The roles of glutamate dehydrogenase, glutamine synthetase and three forms of glutamate synthase in nitrogen assimilation in various organs of *Pisum arvense* L.

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Abstract

The activities of GDH, GS and three forms of GOGAT (NADH, NADPH or ferredoxin-dependent) were studied in the leaves, stems and roots of the *Pisum arvense*. GS and the individual forms of GOGAT dominated in the leaves of 7 day-old plants which were taking up NO_3^- or NH_4^+ ions, while NADH-GDH dominated in the roots of these plants. In comparison with HNO_3^- , NH_4^+ ions stimulated the activity of most of the enzymes of the GS/GOGAT and GDH pathways in stems and roots, while in leaves this effect was age-dependent. The Fd-GOGAT located in leaves and stems was not regulated by NH_4^+ , which indicates that this enzyme is not likely to be directly involved in the assimilation of NH_4^+ ions that have been taken up. The obtained data indicate that at lower tissue NH_4^+ concentration a decisive role in nitrogen assimilation in leaves and stems is played by the GS/GOGAT pathway, while in the roots — by GDH and in less degree by GS/GOGAT. High amounts of accumulated NH_4^+ ions set off a detoxication mechanism which includes NADH-GDH, common to all tissues. Only in 7 day-old leaves did the detoxication of NH_4^+ take place with the involvement of NADH-GOGAT and NADPH-GOGAT.

Key words: assimilation of NH_4^+ , glutamate dehydrogenase, glutamine synthetase, glutamate synthase

INTRODUCTION

The assimilation of ammonium ions in the tissues of higher plants may occur via the GS/GOGAT or GDH pathways. The low K_m of glutamine synthetase for NH_4^+ , which equals approximately $20 \mu\text{M}$ (Mifflin and Lea 1967) suggested that this enzyme is, along with glutamate synthase, the main route of NH_4^+ assimilation. The role of glutamate dehydrogenase in this process was questioned (Probyn and Lewis 1979, Rhodes et al. 1980) because of its low affinity for NH_4^+ (K_m GDH for NH_4^+ is approx. $10\text{--}50 \text{ mM}$, Mifflin and Lea 1977). The studies of Pahlisch and Gerlitz (1980) and Mazurów (1986) carried

out on purified pea and lupine enzymes have shown that GDH may adapt itself to low NH_4^+ concentrations and thus fulfil an assimilatory role in the binding of ammonium ions (Singh and Srivastava 1982, Bielański and Kączkowski 1984, Loyola-Vargas and Sanches de Jimenez 1984, Zelmer and Gunther 1988). It seems that the level of the substrate in plant tissues may be the factor determining the participation of the GS/GOGAT and GDH pathways in the assimilation of the ammonium ions taken up.

In the study presented here the participation of various enzymes of the GS/GOGAT and GDH pathways in the assimilation of NH_4^+ taken up by plants from the culture medium or arising from the reduction of nitrates in leaves, stems and roots of the *Pisum arvense* was studied.

MATERIAL AND METHODS

Pea (*Pisum arvense* L. var. Nieznaniecki) seeds were germinated for 2 days at 27°C in the dark. The seedlings were then transferred to a nitrate (in mM: 5 $\text{Ca}(\text{NO}_3)_2$, 5 KNO_3 , 1 KH_2PO_4 , 1 MgSO_4) or ammonium medium (in mM: 15 NH_4Cl , 5 CaSO_4 , 1 KH_2PO_4 , 2.5 K_2SO_4 , 1 MgSO_4). The mediums contained microelements as described previously (Kubik-Dobosz 1989). Fresh medium was supplied every two days. The plants were grown in 17 hours of light (15.5 W. m^{-2}) and 7 hours of darkness. The leaves, stems and roots of 7 and 14 day-old plants were used for analysis. The plant material was homogenized in a mortar using 2 cm^3 of 50 mM KH_2PO_4 -KOH pH 7.5, 1 mM EDTA, 10 mM MgCl_2 and 2 mM dithiothreitol per gram of material. The homogenate was filtered through four layers of gauze and centrifuged 15 min. at $21000 \times g$. The activities of GS, NADH-GDH, NADH-GOGAT, NADPH-GOGAT and Fd-GOGAT were determined in the supernatant.

The activity of GS was assayed using the transferase method of Hipkin and Syrett (1977) in a reaction medium containing tris-HCl (0.05 M pH 7.2), ADP (1 mM), glutamine (47 mM), sodium arsenate (20 μM) and hydroxylamine (13 mM). The activities of NADH-GOGAT, NADPH-GOGAT and Fd-GOGAT were determined according to Mätsch and Takahashi (1982) in the presence of 50 mM KH_2PO_4 -KOH pH 7.5, 10 mM 2-oxoglutarate, 10 mM glutamine and, appropriately, 0.6 mM NADP/H or 0.23 mg ferredoxin and 12 mM $\text{Na}_2\text{S}_2\text{O}_4$ and 13.5 mM NaHCO_3 . After stopping the reaction by immersing in a boiling water bath for 1 min. the samples were centrifuged 10 min at $10000 \times g$. The supernatant was applied to a column packed with Dowex 1×8 . And glutamic acid was eluted and then assayed by the ninhydrin method (Mätsch et al. 1979, 1980). The activity of NADH-GDH was analyzed according to Duke et al. (1975) in the presence of 0.33 M 2-oxoglutarate, 3M NH_4Cl , 3 mM NADH, 0.1M tris-HCl pH 8.0. The NH_4^+ ion content was determined according to the method of McCullough (1967) after staining an aqueous extract of the plant material with the phenol-hypochlorite reagent.

The presented results are the means of three independent experiments. The data obtained in the individual experiments all showed the same type of changes.

RESULTS

As can be seen from Fig. 1, the activity of GS dominated in leaves, regardless of the age of the plants (7 or 14 days) and the form of nitrogen taken up (NO_3^- , NH_4^+). The activity of GS was very low in the stems and roots and did not usually exceed 20% of GS activity in leaves. In the presence of NH_4^+ increases by 27-74% in GS activity were seen in most of the organs of all of the plants.

Fig. 1. The activity of GS and NADH-GDH in leaves, stems and roots of *Pisum arvense*. The plants were grown for 7 days (A) or 14 days (B) in a nitrate (NO_3^-) or ammonium (NH_4^+) medium. The activity of the enzyme was measured in leaves (light bars), stems (dotted bars) and roots (dark bars)

The data contained in Fig. 1A suggest that NADH-GDH was most active in the roots of plants from both NO_3^- and NH_4^+ mediums and that it showed negligible activity in leaves and stems. However, after 14 days, the activity of NADH-GDH in the leaves of plants growing in the presence of NH_4^+ rose dramatically (Fig. 1B). A similar, although, somewhat smaller effect related to ammonium ions was also observed in the stems and roots of 14-day-old plants.

Fig. 2. The NH_4^+ content (a) and activity of NADPH-GOGAT, NADH-GOGAT and Fd-GOGAT (b) in leaves, stems and roots of *P. arvense*. Remaining legend as in Fig. 1

The leaves and stems of all of the studied plants were characterized by a high Fd-GOGAT activity, whereas in the roots the activity of this enzyme was low (Fig. 2). In comparison with the N- NO_3^- combination, the ammonium nitrogen increased the activity of Fd-GOGAT in the roots but did not cause a change in

the activity of this enzyme in the remaining organs. The results presented on Fig. 2A also indicate that after 7 days of growth of the plants, NH_4^+ ions increased the activities of NADH-GOGAT and NADPH-GOGAT in all of the organs, especially in the leaves. However, after 14 days the activities of NADH-GOGAT and NADPH-GOGAT in leaves, stems and roots remained on a similarly low level (Fig. 2B) in spite of the fact that NH_4^+ ions stimulated the activity of both of these enzymes in the stems by 100-138%.

Regardless of the form of nitrogen in the medium, the roots of 7 day-old plants accumulated similar amounts of NH_4^+ ions (Fig. 2A). In the plants from the NO_3^- combination, ammonium ions were not found in stems and leaves. However, in the plants growing in the ammonium medium, NH_4^+ ions were also found in leaves and stems and their amount increased during cultivation. The highest amounts of free ammonium ions accumulated in the leaves of 14 day-old plants from the NH_4^+ combination (Fig. 2B).

Methionine sulfoximine (MSO) — a specific inhibitor of GS — blocks the assimilation of NH_4^+ in the GS/GOGAT pathway. If plants from the nitrate or ammonium mediums were treated with this inhibitor for 24 hours, then high amounts of ammonium ions were detected in the stems and leaves of such plants, reaching values of 5.3-26.8 $\mu\text{moles/g}$ fresh weight (Table 1). The increase in the level of NH_4^+ in response to MSO in the roots was small and did not exceed 1.1-1.8 $\mu\text{moles/g}$ fresh weight.

Table 1

The effect of methionine sulfoximine (MSO) on the NH_4^+ ion content ($\mu\text{moles NH}_4^+ \times \text{g}^{-1}$ fr.w) in leaves, stems and roots of *Pisum arvense*

Treatment	Leaves	Stems	Roots
NO_3^-	0.0	0.0	2.0
$\text{NO}_3^- + \text{MSO}$	23.0	5.3	3.8
NH_4^+	2.9	1.9	3.6
$\text{NH}_4^+ + \text{MSO}$	26.8	19.6	4.7

The plants were grown for 5 days in nitrate (NO_4^-) or ammonium (NH_4^+) medium, and then for 24 hours with 2.5 mM MSO added to the mediums.

Table 2

The effect of methionine sulfoximine (MSO) on the activity of NADH-GDH ($\mu\text{moles NADH} \times \text{g}^{-1}$ fr. w. $\times \text{h}^{-1}$)

Treatment	Leaves	Stems	Roots
NH_4^+	1.0	1.4	25.3
$\text{NH}_4^+ + \text{MSO}$	1.1 (110)	0.9 (64)	19.3 (76)

The plants were grown for 5 days in an ammonium (NH_4^+) medium, and then for 24 hours in the presence of 2.5 mM MSO. The values in parentheses show percent of control.

As can be seen from the data presented in Table 2, MSO caused a drop in the activity of NADH-GDH in the stems and roots, whereas it did not affect the level of activity of this enzyme in the leaves.

DISCUSSION

Although there is sufficient evidence that there are two metabolic pathways (GS/GOGAT and GDH) incorporating NH_4^+ into amino acids in plant tissues

(Mifflin and Lea 1976), the role of the individual enzymes of both pathways in the assimilation of ammonium ions in the various tissues is still insufficiently documented. The matter is further complicated by the fact that some of the enzymes, such as glutamate synthase, may occur in several molecular forms to which various physiological roles are attributed. Marquez et al. (1986) suggest that Fd-GOGAT is responsible for the photosynthetic and photorespiratory assimilation of ammonium and that NADH-GOGAT assimilates NH_4^+ arising from the reduction of nitrates or from the metabolism of organic nitrogen compounds. In contrast with the data obtained for other species of plants (Arima 1978, Stewart and Rhodes 1978, Bielański and Kączkowski 1984), we found all of the enzymes of the GS/GOGAT and GDH pathways in the above-ground and underground parts of *Pisum arvense*. However, the activities of these enzymes were significantly dependent on the organ and age of the plant. Similarly as in barley or rye seedlings (Lewis et al. 1982, Bielański and Kączkowski 1984), NADH-GDH had the highest activity in the roots of *P. arvense*. Significant amounts of ammonium ions taken up directly from the medium or arising from the reduction of nitrates were found in these organs. *P. arvense* roots seem to be well adapted to the binding of NH_4^+ in the NADH-GDH pathway, while the GS/GOGAT pathway is not so important here. In leaves and stems the GS/GOGAT system seems to be preferred. The NAD-GDH activity in roots of 7 and 14 days-old plants was very slight stimulated by NH_4^+ ions (data not presented).

The experiments conducted with MSO have supplied additional evidence confirming the role played by the GS/GOGAT pathway in the assimilation of ammonium ions in the stems and leaves of *P. arvense* and of GDH in the roots. This inhibitor effectively inhibits GS (Martin et al. 1983), blocking the binding of NH_4^+ in the GS/GOGAT pathway. As is seen from Table 1, the intense accumulation of NH_4^+ under the influence of MSO took place only in the leaves and stems. A rise in the amount of accumulated NH_4^+ in the roots of MSO-treated plants was only from 30–90%, which corresponds roughly to the fall in NADH-GDH activity in these organs (Table 2).

Under conditions of a high supply of NH_4^+ from the medium, *P. arvense* tissues began to put to use a mechanism detoxicating the excess ions. In the roots, this mechanism encompassed mainly NADH-GDH, which agrees with the data presented earlier in a paper by Giván (1979). However, in the leaves and stems of 7 day-old plants which were taking up NH_4^+ , strong stimulation of NADH-GOGAT and NADPH-GOGAT was observed while at the same time the activity of NADH-GDH remained unchanged. The physiological role of NADH-GOGAT decreased however with the age of the plants, leading to a change in the proportions of NADH-GOGAT to Fd-GOGAT in the developing leaves. Matoh and Takahashi (1982) suspect that this change results from developmental changes in plastids and a correlation between light-processes and the activity of Fd-GOGAT. The rapid detoxication of the

ammonium ions accumulated in the leaves and stems of 14 day-old plants in the NH_4^+ combination was made possible by NADH-GDH, whose activity rose abruptly. The NH_4^+ ions did not, however, affect the activity of Fd-GOGAT located in leaves and stems of *P. arvense*. As can be seen from studies (Botella et al. 1988), almost all of the Fd-GOGAT activity in photosynthesizing tissues is associated with chloroplasts. The data presented in the paper by Ogren (1984) show that this enzyme is necessary for the assimilation of the NH_4^+ released during photorespiration. We suggest that the role of leaf Fd-GOGAT in the assimilation of ammonium ions taken up from an external solution is insignificant.

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Udział dehydrogenazy glutaminianowej, syntetazy glutaminowej i trzech form syntazy glutaminianowej w asymilacji azotu w różnych organach Pisum arvense L.

Streszczenie

Badano aktywność GDH, GS i trzech form GOGAT (zależnej od NADH, NADPH lub ferredoksyny) w liściach, łodygach i korzeniach grochu polnego (*Pisum arvense*). W liściach 7-dniowych roślin pobierających jony NO_3^- lub NH_4^+ dominowała GS i poszczególne rodzaje GOGAT, natomiast w korzeniach — NADH-GDH. W stosunku do NO_3^- , jony NH_4^+ stymulowały aktywność większości enzymów szlaków GS/GOGAT i GDH w łodygach i korzeniach, natomiast w liściach efekt zależał od wieku roślin. Fd-GOGAT zlokalizowana w liściach i łodygach nie była regulowana przez NH_4^+ co wskazuje, że ten enzym raczej nie uczestniczy bezpośrednio w asymilacji pobranych jonów NH_4^+ . Jak wynika z uzyskanych danych, przy mniejszej zawartości NH_4^+ w tkankach, decydującą rolę w asymilacji azotu w liściach i łodygach odgrywa szlak GS/GOGAT, natomiast w korzeniach głównie GDH i w mniejszym stopniu GS/GOGAT. Duże ilości zakumulowanych jonów NH_4^+ uruchamiają wspólny dla wszystkich tkanek mechanizm detoksykujący z udziałem NADH-GDH. Jedynie w liściach 7-dniowych roślin detoksykacja NH_4^+ zachodzi przy udziale NADH-GOGAT i NADPH-GOGAT.