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COMPARATIVE STABILITY OF AMMONIUM- AND NITRATE-INDUCED NITRATE REDUCTASE ACTIVITY IN MAIZE LEAVES

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Key Word Index—*Zea mays*; Gramineae; maize leaves; nitrate reductase; ammonium salt effects.

Abstract—Ammonium sulfate (5 mM) had no effect on nitrate reductase activity during a 3 hr dark incubation, but the enzyme was increased 2.5-fold during a subsequent 24 hr incubation of the maize leaves in light. The enzyme activity induced by ammonium ion declined at a slower rate under non-inducing conditions than that induced by nitrate. The decline in ammonium stimulated enzyme activity in the dark was also slower than that with nitrate. Further, cycloheximide accelerated the dark inactivation of the ammonium-enzyme while it had no effect on the nitrate-enzyme. The experiments demonstrate that increase in nitrate reductase activity by ammonium ion is different from the action of nitrate action.

INTRODUCTION

Induction of nitrate reductase (NR, EC 1.6.6.1) activity (NRA) by nitrate, has been examined in many plant systems [1]. Ammonium ion as an end product of nitrate assimilation inhibits the substrate induction of the enzyme in *Lemna* [2–4] and roots of barley [5] and cotton [6], although in other systems it has no effect on the enzyme [7, 8]. On the other hand, ammonium ion increases NRA in cultured rose [9] and wheat [10] cells and in mung bean seedlings [11, 12]. In a preliminary experiment with maize seedlings it was observed that ammonium ion induces NRA in maize leaves even in the absence of any exogenous nitrate [13]. Since, ammonium ion is an end product rather than the substrate of the enzyme, it would be of interest to see if the 'ammonium ion-induced' enzyme was similar to that induced by nitrate. With this in mind, some properties of the ammonium ion-induced enzyme (NH_4-NRA) in maize leaves were compared to that induced by nitrate (NO_3-NRA).

RESULTS

Effect of ammonium ion on nitrate reductase activity

As reported earlier, addition of 5 mM $(NH_4)_2SO_4$ increased NRA by about 2.5-fold (over the control) in the primary leaves of maize (Table 1). The ammonium ion increased enzyme activity slightly in the shoots also, but inhibited that in the roots. Cycloheximide prevented any increase in enzyme activity by ammonium ion in the leaves. The control also had an appreciable level of NRA, possibly due to mobilization of endogenous nitrate from the storage pool.

In vivo *stability of the ammonium- and nitrate-induced enzyme*

Maize leaves treated with either ammonium or nitrate ions for 24 hr were transferred to non-inducing conditions to examine the stability of the NRA enzyme. As shown in Fig. 1, NH_4-NRA increased up to 2 hr when leaf segments were transferred to a fresh ammonium medium. The

Table 1. Effect of ammonium sulfate on nitrate reductase activity in maize seedlings

Seedling part	Nitrate reductase activity (μmol NO_2 hr/g/fr.wt)		
	Control	Ammonium treated	% increase
Root	1.80	1.45	−22
Shoot	0.42	0.51	+21
Leaf	1.67	4.15	+146

Seedling parts were incubated in 10 mM $CaCl_2$ containing either no (control) or 5 mM ammonium sulfate (treated) for 24 hr in light.

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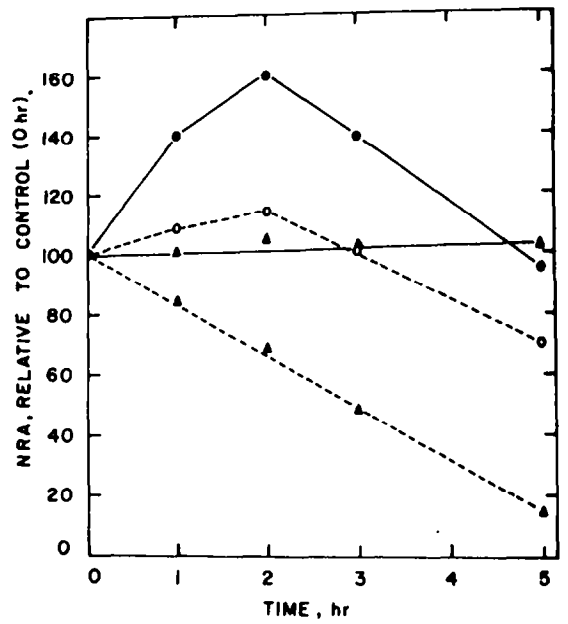

Fig. 1. Stability of ammonium- and nitrate-induced nitrate reductase activity. Leaf segments were induced with either 5 mM $(NH_4)_2SO_4$ or 10 mM KNO_3 for 24 hr in the light. Then the leaves were transferred to 10 mM $CaCl_2$ containing desired nitrogen salt as required. The enzyme was assayed after different time intervals. Circles—NH_4-NRA; triangles—NO_3-NRA; Dashed lines—minus nitrogen; Solid lines—plus nitrogen.

activity started declining thereafter and after 5 hr was almost equal to the 0 hr value. When the leaves were transferred to non-inducing condition (−nitrogen), the enzyme was more or less stable up to 3 hr and then started declining.

In contrast, NO_3-NRA did not change up to 5 hr when the leaves were transferred to a fresh nitrate medium. In −nitrogen (non-inducing) condition, however, the enzyme activity started rapidly declining and after 5 hr only about 15% of the original activity was observed.

Effect of cycloheximide on short term induction and stability of ammonium- and nitrate-induced enzyme activity

As shown in Table 2, a 3 hr incubation with ammonium ion had no effect on NRA while nitrate increased activity by about 2.3-fold during same period. This increase in enzyme activity was inhibited by cycloheximide indicating thereby that increase in NRA by nitrate involves *de novo* protein synthesis.

When the leaves induced with either ammonium or nitrate ions for 24 hr were incubated with cycloheximide, the enzyme activity declined sharply in 5 hr. There was no difference between the enzymes as far as the effect of cycloheximide was concerned. However, the decline in NH_4-NRA was slightly lower than that in NO_3-NRA when the respective salt was also included in the cycloheximide medium.

Dark inactivation of the enzyme

Both NH_4-NRA and NO_3-NRA were quite stable during a 5hr incubation with the respective salts in the light (Table 3). In the dark, however, the rate of decline in activity was higher for NO_3-NRA than for NH_4-NRA. Further, when cycloheximide was also included in the medium, dark inactivation of NO_3-NRA was hardly affected while the inactivation of NH_4-NRA was accelerated.

DISCUSSION

The experiments described demonstrate that the increase in NRA either by ammonium or by nitrate ions is dependent upon protein synthesis, although ammonium ion is able to increase NRA only after a long incubation period. That substrate induction of NRA involves *de novo* synthesis has also been demonstrated in *Lemna minor* [3] rice seedlings [14] and barley [15]. The requirement of protein synthesis in enzyme induction by ammonium ion, however, does not appear to be necessary for direct *de novo* synthesis, but might involve the synthesis of an effector molecule essential for NRA and, therefore, a relatively longer time is required for an increase in enzyme activity by ammonium ions. In the present investigation, the *in vivo* stability of the enzyme also appeared to be dependent upon

Table 2. Effect of cycloheximide on induction and stability of ammonium- and nitrate-induced nitrate reductase activity in maize leaves

Treatment	Nitrate reductase activity (μmol NO_2/hr/g/fr.wt)	
	NH_4-NRA	NO_3-NRA
(A) 3 hr induction		
−Nitrogen*	0.51 (100)	0.51 (100)
+Nitrogen	0.53 (104)	1.20 (236)
+Nitrogen +cycloheximide	0.40 (79)	0.48 (94)
(B) Stability after 5 hr		
+Nitrogen	3.66 (100)	4.48 (100)
−Nitrogen	2.69 (75)	0.92 (20)
+Nitrogen +cycloheximide	1.24 (34)	0.93 (20)
−Nitrogen +cycloheximide	0.67 (18)	0.81 (18)

Leaf segments were floated on desired nitrogenous salt and cycloheximide as required. The nitrogen in the case of NH_4-NRA was 5 mM $(NH_4)_2SO_4$ while in the case of NO_3-NRA it was 10 mM KNO_3.* $CaCl_2$ (10 mM) was included as control. The percentage of control value is given in parentheses.

Table 3. Dark inactivation of ammonium- and nitrate-induced nitrate reductase activity in maize leaves

Treatment	Nitrate reductase activity (μmol NO_2 hr/g/fr. wt)	
	NH_4-NRA	NO_3-NRA
Control	3.81 (100)	4.40 (100)
After 5 hr in nitrogen in.		
Light	3.66 (96)	4.48 (100)
Dark	3.08 (81)	2.88 (65)
Dark + cycloheximide	1.22 (32)	2.53 (56)

Leaf segments floated on either 5 mM $(NH_4)_2SO_4$ (NH_4-NRA) or 10 mM KNO_3 (NO_3-NRA) for 24 hr in light were assayed for enzyme activity (control). Then they were transferred to the same salt containing cycloheximide as required and incubated for 5 hr either in the dark or in the light. The percentage of control value is given in parentheses.

protein synthesis. However, NH_4-NRA appeared to be more stable in a minus ammonium medium than NO_3-NRA in a minus nitrate medium. The NH_4-NRA started declining only after 3 hr while there was a linear decline in NO_3-NRA from the very beginning, which is consistent with earlier observations in many other systems [16–18].

The two enzymes also differed in the process of their dark inactivation and also in the effect of cycloheximide on the process. While cycloheximide may inhibit cytoplasmic protein synthesis with equal efficiency in light and dark, its effect on the specific proteins studied (NH_4-NR and NO_3-NR) may be either the same or different in light and dark, depending upon the mechanism of dark inactivation of the two enzymes. Although several hypotheses have been advanced for the effect of light/dark on NRA, the one proposed by Jolly and Tolbert [19] seems to be the most convincing [1]. The enzyme activity is regulated by a dark-active specific inhibitor which is reversibly inactivated by light [19]. Since light/dark changes cause only inactivation/activation of the presynthesized molecule, an inhibitor of protein synthesis such as cycloheximide does not have any effect on the dark inactivation of nitrate-induced enzyme (Table 3). On the other hand, dark inactivation of NH_4-NRA involved some other mechanism, which was accelerated in the presence of cycloheximide. Apparently, NH_4-NRA required protein synthesis for its maintenance in the dark also.

Although more experiments are required for understanding the induction of NRA by ammonium ions, it is clear that the induction is not via endogenous nitrate as reflected in the difference in the nature of the two enzymes. The enzyme activity in the present experiments has been expressed as total activity and it may be proposed that ammonium ions increase NRA by increasing general protein synthesis. However, incorporation of [^{14}C]-lysine into protein and activity of glucose 6-phosphate dehydrogenase in pea was either inhibited or unaffected by ammonium ions [20] and therefore the above proposal does not seem to be valid.

EXPERIMENTAL

Seeds of *Zea mays* L. cv Ganga safed-2 were used in the present investigation and purchased from National Seed Corporation, New Delhi. Seedlings were raised either on moist filter paper for 5 days (for roots and shoots) or in small pots containing washed sand for 8 days (for leaves) in light of *ca* 5 klx at 25 ± 2°. The seedlings were watered with half strength modified Hoagland solution without any nitrogen. In each case, primary leaves from uniformly grown seedlings were used.

For treatment of the roots, shoots or leaves, they were excised into small (*ca* 5 mm) pieces and floated on the desired solution. The pH in each case was 6.0 and incubation was carried out at 25 ± 2° in light of *ca* 5 klx when required. Cycloheximide was used at a final concn of 0.01 mM. In earlier experiments, chloramphenicol (1 mg/50 ml) was added to prevent any bacterial contamination. Since it did not have any effect on enzyme activity, its use was discontinued later on.

In vivo activity of nitrate reductase was determined as described earlier [21]. Data presented in this paper are the average of at least three replicate experiments.

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