Symposium 2 Newer aspects of micronutrients in at risk groups

New metabolic roles for selenium

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Adequate supplies of the micronutrient Se are required for normal health in both man and animals. Since the recognition of the essentiality of Se, decreased dietary Se supplies have been associated with a large number of clinical conditions. Additionally, Se supplementation of rodents has a beneficial role in the prevention of certain chemically-induced cancers. These findings have been the subject of a number of recent publications and are summarized in Table 1.

The first functional selenoprotein to be identified was cytosolic glutathione peroxidase (cGSHPx; Rotruck *et al.* 1973). Despite the recognition that a number of proteins could be specifically-labelled with ^{75}Se, for several years cGSHPx remained the only identified, functional selenoprotein (Behne *et al.* 1988; Evenson & Sunde, 1988; Bansal *et al.* 1991). Consequently attempts were made to explain the role of Se in the prevention of many apparently unrelated diseases as antioxidant effects of cGSHPx. Also, since cGSHPx in blood and tissues reflects dietary Se intake the enzyme activity has been used as an index of nutritional Se status (Hoekstra, 1975; Combs & Combs, 1986).

In recent years, however, several more selenoproteins have been characterized either by purification and sequencing of the protein and/or by cloning and sequencing of cDNAs. The purpose of the present short review is to describe some of these newly identified selenoproteins and how they may function in conferring the nutritional essentiality of Se.

Table 1. *Some conditions and functional changes associated with selenium*

1. Nutritional myopathy and other deficiency diseases in farm animals
2. Cardiomyopathy and other deficiency diseases in humans (Keshan disease and Kashin Beck disease)
3. Thyroid hormone and I metabolism
4. Deficiency caused by TPN
5. Effects on the immune response?
6. Anti-cancer effects of supplementation?
7. Relationships to CHD etc.?
8. Changes in mood and well-being?

For further details see: Boyne & Arthur, 1986; Combs & Combs, 1986; Arthur & Beckett, 1989; Bansal *et al.* 1990, 1991; Benton & Cook, 1990, 1991; Turner & Finch, 1990, 1991; Vanderpas *et al.* 1990, 1993; Arthur, 1991; Arthur *et al.* 1993; Corvilain *et al.* 1993; Lanfear *et al.* 1993; Ip & Lisk, 1994.

CHD, coronary heart disease; TPN, total parenteral nutrition.

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Table 2. *Selenoproteins and selenium-binding proteins which have been purified and/or cloned*

1. GSH peroxidase: (a) Cytosolic, RBC
(b) Plasma
(c) Phospholipidhydroperoxide
(d) Gastrointestinal
2. Selenoprotein P
3. Iodothyronine 5'-deiodinase
4. Sperm capsule selenoprotein
5. Selenoprotein W
6. 58, 56 and 14 kDa Se-binding proteins

For further details see: Rotruck *et al.* 1973; Takahashi *et al.* 1987, 1990; Yang *et al.* 1987; Avissar *et al.* 1989, 1994*a*; Arthur *et al.* 1990*a*,*b*, 1991, 1993; Berry *et al.* 1991; Hill *et al.* 1991; Karimpour *et al.* 1992; Burk & Hill, 1993; Lanfear *et al.* 1993; Sinha *et al.* 1993; Sunde *et al.* 1993; Vendeland *et al.* 1993; Akesson *et al.* 1994; BrigeliusFlohe *et al.* 1994.

GSH, glutathione; RBC, erythrocyte.

SELENOPROTEINS

Studies using *in vivo* labelling with ^{75}Se have shown that there may be up to thirty proteins which retain Se during subsequent purification and separation using sodium dodecylsulphate (SDS) polyacrylamide-gel electrophoresis. The ability of Se to remain bound at 100° under reducing conditions is taken as evidence of covalent or very strong bonds between trace element and protein rather than a non-specific interaction (Behne *et al.* 1988; Evenson & Sunde, 1988; Sunde, 1990, 1994). Ten of these ^{75}Se-containing proteins have been further characterized, eight of which contain Se as selenocysteine. The form of Se in Se-binding proteins is not known (Table 2). The range of selenoproteins now identified is consistent with multiple biochemical functions for Se and these are discussed in the rest of the present review.

GLUTATHIONE PEROXIDASES

The discovery that there are four distinct glutathione peroxidases may go some way to explain the involvement of Se deficiency in the pathogenesis of apparently unrelated clinical conditions. The glutathione peroxidases function in different subcellular compartments and each of the four are impaired by Se deficiency to different degrees. Thus, depending on the sensitivity of each GSHPx to Se supply, loss of activity from a particular tissue or cell compartment could cause a specific organ-related disease (Arthur *et al.* 1987*a*).

Cytosolic glutathione peroxidase

cGSHPx was the first well characterized biochemical function for Se (Rotruck *et al.* 1973). Since cGSHPx can metabolize H_2O_2 and lipid hydroperoxides it is thought to be a component of cell antioxidant systems (Hoekstra, 1975; Sunde, 1994). However, many

pathophysiological consequences of Se deficiency are not inextricably linked to changes in cGSHPx activity, and in Se-deficient rats hepatic cGSHPx activity may fall to <1% of control values without any obvious adverse clinical effects (Reiter & Wendel, 1983, 1984, 1985; Arthur *et al.* 1987*a*,*b*; Burk, 1989). These observations led to proposals that there were 'non-glutathione peroxidase' functions of Se and that the enzyme may represent a storage form of the trace element (Burk, 1991; Sunde, 1994). However, under specific circumstances cGSHPx can have an antioxidant function. Transgenic cells over-expressing cGSHPx activity are more resistant to peroxide or drug challenge than wild-type cells and the ability of mouse neutrophils to kill ingested *Candida albicans* correlates with cGSHPx activity in the neutrophils over a 'normal' range of Se status (Arthur *et al.* 1986; Doroshaw *et al.* 1991; Mirault *et al.* 1991). Thus, cGSHPx may only have antioxidant functions under conditions where relatively large amounts of H_2O_2 or lipid hydroperoxides are produced in the cell cytosol. Current evidence, therefore, indicates that cGSHPx has both a Se storage and an antioxidant function.

Plasma glutathione peroxidase (plGSHPx)

plGSHPx, also called extracellular GSHPx, is distinct from cGSHPx both in structure and site of function. Antibodies to purified plGSHPx will not cross react with cGSHPx (Takahashi & Cohen, 1986; Avissar *et al.* 1991). Human and rat plGSHPx have been cloned, and hybridization studies indicate that the kidney and lung are the major sites of synthesis (Takahashi *et al.* 1990; Yoshimura *et al.* 1991; Avissar *et al.* 1994*a*,*b*). This is supported by studies which show that anephric patients have very low plGSHPx activities with apparently normal plasma Se concentrations. plGSHPx activities are returned to normal after renal transplantation of these patients, without any change in plasma Se concentrations (Avissar *et al.* 1994*b*). More detailed hybridization studies showed that plGSHPx mRNA occurred in the proximal tubular epithelial cells and the parietal epithelial cells of Bowman's capsule (Avissar *et al.* 1994*b*). Despite the detailed knowledge of plGSHPx structure and synthesis, its function is not known. The glutathione (GSH) substrate for plGSHPx occurs in very low concentrations in the plasma which has led to the suggestion that the enzyme may function other than as a glutathione peroxidase. The concentrations of GSH in the kidney would allow the enzyme to act as a peroxidase and, thus, it may have a specific function in the renal proximal tubules. However, plGSHPx cDNA has a signal sequence and the enzyme is glycosylated, indicating that it is a secretory protein consistent with a function in the extracellular space (Takahashi *et al.* 1990; Avissar *et al.* 1994*b*).

Gastrointestinal glutathione peroxidase (giGSHPx)

giGSHPx has been identified by expression of a cDNA isolated from human hepatoma cells. Antibodies to giGSHPx do not cross react with either cGSHPx or plGSHPx; however, the properties and the structure of giGSHPx are very similar to those of cGSHPx. mRNA for giGSHPx is found in human liver and colon but not in other tissues; in rats the mRNA is detected only in the gastrointestinal tract. The function of this form of glutathione peroxidase has yet to be established but its location suggests a role in protecting against adverse effects of ingested hydroperoxides (Chu *et al.* 1993).

Phospholipid hydroperoxide glutathione peroxidase (PGSHPx)

PGSHPx is different from the other glutathione peroxidases in that it is a monomer of approximately 20 kDa, it is less specific with regard to GSH as its reducing substrate, and it is closely associated with intracellular membranes (Ursini *et al.* 1985; Thomas *et al.* 1990; Maiorino *et al.* 1991*a*). Unlike cGSHPx, PGSHPx will react with phospholipid hydroperoxides which are likely to occur in cell membranes undergoing oxidative stress (Maiorino *et al.* 1991*b*). PGSHPx activity is better preserved in Se deficiency than are the other glutathione peroxidases, indicating that it may have a more important antioxidant function (Weitzel *et al.* 1990). The basis of the biochemical and nutritional interactions between Se and vitamin E may be the action of PGSHPx and the vitamin as antioxidants, preserving membrane lipid integrity.

The tissue distribution of PGSHPx is different from that of cGSHPx in particular; it is abundant in the testes and may be regulated by gonadotrophins (Roveri *et al.* 1992). PGSHPx also has a phosphorylation site which may have a role in regulation of enzyme activity (Schuckelt *et al.* 1991; BrigeliusFlohe *et al.* 1994). Potentially, therefore, PGSHPx may have a function in controlling metabolism rather than just being an antioxidant protecting against lipid peroxidation. Regulation of the levels of eicosanoid hydroperoxides by PGSHPx would influence many cell functions (Bryant *et al.* 1983; Cao *et al.* 1992; Weitzel & Wendel, 1993).

SELENOPROTEIN P

Of plasma Se in humans and rodents 60–80% occurs as selenoprotein P. The protein has been purified from rat and human plasma and cDNA clones have been prepared and sequenced (Yang *et al.* 1987; Hill *et al.* 1991, 1993; Akesson *et al.* 1994). In both cases the cDNA contains ten UGA codons in the open reading frame specifying ten selenocysteine residues. Additionally, selenoprotein P contains twenty-three histidine residues and seventeen cysteine residues, indicating a great potential for binding free transition metals (Hill *et al.* 1991).

Despite detailed knowledge of its structure, the function of selenoprotein P has not been established. When Se-deficient rats are treated with Se the first selenoprotein synthesized is selenoprotein P (Burk *et al.* 1991). This corresponds with protection against toxic effects of diquat, which causes hepatic necrosis in Se-deficient rats. Thus, it has been suggested that selenoprotein P may have an antioxidant function, although initially it was hypothesized to be a Se-transport protein (Hill & Burk, 1994). In Se-deficient rats there seems to be some selective uptake of injected selenoprotein P by the brain but there is no other direct evidence to support a transport function (Burk *et al.* 1991). It would be unusual to have a Se transport protein with such potential antioxidant properties and, furthermore, the energy required for its synthesis would be wasted since the selenocysteine in the protein would have to be returned to an inorganic form for synthesis of other selenoproteins. The same argument about waste of energy would apply to the hypothesis that cGSHPx is a Se storage protein. However, both selenoprotein P and cGSHPx may keep Se in a chemically-inactive form which will not interfere with redox-active thiols in the cell.

IODOTHYRONINE 5′-DEIODINASE (IDI)

The observation that, in rats, plasma thyroxine (T4) concentrations increased and plasma 3,3′,5-triiodothyronine (T3) concentrations decreased in Se deficiency led to the demonstration that this resulted from decreased hepatic type I IDI activity (Arthur *et al.* 1987*b*, 1990*b*; Beckett *et al.* 1987, 1989, 1990, 1992). This work continued with the identification of IDI as a Se-containing protein (Arthur *et al.* 1990*a*, 1991, 1993). Conventional protein chemical techniques were used to prove that IDI contained one Se in each substrate-binding subunit and, using an expression cloning system, Berry and Larsen (Behne *et al.* 1990; Berry *et al.* 1991; Berry & Larsen, 1992) demonstrated that IDI contained selenocysteine inserted via a UGA stop codon in a similar fashion to that of other selenoproteins. IDI activity in liver and kidney is regulated by Se supply in the normal nutritional range; thus, Se plays an important role in the control of thyroid hormone metabolism (Arthur & Beckett, 1994). Decreases in plasma T3 concentration in Se deficiency, however, are less than might be predicted, which indicates that compensatory mechanisms ameliorate some of the adverse effects of the loss of IDI activity (Beckett *et al.* 1992, 1993*b*; Chanoine *et al.* 1992, 1993; Arthur *et al.* 1993). Nevertheless, in conditions such as I deficiency, where thyroid hormone metabolism is impaired, Se deficiency may provide an additional stress with possible adverse effects on growth development, thermogenesis and neonatal survival (Geloen *et al.* 1990; Beckett *et al.* 1993*b*; Nicol *et al.* 1994).

An important compensatory mechanism which preserves T3 in Se-deficient rats is increased thyroidal T3 production, probably coming from induction of thyroidal IDI, despite its being a selenoenzyme whose activity would be expected to decrease (Beckett *et al.* 1993*a*; Chanoine *et al.* 1993). Human thyroid also contains IDI activity and, thus, the potential to maintain T3 production from T4 when Se supplies are limiting (Beech *et al.* 1993). However, many species including ruminants and pigs do not express IDI in the thyroid and, thus, there may be major differences in the response of thyroid hormone metabolism to Se deficiency (Beech *et al.* 1993). Unless other mechanisms exist to preserve thyroidal T3 production, lack of IDI activity in the gland may confer greater susceptibility to the effects of Se deficiency on thyroid hormone metabolism.

SPERM CAPSULE SELENOPROTEIN

Se deficiency causes abnormal sperm development in the rat. Compared with normal rats, Se-deficient rats produce fewer spermatozoa and these have abnormal tails and are immobile (see Calvin *et al.* 1987). The abnormalities have been associated with more fragile mitochondrial capsules, which contain a major structural selenoprotein. The cDNA which codes for mouse mitochondrial capsule selenoprotein has three inframe UGA codons that probably code for selenocysteine and the predicted molecular weight of the protein is 21·1 kDa (Karimpour *et al.* 1992). This is the first protein subunit found, other than selenoprotein P, which contains more than one selenocysteine. Sperm capsule selenoprotein also has six Pro-Cys-Cys-Pro sequences with eighteen to twenty cysteine residues and twenty-three to twenty-seven proline residues in total, which supports a structural role for the selenoprotein, since the intramolecular sulphydryl bonds will confer stability. Loss of this structure in Se deficiency would explain adverse effects on sperm function and lower fertility. Much work remains to be done, however, to confirm the function of the protein.

SELENOPROTEIN W

Selenoprotein W is a low-molecular-weight protein (9·5–10·0 kDa), of unknown function, which has been purified from rat muscle. It is believed to be similar to the low-molecular-weight selenoprotein that occurs in lamb muscle and which decreases in concentration during the onset of myopathy in combined Se and vitamin E deficiency. Selenoprotein W contains approximately 1 g atom Se/mol, as selenocysteine, at position 12 in its sequence. A partial amino acid sequence is not similar to any previously published sequences, indicating that selenoprotein W is not a fragment or subunit of one of the selenoglutathione peroxidases (Vendeland *et al.* 1993). Since selenoprotein W contains redox-active selenocysteine and occurs at concentrations similar to those of cGSHPx in heart and muscle it has been proposed that it may be an antioxidant. Until further investigations such as the response of the protein to Se depletion and repletion and further structural analysis are carried out, the antioxidant function can not be confirmed.

SELENIUM-BINDING PROTEINS

Proteins of 14 kDa and 56/58 kDa bind ^{75}Se both *in vivo* and in cell culture systems. Fatty acid-binding protein has been identified as the 14 kDa Se-binding protein, and the 56 kDa protein is closely related to proteins which bind many drugs and their metabolites. The 58 kDa-binding protein shows sequence homology with protein disulphide isomerase. However, in mouse mammary epithelial cells in culture, changing medium Se concentration did not affect levels of the 58 kDa protein (Sinha *et al.* 1993). Furthermore, Se does not regulate protein disulphide isomerase activity either in rats or in cultured cells (Arthur *et al.* 1991; Sinha *et al.* 1993). The Se-binding proteins have been hypothesized to have an anti-cancer effect; labelling of the 58 kDa protein with ^{75}Se corresponded to inhibition of DNA synthesis by Se added to cell cultures and the 56 kDa protein (SP56) may be involved in the regulation of cell growth by modulating regulatory proteins (Bansal *et al.* 1990; Sinha *et al.* 1993). However, since levels of the Se-binding proteins are not apparently regulated by availability of Se, more experimental information is required to define their function *in vivo*.

CONCLUSIONS

The identification, sequencing and cloning of several selenoproteins has indicated a hitherto unrecognized complexity in the biochemical roles of Se. Since dietary Se intake can affect many biological functions and processes, the challenge is now to relate these to the biochemistry of selenoproteins.

Se has clearly defined roles in antioxidant systems and in thyroid hormone metabolism but the precise functions of selenoprotein P, sperm capsule selenoprotein, selenoprotein W and the Se-binding proteins remain to be established. Additionally, *in vivo* and cell culture-labelling studies with ^{75}Se indicate that there are at least ten more selenoproteins to be characterized. Thus, although much is now known about the nutritional biochemistry of Se, the functions of many selenoproteins remain to be established. Only when this has been achieved will all consequences of changes in Se status be understood.

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