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Selenoprotein P receptor from rat

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Radioreceptor assay technology was used to show the presence in the rat of a receptor that binds selenoprotein P, a selenocysteine-containing rat plasma protein. ^{75}Se-labeled selenoprotein P bound to testis, kidney, and liver membranes. The binding was specific in that increasing amounts of partially-fractionated rat plasma specifically displaced the binding of ^{75}Se-labeled selenoprotein P to testis membrane in a competitive manner. ^{75}Se-labeled selenoprotein P binding was saturable in the presence of increasing amounts of testis membranes. The binding of ^{75}Se-labeled selenoprotein P was optimal at about pH 4.2. Several proteins and blood fractions had little or no significant effect on binding of ^{75}Se-labeled selenoprotein P to testis membranes. All plasma sources tested specifically displaced ^{75}Se-labeled selenoprotein P from testis membrane, indicating that selenoprotein P-related proteins may be widespread in nature. The study indicated that selenoprotein P has a receptor and is involved in selenium transport.

Introduction

The possible mechanism of selenium transport has been discussed [1–3]. In 1977, Hermann [4] found evidence for a plasma protein that specifically incorporated physiological amounts of selenium. Similar plasma selenoproteins were found in the rat and in the monkey (*Mucaca mulata*) [5], and recently in sheep [6], mice [7], and hamsters (Motchnik and Tappel, unpublished data). In the rat and monkey, selenium in the protein was in the form of selenocysteine, which is the form also found in glutathione peroxidase (glutathione : hydrogen peroxide oxidoreductase, EC 1.11.1.9). Rat plasma selenoprotein (selenoprotein P) is probably synthesized in the liver, and the synthesis is sensitive to cycloheximide [8]. The molecular weight of native selenoprotein P was reported [9] to be approx. 80000 as determined by gel filtration, and Yang et al. [10] determined by SDS-PAGE that the molecular weight of the purified protein is 57000. This difference in molecuar weight was ascribed either to the fact that selenoprotein P is highly glycosylated or that a second subunit is present that does not stain well [10].

Motsenbocker and Tappel [3] suggested that selenoprotein P may be a selenium transport protein, and that it may have an important role in the transfer of selenium from liver to various tissues. Selenium distribution within an animal is dependent on the selenium nutritional status of the animal. Since evidence suggested that selenoprotein P is a selenium transport protein, radioreceptor assay technology was used to determine the existence of a specific receptor for the protein.

Materials and Methods

Preparation of ^{75}Se-labeled selenoprotein P. Male Sprague-Dawley rats weighing 240–280 g (Simonsen Laboratories) were fed a Torula yeast selenium-deficient diet (Teklad Test Diets) for 4–5 weeks. Each rat was injected intraperitoneally with 0.25 mCi of ^{75}Se as selenious acid (ICN Radiochemicals) with a specific activity of 70 mCi/mg Se in 0.25 ml 25 mM potassium phosphate buffer (pH 7.2)/0.9% sodium chloride. After 3–4 h, the rats were either anesthetized with ether or injected intraperitoneally with sodium pentobarbital, and blood was collected via cardiac puncture using syringes that contained 0.8 ml 25 mM potassium phosphate buffer (pH 7.2)/1.5% EDTA. The blood was centrifuged at 3500 × *g* for 20 min at 4°C, and the resulting plasma was fractionated by 35 and 50% ammonium sulfate precipitation [11]. The 50% ammonium sulfate precipitate was resuspended in a minimal volume of 10 mM Tris buffer (pH 7.6)/1 mM glutathione/0.1 mM EDTA. The resuspended precipitate was applied to

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an Affi-Gel blue column (2.5 × 35 cm). The column was washed for 3 h with 50 mM Tris buffer (pH 8.5)/0.1 mM EDTA and then proteins were eluted with 50 mM Tris/0.4 M sodium trichloroacetate buffer (pH 8.5)/0.1 mM EDTA. The eluted proteins were monitored by measurement of absorbance at 280 nm, the flow rate was 0.45 ml/min, and 20-min fractions were collected. Fractions with the highest ^{75}Se activity were analyzed by SDS-PAGE and autoradiography. Fractions that contained the 55000 M_r ^{75}Se-labeled selenoprotein P were pooled, dialyzed against distilled water, and filtered using PM-10 Diaflo ultrafiltration membranes (Amicon). The small volume concentrate was diluted in 10 volumes of 100 mM Tris/0.5 M acetate buffer (pH 7.4) and filtration was repeated. The concentrated sample (1000–3000 cpm/μl) was stored at −20 °C.

Partial fractionation of plasma. Male Sprague-Dawley rats fed a selenium-deficient diet for 4 or more weeks were injected intraperitoneally with selenium as sodium selenite (200 μg/kg body weight) in 25 mM potassium phosphate buffer (pH 7.2)/0.9% sodium chloride. Blood collected after 4 h was centrifuged at 3500 × *g* for 20 min at 4 °C. The plasma was fractionated using the ammonium sulfate fractionation procedure described above. The 50% ammonium sulfate precipitate (partially-fractionated plasma) was resuspended in a minimal volume of 100 mM Tris/0.5 M acetate buffer (pH 7.2). The 35% ammonium sulfate precipitate and the 50% ammonium sulfate supernatant were assayed for competition with ^{75}Se-labeled selenoprotein P for testis membranes.

Preparation of membrane fractions. Kidney, liver, and testis membranes were prepared as follows. Tissues were rinsed in ice-cold 0.25 M sucrose/20 mM Tris/1 mM phenylmethylsulfonyl fluoride (pH 8.0) (Buffer A). Kidney and liver were minced and the encapsulating sac was removed from testes. Each tissue was homogenized in 2 volumes of Buffer A with six slow strokes of a motor-driven Potter-Elvehjem homogenizer. Homogenates were centrifuged at 2000 × *g* for 10 min, and the resulting supernatant fractions were centrifuged at 100000 × *g* for 60 min at 4 °C. The 100000 × *g* pellet from each tissue was resuspended in 20 mM Tris buffer (pH 7.5) at a final protein concentration of 10–20 mg/ml, as determined by a dye-binding assay [12]. The membrane fractions were stored at −20 °C.

^{75}Se-labeled selenoprotein P binding to kidney, liver, and testis membranes. Kidney, liver, and testis membrane fractions (1 mg protein) prepared from rats fed a stock diet (Purina rat chow) or a selenium-deficient diet were incubated in a final volume of 0.3 ml with $2.0 \cdot 10^4$ cpm of ^{75}Se-labeled selenoprotein P in the absence or presence of partially-fractionated plasma (1.5 mg protein) in Buffer B. Incubation was for 4 h before the reaction was terminated by centrifugation at 7000 × *g* for 15 min.

Competition of partially-fractionated plasma with ^{75}Se-labeled selenoprotein P for binding to testis membranes. An aliquot of the membrane fraction from rats fed a selenium-deficient diet (900 μg protein) was incubated in a final volume of 0.3 ml with $2.0 \cdot 10^4$ cpm ^{75}Se-labeled selenoprotein P in the absence or presence of increasing amounts of partially-fractionated plasma in 0.1 M Tris/0.5 M acetate buffer (pH 7.6)/0.1% bovine serum albumin (BSA) (Buffer B). Incubation was done in 1.5-ml polyethylene Eppendorf tubes at room temperature with intermittent hand mixing. After 4 h, the separation of bound from free radioactivity was accomplished by centrifugation at 7000 × *g* for 15 min in a Beckman microfuge 12. The supernatant from each tube was removed and radioactivity in each membrane pellet was counted in a Packard gamma counter (30% counting efficiency). The same procedure was performed for each fraction obtained from the ammonium sulfate fractionation and for each subsequent assay(s), unless otherwise stated.

Association of ^{75}Se-labeled selenoprotein P with membranes as a function of time. Testis membrane fraction from rats fed a selenium-deficient diet (450 μg protein) was incubated with $1.4 \cdot 10^4$ cpm ^{75}Se-labeled selenoprotein P in the absence (total binding) or presence (nonspecific binding) of excess unlabeled selenoprotein P in the form of partially-fractionated plasma in Buffer B. Incubation was carried out for 15 min to 18 h before the reaction was terminated by centrifugation at 7000 × *g* for 15 min.

pH optimum for ^{75}Se-labeled selenoprotein P binding to membrane. Testis membrane fraction from rats fed a selenium-deficient diet (900 μg protein) was incubated in a final volume of 0.6 ml with $1.9 \cdot 10^4$ cpm ^{75}Se-labeled selenoprotein P in the absence or presence of partially-fractionated plasma (1.5 mg protein) in Buffer B with the pH adjusted over the range of 0.3–12.2 with HCl or sodium hydroxide. Incubation was for 4 h before the reaction was terminated by centrifugation at 7000 × *g* for 15 min. The final pH of each reaction mixture was determined by preparing duplicate mixtures without ^{75}Se-labeled selenoprotein P.

Determination of ^{75}Se-labeled selenoprotein P binding saturability. Increasing amounts of testis membranes from rats fed a stock diet (0.1–2.0 mg protein) were incubated in a final volume of 0.5 ml with $2.5 \cdot 10^4$ cpm ^{75}Se-labeled selenoprotein P in the absence or presence of 6.0 mg of partially-fractionated plasma in 0.1 M Tris/0.5 M acetate buffer (pH 4.2)/0.1% BSA. Incubation was for 4 h and the reaction was terminated by centrifugation at 7000 × *g* for 15 min.

Competition of various blood fractions, plasma samples, and proteins with ^{75}Se-labeled selenoprotein P for binding to testis membranes. Testis membrane fraction from rats fed a stock diet was incubated in a final volume of 0.8 ml with $1.9 \cdot 10^4$ cpm ^{75}Se-labeled

selenoprotein P in the presence of BSA (16 mg) or plasma (16 mg protein) from various species (Sigma Chemical Co.) in 0.1 M Tris/0.5 M acetate buffer (pH 4.2). Incubation was done for 1 h and the reaction was terminated by centrifugation at 7000 × *g* for 15 min. The same protocol was followed to test for competition for binding by human Cohn fractions I, II, III, IV-1, IV-4, and V (10 mg protein) (Sigma Chemical Co.) and by hemoglobin (0.2–0.4 mg), transferrin (0.1–0.2 mg), selenium-glutathione peroxidase (20 μg), and BSA (0.1–7.0 mg). As a control for competitive binding, 1.5 mg of partially-fractionated plasma was used in the assay.

Subcellular fractionation of testis to determine fraction(s) with binding sites. To determine the subcellular fraction(s) with which the binding sites are associated, testes from rats fed a stock diet were prepared as described above, and the 2000 × *g* membrane pellet was fractionated as described by Aronson and Touster [13], with some modifications. The discontinuous sucrose gradients were centrifuged at 66000 × *g* for 4 h in a Spinco SW 25.1 rotor. Each visible band was separated, the membranes in each band were centrifuged [13], and the resulting pellets were resuspended in 20 mM Hepes buffer (pH 7.2). Each band was assayed for the marker enzymes, phosphodiesterase I [13] (plasma membrane), glucose-6-phosphatase [13] (microsomes), and *N*-acetyl-glucosaminidase [14] (lysosomes). Using difference spectra [15], mitochondria were detected. Aliquots from each band were incubated in a final volume of 0.5 ml with ^{75}Se-labeled selenoprotein P in the absence or presence of partially-fractionated plasma (6.0 mg) in 20 mM Hepes buffer (pH 7.2)/0.1% BSA. Incubations were done for 2 h, and the reaction was terminated by centrifugation at 7000 × *g* for 15 min.

Results

Preparation of ^{75}Se-labeled selenoprotein P

Fig. 1 depicts the elution profile of the proteins and the ^{75}Se radioactivity from the Affi-Gel blue column. Only one major ^{75}Se peak of about 10^6 cpm was observed, and it coincided with a small 280 nm absorbance. SDS-PAGE followed by autoradiography of the fractions that contained the highest amounts of ^{75}Se showed one selenoprotein with 55000 M_r. Selenoprotein P was 50–100-fold purified, with a yield of about 20–25%, a result that is similar to results in a previous report [11]. The specific activity of six preparations ranged from 2000 to 5000 cpm ^{75}Se/μg protein.

^{75}Se-labeled selenoprotein P binding to kidney, liver, and testis membranes

The percentage binding of ^{75}Se-labeled selenoprotein P to membrane fractions prepared from kidney, liver, and testes from rats fed a selenium-deficient diet or rats fed a stock diet is shown in Table I. More ^{75}Se-labeled selenoprotein P bound to testis membrane fractions prepared from rats fed a selenium-deficient diet than to any other membrane fraction tested. The experiment was done twice with the same result.

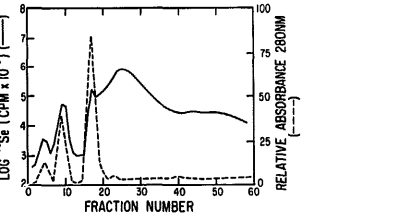

Fig. 1. Affi-Gel blue chromatography of partially-fractionated plasma obtained from selenium-deficient rats 3–4 h after injection with ^{75}Se. After application of the sample, the column was washed with 50 mM Tris/0.1 mM EDTA buffer (pH 8.5) for 3 h. Proteins, shown as absorbance at 280 nm, were eluted with 50 mM Tris/0.4 M trichloroacetic aicd/0.1 mM EDTA buffer (pH 8.5).

Competition of partially-fractionated plasma with ^{75}Se-labeled selenoprotein P for binding to testis membranes

Addition of increasing amounts of partially-fractionated plasma (50% ammonium sulfate precipitate) to ^{75}Se-labeled selenoprotein P and testis membrane fraction decreased the amount of ^{75}Se bound to the membranes (Fig. 2). The binding decreased in a concentration-dependent competitive manner. At the IC_{50}, the approximated concentration of ^{75}Se-labeled selenoprotein P and the concentration of the displacer selenoprotein P were similar. The data fit the preliminary test for competitive inhibition as suggested by Williams and Wood [16]. The competition assay was done twice with the same result. Only the 50% ammonium sulfate precipitate competed with the ^{75}Se-labeled selenoprotein P, while the 35% ammonium

TABLE I

^{75}Se-labeled selenoprotein P binding to membranes from rat testis, kidney, and liver

Rats were fed either a stock diet or a selenium-deficient Torula yeast-based diet. Each tissue preparation was a pool of membranes from 4–6 rats. Values are expressed as percentage of total ^{75}Se-labeled selenoprotein P in the assay that bound to the membranes.

Tissue	% ^{75}Se bound	
	rats fed stock diet	rats fed selenium-deficient diet
Testis	8.6	16.8
Kidney	11.0	6.6
Liver	8.4	6.2

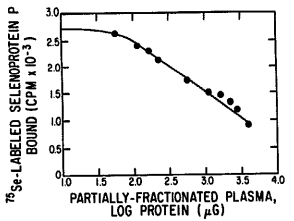

Fig. 2. Competitive binding by partially-fractionated rat plasma. ^{75}Se-labeled selenoprotein P was incubated with testis membranes in the presence of increasing amounts of partially-fractionated plasma obtained from selenium-deficient rats 4 h after injection with 200 μg selenium/k weight.

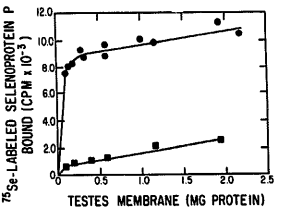

Fig. 4. Saturability of ^{75}Se-labeled selenoprotein P binding to testis membranes. ^{75}Se-labeled selenoprotein P was incubated with increasing amounts of testis membranes in the absence (total binding, ●) or presence (nonspecific binding, ■) of partially-fractionated plasma (6.0 mg protein) for 2 h at pH 4.2. Plotted values are from two determinations.

sulfate precipitate and the 50% ammonium sulfate supernatant each had less than 20% of the competition activity of the 50% ammonium sulfate precipitate. The competitive binding was also done at pH 4.2, where the binding was greater than that shown in Fig. 2. The shapes of the binding curves were the same at pH 4.2 and 7.6, and the stoichiometry was about the same.

Association of ^{75}Se-labeled selenoprotein P with testis membrane as a function of time

More than 70% of the binding of ^{75}Se-labeled selenoprotein P to the testis membrane fraction occurred within 15 min. Maximum binding of the ^{75}Se-labeled selenoprotein P occurred 3–4 h after initiation of the reaction. Maximum binding was constant up to about 18 h of incubation.

^{75}Se-labeled selenoprotein P testis membrane binding pH optimum

Fig. 3 shows the specific binding by 1.5 mg of partially-fractionated plasma at different pH levels. The maximum specific binding by 1.5 mg of partially-fractionated plasma occurred at about pH 4.2. The pH profile for binding of ^{75}Se-labeled selenoprotein P to testis membrane fraction was bell-shaped and extended over a wide pH range. The experiment was done three times with the same results.

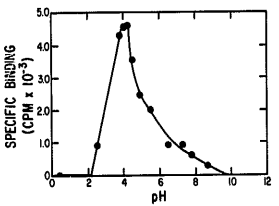

Fig. 3. Effect of pH on binding of ^{75}Se-labeled selenoprotein P to testis membranes. ^{75}Se-labeled selenoprotein P was incubated with testis membranes in the absence or presence of 1.5 mg partially-fractionated plasma for 4 h at varying pH.

Determination of ^{75}Se-labeled selenoprotein P binding saturability

^{75}Se-labeled selenoprotein P binding to testis membrane fractions was saturable (Fig. 4). More than 80% of the total amount of ^{75}Se-labeled selenoprotein P bound to the testis membrane fraction was specifically bound, as shown by addition of 6 mg of partially-fractionated plasma. The ^{75}Se-labeled selenoprotein P that could not be competitively removed by large amounts of partially-fractionated plasma was nonspecifically bound to the membranes. Fig. 4 shows that nonspecific binding increased linearly with increased amounts of membrane.

Competition of blood fractions, plasma, and proteins with ^{75}Se-labeled selenoprotein P for binding to testis membrane fraction

Plasma for all species tested significantly decreased the binding of the ^{75}Se-labeled selenoprotein P to the testis membrane fraction, and BSA had no effect on the binding (Table II). The percentage of specific displacement is the amount of decrease in binding of ^{75}Se-labeled selenoprotein P to testis membrane fractions as compared to controls without protein and BSA. Plasma from avian species seemed to have the highest specific displacement. Evolutionarily-related species, such as human and baboon, and hamster and guinea pig, had similar values for specific displacement.

BSA, hemoglobin, transferrin, and selenium-glutathione peroxidase did not decrease the binding of ^{75}Se-labeled selenoprotein P to testis membrane fractions.

TABLE II

Competition of plasma from various species with ^{75}Se-labeled selenoprotein P for binding to testis membrane fraction

The percentage specific displacement is the percentage decrease of ^{75}Se-labeled selenoprotein P bound to testis membranes at pH 4.2 in the presence of BSA (16 mg) or plasma (16 mg protein) from various species As a control, 1.5 mg of partially-fractionated rat plasma was assayed. Data were analyzed by a two-tailed *t* test.

Species	% Specific displacement	*P*
None	0.0	–
BSA	0.0	–
Human	15.0 ± 1.8 [a]	< 0.01
Baboon	18.1 ± 2.8	< 0.01
Hamster	23.4 ± 2.2	0.001
Guinea pig	31.5 ± 1.8	< 0.01
Bovine	34.1 ± 3.0	< 0.01
Goat	34.4 ± 2.0	0.001
Horse	18.4 ± 1.5	< 0.01
Dog	21.0 ± 1.8	< 0.01
Pig	27.0 ± 2.9	< 0.01
Chicken	29.4 ± 2.3	< 0.01
Pigeon	51.1 ± 1.7	< 0.001
Turkey	64.4 ± 1.6	< 0.001
Rat, partially-fractionated	40.9 ± 2.0	< 0.01

[a] Mean ± S.D. and $N = 3$.

Partially-fractionated rat plasma was used as a control, and it decreased the amount of binding of ^{75}Se-labeled selenoprotein P to testis membrane fractions in a competitive manner.

Selenium-deficient rat plasma, which assays low in selenoprotein P, did not compete as well with ^{75}Se-labeled selenoprotein P for binding as did plasma from selenium-sufficient rats.

The human blood fractions, Cohn fractions I, II, III, and V, did not compete with the ^{75}Se-labeled selenoprotein P for binding to testis membrane fractions. Cohn fractions IV-1 and IV-4 had less than 4% of the competitive binding of partially-fractionated plasma.

Subcellular fractionation of testes to determine binding sites

After centrifugation of discontinuous sucrose gradients, four bands, as described by Aronson and Touster [13], were observed. Band 2 from the top of the gradient had the highest specific activity of the plasma membrane marker, phosphodiesterase I, and the highest ^{75}Se-labeled selenoprotein P binding activity. This band was localized at the interface of the 37.2% sucrose and 0.25 M sucrose layers. Band 2 contained 1.2% microsomes (calculated from the total homogenate) and no detectable mitochondria. Band 2 was composed mostly of plasma membrane, which is in agreement with the results of Aronson and Touster [13].

Discussion

The protocol for preparation of partially-purified ^{75}Se-labeled selenoprotein P was developed using the information obtained from a time-course study of the incorporation of ^{75}Se into blood plasma and tissue proteins [8]. The study indicated that maximum selenoprotein P labeling occurred 3–4 h after injection of ^{75}Se, and the maximum amount of labeling was dependent on the selenium nutritional status of the rats. The in vivo labeling of the selenoprotein P with ^{75}Se is specific, since selenoprotein P is by far the major selenoprotein produced early after administration of ^{75}Se to selenium-deficient rats. For receptor studies in vivo labeling, such as with ^{75}Se, has some important advantages over in vitro labeling, such as labeling of proteins by the lactoperoxidase and chloramine T methods [17,18]. In vitro labeling requires that the protein of interest be pure, and the process may damage the protein as well as change its affinity for binding sites as a result of introducing iodine into the molecule. It is not necessary for selenoprotein P to be pure, since binding assays work in the presence of extraneous proteins. The only requirement is that it be the only labeled protein in the assay. As long as this criterion is satisfied, the ^{75}Se-labeled selenoprotein P can be used as a tracer in binding studies to determine receptor sites for selenoprotein P.

The use of membrane preparations as the source of receptor follows the practice used in the field of receptor research. The majority of studies of ligand binding to receptors have used homogenates or membrane preparations as the source of receptors [19]. These types of preparations were used, for example, for studies of insulin receptor [20].

The two main criteria to show the existence of a receptor are that the binding be specific for the radioligand and that the binding of the radioligand be saturable. Other criteria give further support for the existence of a receptor; for example, increased association of the radioligand over a period of time, pH dependence of binding of the radioligand, and the decrease in binding activity of membranes due to harsh preparation and storage.

The binding of ^{75}Se-labeled selenoprotein P was shown to be specific in that various protein and blood fractions, the 35% ammonium sulfate precipitate, and the 50% ammonium sulfate supernatant of rat plasma did not significantly decrease the binding of ^{75}Se-labeled selenoprotein P to testis membrane fractions. Only the selenoprotein P-rich fraction (50% ammonium sulfate precipitate) competed with the ^{75}Se-labeled selenoprotein P in a concentration-dependent manner.

The ^{75}Se-labeled selenoprotein P binding was saturable and specific. Some of the ^{75}Se-labeled selenoprotein P could not be displaced even by a large amount of

partially-fractionated rat plasma. This nonspecific binding increased in direct proportion to the amount of membrane in the assay.

^{75}Se-labeled selenoprotein P associated with the testis membranes rapidly in a manner similar to the rapid association of epidermal growth factor with its binding sites [21]. For unknown reasons the pH for optimum binding of ^{75}Se-labeled selenoprotein P was unusually low.

^{75}Se-labeled selenoprotein P bound to membrane preparations from various tissues. The highest percentage binding of ^{75}Se-labeled selenoprotein P was to testis membrane fractions from rats fed a selenium-deficient diet. The higher binding of selenoprotein P to testis membranes and lesser binding to liver and kidney membranes correlates with the increased incorporation of ^{75}Se into the testes and its decreased incorporation into liver and other tissues of selenium-deficient rats [8]. The increased selenium content in testes of selenium-deficient rats may be a result of increased binding of selenoprotein P due to an increase in either receptor number or affinity. The increased selenoprotein P binding to membranes from testes of selenium-deficient rats may be related to the importance of selenium in reproduction. Selenium is essential in spermatogenesis in the rat [22–24] and mouse [25]. A selenoprotein is localized in the midpiece of the rat sperm tail, and this protein may be important in assembly and motility of sperm [26,27]. Selenoproteins with 47000 and 54000 M_r were found in the testes between 5–24 h after injection of rats with ^{75}Se [27]. These selenoproteins appeared before the selenoprotein was found in the midpiece of the sperm tail. It is possible that these selenoproteins derive from selenoprotein P.

Various plasma samples competed with ^{75}Se-labeled selenoprotein P for binding to testes membranes, indicating that selenoprotein P is widespread in nature. Further evidence for the conclusion that selenoprotein P is widespread is shown by the presence of a plasma selenoprotein in monkey [5], sheep [6], and mouse [7].

The widespread nature of selenoprotein P and the existence of a receptor in the rat are evidence that selenoprotein P is a selenium-transport protein. Selenoprotein P does not fit the definition of a classical transport protein [28], so it may be an exception to the rule. The energy cost of transporting selenium would seem to be high and very inefficient, as pointed out by Yang et al. [10]. Although regulation costs energy, the energy cost is not so high since selenium is a trace element and only very small amounts of selenoprotein P would be transported. The only energy cost would be that for synthesis of the protein, since the selenoprotein P can be broken down by the recipient tissue and the amino acids can then be used for energy or synthesis of other proteins. Distribution of selenium to tissues appears to be regulated, and this regulation may be controlled by a receptor as is the case for the regulation of iron transport by transferrin [29]. Modulation of number and/or affinity of the receptor on the plasma membrane can be used to control the transport of selenium to the tissues that require it.

The radioreceptor assay for detection of selenoprotein P has a great many applications. The radioreceptor assay is applicable to detection of selenoprotein P in plasma of many animals because of the cross reactivity of this protein from different species with the receptor from the rat testes. The materials required for the radioreceptor assay are easy to prepare.

The results from previous studies [5,8] and those presented in this paper support the hypothesis that selenoprotein P is involved in selenium transport. Selenium distribution may be controlled by the selenoprotein P specific receptor. It would be of interest to determine what happens to selenoprotein P after it binds to the receptor and what biochemical signals from tissues alter selenoprotein P binding.

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