The Biosynthesis of Protein Amino Acids in Plant Tissue Culture I. Isotope Competition Experiments Using Glucose-U-\(^{14}\)C and the Protein Amino Acids

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Introduction

Most investigators have used excised tissue, cell-free preparations, or whole plants for studies of metabolism in angiosperms. Each of these systems represents a different state of the cells or tissues. Another state of plant cells, that of exponential growth, has been obtained by tissue culture techniques (2, 5). Studies of the metabolism of cells growing exponentially would provide information which is complementary to that already available for plants. Such studies would allow direct comparison of metabolism of higher plant cells with that of microorganisms also growing exponentially. When specific information is not available, it is generally assumed that the metabolism in plant cells is the same as that in micro-organisms. This assumption may be tested using exponentially growing plant cells.

For use in studies of metabolism a culture system in which plant cells are growing exponentially should have several features. The media used should have a known composition. The tissue should be grown in suspension as very small clumps or single cells. The use of media of known composition makes it possible to manipulate the nutrients at will and to avoid the presence of unrecognized compounds which occur in media supplemented with natural extracts. The growth of tissues as suspensions of single cells or small clumps of cells prevents the formation of the diffusion gradients which occur in larger pieces of tissue. Rapid agitation of the suspension keeps the cells in equilibrium with the medium and the medium in equilibrium with its gaseous atmosphere. Such desirable features can now be found in at least 2 plant tissue cultures. These are cultures established from Paul's Scarlet Rose by Dr. W. Tulecke (Tulecke, private communication) and from Nicotiana tabacum var. Xanthi by Dr. P. Filner (22).

Methods established for growth of microbial cultures have been successfully adapted to the culture of plant cells. For example, plant tissues have been maintained on solid media as callus. Single plant cells have been plated in agar to yield visible colonies (8). Also, plant tissues have been maintained as suspensions of cells and clumps in liquid media of known or unknown composition (2) in shake flasks or large carboys (29). The close parallels in methods of growth suggest that methods developed to study the metabolism of microbial cells in culture might also be readily adapted to the study of plant cell metabolism. Several studies (10, 18, 34) of plant metabolism have employed the isotope competition method (25). This paper is to report the application of the isotope competition method to the study of amino acid biosynthesis in suspensions of plant cells growing exponentially in media of known composition.

Methods

Materials. Only the L-amino acids were used (Nutritional Biochemicals Company). The glucose-U-\(^{14}\)C was obtained from New England Nuclear Corporation. When Dowex 50 was used it was Dowex 50-X4 (200-400 mesh) in the hydrogen form. The settled volume used is given.

Cultivation of the Tissue. The Paul's Scarlet Rose tissue used in these experiments was obtained from Dr. Walter Tulecke. This tissue differs from that used by Weinstein et al. (32) in that, subsequent to that report, the tissue was maintained on the defined medium of Tulecke (28). The stock cultures were kept at 30° in conical flasks containing 50 ml of defined medium. Suspension cultures were grown in the defined medium (28) with agar omitted and 2% glucose substituted for sucrose. Unless specified, the medium contained 200 mg/liter of glutamine.

Inocula for experiments were obtained as follows: tissue grown on solid medium was transferred to 250 ml conical flasks containing 50 ml of medium and shaken at 180 rpm in a New Brunswick gyratory shaker at 30°. After 7 to 10 days, aliquots of the suspension were used to inoculate 250 ml conical
flasks containing 50 ml of medium and a 300 ml nephelo-culture flask (Bellco Glass, Inc.) containing 25 ml of medium. Usually a 5 to 10% inoculum was used. The OD of the suspension in the nephelo-culture flask was measured at 600 mμ using a Coleman Junior colorimeter. It was assumed that measurement of the growth of the tissue in the nephelo-culture flask provided an indication of the growth in the 50 ml cultures. Towards the end of exponential growth, the 50 ml cultures were either transferred again to conical and nephelo-culture flasks and the growth followed or used to inoculate flasks of an experiment.

Isotope Competition Experiments. Nephelo-culture flasks containing 25 ml of medium were used for the growth phase of the isotope competition experiments. Glucose concentration in the medium was reduced to 500 mg/liter. Within an experiment, a constant amount of glucose-U-C14, either 2 or 5 μcuries, was added to each flask. Two control flasks were un-supplemented. To each of the remaining flasks 1 compound to be studied was added. Each flask was inoculated with 2.5 ml of exponentially growing culture and the OD followed. At the termination of an experiment a loopful of the suspension from each flask was streaked on slopes of potato-dextrose agar (Difco) and nutrient agar (Difco). The slopes were incubated at 30° for 2 to 3 weeks and then examined for bacterial or fungal colonies. Results from any flask were discarded if it was found contaminated by bacteria or fungi.

Fractionation of the Tissue. Each culture was centrifuged at 1200 X g for 20 minutes, the pellet suspended in 20 ml of culture medium without glucose and re-centrifuged. The culture medium and washings were combined to give the fraction referred to as culture medium. The tissue was extracted successively with 5% trichloroacetic acid at 0°, 50% ethanol-ether at 0°, 5% trichloroacetic acid at 90° and 50% ethanol-ether at room temperature. Each extraction was performed twice with 20 ml of solvent for 20 minutes. The residue was vacuum dried to remove the remaining alcohol and ether and then hydrolysed for 72 hours at 100° with 1.5 ml of Dowex 50 and 5 ml of water. After hydrolysis, the mixture was poured into a 1 X 20 cm column containing 1.0 ml of Dowex 50. The resin was washed with 40 ml of water to elute the nonanionic fraction. The protein amino acids were then eluted with 40 ml of 0.2 N NH4OH.

The protein amino acid eluates were stored over concentrated H2SO4 to remove ammonia and then dried at 35° using a rotary evaporator. Each was treated with 10 ml of 0.1 N piperidine and dried a second time. Each fraction was dissolved in 5 ml of water and sampled for determination of amino acids and radioactivity. All aliquots for determination of total amino acids were treated with 0.2 ml of 0.1 N NaOH and dried in an evacuated desiccator over concentrated H2SO4. The NaOH was neutralized with acetic acid prior to analysis. The method of Moore and Stein (16) was used to determine total amino acids with leucine as a standard. Each protein amino acid fraction was dried in vacuum and dissolved in 0.2 N sodium citrate buffer at pH 2.2 (15) to give a solution, 7.5 mm with respect to amino acids, for column chromatography.

When the amino acids in the trichloroacetic acid extracts were to be recovered, the trichloroacetic acid was removed by 2 extractions with an equal volume of chloroform. The combined chloroform extracts were washed with 10 ml of water. The extracted aqueous solution and the washings were combined and passed through a 1 X 20 cm column containing 8 ml of Dowex 50. The column was washed with 20 ml of water and then eluted with 160 ml of 0.2 N NH4OH. This eluate was then treated in the same way as the eluates containing the protein amino acids.

To isolate the amino acids from the culture medium, a 20 ml portion was passed through a column containing 8 ml of Dowex 50. The column was washed and eluted as above. The amino acids recovered from the culture medium and the cold trichloroacetic acid extracts were heated in 0.1 N HCl at 100° for 2 hours to hydrolyse glutamine and asparagine before chromatography.

Determination of Radioactivity of Tissue Fractions. Aliquots of solutions for counting were dried in vacuum, dissolved in 0.2 ml of n hyamine hydroxide in methanol, diluted with 18 ml of a toluene solution containing 5 g/liter of 2,5-diphenyloxazole and 0.3 g/liter of 2,2'-phenylene-bis-(5-phenyloxazole) and counted in a Tri-Carb liquid scintillation counter. With a C14-toluene standard, the counter had an efficiency of 61%.

Determination of Radioactivity of Amino Acids. The separation of the amino acids in 2.0 ml samples of the protein amino acid fractions, the amino acids from the cold trichloroacetic acid extracts or from the culture medium was performed on ion exchange resin columns (26). The effluent from the columns was passed through a Packard Model 317 Tri-Carb flow detector at 30 ml per hour.

The area under the peak recorded for each amino acid was determined with a planimeter and corrected for background. The area under each peak was expressed as a percentage of the mean area under the peaks of the same amino acid in the control samples. The percentages were rounded to the nearest 10%.

Quantitative Determination of Amino Acids. Determinations of the individual amino acids were performed by the method of Moore and Stein (16) on 2.0 ml fractions of the effluent from the columns. The Beckman amino acid analyser, Model 120B was used for separation and determination of the amino acids in the cold trichloroacetic acid extracts and in some of the protein amino acid fractions. The method of Prokop and Udenfried (23) was used for the determination of hydroxyproline.
Results

The results given in this paper are derived from 7 experiments. Each experiment included analyses of 2 unsupplemented control cultures and other cultures, each supplemented with a single protein amino acid. The effects of each amino acid except glutamic acid were examined twice in 2 entirely separate experiments.

Growth of the Tissue. The usual concentration of amino acid used to supplement the culture medium in these experiments was 100 mg per liter. In cases where this concentration of amino acid inhibited growth, the amount was reduced until the growth of the culture as followed by OD at 600 mμ was not affected. In some cases it was necessary to increase the concentration of specific amino acids to amplify their effects. In these cases the levels used were not inhibitory to growth.

The time required for the OD of the culture medium in the control flasks to double ranged from 62 and one-half to 89 hours with a mean of 74 hours. In 2 experiments a lag phase of 1 to 1 and one-half days was observed. The growth phase of the experiments was terminated after about 4-fold increase in OD while the cells were still growing exponentially but when most of the glucose had been utilized. Some details of the growth of tissue in an experiment are given in table I.

Fractionation of the Tissue and Distribution of Radioactivity. Between 40 and 50% of the radioactivity provided as glucose-U-C14 in the culture medium was recovered (table I). No effort was made to measure CO2 or other volatile compounds produced by the cultures. During hydrolysis of the residue left after extraction of the tissue, some did not dissolve and was retained in the resin.

The distribution of the recovered radioactivity between the tissue fractions (table I) is similar in all the treatments except in the culture from which glutamine was omitted. The data for this culture show several signs that the growth of the tissue was restricted by the omission of glutamine.

Amino Acid Analyses. The approximate quantities of amino acids found in the cold trichloroacetic acid extract of the tissue are shown in table II. The identification of the peaks was based on the position in the elution curve of known amino acids.

Initially the amount of hydroxyproline in the protein amino acid fraction was found to be small. No radioactive peak due to hydroxyproline could be seen preceding the peak due to aspartic acid when the 150-cm columns were maintained at 30° during elution with the pH 3.25 buffer. In subsequent experiments the 150-cm columns were maintained at 50° to decrease the time required for the determination of the radioactivity of the protein amino acid samples. Under these conditions aspartic acid and hydroxyproline do not separate.

During the manipulation of most of the samples, methionine was oxidized to methionine sulfoxides. The values for the radioactivity of methionine were obtained by adding the radioactivities of methionine and methionine sulfoxides.

Dry weight was determined on the residue left after cold ethanol-ether extraction. The control flasks were the only ones sampled for determination of initial radioactivity.

Table I. The Growth of Paul’s Scarlet Rose Tissue, Yield of Tissue, Yield of Amino Acids and the Distribution of Radioactivity from U-C14 Glucose Among the Tissue Fractions in an Isotope Competition Experiment

<table>
<thead>
<tr>
<th>C12 Amino acid added</th>
<th>Control</th>
<th>MET</th>
<th>MET</th>
<th>ILEU</th>
<th>GLY</th>
<th>ASP.NH3</th>
<th>LYS</th>
<th>GLU.NH2</th>
<th>GLU.NH3</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/liter added</td>
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<tr>
<td>Initial OD (×100)</td>
<td>19 20</td>
<td>19 21</td>
<td>18 17</td>
<td>21 18</td>
<td>21 18</td>
<td>21 21</td>
<td>20 20</td>
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<tr>
<td>Final OD (×100)</td>
<td>93 86</td>
<td>81 89</td>
<td>85 89</td>
<td>99 91</td>
<td>90 90</td>
<td>76 76</td>
<td>76 76</td>
<td>76 76</td>
<td>76 76</td>
</tr>
<tr>
<td>Fr wt (mg)</td>
<td>5120 5800</td>
<td>5020 4790</td>
<td>3990 4880</td>
<td>4870 4870</td>
<td>4660 4660</td>
<td>4540 4540</td>
<td>4540 4540</td>
<td>4540 4540</td>
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<tr>
<td>Dry wt (mg)</td>
<td>54 52</td>
<td>46 47</td>
<td>45 53</td>
<td>52 52</td>
<td>46 48</td>
<td>47 47</td>
<td>47 47</td>
<td>47 47</td>
<td>47 47</td>
</tr>
<tr>
<td>Amino acid in hydrolysate (amoles)</td>
<td>76.0 83.5</td>
<td>73.3 74.8</td>
<td>63.5 74.8</td>
<td>84.0 73.2</td>
<td>75.2 56.5</td>
<td>56.5 56.5</td>
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<tr>
<td>cpm/amoles amino acid</td>
<td>7500 7930</td>
<td>7550 7650</td>
<td>7280 8050</td>
<td>7380 6710</td>
<td>7280 8160</td>
<td>8160 8160</td>
<td>8160 8160</td>
<td>8160 8160</td>
<td>8160 8160</td>
</tr>
<tr>
<td>Initial radioactivity (cpm × 10-5)</td>
<td>94.9 94.1</td>
<td>94.9 94.9</td>
<td>94.9 94.9</td>
<td>94.9 94.9</td>
<td>94.9 94.9</td>
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<td>94.9 94.9</td>
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<tr>
<td>Radioactivity recovered (cpm × 10-5)</td>
<td>38.0 41.2</td>
<td>46.5 47.0</td>
<td>51.2 43.9</td>
<td>44.6 44.2</td>
<td>45.1 45.1</td>
<td>48.0 48.0</td>
<td>48.0 48.0</td>
<td>48.0 48.0</td>
<td>48.0 48.0</td>
</tr>
<tr>
<td>Radioactivity remaining in the culture medium (cpm × 10-5)</td>
<td>9.9 11.4</td>
<td>20.8 21.6</td>
<td>29.4 14.8</td>
<td>19.7 21.5</td>
<td>22.2 20.3</td>
<td>20.3 20.3</td>
<td>20.3 20.3</td>
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<tr>
<td>Distribution of radioactivity among the tissue fractions (% of that recovered in the tissue)</td>
<td>32 34</td>
<td>35 34</td>
<td>26 23</td>
<td>33 23</td>
<td>20 20</td>
<td>19 19</td>
<td>19 19</td>
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<td>Cold trichloroacetic acid extract</td>
<td>17 17</td>
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<tr>
<td>Cold ethanol-ether extract</td>
<td>15 15</td>
<td>15 15</td>
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<td>15 15</td>
<td>15 15</td>
<td>15 15</td>
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<tr>
<td>Hot ethanol-ether extract</td>
<td>10 10</td>
<td>10 10</td>
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<td>10 10</td>
<td>10 10</td>
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<td>Nonprotein fraction of hydrolysate</td>
<td>12 12</td>
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<tr>
<td>Protein amino acid fraction</td>
<td>20 22</td>
<td>21 21</td>
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</table>
and methionine sulfoxides. The recovery of methionine was quite variable.

A radioactive peak due to cystine was observed only occasionally during chromatography of the protein amino acids.

The quantities of individual amino acids remaining in the culture medium from a control flask, an aspartate treatment, and a methionine treatment were determined. In the case of the methionine treatment in which growth was about half of that in the control flasks, free methionine (370 μg), methionine sulfoxide (350 μg), aspartic acid (280 μg), glutamic acid (1900 μg), serine (160 μg), glycine (29 μg), and alanine (26 μg) were detected. These amino acids contained C14. About 60% of the glutamine and about 70% of the methionine were no longer present in the culture medium. In the culture medium of the control and of the aspartic acid treatment only traces of serine, glutamic acid, alanine, and glycine were present. The amounts of aspartic acid, the only other amino acid found in the medium, were 90 μg and 70 μg, respectively. The uptake of both glutamine and aspartic acid in the aspartate treatment and the uptake of glutamine in the control were virtually complete.

Effects of Exogenous Amino Acid on the Incorporation of Radioactivity into Protein Amino Acids. In the determination of the radioactivity of the amino acids in the protein amino acid fraction, the coefficient of variation for the determination of the area under each peak was 3.7%.

The radioactivities of each amino acid of the protein of tissue grown in the presence of an added amino acid were expressed as a percentage of the mean radioactivity of the same amino acid in the 2 controls. This percentage has a variability of at least 10%. This variability is to be expected from the coefficient of variation of the measurement of area under the peaks of radioactivity.

The effects of the various amino acids on the incorporation of radioactivity from glucose into protein amino acids may be classified in 2 ways. Firstly, an exogenous amino acid may affect the radioactivity of the corresponding amino acid in the protein. The percentage of an amino acid synthesized from glucose when that amino acid was provided exogenously was: lysine (100 mg/liter) 0, threonine (200 mg/liter) 10, isoleucine (100 mg/liter) 0, methionine (50 mg/liter) 20, arginine (100 mg/liter) 20, valine (50 mg/liter) 60, leucine (50 mg/liter) 40, serine (100 mg/liter) 80, glycine (50 mg/liter) 80, histidine (100 mg/liter) 0, phenylalanine (100 mg/liter) 0, tyrosine (100 mg/liter) 0. In 2 experiments with proline (100 mg/liter) 70 and 100% of the protein proline was synthesized from glucose. In the presence of asparagine (200-400 mg/liter), 70% of the protein aspartic acid was synthesized from glucose. Ammonium aspartate (100-500 mg/liter), ammonium glutamate (500 mg/liter), alanine (200 mg/liter) and cystine (50 mg/liter) had no effect on the biosynthesis of protein amino acids from glucose. Secondly, an exogenous amino acid may affect the radioactivity of other amino acids in the protein. In the presence of the amino acid in parenthesis the percentages of the following amino acids synthesized from glucose were: isoleucine 50% (threonine, 200 mg/liter), leucine 80% (valine 50 mg/liter), glycine 80% (serine 100 mg/liter) and serine 80% (glycine 50 mg/liter).

A range of glutamine concentrations from 0 to 1000 mg/liter was used to explore the possibility that glutamine was used as an alternative source of carbon to glucose in the cultures. No effect of glutamine on the distribution of C14 from glucose in the protein amino acids was found.

Discussion

Exogenous Amino Acids which provide Carbon to Protein Amino Acids. The results of the isotope competition experiments performed with cells of Paul's Scarlet Rose show that with respect to many amino acids these cells behave in the same way as many micro-organisms. When either lysine, arginine, histidine, methionine, threonine, isoleucine, phenylalanine or tyrosine is provided in the culture medium the plant cells use the amino acid for the synthesis of protein in preference to glucose or any compound derived from glucose. These experiments were performed under conditions in which net synthesis of protein occurred. The results obtained provide strong support for the hypothesis that amino acids are incorporated, as such, into protein and they
are not consistent with the hypothesis that carbohydrates are the preferred source of carbon for protein synthesis. The evidence for these 2 hypotheses in plants is reviewed by Yemm and Folkes (33) and commented on by Hellebust and Bidwell (9).

In the cases where an external supply of an amino acid is used preferentially for protein synthesis, the supplied amino acid virtually prevents its own biosynthesis from glucose. In microorganisms the end product of a metabolic sequence will sometimes inhibit the first enzyme of the sequence and thus prevent excessive enzymic production of the product. End-product inhibition of several isolated plant enzymes has been reported (4, 17, 30). In view of the fact that an external supply of either lysine, arginine, histidine, methionine, threonine, isoleucine, phenylalanine or tyrosine prevents its own biosynthesis, it is reasonable to propose that each of these amino acids inhibits the first enzyme of their respective biosynthetic pathways.

Oaks (18) showed by experiments in which a mixture of unlabeled amino acids and acetate-2-C14 was supplied to maize root tips that the biosynthesis of threonine, valine, leucine, lysine, arginine and proline was inhibited and suggested that end-product inhibition occurred. No effect on the biosynthesis of histidine, tyrosine and phenylalanine was observed. This may be related to the use of acetate as a source of C14. Oaks (21) has since shown that exogenous leucine specifically inhibits the biosynthesis of leucine.

**Exogenous Amino Acids which provide a Portion of the Carbon to Protein Amino Acids.** Asparagine, valine, leucine, serine and glycine when provided in the culture medium cause a 25% decrease of incorporation of C14 from glucose into protein amino acids. While mixing of exogenous and endogenous amino acid would account for the partial effects, other explanations are possible. Asparagine, for example, at 2 concentrations gives a 30% inhibition of incorporation of glucose carbon into protein aspartic acid. This indicates that the quantity of asparagine supplied does not limit the effect. The aspartic acid present in the protein amino acids fraction represents both protein aspartic acid and protein asparagine. An explanation of the partial effect of asparagine is that exogenous asparagine completely suppresses the incorporation of carbon from glucose into protein asparagine but has no effect on the incorporation into protein aspartic acid.

The partial effects of valine and leucine may be considered together because valine contributes carbon to leucine and because they both inhibit the growth of the tissue. These inhibitory effects on growth necessitate reduction of the levels of these compounds in the medium. The partial effects on isotope incorporation could be associated with the low concentrations of the compounds provided. If the inhibitory effects were relieved, then it should be possible to increase the levels of valine and leucine in the culture medium and observe a further decrease of the biosynthesis of valine and/or leucine from glucose. In the presence of 50 mg/liter of valine, 60% of protein valine and 80% of protein leucine is synthesized from glucose. In contrast, in a single experiment using 100 mg/liter of isoleucine to reverse the growth inhibition by 100 mg/liter of valine, 5% of the protein valine and 40% of the protein leucine was synthesized from glucose. With modification of these experiments it may be possible to show that exogenous asparagine, valine and leucine are also used in preference to the biosynthesized amino acids for protein synthesis.

The 2 remaining amino acids, glycine and serine, differ from asparagine, valine and leucine in that they provide carbon to each other. Glycine is an inhibitor of cell growth and was therefore provided at a reduced concentration. Serine inhibits growth at concentrations greater than 100 mg/liter. To speculate on the incomplete competition by both serine and glycine seems unwise in the absence of additional information.

**Exogenous Amino Acids which did not provide Carbon to Protein Amino Acids.** Alanine, glutamine, aspartic acid, glutamic acid and perhaps proline do not affect the incorporation of radioactivity into the protein. In this respect these plant cells differ from *Escherichia coli* (25). To interpret these results one must examine the 2 assumptions, of the isotope competition method. These are that an exogenous compound first enters the cell and then mixes with the biosynthesized compound. The assumptions hold in the case of the 13 amino acids which do compete with glucose. An interpretation of the lack of competition observed with 5 of the amino acids is that either the first or the second assumption is not obeyed and that glucose remains the only source of carbon for the biosynthesis of these 5 amino acids for protein synthesis.

The estimations of free amino acids in the culture medium at the termination of growth show conclusively that the plant cells are permeable to alanine, glutamine, and aspartic acid. The cells are permeable to the 13 amino acids which compete with glucose. It is reasonable to assume that glutamic acid and proline can also enter the cells. One must conclude that alanine, aspartic acid, glutamic acid and probably proline do not mix with the corresponding pool from which the amino acid for protein synthesis is drawn. Similarly, one must conclude that glutamine does not mix with the pool from which protein glutamine is drawn nor does it contribute glutamic acid to the pool from which protein glutamic acid is drawn.

The phenomenon of 2 sources of a compound contributing to 2 separate and independent metabolic pathways has been termed physiological channeling, metabolic channeling or compartmentation. In plant cells or tissues evidence for compartmentation of
glutamic acid (1, 9, 12, 27), aspartic acid (1, 9, 12, 27), glutamine (27), serine (1, 9, 27), glycine (1, 9), alanine (1, 9, 27), threonine (1) and leucine (20) has been obtained.

Pathways of Biosynthesis of Amino Acids. Evidence for the biosynthesis by plant cells of isoleucine from threonine and of leucine from valine is provided by this study. Evidence for these pathways in other higher plants has been obtained (3, 11, 19).

Several studies of the metabolic interconversions of serine and glycine (14, 24, 31) and phenylalanine and tyrosine (6, 7, 13) in higher plants have been made. In Paul's Scarlet Rose tissue serine and glycine contribute carbon to each other and phenylalanine and tyrosine do not.

Summary

Isotope competition experiments were performed with cell suspensions of Paul's Scarlet Rose tissue growing exponentially in a defined medium. Nineteen amino acids usually present in protein were studied for their effects on the incorporation of radioactivity from glucose-U-14C into protein amino acids. Exogenous lysine, histidine, arginine, threonine, methionine, isoleucine, phenylalanine or tyrosine was used in total preference to the biosynthesized amino acid for protein synthesis. End product inhibition is proposed as the explanation of these effects.

Asparagine, valine, leucine, serine and glycine caused partial inhibition of the incorporation of C14 from glucose-U-14C into these protein amino acids. Some features of the studies of these amino acids provide possible explanations of the partial inhibitions.

Exogenous aspartic acid, glutamic acid, glutamine, alanine and probably proline did not affect the radioactivity of the corresponding amino acids. This lack of effect is explained in terms of compartmentation.

Acknowledgments

The excellent technical assistance of Miss Barbara Bock is gratefully acknowledged.

Literature Cited

Calcium Activation of Orthophosphate Absorption by Barley Roots

James E. Leggett, Raymond A. Galloway, and Hugh G. Gauch

Introduction

Calcium, an essential element for plant growth, is involved in a wide spectrum of events including development of the meristematic regions (1) and absorption rates of other ions (3, 7). Other alkaline earth cations do not completely substitute for calcium in fulfilling this growth requirement (1, 8).

The presence of Ca++ in the nutrient media of plants has been observed to influence the absorption of other ions. In general, the effect was one of inhibiting the rate of sodium absorption (4), but of enhancing the rate for potassium (4, 10, 11, 14, 21), sulfate (13), chloride (17, 20), and phosphate (18, 19). Thus, Ca++ affects the rate of absorption of a specific salt although the absorption rate of the cation or anion is usually considered to be independent of each other.

Although its general influence has been known for several years, the mechanism of action of Ca++ on ion absorption is not fully understood. This investigation considers the mode of Ca++ activation of phosphate absorption by kinetic analysis of measurements of steady-state absorption.

Materials and Methods

Roots of 2 varieties of Hordeum vulgare L., Atlas 46, and Trebi, were obtained from plants grown essentially as described by Epstein and Hagen (5). Seeds (30 g) were soaked for 24 hours in 600 ml of continuously-aerated demineralized water and distributed on cheesecloth supported on a stainless steel screen. The screen was placed on top of a 4-liter beaker containing $2 \times 10^{-4}$ m CaSO$_4$ solution, so that the seeds were 1 cm above the solution.