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Glycine Supports *in Vivo* Reduction of Nitrate in Barley Leaves

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ABSTRACT

Glycine, a photorespiratory intermediate, enhanced the *in vivo* reduction of nitrate in barley (*Hordeum vulgare* L.) leaf slices, when included in the assay medium. Isonicotinyl hydrazide, an inhibitor of glycine oxidation, partially reduced NO_2^- production. The enhancement caused by glycine treatment was reversed by isonicotinyl hydrazide when both were present together in the medium. Similar effects were observed when the excised leaves were preincubated with the metabolite and the inhibitor. Glycine also partially relieved the inhibition of nitrate reduction caused by malonate, an inhibitor of the tricarboxylic acid cycle. The results support the hypothesis that glycine decarboxylation activity is a source of NADH for nitrate reductase activity.

NR[1](EC 1.6.6.1) reduces nitrate to nitrite utilizing NADH as reducing power (2). Earlier studies by Klepper *et al.* (8) have shown that NADH for nitrate reduction in leaves is derived from photosynthetically produced glyceraldehyde 3-P, which after migrating from chloroplasts, generates NADH in the cytoplasm on being oxidized to PGA by triosephosphate dehydrogenase. On the other hand, Sawhney *et al.* (11) have also demonstrated that NADH generated in the TCA cycle can be used for the reduction of nitrate. A third source of NADH could be the process of glycine oxidation in mitochondria. Green leaves of C_3 plants are known to synthesize substantial quantities of glycine during photorespiration (15). Two molecules of glycine form one molecule each of serine, CO_2 and NH_3 with concomitant reduction of one molecule of NAD^+ by the action of glycine decarboxylase complex in the mitochondria (7). The turnover of glycine in glycolate pathway is quite rapid, often attaining rates of about 80 μmol of serine formed per g tissue per h (16). In this report we show that glycine can act as a source of reducing power for nitrate reduction in leaves.

MATERIALS AND METHODS

Barley plants (*Hordeum vulgare* L. cv DL-157) grown in sandy loam soil in pots were used. Third and fourth leaves from 15 to 20 d old plants were selected. Nitrate reductase *in vivo* was assayed according to Klepper *et al.* (8) with slight modification (6). In experiments where various metabolites and inhibitors were included in the assay medium, vacuum infiltration was done. Wherever preincubation was involved, the excised leaves were incubated with different treatment solutions for 3 h under light (800 $\mu E\ m^{-2}\ S^{-1}$) at 30°C.

[1] Abbreviations; NR, Nitrate reductase; PGA, glycerate 3-phosphate; INH, isonicotinylhydrazide; α-HPMS, α-hydroxy-2-pyridine methane sulfonic acid.

RESULTS AND DISCUSSION

Glycine enhanced nitrate reduction in barley leaves when included in the *in vivo* assay medium (Fig. 1). The maximum enhancement was attained at 10 mM concentration. Three possible explanations could be attributed to explain this enhancement: a. possible induction of NR by the amino acid; b. increased stability of the enzyme in the presence of ammonium (13) released during glycine oxidation; c. increased supply of reducing power to the enzyme. It has been observed by many workers that amino acids either repress the synthesis or reduce the activity of NR (10). We observed no considerable change in NR activity by using a wide range of amino acids other than glycine in the

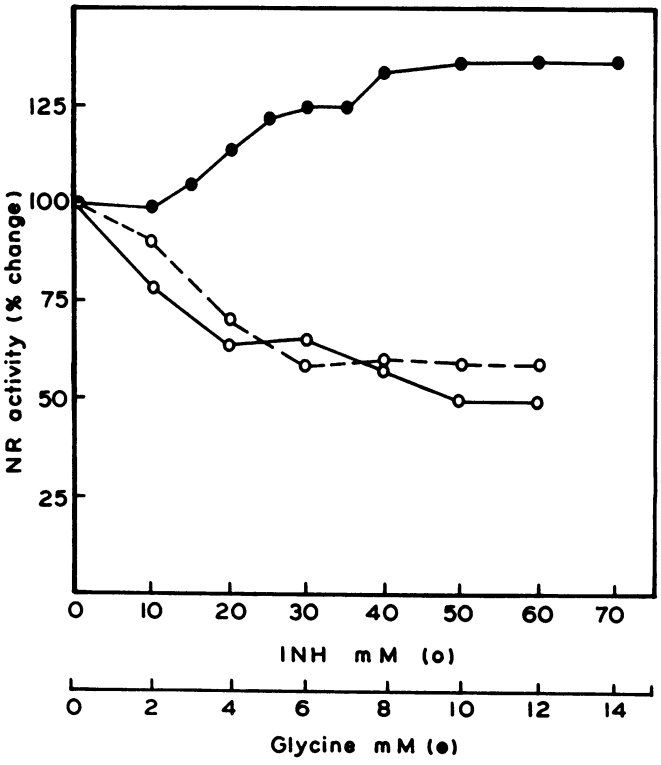

FIG. 1. Changes in NR activity (% of control) caused by glycine and INH when included in the preincubation medium (---) or in the assay medium (——). Assay medium consisted of 0.1 M phosphate buffer (pH 6.0) + 0.2 M KNO_3.

Table I. *NR Activity in Vivo in Barley Leaf Slices as Affected by Glycine*

Preincubation medium in the case of control was distilled water. Assay medium consisted of 0.1 M phosphate buffer (pH 6.0) with 0.2 M KNO_3.

Treatment	NR Activity			
	Preincubation medium		Assay medium	
	μmol NO_2^- g^{-1} fresh wt h^{-1}	*% of control*	*μmol NO_2^- g^{-1} fresh wt h^{-1}*	*% of control*
Control	2.57	100	2.43	100
INH, 35 mM	1.19	46	1.22	60
Glycine, 10 mM	3.21	125	3.02	124
Glycine 10 mM + INH, 35 mM	1.58	61	1.57	64

Table II. *NR Activity in Vivo in Barley Leaf Slices as Affected by Glycolate and α-HPMS*

Glycolate and α-HPMS were included in the assay medium consisting of 0.1 M phosphate buffer (pH 6.0) and 0.2 M KNO_3.

Treatment	NR Activity	
	μmol NO_2^- g^{-1} fresh wt h^{-1}	*% of control*
Control	2.67	100
α-HPMS, 10 mM	0.86	33
Glycolate, 10 mM	2.97	116
Glycolate, 10 mM + α-HPMS, 10 mM	1.39	54

Table III. *NR Activity in Vivo in Fresh and Dark (24 h) Treated Barley Leaves as Affected by Malonate, Glycine and INH*

Malonate (5 mM), glycine (10 mM), and INH (35 mM) were included in the assay medium consisting of 0.1 M phosphate buffer (pH 6.0) and 0.2 M KNO_3.

Treatment	NR Activity			
	Fresh leaves		Dark-treated leaves	
	μmol NO_2^- g^{-1} fresh wt h^{-1}	*% of control*	*μmol NO_2^- g^{-1} fresh wt h^{-1}*	*% of control*
Control	2.47	100	1.16	100
Malonate	1.03	42	1.07	92
Malonate + glycine	1.90	77	1.73	149
Malonate + glycine + INH	0.52	21	0.55	47

assay medium (results not shown). Second, free ammonium content in the leaves does not alter considerably even after incubation of leaves with glycine (9). This is due to the presence of an efficient system of reassimilation of photorespiratory ammonia by glutamine synthetase (14). The third possibility has been examined by using INH, a potent inhibitor of glycine oxidation. Figure 1 shows the effect of INH on NR activity. There has been a 40 to 50% reduction in the enzyme activity by either mode of treatment. Table I shows further that the enhancement of NR activity by glycine is effectively reversed by INH when it is included along with glycine. This clearly shows that glycine oxidation is responsible for the increase in NR activity. Similar results have been obtained using leaves kept in dark for 24 h to deplete the carbohydrate reserves (data not shown).

In another experiment we have provided glycolate (10 mM), a precursor of glycine, to the excised leaves. Glycolate treatment slightly enhanced NR activity (Table II). α-HPMS, an inhibitor of glycolate oxidation, reduced nitrate reduction when provided alone or together with glycolate.

Table III shows the effect of malonate, a competitive inhibitor of succinate dehydrogenase, on nitrate reduction in fresh and 24 h dark treated leaves. Malonate inhibited nitrate reduction in fresh leaves by 60%. This inhibition was substantially reversed by the addition of glycine. Malonate, glycine, and INH together reduced NR activity by 80%. This clearly demonstrates that glycine partially restores the NR activity inhibited by malonate. This restoration is nullified by INH.

In dark treated leaves NR activity persisted though at a reduced level. This activity was resistant to malonate which indicate sources of NADH other than the TCA cycle. The malonate resistant component was found in fresh leaves as well. Glycine substantially increased nitrate reduction when added with malonate. INH effectively inhibited this enhancement.

It is now well established that in green leaves in light, NADH for nitrate reduction can be provided by two sources *viz.*, metabolism of glyceraldehyde 3-P and oxidation of TCA cycle intermediates (1, 11). In the present investigation we demonstrate that glycine oxidation also supports the *in vivo* reduction of nitrate by providing reducing power. This is supported by the partial inhibition of nitrate reduction by INH and α-HPMS, inhibitors of glycine and glycolate oxidation, respectively. Exogenous supply of glycine to the leaves enhanced nitrate reduction and this enhancement was curtailed by INH. Further evidence is provided by the experiment wherein glycine reversed, though not completely, the inhibition of nitrate reduction by malonate and INH blocked this reversal.

It has been suggested that glycine oxidation is coupled to the mitochondrial election transport (5). However, high phosphorylation potential which exists in the cell during photosynthesis may be inhibitory to the mitochondrial electron transport (4) thus making NADH available for nitrate reduction (12). Thus, the NADH generated during glycine oxidation can also be available for nitrate reduction in the cytosol. The export of NADH out of mitochondria could be via the malate-oxaloacetate shuttle proposed by Woo *et al.* (17) or via the transmembrane transhydrogenase system present in plant mitochondria (3). Woo *et al.* (18) have demonstrated that glycine can support nitrate reduction in a reconstituted system, but they held the view that glycine oxidation is not linked to nitrate reduction *in vivo*. Our results conclusively prove that glycine oxidation can also support nitrate

reduction *in vivo*, thus making it yet another source of reducing power for NR. This raises the question again whether photorespiration long considered to be a wasteful process is really so.

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