A Method for the Determination of Some Amino Acid Decarboxylases*

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Mammalian organs are known to decarboxylate a number of L-amino acids. Of the mammalian decarboxylases, 3,4-dihydroxyphenylalanine decarboxylase is probably the most studied. Blaschko (1) reported values for DOPA \(^1\) decarboxylase in liver and kidney of several animal species. The enzyme was shown to be deactivated by dialysis and its activity restored by the addition of pyridoxal phosphate (2, 3). Interest in DOPA decarboxylase has increased. New studies of the properties of this enzyme have been reported (4, 5). Some preparations of DOPA decarboxylase act on other substrates. Blaschko (6) has shown that o-tyramine could be formed from o-tyrosine in the animal. He has also demonstrated that the enzyme can act on m-tyrosine but not on p-tyrosine (7). With the discovery of 5-hydroxytryptophan decarboxylase, the problem has become more complex. This enzyme appears to be difficult to separate from DOPA decarboxylase (8). The method most frequently used to measure DOPA decarboxylase and other amino acid decarboxylases has been manometric. Unfortunately the measurement of CO\(_2\) is not sufficiently sensitive to permit measurement of low activities. Measurement of the resulting product, the amine, could increase the range considerably. This is done in many cases, the amine is measured by physical, chemical, or biological methods. Dietrich (9) has used Permunit successfully to separate the amine from the amino acid and presented a rapid method for the determination of DOPA decarboxylase. Recently we reported (10) that Amberlite CG-50 H\(^+\) can separate amino acids and amines quantitatively and proposed the use of this procedure as a method for measuring amino acid decarboxylases. We are reporting the details of the method in this paper. With the method reported, we have measured four decarboxylases, viz. o-tyrosine, m-tyrosine, 3,4-dihydroxyphenylalanine, and 5-hydroxytryptophan. Eight different organs from three different animal species were studied and all were found to possess activity with all four substrates.

**EXPERIMENTAL**

**Materials and Methods**

Amino acids were obtained from the California Corporation for Biochemical Research. Amberlite CG-50, type 2, 200–400 mesh was purified by the method of Hirs et al. (11), and used in the H\(^+\) form. The animals used were white male Holtzman rats, male New Zealand rabbits, and male guinea pigs of unknown strain. The rats weighed about 200 g, the rabbits about 2500 g, and the guinea pigs about 500 g.

The animals were killed by decapitation. The organs to be studied were quickly removed, chilled over ice, and 10 or 20% water homogenates were prepared with Potter-Elvehjem homogenizers. All reactions were carried out in duplicate. The reaction mixture consisted of 1 ml of the homogenate, 0.5 ml of 0.02 M phosphate buffer (pH 6.9 for DOPA, 8 for 5-hydroxytryptophan, and 7.3 for o- and m-tyrosine) which contained 10 \(\mu\)g of pyridoxal phosphate, and 10 \(\mu\)moles of the substrate dissolved in 0.5 ml of the appropriate 0.02 M phosphate buffer. The reactions were carried out in culture tubes. Air was excluded by flushing the tubes with nitrogen before addition of substrate and during the incubation. To accomplish this, the culture tubes were tightly stoppered with a rubber stopper with two holes. The holes were small enough to be well covered by the base of a hypodermic needle. Two needles, a 20-gauge 1/4 inch and a 25-gauge 1 inch, were introduced into the stopper and their bases forced into the holes. The 20-gauge needles were connected to a nitrogen gassing manifold. Incubations were carried out at 37\(^\circ\)C in a water bath provided with a shaking device. All tubes were incubated for 5 minutes before the substrate was added. All substrate additions were made by removing the 25-gauge hypodermic needles and then quickly replacing them after the addition was made with a 1 ml syringe. Incubations were carried out for 20 minutes. The reaction was stopped by adding 7 ml of absolute ethanol and heating the tubes in boiling water for 1 minute. The tubes were centrifuged for 5 minutes at 2000 r.p.m. The supernatant and two rinses with 3 ml distilled water were transferred to 50-ml Erlenmeyer flasks containing 1 g of Amberlite CG-50 H\(^+\). The flasks were flushed with nitrogen gas, covered with Parafilm, and shaken for 1 hour in a mechanical shaker. The resin and solution were transferred to a Pyrex tube (1 X 25 cm) with narrow bottom which was plugged with glass wool and which contained 1 g of the resin. The solution was allowed to flow through the column. The flasks were rinsed with two portions of 5 ml of distilled water and the rinses transferred to the column. The columns were washed with 100 ml of water. This operation was simplified by the use of a manifold connecting the tubes with a reservoir of water. The amines retained by the resin were eluted with 20 ml of 4 N acetic acid. The amine concentration of the eluate was determined. Since the four amines studied absorb strongly at 279 m\(\mu\), they were readily determined by means of ultraviolet spectrophotometry.
The reliability of the method was tested by determining the recovery of added amines to Amberlite CG-50 H+, and the ability of the resin to separate the amine from amino acids. Tables I and II, taken from a paper still in press (10), show that both expectations were realized. It is important that the separation be complete, particularly when the activity of the enzyme is low.

A series of determinations were carried out with rat liver suspensions, to establish the best incubation period. Reaction velocity plots are shown in Fig. 1. Linearity is maintained in all instances for more than 20 minutes except in the case of DOPA decarboxylase which begins to fall off after 20 minutes. In Fig. 2 are shown plots of enzyme concentration against activity. Of the eight organs studied, the kidney was the most active toward the four substrates (Tables III, IV, and V). All organs were active though some had very low activity. The order of activity was the same regardless of the source of enzyme. In each case.

**RESULTS AND DISCUSSION**

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Effect of incubation time on decarboxylating activity of rat liver homogenate. Substrates: o-tyrosine, m-tyrosine, DOPA, 5-hydroxytryptophan. Activity is expressed as pmoles of amine formed per g dry weight tissue.

o-tyrosine was the substrate most actively decarboxylated followed by m-tyrosine. DOPA was decarboxylated less and 5-hydroxytryptophan least. Since crude tissue suspensions were used here it is not possible to tell if one or more enzymes were present. In no instance was a reverse order of activity observed for the four substrates. There was, however, great variability in the values obtained from different species.

o-Tyrosine is the most actively decarboxylated substrate. This amino acid is known to be decarboxylated in vivo (12) and also appears to be the source of o-hydroxyphenylacetic acid (13, 14) which is found in human urine. Both o-tyrosine and m-tyrosine have been reported to exist or suspected to exist in nature (15, 16). Recently a "metatyramine-like" substance was detected in human urine after administration of a monoamine oxidase inhibitor (17). None of these factors explains the degree of activity of tissues with o-tyrosine and m-tyrosine. Other substrates tested were tryptophan and p-tyrosine but none of the tissues had decarboxylase activity with these two compounds.

The present method is applicable to the measurement of other o-amino acid decarboxylases. It cannot be applied to the measurement of basic or dicarboxylic amino acid decarboxylases since in the first case separation is not possible and in the second case w-amino acids are formed rather than amines. The method when applicable has some advantages over the manometric method. The main advantage is that one measures the amine and it is demonstrable by chromatographic analysis. The effluent from the resin has a low salt concentration. The acetic acid is readily evaporated and the residue is suitable for paper chromatography when taken up in water. Finally, the range of measurement can be increased to allow measurement of very low concentrations of amines by the use of fluorometric methods and bioassay.

**SUMMARY**

A method for the determination of some amino acid decarboxylases is reported. The amino formed is separated from the parent amino acid by passage through Amberlite CG-50 H+ and measured spectrophotometrically. With this method, four amino acid decarboxylases were determined, viz. o-tyrosine, m-tyrosine, 3,4-dihydroxyphenylalanine, and 5-hydroxytryptophan decarboxylases. Activity was found in eight different organs of three different species. All were active to different degrees with the four substrates.

**REFERENCES**


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