Regulation of selenoprotein gene expression and thyroid hormone metabolism


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Introduction

Adequate dietary intakes of selenium are essential for normal health in man and animals. When insufficient selenium is available, a wide range of clinical conditions and diseases can occur, consistent with several biochemical functions of the trace element [1]. These functions are associated with selenoproteins, estimated to number between 20 and 100 in mammalian cells. In vitro labelling of rats with $^{75}$Se and subsequent separation of proteins by SDS/PAGE under reducing conditions has detected 20–30 selenoproteins [2,3]: reduction and boiling of proteins before electrophoresis removes selenium that is non-specifically bound but not that bound as the amino acid selenocysteine. In addition, Burk and Hill [3] have estimated that, since there are 10 minor proteins for each abundant protein in the genome and since 10–15 abundant selenoproteins have been discovered, the existence of 100 selenoproteins is not unreasonable. Most known selenoenzymes probably use selenocysteine as a redox catalyst since at physiological pH, the amino acid -SeH group is more likely to be ionized than an equivalent -SH group. This allows selenium to be a more active catalyst than sulphur. Cytosolic glutathione peroxidase, the first selenoprotein to be identified, was originally thought to have a 'hyperactive sulphur' at the active site [4]. As well as in selenoproteins, selenium has also been detected in some bases in tRNA from both prokaryotes and eukaryotes; however, the biological function of this selenium is not known [5].

Selenium function is regulated through selenoprotein gene expression and this can be controlled in two important ways: by the availability of dietary selenium for incorporation into selenocysteine and by metabolic changes that influence biochemical pathways in which selenoproteins play a crucial role. For example, factors such as oxidative stress can increase selenium-containing glutathione peroxidase activities, and hyperthyroidism will decrease selenium-contain-

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ing type-1 thyroid hormone deiodinase activity [6–8]. This review concentrates on the influence of selenium supply and possible mechanisms whereby this can control the expression of selenoenzymes. This is a complex process with the large numbers of selenoproteins that exist and the requirement to preserve the most important activities when supplies of selenium are limiting. Thus in selenium deficiency some selenoenzyme activities can increase whereas others will decrease to less than 1% of control. The relative preservation of the different selenoproteins in deficiency is specific to different organs and is most efficient in tissues with an endocrine function and in the brain [9].

**Selenoproteins**

In animals nine selenoproteins have been characterized by cDNA cloning and full or partial purification (Table 1). The first selenoprotein to be identified was cytosolic glutathione peroxidase. This is the major selenoprotein found in mammalian cells accounting for approx. 60% of total selenium in selenium-adequate animals. However, in selenium-deficient rats, hepatic cytosolic glutathione peroxidase activity can fall to less than 1% of control levels without any ill effects. This has led to the hypothesis that cytosolic glutathione peroxidase may be a buffer to provide selenium for other selenoproteins with functions that are more important for maintaining normal health [32].

A glutathione peroxidase that is very similar to the cytosolic enzyme has been identified by cloning and its mRNA is expressed mainly in gastrointestinal tissue [15]. Thus this glutathione peroxidase is thought to have an antioxidant function specific to intestinal tissue. Phospholipid hydroperoxide glutathione peroxidase is a monomeric selenoprotein that is associated with cell membranes and is involved, along with vitamin E, in the protection of membranes against oxidative damage [33]. A fourth (extracellular) glutathione peroxidase is a glycoprotein that is synthesized mainly in the kidney and is thought to protect extracellular spaces from oxidative damage caused by peroxides and subsequent oxygen-based radical formation [34]. Other selenoproteins that have been postulated to have antioxidant functions are selenoprotein P and selenoprotein W [20,35]. Selenoprotein P accounts for up to 70–80% of plasma selenium and its gene has ten TGA codons in the open reading frame for incorporation of selenocysteine. Selenoprotein W is expressed in skeletal muscle and loss of this protein is associated with

<table>
<thead>
<tr>
<th>Protein</th>
<th>References</th>
<th>Selenocysteine?</th>
<th>Tissue distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosolic glutathione peroxidase</td>
<td>10, 11</td>
<td>Yes (1)</td>
<td>All*</td>
</tr>
<tr>
<td>Phospholipid hydroperoxide glutathione peroxidase</td>
<td>12–14</td>
<td>Yes (1)</td>
<td>All*</td>
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<td>Gastrointestinal (GI) glutathione peroxidase</td>
<td>15</td>
<td>Yes (1)</td>
<td>GI tract</td>
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<tr>
<td>Extracellular glutathione peroxidase</td>
<td>16, 17</td>
<td>Yes (1)</td>
<td>Plasma†</td>
</tr>
<tr>
<td>Selenoprotein P</td>
<td>18, 19</td>
<td>Yes (10)</td>
<td>Plasma†</td>
</tr>
<tr>
<td>Selenoprotein W</td>
<td>20, 21</td>
<td>Yes (1)</td>
<td>Muscle</td>
</tr>
<tr>
<td>Type-1 iodothyronine deiodinase</td>
<td>22–24</td>
<td>Yes (1)</td>
<td>Liver, kidney, thyroid, BAT‡</td>
</tr>
<tr>
<td>Type-2 iodothyronine deiodinase</td>
<td>1, 8, 25</td>
<td>Yes (1)</td>
<td>CNS, pituitary, BAT‡</td>
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<tr>
<td>Type-3 iodothyronine deiodinase</td>
<td>26</td>
<td>Yes (1)</td>
<td>CNS, placenta</td>
</tr>
<tr>
<td>Sperm capsule selenoprotein</td>
<td>27</td>
<td>Yes (3)</td>
<td>Sperm tail</td>
</tr>
<tr>
<td>Selenium-binding proteins (58, 56, 14 kDa)</td>
<td>28, 29</td>
<td>No</td>
<td>All*</td>
</tr>
</tbody>
</table>

*Although these proteins are found in all tissues, their abundance may vary 10–20-fold.
†These proteins are found in plasma and extracellular space, although their mRNAs are detected in specific organs [17, 18].
‡The distribution of type-1 and type-2 iodothyronine deiodinases in brown adipose tissue (BAT) varies between animal species [31].§Type-2 iodothyronine deiodinase has only been cloned from amphibian tissue, showing that it contains selenium. Indirect evidence that mammalian type-2 iodothyronine deiodinase is a selenoprotein is, as yet, contradictory [1, 4, 25, 30].
myopathy in selenium deficiency [20]. Although it has been hypothesized to have an antioxidant function its true function remains to be identified.

Type-1, type-2 and type-3 iodothyronine deiodinases which are involved in thyroid hormone metabolism are a 'non-antioxidant' class of selenoprotein [8,22,23,25,26,36,37]. Despite having selenocysteine at their active sites, the enzymes have very different sensitivities to inhibitors such as propylthiouracil and gold-thioglucose [25,26]. A selenoprotein specific to sperm capsule has been cloned and shown to have three in-frame TGA codons. This protein may have a structural role in the sperm tail. Prolonged selenium deficiency causes abnormal sperm formation and infertility in male rats [27]. In addition to these selenoproteins which have been cloned, there are at least three selenium-binding proteins. The form of selenium in these proteins is not selenocysteine and has not been identified; however, it remains attached during SDS/PAGE [28]. The functions of these binding proteins are not clear although they may mediate some anti-cancer effects of selenium [28,29].

Synthesis of selenoproteins

The incorporation of selenocysteine into proteins is well characterized for bacterial systems. Four gene products are involved, including a specific tRNA which binds serine which is subsequently converted through selenophosphate into selenocysteine [38,39]. Synthesis of selenocysteine in mammalian proteins is less well characterized [40], although the human selenophosphate synthetase has now been cloned [41]. The incorporation of selenium into selenocysteine is a potential site for the regulation of selenoprotein expression, particularly since the process may require cofactors such as vitamin Bi [40]. Thus the dietary availability of a potential cofactor could modulate the overall expression of selenoproteins but not specific selenoproteins.

Selenocysteine in proteins is encoded by the TGA triplet which is normally a stop codon [11]. The reading of this codon as selenocysteine requires stem-loop structures in the 3'-untranslated regions of the selenoprotein genes [24,42]. These stem-loop structures vary between the selenoproteins but despite this, transfection studies with constructs made with cytosolic glutathione peroxidase 3'-untranslated region and type-1 deiodinase-coding region show that these chimaeric transcripts can still be translated to express deiodinase activity [24]. The structure of the stem-loop may provide a mechanism whereby selenium is incorporated into the different selenoproteins with variable efficiency. For instance, the 3'-untranslated region of selenoprotein P allows more efficient production of deiodinase in Xenopus laevis oocytes than does the normal deiodinase untranslated regions [42]. Work on the identification of essential structural elements of the 3'-untranslated regions of selenoprotein genes has been recently reviewed [42,43].

Selenoprotein gene expression

The availability of cDNAs for selenoproteins has allowed the study of mRNA levels and the relative importance of transcriptional and post-transcriptional control in the regulation of selenoprotein expression. Initial studies showed decreased of 80–90% in cytosolic glutathione peroxidase mRNA in liver from selenium-deficient animals [2,32]. Coupled with lower liver selenium levels, these changes in mRNA can account for hepatic cytosolic glutathione peroxidase activity of less than 1% of control in selenium deficiency. More recent work has demonstrated that hepatic phospholipid hydroperoxide glutathione peroxidase mRNA levels are unaffected by selenium deficiency, whereas selenoprotein P mRNA decreases but is better retained than type-I deiodinase mRNA which in turn is better maintained than cytosolic glutathione peroxidase mRNA [32,44,45]. Nuclear transcription assays with hepatic nuclei show that selenium deficiency does not affect transcription of the cytosolic glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase or type-I deiodinase genes [32,45]. As there is no effect of selenium deficiency on the transcription of the genes for these proteins, the different stabilities of their mRNAs would account for the variation in their expression in selenium deficiency [32,44,45]. Total hepatic phospholipid hydroperoxide mRNA levels are unchanged by selenium deficiency, enzyme activity falls and enrichment of the mRNA in polysomes is decreased, consistent with reduced translation. The decreased translation of selenoprotein mRNA may in some cases (e.g. cytosolic glutathione peroxidase mRNA) be associated with mRNA degradation. This hypothesis is at present being investigated using a cell culture model [46] in which it is possible to estimate mRNA degradation in conditions of selenium adequacy and deficiency.
Although differential mRNA stability may regulate selenoprotein synthesis in the liver, additional factors may be involved in other organs and recent studies have highlighted the differential control of selenoprotein expression in various tissues [45]. In selenium deficiency, endocrine organs such as the thyroid retain selenium more efficiently than the liver [9]. In contrast with the liver, thyroidal type-1 deiodinase activity and mRNA increase in selenium-deficient rats. In addition, phospholipid hydroperoxide glutathione peroxidase activity is unchanged and its mRNA increases in selenium-deficient thyroid. Cytosolic glutathione peroxidase activity and mRNA increase in selenium-deficient rats. In addition, phospholipid hydroperoxide glutathione peroxidase expression is also under the control of trophic hormones in rat testis [49].

**Selenium and thyroid hormones**

Selenium plays an important role in thyroid hormone metabolism as an essential component of the three deiodinases, which regulate interconversion of active and inactive forms of iodothyronines. Extracellular glutathione peroxidase may also control the levels of H₂O₂ necessary for activation of iodine for hormone synthesis in the thyroid gland [50]. Dietary selenium deficiency decreases expression of hepatic and renal type-1 iodothyronine deiodinase and impairs thyroid hormone metabolism despite compensatory increases in thyroidal type-1 deiodinase [1,4,36,37,45]. Type-2 and type-3 deiodinases are less sensitive to selenium deficiency, indicating that their functions may be more essential than those of type-1 deiodinase. The importance of deiodinase in thyroid and brain is emphasized in iodine-deficient rats where type-1 and type-2 activities are induced to compensate for lower thyroid hormone synthesis. In rat thyroid, induction of type-1 deiodinase is associated with 3-5-fold increases in its mRNA. Surprisingly a concurrent selenium deficiency further increases the induction of selenium-containing deiodinase activities in brain and thyroid above that observed in iodine deficiency (J. H. Mitchell, F. Nicol, G. J. Beckett and J. R. Arthur, unpublished work).

The continuing characterization of 'new' selenoproteins which have unique organ distributions and regulation by selenium availability emphasize that complex mechanisms exist to control their expression. Varying mRNA stabilities can account for some of this control by selenium availability, particularly in the liver. However, when selenium supply is limited in animals, the preferential synthesis of particular selenoproteins in brain and endocrine organs indicates that more complex mechanisms are required to regulate the process.

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