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Selenoprotein W: a review

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Abstract. Purification of selenoprotein W (Se-W) from rat and monkey muscles was shown to exist in multiple forms: with or without reduced glutathione and/or a 41-Da moiety (identity still unknown). TGA is located at coding position 13 in Se-W complementary DNA (cDNA) from all five species studied (rats, mice, sheep, human and monkey). TGA is also the stop codon in the rodents and sheep cDNA, but TAA is the stop codon in primates. There is an 80% homology of the nucleotide sequence in the coding region among the five species of animals, and the predicted amino acid sequences are 83% identical (rodents identical and primates identical). Se-W levels are highest in muscle, heart and brain from sheep and primates, but very low in rodent hearts. Studies with tissue cultures of muscle and brain cells indicated that selenium influenced Se-W levels. Although the metabolic function of Se-W is unknown, preliminary data suggest that it has an antioxidant function.

Key words. Selenoprotein W; tissue distribution; rodents; primates; sheep; amino acid and nucleotide sequences.

Background

White muscle disease (WMD) was first shown to be a selenium-responsive myopathy at Oregon State University [1]. One of the primary lesions of WMD is calcification of skeletal and cardiac muscle [2]. Even with extensive research in this area, the reason for calcification in this disorder is still not known. The sarcoplasmic reticulum membranes from muscle of WMD animals have lost their ability to sequester calcium [3], but how selenium is involved in maintaining these membranes in a state to sequester the calcium still remains a mystery. Selenium is incorporated into these membranes as selenocysteine, and this selenoamino acid is suspected to be the metabolic active form.

Selenium was tested with WMD because in prior studies selenium was reported to prevent liver necrosis in rats fed a diet low in vitamin E and sulfur amino acids [4]. Until that time the only significance of selenium was thought to be its toxicity. This remarkable discovery was the beginning of the long and challenging effort to change the image of selenium. It is interesting that this same year in which selenium was shown to prevent liver necrosis in rats, the characteristics of a newly discovered enzyme, glutathione peroxidase (GPX), were reported [5]. However, it took one and a half decades to discover that GPX and selenium had a common feature, namely it was a selenoenzyme [6]. In our early work a significantly higher content of nonprotein sulfhydryls and reduced glutathione was found in muscle from WMD lambs, suggesting a relationship between selenium and sulfhydryls [7]. The discovery that GPX was a selenoenzyme started intensive research on selenium biochemistry, and a number of mammalian selenoproteins are now known, as discussed by others in this series of articles.

Tissue labeling

Radioactive selenium was used to label the proteins in WMD in selenium-supplemented lambs, and various tissues were removed and prepared for gel filtration. The results of these original studies were published as an abstract in 1969 [8], and more detailed reports were also published subsequently [9, 10]. The results of those labeling studies provided the basis to search for a selenoprotein now known as selenoprotein W (Se-W). As shown in figure 1, there is a low molecular weight selenium-containing peak present in hepatic cytosols from selenium-supplemented lambs which is missing or very low in WMD lambs [11]. Similar patterns were

found for skeletal muscle cytosols, but no differences between WMD and supplemented lambs were found with cytosols from liver, kidney or pancreas (and plasma). The restriction of these differences to hepatic and skeletal muscles was intriguing because these are the tissues that are affected in WMD.

Purification of Se-W

Since this low molecular weight selenium-containing protein was present in tissues from selenium-supplemented lambs but absent or very low in tissues from WMD animals, it was decided to purify this protein from the muscle of selenium-supplemented animals. Although initial studies were conducted with lambs [9, 12], later studies were performed with rats, mostly for convenience. After techniques were developed with rats, the properties of the ovine protein were investigated. It was of concern that this low molecular weight binding protein may be metallothionein with this element 'bound' to it, but the amino acid composition reported in 1972 on the semipurified preparation showed aspartate, glutamate, lysine, glycine and leucine as the predominant amino acids with a very low content of cysteine [12]. This quieted our fears that it was metallothionein, because this protein has a very high content of cysteine [13]. The demonstration that selenium was present in a semipurified preparation as selenocysteine raised our confidence that we were dealing with a selenoprotein [14], which is the same form of selenium at the active site of GPX [15].

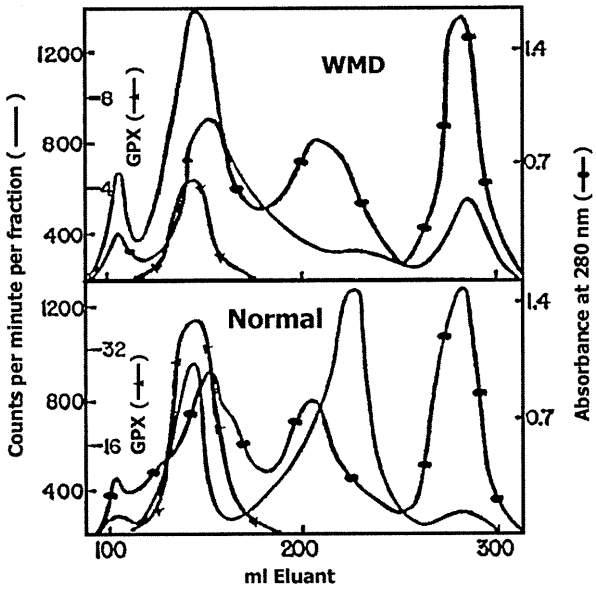

Figure 1. Gel filtration (Sepadex G-150) of cardiac cytosols from WMD and normal lambs injected with radioactive selenium.

There were two major obstacles we had to overcome in order to successfully purify Se-W. We labeled the animals with radioactive selenium and purified the protein of interest with respect to radioactivity. After several purification steps, the radioactivity disappeared, and it was thought that the radioactive selenium was removed from the protein. With this seemingly mysterious disappearance, it was referred to in our laboratory as the 'ghost' protein and is the reason it was initially named the G protein [16, 17]. Since there is now a family of proteins called G proteins that have no relationship to selenium [18], it was decided to change the name of our protein to Se-W (for white muscle disease) in order to avoid confusion [19]. It is now known that Se-W has an affinity for glass, and this was the reason for this mysterious disappearance of selenium [19a]. After this problem was recognized, significance progress was made in the purification of this selenoprotein.

The other obstacle was overcome when it was realized that multiple forms of this selenoprotein existed [19a]. As long as gel filtration steps were used, the radioactivity remained as one major peak, but when ion-exchange resins were used, multiple peaks were obtained. After the first 10 amino acid sequences were shown to be identical on the individual purified peaks, it was obvious that multiple forms of the same protein existed. Se-W was purified by ammonium sulfate fractionation, Sephadex G-50 gel filtration, cation-exchange chromatography on CM-Sephadex, and reverse-phase high-pressure liquid chromatography using C-18 Vydac columns [19a]. Four forms of the protein were separated by the cation exchange and reverse-phase chromatography steps. Molecular weights of the four proteins as determined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI) revealed masses of 9550, 9596, 9858 and 9898. The 9550 form does not contain any moieties; the 9596 form contains a 41-Da moiety, which is still unknown. The 9858 form contains reduced glutathione as the attached moiety [20]. The reduced glutathione can only be removed by dithiothreitol at elevated temperatures, suggesting that this protein must be denatured to remove this tripeptide. The highest molecular weight form contains both glutathione and the unknown 41-Da moiety.

Nucleotide and amino acid sequences

The successful amino acid sequence of most of the first 60 amino acids formed the basis for further work. Rat skeletal muscle Se-W complementary DNA (cDNA) was isolated and sequenced [21]. The isolation proce-

Table 1. Selenoprotein W deduced amino acid sequences in five species of animals.

	1											15				
Primate	Met	Ala	Leu	Ala	Val	Arg	Val	Val	Tyr	Cys	Gly	Ala	Sec	Gly	Tyr	Lys	Ser	Lys	Tyr	Leu
Rodent																	Pro			
Sheep				Val	Val												Pro			

																				30
Primate	Gln	Leu	Lys	Lys	Lys	Leu	Glu	Asp	Glu	Phe	Pro	Gly	Arg	Leu	Asp	Ile	Cys	Gly	Glu	Gly
Rodent				Glu				His					Cys							
Sheep												Ser								

			45																	60
Primate	Thr	Phe	Gln	Ala	Thr	Gly	Phe	Phe	Glu	Val	Met	Val	Ala	Gly	Lys	Leu	Ile	His	Ser	Lys	Lys
Rodent				Val						Thr							Val			
Sheep				Val						Phe							Val			

													75							
Primate	Lys	Gly	Asp	Gly	Tyr	Val	Asp	Thr	Glu	Ser	Lys	Phe	Leu	Lys	Leu	Val	Ala	Ala	Ile	Lys	Ala
Rodent	Arg												Arg				Thr				
Sheep	Gly																				

					88
Primate	Ala	Leu	Ala	Gln	Gly
Rodent				Cys	Gln
Sheep				Ala	

For the rodent and sheep proteins, only the amino acid residues that differ from the primate sequence are shown. The amino sequences for the rat and mice, and the monkey and human are identical.

dure involved the design of degenerate polymerase chain reaction (PCR) primers from reverse translation of a partial peptide sequence. A reverse transcription-coupled PCR product from rat muscle mRNA was used to screen a muscle cDNA library prepared from selenium-supplemented rats. The cDNA sequence confirmed the known protein primary sequence, including a selenocysteine residue encoded by TGA, and the residues identified needed to complete the protein sequence. mRNA-folding algorithms predict a stem-loop structure in the 3–untranslated region of the Se-W mRNA that resembles selenocysteine insertion sequence (SECIS) element identified in other selenocysteine coding cDNAs.

Mouse, sheep and monkey skeletal muscle cDNA libraries were constructed, and a human skeletal muscle cDNA was obtained from a commercial source [22]. Probes were prepared from a cloned rat Se-W cDNA by PCR and used to screen human, monkey, sheep and mouse cDNA libraries. In all species TGA is located at coding position 13. TGA is also the stop codon in the rodents and sheep, but TAA is the stop codon in primates. The sheep is similar to the primates in that the open reading frame is one codon shorter than the rodents. There is about 80% homology of the nucleotide sequence in the coding region among the five species.

The predicted amino acid sequences of the five species are 83% identical (table 1). The complete amino acid sequence for human Se-W is shown and only those that are different in the other species presented. The deduced amino acid sequences for Se-W indicate that the rat and mouse sequences, and the human and monkey sequences are identical. The sheep and primate proteins are shorter by one amino acid than the rodent proteins. There are differences in the five-peptide sequences in only 15 of 88 (17%) amino acid positions. The selenocysteine residue in position 13 and the cysteine residues at positions 10 and 37 are retained in the proteins from all five species. These selenhydryl and sulfhydryl residues are postulated to be essential for either catalysis or maintenance of tertiary structure. The cysteine residues at positions 33 and 87 of the rodent proteins are not retained in the sheep or primate proteins and are assumed to be not essential to the function of the protein.

In addition to a highly conserved coding sequence, the section of the 3′-untranslated region which contains the SECIS element is also highly conserved [22]. It contains a predicted stem loop structure similar to those reported in other selenoprotein cDNAs, such as for cGPX, 5'-deiodinase and selenoprotein P [23–26]. In prokaryotes, stem loops mediating selenocysteine incorporation are

located immediately downstream of the UGA selenocysteine codon, which is in contrast to that required for eukaryotes [25]. A greater distance is required for eukaryotes. The sequence motif occurs in the 5' arm of each putative Se-W SECIS element mRNA, which is a slight deviation from the standard SECIS structure. Aside from the nonprimate Se-W mRNAs, the use of UGA for both termination and selenocysteine incorporation in the same mRNA has been reported only for GPX of *Schistosoma mansoni* [27].

The transcription start site for the rat gene was tentatively identified as either 17 or 16 bases upstream of the 5' end of the longest cDNA [28]. This corresponds to bases 2141 and 2142 of the genomic sequence. There is an apparent TATA box 150 bp earlier (positions 1985–1989). The nuclease protection assays did not indicate the presence of any mRNA originating upstream of position 2141. The Se-W gene for the rat contains five introns and six exons. The coding sequence and SECIS element are much more highly conserved than other regions of the gene.

Antibody production

Since it was not feasible to purify enough Se-W to raise antibodies, an alternate approach was used. From the amino acid sequence of rat Se-W [22], the hydrophilicity was calculated [29]. There are two major hydrophilic regions in Se-W, which are amino acids 13–31 for peptide one and amino acids 51 to 69 for peptide two. Rabbits were immunized with the two synthetic peptides [29], and the resulting polyclonal antibodies from peptide one are routinely used in Western blots to quantitate the Se-W levels in various tissues because the titer for peptide two was not as good as that for peptide one and thus was not used. The antibody raised to the synthetic peptide could be used to detect Se-W in tissues from mice, rats, sheep, cattle, rabbits and guinea pigs, but would not detect this selenoprotein in primate tissues.

Using the same procedures as described for the rat peptide, polyclonal antibodies were raised against a primate peptide from amino acids 13 to 31, but unfortunately for unknown reasons these antibodies would not function in Western blots. Therefore, another approach was used. The human Se-W coding region with the selenocysteine codon (TGA) changed to a cysteine codon (TGT) was fused to six histidine codons (at its 3′ end), cloned into a prokaryotic expression vector (pTrc99a) and the corresponding mutated Se-W expressed in bacteria [30]. The protein was purified by Ni-NTA agarose column and reverse-phase HPLC. Polyclonal antibodies raised from this mutated protein in rabbits were used in Western blots to determine tissue distribution of Se-W in both monkey and human tissues.

Se-W was purified from monkey skeletal muscle to investigate the binding of glutathione. Since the primate protein contains only two cysteines but the rodent protein has four cysteines, it was deemed more efficient to identify the binding site with the primate protein using peptide-mapping studies. Primate Se-W was monitored during purification by slot blots using the antibodies raised against the mutated protein [31]. Similar to the rodents, MALDI revealed that the proteins also existed in multiple forms. The 9635-Da form of the protein was shown to contain bound glutathione that could be released by reduction with dithiothreitol at an elevated temperature. MALDI peptide mapping with endoproteinase Glu-C suggested that glutathione is bound to the 37th amino acid, a cysteine.

Tissue distribution

In our initial studies,Western blots of tissues from rats fed a commercial chow revealed the presence of Se-W in muscle, spleen, testis and brain [29]. Based on densitometic readings, this protein was highest in the muscle and brain and lower in spleen and testis. It was not detected in liver, kidney, intestinal mucosa, lungs, heart, plasma and erythrocytes. Western blots indicated the presence of this selenoprotein in muscles of rabbits, mice, guinea pigs, sheep and cattle. In a second study rats were fed a purified diet with no additional selenium or this diet with 0.1 and 4.0 mg selenium per kilogram diet. Se-W was undetectable in skeletal muscle of rats fed the basal diet, detectable in muscle of those fed the 0.1 mg selenium per kilogram diet, but much higher in muscle of rats fed the highest level of selenium. This indicates that the levels of this selenoprotein are dependent upon the selenium intake. In a more thorough study, the tissue distribution of Se-W was found to be more widely spread than once thought [32]. The influence of deficient, adequate (0.1 mg/kg) or excessive (4.0 mg/kg) levels of dietary selenium on Se-W content was investigated in 28 rat tissues. Se-W was nondetectable in liver, thyroid, pancreas, pituitary and eyes regardless of the level of selenium fed. Se-W was not detected in heart, lungs, prostate, esophagus, small intestine, tongue, skin, diaphragm or skeletal muscle from deficient rats but was present in these tissues when the two higher levels of selenium were fed. In other tissues such as the kidney and seminal vesicles, Se-W was detected only in rats fed the diet with excess selenium.

The effects of eight dietary levels of selenium (deficient to 4.0 mg Se per kilogram) were investigated on the response of Se-W in brain, skeletal muscle, spleen and testis [33]. Se-W levels in muscle did not increase until

0.06 mg selenium per kilogram diet were fed, but a very marked increase occurred with 1.0 mg selenium per kilogram diet where no further increases occurred with higher levels. There was a linear increase of Se-W in brain and spleen with dietary selenium intake up to 0.1 mg selenium per kilogram where a plateau was reached. The testis showed a different pattern in that a marked increase occurred when only 0.01 mg selenium per kilogram diet was fed where an inflection was reached. Except for the muscle, GPX activities reached a plateau in all tissues when diets containing 0.06–0.1 mg selenium per kilogram were fed. The results indicate that in rats, the regulation of Se-W by selenium is different for various tissues and differs from that for GPX.

The influence of selenium on Se-W was examined in sheep fed either a low selenium diet (0.02 mg Se/kg) or a diet supplemented with selenium [34]. Muscle biopsies revealed that Se-W increased over initial levels at 3.5 weeks and afterwards, whereas in sheep consuming the low selenium diet this selenoprotein declined significantly. At the end of the experiment (10.5 weeks) the sheep were killed, and Se-W was found to be significantly lower in skeletal muscle, heart, tongue, lungs, spleen, kidney and liver from the deficient sheep than the supplemented ones. Interestingly, there were no differences in the content of this selenoprotein in brain between deficient and supplemented sheep. Since there was a significant difference in GPX activity and selenium content in brain from deficient versus supplemented sheep, this indicates preferential retention of Se-W in the brain. The Se-W content in heart of the supplemented sheep was similar to the content in muscle, which is different from rats where cardiac levels were very low. It is interesting that the tissues with the highest content of Se-W in supplemented sheep are the ones affected in WMD.

Using the antibodies raised against the mutant protein, Se-W levels were determined in rhesus monkey and human tissues [30]. Se-W was present at highest amounts in skeletal muscle and heart, and lowest in the liver. In contrast to the rat, Se-W was found in all tissues examined (muscle, tongue, heart, brain, spleen, kidney, liver, testis and ovary). Se-W was not detected in liver of rats fed any level of selenium and that in kidney was detected only when excess selenium was fed [32]. There was no correlation between Se-W levels in various tissues and GPX activity or selenium content in primate tissues. Thus, the relative tissue distribution of Se-W in sheep and primates is similar but different from rats with respect to the heart.

In a subsequent study, selenium deficiency resulted in undetectable Se-W levels in heart and muscle from sheep and rats, but the content in brain was unaffected by selenium status [35]. Selenium-depleted and -repleted second-generation rats showed that the expression of Se-W in cortex and cerebellum was not significantly affected by dietary selenium but that selenium increased its levels in thalamus [36]. To further illustrate the different responses in various regions of the brain and to provide comparative results, GPX activities were determined [36]. Selenium had no significant effect upon GPX in cortex but significantly increased the activity in cerebellum and thalamus. Interestingly, GPX activity was significantly higher at 2 weeks and afterward than initially in thalamus, higher at 6 weeks than at other times in cerebellum and higher at 6 and 10 weeks afterwards in cortex from rats fed selenium-deficient diet. The reasons for these observations in deficient rat brain regions are not known, but indicate that selenium metabolism in this organ is poorly understood. Therefore, these results indicate that different regions of the brain should be examined to obtain trends rather than using a composite of the whole brain.

In order to obtain information on the effects of selenium on human tissue levels of Se-W, tissues from spontaneously aborted fetuses were examined from women living in selenium-deficient (Xichang), adequate (Shijiazuang) or excessive (Enhi) regions of China [37]. Tissues from six selenium-adequate adults killed in accidents were also examined. GPX activity was lowest in muscle and brain from low-selenium fetuses but most selenium-adequate adult and fetal values were very similar. Whole tissue selenium correlated with regional selenium status, with the low-selenium fetuses having the lowest tissue selenium content, especially in the muscle, brain and heart. Se-W content in muscle was usually higher in selenium-adequate adults than fetuses, and fetal muscle and heart Se-W closely reflected selenium status. Of interest is the tendency for adult muscle to have higher levels of Se-W but lower selenium content than fetal tissues.

One way selenium regulates Se-W levels is through the alteration of mRNAs. Northern blots indicated that mRNA increased in muscle four- [21] to six- [33] fold in rats fed excess selenium as compared with deficient rats. Northern blots indicated that mRNA levels were highest in monkey muscle and heart (2- to 2.5-fold greater than liver), which is similar to the pattern found with a human multiple tissue Northern blot [30]. Since Se-W protein levels correlate with Se-W mRNA, the relative tissue distribution of this selenoprotein in humans and monkeys appear to be similar.

Cell cultures

Studies with different brain cell cultures indicated that selenium is metabolized differently by various brain cell

types [35]. GPX activity decreased at a faster rate than Se-W levels with neuroblastoma cells, whereas the Se-W content decreased at a faster rate than GPX activity in glial cells when selenium was removed from the media. In contrast, L8 rat muscle cells showed a different pattern in that GPX activity and Se-W levels deceased at about the same rate when selenium was removed from the media.

Three different chemical forms of selenium resulted in various increases of Se-W levels and GPX activity in L8 myotubes [38]. Selenite was more effective than selenocysteine for increasing both Se-W levels and GPX activity, but selenomethionine was less available. Northern blot data indicated that the expression of Se-W mRNA increased significantly when L8 myotubes were cultured with selenium.

Northern blots indicated that there were no significant changes in Se-W mRNA levels during cell proliferation and differentiation [39] in further studies with cultured L8 rat muscle cells. However, reduction of selenium concentration in the medium decreased the Se-W mRNA levels in myoblasts. Nuclear run-on experiments with isolated L8 nuclei showed the same rate of Se-W mRNA synthesis in cells cultured in either low-selenium or selenium-supplemented medium, suggesting that the transcription rate of Se-W gene is independent of selenium. Measurement of Se-W mRNA half-lives in myoblasts treated with the transcription inhibitor, α-amanitin, showed that Se-W mRNA levels decreased over time with an estimated half-life of 57 h for cells grown in low-selenium medium. Selenium treatment increased the Se-W mRNA half-life twofold, suggesting that selenium stabilizes Se-W mRNA but has no effect on transcription.

Gender differences

The relative tissue distribution of Se-W was similar in male and female rats. However, the Se-W content in muscle and skin (and mRNA levels in skin) was significantly higher in female than male rats fed a commercial diet [40]. In second-generation selenium-depleted rats fed a torula yeast-based diet, gender differences were not observed [36]. Whether this lack of difference is due to feeding the purified diet or due to depletion of selenium for two generations is not known. Se-W was high in testes of male rats but very low in ovaries from female rats. Gender differences were also noted in monkeys. Se-W was higher in muscle and heart from female monkeys than in males, but this difference was statistically different only in the muscle from females versus males [30].

What is the metabolic function of Se-W?

Se-W is highest in muscle and heart of supplemented lambs, which are the tissues affected in WMD. Thus, it has been speculated that Se-W is involved in muscle and cardiac metabolism [21, 29, 34]. Most of the selenoenzymes identified thus far are involved in redox reactions, and it is reasonable to suspect that Se-W may have antioxidant functions. This possibility was strengthened when it was demonstrated that glutathione was a bound moiety to the major species of Se-W [20, 31]. A study was undertaken to elucidate the possible antioxidant function of Se-W [41, 42], employing rat glial cells. Using inducible LacSwitch expression vectors, Se-W and its mRNA were respectively overexpressed 22- and 11-fold higher than control in these cells. Se-W expression was also reduced to 20% of the control cells. GPX activity and reduced glutathione levels were not significantly different between induced and control cells. There was a greater survival rate of overexpressed cells when incubated with 2,2′-azobis (2-amidinopropane) dihydrochloride than control cells, suggesting Se-W does have an antioxidant function.

Identification of the 41-Da moiety bound to Se-W may give some clues on its function. Our data suggest that it is bound to an amino acid beyond number 48 [31]. Except for three amino acids, Se-W contains a motif [60–71] that strongly resembles calcium-binding domains found in such calcium-binding proteins such as calmodulin [43]. Since selenium deficiency results in soft tissue calcification, it is attractive to speculate that this low molecular weight moiety is calcium. However, unpublished data have been negative for calcium as this moiety, but possibly the right conditions have not been used. If Se-W could be shown to be involved in the regulation of calcium metabolism, it would help to explain the link between selenium and this element.

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