Transport of Amino Acids to the Maize Root[1]

Ann Oaks[2]
Department of Biological Sciences, Purdue University

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Summary. When 5-mm maize root tips were excised and placed in an inorganic salts solution for 6 hours, there was a loss of alcohol-insoluble nitrogen. The levels of threonine, proline, valine, isoleucine, leucine, tyrosine, phenylalanine, and lysine in the alcohol soluble fraction were severely reduced, whereas those of glutamate, aspartate, ornithine, and alanine were scarcely affected. There was a 4-fold increase in the level of γ-aminobutyrate. Those amino acids whose synthesis appeared to be deficient in excised root tips also showed poor incorporation of acetate carbon. In addition, the results show that asparagine and the amino acids of the neutral and basic fraction were preferentially transported to the root tip region. The results therefore suggest that the synthesis of certain amino acids in the root tip region is restricted, and that this requirement for amino acids in the growing region could regulate the flow of amino acids to the root tip.

The movement of materials in plants is a well-documented phenomenon which has recently been reviewed by Kursanov (13). The general theory evolved indicates that certain tissues, i.e. a leaf participating in photosynthesis or the endosperm of a cereal seed, serve as a source for materials required by a growing region. It has been shown in systems utilizing the movement of the products of photosynthesis (1, 10, 13) that sugars are the principal components transported out of the leaf. It has been assumed that, after their arrival, these transported sugars are extensively metabolized to yield all the cell constituents. Further support for this idea has been derived from observations concerning amino acid interconversions, principally the extensive metabolism of glutamic and aspartic acids in growing tissues (6, 29), and the ubiguity of transaminase reactions (28, 29). However 3 types of experiments are inconsistent with the notion of an extensive metabolism of glucose or interconversion of amino acids in the growing region.

A) When maize embryos are detached from their endosperms and grown under sterile conditions, there is, initially, a decline in the soluble amino acid content and an insignificant increase in embryo protein. This initial deficiency is overcome by the addition of an appropriate mixture of amino acids to the culture medium (21). If glucose were the major precursor for the amino acids and embryo protein, this initial lag in protein synthesis would not be expected.

B) It is usually found that C^{14}-labeled amino acids are recovered from plant tissues mainly in the form in which they were administered (9, 15). Glutamic and aspartic acids, therefore, represent the exception rather than the rule for the idea of an extensive interconversion of amino acids.

C) Both Kursanov (13) and Ziegler (31) have found that the minor components transported in plants vary considerably, when the environmental conditions were altered. Their results are suggestive of a selective transport of certain constituents. The variations in the amino acid content in their experiments could, for example, reflect a restriction in the uptake of specific components into the transport system under certain environmental conditions or they could reflect a changed requirement for components in the receiver region. This latter possibility is preferred since, in at least one instance, the germinating maize seedling (19), the movement of amino acids from the source (the endosperm) was inhibited by the addition of the required amino acids to the sink (the embryo). If the amino acids were normally synthesized from glucose in the receiver region a selective transport of amino acids related to the requirement for amino acids would not be expected.

Thus, it appears that glucose may not be extensively metabolized in a growing region, that the majority of the amino acids may be conserved and used as such and finally that a transport of amino acids to a receiver region could be limited by the amino acid requirement of that region. To test these possibilities it was necessary first to know the amino acid composition of the protein in a region where a net synthesis of protein was occurring. The 5-mm root tip of maize represents such a region (17). As the cells in this region mature there may be minor losses of total protein (4, 12) and a redistribution of existing proteins (3, 25). As new cells are formed, however, the proteins which are required for cell division and elongation must be maintained. The amino acid precursors for these particular proteins may be derived from synthesis and protein turnover within the

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[2] Present address: Research Unit in Biochemistry, Biophysics, and Molecular Biology, McMaster University, Hamilton, Canada.

tip, and by transport from other regions of the seedling. The relative requirement for the amino acids necessary for growth was established by analyzing the amino acid composition of the protein in the 5-mm root tip. Thus, the relative rate of supply of the amino acids from all sources (synthesis, protein turnover, and transport) could be established.

When acetate-2-C^{14} was fed to intact root tips, it was found that the spread of acetate carbon into the amino acids of the root tip protein did not approach the spread that would be required if all the amino acids were made exclusively in the root tip. Thus, for example, the synthesis of glutamic and aspartic acids and glutamine appeared to be relatively easy for the root tip cells; the synthesis of such amino acids as theonine, proline and leucine restricted. When the root tips were excised and grown in culture for 6 hours, the soluble pools of this latter group of amino acids (referred to as the neutral and basic amino acid fraction) was drastically reduced. In another set of experiments, acetate-2-C^{14} was fed to some distant part of the seedling, the scutellum or a region of the root 3 to 4 cm from the tip, and the distribution of C^{14} in the growing region analyzed. In this case the contribution of acetate carbon to amides, chiefly asparagine, and the neutral and basic amino acids was considerable. The results, therefore, suggest that many of the amino acids are not made in the root tip in amounts sufficient to support growth, and that this requirement may, in fact, be influential in determining which amino acids are transported to the root tip.

Materials and Methods

Maize seeds (hyb. var. Wf9 × 38-11) were sterilized briefly with Chlorox (a commercial bleach containing 5 % sodium hypochlorite) rinsed, and allowed to germinate on a thin layer of agar (1.5 %) in the dark at 30°. Seedlings with roots 4 to 5 cm long were used in all experiments.

Identification of the Amino Acid Pools. One hundred 5-mm root tips were either extracted immediately with 80 % (v/v) ethyl alcohol or incubated in a salts solution (18, 21) for 6 hours before extraction. The alcohol soluble fraction and the insoluble residue were each hydrolyzed with 6 N HCl at 100° for 12 hours. Each fraction was then taken to dryness in vacua at 40°. A Technicon amino acid auto-analyzer was used to determine the individual amino acid pools.

Application of Radioactive Acetate. Acetate-2-C^{14} (7.3 mc/μmole) was added to a solution of 1.5 % agar (45°) containing the usual salts (26). The labeled agar was then hardened in molds 2 mm × 50 mm. One mold was placed on a microscope slide, and the critical regions of the intact root (5 per mold) were anchored on the labeled agar with strips of unlabeled agar. Alternatively acetate-2-C^{14} was placed in a small watchglass containing a salts solution and glucose. The exposed scutella of excised embryos were placed directly in the liquid. In the pulse experiments, the scutella were washed with deionized water and placed in fresh salts solution without tracer. After the experiment, the critical regions of the root were excised and treated as described previously (17, 18, 21).

Results

Amino Acid Content of the 5-mm Root Tip. The total amounts of the individual amino acids in the alcohol-soluble and insoluble fractions are shown in table I. In the 5-mm root tips, glutamic and aspartic acids made up half the alcohol-soluble amino acid pool but only 20 % of the alcohol-insoluble amino acid content. It is clear from this data that as the root tip grows and as those proteins characteristic of the root tip are made, the requirement for the amino acids of the neutral and basic fraction as a group is considerably greater than the requirement for glutamic and aspartic acids.

Changes in the levels of the amino acids after excision show more generally which amino acids are made in adequate amounts within the root tip and which may be supplied preferentially by the transport system (table I, columns 3 and 4). The soluble pools of glutamic and aspartic acids and of alanine and ornithine were scarcely affected when the 5-mm tips were excised and placed in a salts solution for 6 hours. The level of γ-amino butyric acid showed a 4-fold increase, whereas the levels of all other amino acids declined during this treatment. The level of each of the neutral and basic amino acids in the alcohol-insoluble fraction was slightly lower in the excised roots. The reduction of proline in the alcohol-soluble and insoluble fractions was more pronounced than with any other amino acid. Thus, as with detached embryos (21), there was, under culture conditions, a preferential loss of certain amino acids in the root tip. During this time there would have been a measurable increase in length in the 5-mm tips had they not been excised and an increase in alcohol-insoluble nitrogen (17). There was, however, a 26 % loss in alcohol-insoluble nitrogen during the 6-hour incubation. The reduced rate of protein synthesis, relative to protein degradation, could be directly related to the reduced supply of amino acids. However, it should be stressed that other reasonable explanations exist.

Kinetics of Incorporation of Acetate-2-C^{14} by the Root Tip. The kinetics of acetate incorporation into the amino acids of the intact root tip are summarized in figure 1. Typically, there was a linear increase in glutamic and aspartic acids and their amides from the earliest times (10 min), while a slight lag preceded a linear increase in glutamic and aspartic acids of the insoluble fraction (fig 1A). When the individual components were isolated on Dowex-1 (acetate) as described previously (14, 18, 21), a lag in the incorporation of acetate carbon into glutamic and aspartic acids and into glutamine was not observed. C^{14}-

labeled asparagine was usually not detected in intact root tips. There was a slight lag before a linear increase in C^{14}-labeled glutamic acid was observed in the residue but no apparent lag for the incorporation of C^{14} into aspartic acid of the residue (fig 1E). An apparent steady state was reached in about 20 minutes for the soluble components (glutamic and aspartic acids and glutamine). The reduction in the rate of incorporation of C^{14} into the residue after 2 hours probably reflects the exhaustion of acetate-C^{14} in the agar strips.

The incorporation of acetate carbon into the amino

Table I. *Alcohol-Soluble and Insoluble Amino Acids in 5-mm Root Tips*

The 5-mm root tips were excised, washed with water, and either placed in a salts solution for 6 hours or extracted directly with 80 % (v/v) ethyl alcohol. The alcohol-soluble and insoluble fractions were hydrolyzed in 6 *N* HCl at 100° for 12 hours. The amino acids were isolated with Dowex-50 H^+ resin and then separated with a Technicon amino acid analyzer.

	Intact tips		Excised tips	
	Soluble μmoles/20 tips	Insoluble μmoles/20 tips	Soluble (% Intact)	Insoluble (% Intact)
	1	2	3	4
Glutamate	2.54	1.71	124	93
Aspartate	1.45	1.32	87	111
Threonine	0.25	0.72	58	98
Serine	0.39	0.96	76	88
Glycine	0.30	1.56	78	88
Alanine	0.87	1.64	93	89
Proline	0.69	0.98	39	68
Valine	0.32	1.35	66	81
Isoleucine	0.12	0.86	68	81
Leucine	0.19	1.59	57	78
Tyrosine	0.08	0.42	57	83
Phenylalanine	0.12	0.65	42	77
Lysine	0.07	1.15	63	87
Histidine	0.17	0.34	80	84
Arginine	0.05	0.83	80	81
γ-Amino butyrate	0.04	...	415	...
Ornithine	0.10	...	108	...
Total nitrogen		502		74
Total GA*	3.99	3.03	...	...
Total NB	3.86	12.05	...	...

* GA represents the μmoles of glutamic and aspartic acids and their amides; NB, the μmoles of all the other amino acids.

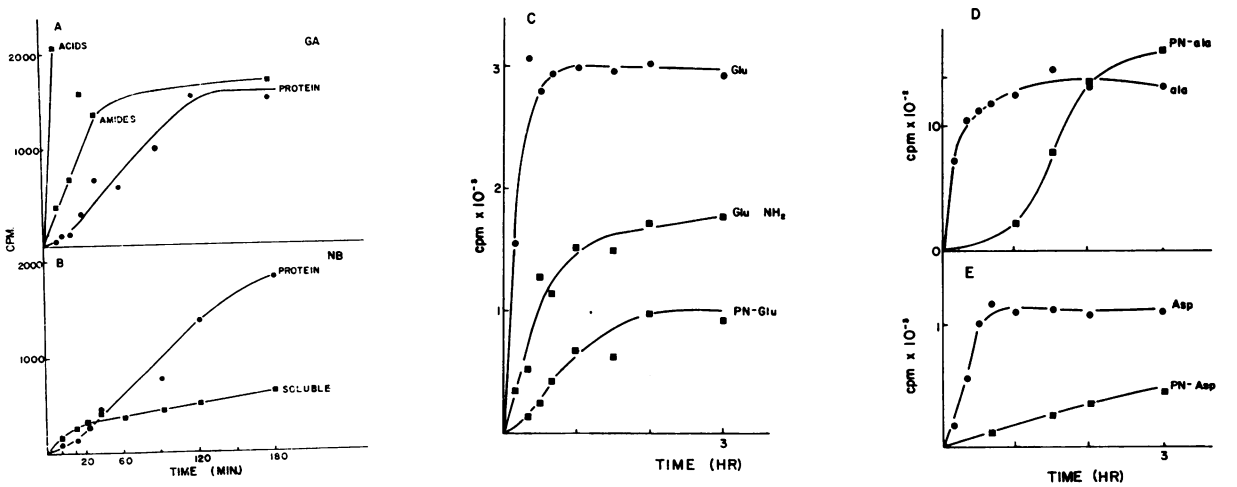

FIG. 1. Kinetics of incorporation of acetate-2-C^{14} into intact maize root tips. A, glutamic and aspartic acids and their amides. B, the neutral plus basic amino acid fraction. C, glu is the soluble glutamic acid; glu NH_2 glutamine; Pn-glu, the glutamic acid of the residue. D, ala is the soluble alanine; Pn-ala, alanine of the residue. E, asp is the soluble aspartic acid; Pn-asp, aspartic acid of the residue.

The initial activity of the acetate-2-C^{14} in 6 ml of agar was 979,500 cpm. Approximately 0.2 ml of agar was pipetted into each mold. Five root tips were placed on each agar strip and 20 root tips were extracted as 1 sample.

acids of the neutral and basic amino acid fraction gave a somewhat different picture (fig 1B). There was a relatively fast initial increase of C^{14} into the soluble fraction during the first 30 minutes, followed by a linear increase after this time. Furthermore, after the first 30 minutes the C^{14} recovered in the residual neutral and basic amino acids exceeded that recovered in the soluble fraction, a situation never observed with glutamic and aspartic acids. The patterns of increase of the individual neutral and basic amino acids could be divided roughly into 2 categories: A) Those in which a steady state in the soluble amino acid was achieved at about the same time as the linear increase of C^{14} into the residue was observed. This is represented in figure 1D for alanine. The synthesis of serine and glycine had similar kinetics. B) Those amino acids in which very little C^{14} was recovered in the soluble fraction. This type of incorporation, characteristic of leucine, proline, threonine, valine, arginine, and lysine, has been discussed extensively with respect to the incorporation of leucine into root tip protein (17). Similar kinetic studies yielding essentially similar results have been performed with regions of the root 3 to 4 cm from the tip.

Relative Rates of Synthesis. Calculations of the rates of synthesis of the individual amino acids are fraught with complications (2, 10, 17). Most serious is the pool phenomena which has already been discussed in detail (17). It is critical to note, however, that when a linear increase in C^{14} in a protein amino acid is observed, a saturation of the protein-precursor pool is assumed. Thus, for example, the incorporation data indicate a small protein precursor pool for glutamic acid since there was a lag of several minutes before a linear rate is achieved; a large protein-precursor pool for alanine since there was a lag of 30 minutes. By this definition the protein-precursor pool for aspartic acid should be very small since no lag was observed. These 3 amino acids should be synthesized efficiently in the root tip since their levels are not appreciably affected by excision (table I). Using either the cpm achieved after a 2-hour incubation with acetate-2-C^{14}, the rate of increase of C^{14} in the insoluble fraction, or the specific activity of the protein precursor amino acid as criteria for the rate of synthesis, it is apparent that the relative rates for aspartic acid and alanine were less than would be predicted from their relative contribution to root tip protein (table II). The reduced rate of incorporation of C^{14} into aspartic acid may be attributed to pool phenomena or to the contribution of asparagine, which is not made in significant amounts in the root tip. The latter alternative is preferred, since the kinetic data suggest that the protein precursor pools for aspartic acid are small. A considerable pool dilution of alanine made from acetate-2-C^{14} is, however, indicated from the kinetic studies (fig 1D), and, accordingly, may be a major factor in the apparent reduced rate of synthesis.

Despite this discrepancy between the incorporation rates and the predicted rates, comparisons of the rates of incorporation of acetate carbon into glutamic acid and leucine of the alcohol-insoluble residue are informative because A) the relative amounts of glutamic acid and leucine in the root tip are similar, B) they are representatives of those amino acids whose synthesis in excised root tips is either adequate or deficient, and C) the formation of acetyl Co.A followed by 4 enzymatic reactions is required in the synthesis of each (30). Comparisons show that when the incorporation of C^{14} into the amino acid of the residue was linear, the rate of incorporation into glutamic acid was 428 cpm per hour for 20 root tips, and into leucine, 284 cpm per hour (table II). It would appear, then, that glutamic acid is made much more rapidly from acetate in the intact root tip. A similar argument could be presented for most of the neutral and basic amino acids (e.g., threonine and proline in table II). However, lack of information about the actual biosynthetic sequence of these amino acids in plant tissues makes the argument less certain.

Transport of Amino Acids to the Root Tip. Wiebe and Kramer (27) have demonstrated, by selective administration of P^{32} and S^{35} to barley roots, a general movement of these inorganic ions to the

Table II. *Relative Contributions of Total Amino Acids and Amino Acids Synthesized from Acetate-2-C^{14} in Root Tip Protein*

The results are calculated from data obtained from the experiments described in tables I and IV and in figure 1. The calculation for the specific activity of the new amino acid incorporated into the root tip protein has been described previously (21).

Amino acid	μmoles	Cpm*	Rate	Specific activity of new amino acid in protein
Glutamate	1	100	100	100
Aspartate	0.77	35.4	52.4	46.4
Alanine	0.96	27.4	13.5	8.0
Threonine	0.42	7.8	12.6	12.9
Proline	0.57	16.6	27.8	17.6
Leucine	0.93	52.5	68.5	27.4

* The cpm in glutamate at 2 hours was 985; the rate after a linear incorporation of C^{14} was achieved was 428 cpm in 1 hour for glutamate, and the specific activity of the new protein glutamate was 5175. These values for glutamate were set at 100 %.

growing region. Furthermore, when they fed these tracers to the root tip little radioactivity moved out of this region. A similar picture pertains when either acetate-2-C^{14} or leucine-1-C^{14} is given to discrete regions of the maize root (table III). In each case, much of the C^{14} was recovered in the region of application, showing that a large part of the tracer was not available to the transport system. When the tracers were given to the root tip, considerable C^{14} was recovered in the 1 cm adjacent to the tip, but very little in other regions of the root or shoot. With more basal applications the C^{14} was more generally distributed throughout the seedling; however, a movement to the growing tip was apparent.

C^{14}-labeled acetate represented an ideal tracer for the experiments illustrated in table IV, because negligible amounts of acetate carbon are converted to sugars in the maize root (8). Acetate-2-C^{14} was fed directly to designated regions of the intact root, and, after the required time, the critical regions of the root were removed and extracted as described previously (17, 18). The results in table IV show that, as with isolated pieces (14), the greater part of the acetate carbon was recovered in the organic acids and in glutamic and aspartic acids when acetate was fed directly to the root tip or to a region of the root 3 to 4 cm from the tip. In contrast to this, most of the acetate carbon transported to the root tip from a region 3 to 4

Table III. *Effect of Region of Application on the Redistribution of Carbon Derived from Leucine-1-C^{14} and Acetate-2-C^{14}*

The tracer was given to the designated region of the root for 2 hours. After this time the critical regions were removed and extracted as described in Materials and Methods.

Tracer applied at		C^{14} Recovered in the alcohol-soluble fraction (cpm/20 pieces) Region* of the root				
		I	II	III	IV	Shoot
Acetate-2-C^{14}						
	Tip	9600**	1560	110	20	n.d.
	2 cm from tip	2275	1497	4699**	3490	685
	4 cm from tip	2160	830	330	6566**	1022
Leucine-1-C^{14}						
	Tip	13,240**	7785	87	38	10
	2 cm from tip	3160	6364	63,640**	1422	162
	4 cm from tip	3018	2124	3064	52,480**	512

* Region I is the 5-mm tip; II, 0.5 to 1.5 cm from the tip; III, 1.5 to 2.5 cm from the tip; IV, 2.5 to 5.0 cm from the tip.

** The radioactivity recovered in the region of application.

Table IV. *Distribution of Carbon Derived from Acetate-2-C^{14}*

Acetate-2-C^{14} (7.3 mc/μmole) was applied to the root in agar strips that were 2 mm wide. Twenty 5-mm tips or sections 3 to 4 cm from the tip were excised at the times indicated, washed with water, and extracted with 80 % (v/v) ethyl alcohol.

	Time (hr)	Total C^{14} in the soluble fraction (cpm in 20 pieces)	% Distribution within the soluble fraction				
			Ether-soluble	Amino acids			
				OAS*	GA	AM**	NB
Tracer given directly to tip (tip sections analyzed)	2	5816	29	42	21	5	3
Tracer given 3 cm from tip (3-cm sections analyzed)	2	4487	9	59	18	10	3.5
Transport to tip (tracer applied 3 cm from tip)	2	540	17	20	11	15	37
	4	1130	14	21	8	21	36
	8	2087	7	25	14	21	33

* OAS represents the organic acid and sugar fractions; GA, glutamic and aspartic acids; AM, glutamine and asparagine; NB, the other amino acids.

** When acetate was fed directly to the tip, all the detectable C^{14} in the amide fraction was in glutamine. In the sections 3 to 4 cm from the tip, the C^{14} was almost equally distributed between glutamine and asparagine. In the transport experiments, approximately 80 % of the C^{14} in the amide fraction was in asparagine.

cm from the tip was recovered in the amide fraction, chiefly asparagine, and in the neutral and basic amino acid fraction. Thus, there does appear to be a selective transport of neutral and basic amino acids and amides, to the root tip region.

Transport of Amino Acids from the Scutellum to the Root. When acetate-C^{14} was fed to a region of the root 3 to 4 cm from the tip, it proved difficult to get sufficient C^{14} to the tip to determine accurately which amino acids were being transported. An alternative approach was to feed acetate-2-C^{14} to the scutellum, and then to determine which amino acids were transported to the root. The results from such an experiment are presented in table V (columns 5–8). Acetate-2-C^{14} was also fed directly to the intact root tip (column 1 and 2) and to a region 3 to 4 cm from the tip (column 3 and 4). The distribution of C^{14} in these experiments represents that which might be expected if glucose, made from acetate-2-C^{14} in the scutellum and transported to the root, served as the major source of amino acids recorded in columns 7 and 8.

In the root tip region (columns 1 and 2), by far the greatest proportion of the acetate carbon was recovered in the soluble glutamic and aspartic acids and glutamine. Very little C^{14} was recovered in asparagine. About half the C^{14} in the insoluble fraction was in glutamic and aspartic acids. Likewise, most of the acetate carbon recovered from a region of the root 3 to 4 cm from the tip (columns 3 and 4) was in glutamic and aspartic acids. In the older regions of the root considerably more asparagine was made, and a greater proportion of the acetate carbon was recovered as γ-amino-butyric acid. Again, most of the C^{14} in the other amino acids was in the alcohol-insoluble residue. Alanine was an exception. In the scutellum most of the C^{14} in the amino acid fraction was recovered in glutamic acid and glutamine (columns 5 and 6). The major difference in this tissue was that a greater proportion of the C^{14} in the other amino acids was found in the soluble fraction. Proline was the most striking example. When the scutella were washed and placed in fresh salts solution and glucose for an additional 10 hours (pulse experiments), over 80 % of the C^{14} was lost from the soluble amino acids (*22*). The exception was asparagine, whose C^{14} content doubled during this time.

At 2 hours most of the C^{14} in the amino acid fraction of the root was in glutamic and aspartic acids. This distribution probably reflects the remarkable ability of the scutellum to make and export sugars (*22*) and the ready equilibrium between these 2 amino acids and the acids of the tricarboxylic acid cycle (14). With time, a greater proportion of the C^{14} was recovered in the neutral and basic amino acid fraction. No special significance is placed on this trend, since the kinetic studies (fig 1, A and B) showed a similar trend with time when acetate was supplied directly to the root. In contrast to local applications to the root, however, considerably more C^{14} was recovered as asparagine, and a greater proportion of other amino acids, notably glycine, serine, alanine, proline, and leucine, was recovered in the soluble fraction. In addition, the distribution of C^{14} in the amino acids of the root did not reflect the distribution in the scutellum, indicating that all the amino acids made in the scutellum were not equally accessible to the transport system.

Table V. *Incorporation of Acetate-2-C^{14} into the Amino Acids of the Maize Scutellum and Root*

The values represent the C^{14} recovered after a 2-hour local feeding at the scutellum the designated region of the root. Values for the transport of amino acids to the root (the whole root was used in this case) are 10 hours after a local feeding at the scutellum. Twenty pieces were used per sample.

	Root (direct application of tracer)				Scutellum (direct application of tracer)		Transport to root (tracer applied at scutellum)	
	5mm tip		3–4 cm from tip					
	Sol*	Res*	Sol	Res	Sol	Res	Sol	Res
	1	2	3	4	5	6	7	8
Glutamate	2080	985	1682	744	101,000	16,850	4940	1482
Aspartate	546	352	338	360	39,800	12,140	720	1050
Glutamine	734	...	546	...	61,000	...	1900	...
Asparagine	20	...	960	...	3210	...	4440	...
Serine-glycine	110	227	n.d.***	81	6840	2990	530	512
Threonine	n.d.	77	n.d.	110	n.d.	n.d.	n.d.	178
Alanine	133	270	205	155	21,950	6340	860	727
Proline	n.d.	163	n.d.	277	2380	2380	891	515
γ-Amino butyrate	55	...	191	...	2935	...	380	...
Valine	n.d.	143	n.d.	127	n.d.	n.d.	n.d.	376
Leucine-isoleucine	104	514	n.d.	280	841	10,050	470	760
Lysine-arginine	66	370	n.d.	237	n.d.	n.d.	n.d.	303
C^{14} in GA (cpm)**	3370	1337	3526	1104	205,010	28,990	12,000	2532
C^{14} in NB (cpm)**	468	1764	387	1219	34,946	21,670	3131	3371

* Sol is the C^{14} in the alcohol-soluble fraction; Res, that in the insoluble fraction.

** The total C^{14} is the acetate-2-C^{14} incorporated into all the cell components; C^{14} in GA is the C^{14} recovered in glutamic and aspartic acids and the amides; C^{14} in NB is the C^{14} recovered in the other amino acids.

*** n.d.: C^{14} too low for detection.

Discussion

Folkes and Yemm have shown that the amino acid composition of reserve proteins in barley endosperm is very different from that of the cytoplasmic proteins of the embryo (5, 30). From their extensive nitrogen balance sheets, they concluded that the major losses in glutamic acid, asparagine, and proline could be accounted for mainly in the significant gains in "other bases" (more recently defined as nucleic acids and their precursors; see ref. 11), in chlorophyll, and in the amino acids aspartate, lysine, arginine, and alanine (6). The other amino acids showed only minor increases or decreases during the germination process. This represented the experimental foundation for the notion of an extensive interconversion of amino acids in the embryo. Further evidence for rapid synthesis or extensive conversion of amino acids in the embryo was derived from the ubiquity of transaminase reactions. The rates of the glutamate-aspartate or glutamate-alanine transaminations were, however, considerably higher than the rates of transamination between glutamate and the other amino acids tested (28, 29). From these observations it might be preferable to place glutamic and aspartic acids, and perhaps proline, in a special category of amino acids undergoing rapid metabolism during germination. The other amino acids stored in the endosperm appear to be conserved and used directly as protein precursors in the embryo. The supply of preformed amino acids to the embryo is apparently a prerequisite for the increases in embryo protein, since embryos grown in culture do not augment their protein content unless an appropriate mixture of amino acids is added to the medium (21). Furthermore, this requirement for amino acids appears to be critical in regulating the degradation of storage protein, since addition of this same mixture of amino acids to the medium delays the disappearance of nitrogen from the endosperm of normal seedlings (19).

Reactions leading to growth in the embryo are concentrated in the limited meristematic regions; that is, in those regions where a net synthesis of macromolecules is occurring. Using the root tip as a guideline is obviously an oversimplification of the growth processes in the embryo, since new proteins are made in other regions of the embryo (3, 25). In fact, the enhanced synthesis of asparagine and γ-aminobutyrate in older regions of the root lends support to this contention. However, using the root tip does permit comparisons which are not possible with the more heterogeneous tissues of the whole embryo. Thus, knowing the amino acid composition of the root tip protein, it is possible to say that for each micromole increase in glutamic acid, an increase of 0.42 μmole of threonine, 0.96 μmole of alanine, 0.57 μmole of proline, and 0.93 μmole of leucine would be required. Ideally, it should be possible to determine the rates of synthesis of each of these components with radioactive tracers; and if various sources are suspected, it should be possible to determine the rates of supply from each of these sources. Such calculations have proved to be useful parameters in bacterial systems (23). However, at present, 2 complications prohibit similar calculations in tissues from higher plants: A) the infinitely more complicated pool structure (2, 17, 24), and B) the inadequate steady state conditions, which are in part the nature of the diversity of the cells involved and in part the less carefully controlled methods of administering both nutrient and tracer. Nevertheless, useful comparisons are possible. The results of the present investigation show clearly, for instance, that acetate carbon is preferentially incorporated into glutamic and aspartic acids in 2 regions of the root, and also in the scutellum. Futhermore, less acetate carbon is incorporated into leucine, an amino acid whose synthesis involves a series of reactions very similar to those required for the synthesis of glutamic acid (30). In cultured roots the level of such amino acids as proline, leucine, and threonine is not maintained. These amino acids show relatively poor incorporation of acetate carbon. Taken together, these observations suggest that the synthesis of many amino acids is deficient in growing tissues. Hence, the entensive interconversion of amino acids previously assumed to be prevalent in plant tissues (2, 5, 24) may be of minor importance. Additional support for this argument is provided by the fact that amino acids such as leucine (17), valine, or lysine (Oaks, unpublished) are not extensively metabolized by root tissue. In contrast to this, those amino acids closely associated with the tricarboxylic acid cycle, glutamic and aspartic acids, do appear to be made in sufficient amounts in embryonic tissues and are extensively metabolized by the root tissue (14).

If the synthesis of many amino acids is, in fact, deficient in growing tissues, 2 types of questions may be asked: A) Does the low rate of synthesis represent the genetic capacity of the plant cell; i.e., can this rate be altered by altering the levels of specific substances in the cytoplasm? B) Does the low rate of synthesis influence the composition of amino acids transported to the growing region from senescing regions? Answers to the first question have been attempted with regard to leucine biosynthesis in maize embryos (20). The results show that instead of inhibiting the development of 3 enzymes in the biosynthetic sequence, a result that might have been predicted from bacterial systems (7), leucine actually caused a slight increase in the recovered activity (20).

The present investigation represents an approach to the second question. Both the altered pool levels in cultured root tips and the incorporation of acetate-2-C^{14} into root tip amino acids suggest that the synthesis of many of the neutral and basic amino acids is deficient. Significantly, this same group of amino acids is preferencially transported to the root tip. Although identification of specific amino acids in the transport system was difficult because the recovery of C^{14} in the root tip was low, it was possible to identify

asparagine as a major component of the transport system. Furthermore, the synthesis of asparagine from acetate-2-C^{14} in the intact root tip was almost completely absent. Thus, in this one instance a specific amino acid, required in the synthesis of root tip protein but not made in the root tip, was supplied by the transport system. In this one case, then, it is reasonable to say that the requirement for a particular amino acid in the growing region could be directing the movement of that amino acid in the transport system.

The experiments concerned with the transport from the scutellum are not as precise, since sugars represent a major component of this system (22). However, the high recovery of many of the neutral and basic amino acids in the soluble fraction of the root is a distinctive feature. It has been suggested that the synthesis of amino acids in the growing regions could be controlled by the supply of amino acids from the transport system (16, 17). This could be invoked by the repression of enzyme formation or by allosteric inhibition of the action of one of the enzymes in the biosynthetic sequence. Demonstration of the first phenomenon has, to date, been elusive. The latter phenomenon, however, appears to be of major importance in the biosynthesis of many amino acids in the maize root tip (16, 18). The involvement of either phenomenon would require the buildup of a part of the pool amino acid. It is therefore significant that the amino acids transported to the root do contribute extensively to the soluble pool.

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