GLUTAMATE DEHYDROGENASE AND GLUTAMINE SYNTHETASE IN RYE SEEDLINGS SUPPLIED WITH AMMONIUM AND NITRATE

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1. Location of glutamate dehydrogenase (EC 1.4.1.2 - 1.4.1.4) in the 21-day-old rye seedlings is confined basically to roots while that of glutamine synthetase to leaves; the bulk of the activity (60 - 80%) of both enzymes was found in the 48 000 g supernatant.

2. Glutamate dehydrogenase is probably the same protein acting with NAD+ and NADP+ (the activity ratio about 6:1), inducible by ammonium; the induction is abolished by cycloheximide. A slight, about 25% increase in the activity of glutamine synthetase was observed irrespective of the kind of nitrogen source supplied.

3. The glutamate/glutamine ratio is in roots about 1 and in leaves about 2.5 despite much higher activity of glutamine synthetase in the former organ. The contribution of glutamate, glutamine and aspartate in the total amino acid pool remains constant irrespective of the kind of nitrogen source applied, except in the roots of the plants supplied with ammonium.

4. The glutamate/2-oxoglutarate ratio and a shift towards reduction of the redox potential follows in general changes in glutamate concentration whereas the aspartate/oxaloacetate ratio shows no such dependence.

There are two routes of primary nitrogen assimilation in higher plants: direct amination of 2-oxoglutarate by glutamate dehydrogenase (EC 1.4.1.2 - 1.4.1.4) and incorporation of ammonium into amide group of glutamine in the ATP-requiring reaction catalysed by glutamine synthetase (EC 6.3.1.2). This latter enzyme coupled with glutamate synthase (glutamine:2-oxoglutarate aminotransferase, NADPH oxidizing, EC 2.6.1.53) provides another, indirect, way for glutamate formation. Other amination systems seem to be of minor importance (Folkes & Sims, 1974).

The incorporation pattern of $^{15}$N, high $K_m$ values for NH$_4^+$ (10$^{-3}$ M) and the results obtained with specific inhibitors of glutamate dehydrogenase suggest that,
at low NH\textsuperscript{+} concentration, this enzyme does not play any major role in assimilation of nitrogen, at least in microorganisms and aquatic plants. Data on terrestrial plants are inconclusive (for review see Miflin & Lea, 1976).

The presented paper is a preliminary report on the response of glutamate dehydrogenase and glutamine synthetase to the changes in the intracellular concentration of ammonium and nitrate in the leaves and roots of rye seedlings. The examination of this response was extended by determination of the amino acid pool, glutamine, glutamate and 2-oxoglutarate — the metabolites participating in assimilation of nitrogen by glutamate dehydrogenase and glutamine synthetase. For comparison purposes we included measurements of the level of oxaloacetate, aspartate and asparagine which are not directly involved in the primary assimilation process.

**MATERIALS AND METHODS**

*Chemicals.* Glutamate dehydrogenase, glutamate oxaloacetate transaminase, NADH, NADPH, NAD\textsuperscript{+}, ATP, pyruvate and aspartate were from Boehringer und Soehne (Mannheim, F.R.G.); malate dehydrogenase, hydroxy pyruvate, ketosialeric acid and cytochrome c were from Sigma (St. Louis, Mo., U.S.A.); Tris(hydroxymethylaminomethane) and 2-mercaptoethanol were from Koch-Light (Colnbrook, England); Sephadex G-25 coarse was purchased from Pharmacia (Uppsala, Sweden) and trichloroacetic acid from Reanal (Budapest, Hungary). Bovine serum albumin and Amberlite IR-120 were from Serva Feinbiochemica (Heidelberg, F.R.G.) and dithiothreitol from Calbiochem (Los Angeles, Calif., U.S.A); other reagents were from Fabryka Odczynników Chemicznych (Gliwice, Poland).

*Materials.* Plants (*Secale cereale* c.v. Dańkowskie Złote) were grown for three weeks in a greenhouse at 14 h photoperiod in 51 plastic pots (15 - 20 seedlings per pot) filled with washed quartz sand. From the 5th day of cultivation, the cultures were supplied daily with 50 ml of the solution containing in a volume of 1 l: 0.428 g NH\textsubscript{4}NO\textsubscript{3}; 0.236 g Ca(NO\textsubscript{3})\textsubscript{2} \cdot 4H\textsubscript{2}O; 0.150 g KNO\textsubscript{3}; 0.102 g (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4}; 0.490 g MgSO\textsubscript{4} \cdot 7H\textsubscript{2}O; 0.006 g FeCl\textsubscript{3} \cdot 6H\textsubscript{2}O. Total volume of the solution added was 750 ml per pot. In the induction experiments plants were grown for 18 days without any fertilizer and were supplied with 50 ml of tap water per pot. Then to the hydroponic system was added the solution containing in a volume of 1 l: 0.520 g KH\textsubscript{2}PO\textsubscript{4}; 0.49 g MgSO\textsubscript{4} \cdot 7H\textsubscript{2}O; 0.060 g CaCl\textsubscript{2} \cdot 6H\textsubscript{2}O; 0.006 g FeCl\textsubscript{3} \cdot 6H\textsubscript{2}O. After 48 h plants were transferred to the induction medium supplemented additionally with 0.660 g (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4} plus 0.340 g (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} or with 0.952 g Ca(NO\textsubscript{3})\textsubscript{2} \cdot 4H\textsubscript{2}O plus 0.766 g KNO\textsubscript{3}.

*Preparation of extracts for enzyme assays.* Leaves and roots frozen in liquid nitrogen were homogenized in a mortar with 0.2 M-Tris/HCl buffer, pH 7.6, containing 1 mm-2-mercaptoethanol (5 ml/g of fresh tissue). The homogenate was centrifuged at 20,000 g for 15 min. The supernatant obtained was freed of the low-molecular compounds using Sephadex G-25 coarse, and assayed for enzymatic activities.

*Preparation of subcellular fractions.* Leaves and roots were washed, rapidly cut and homogenized in a Virtis homogenizer (3 \times 10 s) in the medium (1:4, w/v) containing 0.4 m-sucrose; 0.05 M-Tris/HCl buffer, pH 7.5; 1 mm-MgCl\textsubscript{2}; 5 mm-dithio-
treitol; 5 mM-KCl; and 0.1% bovine serum albumin. The homogenate was filtered through three layers of gauze and the filtrate was centrifuged at 500 g for 5 min. The supernatant after removal of cell debris was centrifuged at 10 000 g for 10 min to obtain the particle fraction. The successive supernatant was then spun down at 48 000 g for 15 min. The 10 000 g and 48 000 g pellets were washed twice with 0.5 M-sucrose containing 0.05 M-Tris/HCl buffer, pH 7.5, spun down as before and resuspended in 3 ml of 0.05 M-Tris/HCl buffer, pH 7.5. The suspensions were homogenized in a mortar in liquid nitrogen. The homogenates were centrifuged at 20 000 g for 15 min, the supernatants obtained were separated from low-molecular compounds using Sephadex G-25 coarse and were used for the enzyme assay and protein determinations. The particle fraction (10 000 g) obtained from roots and leaves contained 90% of cytochrome oxidase and 88% of chlorophyll. The content of cytochrome oxidase and chlorophyll in the 48 000 g supernatant did not exceed 10%, the pellet was free of both markers.

Preparation of extracts for determination of metabolites. To 1 g of washed plant tissue 5 ml of 10% (v/v) perchloric acid and 100 mg of insoluble polyvinyl pyrrolidone, were added and homogenized in a mortar for 3 min. The extract was centrifuged at 20 000 g for 15 min, neutralized with 20% KOH, the potassium perchlorate formed on 30 min standing in ice was centrifuged off, and the supernatant was used for determinations of α-ketoacids.

Free amino acids, ammonium and nitrate were extracted with 80% ethanol as described by Nowakowski & Byers (1972).

Enzyme assays. Glutamate dehydrogenase, alanine dehydrogenase and L-amino-acids: NAD oxidoreductase, deaminating (EC 1.4.1.5) were determined at 30°C by measuring the decrease in absorbance at 340 nm. The modified reaction mixture of Barash et al. (1973) contained in a final volume of 3.1 ml: 100 mM-Tris/HCl, pH 7.6; 25 mM-α-ketoacids; 200 mM-NH₄Cl; 0.25 mM-NAD(P)H, and 0.2 - 0.5 ml of the enzyme extract (1 - 2.5 mg protein).

Glutamine synthetase activity was measured colorimetrically after O'Neal & Joy (1973) basing on the formation of γ-glutamylhydroxamate. The slightly modified incubation mixture contained in a total volume of 2 ml: 100 mM-triethanolamine buffer, pH 7.8; 80 mM-glutamate; 8 mM-ATP; 6 mM-NH₄OH; 30 mM-MgSO₄; 1.5 mM-EDTA; and 0.2 - 0.4 ml of enzyme extract (1 - 2 mg protein). The mixture was incubated for 10 min at 37°C. The reaction was stopped by the addition of 0.5 ml of 0.5 M-trichloroacetic acid. The precipitated protein was centrifuged off and to the 2 ml samples of the supernatant was added 1 ml of the solution containing 0.57 M-FeCl₃ and 1 M-HCl. The amount of γ-glutamylhydroxamate formed was measured at 540 nm. Standard curve was prepared using tyrosine hydroxamate.

Asparagine synthetase was determined according to Black & Wright (1955).

Cytochrome c:NADH oxidase was assayed by the method of Cooperstein & Lazarow (1951).

Other assays. Glutamate was assayed enzymatically with glutamate dehydrogenase after Bernt & Bergmeyer (1962), aspartate dehydrogenase according to
Pfleiderer (1962). 2-Oxoglutarate was estimated according to Bergmeyer & Bernt (1962) and oxaloacetate after Hohorst & Reim (1962). Glutamine was measured as glutamate after hydrolysis of amide group in 1 M-H₂SO₄ for 3 h (Nowakowski & Byers, 1965). Concentration of ammonium and nitrate was measured using the steam distillation method of Bremner & Keeney (1965). The total amino acid pool was estimated colorimetrically by the method of Alberti & Bartley (1963) and protein content according to Lowry et al. (1951). Chlorophyll was assayed by the method of Arnon (1949).

RESULTS AND DISCUSSION

Distribution of glutamate dehydrogenase and glutamine synthetase in rye seedlings

As can be seen from Table 1, activity of glutamate dehydrogenase in rye seedlings is confined basically to roots whereas that of glutamine synthetase to leaves. In these experiments nitrogen was provided in equal portions of ammonium and nitrate. The activity of NAD⁺-dependent glutamate dehydrogenase in roots equalled that of glutamine synthetase in leaves and vice versa. The activity of NADP⁺-dependent dehydrogenase was severalfold lower.

Table 1

The activities of glutamate dehydrogenase and glutamine synthetase in the 21-day-old rye seedlings

For details see Materials and Methods. The values are means of three experiments.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity (nmol/min per mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>Glutamate dehydrogenase (EC 1.4.1.2 - 1.4.1.4)</td>
<td></td>
</tr>
<tr>
<td>NAD⁺-dependent</td>
<td>22.5</td>
</tr>
<tr>
<td>NADP⁺-dependent</td>
<td>5.0</td>
</tr>
<tr>
<td>Glutamine synthetase (EC 6.3.1.2)</td>
<td>175.0</td>
</tr>
</tbody>
</table>

Differences in distribution of glutamate dehydrogenase and glutamine synthetase reflect different functions and metabolism of the two organs and point to the role of specific regulatory metabolites and/or availability of precursors and the effect of the reaction products.

Negligible activity of alanine dehydrogenase was found in roots and that of asparagine synthetase in leaves (2 - 3 nmol/min per mg protein). No activity of L-aminoacids:NAD⁺ oxidoreductase (deaminating) (EC 1.4.1.5) could be detected as examined with hydroxypruvate and 2-oxoisovaleric acid in the reductive amination systems. This is in agreement with the general opinion on the limited importance of the above-mentioned systems in assimilation of nitrogen in higher plants (Folkes & Sims, 1974).
Table 2

Subcellular distribution of the activities of NAD⁺- and NADP⁺-dependent glutamate dehydrogenase and glutamine synthetase in the leaves of 21-day-old rye seedlings

For details see Materials and Methods. Values in parentheses indicate per cent of the total activity of crude extract. Data are the means of three experiments. Specific activity expressed per mg of protein.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Activity (nmol/min)</th>
<th>Glutamate dehydrogenase</th>
<th>Glutamine synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NAD⁺-dependent</td>
<td>NADP⁺-dependent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>total</td>
<td>specific</td>
</tr>
<tr>
<td>Crude extract</td>
<td>710 (100)</td>
<td>25.2</td>
<td>115 (100)</td>
</tr>
<tr>
<td>Particles</td>
<td>232 (33)</td>
<td>115.0</td>
<td>26 (22)</td>
</tr>
<tr>
<td>48 000 g supernatant</td>
<td>469 (66)</td>
<td>17.0</td>
<td>85 (74)</td>
</tr>
</tbody>
</table>

The bulk of the activity (70 - 80%) of glutamate dehydrogenase and glutamine synthetase was found in the 48 000 g supernatant of leaf homogenate (Table 2). However, the specific activity of both enzymes in the particle fraction was distinctly higher than in the organelle-free supernatant. Distribution of the activity of NAD⁺- and NADP⁺-dependent glutamate dehydrogenase in root homogenate (Table 3) was similar to that observed in leaves (Table 2). Although enzymatic activities of particular fractions varied (up to 30%), the relative distribution values never exceeded 10%. The activity of NAD⁺-dependent glutamate dehydrogenase was 5 - 6 times higher than that of NADP⁺-dependent enzyme, their ratio being the same in the particle fraction and in the 48 000 g supernatant both in leaves and roots.

Table 3

Subcellular localization of NAD⁺- and NADP⁺-dependent glutamate dehydrogenase in the roots of 21-day-old rye seedlings

For details see Materials and Methods. Values in parentheses indicate per cent of total activity of crude extract. Data are the means of two experiments. Specific activity expressed per mg of protein.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Glutamate dehydrogenase activity (nmol/min)</th>
<th>Glutamine synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAD⁺-dependent</td>
<td>NADP⁺-dependent</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>specific</td>
</tr>
<tr>
<td>Crude extract</td>
<td>3120 (100)</td>
<td>390</td>
</tr>
<tr>
<td>Particles</td>
<td>1162 (37)</td>
<td>2470</td>
</tr>
<tr>
<td>48 000 g supernatant</td>
<td>1997 (64)</td>
<td>277</td>
</tr>
</tbody>
</table>

The results obtained with rye seedlings are not consistent with those obtained by Gayler & Morgan (1976) in Caulerpa simpliciuscula, in which the bulk of glutamate dehydrogenase activity was found in chloroplasts considered as a site of the
anabolic NADP⁺-dependent glutamate dehydrogenase cooperating with nitrite reductase. Although specific activity of the particle fraction of rye seedlings exceeds that in cytosol, especially in roots (mitochondria?), high activity of glutamate dehydrogenase and glutamine synthetase in the organelle-free supernatant suggests that cytosol is the main site of primary amino acid synthesis both in leaves and roots.

The amino acid pool and the glutamate/2-oxoglutarate and aspartate/oxaloacetate ratios

Rye seedlings starved of nitrogen for 48 h were transferred from pots to the liquid medium containing ammonium or nitrate as the nitrogen source, and kept in this medium for another 72 h. Data given in Fig. 1 illustrate time-course of changes in the intracellular concentration of ammonium and nitrate in leaves and roots following nitrogen supply. The data presented are comparable since one gram of both fresh leaves and roots corresponded to 0.12 g of dry wt. The level of nitrate in the seedlings grown on ammonium medium was not determined. In plants not supplemented with nitrogen, changes in concentration of ammonium and nitrate were negligible and during the 72 h experimental period the level of ammonium varied from 7.2 to 6.1 in roots and from 6.5 to 5.5 μmol/g of fresh wt. in leaves; concentration of nitrate decreased from 0.4 to 0.1 and from 0.25 to 0.1 μmol/g fresh wt., respectively.

![Graphs showing concentration vs time](image)

Fig. 1. Changes in the intracellular concentration of ammonium and nitrate in the 3-week-old rye seedlings kept on the medium containing ammonium (A) and nitrate (B) as a nitrogen source. The 18-day-old seedlings were kept for 72 h in the liquid medium containing ammonium or nitrate at concentration of 25 mM. Concentration of ammonium (circles) and nitrate (triangles) in the leaves (open) and roots (shaded).

As can be seen from the data presented, the uptake of ammonium was far more rapid than that of nitrate but the concentration of nitrate in roots after 72 h of incubation was practically the same as that of ammonium. Due to the time needed for
translocation, concentration of both forms of nitrogen in leaves increased slower but after 24 h the ammonium level in leaves equalled or even surpassed the level in roots. Concentration of nitrate in leaves remained considerably lower than in roots. In plants kept in the nitrate medium, concentration of ammonium was raised after 72 h in roots by about 60% and in leaves by about 25%. The increase in ammonium concentration following the 8 h lag period reflected the induction of nitrate reductase responsible for the formation of ammonium.

Under conditions of the increased intracellular concentration of ammonium and nitrate, we examined changes in the size of amino acid pool and in the concentration of 2-oxoglutarate, glutamine and glutamate.

The size of total amino acid pool in roots was half that in leaves (Fig. 2). In comparison with the control grown without a nitrogen source, the pool was increased in both organs by about 5 μmol/g wet weight, i.e. only by 10 - 30% on the medium containing nitrate, whereas on the ammonium medium the pool was raised twofold in leaves and about threefold in roots, i.e. by 30 and 35 μmol/g fresh wt., respectively.

![Graph showing amino acid concentration over time](image)

Fig. 2. The pool of free amino acids in the 21-day-old rye seedlings, supplied with ammonium (circles), nitrate (triangles) or starved of nitrogen (squares). Leaves (open), roots (shaded). For growth conditions see legend to Fig. 1 and Materials and Methods.

Data given in Fig. 3 show that, in plants not supplemented with nitrogen, concentration of free glutamate was about twice that of aspartate; the level of both amino acids was higher in leaves than in roots, but, due to a large difference in the size of amino acid pool, glutamate constituted only 7% of total free amino acids in leaves and up to 14% in roots. Concentration of asparagine was by one order of magnitude lower than of glutamine and the distribution pattern of both amides was different: asparagine level was higher in leaves than in roots whereas concentration of glutamine was higher in roots. The glutamate/glutamine ratio was about 1 in roots and about 2.5 in leaves.

The transfer to the nitrogen-containing medium resulted in the increase of the level of all four amino acids studied. In leaves the increase in concentration of gluta-
Fig. 3. Concentration of glutamate (A), glutamine (B), aspartate (C) and asparagine (D) in the 3-week-old rye seedlings supplied with ammonium (circles), nitrate (triangles) or starved of nitrogen (squares). Leaves (open), roots (shaded). 18-day-old seedlings were transferred to the liquid medium containing no nitrogen, or either ammonium or nitrate at concentration of 25 mM. For details see Methods.

Glutamate, glutamine and aspartate was in general parallel to the changes in the size of total amino acid pool irrespective of the kind of nitrogen source applied. Thus, in spite of almost doubled concentration of glutamate and aspartate (Fig. 3A, C) in plants provided with ammonium, their percentage contribution in the amino acid pool remained practically unaltered. The profile of changes in roots of the plants kept on nitrate medium was similar to that observed in leaves. Growth on the ammonium medium resulted in a distinctly lower rate of accumulation of glutamate and glutamine as compared with the increase in total amino acid pool. In consequence, the glutamate/glutamine ratio remained constant but contribution of these two amino acids to the pool was reduced. This indicates preferential formation of other amino acid(s) in roots in the presence of ammonium. This amino acid is neither aspartate, contribution of which to the pool was decreased similarly as that of glutamate and glutamine, nor asparagine, concentration of which in rye seedlings was very low and practically unchanged (Fig. 3D).

High stability of the glutamate/glutamine ratio in leaves and roots of rye seedlings under various experimental conditions is of considerable interest, and inclined us
to examine the level of 2-oxoglutarate, a specific acceptor of ammonium both in the dehydrogenase and synthetase systems. The increase in concentration of glutamate and aspartate in plants supplied with nitrogen was associated with reduced concentration of both 2-oxoglutarate and oxaloacetate (Table 4). The decrease in concentration of these ketoacids in Chlorella transferred to the nitrogen-containing medium was observed by Satilov et al. (1967).

**Table 4**

*Content of 2-oxoglutarate and oxaloacetate (and the glutamate/2-oxoglutarate and aspartate/oxaloacetate ratios) in the rye seedlings supplied with ammonium or nitrate*

The 18-day-old seedlings were first starved of nitrogen, then kept in the medium containing alternately ammonium (25 mM) or nitrate (25 mM) as described under Methods. Data are means of two experiments.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Time after transfer (h)</th>
<th>Concentration (nmol/g. wet wt.)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2-oxoglutarate</td>
<td>oxaloacetate</td>
</tr>
<tr>
<td>Roots (\text{NH}_4^+) medium</td>
<td>0</td>
<td>147</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>67</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>55</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>147</td>
<td>92</td>
</tr>
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<td></td>
<td>4</td>
<td>109</td>
<td>85</td>
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<td></td>
<td>12</td>
<td>100</td>
<td>82</td>
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<tr>
<td></td>
<td>24</td>
<td>90</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>78</td>
<td>52</td>
</tr>
<tr>
<td>Leaves (\text{NH}_4^+) medium</td>
<td>0</td>
<td>128</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>81</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>74</td>
<td>61</td>
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<td></td>
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<td>64</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>NO(_3^-) medium</td>
<td>0</td>
<td>128</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>121</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>101</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>85</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>51</td>
<td>34</td>
</tr>
</tbody>
</table>

Data presented in Table 4 show that the decrease in concentration of 2-oxoglutarate was more pronounced on ammonium than on nitrate media consistently with the rate of glutamate accumulation; this regularity is also reflected by the glutamate/2-oxoglutarate ratio. In contrast, concentration of oxaloacetate dropped by half independently of the experimental conditions, and was not related to the changes in concentration of aspartate. The decrease in concentration of 2-oxoglutarate was more rapid than accumulation of glutamate, especially within the first
4 h of the experiment; this indicates a flow of amino group via glutamate in the transamination process.

The convergence of changes in concentration of 2-oxoglutarate and glutamate and the stability of the glutamate/glutamine ratio proves that under our experimental conditions neither the availability of 2-oxoglutarate nor of reducing equivalents limited primary assimilation of nitrogen. To get a better insight into this process we measured the time-course of changes in the activities of glutamate dehydrogenase and glutamine synthetase in plants supplied with ammonium and nitrate.

Effect of ammonium and nitrate on the activity of glutamate dehydrogenase and glutamine synthetase

In the plants non-supplemented with nitrogen the activity of glutamate NAD+ dependent dehydrogenase in leaves was about 12 nmol/min per mg protein and in roots about 50 nmol/min per mg protein (Fig. 4). The values reported for the activity of this enzyme in plants differ widely depending on the species and environmental conditions. According to Stewart & Rhodes (1977) the activity of glutamate dehydrogenase in * Lemma minor* amounted up to 100 nmol/min per mg protein while in *Platynomonas striata* (Edge & Ricketts, 1978) it was only 0.1 nmol/min per mg protein. In roots of *Pisum sativum* it was 21 nmol/min per mg protein, i.e. of the same order of magnitude as in the roots of rye seedlings.

![Fig. 4. Glutamate dehydrogenase activity in the leaves (A) and roots (B) of the 3-week-old rye seedlings. NAD+ dependent (open symbols) and NADP+ dependent (shaded symbols) activities of glutamate dehydrogenase in plants supplied with ammonium (circles), nitrate (triangles) or starved of nitrogen (squares). For growth conditions see legend to Fig. 3 and Materials and Methods.](image)

The activity of glutamate dehydrogenase was increased in leaves (Fig. 4A) after a 12 h lag period irrespective of the type of nitrogen source; by this time ammonium was formed by nitrate reductase in plants kept on nitrate medium (Fig. 1). On the ammonium medium, dehydrogenase activity was about twice as high as in plants...
non-supplemented with nitrogen. The action of the inducible glutamate dehydrogenase is probably responsible for the gradual decrease in the intracellular ammonium ion concentration. On the nitrate medium, the increase in dehydrogenase activity did not exceed 50%. A very similar induction pattern was observed in roots; the only difference was a shorter lag period. The induction of NADP⁺-dependent glutamate dehydrogenase followed the same pattern as the induction of the NAD⁺-dependent form. The ratio of the activities with both coenzymes was the same as that observed in subcellular fractions examined both in leaves and roots (Table 3). This strongly supports the conclusion that in rye seedlings, contrary to Chlorella (Meredith et al., 1978), glutamate dehydrogenase is the same protein showing the activity with both nucleotides.

![Graph](image1)

**Fig. 5**

Induction of glutamate dehydrogenase in the leaves of 3-week-old rye seedlings by ammonium. NAD⁺-dependent (circles) and NADP⁺-dependent (triangles) activity of glutamate dehydrogenase following the addition of ammonium (25 mM) (1); ammonium (25 mM) plus cycloheximide (10 μg/ml) (2); and controls without additions (3). From the 3-week-old seedlings grown on sand in pots under conditions described in Materials and Methods, leaves were cut off and transferred for 24 h to hydroponic systems containing the nutrient medium (see Methods) supplemented as indicated.

![Graph](image2)

**Fig. 6**

Glutamine synthetase activity in the leaves of 3-week-old rye seedlings, supplied with ammonium (O), nitrate (△), or starved of nitrogen (□). Conditions as in the legend to Fig. 3 and in Materials and Methods.

The inducible character of glutamate dehydrogenase in rye was confirmed in the experiment in which ammonium was added together with cycloheximide (Fig. 5), an antibiotic inhibiting the action of 60S ribosomal subunit in protein biosynthesis. The addition of the antibiotic completely abolished induction of the NAD⁺- and NADP⁺-dependent activity of glutamate dehydrogenase. The same results were obtained by Barash et al. (1973) with oat seedlings.

Under the same conditions of nitrogen supply, the changes in the activity of glutamine synthetase (Fig. 6) were rather small: on nitrate medium the activity
increased only by about 25% and on addition of ammonium a slight but consistently observed decrease was followed by an about 20% increase in the activity. Since concentration of glutamine was simultaneously increasing, the inhibitory effect of glutamine on glutamine synthetase should be excluded. This is at variance with the earlier observations of Rhodes et al. (1976) on Lemma minor but is in agreement with the results of Rowell et al. (1977) for blue-green algae.

The results presented in this paper show a higher rate of assimilation of nitrogen into amino acids, when nitrogen is administered in the form of ammonium; the role of inducible glutamate dehydrogenase in this process was also evidenced. However, a larger size of amino acid pool does not necessarily mean higher conversion of nitrogen to proteins. In rice seedlings, in spite of faster uptake and assimilation, ammonium ion is converted to proteins at the same rate as nitrate (Oji & Izawa, cf Sasakawa & Yamamoto, 1978). The evaluation of the role of the second route of nitrogen assimilation in cereals via glutamine synthetase requires information on glutamate synthase — the second enzyme of the pathway. The preliminary observations indicate that the activity of the ferredoxin-dependent glutamate synthase in leaves is of the same order of magnitude as that of glutamine synthetase (Bielawski, unpublished).

The preferential location of glutamate dehydrogenase in roots and of glutamine synthetase in leaves is not reflected in the composition of the amino acid pool in both organs. It is not clear why the concentration of glutamine in roots is higher than in leaves although in roots the activity of glutamine synthetase is hardly detectable; and what is the mechanism of precise regulation of the glutamate/glutamine ratio, stable in both organs under various conditions of nitrogen supply. It is possible that the energy charge of the cell is responsible for this status since it is known that ATP inhibits glutamate dehydrogenase and stimulates glutamine synthetase (Stewart & Rhodes, 1977).

REFERENCES


DEHYDROGENAZA GLUTAMINOWA I SYNTETAZA GLUTAMINOWA W SIEWKACH ŹYTA: WŚYW JONÓW AMONOWYCH I AZOTANOWYCH

Streszczenie

1. W 21-dniowych siewkach źыта niemal całą aktywność dehydrogenaz glutaminowej stwierdzono w korzeniach, natomiast syntetyzy glutaminowej — w liściach; 60 - 80% aktywności obydwu enzymów znajdowało się w supernatancie uzyskanym przez wirowanie przy 48 000 g.

2. Dehydrogenaza glutaminowa prawdopodobnie stanowi cząsteczkę białkową współdziałającą zarówno z NAD+ jak NADP+ (stosunek aktywności około 6:1), indukowaną przez jony amonowe. Indukcja enzymu znoszona jest przez cykloheksimid. Nieznaczny, ok. 25% wzrost aktywności syntetyzy glutaminowej obserwowano niezależnie od źródła dostarczonego azotu.
3. Stosunek glutaminianu do glutamininy wynosi w korzeniach ok. 1, a w liściach ok. 2,5, pomimo że aktywność syntetyz glutaminowej w liściach jest znacznie wyższa. Udział glutaminianu, glutamininy i asparaginianu w puli aminokwasowej pozostaje taki sam niezależnie od formy podanego azotu, z wyjątkiem korzeni roślin, którym dostarczono NH₄⁺.

4. Stosunek glutaminianu do α-ketoglutaramianu i przesunięcie potencjału redox w kierunku redukcji zmienia się ze zmianą stężenia glutaminianu; brak tej zależności w odniesieniu do układu asparaginian/szczawioocutan.

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