OXIDATION AND DECARBOXYLATION OF AMINO ACIDS BY SQUASH PREPARATIONS[1,2]

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Only a moderate amount of information is presently available on the oxidation and decarboxylation of amino acids by higher plant tissues. In conjunction with other studies on amino acids, it became desirable to find out whether amino acids in plant tissues undergo degradation at rates which vary sufficiently to account for the results of Wood and Cruickshank (17) and Kemble and Macpherson (6). These investigators have shown that certain amino acids are degraded in detached leaves at rates which make it unlikely that these amino acids are available for normal protein synthesis. The dehydrogenase which catalyzes the oxidative deamination of glutamic acid has been found in many plants (2) and a tryptophan oxidase has been reported to occur in pea-seedling tissue (16). Only glutamic acid has been demonstrated to be decarboxylated by plant tissue preparations (12), although the occurrence in plant tissues of histamine, hydroxytyramine, and putrescine, the decarboxylation products respectively of histidine, dihydroxyphenylalanine, and ornithine, has been reported (10, 17).

The enzyme source in the present study was an extract of acorn squash prepared essentially by the method of Schales et al (11). The ovary wall of the squash was homogenized in a Waring blendor at 4° C in M/15 phosphate buffer of pH 5.8. The brei was filtered through muslin to remove cell wall debris, and the filtrate used without further treatment.

Decarboxylation and oxidation rates were measured manometrically at 35° C by standard techniques (13). For decarboxylation studies, the reaction system consisted of 4 ml of squash extract containing 20 mg of protein, and 1 ml of a solution containing 33 micromoles of the desired substrate. Both aerobic and anaerobic (nitrogen) conditions were employed. For studies on oxidation, the protein concentration was two times that noted above. The reaction mixture consisted of 2 ml of squash extract, 0.5 ml of a solution containing 33 micromoles of the desired substrate, 1 ml of phosphate buffer of pH 7.0, and 0.2 ml of 10 % KOH in the center well of the Warburg vessel.

In order to determine the cellular location of glutamic decarboxylase, the enzyme preparation was fractionated by centrifugation into three portions: material sedimented for 15 minutes at 10,000 × g, probably nuclear and mitochondrial (7), material sedimented for 15 minutes at 100,000 × g which is designated the microsomal fraction (5), and the supernatant from this last separation. The reaction mixture consisted of 0.5 ml of a solution which contained 33 micromoles of glutamic acid, 1 ml of phosphate buffer of pH 5.8, and 1 ml of a water solution of one of the fractions or a combination of the fractions. Measurements of decarboxylation were carried out in the manner reported above.

The decarboxylation and oxidation results obtained for a number of substrates are shown in table I. It is clear that the relative rates of decarboxylation and oxidation of this series of amino acids by the whole squash preparation vary greatly.

Glutamic acid decarboxylase is by far the most

TABLE I

RATES OF METABOLISM OF VARIOUS SUBSTRATES BY A SQUASH EXTRACT

SUBSTRATE	DECARBOXYLATION μL OF CO_2 EVOLVED/HR × MG OF SQUASH PROTEIN		OXIDATION μL OF O_2 ABSORBED/HR × MG OF SQUASH PROTEIN
	AIR	NITROGEN	AIR
α-L-Alanine	2.0	0	0.6
β-Alanine	0	0	0
α-Aminobutyric acid	0	0.2	0.5
γ-Aminobutyric acid	0	0.2	0.4
L-Arginine	2.3	0	0.5
L-Asparagine	1.9	0	0.5
L-Aspartic acid	4.0	0.2	0.2
Canavanine	0.9	...	0.2
DL-Citrulline	0	0	0.2
L-Cysteic acid	0	0.8	0.5
L-Cysteine	4.0	0	2.5
L-DOPA	0	0	0
L-Glutamic acid	19.6	15.2	0.4
L-Glutamine	6.5	0.2	0.7
Glycine	1.6	0	0
L-Histidine	0.9	0	0
Hydroxy-L-proline	3.6	0.3	0.2
L-Isoleucine	2.1	0	0.4
L-Leucine	0.9	0	0
L-Lysine	2.2	0.2	0.3
L-Methionine	1.6	0.2	0
L-Ornithine	5.2	0	0.4
L-Phenylalanine	0	0	0.3
L-Proline	1.6	...	0.3
L-Serine	0	0	0
L-Threonine	0	0	0
L-Tryptophan	0.9	0	0
L-Tyrosine	1.4	0	0
DL-Valine	0.7	0	0
α-Ketobutyric acid	3.8	...	0.5
α-Ketoglutaric acid	0	0	0.5
Glyoxylic acid	0	0	0
Glutaric acid	0	0	..
Pyruvic acid	5.6	0	0
Urea	3.3	...	0

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active of the decarboxylases present in the preparation. Glutamic acid is decarboxylated at a high rate under both aerobic and anaerobic conditions. The amino group appears to be necessary for decarboxylation, since α-ketoglutarate is not decarboxylated under the conditions used. The presence of glutamic acid decarboxylase in the soluble fraction of the squash preparation is evident on the basis of the data shown in table II. This finding is in contrast to those of Okunuki (9) and of Morrison (8) who were unable to find a soluble enzyme in any of a variety of plant tissues.

The decarboxylation of glutamine is somewhat peculiar in that the evidence suggests that deamidation does not take place prior to decarboxylation. If deamidation, which does not require oxygen, were to take place before decarboxylation, then one would expect glutamine to show a substantial rate of decarboxylation under anaerobic conditions. This did not occur. It seems possible that glutamine is oxidatively deaminated to α-ketoglutaramide, which may

TABLE II

INTRACELLULAR LOCATION OF GLUTAMIC ACID DECARBOXYLASE IN SQUASH

FRACTION	DECARBOXYLATION μL OF CO_2 EVOLVED/HR
Mitochondrial	5
Microsomal	5
Supernatant	211
Supernatant plus microsomal	225
Supernatant plus microsomal plus mitochondrial	228

then undergo oxidative decarboxylation to form the amide of succinic acid.

In bacterial (4) and mammalian (1) preparations amino acids are decarboxylated equally well under both aerobic and anaerobic conditions. In the squash preparation this does not occur. Instead, all the amino acids, with the exception of glutamic, are either not decarboxylated anaerobically, or show very low rates of decarboxylation under anaerobic conditions. Apparently they are preferentially oxidatively deaminated to the respective keto acids and are then decarboxylated.

The rates of oxidation by the present preparation are quite low as compared to rates obtained in similar experiments with *Neurospora crassa*, rat kidney, *Proteus vulgaris*, and cobra venom (3). The data suggest that deamination of amino acids in higher plants is largely by transamination and is not primarily an oxidative process. This is in accord with the work of Wilson et al (15) who have demonstrated both the presence of highly active transamination systems in a variety of plant tissues and a low capacity of these tissues for reductive amination. It is probable that oxidative deamination may be the limiting step in the aerobic amino acid decarboxylation illustrated in table I.

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