Selenium: Biochemical Role as a Component of Glutathione Peroxidase

Abstract. When hemolysates from erythrocytes of selenium-deficient rats were incubated in vitro in the presence of ascorbate or H2O2, added glutathione failed to protect the hemoglobin from oxidative damage. This occurred because the erythrocytes were practically devoid of glutathione-peroxidase activity. Extensively purified preparations of glutathione peroxidase contained a large part of the 35Se of erythrocytes labeled in vivo. Many of the nutritional effects of selenium can be explained by its role in glutathione peroxidase.

Although the nutritional importance of selenium and its relation to vitamin E are well known (1), definition of a specific biochemical role for selenium has so far proved elusive. We present evidence here for a role of Se in glutathione (GSH) peroxidase (glutathione: H2O2 oxidoreductase, E.C. 1.11.1.9; 2GSH + H2O2 = GSSG + 2H2O) which provides further evidence for the essentiality of Se for animals and for one of its biochemical functions, and provides a plausible explanation for its relation to vitamin E.

Earlier, we found that dietary Se helped prevent oxidative damage to rat erythrocytes incubated in vitro, as evidenced by decreased hemolysis and decreased hemoglobin oxidation (2). These effects of dietary Se were dependent on the addition of glucose in vitro, and the well-known protection against hemolysis and hemoglobin oxidation afforded by glucose (3) was virtually absent in erythrocytes from rats deficient in Se. A related dietary inhibitor of oxidative damage, α-tocopherol (vitamin E), protected against hemolysis whether or not glucose was present, but did not protect against hemoglobin oxidation. This result demonstrated that the effect of Se was specific and distinct from that of vitamin E (2).

Protection by glucose against oxidative damage to red blood cells has been attributed to the maintenance of the intracellular concentration of reduced glutathione (GSH) through the combined actions of the enzymes, glucose-6-phosphate dehydrogenase [which generates reduced nicotinamide adenine dinucleotide phosphate (NADPH)] and glutathione reductase [which generates GSH from NADPH and oxidized glutathione (GSSG)]. At least in part, GSH acts by destroying hydrogen peroxide and fatty acid hydroperoxides through reactions catalyzed by GSH peroxidase (3, 4). In our earlier work (2) we found that the GSH concentration was higher in Se-deficient than in Se-sufficient erythrocytes and that it was as effectively maintained during incubation in vitro. This suggested that the defect in Se deficiency was not in the maintenance of GSH but rather in the utilization of GSH in protecting the cell (2). We therefore focused on a possible role for Se in the GSH protection against hemoglobin oxidation and ultimately on the enzyme (GSH peroxidase).

All experiments were performed with selenium-deficient rats.
erythrocytes from rats fed either a Se-deficient (Se content, < 0.01 ppm) or Se-supplemented (Se content, 0.5 ppm; supplied as sodium selenite) diet (2). Erythrocyte hemolyzates prepared from rats receiving the respective diets are called (for convenience) Se-deficient or Se-sufficient hemolyzates. Because previous experiments showed that the effects of Se in preventing oxidative damage were independent of vitamin E (2), all diets in our studies contained 50 mg of dl-a-tocopherol per kilogram of diet. The rats received the respective diets for at least 6 weeks before blood samples were taken. Erythrocytes were centrifuged from heparinized blood taken by heart puncture, the buffy coat was removed by aspiration, and the erythrocytes were washed three times with isotonic saline-phosphate buffer at pH 7.4 (2). The cells were then hemolyzed with an equal volume of water; hemoglobin content was measured by the cyanmethemoglobin method (3), and hemoglobin oxidation was followed by the methods described by Mills (6); GSH was measured as described previously (2).

Ascorbate- and H_{2}O_{2}-induced hemoglobin oxidations were quantitated in hemolyzates incubated in vitro by measuring, respectively, choleglobin formation and increase in absorbance at 627 nm by slight modifications of methods described by Mills (6). As shown in Table 1, GSH decreased both ascorbate- and H_{2}O_{2}-induced oxidations of hemoglobin in Se-sufficient hemolyzates, but GSH did not decrease hemoglobin oxidation in Se-deficient hemolyzates. Without added GSH, hemoglobin oxidations were similar in Se-sufficient and Se-deficient hemolyzates. The oxidation of hemoglobin by H_{2}O_{2} was easily visible as a brownish-green discoloration within 2 minutes after H_{2}O_{2} was added. These studies suggested that the activity of the GSH peroxidase may be much lower in Se-deficient hemolyzates.

It has been shown that Se-deficient hemolyzates have much lower GSH peroxidase (method in legend of Fig. 1) as compared to Se-sufficient hemolyzates [for example, at 66 days on experiment the value was 10.0 ± 0.5 (S.E.) compared to 55.5 ± 2.1 enzyme units per milligram of hemoglobin (7)].

The Se-dependent GSH peroxidase activity could not be restored to the Se-deficient hemolyzates by incubating for 30 minutes with either 6.3 μM sodium selenite alone, or combined with 25 mM GSH or 25 mM dithiothreitol, suggesting that the enzyme had not simply been oxidatively deactivated in Se-deficient hemolyzates. Enzyme activity did not disappear upon dialysis against any of the following: saline-phosphate buffer (2), 5 mM GSH in buffer, or 5 mM GSH plus 1 mM EDTA in buffer. Also the enzyme activity was not retarded on G-25 Sephadex. These observations suggested that Se may be an integral part of GSH peroxidase rather than a loosely bound cofactor of low molecular weight.

To test the hypothesis that Se is a component of GSH peroxidase, we partially purified GSH peroxidase from erythrocytes of Se-adequate rats which 2 or 4 weeks earlier had been injected with ^75Se as sodium selenite. By DEAE-Sephadex chromatography (Fig. 1, top), GSH peroxidase was separated from the large hemoglobin peak and some minor erythrocyte proteins. Approximately 60 percent of the ^75Se in the initial hemolyzate cochromatographed with the GSH peroxidase activity. There were no other substantial ^75Se-containing peaks. Fractions containing GSH peroxidase were concentrated and rechromatographed on Sephadex G-150 (Fig. 1, bottom). Two ^75Se-containing peaks were obtained corresponding to approximate molecular weights of 90,000 and 25,000. The lower-molecular-weight peak, which has not been identified, contained insignificant GSH peroxidase activity. The peak of 90,000 molecular weight corresponded to the GSH peroxidase activity and contained about 70 percent of the ^75Se applied to

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**Table 1. Effect of glutathione (GSH) on the oxidation of hemoglobin (Hb) by ascorbate and by H_{2}O_{2} in Se-sufficient (Se+) and Se-deficient (Se−) erythrocyte hemolyzates.**

<table>
<thead>
<tr>
<th>Source of hemolyzate</th>
<th>GSH</th>
<th>Ascorbate-induced oxidation (%)</th>
<th>H_{2}O_{2}-induced oxidation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se−</td>
<td>−</td>
<td>19.4</td>
<td>0.107</td>
</tr>
<tr>
<td>Se+</td>
<td>+</td>
<td>7.7</td>
<td>0.114</td>
</tr>
<tr>
<td>Se−</td>
<td>+</td>
<td>20.4</td>
<td>0.100</td>
</tr>
<tr>
<td>Se+</td>
<td>+</td>
<td>6.5</td>
<td>0.036</td>
</tr>
</tbody>
</table>

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**Fig. 1. Purification of GSH peroxidase.** (Top) Erythrocytes separated from 3 ml of heparinized blood from a rat injected 2 weeks earlier with 10 μg of sodium [^75Se]selenite were hemolyzed with water. Approximately 90 percent of the hemoglobin precipitated on overnight storage at 4°C, and was used to determine GSH-peroxidase activity. The supernatant containing 75 percent of the ^75Se was chromatographed on DEAE-Sephadex A-50, eluting with 0.05 M tris buffer, pH 8.0, and a linear gradient of 0.0 to 0.5 M NaCl (dashed line). The GSH peroxidase was assayed by a modification of Mills' procedure (1/). The incubation mixture at 37°C contained 0.08 M sodium phosphate (pH 7.0), 0.08 M EDTA, 1.0 mM sodium azide, 0.40 mM GSH and 0.25 M H_{2}O_{2}. GSH was determined at 3-minute intervals (15). An enzyme unit represents a decrease in GSH concentration of 0.001 log unit per minute, after subtraction of the nonenzymatic rate. The ^75Se was measured with a crystal scintillation spectrometer. (Bottom) The DEAE-Sephadex A-50 column fractions containing GSH peroxidase activity from 3 ml of blood from a rat injected 4 weeks earlier with 100 μg of ^75Se were pooled, concentrated by ultrafiltration, and rechromatographed on Sephadex G-150, eluting with 0.05 M tris buffer, pH 8.0. OD, optical density; Vv, void volume.
the column. At the concentration present in the eluate, no protein could be detected in the active peak by absorbance at 280 nm, suggesting that extensive purification of the enzyme had been achieved. Although we have not yet completely purified the rat enzyme, cochromatography of 125Se with GSH peroxidase activity through two highly effective purification steps suggests that Se is an integral and necessary part of the enzyme. In our laboratory the enzyme from ovine blood has been purified, yielding a preparation containing at least 2 gram-atom of Se per mole of enzyme (8).

Flohé (9) reported that bovine erythrocyte GSH peroxidase contains no non-protein prosthetic group; however, a Se moiety would probably not be detected by the spectrophotometric methods used by Flohé. Selenite (10) or Se-amino acids (11) enhance the reducing ability of GSH in model systems, and SH groups in GSH peroxidase appear to change their redox state during the catalytic process (9). Whether Se in the enzyme participates in these redox reactions or has some other function is not known.

Several groups of investigators (3) have emphasized the role of GSH peroxidase as the primary mechanism for degrading low levels of H₂O₂ in cells. Since GSH peroxidase also acts on hydroperoxides of unsaturated fatty acids (4), the enzyme plays an important role in protecting membrane lipids, and thus the cell membranes, from oxidative disintegration. Failure of peroxide destruction can explain the hemolysis in vitro and oxidative damage to hemoglobin and possibly the wide variety of degenerative conditions that occur in Se deficiency. A role for Se in GSH peroxidase may also account for the apparent "antioxidant" effects of dietary Se observed by previous workers (12).

Our work offers new insight into the interactions of Se, vitamin E, and the sulfur-containing amino acids in preventing some of the same nutritional diseases. If vitamin E prevents fatty acid hydroperoxide formation, and the sulfur amino acids (as precursors of GSH) and Se are involved in peroxide destruction, these nutrients would produce a similar biochemical result—that is, lowering of the concentration of peroxides or peroxide-induced products in the tissues. Protection against oxidative damage to susceptible nonmembrane proteins by dietary Se but not by vitamin E (2) might explain why some nutritional diseases respond to Se but not to vitamin E (7). On the other hand, certain tissues or subcellular components may not be adequately protected from oxidant damage because they are inherently low in GSH peroxidase even with adequate dietary Se. Damage to such tissues would be expected to be aggravated by diets high in unsaturated fatty acids and to respond adequately to vitamin E but not to Se.

Measurement of GSH peroxidase may provide a useful means for defining Se requirements and for identifying Se deficiency in animals and humans. With purified GSH peroxidase it should be possible to identify the active form of Se and further clarify its role.

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References and Notes
dine, O. H. Muth, Ed. (American, West
7. R. A. Sundeen, W. G. Hoekstra, D. G. Hafe
m, in preparation.
12. J. G. Bier, H. Dam, C. Prange, E. Sonder
13. For ascorbate-induced oxidations, the incuba
tion mixture contained: 0.5 mg of hemo
globin per 0.5 ml GSH (when added), 3.5 mM GSHSe (when added), 3.5 mM GSHSeSe and 0.5 mM sodium azide. Incubations were carried out at 37°C for 60 minutes; cheloglobin formation, expressed as percentage of the total hemoglobin, was deter
ded immediately, as described by Mills (6). For H₂O₂-induced oxidations, the incubation mixture contained: 0.5 mg hemoglobin per milliliter, 0.5 mM GSHSeSe (when added), 0.1 mM H₂O₂, and 1 mM sodium azide. Incubations were carried out at 37°C for 10 minutes. In
crease in optical density at 627 nm measured hemoglobin oxidation (6). The data in the table represent the respective hemolyses from one Se-deficient and one Se-supplemented rat; however, the effect has been confirmed repeatedly by visual observation of hemo
globin discoloration as well as by quantitation of GSH peroxidase activity in this text.
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Haptic Illusion: Apparent Elongation of a Disk Rotated between the Fingers

Abstract. A disk (coin) turned over end over end between thumb and forefinger feels longer to the turning hand. The illusion grows rapidly for 30 seconds but does not become asymptotic within 60 seconds. The illusion increases with coin size and turning rate, and is independent of holding pressure. It appears to involve illusory mechanisms in both hands.

This report describes an illusion which I first observed while idly turning a penny end over end between my thumbs and forefingers, using two hands. The coin was held on edge so that the balls of the thumb and forefinger of the holding hand were separated by its diameter. This diameter served as the axis about which the same digits of the other hand turned the coin. The thumb of the turning hand pushed on one rim and the forefinger pulled the opposite rim around. As the fingers came together, the thumb slid back so that it could engage the other rim while the index finger moved forward to catch the edge released by the thumb. This operation was repeated over and over, turning the coin end over end. The coin seemed to stretch