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Nitrogen Metabolism of Pea and Alder

Transamination of γ-Aminobutyric Acid and L(+)-Citrulline with α-Ketoglutaric Acid

JORMA K. MIETTINEN and ARTTURI I. VIRTANEN

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

Occurrence of γ-aminobutyric acid and L(+)-citrulline in free form in tissues of alder(*Alnus*) [1] and of γ-aminobutyric acid and β-alanine in pea plant (*Pisum*) [2,3] has arisen interest in the metabolic fate of these non-protein amino acids in plants. Our attention has been specially devoted to citrulline as high concentrations of this amino acid are found in alder tissues but the reactions by which it is connected with the plant metabolism are evidently incompletely known. In a previous paper [4] possible occurrence of arginase and L(+)-glutamic acid decarboxylase in these plants was studied. Arginase activity could not be shown to be present in alder, which means that the urea cycle evidently could not operate. Tissue homogenates of pea effected heavy formation of γ-aminobutyric acid from L(+)-glutamic acid by a soluble decarboxylase, homogenates of alder tissues effected only slight formation by an insoluble decarboxylase.

As an extension of these studies occurrence of transaminases for these amino acids in pea and alder has now been studied. Transamination of γ-aminobutyric acid and β-alanine with α-ketoglutaric acid has been recently shown in animal tissues [5,6], but could not be shown in two plant tissues, ripening avocado and fresh pepper, when studied by Roberts and Bregoff [6]. We have found in pea roots strong transamination of γ-aminobutyric acid and in them and alder leaves slight transamination of L(+)-citrulline with α-ketoglutaric acid, but no reaction could be shown between β-alanine and the keto acid in these tissues.

EXPERIMENTAL

The plant samples studied were harvested at the beginning of August: 1) roots of pea (test plants grown on field, age 6 weeks, at the beginning of flowering, nodules big and red); 2) leaves and 3) root nodules of gray alder *(Alnus incana)*, grown in greenhouse. The experiments with the roots of pea were made as follows:

Homogenate was made by crushing 10 g of fresh, nodulated roots in 5 ml *M*/7.5 phosphate buffer, pH 7.5, to a pipettable suspension. α-ketoglutaric acid solution was made

by dissolving sodium α-ketoglutarate (Hoffman La Roche) in the abovementioned buffer to contain 20 mg of the acid per ml.

Reaction mixtures 1—8 were made by mixing 0.2 ml plant suspension and 0.1 ml ketoglutaric acid solution and adding the other reagents in dry form as follows:

1 20 mg DL-alanine
2 20 mg β-alanine
3 20 mg γ-aminobutyric acid
4 20 mg L(+)-citrulline
5 20 mg γ-aminobutyric acid + 2 mg NH_4Cl
6 20 mg L(+)-citrulline + 2 mg NH_4Cl
7 no addition
8 2 mg NH_4Cl

Reaction mixture No. 9 was made by mixing 0.2 ml plant suspension and 0.1 ml buffer, No. 10 by mixing 0.2 ml *boiled* plant suspension + 0.1 ml buffer.

Reaction mixtures Nos. 11—15 were made as No. 7 and Nos. 1—4 except that prior to mixing the plant homogenate was kept in a test tube in boiling water bath for 5 min. to inactivate all enzymes.

11 no addition
12 20 mg DL-alanine
13 20 mg β-alanine
14 20 mg γ-aminobutyric acid
15 20 mg L(+)-citrulline

The reaction mixtures, with a final phosphate concentration of *ca.* *M*/15 and pH 7.4, were incubated at 38° C for 4 hrs with occasional shaking after which initial spots of 0.01 ml were pipetted for chromatographing on Whatman No. 1 paper. Watersaturated phenol in NH_3-athmosphere (20° C, 40 hrs) was used for irrigation and ninhydrin for colour development.

The experiments with leaves and nodules of alder were made in the same way as those described above, except that the homogenate was made by using for 10 g of fresh plant tissue 20 ml buffer.

With all three tissues two other incubation series were made where the plant homogenate was fortified before mixing with other reagents with 2 mg $MgCl_2 \cdot 6\,H_2O$ and 2 mg pyridoxal phosphate per ml in one series and 2 mg of $MgCl_2 \cdot 6\,H_2O$ + 2 mg pyridoxamine phosphate per ml in the other series. These additions had no detectable effect on the results.

RESULTS

The following results were obtained with *pea roots*. Even addition of α-ketoglutaric acid alone (chromatogram No. 7 in Fig. 1) led to slight formation of glutamic acid from amino acids present in small amounts in the pea plant suspension (No. 9), especially from aspartic acid which reacted quantitatively. A highly significant increase of glutamic acid above this level was obtained with addition of alanine (No. 1) or γ-aminobutyric acid (No. 3). Addition of citrulline also led to a small increase of glutamic acid (No. 4), but addition of β-alanine evidently did not lead to any increase. The amounts of glutamic acid present in the chromatograms (in μg) are, based on visual comparisons with a series of known dilutions: No. 9 = 0.5; Nos. 8, 7, and 2 = 1; Nos. 6 and 4 = 1.5; Nos. 5 and 3 = 10; No. 1 = 20.

A homogenate of *alder leaves* effected with alanine a heavy formation of glutamic acid from α-ketoglutaric acid (ca 30 μg/10 μl), but only a weak although clearly positive formation was obtained with citrulline (1 μg/10 μl). With γ-aminobutyric acid and β-alanine negative results were obtained.

In a homogenate of *alder nodules* negative results were obtained with all abovementioned amino acids and also with aspartic acid.

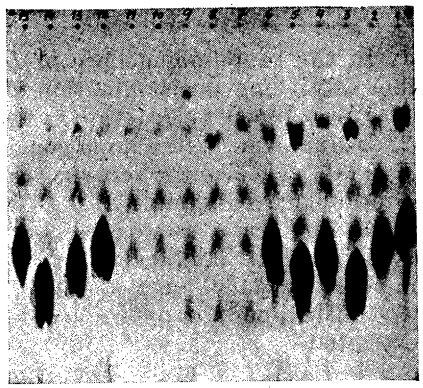

Fig 1. Paperchromatograms prepared from different reaction mixtures (plant suspension + α-ketoglutaric acid + different amino acids etc.) Cf. *composition of the reaction mixtures, and other details in experimental part p. 1244.*

Addition of NH_4Cl (*e.g.* Nos. 5, 6 and 8 in Fig. 1) had in no case any effect upon the amount of glutamic acid formed. The slightly higher mobility and elongation of the glutamic acid spot in these chromatograms is due to a local change of pH, caused by the chloride spot. It is therefore evident that the reactions obtained have not been due to a reductive amination of α-ketoglutaric acid by ammonia liberated from the amino acid added.

Addition of α-ketoglutaric acid only, or this acid and amino acids, to the boiled extract (Nos. 10—15) did not lead to any increase of glutamic acid.

DISCUSSION

The results of the present study show that the roots of the pea plant contain an enzyme system catalyzing transamination between γ-aminobutyric and α-ketoglutaric acids. Whether the reaction in pea is reversible remains to be shown. By this reaction γ-aminobutyric acid may enter the normal metabolic cycles. The only reactions where this acid is known to partake are, so far, its formation from glutamic acid by decarboxylation or from glutamic acid and succinic semi-aldehyde by transamination [5] and its conversion back to the last-mentioned compounds by transamination with α-ketoglutaric acid. Whether it still has some more specific function, is unknown. There seems to be great difference between different plants in respect to this transaminase. The pea plant contains considerable activity, but avocado and fresh pepper [6] nil. As all these plants are rich in L-glutamic acid decarboxylase, there seems to be no correlation between these two enzyme activities either.

The results of the present study suggest also weak transamination of citrulline with α-ketoglutaric acid in pea root and alder leave homogenates. This reaction may play a role in mobilization of citrulline nitrogen in the leaves of alder. The presence of this enzyme system in leaves and its absence from the nodules would be in conformity with the role ascribed to citrulline in alder: transformation of nitrogen, fixed in the nodules, to the other parts of the plant.

SUMMARY

1. In the root homogenate of *Pisum* has been demonstrated an enzyme system catalyzing transamination of γ-aminobutyric acid with α-ketoglutaric acid. The rate of the reaction is about half of that found with alanine.

2. In the same tissue homogenate could be demonstrated weak formation of glutamic acid from α-ketoglutaric acid by L(+)-citrulline, but no reaction was obtained by β-alanine.

3. In the leave homogenate of *Alnus incana* was demonstrated a weak formation of glutamic acid from α-ketoglutaric acid by citrulline, but not by γ-aminobutyric acid or β-alanine.

4. Crushed nodules of *Alnus* did not catalyze any transamination reactions investigated.

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