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A Glutathione *S*-transferase Elutes with Glyoxalase-I (Gly-I) During Purification of Gly-I from Maize (*Zea mays* L.) Seedlings

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Abstract: In this study an attempt was taken to purify Glyoxalase-I (Gly-I: E.C., 4.4.1.5), from maize seedlings. Both green and non-green parts of 7 day old maize seedlings were used as plant materials. Crude proteins were precipitated by 65% $(NH_4)_2SO_4$, and dialyzed overnight. The dialyzate was applied on DEAE-cellulose chromatography and eluted with linear gradient of KCl from 0 to 0.2 M. In both cases, Gly-I eluted at approximately 85 mM of KCL. The active Gly-I fractions were pooled and applied on a hydroxylapatite chromatography and eluted with 0-40 mM potassium-phosphate buffer, but the eluted fractions showed very poor activity. Therefore, the active pooled fraction of DEAE-chromatography was then applied directly on affinity chromatography (*S*-hexyl glutathione-agarose) for final purification and eluted with 1.2 mM of *S*-hexyl glutathione. The purified protein from green and non-green part had specific activity of 33.23 and 39.25 μmol min^{-1} mg^{-1} protein, respectively, along with recovery of 1.47 and 162, respectively, and yield of 83.11 and 68.15, respectively. In SDS-PAGE, the active purified affinity fraction was found to move with another protein. The spectrophotometric analysis of high active Gly-I fractions from DEAE-cellulose and affinity chromatography showed GST [another detoxifying enzyme (E.C., 2.5.1.18)] activity. This result suggested that one of the adjacent protein bands in SDS-PAGE was due to presence of a GST in Gly-I fraction.

Keywords: Glyoxalase-I Purification, Glutathione *S*-transferase, Simultaneous Elution, Maize

1. Introduction

Growth of plants are continuously hampered by abiotic stresses such as salinity, drought, heavy metal toxicity and extreme temperatures that reduce crop yield by more than 50% worldwide [1]. The scenario is likely to be more aggravated by the predicted forthcoming global changes in climate. Understanding the importance of developing stress-resistant crops with sustainable growth and productivity under stress condition, stress tolerance mechanism can help in developing stress-resistant crops. However, abiotic stress tolerance is multigenic origin of adaptive response and still not fully understood [2, 3]. Under abiotic stresses, over production of reactive oxygen species (ROS) and methylglyoxal (MG) is a common phenomenon in plants [4-12]. ROS can damage cells as well as initiate responses such as new gene expression to protect from oxidative damage. On the other hand, MG produced under abiotic stress condition is highly toxic to plant cell. It can damage and modify proteins, lipids, carbohydrates and DNA which ultimately results in cell death. Therefore, MG must be detoxified in plant cell to survive. MG metabolism in eukaryotes by the glyoxalase system comprises of two enzymes, glyoxalase-I (Gly-I) and glyoxalase-II (Gly-II) (Fig. 1). Gly-I catalyzes the formation of *S*-lactoylglutathione (SLG) from MG in presence of reduced

glutathione (GSH), while Gly-II catalyzes the hydrolysis of SLG to regenerate GSH and liberate D-lactate [8].

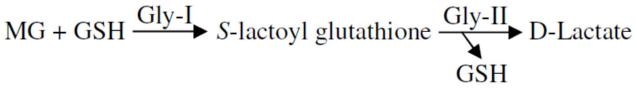

Fig. 1. *MG detoxification mechanism of glyoxalases.*

Recent investigations in plants have brought new developments in the involvement of the glyoxalase system in stress tolerance and its involvement with oxidative defense systems. This pathway has been reported from a diverse group of organisms, including humans, mice, protozoa, fungi, bacteria and plants. Recently, it has been reported that MG levels were increased significantly in plants in response to salinity, drought and cold stresses [6-8, 12]. External stimuli like hormones (auxins, cytokinins, etc.) and blue light also increase Gly-I activity [13]. Conversely, inhibition of cell growth resulted in lower levels of Gly-I activity [14-16]. Gly-I from tomato and Brassica were shown to be upregulated under salt, water and heavy metal stresses [4, 12, 17]. Though, the physiological role of glyoxalase system in higher plant has been studying in different plants under abiotic stress [4, 6, 7, 18-22], the number of purification report on Gly-I is very few. Recently, role of glyoxalse under salinity has been reported in maize [12], but Gly-I has never been considered to purify from maize. To examine the accumulation of the protein in maize under salinity, purification and production of polyclonal antibody are important. With this view, first step was undertaken to purify Gly-I from maize seedlings.

2. Materials and Methods

2.1. Plant Materials

The green part (leaf) and non-green part (except leaf) of 7 day old maize seedlings of BARI Hybrid Maize 7 were as plant materials.

2.2. Extraction of Crude Protein for Gly-I Purification

Fifty gram tissue (green part and non-green part separately) were homogenized in an equal volume of 25 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, 1% (w/v) ascorbate and 10% (w/v) glycerol with Waring blender. The homogenates squeezed in a nylon cloth and was centrifuged at 11500 × *g* for 15 min, and the supernatant was used as crude enzyme solution.

2.3. DEAE-Cellulose Chromatography

Proteins were precipitated by ammonium sulfate at 65% saturation from the supernatant and centrifuged at 11,500 × *g* for 10 minutes. The proteins were dialyzed against 10 mM Tris-HCl buffer (pH 8) containing 0.01% (w/v) β-mercaptoethanol and 1 mM EDTA (buffer A) overnight to completely remove low molecular inhibitors. The dialyzate was applied to a column (1.77 cm i.d. × 20 cm) of DEAE-cellulose (DE-52, Whatman, UK) that had been equilibrated with buffer A and eluted with a linear gradient of 0 to 0.2 M KCl in 600 ml of buffer A. The active eluted Gly-I peak was collected for further purification.

2.4. Hydroxylapatite Chromatography

The high active pooled sample of Gly-I, separated by DEAE-cellulose column chromatography, was applied on a hydroxylapatite column (1.5 cm i.d. × 5.5 cm) that had been equilibrated with buffer A. The column was eluted with a 300 ml linear gradient of potassium phosphate (K-P) buffer from 0 to 40 mM (pH 7.0) in buffer A.

2.5. Affinity Chromatography

The collected sample was applied to a column (0.76 cm i.d. × 4.0 cm) of *S*-hexyl glutathione-agarose that had been equilibrated with 10 mM Tris-HCl buffer (pH 8.0) containing 0.01% (v/v) β-mercaptoethanol (buffer B). The column was washed with buffer B containing 0.2 M KCl and eluted with buffer B containing 1.2 mM *S*-hexyl glutathione. The high active protein fractions eluted with *S*-hexyl glutathione were combined and dialyzed against buffer B, and the dialyze was used as the purified Gly-I. The activity and absorbance (A_{280}) were taken.

2.6. Enzyme Assay and Protein Quantification

Gly-I and glutathione *S*-transferase (GST*:* EC, 2.5.1.18) activities were assayed following the method of Rohman et al. [12] spectrophotometrically (Shimadzu, UV-1800) and were calculated using the extinction coefficient of 3.37 $mM^{-1}cm^{-1}$ and 9.6 $mM^{-1}cm^{-1}$, respectively. Protein concentration was estimated following the method of Bradford [23] using BSA as protein standard.

2.7. SDS-PAGE and Silver Staining

The homogeneity of purified Gly-I enzyme, SDS-PAGE was done in 12.5% (w/v) gel containing 0.1% (w/v) SDS by the method of Laemmli [24] followed by silver staining.

3. Results and Discussion

In extracts of crude protein from green part and non-green part, it was found that the Gly-I had higher specific activity in protein extract from non-green part (Table 1). During separation of protein by DEAE-cellulose chromatography, the Gly-I eluted at approximately 85 mM of KCl for both cases (Fig. 2). In DEAE-cellulose fractions from green and non-green part contained specific activity of 6.43 and 8.73 μmol min^{-1} mg^{-1} protein, respectively. The pooled Gly-I fractions was applied of hydroxylapatite chromatography, but the elution showed very poor activity. Therefore, the active Gly-I fractions of DEAE-cellulose chromatography were directly applied to affinity chromatography (*S*-hexyl glutathione-agarose) for final purification and eluted with 1.2 mM of *S*-hexyl glutathione.

The purified protein from green and non-green part had

specific activity of 33.23 and 39.25 µmol min^{-1} mg^{-1} protein, respectively, along with recovery of 1.47 and 1.62, respectively, and yield of 83.11 and 68.15, respectively (Table 1). The fraction of affinity chromatography was applied to examine the purity of Gly-I by SDS-PAGE.

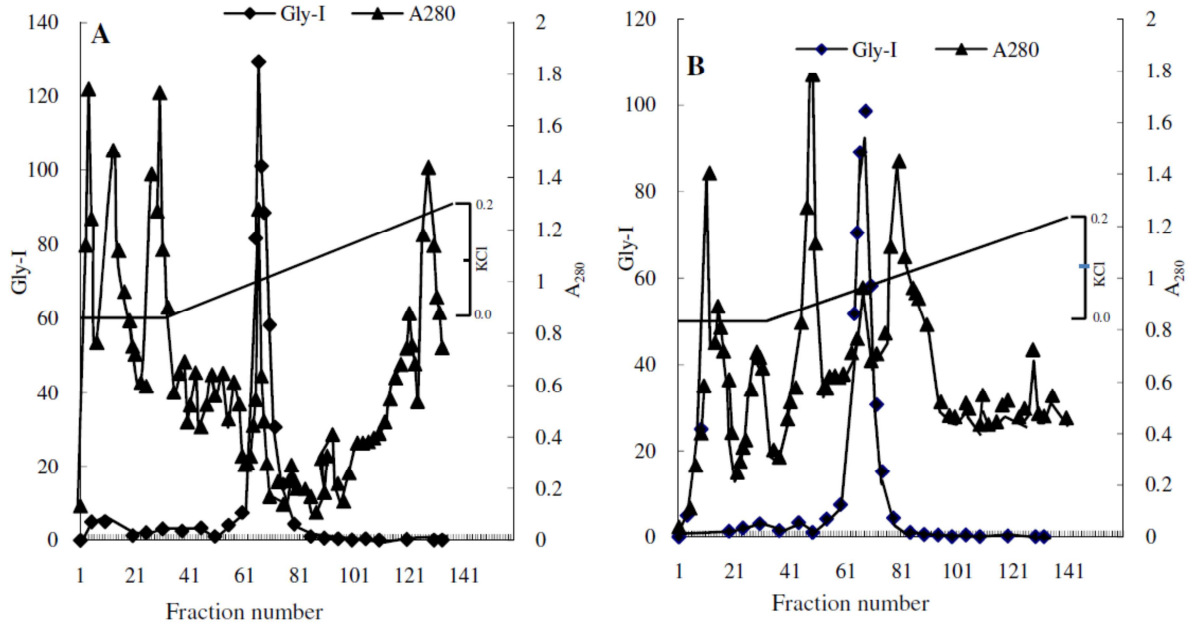

Fig. 2. *Typical column chromatography of DEAE-cellulose of soluble proteins prepared from 50 g of green part (A) and non-green part (B) of maize seedlings. For each fraction, absorbance at 280 nm (●) and Gly-I activity (♦) were determined. Activity is expressed as µmol min^{-1} ml^{-1}. The curve shows the gradient solution of KCl (0-0.2 M).*

Table 1. *Summary of purification of Gly-I from maize seedlings.*

Fraction	Specific activity (µmol min^{-1} ml^{-1})		Total activity (mmol min^{-1})		Total protein(mg)		Recovery (%)		Yield	
	GP	NGP	GP	NGP	GP	NGP	GP	NGP	GP	NGP
Crude protein	0.40	0.57	447.9	424.2	920.1	740.8	100.0	100.0	1.00	1.00
$(NH_4)_2SO_4$	0.78	0.79	382.9	263.4	489.8	423.3	85.49	62.10	1.96	1.36
DEAE-Cellulose	6.43	8.73	44.94	50.60	6.99	5.80	10.03	11.93	16.07	15.25
Affinity	33.23	39.25	6.57	6.87	0.20	0.17	1.47	1.62	83.11	68.55

GP= Green part, NGP= Non-green part.

In silver staining of SDS-PAGE, the active purified Gly-I fraction was found to move with another protein (Fig. 3). Since in purification of Gly-I and GST, the chromatographical uses are almost similar [25], the additional protein band might be due to presence of GST. Therefore, the fractions of DEAE-cellulose and affinity chromatography were also subjected to examine GST activity.

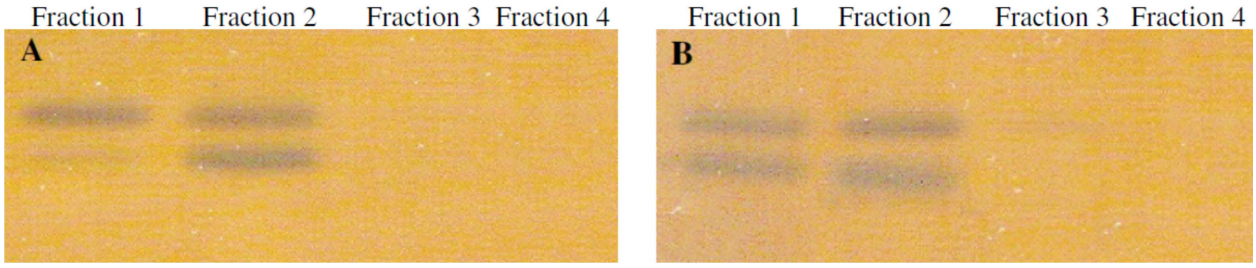

Fig. 3. *Silver staining of active Gly-I fractions eluted from affinity chromatography by S-hexyl glutathione from green part (A) and non-green part (B) of maize seedlings.*

The spectrophotometric assay of Gly-I and GST activities in DEAE eluted fractions showed that the active Gly-I fractions had also GST activity (Fig. 4). The GST activity started increasing under the Gly-I peak. It should be mentioned that in our previous study, three GSTs were reported in this maize variety [26]. Among them, the 1st eluted GST (91.7 mM KCl) had very close elution point to that of Gly-I (85 mM KCl) in the same chromatography. Thus, the 1st GST overlapped the Gly-I in maize seedlings (Fig. 4).

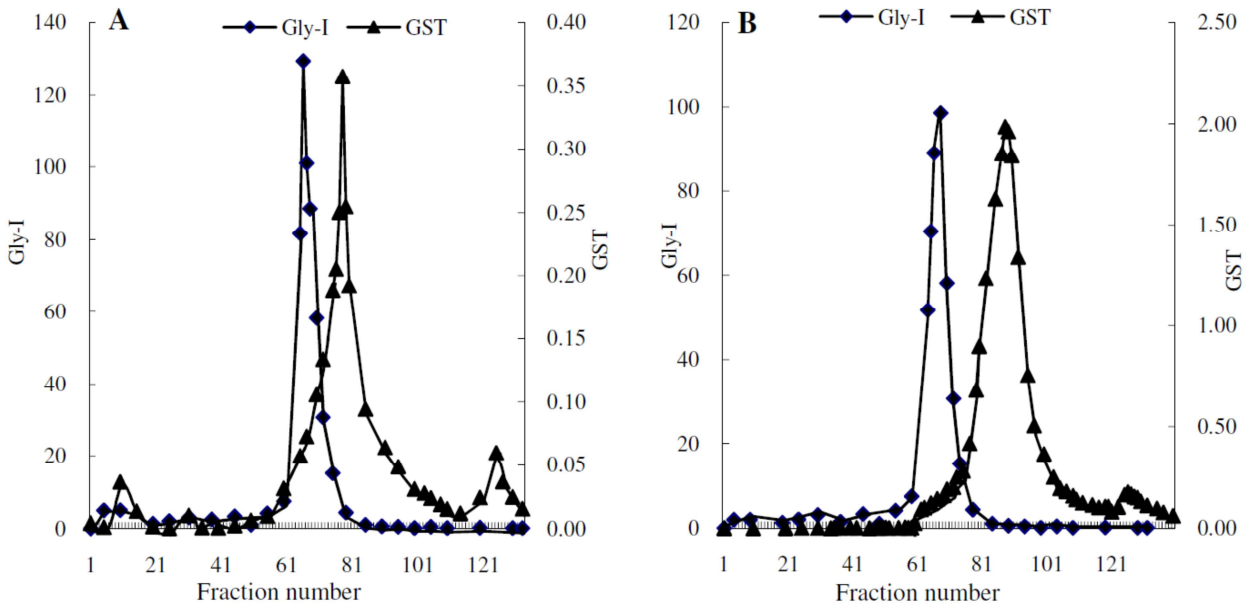

Fig. 4. *Gly-I (♦) and GST (▲) activities in DEAE-cellulose and affinity chromatography fractions from green part (A) and non-green part (B) of maize seedlings.*

The fractions eluted from affinity chromatography were also subjected to examine GST activity (Fig. 5). Interestingly, the affinity fractions of both green and non-green part also showed the activities of Gly-I and GST in the same fraction. The both cases, the highest activities were obtained in fraction 2.

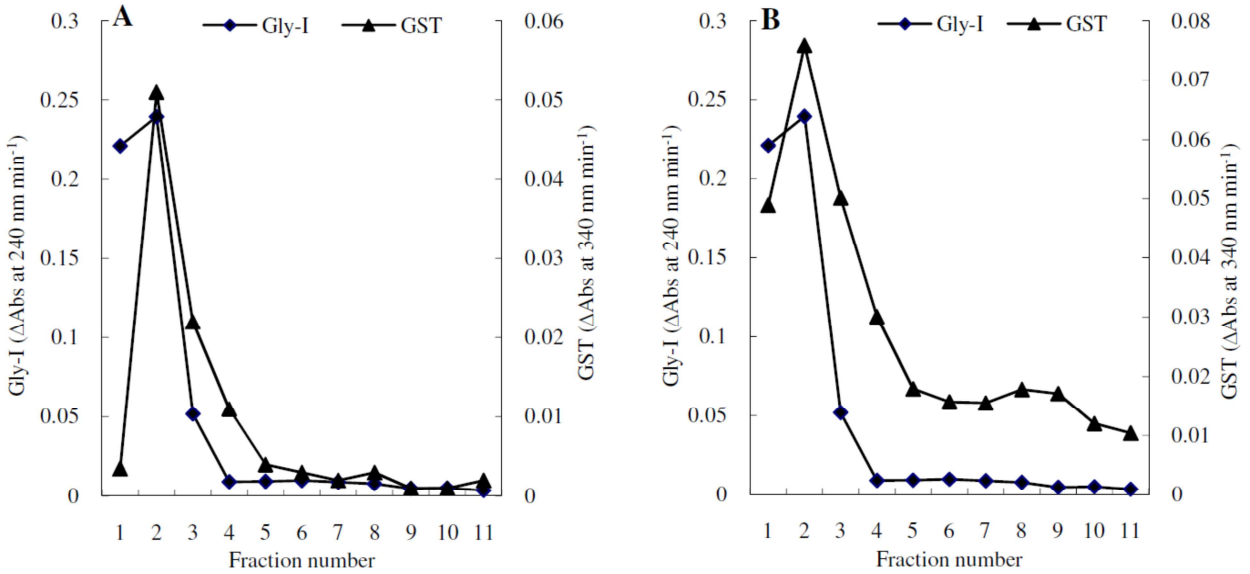

Fig. 5. *Gly-I (♦) and GST (▲) activities in fractions eluted by S-hexyl glutathione from affinity chromatography in green part (A) and non-green part (B) of maize seedlings.*

Therefore, it is clear that a GST eluted with Gly-I during purification of Gly-I from maize seedlings and the additional protein band in SDS-PAGE was due to presence of the GST protein. Previously, Deswal and Sopory [27] found two adjacent two band during gly-I purification from *Brassica juncea* which was due to shifting in mobility. Recently, Islam et al. [28] reported a GST and Gly-I protein in same fraction eluted from affinity chromatography during purification of GST from onion bulb.

4. Conclusion

In this study, during purification of Gly-I from maize seedlings, a GST protein eluted with Gly-I. In active Gly-I fractions from DEAE-cellulose contains GST activity also. Similarly, affinity fraction also contained the two proteins. Therefore, the two adjacent protein bands in SDS-PAGE are due two presence of Gly-I and GST. From this study, it could be suggested that during purification of a target protein, the other related proteins should also be examined.

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