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The Activity of Glutamic Acid Decarboxylase from Intact Wheat Roots

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Abstract. Experiments with isolated wheat roots and with intact wheat plants showed that glutamic acid decarboxylase from the roots takes part in the transformation of substance in the medium and that its activity is influenced by the medium. Glutamic acid decarboxylase is thus a factor taking part in the formation of substances excreted by the plants roots.

In the study of the excretion of amino acids by the roots of wheat in water culture it was found that excretions were composed of a whole series of amino acids, qualitatively similar and to a certain extent quantitatively proportional to free amino acids in the roots (TESAŘ, KUTÁČEK 1955). γ-amino butyric acid (γ-NH_2but) and proline showed deviations from this proportionality, being present in the excretions in quite large amounts.

In the present work we therefore investigated the activity of glutamic acid decarboxylase (DAG) from wheat roots and particularly the decarboxylation of glutamic acid in solution by the intact roots of wheat, i.e. the effect of roots on their surroundings.

In preliminary experiments with tissue pulp preparations from the roots of six-day wheat plants we found that the optimum pH for DAG from roots ranges from 5·6—5·7 and optimum temperature without the addition of the coenzyme, pyridoxal-5-phosphate (P5P), is 30° C. On adding P5P (10^{-4} M) the temperature optimum is raised (50° C). This can be explained either by the protective effect of the added coenzyme or by the more rapid reaching of

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higher concentrations of γ-NH_2 but during enzymatic degradation of glutamic acid. In dialysed enzyme preparations multivalent cations were found to be ineffective in the activation of DAG from the roots, with the exception of heavy metals which destroyed the enzyme. EGGLESTON (1958) obtained similar results with decarboxylases of plant origin, as opposed to bacterial decarboxylases which are activated by multivalent cations. Substances reacting with aldehyde groups (hydroxylamine, cyanide, sodium azide and isonicotinic acid hydrazide) acted as typical inhibitors. p-Chloromercuribenzoate and Cu ions also inhibited enzyme activity. But monoiodoacetic acid reacted only weakly.

In our work, main interest was directed to experiments with the isolated roots of six-day wheat plants and to experiments with intact plants. Isolated roots or parts of them (apical and basal) were placed in 6 ml. M/15 phosphate buffer (pH 5·6) containing glutamic acid, coenzyme or inhibitor which were added with the substrate. A few drops of toluene were added too. In the single experiments the fresh root weight was 0·2 g. In our experiments, the apical part formed about $^1/_3$rd of the length of the roots and included the root cap and meristematic zone (see "Streckungszone" — FRENZEL 1957). The basal part primarily included the zone with root hairs (see "Wurzelhaarzone" — FRENZEL 1957). The samples were incubated in a thermostat at 30° C for 12 hours.

Intact plants were cultivated under aseptic conditions in test tubes with their roots immersed in water (TESAŘ, KUTÁČEK 1955). After six days the water was exchanged for sterile neutralized culture medium which was analysed after contact for 72 hours with the roots. Activity was evaluated from the amount of γ-amino butyric acid formed (in μg. per gram dry weight of roots). The γ-amino butyric acid was isolated chromatographically and determined

Table 1

DAG Activity in isolated wheat roots and their parts

Experiment A

Activity of whole isolated roots

	μg γ-NH_2but/g dry weight
Whole roots (roots excreted γ-NH_2but)	21
Whole roots + glutamic acid solution $3 . 10^{-3}$ M	63
Whole roots + glutamic acid solution $3 . 10^{-3}$ M + P5P 10^{-4} M	86
Whole roots + glutamic acid solution $3 . 10^{-3}$ M + hydroxylamine $5 . 10^{-4}$ M	19

Experiment B

Activity of part of isolated roots

	μg γ-NH_2but/g dry weight
Basal part of roots + glutamic acid solution $3 . 10^{-3}$ M	27
Basal part of roots + glutamic acid solution $3 . 10^{-3}$ M + P5P 10^{-4} M	40
Basal part of roots + glutamic acid solution $3 . 10^{-3}$ M + hydroxylamine $5 . 10^{-4}$ M	17
Apical part of roots + glutamic acid solution $3 . 10^{-3}$ M	36
Apical part of roots + glutamic acid solution $3 . 10^{-3}$ M + P5P 10^{-4} M	50
Apical part of roots + glutamic acid solution $3 . 10^{-3}$ M + hydroxylamine $5 . 10^{-4}$ M	18

colorimetrically by the method of Giri and Rao (see ROHRLICH and RASMUS 1956).

In the experiment with isolated whole roots it was found that the roots acted on the decarboxylation of glutamic acid in the solution. The addition of P5P to the medium increased decarboxylation activity, the addition of hydroxylamine had a considerable inhibitory effect (Tab. I, experiment A). In a separate experiment with other plant material the decarboxylating effect of the apical and basal parts of roots were compared. The decarboxylating effect of the apical part of the roots was greater than that of the basal parts (Tab. I, experiment B). The results are the average of five experiments.

The roots of intact plants also decarboxylated glutamic acid in the cultivation medium. The addition of P5P to the medium increased decarboxylation of glutamic acid, the addition of hydroxylamine to the medium inhibited the reaction (Tab. I, experiment C). The results are the average of three experiments. The excretion of decarboxylase into the cultivation medium was not demonstrated. RATNER and SAMOYLOVA (1955, 1958) obtained similar results when studying the phosphatase activity of sunflower roots.

Table 2

DAG Activity in wheat roots in intact plants cultivated under aseptic conditions

Experiment C

Cultivation medium	μg γ-NH_2but/g dry weight
Distilled water (roots excreted γ-NH_2but)	6
Glutamic acid solution $5 . 10^{-3}$ M	22
Glutamic acid solution $5 . 10^{-3}$ M + P5P 10^{-4} M	63
Glutamic acid solution $5 . 10^{-3}$ M + hydroxylamine 10^{-3} M	11

According to ROGERS (1955), DAG is almost exclusively present in the non-sedimenting part of the protoplasm of squash, whereas the cell membranes did not show enzyme activity. If we apply these findings to our results that both isolated and intact roots of wheat decarboxylate glutamic acid added to the culture medium, we reach the conclusion that this enzymatic transformation of glutamic acid evidently takes place inside the root cells. In other words, the space inside the roots take part in the formation and degradation of substances excreted by the roots. This conception is also in agreement with the opinion of other authors that part of the cells space (termed by some authors the "apparent free space") is freely accessible to substances from the outside and vice versa (BRIGGS and ROBERTSON 1957, LÜTTGE and WEIGL 1962, 1964). A further interesting finding was the difference in DAG activity between the basal and apical parts of the roots. This may have a qualitative and quantitative effect on substances excreted by different parts of the roots (cf. BROWN and ROBINSON 1955, FRENZEL 1957).

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Naše pokusy s izolovanými kořínky pšenice a pokusy s intaktními rostlinami dokazují, že dekarboxyláza kyseliny glutamové z kořínků se zúčastňuje přeměn látek vnějšího prostředí a zároveň její aktivita je vnějším prostředím ovlivňována. Je tedy dekarboxyláza kyseliny glutamové faktorem zúčastňujícím se tvorby látek vydávaných kořeny rostlin.

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Наши опыты с изолированными корнями пшеницы и опыты с интактными растениями указывают на то, что декарбоксилаза глютаминовой кислоты из корешков участвует в обмене веществ внешней среды, а также что ее активность подвергается влияниям внешней среды. Следовательно, декарбоксилаза глютаминовой кислоты является фактором, участвующим в образовании веществ выделяемых корнями растений.