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# Effects of Diallyl Disulfide on the Hepatic Glutathione Peroxidase Activity in Rat

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## ABSTRACT

Glutathione peroxidase might play an important role in the protection of cellular structures against oxidative challange by hydrogen peroxide and several organic hydroperoxides.

It is widely accepted that allicin is biological active component of garlic, and allicin is easily degraded to diallyl disulfide and other components.

This study was attempted to elucidate the effect of diallyl disulfide on some biological activities.

It was observed that the activity of serum transaminase and glutathione level in liver were not changed by the treatment of diallyl disulfide. The liver cytosolic glutathione peroxidase activity was significantly enhanced. Whereas, mitochondrial enzyme activity was slightly increased. In the presence of diallyl disulfide in vitro, $V_{max}$ value of glutathione peroxidase for hydrogen peroxide was increased. On the other hand, Km value was not changed.

**Key Words:** Allicin, Diallyl disulfide, Glutathione, Glutathione peroxidase

## INTRODUCTION

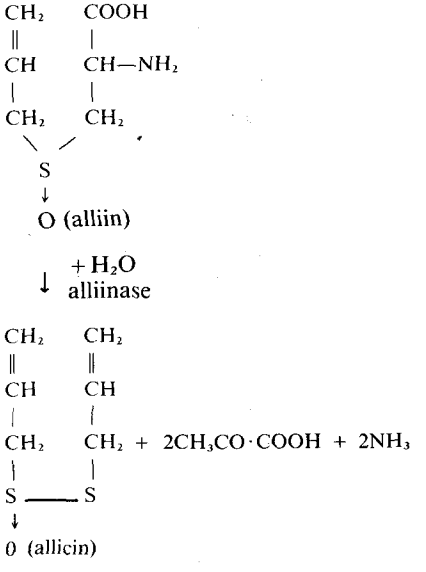
Glutathione peroxidase (EC 1.11.1.9) is found in a wide variety of organisms and tissues (Little *et al.*, 1970; Awasthi *et al.*, 1975).

This enzyme protects the cell from hydrogen peroxide and several organic hydroperoxides by reuction with concomitant oxidation of reduced glutathione (Flore *et al.*, 1973; McCay *et al.*, 1971; Rotrck *et al.*, 1973).

It is well known that diallyl disulfide is a breakdown product of allicin which is biological active component of garlic (Wills, 1956).

In the previous report in this laboratory, it was recognized that garlic water extracts increased hepatic glutathione peroxidase activity (Huh *et al.*, 1985). However, the action mechanism of garlic components have not been completely elucidated yet.

Therefore, this study was undertaken for further investigation of garlic components on the hepatic glutathione peroxidase activity.

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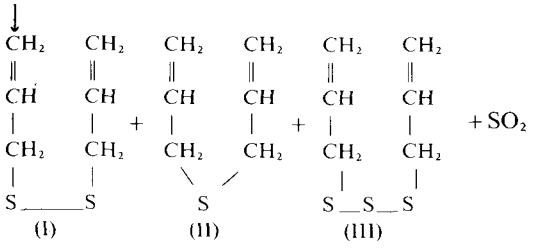

(I), diallyl disulfide; (II), diallyl sulfide; (III), diallyl trisulfide.

Degradation pathway of alliin.

## MATERIALS AND METHODS

### Chemicals

Bovine serum albumin, glutathione reductase and NADPH were obtained from Sigma chemical Co. 5,5'-dithiobis (2-nitrobenzoic acid) was purchased from Nakarai chemical Co. Diallyl disulfide was obtained from Tokyo Kasei chemical Co. and reduced glutathione from Fluka AG. The other reagents used were of reagent grade.

### Animals

Male Sprague-Dawley rats weighing 250g were used for all experiments. They were divided into 4 groups. One groups, the control, were given olive oil subcutaneously. The other groups were injected diallyl-disulfide (80, 120, 160mg/kg respectively) subcutaneously once daily for 3 days. All experimental animals were freely given water and commercial laboratory chow but deprived of the 16 hours prior to sacrifice.

### Preparation of mitochondrial and cytosolic fraction

The animals were sacrificed by exsanguination from the abdominal aorta. The liver exhaustively perfused with cold 0.15 M sodium chloride solution through the portal vein until uniformly pale and quickly removed. After trimmed and minced, the pieces of liver were homogenized with 4 volumes of cold 0.25 M sucrose solution. Each homogenate was centrifuged at 600×g for 10 min. The pellet was discarded and supernatant was centrifuged at 10,000×g for 20 min. The pellet was collected as mitochondrial fraction. The postmitochondrial fraction was further centrifuged at 105,000×g for 1 hour and resultant supernatant was used as the cytosolic fraction.

### Enzyme assay

Glutathione peroxidase activity was assayed spectrophotometrically under the conditions described by Paglia and Valentine (1967).

Enzyme activity defined as decreased NADPH n mole per mg protein per min at 25°C. Protein content was determined by the method of Lowry *et al.*, (1951) using bovine serum albumin as standard.

Serum transaminase activity was estimated according to the procedure described by Reitman and Frankel (1957). A unit of transaminase is expressed as the Karmen (1955) unit per ml of serum.

Glutathione content was determined by the methods of Owens *et al.*, (1965) and Mitchell *et al.*, (1973).

## RESULTS

### Effect of dially disulfide on the serum transaminase activity

Serum transaminase (ALT, AST) activities were not changed by the treatment of various concentration of diallyl disulfide compared with control group (Fig. 1).

### Effect of diallyl disulfide on the content of glutathione in liver

Fig. 2 shows the effect of diallyl disulfide on the glutathione level in the liver. In this figure, glutathione content was not altered by the treatment of diallyl disulfide for 3 days.

### Effect of diallyl disulfide on the glutathione peroxidase activity

In the liver cytosol of diallyl disulfide injected-rats, glutathione peroxidase activities were significantly increased by the injected concentration of diallyl disulfide (Fig. 3).

Meanwhile, the activity of mitochondrial enzyme was increased by the treatment of high concentration (160 mg/kg) of diallyl disulfide. Whereas, in the low concentration (below 120mg/kg) treated-rats, glutathione peroxidase activities were not induced (Fig. 4).

### Effect of diallyl disulfide on the activity of liver cytosolic glutathione peroxidase in vitro

The activities of liver cytosolic glutathione peroxidase were not affected by low concentration (below

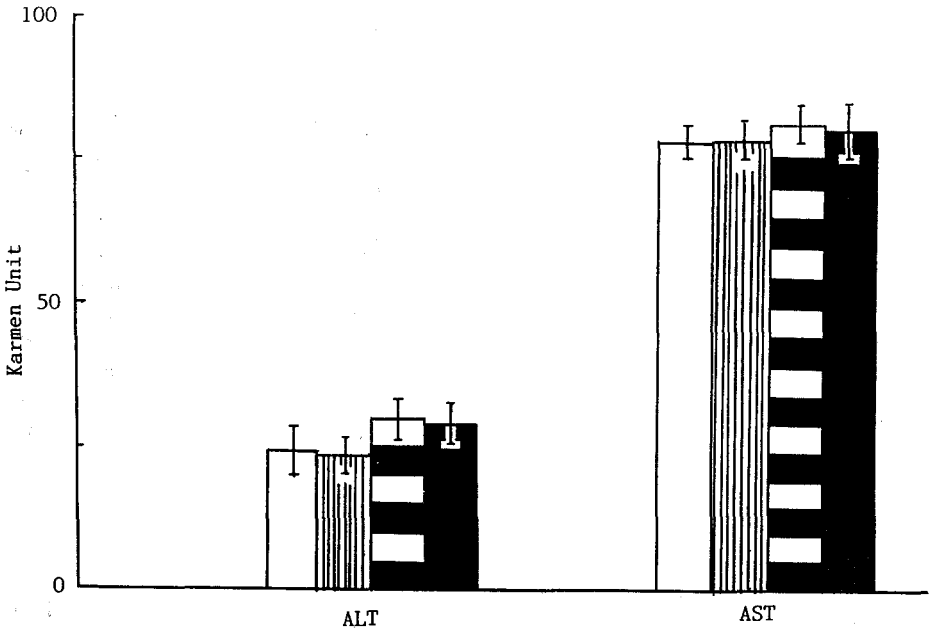


**Fig. 1.** Effect of diallyl disulfide on the serum transminase activity in rat. The assay procedure was described in the text. Values are mean ± SE of 5 animals in each group. ▯; Control, ▩; Diallyl disulfide 80 mg/kg, ▬; Diallyl disulfide 120 mg/kg, ■; Diallyl disulfide 160 mg/kg.

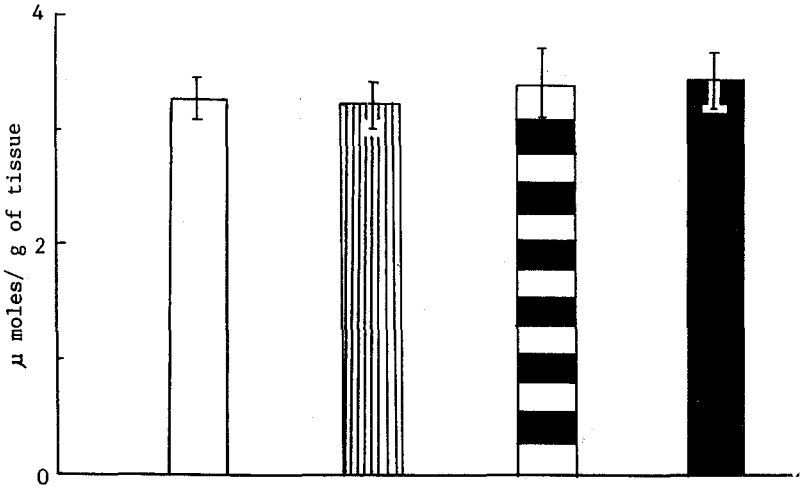


**Fig. 2.** Effect of diallyl disulfide on the content of glutathione in liver. The assay procedure was described in the text. The other conditions are the same as described in Fig. 1.

10 μg/ml) of diallyl disulfide. Whereas, in addition of higher concentration (above 15 μg/ml), glutathione peroxidase activities were powerfully increased (Fig. 5).

**Effect of diallyl disulfide on the kinetics of liver cytosolic glutathione peroxidase**

Fig. 6 shows the double reciprocal plots of glutathione peroxidase activity versus hydrogen peroxide concentration.

The $V_{max}$ value of glutathione peroxidase was in-

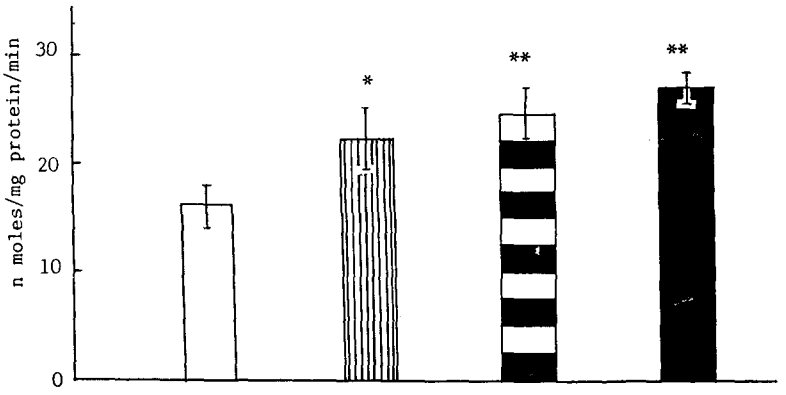


**Fig. 3.** Effect of diallyl disulfide on the liver cytosolic glutathione peroxidase activity in rat. The assay procedure was described in the text. The other conditions are the same as described in Fig. 1. *; $p<0.05$, **; $p<0.01$. Significantly different from control group.

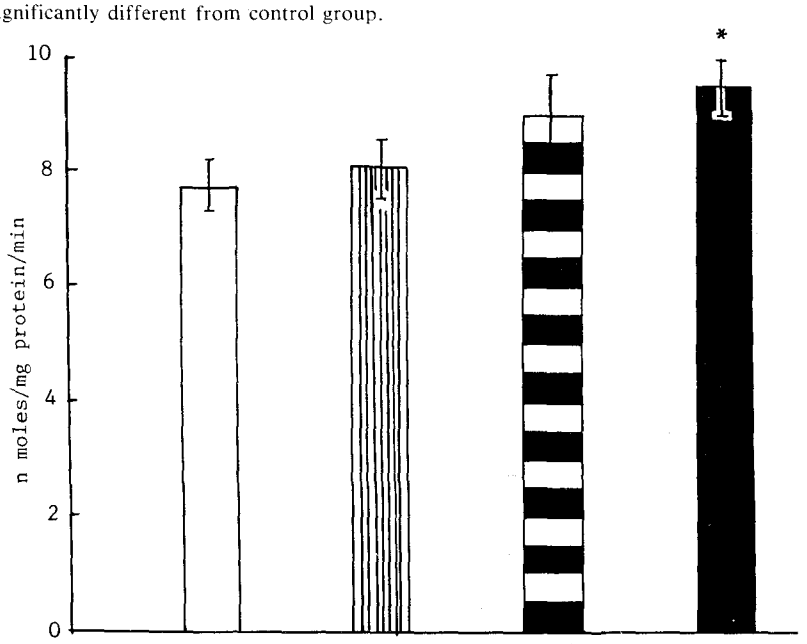


**Fig. 4.** Effect of diallyl disulfide on the liver mitochondrial glutathione peroxidase activity in rat. The other conditions are the same as described in Fig. 3.

creased to 67.7 nmoles/mg/min in the presence of diallyl disulfide (20μg/ml compared with control (45.5 nmoles/mg/min). On the other hand, Km Value was not changed (Fig. 6)

## DISCUSSION

It was observed that serum transaminase activity was not changed by the treatment of diallyl disulfide. Even though data were not published, histological changes were not found in these experiments. This result indicated that diallyl disulfide treatment to rat would not induced hepatocellular damage in these experimental conditions.

When diallyl disulfide was given for 3 days, liver cytosolic glutathione peroxidase activity was powerfully enhanced. As mentioned above, glutathione peroxidase not only protected cell organelles from hydrogen peroxide toxicity, but also prevented the

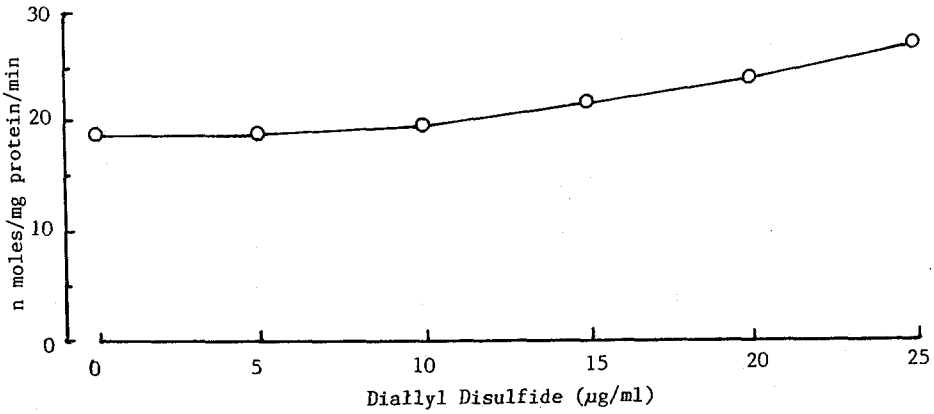


**Fig. 5.** Effect of diallyl disulfide on the hepatic glutathione peroxidase activity in vitro. The reaction mixture contained 0.1 M Tris-Hcl buffer, pH 7.2, various concentration of diallyl disulfide, 1 mM glutathione, 0.2 mM NADPH, 0.25 mM hydrogen peroxide, 2 IU glutathione reductase and cytosolic enzyme. Values are mean of 3 separate experiments.

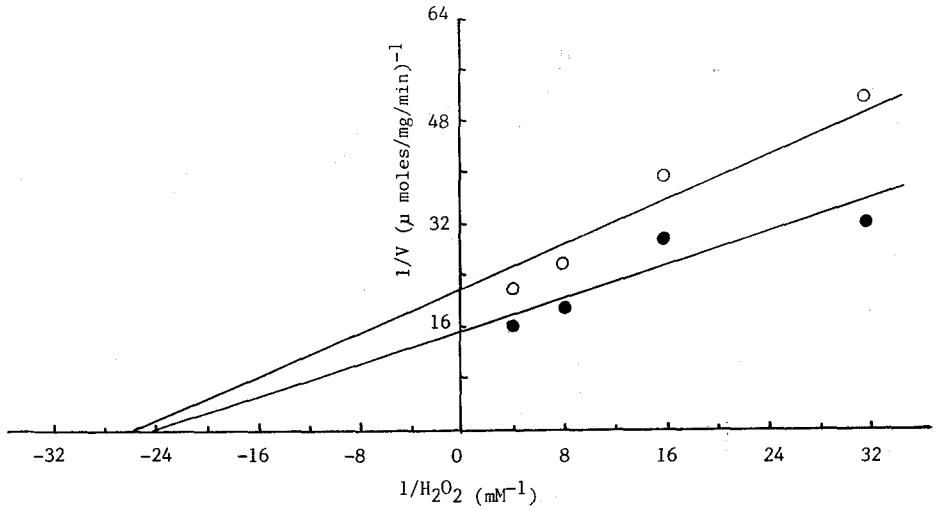


**Fig. 6.** Lineweaver-Burk plots of the hepatic glutathione peroxidase activity with hydrogen peroxide. The reaction mixture contained 0.1 M Tris-Hcl buffer, pH 7.2, various concentration of hydrogen peroxide, 1 mM glutathione, 0.2 mM NADPH, 2 IU glutathione reductase, diallyl disulfide (20 μg/ml) and cytosolic enzyme. Values are mean of 3 separate experiments. —○— Control, —●—; Diallyl disulfide 20 μg/ml.

autooxidation of structural lipids by cleaving lipid hydroperoxides, resulting in the disruption of the autocatalytic chain of lipid peroxidation (Ganther *et al.*, 1976; Lawrence *et al.*, 1976; McCay *et al.*, 1976; Prohaska *et al.*, 1977; Burk *et al.*, 1980; Reddy *et al.*, 1981). This result revealed that diallyl disulfide might prevent the cell damage due to oxidative stress by hydrogen peroxide and organic hydroperoxides. Data were not shown in this paper, it was also observed that hepatic lipid peroxide content and xanthine oxidase activity, superoxide generating system (Fridovich *et al.*, 1961, 1972; Kellogg *et al.*, 1975) were decreased by the treatment of diallyl disulfide. Thus, these observation strongly suggested that diallyl disulfide inhibited superoxide generating system and induced lipid hydroperoxide scavenging system. Meanwhile, glutathione is two types such as protein-bound and

free form in tissues (Kaplowitz *et al.*, 1979; Higashi *et al.*, 1985). In these experimental conditions, free glutathione content in the liver was not changed after administration of diallyl disulfide, indicating that the increase of glutathione peroxidase activity has no relation with the concentration of glutathione.

By the way, glutathione peroxidase activity was increased by the addition of daillyl disulfide in vitro. Further, $V_{max}$ without affecting the Km value for hydrogen peroxide was increased by the addition of diallyl disulfide. These increasing effects indicated that diallyl disulfide might affect the other site including allosteric effect in this enzyme.

In summary, the observation led us to conclude that the diallyl disulfide might modulate the hepatic glutathione peroxidase activity.

These findings also indicated that the action mechanism of garlic components might be associated with diallyl disulfide, one of the breakdown product of allicin. But more active researches in this field are needed.

## ACKNOWLEDGEMENT

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=국문초록=

## 흰쥐 간 Glutathione peroxidase 활성에 미치는 Diallyl disulfide의 영향

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허 근, 이상일, 박종민

마늘성분중의 하나인 diallyl disulfide가 간 glutathione peroxidase 활성에 어떠한 영향을 주는가를 관찰하여 다음과 같은 성적을 얻었다.

여러 농도의 diallyl disulfide를 흰쥐에 투여하였을때 혈청 transaminase의 활성은 변동이 없었으며 또한 간 glutathione의 함량도 변화되지 않았다. 그러나 간 가용성분획의 glutathion peroxidase 활성은 현저히 증가되었다. 시험관 내에서 간 가용성분획의 glutathione peroxidase의 활성은 15㎍/mℓ 이상의 diallyl disulfide를 첨가하였을때 현저히 증가되었으며, 동력학적인 측면에서 관찰하였을때 diallyl disulfide(20㎍/mℓ)에 의해 Km치는 별 변화가 없었으나 Vmax치는 현저히 증가되었다.

이상의 실험결과에서 마늘 성분중에 glutathione peroxidase 활성을 증가시키는 성분은 allicin의 분해산물인 diallyl disulfide에 기인된 것으로 사료되며 또한 이 성분은 효소의 기질결합 부위가 아닌 다른 부위에서 효소의 활성을 조절해 줄 것으로 생각되어진다.