— **Minireview** —

Selenoprotein P: Its Structure and Functions

Yoshiro Saito and Kazuhiko Takahashi *

Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita 12, Nishi 6, Kita-ku, Sapporo 060–0812, Japan

(Received June 30, 2000)

Selenoprotein P (SeP: the "P" denotes its presence in plasma) is the major selenoprotein in plasma. All selenoproteins so far reported contain one atom of selenium (Se) as selenocysteine per molecule except SeP, which is thought to contain ten atoms of Se as selenocysteine per molecule. SeP contains 50% of the total Se in plasma. The function of SeP is currently unknown, although several indirect lines of evidence suggest that SeP is a free radical scavenger or Se transporter. Recently, we reported that SeP is capable of reducing phospholipid hydroperoxide in the presence of glutathione, and characterized the enzymatic nature of SeP. SeP is also reported to function as a peroxynitrite scavenger or cell survival factor in primary culture of neurons. In this minireview, we discuss the relationship between the structure and function of SeP.

Key words —— selenium, selenocysteine, selenoprotein, glutathione peroxidase

INTRODUCTION

Se is an essential micronutrient, and is incorporated into proteins in the form of selenocysteine. These proteins containing selenocysteine (Sec) are termed selenoproteins.[1)] Each of the cDNA clones of a selenoprotein contains one TGA codon, which corresponds to UGA in mRNA, in the open reading frame.[1,2)] This UGA, known formerly only as a stop codon, encodes and directly incorporates Sec into these selenoproteins.[1,3)] The Sec insertion sequence (SECIS) element forming the stem-loop structure is located in the 3′-untranslated region of mammalian selenoprotein mRNA. The presence of SECIS is necessary for the recognition of UGA as a signal for Sec insertion (reviewed in Ref. 4, 5).

The first identified mammalian selenoprotein was glutathione peroxidase (GPx). GPx catalyzes the reduction of hydrogen peroxide and organic hydroperoxide by glutathione (GSH) and functions in the protection of cells against oxidative damage. At least four forms of GPx are reported to exist, and these differ in many properties, including their localization, subunit structure, primary structure, and enzymatic nature. The classical cellular GPx (cGPx), found in the cytosol of various tissues and blood cells, reduces hydrogen peroxide and organic hydroperoxides, but cannot reduce phospholipid hydroperoxides. A second GPx, phospholipid hydroperoxide GPx (PH-GPx), is believed to be mostly cytosolic and partly associated with mitochondria.[6,7)] This enzyme is able to reduce phospholipid hydroperoxide in addition to hydrogen peroxide.[6–8)] The function of PHGPx is reviewed by Nakagawa in this issue.[9)] A third GPx in plasma, now called extracellular GPx (eGPx), is reported to reduce both hydrogen peroxide and phospholipid hydroperoxides.[10,11)] A fourth GPx, originally called GPx-related selenoprotein,[12)] is now called gastrointestinal GPx (GI-GPx) because of its high level of expression in the gastrointestinal tract mucosal epithelium.[13)] Furthermore, type I, type II and type III iodothyronine deiodinase[14)] and thioredoxin reductase[15)] were identified as selenoproteins. In all mammalian Sec-containing enzymes belonging to the oxidoreductase class, the selenocysteine residue is a catalytically active site. Further mammalian selenoproteins with as-yet-unidentified functions have been reported, such as selenoprotein P (SeP) from plasma,[16,17)] and selenoprotein W from rat muscle.[18)] All selenoproteins so far reported contain one atom of Se as selenocysteine per polypeptide except SeP. The present review will discuss the structure and function of this distinctive selenoprotein, SeP.

*To whom correspondence should be addressed: Graduate School of Pharmaceutical Sciences, Hokkaido University, Kita 12, Nishi 6, Kita-ku, Sapporo 060–0812, Japan. Tel.: +81-11-706-3244; Fax: +81-11-706-4990; E-mail: kazu@pharm.hokudai.ac.jp

Structure of SeP

Raymond Burk reported the existence of Se-containing protein in rat plasma in 1973.[19] Ten years later, Motsenbocker and Tappel showed that this protein contains Se as Sec, and named it selenoprotein P (SeP: the "P" denotes its presence in plasma).[20] Finally, SeP has been purified from humans and rats,[21,22] via a procedure which includes immuno-affinity chromatography, and was characterized as containing 7–8 Se atoms as selenocysteine per molecule. Until now, the cDNA of SeP and its analog have been cloned from rat, human, bovine, and murine cDNA libraries.[23–26] The sequence of the cloned DNA suggests that SeP contains 10 to 12 selenocysteines encoded by UGA stop codons in the open reading frame of its mRNA, and two SECIS elements in the 3′-untranslated region of its mRNA. SDS-PAGE of purified human SeP revealed an apparent molecular mass of 67 kDa, though a mass of 41 kDa was predicted from human SeP cDNA. This difference is largely due to five *N*-glycosylation sites predicted from cDNA and a number of *O*-glycosylation sites. The presence of a signal peptide sequence supports the fact that SeP is an extracellular protein. It is believed that SeP is mainly synthesized and secreted by the liver in the same manner as most plasma proteins.

A summary of the gross structures of three (human, rat and mouse) forms of SeP is shown in Fig. 1. While there is only one Sec residue present per polypeptide in all other selenoproteins, SeP contains 10 Sec residues per polypeptide. Furthermore, the bovine SeP analogue contains 12 Sec residues per polypeptide. Only one Sec residue is located in the N-terminal region, while the remaining 9 Sec residues are predicted to be located in the C-terminal third part. When this C-terminal third is compared with the other ones, not only Sec but also Cys residues are rich and relatively well-conserved. If Sec and Cys are considered together, an almost complete conservation of Sec and Cys residues is indicated. SeP has two His-rich domains; one of which includes a run of four His residues followed by a Lys, His, Lys for a total of seven consecutive basic amino acid residues. Thus, SeP is rich in Sec, Cys and His, indicating that SeP is capable of binding a metal via these amino acids. Actually, SeP binds strongly to immobilized nickel chelate chromatography, and is eluted by competition with imidazole, a histidine analogue.[27]

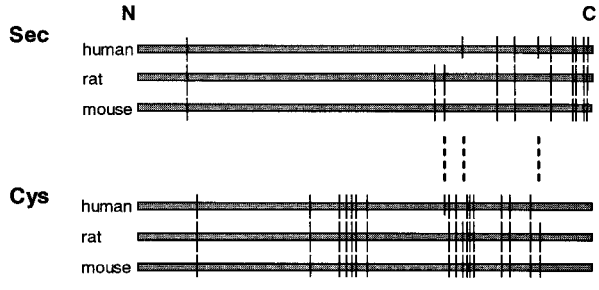

Fig. 1. Structure of SeP from Three Species

The vertical line represents a Sec residue or a Cys residue predicted from the human (24), rat (23) and mouse (26) cDNA sequence.

Burk *et al*. reported the purification of SeP from human and rat plasma[21,22] using immunoaffinity chromatography. Though Eberle and Haas purified human SeP without immunoaffinity chromatography, their final preparation reportedly contained some contaminants.[28] Recently, we established a procedure for the isolation of SeP using conventional means.[27] The purified SeP yielded a single band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The amino acid composition and amino-terminal amino acid sequence of the final preparation is similar to those postulated for the deduced polypeptide, confirming the homogeneity of our final preparation. Multiple forms of rat and human SeP are reportedly present in plasma.[29,30] In the case of rat SeP, it is known that one form (47 kDa) terminates at the second UGA while the other form (57 kDa) terminates at a UAA stop codon after all 10 UGAs have been translated as Sec.[31] Incubation of partially purified fractions results in the fragmentation of human SeP, when detected by immunoblot method (Y. Saito, unpublished observations). When a potent serine protease inhibitor, diisopropyl fluorophosphate, is added to the incubation mixture, no cleavages of human SeP are observed. Furthermore, immunoprecipitation and immunoblot analysis reveals that only one form of SeP is detected from human plasma. These results indicate that some serine proteases cleave SeP when activated in the purification process. It is likely that SeP may be cleaved under some pathological conditions, and these smaller forms may function in an unknown fashion. Only 6.3 mol of Se were found in the Se and quantitative amino acid analysis of purified human SeP, even though 10 mol were predicted from its cDNA. Under the same analysis conditions, 1.1 mol of Se were detected in the purified eGPx preparation, as expected. This discrepancy was also observed in a full-length form of SeP purified from rat plasma.[31] Further structural analysis of purified

SeP is necessary to clarify the cause of this discripancy.

Function of SeP

Selenoproteins with known enzymatic activity are redox enzymes, and contain selenocysteine in their active sites. A key issue concerning SeP that remains unresolved is its catalytic function. Previous reports show that SeP does not reduce hydrogen peroxide in the presence of GSH.[32] As we could prepare a highly purified SeP without denaturation, it apppeared worth reexamining the GPx-like activity of SeP. First, we confirmed that SeP lacks the enzymatic activity of cGPx. Next, we investigated the PH-GPx activity of SeP. We used a synthetic phospholipid hydroperoxide, 1-palmitoyl-2-(13-hydroperoxy-*cis*-9, *trans*-11-octadecadienoyl)-3-phosphatidylcholine hydroperoxide (PLPC-OOH) as a substrate. Using two different methods, coupled assay and HPLC assay, it was shown that SeP reduces the same amounts of PLPC-OOH in the presence of GSH.[27] The conversion rate (2.03 μmol per minute per mg of protein) of PLPC-OOH to PLPC-OH by SeP directly determined by HPLC, was equal to that indirectly determined by coupling assay, in which PH-GPx activity is coupled to the oxidation of NADPH by GSH reductase. Immunoprecipitation with monoclonal antibodies against SeP results in the almost total loss of PH-GPx-like activity in the supernatant of the purified preparation. This rules out the possibility of contamination by another enzyme in our final preparation.

The highest level of PH-GPx activity for SeP was observed in the presence of 0.025% Triton X-100 and 0.3 mM deoxycholate. The addition of Triton X-100 to the assay mixture resulted in the transformation of the lipid bilayer into an optically clear solution of mixed micelles. This suggested that SeP catalyzes a reaction at the lipid-water interface, as in the case of PH-GPx.[33] Deoxycholate reportedly can stimulate PH-GPx activity in the presence of Triton X-100.[34] The same stimulatory effect by deoxycholate was observed in our experiments using SeP.

A difference in peroxide specificity in the GPx family was reported. cGPx reduces hydrogen peroxide and *t*-butyl hydroperoxide, but does not reduce phospholipid hydroperoxide. PH-GPx reduces phospholipid hydroperoxide, and is also reactive against hydrogen peroxide,[6,7] while eGPx reduces hydrogen peroxide, *t*-butyl hydroperoxide and phospholipid hydroperoxides.[10,11] We found that SeP reduces phospholipid hydroperoxide but has no activity against hydrogen peroxide and *t*-butyl hydroperoxide. The peroxide specificity of SeP was more similar to that of PH-GPx than to that of cGPx or eGPx. Approximately one-hundred-fold lower k_{+1} and k'_{+2} values were observed in human SeP as compared to pig PH-GPx,[33] suggesting that SeP is less reactive to PLPC-OOH, an artificial substrate, than is PH-GPx.

Previous reports indicated that PH-GPx, unlike cGPx, did not exhibit a strict requirement for glutathione as a reducing agent. Using SeP as an enzyme, we observed that 1,4-dithiothreitol has a stronger activity than does glutathione. Other thiols, including 2-mercaptoethanol, cysteine and homocysteine, also serve as a reductant, as is already known in the case of PH-GPx.[6,35]

The N-terminal fragment, which contains only the first isolated Sec, is capable of reducing PLPC-OOH in the presence of GSH (Y. Saito, unpublished observations). Selenoproteins with enzymatic activity contain Sec in their active sites. These suggest that the first isolated Sec might be the active site of this enzyme.

It is currently unknown whether SeP is able to react with certain phospholipid hydroperoxides under physiological conditions. Using Se–deficient rats supplemented with Se, it was demonstrated that SeP appearance correlated with the disappearance of diquat–induced lipid peroxidation.[36] Furthermore, SeP was reportedly associated with protection against oxidant injury from GSH depletion in the Se–deficient rat.[37] These *in vivo* studies and our *in vitro* results described above strongly suggest that SeP serves to protect the plasma membrane from oxidative damage in the presence of GSH. Even though the GSH concentration in plasma is low, it is released continuously from the cells,[38] and it may represent a significant source of reductant for the enzymatic activity of SeP. SeP and eGPx are located in the extracellular fluids, and cGPx, PH-GPx and GPx-GI found in the cytosol. These enzymes exhibit different substrate specificities, and collaborate to protect biological molecules from oxidative stress inside and outside the cells, respectively.

It is well known that Se is essential for cell growth culturing using serum-free medium, but not using serum-containing medium. Serum-free culture medium for immune cells and neuron contains insulin (as a growth factor), transferrin (as an Fe supplier), sodium selenite (as a Se supplier) and albumin. Without these compounds, cells can neither

survive nor proliferate. This suggests the existence of a Se supplier (or transporter) in serum. Identification of the Se transporter is critical for understanding the distribution of Se in the body. There are three serum Se-containing proteins that are considered to be candidates for the Se–transporter, SeP, eGPx and albumin. SeP is present in human plasma at a concentration of approximately 5 μg of protein per ml. Immunoprecipitation studies indicate that SeP contains 50% of the total Se in human plasma. SeP is also associated with the endothelial membranes of liver, kidney and brain.[39] These indicate a possible role of SeP in the transport of Se. Motsenbocker and Tappel proposed in 1982 that SeP is synthesized in the rat liver and transfers Se from the liver to extrahepatic tissues.[20] Furthermore, the presence of a cell membrane receptor that binds SeP was demonstrated in the rat.[40] When ^{75}Se-labeled SeP is injected into the rat, it was shown that SeP disappeared more rapidly from plasma.[41] Se deficiency in rat did not significantly affect ^{75}Se disappearance from the plasma. At 2 h, the brain, but not other tissues, took up more ^{75}Se in Se–deficient rats than in control rats. This suggests that the brain has a specific uptake mechanism for Se given in the form of SeP. Interestingly, SeP has recently been reported to promote the survival of cultured rat neurons.[42] Purified SeP supported neuronal survival more effectively than inorganic Se. These results suggest that SeP contributes to the neuronal survival-promoting activity of serum. On the other hand, many studies suggest that an adequate intake of Se is required to ensure optimal immune functions, and that Se deficiency depresses the effectiveness of immune cells.[43] It has been observed that plasma Se and GSH levels are subnormal in HIV-infected individuals.[44] Under these conditions the survival rate of AIDS patients is reduced significantly. Taking these together, we propose that SeP is not only an inert carrier of Se but that it functions as a Se transporter, especially for both neuronal and immune cells. As Se is covalently bound to SeP, the breakdown of SeP is necessary for the release of its Se. Only 6.3 mol of Se were detected, even though 10 mol of Se were predicted from human SeP cDNA. It is probable that some Se in the C-terminal Sec-rich part of SeP may be released by an unknown mechanism. We are currently in the progress of testing this hypothesis. Our preliminary results indicate a possible role of SeP as a Se–transporter.

Finally, recent work suggests that SeP in plasma diminishes the oxidizing and nitrating reactivity of peroxynitrite, a reactive intermediate formed by the reaction of nitrogen monoxide and superoxide anion.[45] Due to the association of SeP with endothelial membranes, it is speculated that endothelial cells are protected against peroxynitrite toxicity by SeP.

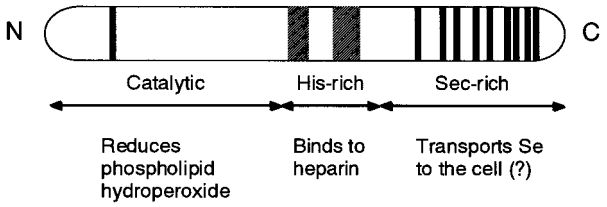

Fig. 2. Domain Structure of SeP and Its Function

The vertical line represents a Sec residue. The shadow box represents His-rich region.

CONCLUSION

The structure-function relationship of SeP is shown in Fig. 2. We propose that SeP is composed of two domains, the N-terminal domain which contains one Sec residue and the C-terminal domain containing multiple Sec residues, which is connected with a bridge containing two His-rich regions. The former displays enzymatic activity, reducing phospholipid hydroperoxide in the presence of thiol, while the latter may demonstrate Se carrier activity, delivering Se to cells. Thus, SeP has a unique protein structure, and is proposed to be a multi-functional protein. Further studies are required to prove the above hypothesis and to establish the physiological roles of this interesting protein.

REFERENCES

1) Stadtman T.C., *Annu. Rev. Biochem.*, **65**, 83–100 (1996).
2) Burk R.F., Hill K.E., *Annu. Rev. Nutr.*, **13**, 65–81 (1993).
3) Hatfield D., Diamond A., *Trends Genet.*, **9**, 69–70 (1993).
4) Mizutani T., Goto C., Totsuka T., *J. Health Sci.* **46**, 399–404 (2000).
5) Lescure A., Gautheret D., Fagegaltier D., Carbon P., Krol A., *J. Health Sci.*, **46**, 405–408 (2000).
6) Roveri A., Maiorino M., Nisii C., Ursini F., *Biochim. Biophys. Acta*, **1208**, 211–221 (1994).
7) Pushpa-Rekha T.R., Burdsall A.L., Oleksa L.M., Chisolm G.M., Driscoll D.M., *J. Biol. Chem.*, **270**, 26993–26999 (1995).

8) Thomas J.P., Maiorino M., Ursini F., Girotti A.W., *J. Biol. Chem.*, **265**, 454–461 (1990).
9) Nakagawa Y., Imai H., *J. Health Sci.,* **46**, 414–417 (2000).
10) Takahashi K., Avissar N., Whitin J. , Cohen H., *Arch. Biochem. Biophys.*, **256**, 677–686 (1987).
11) Yamamoto Y., Takahashi K., *Arch. Biochem. Biophys.*, **305**, 541–545 (1993).
12) Akasaka M., Mizoguchi J., Takahashi, K., *Nucleic Acids Res.*, **18**, 4619 (1990).
13) Esworthy R.S., Swiderek K.M., Ho Y.S., Chu F.F., *Biochim. Biophys. Acta*, **1381**, 213–226 (1998).
14) Davey J.C., Becker K.B., Schneider M.J., St Germain D.L., Galton V.A., *J. Biol. Chem.*, **270**, 26786–26789 (1995).
15) Tamura T., Stadtman T.C., *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 1006–1011 (1996).
16) Read R., Bellew T., Yang J.G., Hill K.E., Palmer I.S., Burk R.F., *J. Biol. Chem.*, **265**, 17899–17905 (1990).
17) Burk R.F., Hill K.E., *J. Nutr.*, **124**, 1891–1897 (1994).
18) Vendel S.C., Beilstein M.A., Yeh J.Y., Ream W., Whanger P.D., *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 8749–8753 (1995).
19) Burk R.F., *Proc. Soc. Exp. Biol. Med.*, **143**, 719–722 (1973).
20) Motsenbocker M.A., Tappel A.L., *Biochim. Biophys. Acta*, **719**, 147–153 (1982).
21) Akesson B., Bellew T., Burk R.F., *Biochim. Biophys. Acta*, **1204**, 243–249 (1994).
22) Yang J.G., Morrison-Plummer J., Burk R.F., *J. Biol. Chem.*, **262**, 13372–13375 (1987).
23) Hill K.E., Lloyd R.S., Yang J.G., Read R., Burk R.F., *J. Biol. Chem.*, **266**, 10050–10053 (1991).
24) Hill K.E., Lloyd R.S., Burk R.F., *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 537–541 (1993).
25) Saijoh K., Saito N., Lee M.J., Fujii M., Kobayashi T., Sumino K., *Brain. Res. Mol. Brain Res.*, **30**, 301–311 (1995).
26) Steinert P., Ahrens M., Gross G., Flohe L., *Biofactors*, **6**, 311–319 (1997).
27) Saito Y., Hayashi T., Tanaka A., Watanabe Y., Suzuki M., Saito E., Takahashi K., *J. Biol. Chem.*, **274**, 2866–2871 (1999).
28) Eberle B., Haas H.J., *J. Trace Elem. Med. Biol.*, **9**, 55–57 (1995).
29) Chittum H.S., Himeno S., Hill K.E. , Burk R.F., *Arch. Biochem. Biophys.*, **325**, 124–128 (1996).
30) Mostert V., Lombeck I., Abel J., *Arch. Biochem. Biophys.*, **357**, 326–330 (1998).
31) Himeno S., Chittum H.S., Burk R.F., *J. Biol. Chem.*, **271**, 15769–15775 (1996).
32) Motchnik P.A., Tappel A.L., *Biochim. Biophys. Acta*, **993**, 27–35 (1989).
33) Ursini F., Maiorino M., Gregolin C., *Biochim. Biophys. Acta*, **839**, 62–70 (1985).
34) Roveri A., Maiorino M., Ursini F., *Methods Enzymol.*, **233**, 202–212 (1994).
35) Bao Y., Chambers S.J., Williamson G., *Anal. Biochem.*, **224**, 395–399 (1995).
36) Burk R.F., Hill K.E., Awad J.A., Morrow J.D., Kato T., Cockell K.A., Lyons P.R., *Hepatology*, **21**, 561–569 (1995).
37) Burk R.F., Hill K.E., Awad J.A., Morrow J.D., Lyons P.R., *Lab. Invest.*, **72**, 723–730 (1995).
38) Christison J., Sies H., Stocker R., *Biochem. J.*, **304**, 341–345 (1994).
39) Burk R.F., Hill K.E., Boeglin M.E., Ebner F.F., Chittum H.S., *Histochem. Cell Biol.*, **108**, 11–15 (1997).
40) Gomez B., Jr., Tappel A.L., *Biochim. Biophys. Acta*, **979**, 20–26 (1989).
41) Burk R.F., Hill K.E., Read R., Bellew T., *Am. J. Physiol.*, **261**, E26–30 (1991).
42) Yan J., Barrett J.N., *J. Neurosci.*, **18**, 8682–8691 (1998).
43) McKenzie R.C., Rafferty T.S., Beckett G.J., *Immunol. today*, **19**, 342–345 (1998).
44) Baum M.K., Shor-Posner G., Lai S., Zhang G., Lai H., Fletcher M.A., Sauberlich H., Page J.B., *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.*, **15**, 370–374 (1997).
45) Arteel G.E., Mosterrt V., Oubrahin H., Briviba K., Abel J., Sies H., *Biol. Chem.*, **379**, 1201–1205 (1998).