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# Studies on Inhibition of Glyoxalase II by Synthetic and Natural Products

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Abbreviations: GLO, glyoxlase; S-LG, *S*-lactoylglutathione

## Abstract

**Inhibitors of glyoxalse I and II have been investigated as a possible approach to development of anticancer agents. In this study the effects of S-blocked glutathione derivatives and methanol-extracts of edible plants and medicinal herbs on glyoxalase II activity from bovine liver were assayed. It was found that the extracts of plants such as chestnut, walnut and wild walnut have strong inhibitory activity against glyoxalase II.**

**Keywords**: anti-tumor agent, glyoxalase II, glyoxalase inhibitors

## Introduction

The glyoxalase (GLO) system consisting of two enzyme components, GLO I (EC 4.4.1.5, lactoylglutathione methylglyoxal lyase) and II (EC 3.1.2.6, hydroxyacylglutathione hydrolase) catalyzes the conversion of methylglyoxal to D-lactate through an intermediate, *S*-D-lactoylglutathione (S-LG) (Neuberg, 1913; Dakin and Dudley, 1913; Racker, 1951). The physiological function of the GLO system is suggested to remove cytotoxic methylglyoxal from cells as D-lactate, but the exact function is still unknown. Methylglyoxal, the substrate of GLO I is produced as an unavoidable byproduct of normal metabolism (Richard, 1991). Vince and Daluge (1971) proposed that inhibitors of GLO I might function as anti-cancer agents by inducing high levels of methylglyoxal which has antiproliferative activity in cells (Apple and Greenberg, 1967; Egyad and Szent-Gyogyi, 1968; Jerzykowski *et al.*, 1970; Conroy, 1979; Raiffen and Schneidler, 1984). However, cancer chemotherapy with GLO I inhibitors is not sucessful yet, because specific GLO I inhibitors have not been developed thus far.

It has been reported that GLO II activity in tumor cells is markedly lower than in non-tumor cells and that S-LG, a substrate of GLO II, is toxic to human leukemia 60 cells in culture but not to differentiated neutrophils (Jerzykowski *et al.*, 1978; Thoralley, 1990). Accordingly it was suggested that S-LG and GLO II inhibitors may be prospective antitumor agents (Thornalley, 1990; Lo and Thornalley, 1992). Inhibition study on GLO II with natural product for development of antitumor agent has not been done so far. In this study, as a possible approach to development of antitumor agents from natural products, we describe inhibitory activity of methanol-extracts of some edible plants and medicinal herbs as well as synthetic S-blocked glutathione derivatives against GLO II of bovine liver.

## Materials and Methods

### Chemicals

S-LG, *S*-methylglutathione, *S*-hexylglutathione, *S*-propylglutathione, and *S*-(*p*-nitrobenzyl)glutathione were purchased from Sigma (St. Louis, U.S.A.).

### Methanol extraction of plants

Fifty to 300 g of 17 kinds of edible plant, such as walnut (*Juglans siebolidocha*), perilla (*Perilla frulescens Bitt. Var. awia*), chestnut (*Castanea crenata*), field murstard (*Brassica compestis*), *Ganoderma lucidum*, pepper (*Zanthoxylum piperitum*), sesame (*Sesamum indicum*), ginger (*Zingiber officinale*), soybean (*Glysine max*), eschallot (*Allium assalonicum*), lettuce (*Lantuca Saliva L. var. crispa*), lotus root (*Nolumbo nucifera*), cauliflower (*Brassica oleracea, L. var. botrytis*), bracken (*Ptieridium aquilinum var. japonicum*), ginko nut (*Ginko biloba*), *Codonopsis lanceolata*, and banana (*Musa paradisiaca var. sapientum*) were extracted with methanol at 90°C for 10 h. The extracts were filtered and concentrated (0.7 to 13 mg of raw material/μl). Dried methanol extracts of 11 kinds of medicinal herbs, poney (*Paenonia japonica*), barbersy root (*Jeffersonia dubia*), *Aralia continentalis*, sweet blay (*Acorus gramineus*), *Liriope platyphylla*, wild walnut (*Potamogeton fromchetii*), *Pogostemon cablin*, Ginseng (*Panax Schinseng*), *Disocorea batatas*, *Uncaria rhnchophylla* and myrsh (*Commiphora myrrha*) were kindly obtained from Institute of Natural Medicine, Hallym University.

### Assay of GLO II acivity and inhibitory effect

The activity of GLO was dertermined according to the method of Uolita (1973) or Principato *et al.* (1987) using purified bovine liver GLO II purified previously (Yang *et al.*, 1995) as an enzyme source in the presence or absence of *S*-blocked glutathione derivatives and the methanol-extracts. The inhibitory activity was expressed as $I_{50}$ which represents mg of raw materials (edible plant) or μg of dried extract (medicinal herbs) required for 50% inhibition under the standard assay conditions (pH 7.2, 100 mM Tris buffer and 2 mM S-LG) and is calculated by plotting $V_o$ (activity without extract)/$V_i$ (activity with extract) versus inhibitor concentration.

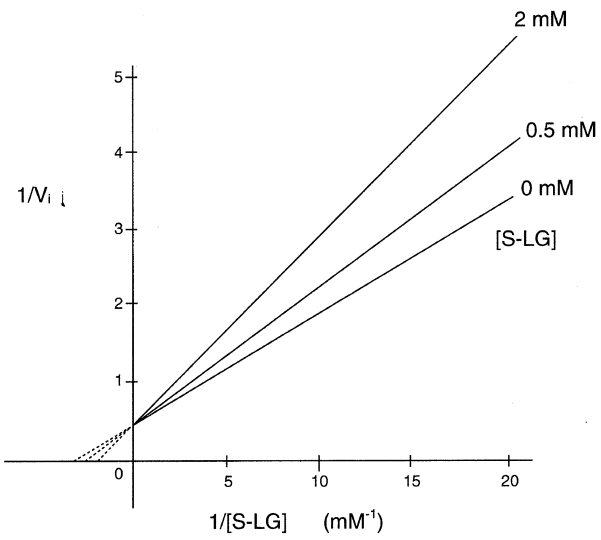


**Figure 1.** Lineweaver-Burke plot of the inhibition of GLO II by S-methylglutathione

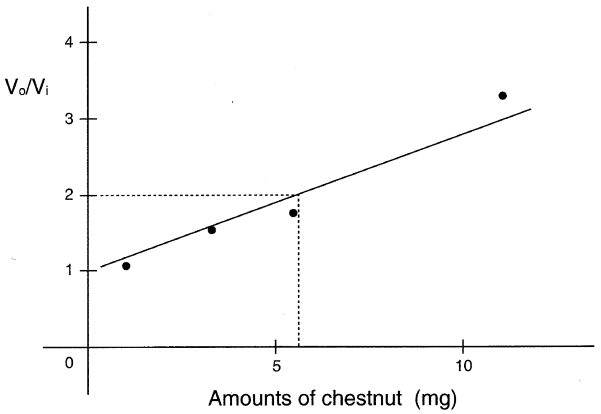


**Figure 2.** Inhibition of GLO II by methanol-extract of chestnut $V_o$ and $V_i$ represent the enzyme activity in the absence and presence of the extract, respectively.

## Results and Discussion

### Inhibition by S-blocked glutathione derivatives

Effects of S-blocked glutathione derivatives, *S*-methylglutathione, *S*-propylglutathione, *S*-hexylglutathione, and *S*-(*p*-nitrobebzyl)glutathione on GLO II of bovine liver were examined under the standard conditions.

As shown in Figure 1, *S*-methylglutathione inhibited competitively the enzyme activity with $K_i$ 1.33 mM. Other S-blocked glutathione derivatives except *S*-(*p*-nitrobenzyl)glutathione also inhibited similarly the enzyme activity (data not shown). It has been reported that GLO II from zea may is not inhibited by *S*-methylglutathione but inhibited by *S*-(*p*-nitrobenzyl) glutathione (Norton *et al.*, 1989) and that GLO II from rat liver is inhibited by *S*-methylglutathione with $K_i$ 2.6 mM, but not by *S*-(*p*-nitrobenzyl)glutathione (Principato *et al.*, 1989). These reports and our results indicate that the kinetic property of GLO II from plant is different from that of animals.

### Inhibition of GLO II by methanol-extracts

To screen natural inhibitors from plants we tested effects of methanol-extracts of various edible plants and medicinal herbs on GLO II activity. As shown in Figure 2 and 3, and Table 1 and 2, the extracts of several plants inhibited strongly GLO II activity. In the case of chestnut the $I_{50}$ calculated by plotting $V_o/V_i$ was the amount of extract corresponding to 5.6 mg of raw chestnut (Figure 2). High inhibitory activity was found in 7 species: walnut, chestnut, mustard, perilla seed, Ganoderma lucidum, black pepper and sesame (Table 1). Koshimizu *et al.* (1988) has screened edible plants for possible anti-tumor promoting activity by an *in vitro* short-term assay system of Epstein-Barr activation

**Table 1.** Inhibitory effects of methanol-extracts of some edible plants against GLO II

| Plant | $I_{50}$ | Plant | $I_{50}$ |
|---|---|---|---|
| Walnut | 4.2 | Ginger | 456 |
| Chestnut | 5.6 | Soybean | 480 |
| Mustard | 28.5 | Lotos root | 540 |
| Perilla (seed) | 44.8 | Banana | 546 |
| Ganoderma lucidum | 94 | Lettuce | 592 |
| Black pepper | 94 | Ginko nut | 672 |
| Sesame | 34 | Eschalot | 1460 |
| bracken | 231 | Cauliflower | 1700 |
| Codonopsis lanceolata | 451 | | |

$I_{50}$ is mg of raw materials required for 50% inhibition under the standard assay conditions and calculated by ploting Vo/Vi versus inhibitor concentration.

induced by a phorbol-ester promotor and found that the methanol-extracts of 14 species of the edible plants such as curled lettuce, field mustard, perilla, Japanese pepper, ginger, chestnut, and walnut strongly inhibited the activation. Extracts of some plants, such as chestnut, walnut, mustard and perilla showed both GLO II inhibitory effect and anti-tumor promoting activity. However, at present it is not clear whether there is any correlation between the inhibitory activity against GLO II of some plants in this study and the antitumor promoting activity of edible plants reported by Koshimuzu *et al.* (1988). However, our results suggested that inhibitors of GLO II, which would have anti-tumor acitivity, occur in a wide variety of edible plants.

We also tested the effects of methanol-extract of various medicinal herbs on GLO II activity. As shown in Figure 3, the extract of wild walnut strongly inhibited the enzyme activity ($I_{50}$ = 36 μg). Besides wild walnut, several medicinal herbs showed the inhibitory activity against GLO II (Table 2).

Several compounds, such as nucleotides, porphyrins, hydroxylated aromatic compounds and synthetic *S*-bloked glutathione derivatives have been studied as potent inhibitors of GLO (Kraus *et al.*, 1988; Bernard and Honek, 1989; Norton *et al.*, 1989; Lo and Thornalley, 1993). If any potent, natural inhibitor of GLO I and II could be obtained from edible plant and medicinal herbs, the inhibitor would be more desirable as anti-cancer agent than synthetic inhibitor, such as *S*-blocked glutathione derivatives.

This study showed that some plants, such as walnut, chestnut and wild walnut contain some compounds which have strong inhibitory activity against GLO II. However, the chemical nature of the compounds remains to be solved. Further studies on the purification of inhibitory compounds from the edible plants and medicinal herbs, and the effect on cancer cell growth would be worthy to be performed.

**Table 2.** Inhibitory effects of methanol-extract of some medicinal herbs against GLO II

| Herbs | $I_{50}$[a] | Herbs | $I_{50}$ |
|---|---|---|---|
| Wild walnut | 36 | Discorea batatas | 10920 |
| Poney | 204 | Myrsh | 17600 |
| Pogostemon cablin | 250 | Barbersy root | 19400 |
| Sweet blay | 2040 | Uncaria rhnchophylia | 28500 |
| Liriope platyphylla | 3540 | Ginseng | 75000 |
| Aralia continentalis | 6360 | | |

[a] mg of dried extract for 50% inhibition.

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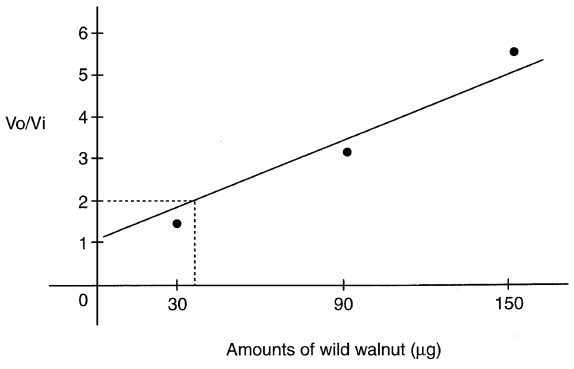


**Figure 3.** Inhibition of GLO II by methanol-extract of wild walnut

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