GLUTATHIONE PEROXIDASE: A SELENOENZYME*

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1. Introduction

Selenium was discovered as an essential trace element for mammals by Schwarz and Flotz [3] in 1957. The pathological symptoms of selenium deficiency closely resemble those seen in tocopherol deficient animals. In addition, growth depression, increased mortality, myopathies and decreased fertility developed in tocopherol deficiency could be improved by application of dietary selenium [4]. The synergistic effects of dietary selenium and tocopherol suggest a common physiological target of these two compounds. The biochemical mechanism by which selenium acts as an antioxidant remained obscure until Rotruck and coworkers [5] presented some experimental evidence for the involvement of selenium in the GSH-dependent metabolism of hydroperoxides. The authors described an incorporation of intraperitoneally injected ^{75}Se into a protein fraction which after partial purification showed GSH peroxidase activity. It was assumed that selenium might function as a constituent of GSH peroxidase.

In order to check this hypothesis we determined the selenium content of GSH peroxidase isolated in homogeneous and crystalline state from bovine blood by neutron activation analysis. We actually found four gram atoms of selenium per mole of enzyme, which means most probably one atom of selenium per subunit.

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2. Methods

2.1. Preparation of GSH peroxidase

GSH peroxidase was prepared from bovine blood essentially as described in [1]. The enzyme appeared to be homogeneous as judged by rechromatography, ultracentrifugation studies and discontinous gel electrophoresis [6]. The specific activity of the sample was 500 U_{37}/mg [1]. The enzyme was crystallized from 1.2 M potassium phosphate buffer. A charge of enzyme was dissolved in 5 mM potassium phosphate and lyophilized before use.

2.2. Determination of selenium by neutron activation analysis [7]

0.69 mg GSH peroxidase lyophilized in phosphate buffer and a set of suitable standards containing DL-selenomethionine (A grade, Calbiochem) were sealed in polyethylene tubes, wrapped in aluminium foil and irradiated in the reactor FR-2 of the Gesellschaft für Kernforschung (Karlsruhe) at a flux of 6×10^{13} n cm^{-2} sec^{-1} for 24 hr. After a cooling period of ten days the γ-spectra of the samples were obtained by means of a Ge (Li) detector. The selenium content of the samples was evaluated at the 264.6 KeV line of ^{75}Se ($T_{1/2}$ = 120.4 days). The experimental errors listed in the table consider the statistical errors of peak integration, the deviation of the selenium standards and the difficulties in handling the small amounts of protein.

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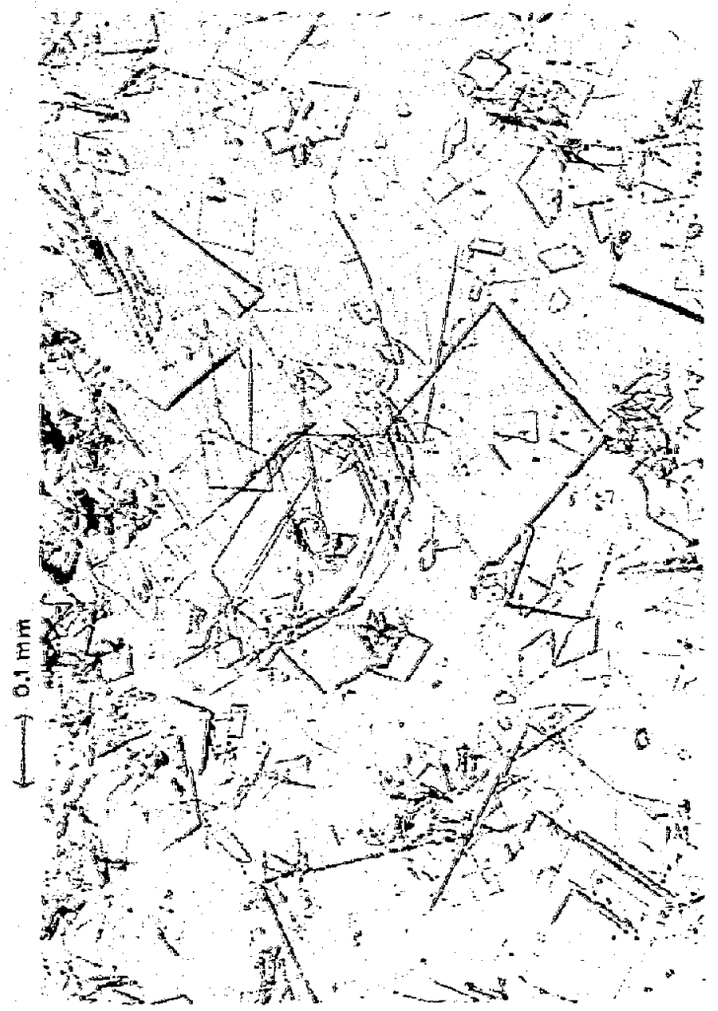

Fig. 1. Microphotograph of crystalline GSH peroxidase from bovine blood suspended in 1.2 M potassium phosphate buffer. The picture was kindly taken by Prof. Dr. H. Haselmann (Institut für Wissenschaftliche Mikroskopie der Universität Tübingen).

3. Results

As can be seen from the table, glutathione peroxidase contains about four gram atoms of selenium per mole. This value is calculated assuming a molecular weight of 84 000 daltons for the native enzyme [6]. GSH peroxidase consists of four subunits of 21 000 daltons [6] which most probably are functionally equivalent [8]. We may therefore conclude that each subunit of the enzyme contains one gram atom of selenium. The γ-spectrum of the irradiated enzyme sample revealed that no other metal component detectable by the method employed was present in the preparation.

Table 1
Determination of selenium content of GSH peroxidase by neutron activation analysis.

Sample	Se (μg)	Se (gram atoms) / enzyme (moles)
Empty polyethylene tube	< 0.02	
2.2 mg potassium phosphate buffer (Merck, p.A.)	< 0.08	
0.62 mg bovine serum albumin (Serva, purum)	0.038 ± 0.02	
0.69 mg GSH peroxidase (= 8.2 nmoles) + 2.2 mg potassium phosphate buffer	2.62 ± 0.02	4.04 ± 10%

4. Discussion

To the best of our knowledge the present report for the first time describes the presence of selenium in a protein in stoichiometric amounts. In addition, the observation of Rotruck et al. [5] that selenium deficient rats exhibit decreased glutathione peroxidase activity indicates that selenium must be considered as an essential component of the enzyme. Since GSH peroxidase does not contain heme or flavine and no conclusive evidence for any other functional group has been supplied so far, we may tentatively assume that selenium undergoes an oxidation–reduction cycle during catalysis. The catalytic function of the selenium, however, remains to be established.

Our finding that GSH peroxidase is a selenoenzyme can explain many manifestations of selenium deficiency. According to Underwood [4] most of the pathological conditions of selenium deficiency are related to peroxidation of unsaturated lipids in biological membranes. It is a well established physiological function of GSH peroxidase to prevent lipid peroxidation by either elimination of low molecular hydroperoxides or reduction of lipid hydroperoxides [9]. Thus, the antioxidative effects of alimentary selenium could be well attributed to the fact that it functions as an integral component of GSH peroxidase.

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