Short Communication

Glutamate Dehydrogenase Activity in Soybean,
and the Effect on it of Amino Acids
and Growth Substances

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Summary. Glutamate dehydrogenase (GDH) is largely particulate in soybean, and its specific activity is much higher in roots than in leaflets. The specific activity of GDH in soybean callus is considerably lowered by glycine and leucine and is markedly increased by glutamate, tyrosine, phenylalanine, and especially serine. Increasing concentrations of indoleacetic acid increase the specific activity of GDH in callus far more than they increase either fresh weight or specific activity of malate dehydrogenase. Increasing kinetin also causes an increase in the specific activity of GDH, but mainly at low indoleacetic acid concentrations.

The enzyme glutamate dehydrogenase (GDH) presumably plays an important role in plant metabolism, since reductive amination of α-ketoglutarate to form glutamate probably provides ultimately the various nitrogen-containing organic molecules which are required for differentiation and subsequent development, among other things. GDH may thus play a regulatory role in growth and development of a plant. This possibility has led us to look at the activity of GDH in various parts of soybean plants and in soybean callus under different conditions of growth.

We grew soybean plants [Glycine max (L.) Merr. cv. Chippewa 64] for 30 days in an aerated nutrient solution in a 12-h-day (29°), 12-h-night (21°) cycle, with a light intensity of 1,200 ft-c. We grew callus in the dark for 4 weeks at 28° on the medium of Murashige and Skoog (1962).

We ground the plant materials in a mortar and pestle with acid-washed quartz sand in a suspending medium of 0.5 M sucrose, 0.025 M phosphate at pH 7.3, with 1 mg/ml of ascorbate. We added per g fresh weight about 1 g of the insoluble polyvinylpyrrolidone “Polycar A7” (General Aniline and Film Corp., New York). After passing the material through cheesecloth, we centrifuged the extract as indicated in the tables, resuspending pellet fractions in suspending medium less
ascorbate. Pellet fractions were assayed as soon as possible after preparation, supernatant fractions after 5—10 h dialysis against 0.001 M phosphate, pH 6.7. Protein estimation was on trichloroacetic-acid-precipitable material, using either the biuret method (Gornall et al., 1949) or the method of Lowry et al. (1951). The assay for GDH, using essentially the method of Bulen (1956), involved measuring the ammonia-dependent oxidation of NADH in the presence of α-ketoglutarate (with the use of 0.033 M phosphate, pH 8.0, and with the control cuvette lacking NADH; final pH was 7.7—7.8). In most of our extracts, the rates observed increased linearly (within 10—15%) with increased amount of extract.

Table 1 shows that GDH in soybean is largely particulate (in agreement with other literature data) and that its specific activity is much higher in the roots than in the leaves. These results suggest the possibility that amino acid synthesis in soybean may be largely dependent

Table 1. Specific activity of GDH in fractions prepared from soybean roots and leaflets

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Pellet</th>
<th>Supernatant</th>
</tr>
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<tbody>
<tr>
<td>Root</td>
<td>176</td>
<td>32</td>
</tr>
<tr>
<td>Leaflet</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

The fractions analyzed were those resulting from centrifugation at 10,000 × g for 15 min of the supernatant fraction from a 10 min centrifugation at 1,000 × g. Rates are nmols NADH oxidized/(min × mg protein) and are averages of two experiments.

on the activity of GDH in roots. Tracer studies with C¹⁴ by Pate (1966) and Joy (1967) have suggested that the root system in nodulated peas and in sugar beets is responsible for the synthesis of the glutamate required by the shoots of the plants. (Due to uncertainty about the extent of extraction and denaturation of various proteins from plant materials, we feel that the use of the term “total activity” merits further study. Preliminary results indicate that the apparent total activity of GDH may be 2—4 times greater in soybean roots than in leaflets.)

Addition of a variety of amino acids to soybean callus gave the results shown in Table 2. The effects range from a stimulation of GDH activity with tyrosine, phenylalanine, glutamate, and especially serine, to considerable inhibition with glycine and leucine.

Table 3 shows the effect of kinetin and indoleacetic acid (IAA) on the specific activity of GDH in soybean callus. At a low IAA concentration the specific activity of GDH rose with increasing kinetin. Callus grown at all kinetin concentrations showed an increased specific activity of GDH with increasing IAA concentration. Over the range from the lowest to the highest concentrations of both hormones, the specific activity of GDH showed an increase of over 40-fold, while that of