

BRIEF COMMUNICATION

The Effect of Exogenously Supplied Hydroxylamine on Glutamate Dehydrogenase, Nitrate Reductase, and Nitrite Reductase in Isolated Pea Roots

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Received October 3, 1973

Abstract. Hydroxylamine added to the nutrient medium in sublethal concentrations (0.2 to 1.0 mN) enhanced NADH₂ dependent glutamate dehydrogenase activity in isolated pea roots. The increase in activity depended on proteosynthesis and was lower in the presence of NO₃⁻ and NH₄⁺ ions. The induction of nitrate reductase and of nitrite reductase was partly inhibited by sublethal hydroxylamine concentrations.

It was established in previous experiments (SAHULKA 1973) that NADH₂ dependent glutamate dehydrogenase (GDH) activity was enhanced by exogenously supplied nitrite. The results presented in this paper indicate that another supposed metabolite of nitrate assimilation, hydroxylamine, when added to the nutrient medium, causes in sublethal concentrations an increased synthesis of NADH₂ GDH and simultaneously depresses the induction of nitrate and nitrite reductases.

TABLE 1

THE EFFECT OF ACTIDIONE AND HYDROXYLAMINE added to the nutrient solution (c) on NADH₂ dependent GDH activity in excised pea roots after 24 h incubation, expressed in % of the control (no actidione, no hydroxylamine). Mean values of three experiments

No hydroxylamine No actidione	Hydroxylam. 1.0 mN No actidione	No hydroxylamine Actidione 1 × 10 ⁻⁴ M	Hydroxylamine 1.0 mN Actidione 1 × 10 ⁻⁴ M
100	242.3	73.6	82.9

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TABLE 2

THE EFFECT OF THE PRESENCE OF NO_3^- AND NH_4^+ in the nutrient medium on the increase in the activity of NADH_2 dependent GDH ($\Delta\text{E min}^{-1} \text{g}^{-1} \text{f.w.}$) caused by hydroxylamine in isolated pea roots. Both the differences caused by hydroxylamine and the differences caused by NO_3^- and NH_4^+ ions in the presence of hydroxylamine are statistically significant, $P < 0.05$

Nutrient sol. Variant	with nitrate (a)	without other nitrogen source (c)	with NH_4NO_3 (d)	without other nitrogen source (e)
No hydroxylamine	0.795	0.765	0.770	0.766
0.5 mN hydroxylamine	1.285	1.585	1.330	1.628

3 cm long apical segments of primary roots isolated from 6 days old seedlings germinated under aseptic conditions were grown for 24 h under aseptic conditions in nutrient solutions of this composition: (a) with nitrate nitrogen: $\text{Ca}(\text{NO}_3)_2$ 1.02 mM, KNO_3 0.85 mM, MgSO_4 0.17 mM, KCl 0.82 mM, KH_2PO_4 0.15 mM; (b) with ammonium nitrogen: $(\text{NH}_4)_2\text{SO}_4$ 1.5 mM, MgSO_4 0.17 mM, KCl 0.82 mM, KH_2PO_4 0.15 mM, CaCl_2 1.0 mM, K_2SO_4 0.42 mM; (c) without any other source of nitrogen: MgSO_4 0.17 mM, KCl 0.82 mM, KH_2PO_4 0.15 mM, CaCl_2 1.0 mM, K_2SO_4 0.42 mM; (d) with both nitrate and ammonium nitrogen: NH_4NO_3 1.5 mM, MgSO_4 0.17 mM, KCl 0.82 mM, KH_2PO_4 0.15 mM, CaCl_2 1.0 mM, K_2SO_4 0.42 mM. Hydroxylamine sulfate was added to these basic nutrient solutions in the following concentrations: 0; 0.1; 0.2; 0.5; and 1.0 mN respectively. In addition, all the solutions contained 20 g sucrose, 1 mg ZnSO_4 , 1.5 mg H_3BO_3 , and 0.3 mg $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$ in 1000 ml. Fe^{3+} was omitted because it is known that hydroxylamine can be oxidized under acid conditions by Fe^{3+} (CHAO and KROONTJE 1969). Toxic effects of hydroxylamine could be observed in some experiments already at 0.2 mN hydroxylamine concentration, the 2.0 mN concentration being lethal.

After the incubation the roots were rinsed, weighed, and homogenized with a fivefold amount of 0.066 M tris-HCl buffer pH 8.0. Homogenates were cleared by centrifugation at 15 000 g and the supernatants obtained were used for the determination of enzyme activities both without further purification and after filtrating them through a Sephadex G 25 column to separate the enzymes from low-molecular-weight substances.

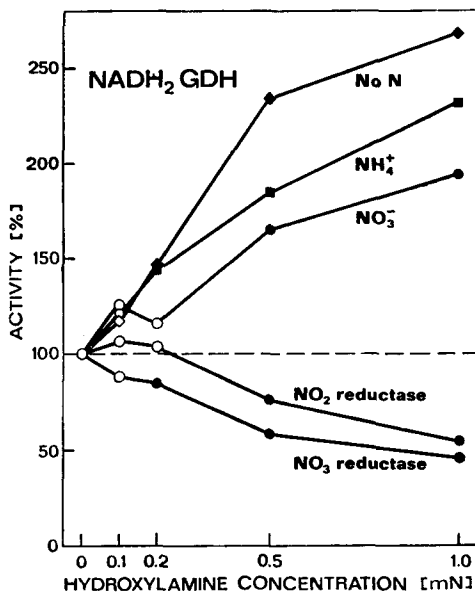
GDH activity was determined spectrophotometrically when recording NADH_2 oxidation or NAD^+ reduction, nitrite reductase activity was determined on the basis of nitrite disappearance from the assay mixture containing benzyl viologen as electron donor, and in nitrate reductase assay, the production of nitrite was followed in an assay mixture with NADH_2 as electron donor for the complete oxidation of which endogenous malate dehydrogenase was used. The results were statistically evaluated using the t-test.

Hydroxylamine when added to the nutrient solution in sublethal concentrations caused an increase in NADH_2 dependent GDH activity in the roots (Fig. 1). The increase in activity was inhibited by actidione (Table 1) which indicates that it depended on proteosynthesis (higher amounts of hydroxylamine were found in the roots incubated with actidione than without it), and was lower in the presence of NO_3^- and NH_4^+ ions (Fig. 1, Table 2). The deaminating GDH activity was increased only slightly with the increase being not statistically significant. The induction of nitrite reductase and of nitrate reductase was partly inhibited by toxic hydroxylamine concentrations (Fig. 1). *In vitro*, hydroxylamine influenced in concentrations which could occur in the extracts only the NAD^+ dependent GDH activity (Table 3).

Thus hydroxylamine is another metabolite of nitrate assimilation which enhances NADH_2 dependent GDH activity in isolated pea roots. In contrast to nitrite which probably did not influence the synthesis of GDH as its

Fig. 1. The effect of rising hydroxylamine concentrations present in nutrient solutions on NADH₂ dependent glutamate dehydrogenase (NADH₂ GDH), nitrate reductase (NO₃Red), and nitrite reductase (NO₂Red) activities [%] in excised pea roots after 24 h incubation. Hydroxylamine concentrations given in the figure are the concentrations at the beginning of the experiments.

○ nitrate containing nutrient solution (a), □ ammonium sulfate containing nutrient solution (b), ◇ nutrient solution without other nitrogen source (c). Full points ●, ■, ◆ — difference from the control (no hydroxylamine) is statistically significant, starting at the $P = 0.05$ level.



effect was not dependent on proteosynthesis, hydroxylamine may induce an enhanced *de novo* synthesis of this enzyme despite the fact that the toxicity of hydroxylamine is higher than that of nitrite; however, exogenously supplied hydroxylamine can be metabolized and its nitrogen can be incorporated into proteins (*e.g.* KRETOVICH *et al.* 1964). Simultaneously with the possible increase in GDH synthesis hydroxylamine depresses the induction of nitrate reductase and of nitrite reductase. A similar effect of hydroxylamine on nitrate reductase was observed in *Neurospora crassa* by SUBRAMANIAN and SARMA (1969). The effect of hydroxylamine on nitrate reductase in isolated pea roots is similar to that of toxic concentrations of NH₄⁺ ions; however toxic concentrations of NH₄⁺ ions cause only a slight increase in NADH₂ dependent GDH activity (21 mM NH₄⁺ in the nutrient solution caused a 22 % enhancement of NADH₂ dependent GDH activity and a 54 %

TABLE 3

In vitro EFFECT OF HYDROXYLAMINE ON NITRATE REDUCTASE ACTIVITY (NO₃Red), NADH₂ dependent glutamate dehydrogenase activity (NADH₂ GDH), and on NAD⁺ dependent glutamate dehydrogenase activity (NAD⁺ GDH). Hydroxylamine sulfate was added to the extracts up to the concentration given in the table. Mean values of 4 replicates. Activity expressed in %.

Enzyme	Hydroxylamine concentration		
	0	1.0 mM in the roots	1.0 mM in the extract
NO ₃ Red	100	101.1	102.1
NADH ₂ GDH	100	98.8	98.3
NAD ⁺ GDH	100	92.2	57.3

inhibition of nitrate reductase induction in excised pea roots, 9 mM NH_4^+ did not produce any macroscopically apparent symptoms of toxicity, did not influence NADH_2 dependent GDH activity, and caused only a 13 % inhibition of nitrate reductase). The results presented in this paper confirm earlier observations (references see SAHULKA 1973) that in pea roots GDH activity is rather enhanced than depressed under toxic conditions and under conditions unfavourable to growth.

Acknowledgement

The author wishes to thank Miss J. Smutná for her assistance.

References

- CHAO, T. T., KROONTJE, W.: Inorganic nitrogen transformations through the oxidation and reduction of iron. — Proc. Soil Sci. Soc. Amer. **30** : 193—196, 1966.
- KRETOVICH, V. L., BUNDEL, A. A., BOROVIKOVA, N. V.: [Incorporation of ^{15}N -hydroxylamine into the proteins of wheat seedlings]. In Russ. — Fiziol. Rast. **11** : 31—37, 1964.
- SAHULKA, J.: The regulation of glutamate dehydrogenase, nitrite reductase, and nitrate reductase in excised pea roots by nitrite. — Biol. Plant. **15** : 298—301, 1973.
- SUBRAMANIAN, K. N., SARMA, P. S.: Regulation of nitrate reductase activity in *Neurospora crassa*. — Indian J. Biochem. **6** : 203—207, 1969.