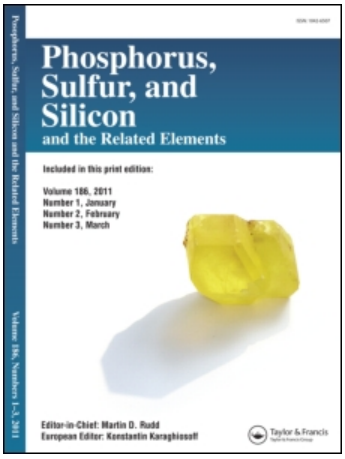

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BIOCHEMICAL FUNCTIONS OF SELENIUM

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Abstract The metabolic role of selenium is largely determined by the reactions catalyzed by selenium-dependent enzymes. At present, two Se-containing glutathione peroxidases are known, one of which exclusively reacts with H_2O_2 and with a large variety of other hydroperoxides, however not with esterified phospholipid hydroperoxides. The second GSH peroxidase is an interfacial enzyme acting on lipid hydroperoxides including phosphatidyl-choline- and cholesterol-hydroperoxide. Recently, a 5-deiodinase activity has been identified as a selenoenzyme which is responsible for the production of active thyroid hormone (T_3) from its pro-hormone (T_4). A plasma selenoprotein P has been isolated and sequenced which contains as many as 10 selenocysteine residues per molecule. Its function is still unknown.

In spite of this increasing information , selenium deficiency in animals and man cannot be simply explained on an enzymological basis. Animal experiments showed that selenium is depleted from these proteins with different rates. Upon resupplementation with selenium, enzyme activities are restored with a different kinetics and a different selenium requirement. Also metabolic changes affecting protein turnover, CO_2 exhalation and protein processing have been described in animals. The pattern of inflammatory mediators released upon a stimulus is also much greater in selenium-deficient white blood cells than in controls. These observations suggest a delicate network of interactions of selenium-dependent processes in the metabolism of animals which is by far more complex than previously anticipated.

INTRODUCTION

The essentiality of selenium for mammals is based on the experimental finding of the year 1957, that a selenium-containing factor of a molecular weight of about 300 Dalton (factor 3) was needed to

prevent dietary liver necrosis in rats raised on a diet low in vitamin E as well as in sulfur amino acids.[1] In spite of this clear-cut in-vivo evidence - per definition no artifact if not subject to misinterpretation - the exact nature of this factor 3 has never been elucidated. This illustrates the conflicting progress on the field of metabolic functions of selenium. As elaborated in the following sections of this article, major advances were made in the isolation and characterization of individual selenoproteins. The available knowledge allows to rationally explain selected metabolic pathways such as the reduction of hydroperoxides by glutathione. However, an integrated view of the physiological consequences of selenium shortage, moderate or severe selenium deficiency on the one hand, and on supplementation of supra-optimal amounts of selenium on the other hand are far from being understood. This review will first concentrate on structural data of selenium proteins, on substrate specificities and on kinetic behaviour of selenoenzymes and then try to work out a framework for a partial interpretation of metabolic functions of selenium.

BIOSYNTHESIS OF SELENOPROTEINS

The common form of selenium in bacterial as well mammalian selenoproteins is the amino acid selenocysteine. About one decade ago, two apparently entirely different views existed about how this amino acid is incorporated into proteins: One hypothesis claimed the existence of a tRNA specific for selenocysteine which had been postulated for rat liver without, of course, knowing the anticodon to this RNA.[2] The alternative view was derived from experiments which showed that the carbon of selenocysteine in GSH peroxidase was from serine suggesting a posttranslational modification which converts serine to selenocysteine.[3] Several years of studies aimed to this point as well as serendipity were needed to make clear that either view was correct. When the nucleotide sequence

of glutathione peroxidase became known, it turned out that the triplet TGA (listed in textbooks until then as a stop codon) specified selenocysteine in this enzyme.[4] Further studies in bacterial systems showed that the anticodon sequence of a very special tRNA matches the UGA codon in mRNA (corresponding to TGA sequence in DNA). This unique tRNA is charged with L-serine and serves as the tRNA-bound substrate for an energy-requiring step which converts this amino acid serine to selenocysteine.[5-7] The activation of the seryl-tRNA is likely to occur by phosphorylation catalyzed *via* a kinase.[7] Therefore, in bacteria and animals a highly specific metabolism allows the incorporation of selenium into selected target proteins by a complicated enzymatic machinery which itself seems to be regulated by the presence of selenium.[8]

SELENOPROTEIN P

In rat plasma, the major part of selenium is not associated with glutathione peroxidase, an enzyme which occurs in this compartment in a form that is different from the intracellular species.[9] The plasma selenoprotein designated "selenoprotein P (Se-P)" is a glycosylated protein with a molecular weight on SDS electrophoresis of 57 kD.[10] By amino acid analysis this protein was shown to contain 7-8 selenocysteine residues per molecule. From the sequence of the cloned cDNA a content of 10 selenocysteine residues was deduced because in the open reading frame, this amount of TGA codons was found.[11]

Se-P accounts for most of the selenium in rat serum and represents about 8% of the total selenium in the rat. Se-P was much more slowly depleted in selenium-deficient animals than e.g. GSH peroxidases. On the other hand, when deficient animals were repleted, within 24 hr 75% of control Se-P-content was found while during this interval GSH peroxidase activities increased to only 6% of control.[12] These findings indicate that in the selenium-deficient animal, supplemented selenium is preferentially

incorporated into Se-P. Obviously being biologically a precious protein, the physiological function or biochemical role of Se-P is unknown.

5-DEIODINASE

First observations were made which indicated that the activity of an enzyme catalyzing the 5-deiodination of the prohormone L-thyroxin (T4) to the biologically active thyroid hormone 3,5,3',tri-iodothyronin (T3).[13] This enzyme 5'-deiodinase was labeled with selenium in rats and shown to be identical with the 27 kD substrate binding subunit of type I 5-deiodinase[14], a propylthiouracyl-sensitive oxidoreductase found mainly in liver and kidney. The mRNA of this enzyme contains a UGA-codon for selenocysteine[15], similar to the selenocysteine in the active site of mammalian glutathione peroxidases. Interestingly, substitution of cysteine for selenocysteine resulted in a residual activity of about 20% for the sulfur-substituted selenoprotein. The sequence of 5'-deiodinase showed no obvious analogies to other known selenoproteins. These results explain why selenium is essential in thyroid hormone action, although the metabolic significance of this endocrinological network still awaits clarification.

SELENIUM-CONTAINING GLUTATHIONE PEROXIDASES

Incidentally, the discovery of GSH peroxidase coincides with the discovery of the essentiality of selenium in 1957.[16] The enzyme was purified to homogeneity from bovine blood and studied in great detail with respect to physicochemical parameters and kinetic properties (Reviewed in [17]). This enzyme has insofar unique characteristics among peroxidases as it is extremely specific for the reducing substrate, i.e. glutathione. GSH is the only one among the biological thiols which serves as a substrate. This strict specificity of GSH was later understood when the X-ray structure

of the whole protein and of the active site became known.[18] This work revealed that adjacent to the selenocysteine residue in position 45, two arginyl residues in positions 50 and 177 exist which fix the glutathione molecule with two negative charges on the arginins while the sulfur of GSH is placed in a juxtaposition to the selenium atom of the enzyme. Indeed, it had been demonstrated that during catalysis this selenium atom changes its redox state.[19] With respect to the peroxide substrate, GSH peroxidase shows an opposite substrate specificity: not only H_2O_2, but a large variety of different hydroperoxides are accepted by this enzyme. Physiologically interesting because of their precursor nature for inflammatory mediators, are fatty acid hydroperoxides especially those derived from arachidonic acid. The extrapolated maximum velocities for any of these hydroperoxides are identical for the GSH peroxidase reaction.

The kinetic properties of GSH peroxidase have also important bearings for estimating the physiological consequences of substrate supply: since the apparent k_m-values for GSH are about two orders of magnitude greater than those for the hydroperoxides, at physiological concentrations of substrate (GSH 2-10 mmol, ROOH: <μmol) the actual rate of the reaction depends on the concentration of hydroperoxides and is largely independent of the concentration of GSH. In other words, a reduction of intracellular GSH concentrations by 90% does not slow down the actual rate of GSH peroxidase because 10% residual GSH is still well above k_m. Nutritionally, the enzyme activity depends on the logarithm of dietary selenium with a half-saturation at about 0.25 parts per billion selenium for the mouse liver enzyme.[20]

A detailed proposal for a reaction mechanism of GSH peroxidase has been elaborated. It includes a cycle where first a reduced selenolate reacts extremely fast with a hydroperoxide to a selenenic acid derivative of the selenocysteine residue. This unstable intermediate would easily react then with glutathione to

form a selenadisulfide which upon reaction with a second molecule of GSH regenerates the reduced form of the enzyme. This reaction mechanism is in agreement with the observation that the enzyme can be inhibited by iodoacetate or mercurials or by gold compounds such as aurothioglucose. Also, from the formal enzyme kinetics, different oxidation states of GSH peroxidase were derived which can now be ascribed to the individual species of the tentative reaction mechanism discussed.

During the course of experiments that made clear that GSH peroxidase reduces only free fatty acid hydroperoxides[21] (i.e. under physiological conditions phospholipase activity is needed), another glutathione peroxidase was purified which turned out to be an interfacial enzyme that acts preferentially on esterified phospholipid hydroperoxides.[22] This enzyme which was first termed "peroxidation inhibiting protein, PIP" and received later the name "phospholipid hydroperoxide glutathione peroxidase (PH-GPX)". It was shown to be a monomer of 23 kD MW which contains 1 g atom selenium.[22] A similar protein with similar characteristics was later isolated from rat liver[23]. While the fundamental kinetic properties of this PH-GPX are closely related to the ones of the classical GSH peroxidase, the substrate specificity is markedly different. Many thiols other than glutathione are readily accepted by this enzyme. This can be explained by comparison of the active sites of two enzymes: while most of the structures are conserved in either peroxidase, PH-GPX lacks the arginine in position 50 and contains a threonine instead.[24] Therefore, the substrate GSH is not fixed on two positive charges, but only on one which would allow other thiols to be bound to this PH-GPX enzyme molecule. Surprisingly, there is only little homology between PH-GPX and GSH peroxidase.[24]

An interesting new aspect of PH-GPX with respect to its function is the observation that cholesterol hydroperoxides in erythrocyte ghost membranes are reduced by GSH in the presence of

this enzyme.[25] This may be of important physiological significance for the reduction of oxidized cholesterol which is observed in lipoprotein particles of the peripheral blood during arteriosclerosis.

SIGNIFICANCE OF SELENIUM IN LEUKOTRIENE METABOLISM

Leukotrienes are potent mediators in inflammation and shock. Among them the dihydroxylated products such as LTB_4 exhibit a predominantly leukotactic activity. The so-called cysteinyl leukotrienes which are formed by conjugation to glutathione are characterized by myotropic activities such as broncho- and vasoconstriction. The common precursor of either type of leukotriene is the 5-lipoxygenase product arachidonic acid hydroperoxide 5 (HPETE). In-vitro, this primary product is a substrate of GSH peroxidase as well as of PH-GPX. We addressed the question, which enzyme is responsible for this pathway and whether a dependence on selenium is seen in a system where rat basophilleukemia cells are cultured on selenium-deficient media. When deficient cells were stimulated in the presence of arachidonic acid, they produced a 6-fold amount of 5-lipoxygenase products compared to cells raised in selenium-adequate media.[26] When cell homogenates were subjected to analogous stimulation, a shift of the products in favour of increased HPETE formation was observed in selenium-deficient homogenates. These data showed that granulocytes produce an increased steady-state level of hydroperoxides in selenium-deficiency. Since it is known that the cyclooxygenase as well as the 5-lipoxygenase reaction, i.e. both branches of the eicosanoid metabolism, are subject to a regulation by hydroperoxides ("hydroperoxide tone" concept), these findings suggest that selenium is intimately involved in the regulation of the production of inflammatory mediators. This may have a bearing in one of the pathological conditions in humans that are selenium-responsive, i.e. the so-called Kashin-Beck Disease. This disease,

an endemic osteoarthropathy occurring in China[29], has characteristics typical for a chronic inflammation of the joints.

When mice were held on a severely selenium-deficient diet, containing less than 12 ppb selenium compared to a control diet with 500 ppb selenium, GSH peroxidase activity in the major organs was lost within about 3-4 weeks. In contrast, even after 250 days of depletion, PH-GPX was retained with residual activities ranging from 30-70 % in the different organs.[27] These findings demonstrate that the need for selenium of the two glutathione peroxidases is markedly different in the sense that under the conditions of trace element deficiency, selenium is preferentially recruited for PH-GPX activity. When a selective depletion of GSH peroxidase activity with nearly normal PH-GPX activities was created in RBL 1 cells, these granulocytes maintained their capacity to reduce 5 HPETE to 5- HETE.[28] These data clearly indicate that for this important step in leukotriene biosynthesis, PH-GPX is responsible.

SELENIUM-DEPENDENT CHANGES IN THE INTERMEDIARY METABOLISM OF ANIMALS

First of all, selenium-deficiency affects substrates and enzymes related to selenium-dependent processes themselves. A compensatory increase of GSH transferase activity under this condition has been observed[30]. Several microsomal drug metabolizing enzyme systems were affected by selenium-deficiency[31,32] and an increased release of GSH from liver leading to an increased plasma glutathione level in selenium-deficient rats was observed.[33] Moreover, an increased urinary excretion of ketone bodies in the selenium-deficient rat was found which was interpreted as an impairment in the renal handling of these products.[34] Further observations include an increased CO_2 exhalation in selenium-deficient mice[35] and a dramatically increased DNA synthesis rate in selenium-deficiency after partial hepatectomy in mice.[36]

An obvious question to be raised is whether these manifold metabolic changes caused by increases or decreases of enzyme activities in different metabolic pathways have a common denominator. Further studies in mice provided evidence that severe selenium-deficiency leads to a general increase of protein synthesis, degradation and secretion, i.e. a profound acceleration of protein turnover in general.[37,38] A possible rational to explain all these different findings is to assume that the lack of selenium-dependent glutathione peroxidases *via* increased hydroperoxide steady states leads to a faster oxidation of susceptible amino acids in proteins. It is known for several proteins that oxidation of a single amino acid can trigger proteolytic activities, for instance instantaneous degradation of these proteins.[34] These circumstances then lead to a general increase of protein turnover. *Vice versa*, these findings can be taken as circumstantial evidence for an enhanced steady-state oxidation rate in selenium-deficiency, an event which has to be expected on the basis of theoretical knowledge, but has never been directly shown. For sure, this view, although it reconciles several single observations, is still insufficient to explain the entity of metabolic changes, because the functions of many selenoproteins are still unknown. For instance, in the rat, 13 proteins containing selenium are found[40] and functions can be attributed only to the four ones described in the previous sections.

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