Short Communication

Glutathione Peroxidase from
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There are at least two enzymes showing glutathione peroxidase activity, which catalyze the following reaction (I).

One is selenium-dependent glutathione peroxidase I (EC 1.11.1.9),¹⁾ and the other is selenium-independent glutathione peroxidase II which has been shown to be due to glutathione *S*-transferases (EC 2.5.1.18).²⁾ These enzymes of mammalian origin have been studied extensively, but there have been few reports on these enzymes from microorganisms.³⁾ Our recent investigation showed that *Mucor hiemalis* formed a large quantity of glutathione peroxidase in the mycelium. The present report deals with the purification and some properties of the enzyme from *M. hiemalis*.

Glutathione peroxidase activity was assayed by using cumene hydroperoxide and reduced glutathione as substrates, according to the method of Paglia and Valentine.⁴⁾

For production of glutathione peroxidase, *M. hiemalis* was aerobically cultured at 30°C in a 30-liter jar fermentor containing 15 liters of a medium (pH 6.0) composed of 5% soluble starch, 0.3% yeast extract, 0.3% malt extract, 0.2% KH_2PO_4 , 0.05% KCl and 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. The maximum production was reached in 4~5 days of cultivation.

The purification of glutathione peroxidase from the cell-free extract of *M. hiemalis* was carried out by ammonium sulfate fraction-

FIG. 1. Acrylamide Gel Electrophoretic Pattern of Glutathione Peroxidase from *M. hiemalis*.

The purified enzyme (11 μg) was subjected to electrophoresis at pH 8.3. The gel was stained with 1% Amido black.

TABLE I. DETERMINATION OF SELENIUM CONTENT OF GLUTATHIONE PEROXIDASE FROM *M. hiemalis*

The purified enzyme was dialyzed overnight against 0.01 M potassium phosphate buffer, pH 6.0. The dialyzate (40 ml) was divided into four equal portions. Two of them were lyophilized directly *in vacuo*. To the remaining two was added 5 μl and 10 μl of a standard selenium solution (1000 ppm), and then they were lyophilized. Selenium contents of these four lyophilized samples, which contained 4.1 mg of protein (=91 nmol) respectively, were assayed with an X-ray fluorescence analyzer (Model TEFA-III, Daini-Seikosha Co., Tokyo).

Sample no.	Se added (μg)	Intensity (cps)
1	—	-0.18
2	—	-0.13
3	5	14.4
4	10	25.8

TABLE II. SUBSTRATE SPECIFICITY OF GLUTATHIONE PEROXIDASE FROM *M. hiemalis*

The purified enzyme was assayed for glutathione peroxidase activity and glutathione *S*-transferase activity as described in the text.

Enzyme activity	Substrate	Specific activity ^a
Peroxidase activity	Cumene hydroperoxide	202
	<i>t</i> -Butyl hydroperoxide	182
	Hydrogen peroxide	0
Transferase activity	2,4-Dinitrochlorobenzene	0

^a μ mol of glutathione oxidized/mg of protein/min.

ation (70~90% saturation), and DEAE-Sephadex, Bio-Gel P-100, and hydroxylapatite column chromatographies. The enzyme was purified about 2,000-fold with a recovery of 10%. The purified enzyme gave a single band on polyacrylamide gel electrophoresis (Fig. 1).

The content of selenium in the purified enzyme preparation was measured with an X-ray fluorescence analyzer. There were no detectable amounts of selenium in glutathione peroxidase from *M. hiemalis*, as shown in Table I. Furthermore, this analysis failed to detect other metals such as iron, copper and zinc (<1 ppm). The reduction rates of various hydroperoxides were also examined. The enzyme could reduce organic hydroperoxides such as cumene hydroperoxide and *t*-butyl hydroperoxide, but could not reduce hydrogen peroxide (Table II). It has been reported that selenium-dependent glutathione peroxidase I can use both organic and inorganic hydroperoxides as substrates, whereas selenium-independent glutathione peroxidase II can only use organic hydroperoxides.⁵⁾ *Mucor* glutathione peroxidase is selenium-independent and has the same substrate specificity as selenium-independent glutathione peroxidase II. However, *Mucor* glutathione peroxidase did not show glutathione *S*-transferase

activity, when it was measured by thioester formation between glutathione and 2,4-dinitrochlorobenzene according to the method of Habig *et al.* (Table II).⁶⁾ These results suggest that *Mucor* glutathione peroxidase is a new type of enzyme which differs from both selenium-dependent glutathione peroxidase I and glutathione *S*-transferase exhibiting selenium-independent glutathione peroxidase II activity.

Further characterization of *Mucor* glutathione peroxidase is now under way.

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