Amino Acid and Sucrose Content Determined in the Cytosolic, Chloroplastic, and Vacuolar Compartments and in the Phloem Sap of Spinach Leaves

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ABSTRACT

Amino acid and sucrose contents were analyzed in the chloroplastic, cytosolic, and vacuolar compartments and in the phloem sap of illuminated spinach leaves (Spinacia oleracea L.). The determination of subcellular metabolite distribution was carried out by nonaqueous fractionation of frozen and lyophilized leaf material using a novel three-compartment calculation method. The phloem sap was collected by aphid stylets which had been severed by a laser beam. Subcellular analysis revealed that the amino acids found in leaves are located mainly in the chloroplast stroma and in the cytosol, the sum of their concentrations amounting to 151 and 121 millimolar, respectively, whereas the amino acid concentrations in the vacuole are one order of magnitude lower. The amino acid concentrations in the phloem sap are found to be not very different from the cytosolic concentrations, whereas the sieve tube concentration of sucrose is found to be one order of magnitude higher than in the cytosol. It is concluded that the phloem loading results in a preferential extraction of sucrose from the source cells.

Assimilates are transferred from mature leaf cells to sink tissues by phloem transport. Major constituents of the phloem sap are sugars, mainly sucrose, and amino acids. For an understanding of the partitioning of photosynthetic products between carbohydrates and amino acids, the factors governing the withdrawal of these products from the source cells and their loading into the sieve tubes have to be known. There are various indications that phloem loading of sucrose involves transport against a concentration gradient, probably driven by proton symport (3, 4, 13, 22), and that a proton symport may also be involved in the uptake of amino acids into the sieve tubes (1, 5, 19, 21, 22).

For many plants it has been assumed that phloem loading is an apoplastic process (25). This mechanism implies that for export of substances from a source cell, they first have to be released into the apoplastic space before being taken up into the sieve tubes. This concept of apoplastic loading, derived initially from morphological studies (25), has been recently verified by studies of transformants of Nicotiana tabacum (tobacco) and Solanum tuberosum (potato). In these plants the insertion of yeast acid invertase into the apoplastic compartment of leaves caused the accumulation of sucrose and its precursors in leaves and the disappearance of sucrose in the sieve tube exudate (26) (G Günter, D Heineke, U Sonnewald, I. Willmitzer, HW Heldt, unpublished results). The finding that the hydrolysis of sucrose in the apoplastic space prevented its loading into the sieve tubes, clearly demonstrated that phloem loading with sucrose is an apoplastic process. Unless the loading of amino acids into the phloem is a very different process, for which there is no indication, the transfer of amino acids from the source cells into the sieve tubes might also proceed via the apoplast.

In respect to phloem loading it is not possible with intact leaves in the steady state of photosynthesis to determine the gradient between substrate concentrations in the apoplastic space and the sieve tube compartment, as there are no means for a reliable determination of the apoplastic concentrations in these leaves. But it is possible to assay in intact leaves the cytosolic metabolite levels. A refinement of the nonaqueous fractionation technique, described in this paper, allows the determination of metabolite levels in the vacuolar, chloroplastic and cytosolic compartment of spinach leaves in the steady state of photosynthesis. The overall process of metabolite transfer from the leaf cells to the sieve tubes is subjected to the concentration gradient between the cytosol of the source leaf cells and the sieve tubes. In the present report these gradients of sucrose and amino acids have been evaluated from the nonaqueous fractionation measurements and the analysis of metabolite concentrations in the phloem sap, collected by excised aphid stylets. These results illustrate the specificity of the processes by which products of photosynthesis are withdrawn from the source cells for export by the phloem system.

MATERIALS AND METHODS

Plant Material

Spinach (Spinacia oleracea, U.S. Hybrid 424, Ferry-Morse Co., Mountain View, CA) was grown hydroponically in a climatized chamber (9 h light, 15 h dark, 19°C). The illumination was about 350 μE m⁻² s⁻¹ using tungsten and mercury lighting.

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Freeze Stop, Homogenization and Fractionation of the Tissue

Leaves of 49 d old plants were frozen in liquid N2, while illuminated, 8.5 h after the beginning of the illumination period. Lyophilization, homogenization, and fractionation of the leaf tissue by nonaqueous procedure, the preparation of extracts for the assay of marker enzymes, and the measurements of marker enzyme activities were done according to Gerhardt and Heldt (11) with two alterations. (1) The nonaqueous fluids contained C2Cl4 instead of CCl4 throughout. The layer beneath the exponential density (d) gradient between 1.28 and 1.51 g × cm⁻³ contained 2 mL C2Cl4/C3H110, d = 1.62 g × cm⁻³. (2) For assay of metabolites, chloroform-methanol extracts were prepared (23).

Determination of Sucrose

Sucrose was assayed as previously described (24).

Amino Acid Analysis

The assays were performed by HPLC (Pharmacia/LKB). After precolumn derivatization with o-phthalaldehyde, the amino acid derivates were separated on a 4 μm particle size reversed-phase column (Merck, Darmstadt, Germany) with an acetonitrile gradient in 18 mm potassium phosphate, pH 7.1. The derivates were detected by fluorescence.

Collection of Phloem Sap

For collection of phloem sap the stylets of Myzus persicae (Aphididae) were severed by a laser beam (2, 7, 9). After severing the stylet, about 5 nL exuding sap was collected in a period of 10 to 15 min in a 0.5 μL micropip using a micro-manipulator. The leaf of the plant was illuminated during collection. To avoid evaporation of the stylet exudate, the experiments were done in a climatized chamber with 80% humidity. The stylet exudate was taken up in 30 μL H2O (Suprapure) and stored at -85°C until analysis.

RESULTS

Determination of the Distribution of Amino Acids between Three Subcellular Compartments

Leaves of spinach grown hydroponically in a 9 h light/15 h dark cycle were quenched in their metabolism by freezing them in liquid nitrogen 8.5 h after the start of illumination. From part of the frozen leaf material chloroform-methanol extracts were prepared for metabolite analysis. Table I shows the amounts of amino acids and sucrose assayed in such total leaf extracts.

To determine the distribution of amino acids between subcellular compartments, according to a previously published method (11), the frozen leaf material was lyophilized, homogenized in nonaqueous solvents, and fractionated by density gradient centrifugation. In alteration to the previously published method the toxic carbon tetrachloride was replaced by tetrachlorethylene as constituent of the homogenization and density gradient fluid. After centrifugation the separated material was collected in six or seven fractions, of which aliquots were taken for assay of marker enzymes and of metabolites. Figure 1 shows the distribution of marker enzyme activities in samples of the gradient. Very similar to earlier measurements (11), the chloroplastic material represented by the marker enzyme NADP-GAPDH is found to be concentrated in the middle region of the gradient. The cytosolic material marked by PEPCX activity appears in a lower region, whereas the vacuolar material designated by α-mannosidase activity is mainly found in the fraction with the highest density. Figure 2 shows the results of the corresponding assays of amino acids and sucrose in the various fractions of the gradient. A comparison of the data in Figures 1 and 2 shows that amino acids are widely distributed over all seven fractions, which indicates that they are contained in all three compartments designated by the above marker enzymes.

For the evaluation of the subcellular distribution of metabolites between three compartments, we developed a novel calculation procedure. This procedure is based on the assumption that the metabolites are confined to the stromal, cytosolic, and vacuolar compartment designated by the corresponding marker enzymes. The evaluation is done by a computer program testing all possible cases for the distribution of a certain metabolite between the three compartments at intervals of 1%, e.g.:

(1) Stroma 100%, cytosol 0%, vacuole 0%;
(2) Stroma 99%, cytosol 1%, vacuole 0%;
(3) Stroma 99%, cytosol 0%, vacuole 1%;
(4) Stroma 98%, cytosol 2%, vacuole 0%;
(5) Stroma 99%, cytosol 0%, vacuole 1%;
(6) Stroma 99%, cytosol 0%, vacuole 1.5%;
(1510) Stroma 0%, cytosol 99%, vacuole 1%;
(1511) Stroma 0%, cytosol 0%, vacuole 100%.

In steps of 1% there are 5151 possibilities for the distribution of a metabolite between the three compartments, and it is calculated which of these possible cases yields the best agree-

| Table I. Metabolite Content in Spinach Leaves Illuminated for 8.5 h | 
|------------------|------------------|------------------|
| Metabolite       | Content          | Percent of Total |
|                  | mmoi/mg Chl     | Amino Acids      |
| Asp              | 2.079 ± 0.252   | 25               |
| Asn              | 164 ± 19        | 2                |
| Glu              | 1,840 ± 340     | 22               |
| Gin              | 2,300 ± 385     | 27               |
| Ala              | 345 ± 20        | 4                |
| Ser              | 741 ± 115       | 9                |
| Gly              | 164 ± 53        | 2                |
| Val              | 164 ± 23        | 2                |
| Thr              | 147 ± 14        | 2                |
| Others           | ~410            | ~5               |
| Total amino acids| 8,194           |                 |
| Total amino N    | 10,838          |                 |
| Sucrose          | 7,160 ± 2,290   |                 |

* Values ± sd.

Abbreviations: GAPDH. NADP-glyceraldehyde phosphate dehydrogenase; PEPCX. phosphoenol/pyruvate carboxylase.
Figure 1. Distribution of marker enzymes in a gradient obtained from dried and homogenized spinach leaves. \(\alpha\)-Mann = \(\alpha\)-mannosidase.

Figure 2. Distribution of amino acids and sucrose assayed in an aliquot of the gradient from dried and homogenized spinach leaves of Figure 1.

Figure 3. Comparison between the measured amounts of alanine in the fractions of the gradient of Figure 2 with the results of the three-compartment calculation testing the various possibilities for subcellular distribution. Case A, stroma 20%, cytosol 20%, vacuole 60%, computed data do not fit (first series of bars). Case B, stroma 36%, cytosol 26%, vacuole 38%, best fit (second series of bars). For details see text.

ment with the experimental results. The principle of this evaluation procedure is illustrated in the diagram of Figure 3, where the middle bars represent the relative amounts of alanine assayed in the seven fractions of the density gradient (data from Fig. 2). As an example, one calculation is made for the case that 20% of alanine is located in the stroma, 20% in the cytosol and 60% in the vacuole. It is calculated which proportion of the 20% designated to the stroma would appear in each fraction of the density gradient according to the distribution of the stromal marker enzyme shown in Figure 1. In analogy to this, from the distribution of the cytosolic and vacuolar marker enzymes, the relative amounts of alanine attributed to the cytosolic (20%) and vacuolar (60%) compartment are calculated for each fraction of the gradient. The total amount of metabolites thus calculated for each fraction of the gradient is compared with the amount actually measured in these fractions. After repeating this calculation for all 5151 possible cases it is evaluated which case yields the best fit with the experimental result. As a criterion of the best fit we used the parameter \(Q = \sqrt{\sum \Delta^2}/n - 1\), \(\Delta\) represents the difference between the calculated amount from the measured amount in each fraction and \(n\) the number of fractions (6–7). The best fit of the calculation yields 36% of the alanine located in the stroma, 26% located in the cytosol and 38% located in the vacuole (Fig. 3). To avoid the results being falsified by analytical errors, the calculations are usually based on mean values obtained from measurements of at least two density gradient fractionations of the same leaf material.

Table II lists mean values of subcellular amino acid contents from five individual measurements. Although the volumes of the subcellular compartments of the investigated spinach leaves are not exactly known, commonly employed estimates are 20, 25 and 150 \(\mu L/mg\) Chl for the cytosolic, stromal, and vacuolar compartment, respectively (11). Based on these assigned values, the subcellular metabolite concentrations have
been evaluated from the data of Table II, as shown in Figure 4.

**Determination of the Subcellular Distribution of Sucrose**

Earlier studies of the subcellular distribution of sucrose by nonaqueous fractionation technique carried out in our laboratory clearly indicated that a considerable portion of the total sucrose assayed in spinach leaves could not be ascribed to any of the three subcellular fractions designated by the chloroplastic, cytosolic, and vacuolar marker enzymes and it was discussed that this portion of sucrose may represent the content of sieve tubes (11). As the sucrose concentration in the sieve tubes is one order of magnitude higher than in the cytosol of the mesophyll cells, it is to be expected that a considerable portion of the sucrose analyzed in a leaf is contained in the sieve tubes. Figure 2 shows the distribution of sucrose between the fractions of the density gradient. The bulk of sucrose is found in fraction 7 containing mainly vacuolar material. A close comparison of the distribution of sucrose and the vacuolar marker reveals that especially fractions 6 and 7 contain a considerable amount of sucrose which from its distribution in the gradient cannot be accounted to vacuolar, cytosolic and stromal compartiments. The three-compartment calculation program is therefore not suitable for the evaluation of subcellular sucrose levels. It is possible to evaluate a maximum value for the sucrose content in the cytosolic compartment by allocating all sucrose found in the fractions of the lowest density to the cytosol. The division of the percentage of total sucrose found in fraction 1 and 2 (for the sake of accuracy we base this calculation on two fractions) by the percentage of PEPCX activity found in these fractions yields the maximum value for the percentage of total leaf sucrose present in the cytosol. The data of Figures 1 and 2 yield a value of 34%. Table II lists the mean value from individual measurements for the maximum sucrose content of the cytosolic compartment evaluated as described above. Based on a cytosolic volume of 20 \( \mu L/mg\ Chl\) (11), the mean

![Figure 4. Amino acid concentrations in the stromal, cytosolic, and vacuolar compartment. Data from Table II. Assumed volumes: stroma 25 \( \mu L\), cytosol 20 \( \mu L\), vacuole 150 \( \mu L\) (11).](image-url)

**Table II. Subcellular Contents of Amino Acids in Spinach Leaves Illuminated for 8.5 h, as Evaluated According to the Three-Compartment Calculation Program (Fig. 3)**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Stroma</th>
<th>Cytosol</th>
<th>Vacuole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>920 ± 130*</td>
<td>642 ± 110</td>
<td>510 ± 130</td>
</tr>
<tr>
<td>Asn</td>
<td>74 ± 17</td>
<td>42 ± 17</td>
<td>49 ± 8</td>
</tr>
<tr>
<td>Glu</td>
<td>940 ± 190</td>
<td>580 ± 150</td>
<td>320 ± 100</td>
</tr>
<tr>
<td>Gin</td>
<td>1280 ± 250</td>
<td>690 ± 200</td>
<td>340 ± 80</td>
</tr>
<tr>
<td>Ala</td>
<td>120 ± 20</td>
<td>110 ± 20</td>
<td>120 ± 12</td>
</tr>
<tr>
<td>Ser</td>
<td>280 ± 40</td>
<td>210 ± 80</td>
<td>250 ± 50</td>
</tr>
<tr>
<td>Gly</td>
<td>41 ± 13</td>
<td>52 ± 29</td>
<td>74 ± 28</td>
</tr>
<tr>
<td>Val</td>
<td>57 ± 11</td>
<td>65 ± 18</td>
<td>41 ± 6</td>
</tr>
<tr>
<td>Thr</td>
<td>66 ± 8</td>
<td>28 ± 5</td>
<td>53 ± 7</td>
</tr>
<tr>
<td>Total amino acids</td>
<td>3778</td>
<td>2419</td>
<td>1757</td>
</tr>
<tr>
<td>Total amino N</td>
<td>5132</td>
<td>3151</td>
<td>2146</td>
</tr>
<tr>
<td>Sucrose</td>
<td>&lt;2060 ± 400</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* Values ± sd.

For the evaluation of subcellular amino acid levels a major interference by the sieve tube content is not to be expected, as the amino acids are not concentrated in the sieve tubes (see next paragraph). As the putative sieve tube material appears in the heaviest fractions of the gradient, an interference by the sieve tube content may only result in slight overestimation of the vacuolar amino acid contents. For this reason the vacuolar amino acid concentrations may be even lower than shown in Figure 4.

**Analysis of the Sieve Tube Exudate from Spinach Leaves**

Phloem sap from illuminated spinach leaves was obtained by aphid stylet excision (2). In contrast to monocotyledons like oat (17, 27), barley (27), wheat (8, 14), maize (20, 28), or rice (10) where, after severing of the stylet, the phloem sap can be collected for many hours, with spinach leaves the exudation of the sieve tube sap occurred only for about 10 to 15 min after severing of the stylet. Therefore, only relatively small amounts of phloem sap (about 5 mL) could be collected at a time. The collection of the sieve tube sap was done at
80% humidity. Despite the smallness of the samples, using highly sensitive amino acid analysis and enzymatic assay (see "Materials and Methods"), the relative metabolite contents of the sieve tube sap could be determined with accuracy. Table III shows the average amino acid pattern determined in 10 individual exudate samples. Two thirds of the total amino nitrogen found in the phloem sap were contained in the amino acids glutamate, glutamine and aspartate.

The amino acid concentrations shown in Figure 5 are only estimates, due to the small volume of the collected samples. In 10 samples of phloem sap the average concentration of the sum of amino acids was found to be 160 mM and the average concentration of sucrose accounted to 0.8 M. In phloem sap from maize and barley leaves, the collected samples were much larger (0.2-0.5 \( \mu \)L). In these samples the determination of the sample volumes could be done with reasonable accuracy, and evaporation losses could be corrected for by simultaneous measurements of the evaporation of blank samples. Of both plants the determined average sucrose concentrations in the phloem sap were about 1 M (G Lohaus, manuscript in preparation). Similar sucrose concentrations have been determined by other authors in phloem sap from maize (20) and oat (17). The similarity of these results with the sucrose concentrations assayed in phloem sap from spinach leaves shown here, indicates that the estimation of the sap volume yielded reasonable results and that the amino acid concentrations listed in Figure 5 are realistic values. To avoid uncertainties about volume measurements of the very small phloem sap samples, the concentration values presented in Figure 5 have been evaluated by relating the amounts of amino acids to the amounts of sucrose simultaneously assayed in the phloem sap samples, and by normalizing the data to a sucrose concentration of 0.8 M.

**DISCUSSION**

**On the Analysis of the Subcellular Distribution of Metabolites**

In a mature leaf most of the photosynthetic products are transferred from the source leaf cells into the sieve tubes. The

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**Table III. Metabolites in Phloem Sap from Spinach Leaves**

The samples were collected 8 to 9 h after the beginning of the illumination period. Results of 10 individual measurements. The cytosolic values are calculated from the data of Table II.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Percent of Total Amino Acids in Phloem Sap</th>
<th>Percent of Total Amino Acids in Cytosol</th>
<th>Percent of Phloem in Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>14.7 ± 3.0(^a)</td>
<td>26.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Asn</td>
<td>2.2 ± 1.8</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Glu</td>
<td>39.1 ± 5.8</td>
<td>23.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Gin</td>
<td>10.1 ± 3.7</td>
<td>27.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Ala</td>
<td>7.7 ± 3.1</td>
<td>4.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Ser</td>
<td>9.6 ± 2.4</td>
<td>8.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Gly</td>
<td>4.1 ± 1.2</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Val</td>
<td>4.3 ± 1.1</td>
<td>2.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Thr</td>
<td>2.1 ± 1.0</td>
<td>1.1</td>
<td>1.9</td>
</tr>
</tbody>
</table>

\(^a\) Values ± sd.
phosphate and phosphorylated intermediates in the stroma and in the cytosol are much lower. In both compartments the sum of the concentrations of these compounds was found to be in the range of 25 to 30 mm (12), yielding a total ion concentration below 100 mm. Also the cytosolic concentration of sucrose is below 100 mm (see above), and the cytosolic and stromal concentrations of malate are in the order of $10^{-3}$ M (15). It seems therefore that amino acids are the main solutes in the cytosolic and stromal compartment of a spinach leaf cell. Although 20 to 25% of the total amino acids are found in the vacuolar fraction, because of the relatively large vacuolar volume the evaluated amino acid concentrations (sum of amino acids 12 mm) are one order of magnitude lower than the corresponding concentrations in the cytosol (Fig. 4).

Dietz et al. (6) found with vacuoles aqueously isolated from protoplasts of barley leaves that the portion of the total amino acids of the protoplasts allocated to the vacuoles was relatively high, the total amino acid concentration in the vacuoles amounting to 77 mm. In contrast to these results, analyses by nonaqueous fractionation, done in our laboratory, revealed that in illuminated barley leaves the vacuolar amino acid content was even lower than in spinach leaves (H Winter, manuscript in preparation). Notably, in darkened barley leaves the vacuolar amino acid content appeared higher than in illuminated leaves. These results indicate that the amount of amino acids found in the vacuoles is variable. It is well known that neutral amino acids are able to permeate lipid bilayers by diffusion (30). Even if the rate of permeation is not very high, one should expect that the concentrations of neutral amino acids at either side of the tonoplast equilibrate as the amino acids supposedly are not metabolized in the vacuole. It is feasible that the observed imbalance in neutral amino acid concentrations between the vacuoles and the cytosol may be due to an active extrusion of amino acids across the tonoplast. In vacuoles from barley leaves specific transport of phenylalanine (16) and of alanine, leucine and glutamine (6) has been demonstrated recently. It seems possible that the physiological function of such putative amino acid translocators is not the uptake of amino acids into the vacuoles, but their extrusion. Further experiments are required to verify this hypothesis.

**Relationship between the Relative Amino Acid Contents in the Cytosol and in the Phloem Sap**

Although our model plant spinach is less suitable than other plants for collecting phloem sap by aphid technique, as only about 5 nL of exudate could be obtained from a severed stylet, this amount was sufficient for the determination of amino acids and of sucrose. In the phloem sap from spinach leaves a large number of amino acids are found, similar to phloem sap from wheat (8, 14), oat (17, 27), barley (27), maize (20, 28), and rice (10). Table III shows the relative distribution of amino acids in the phloem sap of spinach and for comparison also the amino acid distribution in the cytosol. In both compartments, the major constituents are glutamine, glutamate and aspartate, containing 66% of the total amino N in the sieve tubes and even 80% in the cytosol. As an indicator of the distribution of amino acids between the two compartments the ratios between the relative amounts of amino acids in the sieve tubes and the cytosol have been listed in Table III. Serine and asparagine yield a ratio of about 1, indicating very similar distribution in both compartments. For glutamine and aspartate the ratio is 0.4 and 0.6, respectively, i.e. there is relatively more of these amino acids in the cytosol than in the sieve tubes. This may indicate that the export of these substances from the source leaf cells to the sieve tubes is to some extent restricted in relative terms. For all other amino acids a ratio of 1.6 to 2.0 is found, which suggests that the export of these substances into the sieve tubes occurs with some slight preference over the export of the other amino acids.

A comparison of the amino acid distribution in whole leaves and in the phloem sap of illuminated maize plants has been made recently in our laboratory (28). Because in spinach leaves the relative distribution of amino acids in the cytosol is similar to the amino acid distribution in whole leaves, as shown above, it is reasonable to assume that this may also apply to maize leaves. Moreover, because of the high density of symplastic connections between the mesophyll and bundle sheath cells, allowing the permeation of substances of a molecular mass up to 900 Da (18), one would expect that in the NADP-malic acid C$_4$ plant maize the amino acid patterns in the mesophyll and bundle sheath cells are not very different, and this has been verified experimentally (29) (H Weiner, HW Heldt, manuscript in preparation). For this reason, in analogy to the data in Table III, the ratio between the relative amounts of amino acids in the phloem sap and in whole maize leaves has been used as an indicator for the specificity of the transfer of amino acids from the source leaf cells to the sieve tubes. The results obtained with maize leaves showed some differences to those with spinach leaves mentioned before. In the phloem sap of maize the bulk of the amino nitrogen was contained in glutamine (28%), alanine (28%), glutamate (13%), and also asparagine (13%). In maize not only the export of aspartate as in spinach but also that of glycine appeared to be restricted. For glutamine, on the other hand, and asparagine, the ratio between the relative amounts in the phloem sap and in the cytosol was found to be 3 and 11, respectively, indicating that these substances were preferentially exported to the sieve tubes.

It remains to be elucidated to what extent the different patterns of amino acid export observed with spinach and maize are characteristics of the different species or caused by different metabolic conditions. Since the loading of amino acids into the phloem can be expected to be an apoplastic process (see introduction) the passage of amino acids from the source leaf cells into the apoplast and from the apoplast into the sieve tubes would require that the amino acids have to traverse two membranes. Specific translocators for the different groups of amino acids may be required for each of these two membranes. Four amino acid symport systems, two for transport of neutral amino acids, one for acidic and one for basic amino acids have been characterized recently in plasma membrane vesicles (19). It is feasible that a variability in amino acid transfer from the cytosol to the sieve tubes in spinach and maize is in part due to differences in the relative activities of these different translocators.
Relationship between the Concentrations of Amino Acids and Sucrose in the Cytosol and in the Phloem Sap

In Figure 5 the concentrations in the cytosol and in the phloem sap are compared for the various amino acids. Although the concentration of glutamine in the sieve tubes appears to be lower, and those of glutamate, alanine, serine, glycine, valine, and threonine higher than in the cytosol, the concentrations in the two compartments differ by less than a factor of three. Irrespective of any possible error in absolute concentrations due to uncertainties of volumes, these results clearly demonstrate that there are characteristic differences in the phloem loading of sucrose and amino acids. Whereas the transfer of sucrose from the cytosol into the sieve tubes involves a concentration increase by a factor of about 10, amino acids are concentrated by less than a factor of 3. Thus, from the cytosol where the concentration ratio of sucrose/total amino acids is less than 0.8, photosynthetic products are extracted to yield a phloem sap with a concentration ratio of sucrose/total amino acids of 5.

Whereas for sucrose the participation of active transport in phloem loading is obvious, in the case of the various amino acids our experiments cannot definitely establish to what extent an active transport is involved, as the amino acid concentrations in the apoplast are not known. Our results clearly demonstrate, however, that the phloem loading of sucrose and amino acids proceeds in such a way that sucrose is preferentially extracted from the source cells.

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LITERATURE CITED