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Ammonia Assimilation in the Roots of Nitrate- and Ammonia-Grown *Hordeum Vulgare* (cv Golden Promise)

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ABSTRACT

^{15}N kinetic labeling studies were performed on seedlings of *Hordeum vulgare* L. var. Golden Promise growing under steady state conditions. Patterns of label incorporation in the pools of nitrogen compounds of roots fed [^{15}N]ammonium were compared with computer-simulated labeling curves. The data were found to be quantitatively consistent with a three-compartment model in which ammonium is assimilated solely into the amide-N of glutamine. Labeling data from roots fed [^{15}N]nitrate were also found to be at least qualitatively consistent with the assimilation of ammonia into glutamine. Methionine sulfoximine almost completely blocked the incorporation of ^{15}N label into the amino acid pools of barley roots fed [^{15}N]nitrate. These observations suggest that ammonia assimilation occurs solely via the glutamine synthetase/glutamate synthase pathway in both nitrate- and ammonia-grown barley roots.

Numerous studies have demonstrated that both roots and leaves of higher plants possess the enzymic potential for the assimilation of ammonia either via the direct reductive amination of 2-oxoglutarate, catalyzed by GDH[2] or via the combined action of GS and glutamate synthase (23). The now widely held belief that the GS/glutamate synthase pathway is solely responsible for ammonia assimilation in these tissues (15) is, however, founded largely on indirect evidence concerning the activities, kinetic properties, and subcellular localization of the assimilatory enzymes. A more direct approach to establish the pathway of ammonia assimilation in higher plants is a quantitative analysis of the kinetics of ^{15}N incorporation into tissue amino acid pools from an isotopically labeled nitrogen source. Such an approach has been successfully applied to cultures of *Candida utilis* growing under steady state conditions (3, 21), demonstrating that in this organism the greater proportion of ammonia taken up from the medium is assimilated via GDH. Comparable studies with cultures of *Lemna minor* gave labeling data consistent with a two-compartment model in which ammonia is assimilated solely via the GS/glutamate synthase route (18).

Data from kinetic labeling studies with root and shoot tissues of land plants have been insufficiently quantitative for conclusions to be reached on the pathway of ammonia assimilation. Thus, ^{15}N-feeding experiments with both root (17, 27) and shoot (8) tissues, and studies using ^{13}N (13, 22) in which glutamine was initially more highly labeled than glutamate, have been interpreted as being consistent with ammonia assimilation via the GS/glutamate synthase route. Similarly, experiments in which glutamate was initially more highly labeled than glutamine (6, 8) have been interpreted as being at least qualitatively consistent with assimilation via GDH. Such conclusions are, however, speculative in the absence of information on the number and relative sizes of the glutamate and glutamine pools in the tissues concerned.

The purpose of these studies was to obtain kinetic labeling data from experiments in which *Hordeum* seedlings, growing under steady state conditions, were fed ^{15}N-labeled ammonia or nitrate. Attempts were then made to apply a quantitative analysis to the ^{15}N incorporation patterns in ammonia-fed barley roots.

MATERIALS AND METHODS

Growth of Plants. The growth system used was a 'continuous flow' water culture system. Seeds of *Hordeum vulgare* L. var. Golden Promise were soaked overnight in running tap water, with aeration, before sowing on sections of Netlon mesh covered with muslin and stretched over the top of lengths of plastic guttering filled with culture solution. Culture solution was cycled through the gutters from a large reservoir by means of a pump. The flow of nutrient solution through the gutters was adjusted to a rate sufficient to prevent the concentrations of nutrient in the gutters differing from those in the reservoir due to uptake by the plants. Seedlings were subjected to a 14-h daylight regime, with a light intensity of 4.95 J cm $^{-2}$ h^{-1} at the level of the plants, and a constant temperature of 23°C. The nutrient solution used was a modified one-quarter strength Long Ashton solution (4). Nitrogen was supplied either as potassium nitrate or ammonium chloride. The volume of the reservoir was chosen to prevent the concentration of ammonia or nitrate in the culture solution from being reduced by more than 10% over a 24-h period. Labeling studies were performed on 9-d-old seedlings. At the start of a ^{15}N-labeling experiment, the pump was stopped, and the gutters were rapidly drained and refilled with culture solution containing the nitrogen source labeled at 96% ^{15}N atom abundance. The reservoir was exchanged for one containing the labeled nitrogen source, and the pump was restarted. The plants were sampled by removing individual sections of Netlon mesh.

Extraction of Soluble Nitrogen Pools. Root and shoot tissues were washed with H_2O, and after blotting and weighing, 1.5 to 3.5 g were frozen in liquid N_2 in a mortar. Soluble nitrogen compounds were extracted in a methanol-chloroform-water mixture as described by Orebamjo and Stewart (16). After evaporating to

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[2] Abbreviations: GDH, glutamate dehydrogenase; GS, glutamine synthetase; MSO, methionine sulfoximine.

dryness at 25°C, residues were taken up in 5 ml H_2O.

Separation of Nitrogen Compounds. NH_3 in plant extracts was separated from nitrate and amino acids by passage through a column (0.5 × 2.0 cm) of Dowex 50W-X8 (400 mesh) sodium form equilibrated with 10 mM sodium acetate, pH 6.2. After washing with 2.5 ml sodium acetate, NH_3 was eluted with 25 ml 50 mM Na phosphate buffer, pH 7.5. In order to separate amino acids from strong anions, including nitrate, the wash fractions from the above column were passed through a column (0.5 × 3 cm) of Dowex 50W hydrogen form. Nitrate was eluted with a further 5 ml H_2O. Amino acids were eluted with 5 ml 1 M NH_4OH. NH_4OH was then removed by evaporating the fractions to dryness. The residues were taken up in 5 ml H_2O, and remaining NH_3 was removed by passage through a further column of Dowex 50W sodium as described above. Neutral and acidic amino acids were then separated on a column (0.5 × 5 cm) of Dowex 1W-X10 (400 mesh) acetate form equilibrated with H_2O. Neutral amino acids were eluted with 3 ml H_2O. Glutamate was eluted with 14 ml 0.1 M acetic acid. Aspartate was then eluted with 8 ml 0.2 M sodium acetate, pH 4.84. The neutral amino acid fraction was incubated with 0.5 unit glutaminase (Sigma, Grade V from *Escherichia coli*) at pH 6.0 and 30°C for 4 h. Glutamine amide-N, as ammonia, was isolated on a column of Dowex 50W sodium as before. After incubation with 0.5 units asparaginase (Sigma Grade V from *E. coli*), asparagine amide-N was isolated as ammonia by a similar procedure. Glutamine and asparagine amino-N were isolated as glutamate and asparatate by passage through a Dowex 1W acetate column as above. Comparisons of nitrate and total N assays (see below) on the nitrate fractions showed these samples to be contaminated less than 5% by other nitrogen compounds and this was considered insufficient to warrant further purification.

Analytical Procedures. α-Amino-N was determined by the ascorbate-ninhydrin procedure (7). NH_3 was determined by the procedure of McCulloch (9). Nitrate was measured by the colorimetric diazo-dye reaction, after reduction to nitrite by cadmium metal (10). Total N in plant tissues was determined as ammonia following Kjehldahl digestion.

^{15}N Analysis of Nitrogen Compounds. Aliquots of each fraction corresponding to approximately 2 μg N were converted directly to N_2 gas by the modified Dumas method, and measurements of ^{15}N isotope abundance in the nitrogen samples were made by the method of emission spectroscopy as described by Fiedler and Proksh (2). The emission spectroscopy apparatus was calibrated by the analysis of nitrogen standards containing a known ^{15}N isotope enrichment.

Enzyme Assays. GS, GDH, and glutamate synthase were extracted and assayed as described by Rhodes *et al.* (19). GS was measured by the synthetase assay.

Mathematical Modeling of Isotope Labeling. The growth of the barley seedlings was measured by sampling roots and shoots at intervals between 7 and 11 d growth. The increase in weight of both tissues over this period was approximately linear with time. The increase in weight over a 4-h period was found to be equal to less than 2% of their total weight. It was decided that the growth of the plants during the labeling experiment could be considered negligible.

Thus, in the barley tissues under study in which growth is ignored, if component b is derived from a with a transfer coefficient k, and is itself utilized in other syntheses with a combined transfer coefficient k_2, then the equation for the increase in ^{15}N label in component b simplifies to:

$$\frac{dB^*}{dt} = \frac{A^*}{A}k_1 - \frac{B^*}{B}k_2$$

where B^* and A^* are the amounts of ^{15}N-labeled molecules of b and a in μmol/g fresh weight and k_1 and k_2 are transfer coefficients measured in μmol/min·g fresh weight.

Under steady state conditions, $k_1 = k_2$. Hence

$$\frac{dB^*}{dt} = \alpha k_1 - \beta k_1$$

where $\alpha = A^*/A$, $\beta = B^*/B$. Thus, the amount of ^{15}N-labeled b at time t is given by

$$B^*_t = B^*_{t-1} + \alpha_{t-1} + \alpha_{t-1}k_1 - \beta_{t-1}k_1$$

and therefore:

$$\beta_t = \beta_{t-1} + \frac{\alpha_{t-1}k_1}{B} - \frac{\beta_{t-1}k_1}{B} \quad (1)$$

Thus, theoretical labeling curves could be derived for tissue ammonia, glutamine-amide and glutamate pools by devising computer programs which reiterated for each time increment dt a series of calculations in which operations analogous to Equation 1 were performed. The time increment dt was routinely set at 1 min, and the programs were set to run for 240 increments. Outputs from the program were in tabular form, from which the theoretical graphs of ^{15}N label incorporation into the various cellular pools could be constructed.

RESULTS

Seed Nitrogen Content. For a quantitative analysis of kinetic labeling data, it is clearly important that the only source from which nitrogen is moving into the root and shoot amino acid pools is the nutrient solution. Total N analysis of the seeds attached to the plants over a 40-h period between 9 and 11 d growth showed that the seed nitrogen content remained constant, thus indicating that by the 9th d of growth, the N reserves of the seed had essentially been exhausted. Removal of the seeds was therefore considered unnecessary.

Patterns of ^{15}N Labeling in Ammonia-Grown Seedlings. The amounts of soluble nitrogen compounds in seedlings grown on 40 nM NH_4Cl, over the period of ^{15}N feeding, are shown in Figure 1. Indications that a 'steady state' was achieved are given by the stability of the levels of individual nitrogen compounds during the experiment. The actual variations seen are within the errors in the recoveries from the purification sequence employed. Figure 2 shows that the pattern of ^{15}N labeling of soluble nitrogen compounds in the roots is at least qualitatively consistent with the GS/glutamate synthase pathway of ammonia assimilation. Thus, ammonia and glutamine amide-N are the most highly labeled components over the time course of the experiment, while glutamate and glutamine amino-N show lower labeling. This conclusion is, however, dependent on the assumption of single pools of glutamine and glutamate in the root, and there are some indications that multiple pools are in fact present. Thus, the labeling of both ammonia and glutamine amide-N appears to become saturated at around 50% ^{15}N abundance, indicating that 'storage pools' of these compounds exist in the roots. Bleeding sap was collected from the stems of plants decapitated 4 h after the addition of ^{15}N label. ^{15}N analysis showed that sap glutamine amide-N was labeled at 60% ^{15}N, while glutamine amino-N was labeled at 40% ^{15}N. The higher labeling of the sap glutamine compared with that of glutamine in the root is again indicative of more than one root glutamine pool. After 2 h of ^{15}N label incorporation, the ^{15}N labeling of root glutamine amino-N exceeds that of glutamate, indicating the existence of at least two glutamate pools.

The patterns of ^{15}N label incorporation in the shoots of ammonia-grown plants (Fig. 3) reflect the forms in which nitrogen is transported from root to shoot. The major nitrogenous constituents of the xylem sap of ammonia-grown barley plants are glutamine and asparagine, very little ammonia being transported (12). Shoot glutamine is initially the most highly labeled compound, while

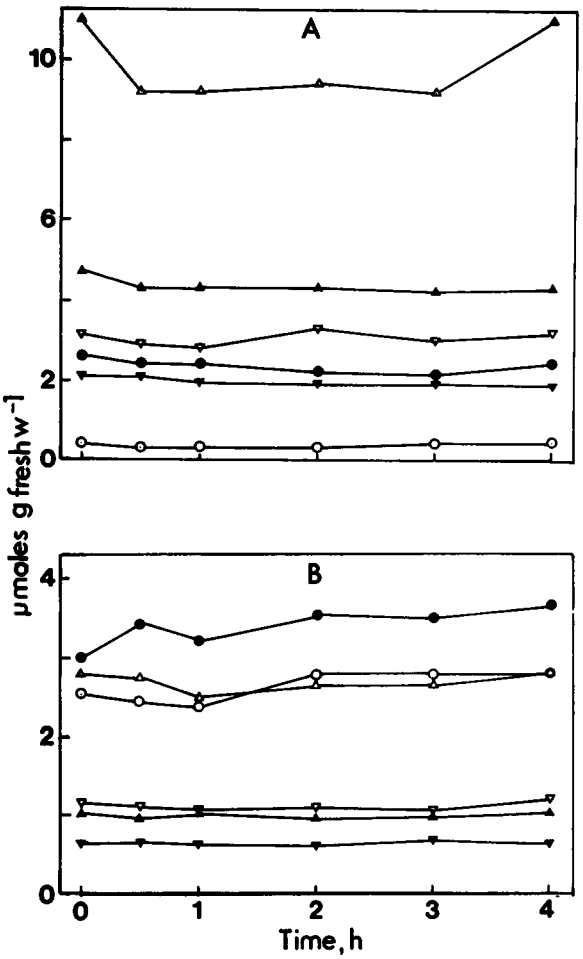

FIG. 1. Amino acid pool levels in ammonia-grown barley seedlings over a period of $^{15}NH_4Cl$ feeding. A, Shoot; B, root; (○) ammonia; (●) glutamine; (△) asparagine; (▲) glutamate; (▼) aspartate; (▽) neutral amino acids.

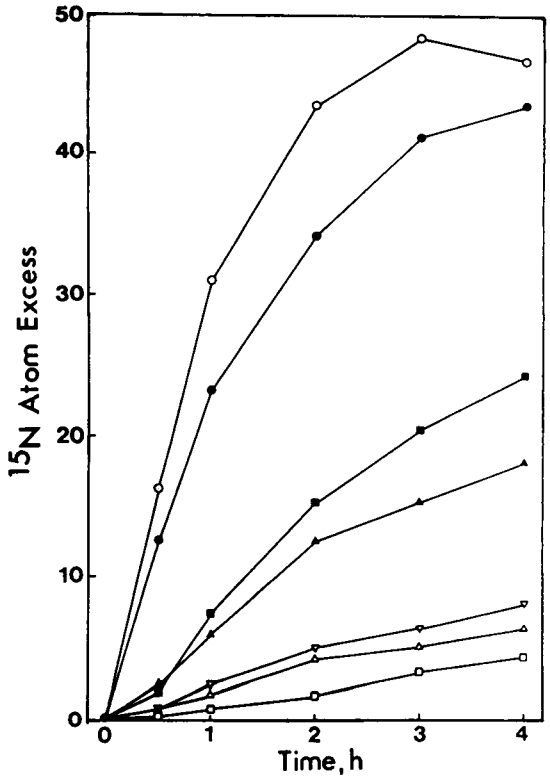

FIG. 2. ^{15}N labeling of amino acid pools in ammonia-grown barley roots over a period of $^{15}NH_4Cl$ feeding. (○) Ammonia; (●) glutamine amide; (△) asparagine amide; (▲) glutamate; (■) glutamine amino; (□) asparagine amino; (▽) neutral amino acids. $^{15}NH_4Cl$ added at zero time.

glutamate becomes highly labeled after an initial lag.

Mathematical Modeling of Root Labeling Data. As a first step in the kinetic analysis of the above labeling data, it was decided to compare theoretical labeling curves for root ammonia, based on a single ammonia pool, with the experimental data. Thus, α in Equation 1 becomes the fractional ^{15}N abundance of ammonia in the culture solution, set at 0.96, while k_1 is the rate of uptake of ammonia from the medium. This rate was calculated by plotting the rate of increase of the total μmol ^{15}N in the soluble nitrogen pools of root and shoot tissues associated with 1 g fresh weight roots (Fig. 4). From the initial linear portion of this curve, the value of k_1 was taken as 0.035 μmol/min·g fresh weight.

Models based on a single ammonia pool, or on a single metabolic pool and a storage pool, failed to fit the experimental labeling data for root ammonia very closely and it was therefore necessary to postulate model systems in which ammonia and amino acids are distributed between at least two metabolic compartments. In evaluating such models, the approach was first to fit the labeling data for root ammonia by deriving computer programs in which the relative sizes of the ammonia pools and the transfer coefficients for movement of ammonia into these pools were varied, but in which the total ammonia pool size and the overall rate of ammonia uptake were maintained at their experimentally derived values. This approach was then applied to the labeling data for glutamine amide-N and glutamate. Of the various models attempted, only one gave a reasonable fit to the experimental labeling data. This model (Fig. 5) comprises three compartments, one of which con-

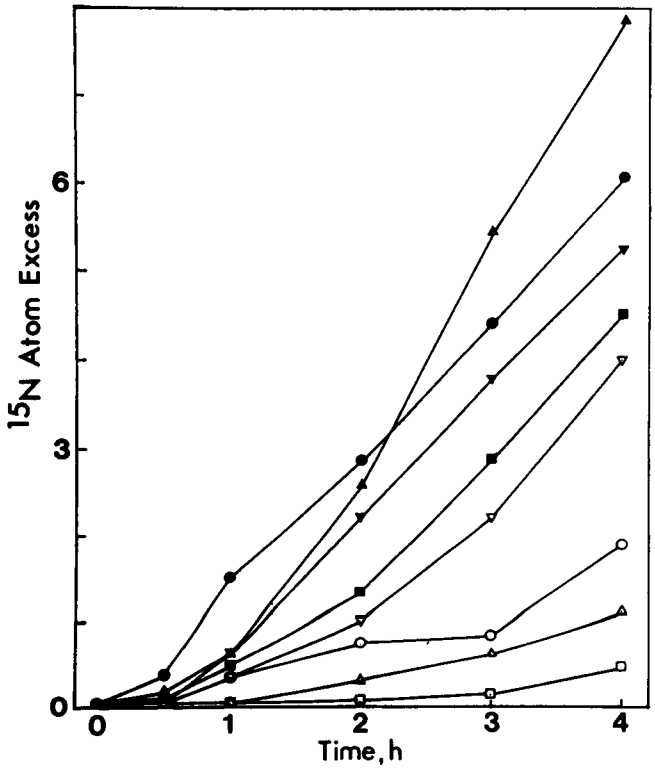

FIG. 3. ^{15}N labeling of amino acid pools in ammonia-grown barley shoots over a period of $^{15}NH_4Cl$ feeding. (○) Ammonia; (●) glutamine amide; (△) asparagine amide; (▲) glutamate; (▼) aspartate; (■) glutamine amino; (□) asparagine amino; (▽) neutral amino acids. $^{15}NH_4Cl$ added at zero time.

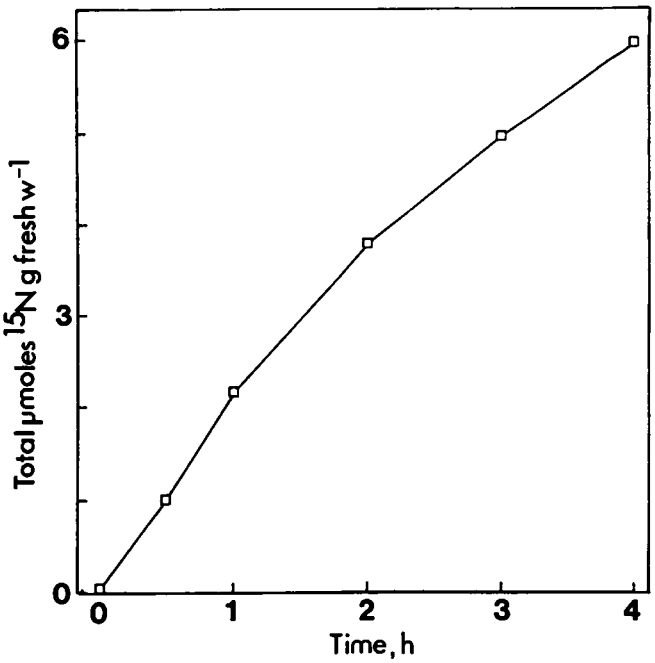

FIG. 4. Increase in total μmol ^{15}N-soluble nitrogen pools of root and shoot tissues with 1 g fresh weight barley roots over a period of $^{15}NH_4Cl$ feeding.

tains storage pools of ammonia and glutamine. Metabolic pools of these components are contained in the other two compartments. One of the metabolic ammonia pools is of a very small size in comparison with the other two ammonia pools. Ammonia entering compartments 1 and 2 of the root from the surrounding medium is assimilated solely into glutamine amide-N as predicted by the GS/glutamate synthase pathway. Figures 6 and 7 show that this model gives a reasonable fit to the experimental labeling data for both ammonia and glutamine amide-N. Attempts were also made to fit models in which ammonia is assimilated into glutamate solely via GDH, to the experimental data. However, it proved impossible to derive such models capable of fitting the labeling data for both glutamate and glutamine amide-N, while accommodating the necessary rates of ammonia assimilation.

Patterns of ^{15}N Labeling in Roots of Nitrate-Grown Seedlings. In a second experiment, seedlings grown on 40 nM KNO_3 were transferred to medium containing ^{15}N-labeled nitrate. The levels of the different nitrogen compounds were found to be constant, indicating that a steady state was maintained over the period of the experiment. The patterns of ^{15}N label incorporation into nitrate-grown roots are again found to be qualitatively consistent with ammonia assimilation via the GS/glutamate synthase pathway. Thus, glutamine amide-N is again the most highly labeled component while glutamate and glutamine amino-N show lower labeling. This interpretation is again complicated, however, by the apparent presence of multiple pools of nitrogen compounds in the roots. Thus, the labeling of glutamine exceeds that of nitrate, indicating the presence of more than one pool of the latter component. Nitrate forms a major component of the nitrogen transported from root to shoot in the xylem sap of nitrate-grown barley plants (12). The large pool size of nitrate in the root, the existence of more than one nitrate pool, and uncertainty with respect to the relative rates of nitrate assimilation in the root and transport to the shoot, made it extremely difficult to attempt a quantitative analysis of the labeling data analogous to that employed with the ammonia-grown seedlings.

Effect of MSO on Label Incorporation from [^{15}N]Nitrate in Barley Roots. In view of the difficulties in applying a quantitative analysis to the kinetic labeling data from nitrate-grown seedlings,

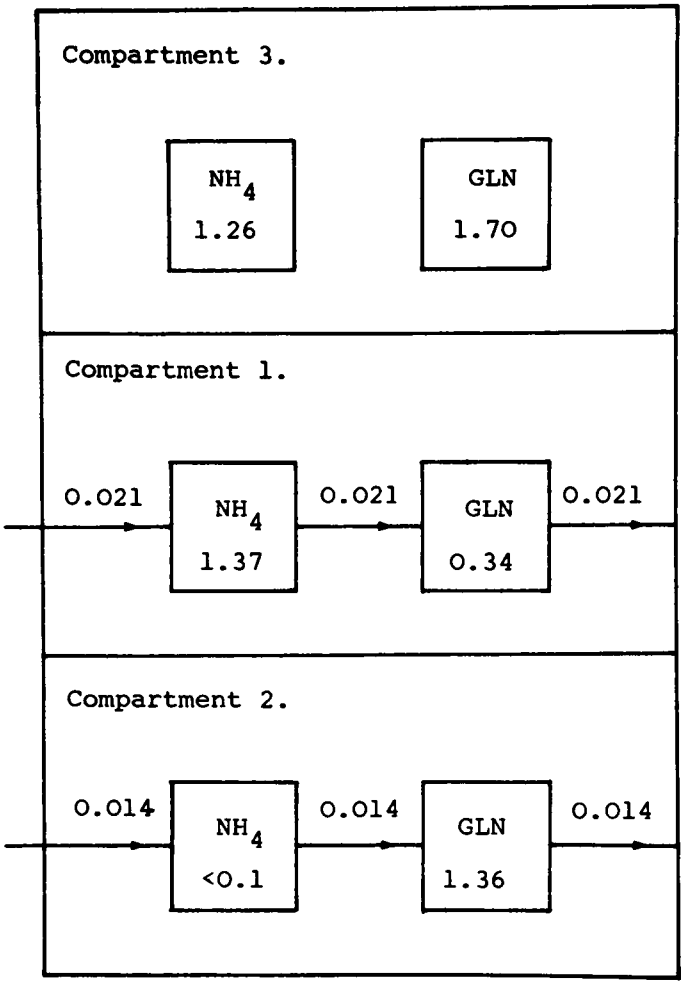

FIG. 5. A model for root ammonia assimilation. The numbers in the boxes show the pool sizes (μmol/min·g fresh weight).

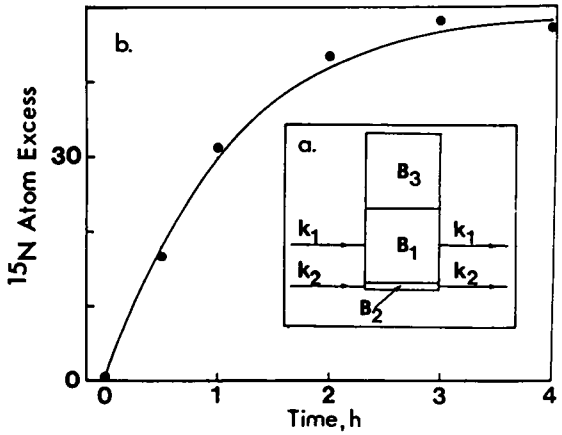

FIG. 6. a, Model for root ammonia comprising two metabolic pools (B_1, B_2) and a storage pool (B_3). b, Computer-simulated labeling curve for root ammonia based on the above model where B_1 is set at 1.37 μmol/g fresh weight, B_3 at 1.26 μmol/g fresh weight, and B_2 is very small. k_1 is set at 0.021 μmol/min·g fresh weight = 0.6k, and k_2 is set at 0.014 μmol/min·g fresh weight = 0.4k.

it was decided to examine the effects of the glutamine synthetase inhibitor MSO (25) on the incorporation of the label from [^{15}N] nitrate into the soluble components of barley roots. Nine-day-old seedlings grown on 0.5 mM KNO_3 were transferred to medium containing 1 mM MSO, and after 2 h the GS activities of MSO-treated and 'control' plants were assayed. While the GS activity of

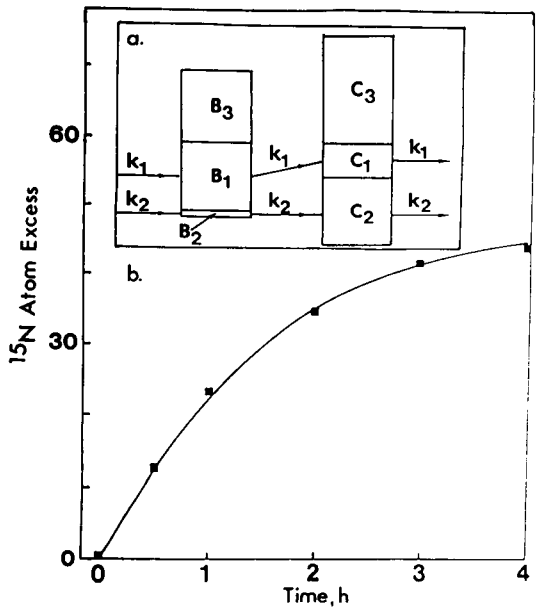

FIG. 7. a, Model for root glutamine comprising two metabolic pools (C_1, C_2) and a storage pool (C_3). b, Computer-simulated labeling curve for root glutamine amide-N based on the above model where the total glutamine pool size CO is 3.39 μmol/g fresh weight and the combined transfer coefficient for glutamine synthesis (k) is 0.35 μmol/min·g fresh weight. B_1 is set at 1.37 μmol/g fresh weight, B_3 at 1.26 μmol/g fresh weight. k_1 is set at 0.021 μmol/min·g fresh weight, k_2 at 0.014 μmol/min·g fresh weight. C_3 is set at 0.5 C, C_1 at 0.1 C, C_2 at 0.4 C. (●) Experimentally derived labeling data.

Table I. *Effect of MSO on Soluble Nitrogenous Compounds and Incorporation of ^{15}N Label in Nitrate-Grown Barley Roots*

Compound	2-h Control + 1-h $K^{15}NO_3$		2-h MSO + 1-h $K^{15}NO_3$ + 1 mM MSO	
	μmol g^{-1} fresh wt	^{15}N atom % excess	μmol g^{-1} fresh wt	^{15}N atom % excess
Nitrate	56	3.2	58	2.4
Ammonia	0.6	7.0	4.8	5.5
Glutamine	0.8		0.1	
Amide-N		8.8		0.3
Amino-N		3.5		0.2
Glutamate	1.2	1.5	1.1	0.04

the control plants remained essentially constant over the 2-h period, the GS activity of the MSO-treated plants was decreased to 4% of its initial level. At the end of this 2-h period, both control and MSO-treated plants were transferred to a medium containing ^{15}N-labeled nitrate. Control plants were sampled immediately prior to the introduction of [^{15}N]nitrate, and both control and MSO-treated plants were sampled after 1 h of ^{15}N feeding.

Table I shows that the amounts of soluble nitrogen compounds in barley roots subjected to a total of 3 h of MSO treatment were markedly different from those in control plants. The ammonia content of MSO-treated roots was substantially increased, while the glutamine content was greatly decreased. While the total amount of ^{15}N incorporation into soluble N in the MSO-treated roots (1.74 μmol/g fresh weight) was only slightly less than that in control plants (1.91 μmol/g fresh weight), Table Ib shows that the patterns of ^{15}N incorporation into individual compounds were very different. Thus, whereas the labeling of nitrate in control and MSO-treated plants was similar, labeled N in the MSO-treated roots accumulated in ammonia, and only very low labeling of amino acids was observed. The amounts of labeled nitrogen incorporated into glutamate and into both N atoms of glutamine in the MSO-treated roots were between 0.3 and 3% of the amounts incorporated in the control roots. The small incorporation of label into glutamate could easily be accounted for by the slight GS activity remaining after MSO treatment. These results therefore are consistent with the assimilation of ammonia solely by the GS/glutamate synthase pathway in nitrate-grown barley roots.

Table II. *Activities of the Enzymes GS, GDH, and Glutamate Synthase in Nitrate- and Ammonia-Grown 10-Day-Old Barley Seedlings*

Seedlings were grown in a nutrient solution containing 1 mM nitrate.

	NO_3-Grown		Ammonia-Grown	
	Root	Shoot	Root	Shoot
	μmol/min/g fresh wt			
GDH	1.1	0.25	1.0	0.36
GS	0.38	1.08	0.48	1.20
Glutamate synthase	0.04	0.39	0.05	0.42

Table III. *Distribution of GS and GDH Activities along the Roots of Nitrate-Grown 10-Day-Old Barley Seedlings*

Distance from Root Tip	GDH	GS
mm	μmol/min/g fresh wt	
0–5	1.30	0.93
5–10	0.58	0.52
10–20	0.54	0.45
20–40	0.45	0.28
40–60	0.45	0.26

Enzyme Activities and Distribution. The results in Table II show that there is very little difference in the activities of GS and GDH between the roots of ammonia- and nitrate-grown barley plants. The activity of GDH is some 2 to 3 times that of GS in root tissue, a finding similar to that obtained in many other studies of root tissue and one which has been used to argue a role for GDH in ammonia assimilation. However, the data obtained here from the ^{15}N labeling studies give no indication of any significant part played by GDH in ammonia assimilation even in ammonia-grown plants where the ammonia content (2.5 μmol/g fresh weight) is some five times greater than that of nitrate-grown plants (0.5 μmol/g fresh weight). Moreover, root activities of GS and glutamate synthase are more than adequate to account for the observed rates of assimilation of nitrate and ammonia. The activities of GS and GDH are the same in different parts of the roots (Table III). Both enzymes have a similar distribution along the root, with largest activities being the root tip and the 5 to 10 mm behind the tip. In older, mature parts of the root, the GS activity is considerably lower than at the tip.

DISCUSSION

Conclusions on the pathway of ammonia assimilation in higher plant tissues have in the past relied heavily upon evidence concerning the levels and kinetic properties of the enzymes GS and GDH. Thus, the high affinity of higher plant GS for ammonia compared with the much lower affinity of GDH (23) together with observed increases in GDH activity in plant tissues fed exogenous ammonia (5, 19, 20) led to the suggestion that ammonia assimilation occurs via the GS/glutamate synthase pathway at low ammonia concentrations, while GDH may operate in the assimilation of high ammonia concentrations (19) in a manner analogous to the algal NADP enzyme (23). There are no indications of any similar alterations in the levels of GS and GDH in barley grown on different nitrogen sources. Although the activity of GDH is always greater than that of GS in barley roots, the evidence based on ^{15}N labeling studies is consistent with the GS/glutamate syn-

thase pathway being the sole route of ammonia assimilation in both nitrate- and ammonia-grown plants.

As the three-compartment system described by the model shown in Figure 7 can account for the observed patterns of isotopic labeling in ammonia-grown barley roots, attempts may be made to equate these theoretical compartments to actual locations within the plant. Inasmuch as ammonia is taken up from the medium into both compartments 1 and 2, it may be argued that these compartments represent two different kinds of root cells. The first 5 mm behind the root tips were found to contain the highest activities of GDH and GS, and there was a decrease in the activities of these enzymes (on a fresh weight basis) farther back along the root. It could therefore be suggested that compartments 1 and 2 represent two populations of root cells, one population consisting of young rapidly metabolizing cells behind the root tip and the other consisting of older more slowly metabolizing cells farther back along the root. It is also possible that one of the metabolic compartments is concerned with the synthesis of glutamine for export to the shoot, while the other compartment is concerned with the synthesis of glutamate for biosynthetic reactions in the root.

In the two-compartment model devised by Rhodes *et al.* (18) on the basis of ^{15}N labeling data in *L. minor*, intracellular localizations were assigned to the compartments. Thus, compartment 1 of their model was identified with the chloroplasts, while compartment 2 was identified with the cytoplasm. The greater complexity of barley root tissues in comparison with *L. minor*, however, means that any interpretation of model systems derived from ^{15}N labeling in barley roots must take into account the heterogeneity of the cells under study. It should also be noted that if the two 'metabolic' compartments postulated for barley roots represented the cytoplasm and the plastids, then the ammonia pools in these compartments would be 'in series,' as ammonia would be transported from the cytoplasm into the plastids, rather than 'in parallel' as shown in Figure 5.

The scheme in Figure 7 therefore suggests a single intracellular site of glutamine synthesis. Consistent with this hypothesis are observations that the GS enzyme of barley roots elutes as a single peak of activity from DEAE-Sephacel corresponding to the cytoplasmic GS isoenzyme of leaves (11). Furthermore, although low proportions of root GS activity have been reported to be associated with the plastids (1, 14), more recent data on maize indicate that root GS activity is wholly cytoplasmic (24). Thus, compartments 1 and 2 in Figure 7 are suggested to represent only the cytoplasmic compartments of different cell types. As appreciable amounts of amino acids and amides have been reported to be located in the cell vacuoles of higher plants (26), it may be suggested that compartment 3 in Figure 7, which contains storage pools of ammonia and glutamine, is located in the vacuoles. It makes little difference, however, to the theoretical labeling curves derived from this model if the vacuolar storage pools are associated with the compartment 1 or compartment 2 cells, or are located in another part of the root.

The kinetic analysis of ^{15}N labeling data from nitrate-grown plants would have been somewhat simpler had the tissue storage pools of nitrate been reduced to a very small size. While this could theoretically have been achieved by greatly reducing the concentration of nitrate supplied to the plants, such an approach would have increased the reservoir of culture solution necessary to maintain the plants in a steady state to a prohibitively large size. Simpler kinetic analysis of labeling data will also result from experiments in which isolated root cells or protoplasts are employed, the techniques for which are now becoming available.

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