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Comparative Investigation of Glutathione S-Transferases, Glyoxalase-I and Alliinase Activities in Different Vegetable Crops

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Abstract

Glutathione *S*-transferases (GSTs, EC 2.5.1.18), glyoxalase-I (EC 4.4.1.5) and alliin lyase (alliinase, EC 4.4.1.4) are important enzyme systems in plant bodies. The first two are mainly detoxifying enzymes that utilize glutathione (GSH) in the defense mechanism, and the last one is mainly involved in secondary metabolism and relevant to sulfur compounds derived from GSH. The activities of the three enzymes have been investigated in soluble extracts of vegetable crops, including pumpkin, cabbage, broccoli, radish, carrot, potato, sweet potato, mungbean, and onion. GST activities were detected in all of the vegetables, and the extract of onion bulb exhibited the highest specific activity (648 nmol/min/mgP). The putative GSTs of most of the vegetables were found to be induced by ethanol. The activities of GSTs in onion bulb were found to be markedly inhibited by S-hexyl glutathione and were also inhibited by S-butyl glutathione and S-propyl glutathione. The anti-*Cm*GSTF1 antiserum recognized a thick band for putative onion GST. The estimated glyoxalase-I activity level was also high in onion bulb (4540 nmol/min/mgP), indicating that the thick band detected by Western blot analysis might result from partial recognition of glyoxalase-I by the antiserum. The specific activities for glyoxalase-I were moderate in radish and carrot, and the extracts of other vegetables had rather low levels of activities. The extract of onion also showed the highest specific activity level for alliinase (2069nmol pyruvate/mgP). The extracts of other vegetables also had alliinase activities, although the estimated values were much lower than that of onion.

Key words: enzymatic activity, glutathione, detoxification, induction, inhibition, vegetables

Introduction

Plants are continuously exposed to toxic compounds of endogenous and exogenous origins. They are also exposed to a combination of unfavorable environmental factors that frequently imposes constraints on growth and development. The ability of plants to cope with a range of environmental as well as chemical stresses is essential for cellular survival. As a result, and in the course of their evolution, plants have developed numerous unique adaptation and defense mechanisms to help them cope with unavoidable stresses that may be imposed upon them. One such form of defense mechanism is the development of an enzyme system for protection against potentially toxic effects of xenobiotics and reactive oxygen species generated during environmental stresses.

Plant glutathione *S*-transferases (GSTs, EC 2.5.1.18) are a family of multifunctional enzymes involved in the intracellular detoxification of xenobiotics and toxic compounds produced endogenously (Mannervik and Danielson, 1988; Edwards et al., 2000). Most of the enzymes are stress-inducible (Marrs, 1996) and play a role in the protection of plants from adverse effects of stresses (Marrs and Walbot, 1997). Basically, GSTs catalyze the conjugation of glutathione (GSH) to electrophilic molecules, which is followed by sequestration into vacuoles where they are further metabolized (Coleman et al., 1997; Walczak and Dean, 2000). Based on sequence similarity and gene organization, plant GSTs can be divided into four main classes (phi, zeta, tau and theta). The phi (F) class GSTFs and the tau (U) class GSTUs are mainly plant-specific; whereas, the zeta and theta classes are more phylogenetically widespread. However, the activities of different GSTs have been detected and characterized in many plants, including maize (Dixon et al., 1997), wheat (Edwards and Cole, 1996), tobacco (Droog et al., 1995), soybean (Andrews et al., 1997), barley (Romano et al., 1993), chickpea (Hunatti and Ali, 1990), peanut (Lamoureux et al., 1981), sorghum (Dean et al., 1991), and sugarcane (Singhal et al., 1991).

Several endogenous ketoaldehydes such as methylglyoxal (MG) are potent cytotoxic compounds that are produced mainly as nonenzymatic by-products of glycolysis (Richards, 1993) and can also be synthesized enzymatically (Martins et al., 2001). Endogenous production of MG has been reported in microorganisms, yeasts, animals and higher plants (Thornally, 1990; Yadav et al., 2005). However, the toxic MG

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in living cells is thereby detoxified by the action of glyoxalase-I (EC 4.4.1.5) through conversion to S-D-lactoylglutathione, which is then converted to D-lactic acid by glyoxalase-II (EC 3.1.2.6) (Racker, 1951). Glyoxalase-I has been purified and studied extensively in microbial (Kimura and Inoue, 1993) and animal systems (Jeryzkowski et al., 1978; Thornally, 1990). It has also been detected in some higher plants (Deswal et al., 1993; Deswal and Sopory, 1998) and in some cases it has been further characterized (Espartero, et al., 1995). On the basis of number of amino acid residues, plant glyoxalases-I are classified into short (upto 190 amino acids, such as, *Brassica juncea*, *Cicer arietinum*, *Glycine max*, etc.) and long (280-295 amino acids, such as, *Oriza sativa*, *Triticum aestivum*, etc.) length types (Johansen et al., 2000).

Garlic, onion, and related *Allium* species a have characteristic smell and taste caused by volatile sulfur containing flavour compounds, alk(en)yl-thio sulfinates. These are formed by the catalytic activity of alliin lyase (alliinase, EC 4.4.1.4) towards odorless and non-volatile cysteine-derived flavour precursors, *S*-alk(en)yl-L-cysteine sulfoxides (Lancaster and Boland, 1990). The alliinase enzymes have been purified to homogeneity and well characterized in various *Allium* species (Tobkin and Mazelis, 1979; Lancaster et al., 2000; Jones et al., 2004). Several members of Cruciferae have also been reported to show alliinase or similar (cystathionine lyase, EC 4.4.1.8) enzymatic activities (Ramirez and Whitaker, 1998; Kiddle et al., 1999).

It has been proposed that the biosynthesis of the flavor precursors proceeds via alk(en)ylation of the cysteine residue in glutathione followed by cleavage and oxidation to form alk(en)yl cysteine sulphoxide (Granroth, 1970). The tripeptide GSH is an important metabolite that can be utilized to detoxify exo- and endogenous toxins by the catalytic activities of some GSH-dependent enzyme such as GSTs and glyoxalase-I. In contrast, GSH conjugates, which are formed by the catalytic activity of GSTs, are rapidly metabolized in the vacuole to L-cystine conjugates (Coleman et al., 1997). Efflux of cysteine conjugates from the vacuole is followed by further metabolism by alliinase or similar enzymes to thiol and other related products in the cytosol (Kiddle et al., 1999). All of these phenomena suggest that there is an interrelationship between GSTs, glyoxalase-I, and alliinase.

A relationship among the above-mentioned three enzymes was revealed in our present studies while we were investigating their activities in soluble extracts of some vegetables. We detected the highest GST activity level in onion, and in Western blot analysis, a thick band for the putative GST was recognized by anti-CmGSTF1 antiserum. However, the band might have partially resulted from onion glyoxalase-I as the soluble extract showed the highest specific activity level for the enzyme. The GSTs and glyoxalase-I are detoxifying enzymes and their functions are relevant to GSH. Therefore, the highest activities of these enzymes are expected in onion bulb and this might suggest the relationship between the two enzymes. Onion bulb also showed the highest activity level for alliinase, a different kind of enzyme but its activity is relevant to sulfur compounds derived from GSH.

The main function of GSTs is detoxification of herbicides and endogenous toxins, and the existence of the proteins in vegetables might therefore increase the quality of food. The quality of food might also be associated with glyoxalase enzymes that detoxify toxic methylglyoxal. On the other hand, there is evidence suggesting that the taste and aroma of *Allium* vegetables are associated with the enzyme alliinase. Though, there are some reports on the activity and characteristics of a particular enzyme in a specific plant but the comparative information among GST, glyoxalase-I and alliinase activities in a plant or a group of plants is scarce. In this paper, we report the levels of activities of the three enzymes in different vegetable crops extracted under same condition. Here we also describe the effect of ethanol vapor on different putative GSTs and their cross-reactivity with anti-pumpkin GST antisera.

Materials and Methods

Plant materials

Mature pumpkin (*Cucurbita maxima*) fruit, cabbage (*Brassica oleracea* var. *capitata*), broccoli (*Brassica oleracea* var. *Italica*), radish (*Raphanus sativus*), carrot (*Daucus carota*), potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatus*), mungbean (*Vigna radiata*) seedling, and onion (*Allium cepa*) bulb were collected from a local market. The outer skin of pumpkin fruit, radish, carrot, sweet potato, and potato were peeled off and disc-shaped pieces (approx. 3mm thick) were used. A portion of the branchlet with floret of the broccoli inflorescence, leaves of cabbage, whole part of mungbean seedlings and a portion of onion bulb were used for extraction.

Preparation of soluble extracts

The plant materials were homogenized with proportional volumes (eg. 5ml for 5g plant material) of 50mM potassium phosphate buffer (pH 7.0) containing 100 mM KCl, 1% (w/v) ascorbate and 10% (w/v) glycerol using a mortar and pestle. The homogenates were centrifuged at 11,500 x g for 10min and the supernatant was used as the crude enzyme solution. All procedures were performed at 0 - 4°C.

Assay of enzymes activities and protein quantitation

GSTs activities were determined spectrophotometrically by the method of Booth et al. (1961) with some modifications. The reaction mixtures contained 100mM potassium phosphate buffer (pH 6.5), 1.5mM reduced glutathione, 1mM 1-choloro-2,4 dinitrobenzene (CDNB), and enzyme solution in a final volume of 0.7ml. The enzyme reaction was initiated by the addition of CDNB, and 340-nm absorbance was monitored at 25°C for 1min.

Glyoxalase-I assay was carried out according to the method of Chakravarty and Sopory (1998) with slight modification. Briefly, the assay mixture contained 100mM sodium phosphate buffer (pH 7.5), 15mM magnesium sulphate, 1.7mM glutathione and 3.5mM methyl glyoxal. The reaction was started by the addition of enzyme solution. The formation of thioester was measured by observing absorption at 240nm for 1min.

Alliinase activity was measured in terms of total pyruvate production using the method described by Fujita et al. (1990). A total of 0.5ml reaction mixture consisted of 50mM potassium phosphate buffer (pH 7), 30mM S-ethyl-L-cysteine sulfoxide as substrate, and an enzyme extract. The mixture was incubated at 30°C for 2min and the reaction was stopped by the addition of 2 N HCl containing 2% (w/v) 2,4-dinitrophenylhydrazine. After incubation at 30°C for 10min, 3ml of 0.6 N NaOH was added to the solution and the absorbance at 420nm was measured. The concentration of total produced pyruvate

was compared against a standard curve of pyruvic acid treated in the same way, except that distilled water was used instead of substrate and buffer solutions.

Protein concentration of each of the soluble extracts was determined by the method of Bradford (1976) with bovine serum albumin as the protein standard.

Ethanol vapor treatment

Equal portions of the above stated plant materials and shoots of six-day-old pumpkin seedlings were arranged on stainless-steel nets. The metal nets were placed inside two plastic boxes lined with paper towels dipped in a small amount of deionized water. One small plastic container with 5ml of 100% ethanol was placed in the middle of the net in one box and another was supplied with 5ml of distilled water to serve as control. The two boxes were covered and incubated at 25°C for 24h. After incubation, the enzyme solution was prepared from each of the plant materials using the previous extraction method.

SDS-PAGE and western blotting

The crude enzyme solution was separated on 12.5% SDS-polyacrylamide gels according to the method of Laemmli (1970). Before loading on to the gel, protein samples were mixed with loading buffer (60mM Tris-HCl containing 2% SDS (w/v), 5% beta-mercaptoethanol and 10% sucrose) and incubated for 2min in a boiling water bath. Proteins were then transferred onto nitrocellulose membranes using an ADVANTEC EB-100 semi-dry transfer unit (Osaka, Japan) in accordance with the conditions suggested by the manufacturer. The membranes were blocked with 5% (w/v) non-fat dry milk in phosphate-buffered saline (0.15 M NaCl, pH 7.5) containing 0.05% (v/v) Tween-20 (PBS-T) for 15min with gentle shaking at room temperature and kept at 4°C overnight in the same solution. The membranes were washed according to the Western Blot Reagents and Protocol (Perkin Elmer Life Sciences, Inc. Boston, MA) and then incubated with a primary antiserum for 1 h at room temperature with gentle shaking. Here, the antisera of pumpkin GSTs (respective anti-rabbit sera) were used as primary antisera (i.e., anti-*Cm*GSTU1, anti-*Cm*GSTU2 and anti-*Cm*GSTF1 antisera). The primary antisera were diluted with PBS-T in ratios of 1:250, 1:1000 and 1:500, respectively (Fujita et al., 1994; Hossain and Fujita, 2002). Horseradish peroxidase-labeled goat anti-rabbit IgG (Jackson Immuno Research, West Grove, PA) diluted to a ratio of 1:1000 with PBS-T was used as the secondary antiserum. After five washes, the membranes were developed using ECL reagents (Perkin Elmer Life Sciences, Inc.) for 1 min and exposed to FUJI X-ray film.

Results and Discussion

GST activities in different vegetable crops

We first examined the GST activities in the soluble extracts of several vegetable crops towards the model substrate CDNB. We found that onion bulb had a higher level of specific GST activity (648 nmol/min/mgP) than the activity levels of other vegetables (Fig.1). Several research groups have detected activities of GSTs in different organs of the onion plant (Fatima and Ahmed, 2005; Lamoureux and Rusness, 1980; Schroder and Stampfl, 1999) but their levels of activities have not been compared with those of other plant species.

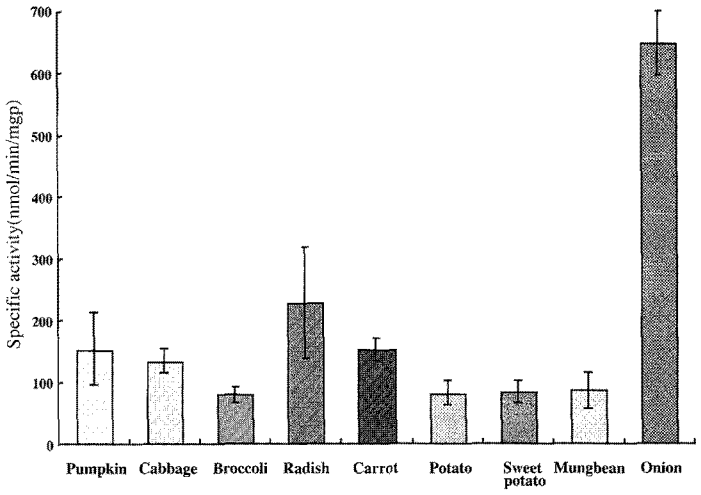

Fig.1. Specific activities of GST in soluble extracts of different vegetable crops. Results were obtained from three independent experiments and bars indicate standard error.

However, in this experiment, the higher GST activity in onion might have resulted from some organosulfur compounds that are present in the soluble extract. Studies have shown that allylsulphides and disulfides or allylcysteine found in garlic and onion are able to induce GSTs (Hatono et al., 1996; Sparnins et al., 1988; Wargovich, 1997).

Next to onion, radish showed a high level of specific activity for GST(229nmol/min/mgP) followed by pumpkin fruit (154nmol/min/mgP), carrot (153nmol/min/mgP), and cabbage (135nmol/min/mgP). The extracts of broccoli, potato, sweet potato and mungbean had rather low and similar level of activities, estimated to be about 80nmol/min/mgP. Detailed reports on physiological properties of GSTs in vegetables are limited; however, their activities have been detected in potato (Hahn and Strittmatter, 1994), broccoli (Lopez et al., 1994), pea (Frear and Swanson, 1973), and French bean (Edwards and Dixon, 1991). In addition, a number of biochemical studies on pumpkin GSTs have been completed in our laboratory (Fujita and Hossain, 2003ab; Fujita et al., 1994, 1998; Hossain and Fujita, 2002).

The high GST activity levels in the cytosol extracts from radish and carrot might be due to the presence of the sulfur compound isothiocyanate which has been reported to be present in crucifers and to detoxify chemical carcinogens through stimulation of GSTs (Wargovich, 1997). Although the specific GST activity level in broccoli was low, the estimated total GST activity level was found to be high (664nmol/min/g fr. wt.) due to presence of an adequate amount of total protein (13-fold greater than that in onion, data not shown) in the extract. The estimated total GST activity level in onion bulb was 395nmol/min/g fr. wt.

The higher level of GSTs activities in vegetables extracts suggested that the enzymes are capable of detoxifying a maximum amount of toxins in the presence of sufficient GSH. Beside detoxification, they also play important role in other physiological functions such as GSH peroxidase activity (Bartling et al., 1993; Mannervik and Danielson, 1988) and intracellular binding and transport of phytochemicals (Edwards et al., 2000).

Onion and related *Allium* species are characterized by a variety of flavor precursors, and their biosyntheses proceed from cysteine via glutathione (Jones et al., 2004). The tripeptide GSH plays a key role in the detoxification of electrophilic substrates by the catalytic activity

of GSTs. Therefore, the existence of GST activity in onion bulb is reasonable. However, the highest specific GST activity level in onion extract is unexpected since the quantity of total GSH in onion has been reported to be less than that in other vegetables (Nakagawa et al., 1986). To check this ambiguous result, we compared the putative GST band of onion with those of other vegetables by Western blot analysis.

Detection of putative GSTs by western blotting

We carried out Western blot analysis to determine the cross reactivity level of anti-pumpkin GST antisera with other vegetable GSTs. An important characteristic of plant GSTs is that most of them are induced by ethanol. Therefore, in this experiment we treated the plant materials with ethanol vapor and compared its effect with the control.

The results (Fig. 2) showed that each anti-pumpkin GST antiserum recognized some protein bands in most of the vegetable extracts that could be referred to as putative GSTs as they were located in a position similar to that of pumpkin GSTs band in SDS-PAGE. The results also revealed that ethanol vapor considerably induced the expression of GSTs in pumpkin seedling and fruit, cabbage, sweet potato and onion. The putative GSTs of other vegetable species were found to be less responsive to ethanol. The role of ethanol in plant GST induction has also been studied in different agricultural crops, including maize (McGonigle et al., 2000) and wheat (Cummins et al., 1997; Dixon et al., 1998).

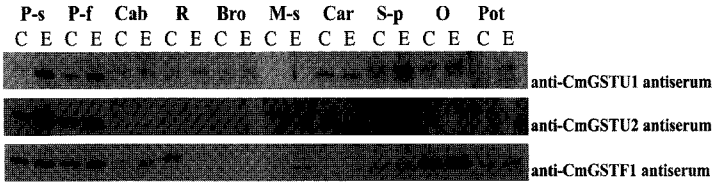

Fig.2. Detection of putative GSTs of various vegetable crops by Western blot analysis. Anti-pumpkin GST antisera were used as primary antisera. Each of the vegetables (P-s: pumpkin seedling; P-f: pumpkin fruit; Cab: cabbage; R: radish; Bro: broccoli; M-s: mungbean seedling; Car: carrot; S-p: sweet potato; Pot: Potato and O: onion) was incubated for 24 h with(E) and without (C) ethanol vapour. Each lane was loaded with 10μg protein.

The GST signals in pumpkin seedling and fruit, cabbage, radish, carrot, sweet potato, potato, and onion were detected by anti-*Cm*GSTU1 antiserum. Some of the species, e.g., sweet potato and potato showed multiple GST bands. The anti-*Cm*GSTU1 antiserum could not bind to any GST in broccoli and mungbean (Fig. 2).

When treated with anti-*Cm*GSTU2 antiserum, the putative GST bands were only detected in pumpkin seedling and fruit. No clear GST bands for either controls or ethanol treatment for any of the other vegetables were detected. The anti-*Cm*GSTF1 antiserum could detect GST bands in pumpkin seedling and fruit, cabbage, radish, mungbean, sweet potato, potato, and onion. In radish, a band could only be detected in the control, while no bands are detected in broccoli either for the control or ethanol treatment (Fig. 2). However, the anti-*Cm*GSTF1 antiserum cross-reacted strongly with the putative GST of onion as its band was found to be much thicker compared to that of other vegetables.

Inhibition of the activities of onion GSTs

The activities of GSTs have been reported to be inhibited by a variety of substances (Droog et al., 1995; Fujita and Hossain, 2003b). A survey report of the recent publications reveals that some derivatives of glutathione act as GST inhibitors (Lucente et al., 1998). We therefore carried out an experiment to check the inhibitory potencies of some derivatives of GSH towards onion GSTs. In this experiment, we used soluble protein from onion bulb precipitated with $(NH_4)_2SO_4$ at 65% saturation followed by dialyzing overnight against 10 mM Tris-HCl (pH 8.0) containing 0.01% (v/v) β-mercaptoethanol.

Among the derivatives, *S*-hexyl glutathione was found to be a potent inhibitor of onion GSTs showing 59.67% inhibition at a concentration of 0.1 mM (Table 1). The same concentrations of *S*-butyl glutathione and *S*-propyl glutathione showed 45 and 24.33% inhibition, respectively. *S*-methyl glutathione and *S*-lactoyl glutathione showed negligible inhibitory effects on activities of onion GSTs. The onion plant has been reported to be a rich source of a variety of sulfur compounds (Jones et al., 1994); therefore, some of those thiols, particularly GSH derivatives, can reduce the xenobiotic (like CDNB) conjugating activities of onion GSTs by acting as physiological substrates of the enzymes.

Table 1. Inhibition of activities of onion GSTs toward CDNB by derivatives of glutathione. Values are means of three independent experiments (±SE).

Chemicals	Inhibition (%)	
	0.05 mM	0.1 mM
S-methyl glutathione	-1.67 (±3.84)	2.33 (±2.73)
S-propyl glutathione	12.33 (±5.90)	24.33 (±5.46)
S-butyl glutathione	26.67 (±5.81)	45.00 (±5.2)
S-hexyl glutathione	47.33 (±5.33)	59.67 (±3.48)
S-lactoyl glutathione	4.33 (±4.41)	7.33 (±2.67)

Glyoxalase-I activities in different vegetable crops

Previously, in our laboratory, during screening of a pumpkin cDNA library using anti-*Cm*GSTF1 antiserum, we found that the antiserum also recognized a short type pumpkin glyoxalase-I (unpublished data) which plays a role in GSH-dependent detoxification of cytotoxic MG, a compound that is synthesized in living cells spontaneously as well as in stress conditions. Therefore, we checked glyoxalase-I activities in different vegetable extracts, including onion extract.

The results (Fig. 3) revealed that all of the vegetables under the study exhibited glyoxalase-I activities, and among them, onion bulb

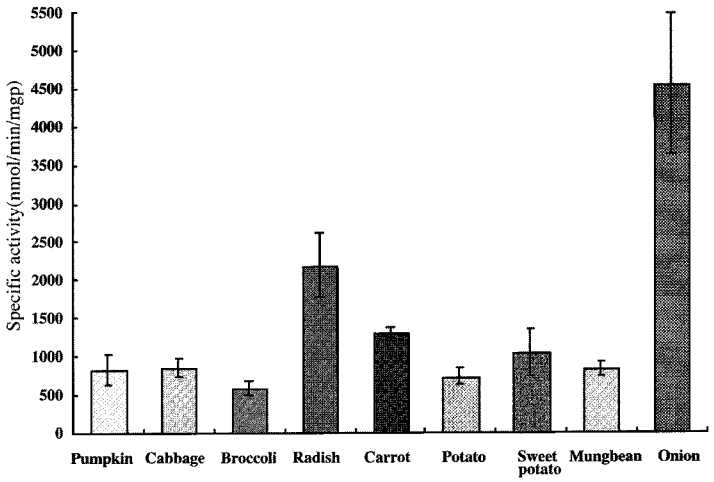

Fig.3. Specific activities of glyoxalase-I in soluble extracts of different vegetable crops. Results were obtained from three independent ex-periments and bars indicate standard error.

showed the highest level of specific activity (4540nmol/min/mgP). Therefore, the thick band of onion detected in Western blot analysis (Fig. 2) might be partially caused by additional recognition of the antiserum for glyoxalase-I. The soluble extracts from radish had the second-highest level of glyoxalase-I specific activity (2196nmol/min/mgP), followed by carrot (1313nmol/min/mgP) and sweet potato (1045nmol/min/mgP). Among all vegetables, broccoli exhibited the lowest level of specific activity (606nmol/min/mgP), but the estimated total activity was even higher (5029nmol/min/g fr.wt.) than that in onion (2969nmol/min/g fr. wt.). Hopkins and Morgan (1945) reported the existence of a glyoxalase system in 19 plant species, but they did not find significant differences among the enzymatic activities of cabbage, cauliflower, radish, potato, and onion. They estimated the combined activities of the enzymes glyoxalase-I and II based on the determination of the rate of CO2 evolution from sodium bicarbonate solution due to the lactic acid production; thus, their findings are not similar to those in our experiment. There have been some biochemical studies on glyoxalse-I in mustard (Deswal and Sopory, 1991, 1998; Veena et al., 1999) and groundnut (Jain et al., 2002), though their activities have been detected in several other higher plants (Thornally, 1990).

The existence of glyoxalase-I activities in vegetables suggest their tolerance mechanisms against MG, which occurs endogenously and is toxic to cells as it arrests growth (Szent-Gyorgi et al., 1967), reacts with proteins and nucleic acids (Thornally, 1996), and inactivates the antioxidant defense system (Martins, 2001). Beside detoxification, the glyoxalase system has been reported to influence cell division and proliferation (Deswal et al., 1993; Paulus et al., 1993; Thornalley, 1993). Glyoxalase-I might also play role in a stressful condition since its level has been reported to be increased by various stresses (Espartero et al., 1995).

Alliinase activities in different vegetable crops

Results of previous studies revealed that the onion bulb has a high level of GST as well as glyoxalase-I activities. Several research groups reported the role of a different kind of enzyme, alliinase, in onion, the activity of which is relevant to sulfur compound derived from glutathione (Nock and Mazelis, 1989; Tobkin and Mazelis, 1979). It is an important enzyme in secondary metabolism that catalyzes the conversion of *S*-alk(en)yl-L-cysteine sulfoxides (flavor precursors) into corresponding thio-sulfinates that are responsible for flavor and taste characteristics. This reaction also produces pyruvate and ammonia as byproducts. Besides onion, some other higher plants have also been reported to have alliinase or similar enzymatic activities (Ramirez and Whitaker, 1998). Therefore, we checked alliinase activities in some vegetable crops including onion.

In this study, we measured the alliinase activities in soluble extracts of different vegetables in terms of total pyruvate production (Fig. 4). Like GST and glyoxalase-I, the extract of onion bulb showed a higher level of specific alliinase activity (2069nmolpyruvate/mgP). The alliinase found in onion has been investigated in detail (Nock and Mazelis, 1987; Tobkin and Mazelis, 1979) and its activity has been found to be sufficient for the conversion of S-alk(en)yl-L-cysteine sulfoxides into the corresponding thio-sulfinates (Clark et al., 1998; Keusgen et al., 2002; Schwimmer and Mazelis, 1963).

Alliinase activity was also detected in other vegetable species (Fig. 4). However, the extracts of radish, pumpkin, carrot and sweet potato

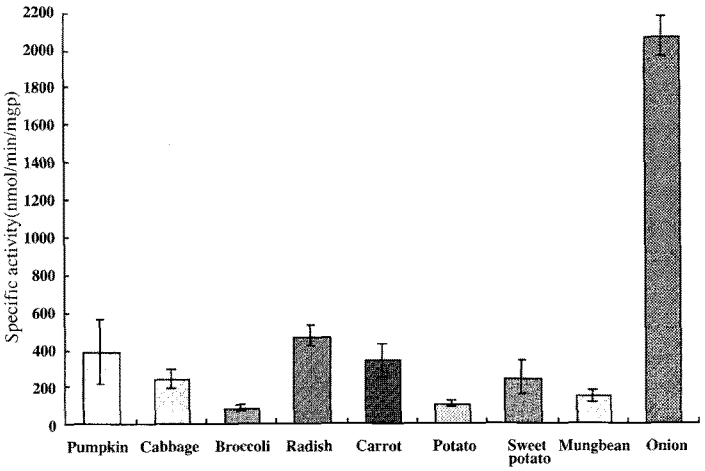

Fig.4. Specific activities of alliinases in soluble extracts of different vegetable crops. Results were obtained from three independent ex-periments and bars indicate standard error.

had only moderate specific activities, and broccoli showed the highest total activity (739nmol pyruvate/g fr. wt.). Several cruciferous vegetables (e.g., cabbage, broccoli, radish, etc.) are abundant in the flavor precursor S-methyl L-cysteine sulphoxide (Kubec et al., 2001) and thus often tend to have high level of alliinase or C-S (cysteine sulfoxide) lyase activities (Hamamoto and Mazelies, 1986; Kiddle et al., 1999; Ramirez and Whitaker, 1998). The levels of alliinase activities in the extracts of other vegetables were much lower than that in onion bulb, but their existence is not unexpected as the vegetables might contain flavor precursors.

Interestingly, most of the vegetables examined do not have an onion-like flavor but they have alliinase activities. The strong pungency of onion, however, is a result of the activity of a second enzyme, lachrymatory-factor synthase, following alliinase action on *S*-transprop-1-enyl cysteine sulphoxide, the major flavor precursor of onion (Imai et al., 2002). *S*-methyl L-cysteine sulphoxide, the common flavor precursor of most vegetables gave odors that are generally described as 'cabbagy' or 'fresh onion' (Jones et al. ,2004).

Alliinase in plants is involved in primary (amino acid biosynthesis) and secondary (non-protein amino acids and xenobiotic) sulfur metabolism. In crucifers and related species, allinase is involved in glucosinolate and cyanogenic glucoside biosynthesis (Kiddle et al., 1999). The roles of the flavor compounds as well as alliinase also include defense against pests and predation, and storage and transportation of carbon, nitrogen, and sulphur (Lancaster and Boland, 1990). They might have a role in prolongation of shelf-life since mild-flavored onions have been reported to have poorer storage properties (Jones et al., 2004). In animals, C-S lyase (an alliinase-like enzyme) is also involved in xenobiotic detoxification, but such information is limited in plant (Lamoureux and Rusness, 1990).

Conclusion

A comparison of the activities of GSTs, glyoxalase-I, and alliinase in various vegetables is described in this paper. The activities of the three enzymes were detected in all of the vegetables; however, their specific activities were highest in onion bulb. The anti-*Cm*GSTF1

antiserum cross-reacted strongly with the putative GST of onion. The putative GSTs of most of the vegetables were induced by ethanol and, contrarily, some derivatives of glutathione were found to be potent inhibitors of onion GSTs. The extracts from radish and carrot showed moderate levels of enzymatic activities, and other vegetables showed low levels of enzymatic activities. These results suggest that different vegetables have different degrees of tolerance to biotic, abiotic and chemical stresses, and have different abilities to convert *S*-alk(en)yl-L-cysteine sulfoxides into the corresponding thiosulfinates. The results of this experiment suggest a more detailed investigation on onion GSTs and glyoxalase-I.

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