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INHIBITION OF NITRATE REDUCTASE INDUCTION BY CANAVANINE IN MAIZE ROOTS

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Key Word Index—*Zea mays*; Gramineae; maize roots; nitrate reductase; canavanine.

Abstract—The induction of nitrate reductase activity in maize root tips was inhibited by canavanine and the inhibition increased with increasing concentration of canavanine between 0·1 and 1 mM. Addition of canavanine to the induced enzyme had little effect on the disappearance of the enzyme when nitrate was removed, and it is likely that the canavanine reduces the activity of the nitrate reductase by inhibiting its synthesis rather than by accelerating its breakdown.

INTRODUCTION

Inhibition of nitrate reductase (E.C. 1.6.6.1) by many amino acids has been shown in higher plants [1–4]. Canavanine is an analogue of the amino acid arginine. In cultured tobacco cells, while arginine is able to enhance the induction of nitrate reductase by nitrate and counteract the repressive action of other amino acids, canavanine inhibits the enzyme induction [3]. Observed inhibition of the enzyme by canavanine may be either due to decreased nitrate uptake or repression of enzyme synthesis or to the inactivation of induced enzyme. In this communication, it has been shown that canavanine inhibits nitrate reductase by repression.

RESULTS

Canavanine at 0·1–1 mM had no effect on the uptake of nitrate by the maize seedlings. The activity of nitrate reductase in the root tips, however, was strongly inhibited by canavanine (Table 1). By increasing the concentration of canavanine between 0·1 and 1 mM inhibition could be increased. With 1 mM canavanine, the activity of the enzyme was only 14% of the original value.

Since in this experiment canavanine was present at all times during induction of the enzyme, the observed inhibition may be either due to repressed synthesis or to increased inactivation of the induced enzyme. In the conditions where there is no induced synthesis due to substrate, the inhibitory effect of canavanine will be due to the inactivation of the enzyme. In order to differentiate between the repression and the inactivation of the induced enzyme, the canavanine was added to the seedlings 3 hr after induction and in the absence of nitrate (Fig. 1). When nitrate was removed from the induction medium, the linear increase in the enzyme was suspended after a small increase for 0·5 hr. This initial increase

Table 1. Effect of canavanine on the induction of nitrate reductase in root tips

Concentration of canavanine (mM	Fr. wt of root mg/root	Enzyme activity units/mg protein
0	53·7	331
0·1	66·5	226
0·2	58·1	152
0·5	56·3	74
1·0	59·8	48
2·0	51·1	43
5·0	58·9	50

Seedlings were preincubated with the required concentration of canavanine for 1 hr before transferring to the induction medium containing the same concentration of canavanine. Induction and pretreatments were performed in the dark at 26°.

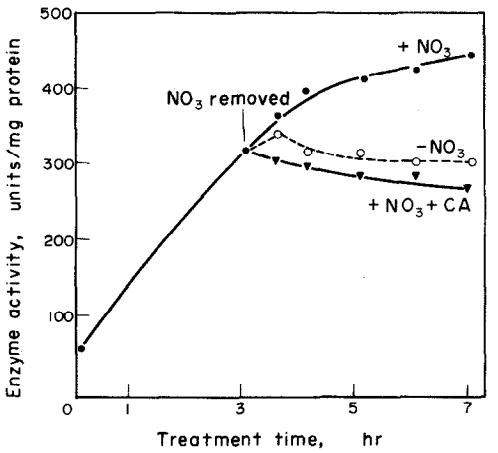

Fig. 1. Effect of canavanine on the stability of nitrate reductase in maize root tips following a 3 hr induction period.

may be the result of the residual endogenous substrate (nitrate). The enzyme level was almost constant for further 3·5 hr. Contrary to this observation, a decrease in nitrate reductase activity was observed by others when nitrate was withdrawn from the medium [5,6].

Figure 1 further shows that the canavanine does not inactivate or accelerate the destruction of nitrate reductase. After 4 hr with canavanine, only a 12% decrease in the enzyme level was observed in the non-inducing conditions. The initial increase observed possibly due to endogenous substrate, however, was not observed with canavanine. Thus, the effect of canavanine is on the synthesis of enzyme.

DISCUSSION

In Filner's experiments with tobacco cells [3], the effect of canavanine on the activity of nitrate reductase was measured after 24 hr in the induction medium, when the enzyme level is in the steady state. Although the inhibitory effects of the amino acids have been described as repression by him, it is difficult to ascertain whether the amino acids are inhibiting the synthesis or accelerating the destruction of the enzyme in some way. In my experiments with root tips, the inhibition by canavanine was observed after 3 hr. Since during this time the enzyme level increases linearly [7], indicating that the rate of synthesis is far greater than the rate of destruction, it is suggested that the canavanine affects the synthesis of enzyme primarily. This is further indicated by the fact that addition of canavanine in the non-inducing condition after 3 hr had no effect on the enzyme level.

In a classical repression [8], canavanine may interact with the product of appropriate regulator gene which in turn may prevent the transcription of the structural gene for the nitrate reductase. Canavanine is incorporated into polypeptide chains during protein synthesis in *Staphylococcus aureus* [9], *E. coli* [10] and rat liver [11]. The altered physicochemical properties of the new protein may reduce the enzymatic activities. In *E. coli*, the substitution of the arginine residue by canavanine in alkaline phosphatase resulted in the accumulation of subunits that were unable to dimerize to form the active enzyme [12]. Incorporation of canavanine into the enzymatic proteins of maize may also interfere with their activities in a similar way. Furthermore, it is also possible that the repression of nitrate reductase by canavanine is the manifestation of nascent ammonia produced by the degradation of canavanine in plant tissues [13].

EXPERIMENTAL

Sterilized seeds of *Zea mays* L. were planted on 0·9% agar dissolved in 1/10 strength Hoagland's soln, which was modified to include either 0 or 10 mM KNO_3 as sole source of N. The agar was contained in large Petri plates. Seedlings were raised at 26° in the dark. When the primary roots were 4–5 cm long (at about 70 hr of planting) suitable seedlings were selected for the induction studies.

Measurement of nitrate. Nitrate uptake from the medium was assayed according to Ref. [14]. Absorbance of the acidified sample from the medium was read at 210 nm. Amount of nitrate was calculated from a standard curve.

Induction of nitrate reductase. The induction medium was contained in a 250 ml beaker covered by a plastic mat. Thirty seedlings were placed on the mat in such a way that their roots were immersed in the medium. The induction medium consisted of 1/10 strength Hoagland soln containing 10 mM KNO_3 and 0·21 μM additional Mo as $Na_2MoO_4 . 7H_2O$ and canavanine as required. Final pH was adjusted to 6. The medium was fully aerated. After the required period of induction, seedlings were washed with cold H_2O and blotted dry. From these seedlings, 0–10 mm root tips were excised for the extraction and assay of the enzyme. The enzyme was extracted and assayed according to Ref. [7]. Protein in the extract was determined by Ref. [15]. Bovine serum albumin was used as a standard. Unit of enzyme activity is defined as nmol of NO_2 produced per hr. L-Canavanine used in the present investigation was obtained from Sigma.

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