

Short Communication

Chloroplast Glutathione Reductase¹

Received for publication November 12, 1976 and in revised form January 12, 1977

MICHAEL SCHAEDEL

Department of Botany and Forest Pathology, State University of New York College of Environmental Science and Forestry, Syracuse, New York 13210

JAMES A. BASSHAM²

Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, 1 Cyclotron Road, Berkeley, California 94720

ABSTRACT

Glutathione reductase (EC 1.6.4.2) activity is present in spinach (*Spinacia oleracea* L.) chloroplasts. The pH dependence and substrate concentration for half-maximal rate are reported and a possible role in chloroplasts is proposed.

Glutathione reductase (NADPH) glutathione oxidoreductase (EC 1.6.4.2) is a ubiquitous enzyme found in animals, bacteria, fungi, and higher plants (2, 4-7, 10, 13, 14). It is usually a highly specific enzyme that utilizes NADPH to reduce oxidized glutathione (GSSG)³ to 2 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). GSH has been implicated in a large number of reactions including the nonenzymic reduction of thial groups, enzymic 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, GSSG reductase has been studied in pea seedlings (6), wheat germ (4), and 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 GSSG reductase in spinach chloroplasts.

MATERIALS AND METHODS

Chloroplasts and stroma enzymes were isolated from spinach (*Spinacia oleracea* L.) leaves by the procedures of Bassham *et al.* (3). Solution Z was modified by replacement of the GSH buffer with tris (tris[hydroxymethyl]aminomethane). The GSH contained at least 0.5% of GSSG, the substrate of the GSSG reductase reaction. Several buffers were tried and all were found to be satisfactory. The GSSG reductase activity when stored at 40 C was stable for more than 4 hr.

A standard reaction mixture of 1 ml at 24 C contained major

reagents at the following concentrations. Tris·HCl, 0.05 M (pH 7.5); NADPH, 0.15 mM; GSSG, 0.5 mM; and MgCl₂, 3 mM. In all cases, enough stroma enzyme preparation (50-100 μl containing 0.15-0.3 mg protein) was added to achieve a rate of NADPH oxidation between 0.05 and 0.08 ΔOD units (at 340 nm)/min.

Protein was determined by the Lowry method (9) using BSA as a standard. Chlorophyll content of the grana fraction was measured using the procedure of Vernon (11). Ribulose-1,5-diphosphate carboxylase was obtained from Calbiochem as a lyophilized powder.

RESULTS AND DISCUSSION

The presence of a GSSG reductase activity in the stroma fraction was first detected during attempts to determine the stoichiometry between CO₂ release and NADP reduction by the glucose-6-P dehydrogenase enzyme found in spinach chloroplast preparations. In controls involving NADPH addition, pyridine nucleotide was rapidly oxidized. Replacement of the GSH buffer with tris eliminated the oxidation of NADPH. This was the result of the presence in commercial GSH of between 0.5 to 1.0% GSSG.

The GSSG reductase activity was specific for NADPH and was inhibited by Zn⁺² (Table I). These characteristics are similar to those of GSSG 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 concentration of the substrate GSSG for half-maximal rate of 0.07 mM (Fig. 2) fell within the range of values reported in the literature (2, 7, 12). The presence of 50 mM GSH did not measurably affect the rate of GSSG reduction, in agreement with the observed irreversibility of the over-all enzymic process.

Since GSSG 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 three times with buffer before lysis. The specific activity of the GSSG reductase activity did not decrease with reference to the protein content, nor with regard to the Chl content of the grana fraction after lysis; in fact, there was a small increase (20%) due perhaps to removal of small amounts of contaminating chloroplast membrane material. This provides strong evidence for the localization of the GSSG reductase within the chloroplast.

The chloroplast stroma enzyme preparation was also able to transfer electrons from NADPH in a ferredoxin-dependent reaction (Table II) (1). After approximately 8 min, the amount of

¹ This work was supported by the United States Energy Research and Development Administration.

² To whom reprint requests should be sent.

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

Table I. Requirements for Glutathione Reductase Activity

Reaction mixture as described under procedure. Stromal preparation 75 μ l added to 1 ml of reaction mixture. Protein concentration of reaction mixture 0.32 mg/ml.

Treatment	Δ OD 340/min
Control I	0.055
-NADPH	0
-Enzyme	0.003
-GSSG	0.003
-NADPH + NADH	0.003
Boiled enzymes	0.003
Control II	0.060
+ZnCl ₂ 10 μ M	0.050
+ZnCl ₂ 50 μ M	0.025
+ZnCl ₂ 0.1mM	0.003

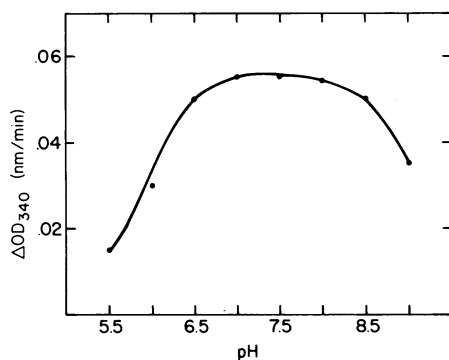


FIG. 1. Effect of pH on the GSSG-dependent NADPH₂ oxidation by stromal preparations. The 0.05 M buffer system used throughout the whole pH range consisted of an equimolar mixture of tris-HCl and HEPES. Protein concentration 0.20 mg/ml of reaction mixture.

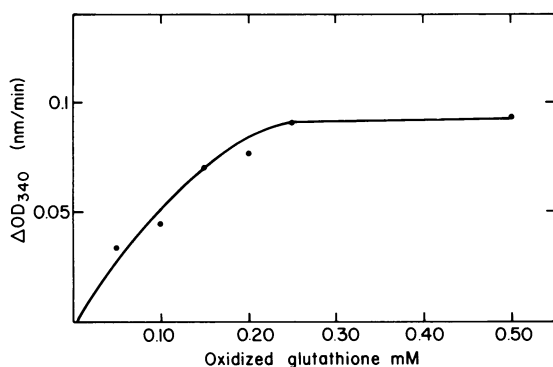


FIG. 2. Effect of GSSG concentration on the rate of NADPH₂ oxidation by chloroplast stromal preparations. Reaction mixture as described under "Materials and Methods." Protein concentration 0.19 mg/ml of reaction mixture.

NADPH oxidized exceeded the amount of ferredoxin added to the preparation (0.034 μ mol). This could be explained by the reoxidation of ferredoxin by O₂. NADPH oxidation with ferredoxin as an electron acceptor was less inhibited by additions of 0.1 mM ZnCl₂.

Tests of commercial RuDP carboxylase preparations showed that they contained high but variable GSSG reductase activity. With 1.5 mg of RuDP carboxylase/ml of reaction mixture instead of stromal enzyme, and other conditions as in Table I, a Δ OD at 340 nm of 0.105 units/min was observed. This activity was specific for NADPH and was completely inhibited by 0.1 mM ZnCl₂. All of the preparations tested may have contained

Table II. Ferredoxin-stimulated NADPH Oxidation

Ferredoxin was 0.4 mg per ml. The commercial preparation of ferredoxin was dialyzed before use against pH 7.5 tris buffer for over 24 hr.

Treatment	Δ OD 340/min
Control	0.070
-E + ferredoxin (Fd)	0.002
-GSSG	0.003
-GSSG + ferredoxin	0.030
-GSSG + ferredoxin 0.1 mM ZnCl ₂	0.015

Table III. NADPH Oxidation by Isolated Stromal Preparation as a Function of Storage Time

Reaction mixture in two experiments as described under procedures. Used 50 μ l of stromal preparation per ml of reaction mixture. Protein concentration 0.28 mg/ml of reaction mixture.

	NADPH oxidized μ mol/ml
Initial fresh enzyme	0.0024, 0.0064
Preparation stored 3 hr air	0.016, 0.037
Preparation stored 3 hr N ₂	0.0048

significant amounts of GSSG 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 without addition of GSSG. This background activity was also specific to NADPH and was sensitive to ZnCl₂. Storage of the preparation under N₂ (Table III) prevented the development of increased NADPH oxidation capacity. This suggests that the GSSG reductase may be part of the mechanism which protects chloroplast components against damage by high O₂ concentrations.

The specific role of the reported GSSG 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/NADP ratios (8) and the higher GSH concentrations may protect sensitive -SH groups in a high oxidant environment. It is also possible that the enzyme could regulate enzyme activity more directly by reduction or oxidation of sulfhydryl groups of proteins.

LITERATURE CITED

- ARNON DI, RK CHAIN 1975 Regulation of ferredoxin catalyzed photosynthetic phosphorylation. Proc Nat Acad Sci USA 72: 4961-4965
- ASNIS RE 1975 A glutathione reductase from *Escherichia coli*. J Biol Chem 213: 77-85
- BASSHAM JA, G LEVINE, J FORGER 1974 Photosynthesis *in vitro*. I. Achievement of high rates. Plant Sci Lett 2: 15-21
- CONN EE, B VENNESLAND 1951 Glutathione reductase of wheat germ. J Biol Chem 192: 17-18
- ERNEST MJ, K-H KIM 1973 Regulation of rat liver glycogen synthetase. Reversible inactivation of glycogen synthetase D by sulfhydryl-disulfide exchange. J Biol Chem 248: 1550-1555
- MAPSON LW, FA ISHERWOOD 1963 Glutathione reductase from germinated peas. Biochem J 86: 173-191
- MIZE CE, RG LANGDON 1962 Hepatic glutathione reductase. I. Purification and general kinetic properties. J Biol Chem 237: 1589-1595
- LENDZIAN K, JA BASSHAM 1975 Regulation of glucose-6-phosphate dehydrogenase in spinach chloroplasts by ribulose 1,5-diphosphate and NADPH/NADP ratios. Biochim Biophys Acta 396: 260-275
- LOWRY OH, NJ ROSEBROUGH, AL FARR, AND RJ RANDALL 1951 Protein measurements with the Folin phenol reagent. J Biol Chem 193: 265-275
- VARANDANI PT, LA SHROYER, MA NOFZ 1972 Sequential degradation of insulin in rat liver homogenates. Proc Nat Acad Sci USA 69: 1681-1684
- VERNON LP 1960 Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Anal Chem 32: 1144-1150
- WILLIAMS CH 1976 Flavin containing enzymes. In PD Boyer, ed. The Enzymes Ed 3 Vol XIII. Academic Press, New York pp 89-173
- WOODIN RS, I SEGEL 1968 Isolation and characterization of glutathione reductase from *Penicillium chrysogenum*. Biochim Biophys Acta 164: 64-77
- YOUNG LDT, EE CONN 1956 The reduction and oxidation of glutathione by plant mitochondria. Plant Physiol 31: 205-211