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A NEW SELENOPROTEIN FOUND IN THE GLANDULAR EPITHELIAL CELLS OF THE RAT PROSTATE

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An inverse relationship between the Se status and the incidence of prostate cancer suggests a significant role of Se in this organ. After labeling of rats with ^{75}Se and sodium dodecyl sulfate-polyacrylamide gel electrophoresis a strongly labeled prostatic 15-kDa protein band was found which was equally distributed among the different lobes. It was localized in the epithelial cells of isolated acini but did not appear in the prostatic secretion. By two-dimensional electrophoresis the band was resolved into three spots with pI-values around 4.5. The most strongly labeled spot stemmed from a cytosolic selenoprotein with an apparent native molecular mass of about 300 kDa which contained Se in the form of selenocysteine. The fact that with insufficient Se intake the element is preferentially incorporated into this compound as compared with glutathione peroxidase implies an important function of this newly found prostatic epithelial selenoprotein (PES).

Pathological changes observed in mammals in Se deficiency show that Se is necessary for the metabolic processes in several tissues such as liver, cardiac muscle, skeletal muscle and testis (1). The results of some studies suggest that the element might also be of importance in the prostate gland. Interest in the role of the element in this organ was roused in particular by the finding of an inverse correlation between the Se status and the incidence of prostate cancer (2,3). Furthermore, there is some indication that the Se present in the seminal plasma might originate mainly from the prostatic secretion (4).

It has now been established that the essential effects of the element are due to several biologically active selenoproteins. So far four glutathione peroxidases (5-8) and the type I and type III iodothyronine deiodinases (9-12) have been identified as mammalian seleno-enzymes. Other compounds which have been completely or partly sequenced are the selenoprotein P in the blood plasma (13), the sperm mitochondrial capsule selenoprotein (14) and the selenoprotein W isolated from the skeletal muscle (15). By in vivo-labeling with ^{75}Se and separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) a larger number of Se-containing proteins have been found in various rat tissues (16). Differences in their distribution patterns indicate that some of the Se compounds are enriched in certain tissues and thus might have tissue-specific sites of action.

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Abbreviations: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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In order to obtain information on specific selenoproteins present in the prostate gland, a study was carried out on rats using in vivo labeling of the Se compounds with ^{75}Se. As it has been found that the incorporation of the labeled element into proteins is the more effective the lower the Se status (16), Se-deficient animals were taken for most of the experiments.

MATERIALS AND METHODS

Animals: Wistar rats (Savo GmbH, Kisslegg, Germany) were fed for several generations either a low Se diet with a Se concentration of 2-5 μg/kg (Se-deficient animals) or the same diet with 300 μg Se/kg added as sodium selenite (Se-adequate animals) and distilled water ad libitum. The composition of the diet and the animal treatment have been described elsewhere (17). For labeling of the Se compounds a dose of 18.5 MBq (500 μCi) of [^{75}Se]selenite containing a Se amount of about 1 μg was injected intraperitoneally into male animals with body masses between 350 and 500 g. They were killed under appropriate anesthesia 5 to 8 days after the tracer injection, and the tissues were taken and homogenized as previously described (16).

Experimental series 1: The labeled Se-containing proteins were investigated in tissue homogenates after separation by SDS-PAGE and autoradiographic determination of the tracer distribution in the gels: **a)** in prostate, lung, brain and heart of a 4-month-old Se-deficient rat; **b)** in the prostate of a 4-month-old Se-adequate and a 5-month-old Se-deficient animal; **c)** in the prostate of the Se-deficient rat used in experiment 1b after preparation of the samples for SDS-PAGE under either reducing or non-reducing conditions.

Experimental series 2: The distribution of Se-containing proteins in several compartments of the prostate was determined by means of fractionation, SDS-PAGE and autoradiography of the labeled compounds: **a)** in the ventral and dorsolateral prostatic lobes collected from a 5-month-old Se-deficient animal; **b)** in the prostatic homogenate, acini and secretion obtained from a 6-month-old Se-deficient animal; **c)** in subcellular fractions of the prostate of an 11-month-old animal fed the Se-deficient diet from the age of 1 month onwards.

Experimental series 3: Some characteristics of the prostatic Se compound in question were determined by means of molecular fractionation procedures using 5- to 8-month-old Se-deficient animals: **a)** by two-dimensional electrophoresis of the homogenate and cytosol of prostatic acini and the homogenates of kidney, liver and lung; **b)** by ultrafiltration and gel chromatography of prostatic acini cytosol; **c)** by analysis of the selenoamino acids in an enriched acinar cytosol fraction.

Subdivision of prostatic compartments: The ventral lobes of the prostate were removed by dissection as described elsewhere (18), and samples of the ventral and dorsolateral lobes were taken. Prostatic fluid was collected by gentle pressure after placing the organ between two glass slides (19) and reduced to a quarter of its original volume by lyophilization. Prostatic acini were isolated from the stroma by partial digestion with collagenase and removal of the tissue fragments by repeated aspiration through pipette tips according to the method of Seitz et al. (20). The prostatic subcellular components were fractionated by means of differential centrifugation as previously described (21) applying the method of Chambers and Rickwood (22).

SDS-PAGE: The samples were separated in 15 % gels as described elsewhere (16) using the Laemmli buffer solution (23) with 50 mM dithiothreitol as the reducing agent. In experiment 1c the separation was carried out with 100 mM dithiothreitol or under non-reducing conditions. The molecular mass was determined by interpolation taking as marker proteins ^{125}I-labeled β-galactosidase (116 kDa), bovine serum albumin (68 kDa), carbonic anhydrase (29 kDa), soybean trypsin inhibitor (20 kDa) and α-lactalbumin (14 kDa).

Two-dimensional electrophoresis: The samples treated with 9 M urea and 50 or 150mM dithiothreitol were separated by isoelectric focusing (carrier ampholytes pH 2-4 and 3-10) and SDS-PAGE using the procedure of Klose (24) and the small gel technique of Jungblut et al. (25).

Determination of the molecular mass: Information on the native molecular mass of the Se compound in the prostatic acini cytosol was obtained by ultrafiltration through a membrane filter with a 100 kDa cut-off and by gel chromatography (fractionation range 8 - 2000 kDa) using

ferritin (440 kDa), catalase (240 kDa), glutathione peroxidase (100 kDa) and cytochrome C (12 kDa) as marker proteins for the estimation of the apparent molecular mass.

Selenoamino acid determination: A method for the analysis of amino acids using separation of the o-phtalaldehyde derivatives by reversed phase HPLC (26) has been modified for the determination of selenocysteine and selenomethionine in ^{75}Se-labeled Se-containing proteins after carboxymethylation and acid hydrolysis (Hammel, C., Kyriakopoulos A., and Behne, D., to be published).

^{75}Se analysis: The ^{75}Se activity in the tissues and tissue fractions was measured by means of a 3 x 3" NaJ(Tl) well-type detector coupled to a multi-channel analyzer. The tracer distribution in the electrophoretic gels was determined autoradiographically either by using an X-ray film as previously described (16) or a photostimulable phosphor plate which in connection with an imaging analyzer (BAS 1000, Fuji Film, Tokyo, Japan) allows quantitative analysis of the ^{75}Se activity in the separated labeled proteins.

RESULTS AND DISCUSSION

The Se-containing proteins in the prostate and some other organs of rats in experiment 1a, labeled in vivo by injection of ^{75}Se-selenite and separated by SDS-PAGE, are shown in Fig. 1. Of the seleno-enzymes already identified, the subunits of the tetrameric glutathione peroxidases and the phospholipid hydroperoxide glutathione peroxidase were located at the molecular mass range between 24 and 25 kDa and at 20 kDa respectively. Considerable differences between the tissues were found in the molecular mass range around 15 kDa. Here two Se-containing bands were visible, with the upper band at about 15.5 kDa being much more strongly labeled in the prostate than in the other tissues.

The tracer distribution among the Se-containing proteins in the prostate homogenate proved to be dependent to a large extent on the Se status of the rats. This was shown in experiment 1b by administering a very small amount of ^{75}Se-selenite either to a Se-deficient or a Se-adequate animal (Fig. 2). In the deficient rat about 50 % of the labeled element was present in the 15 kDa band and only about 10 % in the 24/25 kDa range where the subunits of the tetrameric gluta-

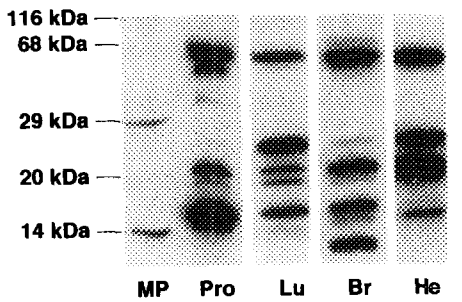

Fig. 1. Autoradiogram of ^{125}I-labeled marker proteins (MP) and ^{75}Se-labeled proteins in homogenates of the prostate (Pro), lung (Lu), brain (Br) and heart (He) of rats. A Se-deficient rat was labeled in vivo with [^{75}Se]selenite. Samples containing 0.3 to 0.5 mg protein and 50 to 100 Bq ^{75}Se were separated by SDS-PAGE and exposed to an X-ray film for 5 days.

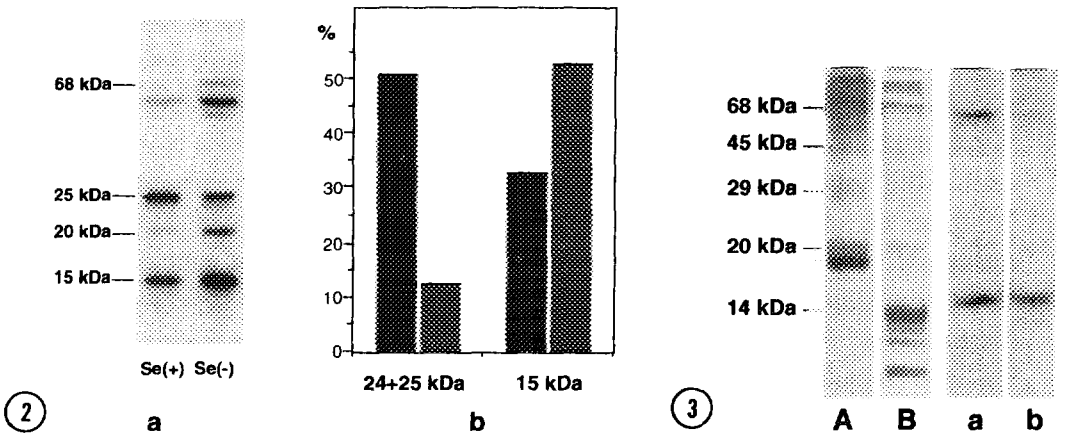

Fig. 2. Effects of the Se status on the incorporation of the element into the prostatic Se-containing proteins. After administration of a small amount of Se (1 μg) in the form of [^{75}Se]selenite to a Se-adequate (Se+) and a Se-deficient (Se-) rat, samples of the prostate homogenates containing about 0.3 mg protein and 100 or 200 Bq ^{75}Se were separated by SDS-PAGE.
a) Autoradiogram of the ^{75}Se-labeled proteins after exposure of the gel to an imaging plate for 18 hours. b) Percentage of the total protein-bound tracer activity in the lane found in the 15 kDa and 24/25 kDa regions of the Se-adequate (black bars) and the Se-deficient animal (shaded bars).

Fig. 3. Comparison of the protein pattern and the Se-containing 15-kDa band in rat prostate homogenate after SDS-PAGE under reducing or non reducing conditions. The two lanes on the left show the silver-stained proteins of the labeled samples (0.15 mg protein, 5 Bq ^{75}Se) after treatment with the Laemmli buffer solution with 100 mM dithiothreitol (A) or without a reducing agent (B), the two lanes on the right the corresponding autoradiograms a and b obtained after exposure of the gel to an X-ray film for 21 days.The transformation of the 20-kDa proteins (A) into smaller compounds (B) was due to fragmentation of the subunits of prostatein, one of the major prostatic proteins (28).

thione peroxidases were situated. In the Se-adequate animal the 15 kDa range was still one of the main Se-containing regions, but it was only second to the 24/25 kDa bands which then took up more than 40 % of the protein-bound ^{75}Se activity present in the prostate homogenate. This indicated that with insufficient Se intake the hierarchy in the distribution of the element among Se-containing proteins observed previously in a large variety of tissues (16) also exists in the prostate gland and that, compared with glutathione peroxidase, the 15 kDa band is preferentially supplied.

As the findings of the two experiments suggest the existence of a Se compound which is specifically enriched in the prostate gland and might play an important role in this organ, the Se-containing protein represented by the 15 kDa band was investigated in more detail.

A prostatic compound with a similar molecular mass of 16 kDa in SDS-PAGE has been described which stemmed from prostatein, one of the major proteins in this organ (27). As after treatment with SDS the prostatein subunits can be further fragmented by cleaving of the disulfide bonds (28), the prostatic homogenate was separated by SDS-PAGE with and without addition of a reducing agent (experiment 1c). From the differences found in the protein pattern between the two samples and the fact that the tracer distribution in the 15 kDa range remained unchanged (Fig. 3), it could be concluded that the Se-containing protein in question is not related to prostatein.

In order to obtain some information on the possible sites of action of the Se compound, in a second series of experiments the prostate was subdivided into various fractions. After separation of the ventral and dorsolateral lobes, which differ with regard to morphology (18) and protein composition (27), and SDS-PAGE of their homogenates, the band was found to be equally distributed among the different prostatic sections (experiment 2a, autoradiograms not presented).

Another kind of subdivision was achieved in experiment 2b by collagenase digestion of the stroma, which mainly consists of smooth muscle cells, and isolation of the prostatic acini from the digested fragments. About 20 % of the prostatic tissue mass was found in the acinar fraction which contained more than 50 % of the total tracer activity. By quantitative determination of the labeled Se-containing proteins in the homogenates of the whole prostate and the acini, the distribution of the 15 kDa band between acini and stroma was estimated. From the results shown in Table 1 it can be seen that only a negligibly small amount of the labeled 15 kDa band was found in the latter and that the prostatic Se-containing protein represented by this band is thus located in the acinar fraction.

In the prostatic secretion, however, relatively little of the labeled element was found to be protein-bound. The comparison of its distribution among the proteins in the secretion and in the acini (Fig. 4) indicated that the Se-containing protein under investigation is not released into the prostatic fluid and most probably only takes part in metabolic processes in the secretory cells.

Information on its distribution among the cellular components was obtained in experiment 2c by quantitative determination of the labeled proteins after SDS-PAGE of aliquots of the subcellular fractions separated by ultracentrifugation. In the animal used in this experiment about 20 % of the total protein-bound ^{75}Se found in the prostate homogenate was present in the 15 kDa region. Similar values of about 20 % were determined in the cytosolic, microsomal and mitochondrial fraction, while in the nuclear fraction only 3 % of the protein-bound tracer was contained in this band. The pattern differed from that of other tissues with regard to the relatively high value in the prostatic cytosol. The distribution of the 15 kDa band among the prostatic compartments was

Table 1. Distribution of the Se-containing 15 kDa band between acini and stroma of the rat prostate[1]

^{75}Se-labeled 15 kDa band	Prostate	Stroma	Acini
Expressed as a percentage of the total protein-bound ^{75}Se in prostate or prostate fraction	40	2	70
Expressed as a percentage of the ^{75}Se-labeled 15 kDa band in whole prostate	100	3	97

[1] Prostatic acini were isolated from the stroma by collagenase digestion (20). Homogenates of prostate and acini were separated by SDS-PAGE and the ^{75}Se-labeled proteins determined quantitatively by means of an imaging plate. The tracer distribution in the stroma was calculated from the differences between prostate and acini.

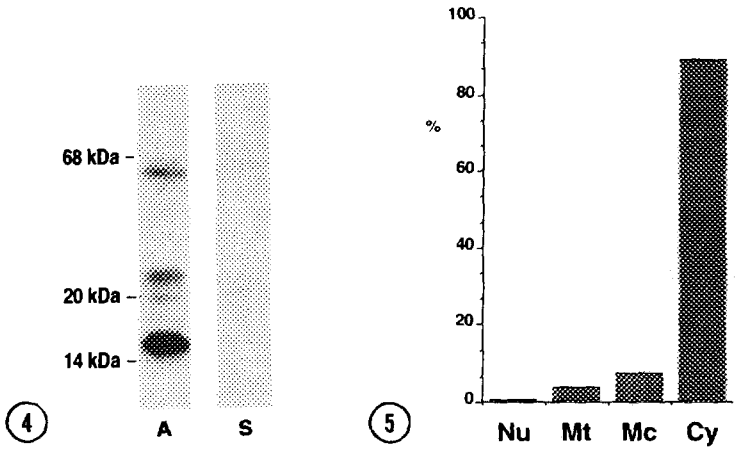

Fig. 4. Autoradiogram of ^{75}Se-labeled proteins in the acini (A) and secretion (S) of the rat prostate. The acini were isolated from the stroma by collagenase digestion (20). The prostatic fluid was obtained from the organ by gentle pressure. Samples of the acini homogenate (0.15 mg, 100 Bq ^{75}Se) and of the fluid, which had been reduced to a quarter of its original volume by lyophilization, were separated by SDS-PAGE and the gel exposed to an imaging plate for 7 days.

Fig. 5. Distribution of the ^{75}Se-labeled 15-kDa band in the nuclear (Nu) mitochondrial (Mt), microsomal (Mc) and cytosolic (Cy) fractions of the rat prostate. The prostate homogenate of a Se-deficient rat, labeled in vivo with [^{75}Se]selenite, was fractionated by ultracentrifugation using the method of Chambers and Rickwood (22). After separation by SDS-PAGE the distribution of the tracer among the fractions was determined quantitatively by means of an imaging plate. With the losses in the separation steps taken into account, the amount of the labeled 15-kDa band in each of the fractions was estimated and expressed as a percentage of its total prostatic amount.

estimated, taking the losses during the different separation steps into account. The results in Fig. 5 show that of the amount of labeled Se found in the 15 kDa range about 5 % was contained in both the mitochondria and microsomes and less than 1 % in the nuclei, and that thus the majority of nearly 90 % was present in the cytosol.

In experiment 3a the proteins of the prostatic acini homogenate were separated by means of two-dimensional electrophoresis (Fig. 6) and compared with the protein patterns of the homogenates of liver, lung and kidney treated in the same way. The prostatic 15 kDa area was resolved into three Se-containing spots with isoelectric points of about 4.5 which consisted, with increasing pI-values, of a weakly labeled prostate-specific spot, a spot specifically enriched in the prostate and a spot found to be similarly labeled in the other tissues. The latter was, however, not dectected in the acinar cytosol. This showed that the strong labeling of the 15 kDa band in the prostate was due to a specific Se compound present in the cytosol of the epithelial cells.

An indication that the 15 kDa protein is part of a much larger molecule was obtained in experiment 3b where after ultrafiltration of acinar cytosol by means of a 100 kDa cut-off filter and SDS-PAGE the labeled compound was detected in the retentate. After gel chromatography and SDS-PAGE this compound was localized in the fraction which contained proteins with molecular masses of about 300 kDa (Fig. 7).

Finally, in experiment 3c the chemical form of Se in the protein was investigated by means of amino acid analysis of the labeled compound. All selenoproteins identified so far have been

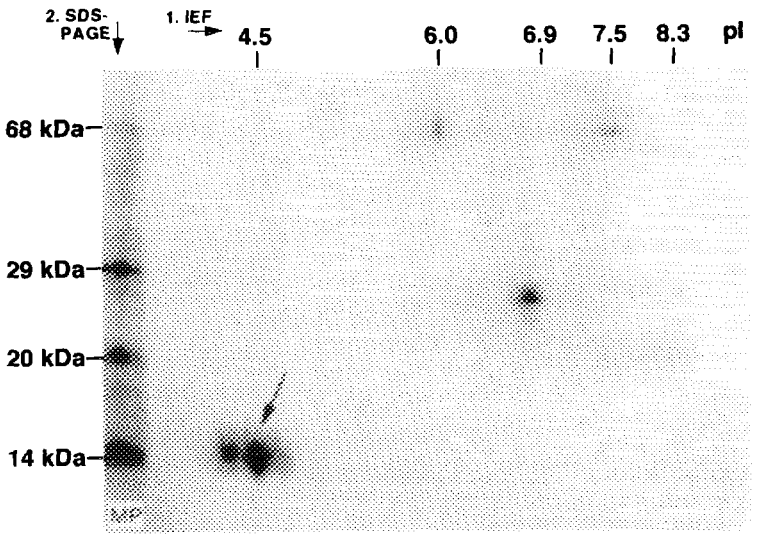

Fig. 6. Autoradiogram of ^{75}Se-labeled proteins in the homogenate of rat prostate acini after two-dimensional separation. The sample was treated with 9 M urea and 150mM dithiothreitol and separated by isoelectric focusing (IEF) with carrier ampholytes pH 2-4 and 3-10 and SDS-PAGE according to the procedures of Klose (24) and Jungblut et al. (25). The molecular mass was determined by means of ^{125}I-labeled marker proteins (MP). The 15-kDa band was resolved into three ^{75}Se-containing spots. The most strongly labeled spot (see arrow) represented a Se compound found to be specifically enriched in the prostatic epithelium.

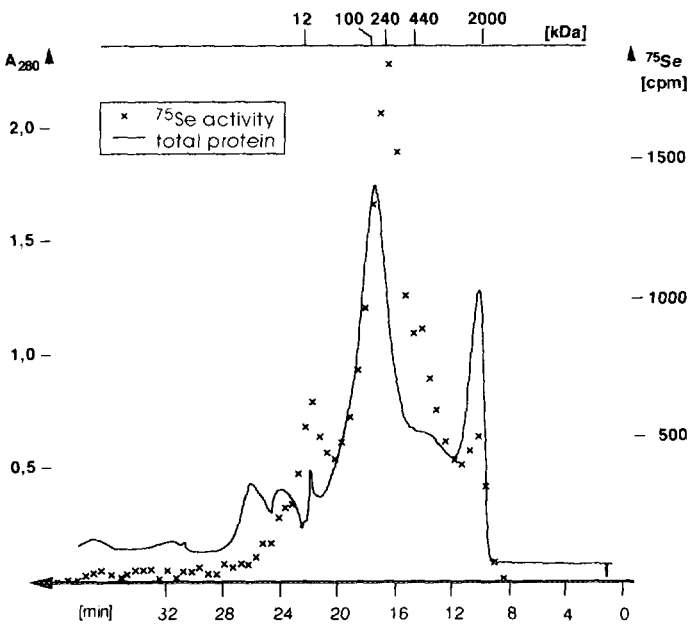

Fig. 7. Gel chromatography (fractionation range 8 - 2000 kDa) of ^{75}Se-labeled prostatic acini cytsol. By means of marker proteins (ferritin, catalase, glutathione peroxidase and cytochrome C with molecular masses of 440, 240, 100 and 12 kDa) an apparent native molecular mass of about 300 kDa was estimated for the Se compound in question.

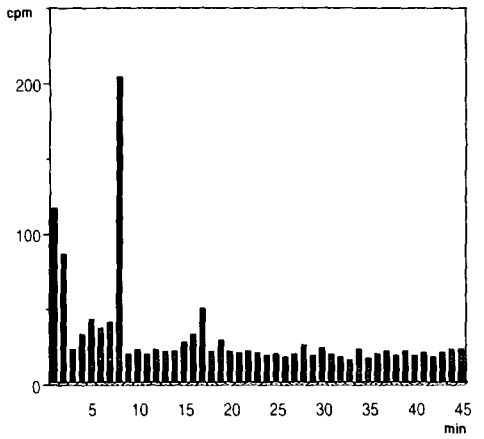

Fig. 8. Determination of the chemical form of Se in the labeled prostatic protein. After separation of the o-phtalaldehyde derivatives by reversed phase HPLC following carboxymethylation and acid hydrolysis of the compound the main part of the tracer was present as selenocysteine in fraction 8. Fractions 1 and 2 contained oxidation products of this amino acid. Selenomethionine would have been found in fraction 38.

shown to contain the element in the form of selenocysteine, and a codon responsible for the incorporation of this amino acid into proteins has been detected (29). From the data in Fig. 8 it can be seen that here, too, Se is present in the form of selenocysteine and that the prostatic Se-containing compound is thus in fact a further selenoprotein.

So far the biological significance of this prostatic epithelial selenoprotein (PES) is still unknown. However, the finding that with insufficient Se supply the incorporation of the element into this compound has priority over that into glutathione peroxidase, suggests an important function of the former, and the identification of its biological role is therefore of great interest. Moreover, the inverse relationship between the Se status and the incidence of prostatic cancer in conjunction with the fact that the protein in question is one of the major Se compounds in the prostatic glandular epithelium is another important factor to be considered in the further investigation of this newly found selenoprotein.

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