ENZYMATIC DECARBOXYLATION OF α-METHYL AMINO ACIDS

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Received August 11, 1960

α-Methyl 3,4-dihydroxyphenylalanine (α-methyl DOPA) and some related α-methyl amino acids[1] were shown to be effective inhibitors of DOPA decarboxylation in mammalian tissues by Sourkes et al. (1954). More recently we have found that purified extracts of mammalian tissues, such as guinea pig kidney, can decarboxylate all of the normal aromatic and cyclic L-amino acids, including DOPA, 5-hydroxytryptophan, tryptophan, tyrosine, phenylalanine and histidine, (Udenfriend et al., 1960) suggesting a general aromatic L-amino acid decarboxylase. α-Methyl DOPA has been found to be an excellent inhibitor of all these decarboxylations. What is of interest though is that α-methyl DOPA and related α-methyl amino acids were themselves found to be decarboxylated by these enzyme preparations. Thus, the suggestion by Mandeles et al. (1954) that the alpha hydrogen is not necessary for amino acid decarboxylase activity is borne out.

EXPERIMENTAL

Although the enzyme from guinea pig kidney has now been purified about 80 fold, a 10-20 fold purified preparation, as described by Clark et al. (1954) for 5-hydroxytryptophan decarboxylase, was employed for these studies. Enzyme activity was determined by measuring amine formation according to the method of Dietrich (1953). Further identification of the enzymatically formed α-methyl amines was established by paper chromatography in several solvent systems.

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[1] The alpha-methyl amino acids were kindly supplied by Merck Sharp and Dohme Research Laboratories.

TABLE I

Decarboxylation of Various Amino Acids and α-Methyl Amino Acids

Substrate	Specific Activity μg/mg. protein/hour
DOPA	8,000
5-Hydroxytryptophan	2,930
Tryptophan	300
Tyrosine	15
α-Methyl DOPA	95
α-Methyl 5-Hydroxytryptophan	38
α-Methyl Tryptophan	16

Incubations, total volume 3 ml, were carried out in 20 ml beakers at 37° and contained enzyme (0.2 to 2 mg protein), 10^{-3} M substrate, 50 μg pyridoxal phosphate, and 250 μmoles Tris buffer pH 8.5.

RESULTS AND DISCUSSION

As shown in Table I several α-methyl amino acids were decarboxylated by the enzyme preparation at about 1/100 the rate of the parent amino acid. Their activity was in the range of the weaker natural substrates for this enzyme such as tyrosine and histidine. A correlation was also observed between the rate of decarboxylation of the α-methyl amino acids and their ability to inhibit the decarboxylation of the natural aromatic amino acids; α-methyl DOPA > α-methyl 5-hydroxytryptophan > α-methyl tryptophan.

Following the elegant isotopic studies of Mandeles et al. (1954) on the mechanism of L-amino acid decarboxylation, Umbreit (1955) reported on attempts to demonstrate the decarboxylation of α-methyl amino acids. Although these experiments were not successful he predicted that with more sensitive and specific methods it should be possible to demonstrate this. The present studies, which

utilize such sensitive procedures, show that α-methyl amino acids are indeed substrates of decarboxylase enzymes. Consistent with the above date is the recent report of Smith (1960) that small amounts of CO_2 were evolved when α-methyl DOPA was incubated with kidney preparations. These findings are in accord with and further support the mechanism of enzyme-pyridoxal phosphate, catalyzed decarboxylation of L-amino acids.

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