The Effect of Carbohydrates and Arginine on Arginine Metabolism by Excised Bean Leaves in the Dark

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ABSTRACT

The effect of carbohydrate on arginine utilization by excised bean (Phaseolus vulgaris L. var. Tendergreen) leaves in the dark was studied by adding arginine to leaves differing in carbohydrate levels, and measuring the arginine content of the leaves at intervals. In nonstarved leaves, the arginine content decreased steadily after vacuum infiltration of 10 mM arginine and was essentially completely utilized by 36 hours after infiltration. In starved leaves, the arginine content did not decrease except for a brief period of about 4 hours after infiltration. The distribution of 14C after adding 14C-arginine to starved and nonstarved leaves indicated that the presence of carbohydrates in the leaves stimulates the utilization of arginine for protein synthesis and conversion to other amino acids, organic acids, and CO2 (catabolism). Adding sucrose along with arginine to starved leaves stimulated this utilization of arginine for both protein synthesis and catabolism. This effect of sugar on catabolism is different than results of similar studies done previously with proline.

Increasing the concentration of added arginine greatly increased arginine catabolism but had a relatively small effect on utilization of arginine for protein synthesis. This result is the same as similar results from adding different concentrations of proline to excised leaves.

Studies on respiration of starved leaves have indicated that the hydrolysis of protein occurs while carbohydrates are present but the subsequent respiration of the hydrolysis products occurs after the depletion of carbohydrates (14). Recently, I observed that proline oxidation and subsequent respiration of the carbon is faster in starved leaves than in nonstarved leaves (9). I showed that proline oxidation is faster when the level of nonprotein proline in the leaf is increased by adding exogenous proline (9) or by allowing proline to accumulate during wilting (10). In the wilting experiments (10), proline oxidation was determined after rehydration.

Experiments reported in this paper were done to determine whether or not the respiration of arginine, which has a catabolic step common to proline oxidation, is subject to the same controls as proline oxidation. The results indicate that in contrast to proline, leaf carbohydrate stimulates arginine catabolism. Arginine catabolism is faster when the level of nonprotein arginine in leaves is increased by adding exogenous arginine, a result that is the same as the effect of proline levels on proline oxidation.

MATERIALS AND METHODS

The methods used in these experiments have been described (9) with arginine replacing proline in the solutions added to the leaves. Nonstarved leaves were from plants (Phaseolus vulgaris L. var. Tendergreen) previously in the light for 16 hr and starved leaves were from plants previously in the dark for 48 hr. The water-soluble portion of the 80% (v/v) alcohol extracts were passed successively through Dowex-50-NH4+ and Dowex-50-H+ (13) and the basic amino acids were chromatographed in one dimension on paper (12). Arginine were determined by the Sakaguchi reaction on the basic fraction (1). All the radioactivity added to the leaves was recovered in the fractions analyzed.

RESULTS

The nonprotein arginine content of excised leaves at various times after infiltration with 10 mM arginine is shown in Figure 1. In nonstarved leaves, there is a relatively rapid loss of arginine during the first 4 hr, followed by a slower, steady decline in arginine content during the 48-hr incubation period. Essentially all the added arginine was utilized. In the starved leaves, there was also a loss of arginine during the first 4 hr, and a slight amount lost between 4 and 8 hr, but during the remainder of the 48-hr incubation period no further loss of arginine occurred and instead, the arginine content increased. Adding 50 mM sucrose along with the arginine to starved leaves did not affect the initial loss, but during the remainder of the incubation there was less increase in arginine compared to the starved leaves receiving no sucrose.

The distribution of radioactivity in various fractions at several times during 4 hr after adding 2 mM uniformly labeled 14C-arginine (specific radioactivity 0.33 μCi/μmol) to nonstarved and starved leaves is shown in Figure 2, A and B, respectively. In the nonstarved leaves, about 50% of the nonprotein 14C-arginine disappeared during the experiment whereas in starved leaves only about 30% disappeared and very little loss occurred after the first few hours. The metabolized arginine was used for protein synthesis and conversion to other amino acids, organic acids, and CO2 (the values for organic acids in Fig. 2B were <2% at all times). The amino acids labeled were mainly proline and glutamic acid with small amounts of 14C in aspartic acid, glutamine, asparagine, ornithine, and γ-aminobutyric acid. The amounts of 14C recovered in other amino acids at 0 time were attributable to a small amount of impurity in the 14C-arginine used and to a rapid incorporation into proline and glutamate during the time the leaves were blotted and weighed. The radioactivity in glutamate decreased during incubation and the radioactivity in proline, aspartic acid, asparagine, and glutamine increased but the total amount of 14C in amino acids other than arginine was relatively constant. The label recovered in other amino acids, organic acids, and CO2 represents catabolism of arginine and oxidation of the carbon in the respiratory pathways, presumably by conversion to glutamic acid and subsequent metabolism in the Krebs cycle. Both incorporation into protein and catabolism were less in starved leaves than in non-starved leaves.
The distribution of radioactivity in various fractions at several times during 24 hr after adding 2 mM UL-$^{14}$C-arginine (specific radioactivity 0.33 $\mu$Ci/umole) together with 0.05 M (Fig. 3A) or 0.1 M (Fig. 3B) sucrose to starved leaves is shown in Figure 3. Comparing the disappearance of $^{14}$C in nonprotein arginine in the experiments represented by Figures 2B, 3A, and 3B, it is clear that additional sucrose along with arginine increases the utilization of arginine. The addition of sucrose increased the catabolism of arginine and the incorporation of arginine into protein.

The effect of arginine concentration on the metabolism of exogenous arginine by nonstarved leaves is shown in Figure 4. In the experiment represented by Figure 4A, high specific radioactivity $^{14}$C-arginine was added which was not sufficient to alter significantly the endogenous arginine content of the leaf (Table I). Labeled arginine was rapidly incorporated into protein under those conditions and very little was catabolized to other amino acids, organic acids, and CO$_2$. In the experiments represented by Figure 4, B and C, 2 mM and 10 mM arginine, respectively, were added. In both cases these amounts significantly increased the endogenous level of arginine. Increasing the arginine concentration increased the amount that was catabolized to other amino acids and CO$_2$. Less $^{14}$C-arginine but more total arginine was incorporated into protein when the supply of arginine was increased. The rates of utilization of arginine for protein synthesis and catabolism were estimated and are tabulated for the three arginine concentrations in Table I. When 2 mM arginine was added to the leaf, a 200-fold greater rate of catabolism was observed than with the addition of high specific radioactivity arginine, whereas only a 50% greater rate of incorporation into protein was observed. When 10 mM arginine was added, a 10-fold greater rate of catabolism was observed than with additional 2 mM arginine. The rate of incorporation into protein was only 2-fold greater for additional 10 mM arginine than for 2 mM arginine.

**DISCUSSION**

The catabolism of arginine is assumed to occur by conversion to ornithine and urea by arginase, transamination of....

Fig. 2. Distribution of $^{14}$C in various fractions in leaves at different times after vacuum infiltration with 2 mM $^{14}$C-arginine. A: nonstarved leaves; B: starved leaves. The average total radioactivity recovered was 475,000 cpm.

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FIG. 3. Distribution of \(^{14}C\) in various fractions in starved leaves at different times after vacuum infiltration with 2 mM \(^{14}C\)-arginine and sucrose. A: 0.05 M sucrose; B: 0.1 M sucrose. The average total radioactivity recovered was 600,000 cpm.

Table I. Rates of Utilization of Arginine for Protein Synthesis and Catabolism at Different Arginine Concentrations

<table>
<thead>
<tr>
<th>Arginine Conc. Added to Leaf</th>
<th>Amount of Arginine Added to Leaf</th>
<th>Rate of Arg Utilization¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmoles/g fresh wt</td>
<td>nmoles/hr·g fresh wt</td>
</tr>
<tr>
<td>0.0015</td>
<td>0.0007</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>0.9</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>4.5</td>
<td>80</td>
</tr>
</tbody>
</table>

¹ The rate of utilization was calculated by plotting cpm/g fresh weight of \(^{14}C\) recovered in protein (protein synthesis) and other amino acids, organic acids, and CO₂ (catabolism) at various times, then dividing the initial slopes (cpm/hr·g fresh weight) by the specific radioactivity of the added arginine (cpm/µmole).

ornithine by ornithine-δ-transaminase to Δ²-pyrroline-5-carboxylic acid which is then oxidized to glutamic acid. Glutamic acid is converted by transamination to α-ketoglutarate which is metabolized by the reactions of the Krebs cycle. This pathway is consistent with previous results (6) and the \(^{14}C\) compounds recovered in these experiments. Arginase (7), ornithine-δ-transaminase (3, 8), and Δ²-pyrroline-5-carboxylic acid dehydrogenase (11) have been demonstrated in extracts from plant tissues. The results in Figures 1, 2, and 3 indicate that the catabolism of arginine was not inhibited by carbohydrates in the leaf as was true of proline oxidation (9). In fact, there was sustained catabolism of arginine only in leaves high in carbohydrates, indicating that carbohydrates stimulate arginine catabolism. Utilization of arginine for protein synthesis was also greater in leaves high in carbohydrates, similar to the result with proline (9). This result is interpreted to mean that in excised leaves in the dark, energy limits protein synthesis. Both protein synthesis and arginine catabolism were less in starved leaves than in nonstarved leaves, accounting for the lack of

Fig. 4. Distribution of \(^{14}C\) in various fractions in nonstarved leaves at different times after vacuum infiltration with \(^{14}C\)-arginine. A: 1.5 µM arginine; B: 2 mM arginine; C: 10 mM arginine. The average total radioactivity recovered was 500,000 cpm.
arginine disappearance in starved leaves (Fig. 1). The increase in arginine content in starved leaves between 8 and 48 hr (Fig. 1) is attributable to a greater rate of formation of nonprotein arginine than utilization. This formation would be from proteinolysis and synthesis from precursors. The slower increase in arginine when sucrose was added to starved leaves (Fig. 1) is explained by the faster catabolism and incorporation into protein (Figs. 2 and 3).

The fact that carbohydrates do not inhibit arginine catabolism but inhibit proline oxidation indicates that the inhibition of proline oxidation occurs before the Δ1-pyrroline-5-carboxylic acid dehydrogenase step, the reaction that is common to both pathways. This means that proline oxidation must be inhibited at the initial oxidation of proline. The only enzyme which has been isolated from plants which catalyzes proline oxidation is proline dehydrogenase (2, 4, 5).

The result (Fig. 4; Table 1) that arginine catabolism occurs much faster when higher concentrations of arginine are added to the leaf is similar to the results obtained with proline (9). Thus, it may be generally true that amino acids are more susceptible to oxidation or catabolism (respiration) when the levels of amino acids are increased either by exogenous or endogenous supplies.

The rate of utilization of exogenous arginine for protein synthesis was not greatly affected by arginine concentration. This result is similar to that observed with proline (9) and is to be expected because it is not likely that the concentration of a single amino acid would limit protein synthesis.

LITERATURE CITED