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CHLOROPLAST GLUTATHIONE REDUCTASE

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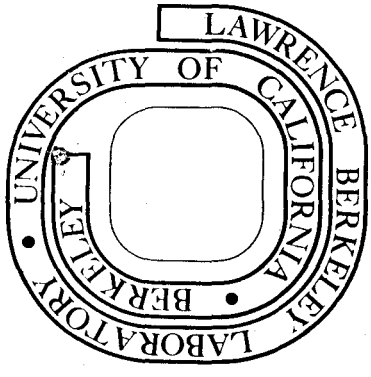

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CHLOROPLAST GLUTATHIONE REDUCTASE

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Chloroplast Glutathione Reductase[1]

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3. Abbreviations: GSSG, oxidized glutathione; GSH, reduced glutathione; RuDP, ribulose 1, 5-diphosphate.

ABSTRACT

Glutathione reductase (E.C. 1.6.42) activity is present in spinach chloroplasts. The pH dependence and apparent binding constant are reported and a possible role in chloroplasts is proposed.

Glutathione reductase ($NADPH_2$) glutathione oxidoreductase (E.C. 1.6.4.2) is a ubiquitous enzyme found in animals, bacteria, fungi and higher plants (2, 4, 5, 6, 7, 10, 13, 14). It is usually a highly specific enzyme that utilizes $NADPH_2$ to reduce oxidized glutathione (GSSG)[3] to two molecules of reduced glutathione (GSH).

Glutathione reductase can maintain a high ratio of GSH/GSSG and by this means plays an important role in the regulation of cell metabolism (12). Reduced glutathione has been implicated in a large number of reactions including the non-enzymatic reduction of thial groups, enzymatic reduction of disulfide bridges of protein with consequent deactivation (10) or activation of the protein, and as a substitute for GSH peroxide (12).

In higher plants glutathione reductase has been studied in pea seedlings (6), wheat germ (4), mitochondria isolated from avocado, mung beans, and peas (14). The mitochondrial preparations were able to couple GSH oxidation with the reduction of dehydroascorbic acid (14).

This paper reports the observation of glutathione reductase in spinach chloroplasts.

Methods

Chloroplasts and stroma enzymes were isolated from spinach leaves by the procedures of Bassham, Levine and Forger (3). Solution Z was modified by replacment of the reduced glutathione buffer with Tris (hydroxymethylaminomethane). The reduced glutathione reductase contained at least 0.5% of oxidized glutathione, the substrate of the glutathione reductase reaction. Several buffers were tried and all were found to be satisfactory. The glutathione reductase activity when stored at 40°C was stable for more than 4 hrs.

A standard reaction mixture of 1 ml contained major reagents at the following concentrations: Tris 0.05 M pH 7.5 $NADPH_2$ 0.15 mM, GSSG 0.5 mM, $MgCl_2$ 3 mM. Enough stroma enzyme preparation (50-100 ul) was added to achieve a rate of $NADPH_2$ oxidation between 0.05 and 0.08 Δ OD units (at 340 nm) per minute.

Protein was determined by the Lowry method (9) using bovine serum albumin as a standard. Chlorophyll content of the grana fraction was measured using the procedure of Vernon (11).

Results and Discussion

The presence of a glutathione reductase activity in the stroma fraction was first detected during attempts to determine the stoichiometry between CO_2 release and NADP reduction by the glucose 6-phosphate dehydrogenase enzyme found in spinach chloroplast preparations. In controls involving $NADPH_2$ addition, pyridine nucleotide was rapidly oxidized. Replacement of the reduced glutathione (GSH) buffer with Tris eliminated the oxidation of $NADPH_2$. This was the result of the presence in commercial reduced glutathione of between 0.5% to 1.0% oxidized glutathione (GSSG). The glutathione reductase activity was specific for $NADPH_2$ (Table 1) and was inhibited by Zn^{++} (Table 2). These characteristics are similar to those of glutathione reductases from fungi (13), wheat germ (4), peas (6), bacteria (2), and animal tissues (7). The enzyme has a broad pH maximum (Fig. 1) between pH 6.5 and 8. In the crude preparation used, the estimated Km for GSSG of 0.07 mM (Fig. 2) fell within the range of values reported in the literature (2, 7, 12). Presence of 50 mM GSH did not measurably affect the rate of glutathione reduction in agreement with the observed irreversibility of the overall enzymatic process.

Since glutathione reductase activity was previously reported in higher plant tissue extracts (4, 6) and plant mitochondria (14) , attempts were made to localize the activity. For this the isolated intact chloroplasts were washed 3 times with buffer before lysis. As seen in Table 3, the specific activity of the glutathione reductase activity did not change with reference to the protein content, nor with regard to the chlorophyll content of the grana fraction after lysis. This provides strong evidence for the localization of the glutathione reductase within the chloroplast.

The chloroplast stroma enzyme preparation was also able to transfer electrons from $NADPH_2$ in a ferredoxin dependent reaction (Table 4) (1). After approximately 8 minutes, the amount of $NADPH_2$ oxidized exceeded the amount of ferredoxin added to the preparation (0.034 µmole). This could be explained by the reoxidation of ferredoxin by O_2. $NADPH_2$ oxidation with ferredoxin as an electron acceptor was less inhibited by additions of 0.1 mM $ZnCl_2$.

Test of high purity commercial RuDP carboxylase preparations showed that they contained high but variable glutathione reductase activity (Table 5). The activity was specific for $NADPH_2$ and was completely inhibited by 0.1 mM $ZnCl_2$. Thus all preparations may have contained significant amounts of glutathione reductase or possibly one of the subunits of RuDP carboxylase may have been responsible for the observed GSSG reduction.

The stroma enzyme preparations on standing acquired an increasing capacity to oxidize $NADPH_2$ without addition of GSSG. This background activity was also specific to $NADPH_2$ and was sensitive to $ZnCl_2$. Storage of the preparation under N_2 (Table 6) prevented the development of increased $NADPH_2$ oxidation capacity. This suggests that the glutathione

reductase may be part of the mechanism which protects chloroplast components against damage by high O_2 concentrations.

The specific role of the reported glutathione reductase activity is unknown. It could, however, regulate the ratio of GSH to GSSG. The concentration of the reduced form should increase during illumination as a result of higher $NADPH_2$/NADP ratios (8) and the higher GSH concentrations may protect sensitive SH groups in a high oxidant environment. It is possible also that the enzyme could regulate enzyme activity more directly by reduction or oxidation of sulfhydro groups of proteins.

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Table 1 Requirements for glutathione reductase activity

Treatment	ΔOD 340/min
Control	0.055
- $NADPH_2$	0
- Enzyme	0
- GSSG	0.003
- $NADPH_2$ + $NADPH_2$	0.003
Boiled enzymes	<0.002

Table 2 Inhibition of glutathione reductase by $ZnCl_2$

Molar conc.	ΔOD 340/min
Control	0.060
10^{-5}	0.050
$2x\ 10^{-5}$	0.043
$5\ x\ 10^{-5}$	0.025
10^{-4}	0 (0.003)

Table 3- Effect of washing chloroplast pellet on glutathione reductase activity*

	µmoles/min* per mg protein	µmoles/min* per mg chlorophyll
1 Wash	0.28	0.43
2 Washes	0.32	0.54
3 Washes	0.34	0.65

*Measured at 340 nm as $NADPH_2$ oxidation.

Table 4 Ferredoxin-stimulated $NADPH_2$ oxidation

Treatment	ΔOD 340/min
Control	0.070
- E + Ferredoxin (FD)*	0.002
- GSSG	0.003
- GSSG + Fd	0.030
- GSSG + Fd 0.1 mM $ZnCl_2$	0.015

Fd* 0.4 mg of Ferredoxin per ml. The commercial preparation of ferredoxin was dialyzed before use against pH 7.5 Tris buffer for over 24 hrs.

Table 5 Glutathione reductase activity of commercial RuDP carboxylase preparatios

	ΔOD 340/min
Control*	0.105
- GSSG	0.020
- GSSG - $NADPH_2$	0
- $NADPH_2$ + $NADH_2$	0.003
Control + 10^{-4} M $ZnCl_2$	0

* 1.5 mg RuDP carboxylase per ml

Table 6 Effect of enzyme storage on $NADPH_2$ oxidation blank

	µmoles $NADPH_2$ oxidized
Initial fresh enzyme	0.0024 - 0.0064
Preparation stored 3 hrs Air	0.016 - 0.037
Preparation stored 3 hr N_2	0.0048

The reaction mixture contained 0.05 M Tris pH 7.5, 0.15 mM $NADPH_2$ and 50 µl of enzyme preparation per milliliter.

FIGURE LEGENDS

Fig. 1. Effect of pH on the rate of $NADPH_2$ oxidation by glutathione reductase.

Fig. 2. Effect of increasing concentration of oxidized glutathione on $NADPH_2$ oxidation by spinach chloroplast stroma enzymes.

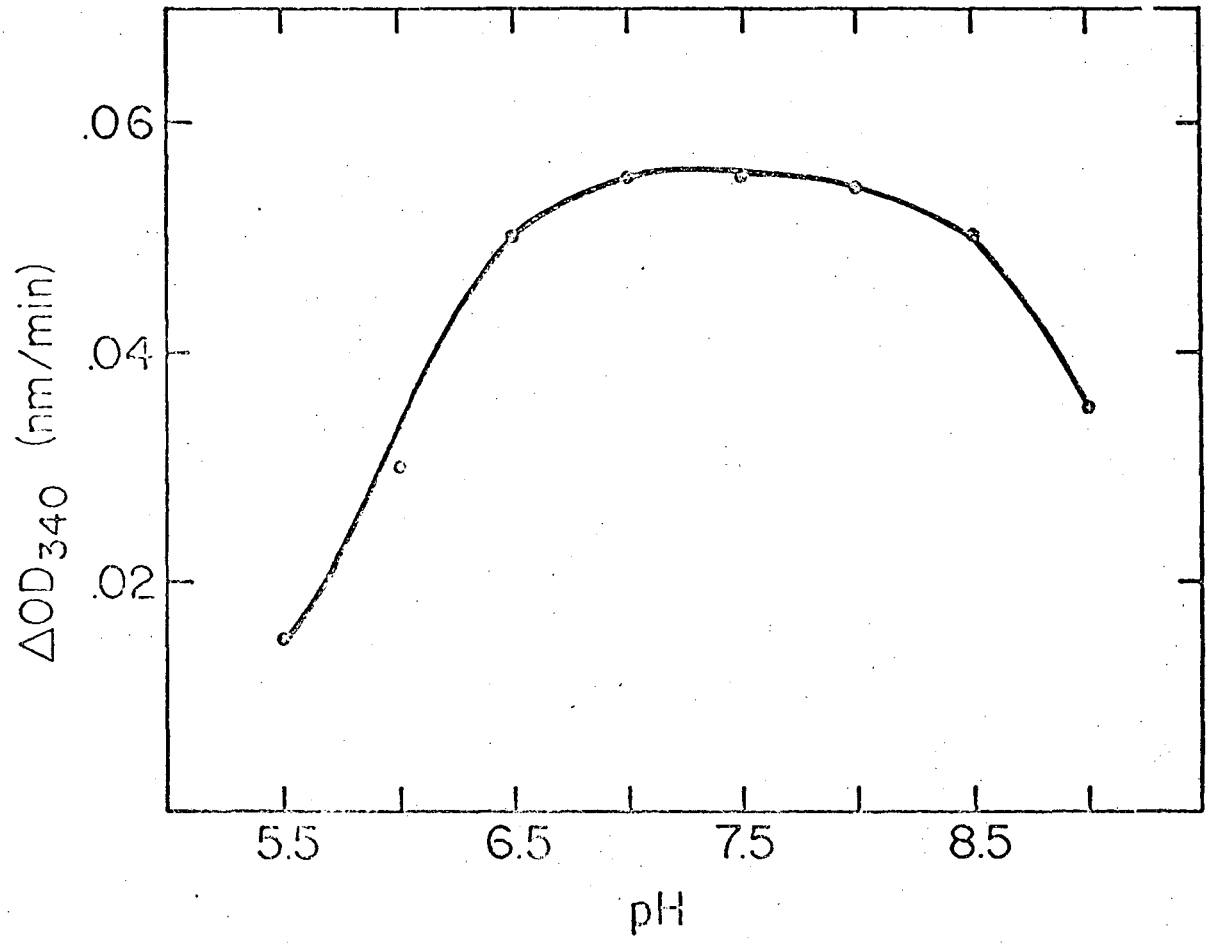

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Fig. 1
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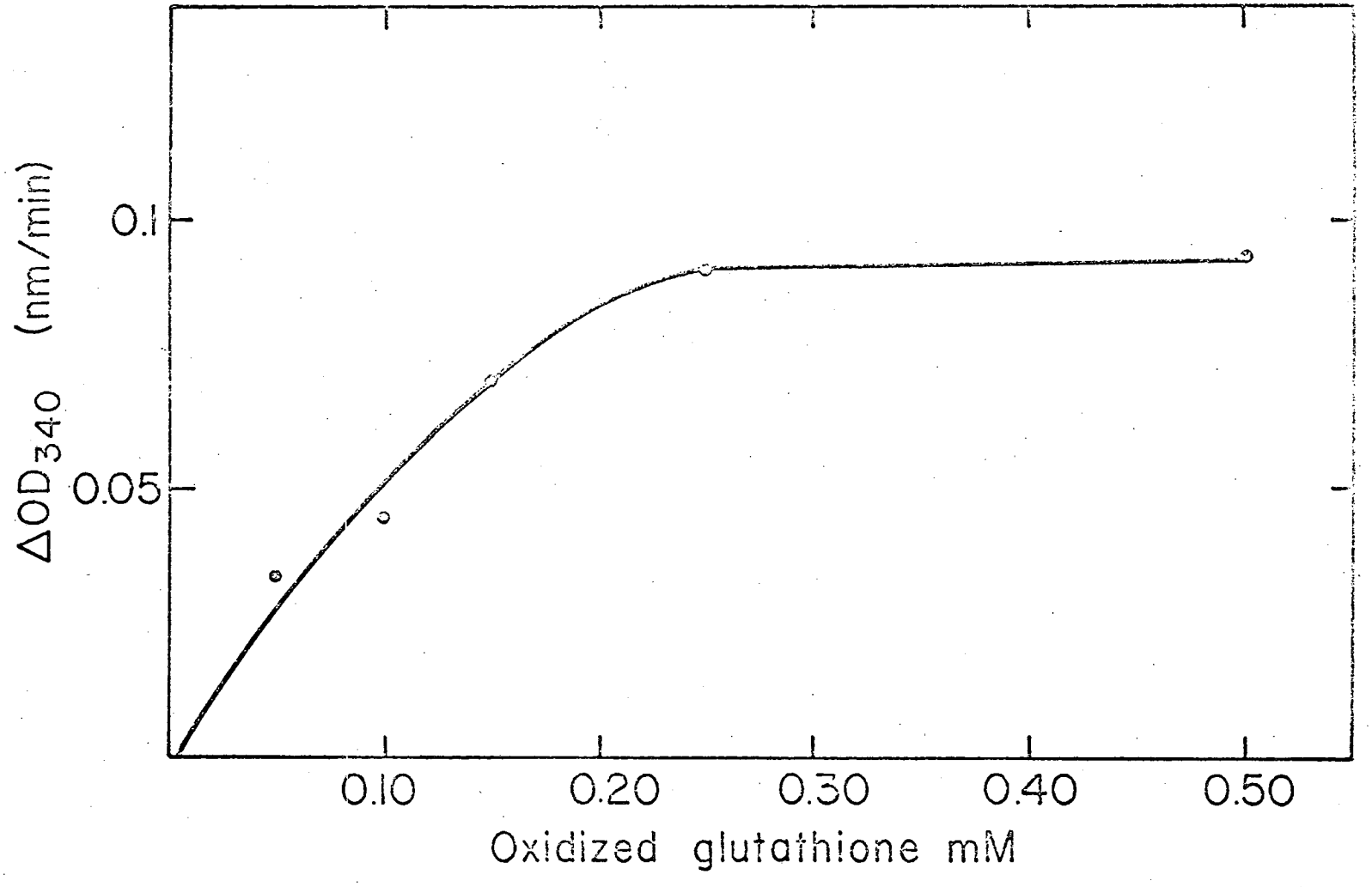

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Fig. 2
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