,20] In addition, Himeno *et al*. found a truncated isoform of rat selenoprotein P, which is terminated at the serine residue prior to the second SeCys, suggesting that the second UGA codon of rat selenoprotein P is used either for translation termination or SeCys insertion.[19] Further analysis on the protein structure of selenoprotein P is required. The role of selenoprotein P has been explained either as a Se carrier or as an antioxidant protein. Burk *et al*. proposed that selenoprotein P protects enterocytes against oxidative injury from outside of cells since selenoprotein P is found to be attached to the plasma membrane of enterocytes[21] probably through the interaction of the histidine-rich region of selenoprotein P with the heparan sulphate of proteoglycans.[22] Saito *et al*. found that human selenoprotein P has an enzymatic activity of PHGPx,[20] but whether the function of selenoprotein P as an antioxidant is ascribed solely to its PHGPx activity remains to be elucidated.

Selenoprotein W is a low–molecular–weight protein (10 kDa), originally found in the muscle of animals.[23] Since white muscle disease in domestic animals was found in a Se–deficient area, the role of selenoprotein W in relation to the etiology of muscle dysfunction needs to be clarified. A 15 kDa selenoprotein was isolated from human Jurkat T cells, but the function of this protein remains unknown.[24] Human homologues of selenophosphate synthetase (*SelD*) gene were cloned and designated as SPS1 and SPS2, the latter containing SeCys.[25] However, whether the products of these genes actually participate in the production of selenophosphate, a precursor for the synthesis of SeCys-tRNA, in mammals has not yet been proved.

Recently, two groups independently cloned several new genes encoding selenoproteins by a computational screening of gene database in search for the genes that contain motifs of SECIS (SeCys insertion sequence) in the 3′-untranslated region of mRNA. One group identified SelT and SelR,[26] while the other group identified SelN and SelX, the latter being similar to SelR.[27] However, the functions of these new selenoproteins remain to be elucidated.

To date, 17 selenoproteins have been identified in mammals (Table 1), but Behne *et al*. predicted the existence of about 30 selenoproteins by means of ^{75}Se-labeling of all selenoproteins in animals.[28] Recently, Bösl *et al*. developed SeCys–tRNA knockout mice, in which the expression of all selenoproteins was hampered, and showed that these mice died at an early phase of embryogenesis.[29] Thus, the synthesis of SeCys–containing proteins is essential for the survival of mice. Which selenoprotein plays the most important role for survival of animals needs to be determined in future studies.

Pharmacological Effects of Se by 'Supranutritional' Supplementation

Since the role of Se as an antioxidant may be primarily mediated by the expression of

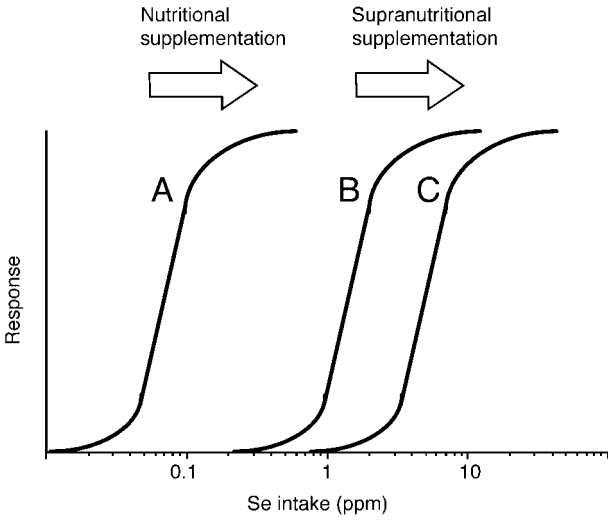

Fig. 1. Dose-Response Relationship Between Se Intake and Biological Functions of Se

A, activities of selenoproteins; B, anticarcinogenic effects; C, toxic effects.

selenoproteins, elucidation of functions of each selenoprotein seems to be warranted. However, the other important role of Se as an anticarcinogenic agent may not be ascribed solely to the expression of selenoproteins.

The anticarcinogenic actions of Se compounds in rats have been extensively investigated by Ip and his coworkers.[30] They developed an animal model of chemical carcinogenesis using dimethylbenz(*a*)anthracene (DMBA) that induces mammary gland tumors in rats. The addition of Se to the diet at the level of 2–5 ppm (as sodium selenite) was shown to be effective in the prevention of DMBA-induced mammary gland tumors. However, these levels of Se were ten times higher than those included in usual commercial diets for animals (0.2–0.4 ppm), and were 20–50 times higher than the minimum dietary requirement level of Se for animals (0.1 ppm). On the other hand, the activities of most selenoenzymes such as cGPx and eGPx are known to reach the plateau level when the dietary level of Se reaches 0.1–0.4 ppm (Fig. 1). Thus, anticarcinogenic effects of Se observed in animals given 2–5 ppm Se may be mediated by other mechanisms than the activation of known selenoproteins. The supplementation of vitamin E modified the anticarcinogenic effects of Se, but did not compensate for the actions of Se, suggesting that the antioxidative actions of Se may not be the major cause for the prevention of carcinogenesis. On the other hand, selenobetaine or Se-methylselenocysteine, which are precursors for methylselenol, have been shown to be as effective as sodium selenite in the protection against DMBA-induced mammary gland tumor formation.[31] Furthermore, simultaneous addition of arsenite, an inhibitor for methylation of Se metabolites, with these precursor compounds enhanced the anticarcinogenic effects, suggesting that partially methylated forms of Se are responsible for anticarcinogenic actions of Se.[32]

Thus, it seems likely that the anticarcinogenic actions of Se observed in animals given 'supranutritional' levels of Se might be due to unknown 'pharmacological' actions such as inhibition of cell growth or induction of apoptosis elicited by methylated Se metabolites. On the other hand, the supplementation of Se to Se-deficient animals or humans will restore the expression of selenoproteins, leading to the recovery of protective ability against oxidative injury. Therefore, this kind of 'nutritional' supplementation of Se towards people suffering from Se deficiency, and 'supranutritional' supplementation of Se towards people taking sufficient amount of Se should be taken as distinctly different ways of Se supplementation. In the latter case, the dose of Se causing pharmacological actions should not be close to the dose exhibiting toxic effects. However, when sodium selenite was added to the diet for rats, the effective doses of Se for anticarcinogenic activity (2–5 ppm) were found to be close to the toxic dose (5 ppm) that causes a loss of body weight gain.[30] The narrowness of the range between pharmacological and toxic doses is the major problem in applying Se to humans at 'supranutritional' level.

In an attempt to develop more effective and less toxic Se compounds for the prevention of carcinogenesis, several novel Se compounds have been synthesized. Among the newly developed Se compounds, *p*-xylylselenocyanate (*p*XSC) appears to be effective for a variety of carcinogenic chemicals including 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone, a specific carcinogen contained in cigarette smoke.[33] However, the mechanism underlying the prevention of carcinogenesis by these newly synthesized Se compounds remains to be elucidated. The detailed data concerning the anticarcinogenic effects of these new Se compounds will be described by Sugie in this issue.[34]

Since the trials for cancer chemoprevention by applying Se compounds in humans have been conducted in U.S. with partial success,[3] the safety evaluation of Se compounds is a more important issue than before. In Enshi county in China, human sele-

nosis occurred among people who took vegetables and cereals grown in the soil containing high concentrations of Se.[35] Epidemiological studies in this region demonstrated that NOAEL (NO Adverse Effect Level) of Se as judged by the occurrence of fingernail damage was 800–850 μg per day.[35] Based on NOAEL reported in China, several international committees have proposed recommendation for the safety dose of Se intake. Workshop on Selenium Compounds in Cancer Chemoprevention Trials in the United States determined a Reference Dose of Se to be 350 μg/d for a 70 kg man (5 μg/kg/d).[36] Reference Dose of Se was defined as 'an estimate (with an uncertainty spanning perhaps an order of magnitude) of a lifetime daily dose to the human population (including sensitive subpopulation) that is likely to be without an appreciable risk of deleterious effects during a lifetime'.[36] The maximum safety level of Se intake was set as 450 μg/d in the United Kingdom,[36] and the upper limit of Se intake was set as 300 μg/d by Nordic Nutrition Recommendations.[37]

CONCLUSION

Since it was shown in 1970s that Se is an essential component of cGPx, various selenoproteins have been isolated and characterized based on the principle that the biological activities of Se are mediated through the expression of selenoproteins. The mode of actions of how Se participates in the redox regulation of various cellular events could be clarified by intensive investigation of the functions of selenoproteins including the newly identified selenoproteins. On the other hand, the anticarcinogenic activities of Se compounds appear to be mediated not only by the expression of selenoproteins but also by hitherto unknown pharmacological actions of Se metabolites. Further studies on both physiological and pharmacological functions of Se are required for understanding whole spectrum of biological roles of Se.

REFERENCES

1) Rotruck J.T., Pope A.L., Ganther H.E., Swanson A.B., Hafeman D.G., Hoekstra W.G., *Science*, **179**, 588–590 (1973).
2) Keshan Disease Research Group, *Chinese. Med. J.*, **92**, 471–476 (1979).
3) Clark L.C., Combs G.F., Jr., Turnbull B.W., Slate E.H., Chalker D.K., Chow J., Davis L.S., Glover R.A., Graham G.F., Gross E.G., Krongrad A., Lesher J.L., Jr., Park H.K., Sanders B.B., Jr., Smith C.L., Taylor J.R., *J.A.M.A.*, **276**, 1957–1963 (1996).
4) Himeno S., Takekawa A., Imura N., *Comp. Biochem. Biophys.*, **B104**, 27–31 (1993).
5) Grossmann A., Wendel A., *Eur. J. Biochem.*, **135**, 549–552 (1983).
6) Ho Y.S., Magnenat J.L., Bronson R.T., Cao J., Gargano M., Sugawara M., Funk C. D., *J. Biol. Chem.*, **272**, 16644–16651 (1997).
7) Nomura K., Imai H., Koumura K., Arai M., Nakagawa Y., *J. Biol. Chem.*, **274**, 29294–29302 (1999).
8) Imai H., Narashima K., Arai M., Sakamoto H., Chiba N., Nakagawa Y., *J. Biol. Chem.*, **273**, 1990–1997 (1998).
9) Nakagawa Y., Imai H., *J. Health Sci.*, **46**, 414–417 (2000).
10) Ursini F., Heim S., Kiess M., Maiorino M., Roveri A., Wissing J., Flohé L., *Science*, **285**, 1393–1396 (1999).
11) Tamura T., Stadtman T.C., *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 1006–1011 (1996).
12) Hill K.E., McCollum G.W., Boeglin M.E., Burk R.F., *Biochem. Biophys. Res. Commun.*, **234**, 293–295 (1997).
13) Zhong L., Arnér E.S., Ljung J., Aslund F., Holmgren A., *J. Biol. Chem.*, **273**, 8581–8591 (1998).
14) Nakamura H., Nakamura K., Yodoi J., *Annu. Rev. Immunol.*, **15**, 351–369 (1997).
15) Sun Q.A., Wu Y., Zappacosta F., Jeang K.T., Lee B.J., Hatfield D.L., Gladyshev V.N., *J. Biol. Chem.*, **274**, 24522–24530 (1999).
16) Burk R.F., Hill K.E., *Bioessays*, **21**, 231–237 (1999).
17) Hill K.E., Lloyd R.S., Yang J.G., Read R., Burk R.F., *J. Biol. Chem.*, **266**, 10050–10053 (1991).
18) Hill K.E., Lloyd R.S., Burk R.F., *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 537–541 (1993).
19) Himeno S., Chittum H.S., Burk R.F., *J. Biol. Chem.*, **271**, 15769–15775 (1996).
20) Saito Y., Hayashi T., Tanaka A., Watanabe Y., Suzuki M., Saito E., Takahashi K., *J. Biol. Chem.*, **274**, 2866–2871 (1999).
21) Burk R.F., Hill K.E., Boeglin M.E., Ebner F.F., Chittum H.S., *Histochem. Cell Biol.*, **108**, 11–15 (1997).
22) Chittum H.S., Himeno S., Hill K.E., Burk R.F., *Arch. Biochem. Biophys.*, **325**, 124–128 (1996).
23) Yeh J.Y., Beilstein M.A., Andrews J.S., Whanger P.D., *FASEB J.*, **9**, 392–396 (1995).
24) Gladyshev V.N., Jeang K.T., Wootton J.C., Hatfield D.L., *J. Biol. Chem.*, **273**, 8910–8915 (1998).
25) Guimaraes M.J., Peterson D., Vicari A., Cocks B.G.,

Copeland N.G., Gilbert D.J., Jenkins N.A., Ferrick D.A., Kastelein R.A., Bazan J.F., Zlotnik A., *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 15086–15091 (1996).
26) Kryukov G.V., Kryukov V.M., Gladyshev V.N., *J. Biol. Chem.*, **274**, 33888–33897 (1999).
27) Lescure A., Gautheret D., Carbon P., Krol A., *J. Biol. Chem.*, **274**, 38147–38154 (1999).
28) Behne D., Kyriakopoeulos A., Weiss-Nowak C., Kalckloesch M., Westphal C., Gessner H., *Biol. Trace Elem. Res.*, **55**, 99–110 (1996).
29) Bösl M.R., Takaku K., Oshima M., Nishimura S., Taketo M.M., *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 5531–5534 (1997).
30) Ip C., *J. Nutr.*, **128**, 1845–1854 (1998).
31) Ip C., Hayes C., Budnick R.M., Ganther H.E., *Cancer Res.*, **51**, 595–600 (1991).
32) Ip C., Ganther H.E., *Cancer Res.*, **50**, 1206–1211 (1990).
33) el-Bayoumy K., Upadhyaya P., Desai D.H., Amin S., Hecht S.S., *Carcinogenesis*, **14**, 1111–1113 (1993).
34) Sugie S., Tanaka T., El-Bayoumy K., *J. Health Sci.*, **46**, 422–425 (2000).
35) Yang G., Zhou R., *J. Trace Elements Electrolytes Health Dis.*, **8**, 159–165 (1994).
36) Patterson B.H., Levander O.A., *Cancer Epidemiol. Biomarkers. Prevent.*, **6**, 63–69 (1997).
37) Sandström B., *J. Nutr.*, **128**, 372S–374S (1998).