Enzymatic Decarboxylation of γ-Hydroxyglutamic Acid to α-Hydroxy-γ-amino-n-butyric Acid

ARTTURI I. VIRTANEN and PENTTI K. HIETALA

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

We have recently isolated γ-hydroxyglutamic acid from Phlox decussata. Attempts to decarboxylate this acid with homogenates of Phlox led to negative results, whereas L-glutamic acid was easily decarboxylated with the same homogenates. Accordingly, Phlox did not contain the specific γ-hydroxyglutamic acid decarboxylase. The same result was obtained with homogenates of pea.

After fruitless experiments with green plants we found decarboxylase activity also with γ-hydroxyglutamic acid by using as enzyme material Escherichia coli, a B_{12} vitamin requiring mutant. α-Hydroxy-γ-amino-n-butyric acid was hereby formed as the decarboxylation product (Fig. 1) which could be isolated and characterized (cf. below). This amino acid could not be detected in E. coli without addition of γ-hydroxyglutamic acid (70% alcohol extract of bacteria, two-dimensional paper chromatography).

According to Umbreit and Heneage, E. coli decarboxylates allo-β-hydroxy-γ-amino-n-butyric acid (synth.). All strains of E. coli capable of decarboxylating this acid contained glutamic acid decarboxylase too, but the results suggested that the two enzymes are different. We have compared the velocities of the decarboxylation of L-glutamic acid, our γ-hydroxyglutamic acid, allo-β-hydroxy-γ-amino-n-butyric acid and allo-γ-hydroxy-β-amino-n-butyric acid using E. coli. The CO₂ evolution was measured in a Warburg apparatus in N₂-atmosphere according to Schales et al. The results are presented in Table 1.

Isolation of the decarboxylation product. 27 mg of γ-hydroxyglutamic acid in 10 ml of 0.1 M acetate buffer (pH 4.9) + 1 g of fresh E. coli (178 mg dry subst.) was kept for 24 h at 35°C with occasional shaking. The bacteria were separated and washed with the buffer. The solution + washwater was passed through an Amberlite IR-120 column. The amino acids remained in the column and were eluted with 1 N ammonia. After evaporation in vacuo

---

Fig. 1a. Two-dimensional paper chromatogram (butanol-acetic acid and phenol-NH₃) of decarboxylation of α-amino-γ-hydroxyglutamic acid (84) by E. coli. Dp = decarboxylation product, 84 = traces of not decarboxylated α-amino-γ-hydroxyglutamic acid, 29 = γ-amino-n-butyric acid.

Fig. 1b. Control experiment without any substrate.

Acta Chem. Scand. 9 (1955) No. 3
Table 1. Decarboxylation rates of hydroxylglutamic acids. Measurements were made with *E. coli* at pH 4.9 in a 0.1 M acetate buffer at 37°C and with *Phlox* and *Pisum* at pH 6.2 in a 0.1 M phosphate buffer at 30°C with a final substrate concentration of about 5.5 μmoles/ml in each case.

<table>
<thead>
<tr>
<th>Agent</th>
<th>QCO₂ (μl CO₂/hour/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-Glutamic acid</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>34 900</td>
</tr>
<tr>
<td><em>Phlox decussata</em></td>
<td>730</td>
</tr>
<tr>
<td><em>Pisum sativum</em></td>
<td>3 000</td>
</tr>
</tbody>
</table>

the solid rest was dissolved in 1 ml of phenol-water-solvent (500:184) and fractionated in a cellulose powder column (1.2 × 37 cm) on the top of which 1 mg methyloorange was placed. 17 fractions of 1.5 ml were collected. The indicator came out in fractions 2—5, γ-aminobutyric acid in fractions 6—11, and the decarboxylation product of γ-hydroxyglutamic acid in fractions 11—17. The combined fractions 12—17 (9 ml) were diluted with alcohol and water, the amino acid separated in an Amberlite IR-120 column, eluted with ammonia and evaporated to dryness. After recrystallization from water the mp. was 199°C.

Characterization of the decarboxylation product.

1. By reduction with HI (d 1.96) and red P at 140°C for 4 h. (1 mg substance + 1 mg P + 6 μl HI) γ-aminobutyric acid was formed (Fig. 2). Accordingly the decarboxylation product was a hydroxyderivative of γ-aminobutyric acid. 2. Analysis: N 11.55. Calc. for a mono-hydroxyaminobutyric acid N 11.75.

3. The decarboxylation product is not homoserine, and does not contain the α-amino group. Accordingly, the α-carboxyl group is split off by decarboxylation, and the decarboxylation product is α-hydroxy-γ-aminono-butyric acid.

\[ \text{HOOC} \cdot \text{CHOH} \cdot \text{CH}_2 \cdot \text{CHNH}_2 \cdot \text{COOH} \]

\[ \xrightarrow{\text{CO}_2} \text{HOOC} \cdot \text{CHOH} \cdot \text{CH}_2 \cdot \text{CH}_2 \text{NH}_2 \]

Transamination: α-ketoglutaric acid + γ-hydroxyglutamic acid \[\xrightarrow{\text{glutamic acid} + \gamma-hydroxy-α-ketoglutaric acid}\] could be found with homogenates of *Phlox*.

As far as we know α-hydroxy-γ-aminono-butyric acid has not earlier been found in any biological material.

We are very grateful to Dr. Karl Pfister, Rahway, New Jersey, for preparations of synthetic β-hydroxy-DL-glutamic acid and allo-β-hydroxy-DL-glutamic acid.

Fig. 2. Paper chromatogram of the pure decarboxylation product after reduction with HI and red P. 1 = reduction products + γ-aminobutyric acid (29), 2 = γ-aminobutyric acid alone, 3 = reduction products, 4 = decarboxylation product alone (86). Solvent phenol-ΝΗ₃. The slow moving spots are probably iodine-containing compounds.


Received February 18, 1955.