PART III

DRUG ACTION

SUBSTRATE SPECIFICITY OF AMINO-ACID DECARBOXYLASES

by

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During the last two years a number of observations on substrates of amino-acid decarboxylases have been recorded from this laboratory. In this review the attempt is made to correlate the results obtained and to arrive at conclusions of a more general character. The experimental data and the methods used have been described elsewhere (BLASCHKO, HOLTON, AND SLOANE STANLEY[1, 2]; BLASCHKO[3]; SLOANE STANLEY[4, 5]).

The decarboxylation of L-3:4-dihydroxyphenylalanine (DOPA) is catalysed by two enzymes: the mammalian L-DOPA-decarboxylase (HOLTZ, HEISE, AND LÜDTKE[6]) and the bacterial L-tyrosine decarboxylase (EPPS[7]). The two enzymes differ in their affinity for L-tyrosine: this is probably the "natural" substrate of the bacterial enzyme, but it is not attacked by the mammalian enzyme. The difference in substrate specificity of the two enzymes has been studied more systematically.

The experimental procedure adopted is easily described. As a source of the bacterial enzyme we used an acetone-dried preparation of *Streptococcus fæcalis* R (ATCC 4083); we owe this strain to Professor I. C. GUNSALUS. The bacteria were usually grown in a medium free of vitamin B_6; in these preparations the tyrosine apodecarboxylase was present, but had to be completed by the addition *in vitro* of pyridoxal and ATP. In some of the experiments we used a "complete" preparation obtained from cells grown in the presence of pyridoxal. As a source of the mammalian DOPA decarboxylase we used fresh tissue extracts, from guinea-pigs kidney or from rats liver.

The enzymic decarboxylation of each amino-acid was measured by following the time course of CO_2 formation manometrically. If an amino-acid was found to be decarboxylated, the contents of the manometer flasks were used for a determination of the pharmacological activity of the amine formed. The activity was tested on the arterial blood pressure of the spinal cat; the pressor activity of the amine formed by enzyme action was compared with that of the synthetic amine.

I. MONOHYDROXYPHENYLALANINES

Our results are summarized on Table I. It was found that *m*-hydroxyphenylalanine (the "meta-tyrosine" of BLUM[8]) was a substrate of the mammalian enzyme; the rate of decarboxylation was slightly less than with 3:4-dihydroxyphenylalanine as substrate. The bacterial preparation also acted on *m*-hydroxyphenylalanine, at about one-third of the rate of decarboxylation of tyrosine.

In the mammalian tissue extracts, *o*-hydroxyphenylalanine (BLUM'S[8] "ortho-tyrosine") was decarboxylated at approximately the same rate as the meta hydroxy

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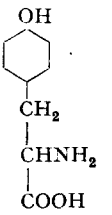
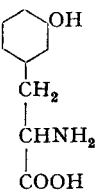
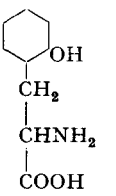
derivative. With the bacterial preparations, the rate of CO_2 formation from *o*-hydroxyphenylalanine was practically zero.

TABLE I

DECARBOXYLATION OF TYROSINE AND ITS ISOMERS

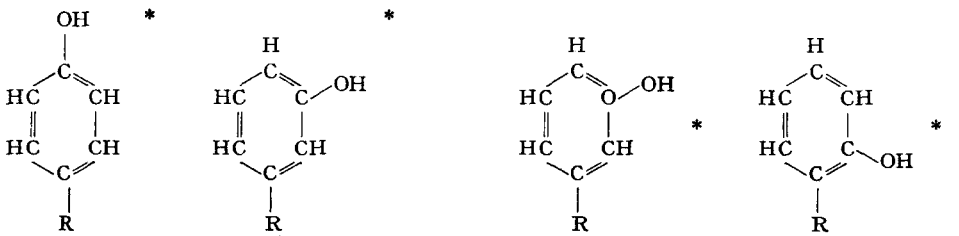
+ signifies decarboxylation
— signifies no decarboxylation

	Substrates		
			
Bacterial preparation	+	+	—
Mammalian preparation	—	+	+

Results of competition experiments suggest that the two enzymes responsible for these decarboxylation reactions are the bacterial tyrosine decarboxylase and the mammalian DOPA decarboxylase. One molecule of each DL-amino-acid gives one-half of a molecule of CO_2 formed; we therefore assume that only one of the two steroisomers, the L-form, is decarboxylated.

These findings demonstrate the importance of the phenolic hydroxyl groups and their positions on the benzene ring for the reaction between enzyme and substrate. It seems safe to assume that these groups react with the protein part of the decarboxylase system.

The nature of the forces which are at work between enzyme protein and substrate is not known. In the case under consideration, it seems possible that the reaction between the phenolic hydroxyl groups and the enzyme involves the formation of a hydrogen bond, with the hydroxyl group either as a "donor" or an "acceptor". At any rate, the results obtained can be understood if it is assumed that the substrate must be held by a group in the enzyme situated so that it can react with a hydroxyl group in



Bacterial enzyme Mammalian enzyme

Fig. 1. The asterisk marks the position of the active group in the enzyme in relation to the substrate.

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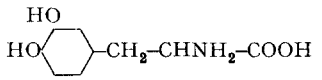
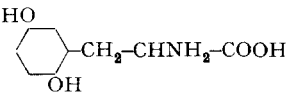
one of two adjacent positions on the benzene ring. The position of this group in the enzyme would be different for the bacterial and the mammalian enzyme, as shown in Fig. 1.

II. 2:5-DIHYDROXYPHENYLALANINE

This amino-acid has recently been synthetized by NEUBERGER[9]. We have examined it and have found that it is a substrate of the mammalian enzyme, but that it is not a substrate of the bacterial enzyme.



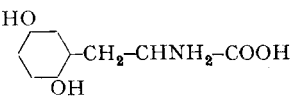
3:4-dihydroxyphenylalanine



2:5-dihydroxyphenylalanine

That 2:5-dihydroxyphenylalanine is a substrate of the mammalian decarboxylase is easily explained by the hypothesis outlined above; the lack of affinity for the bacterial enzyme, however, is not obvious; possibly the presence of the hydroxyl group in ortho position interferes with the attachment to the enzyme.

We have examined both the L and the D forms of this amino-acid; in agreement with expectation, only the L form is a substrate of DOPA decarboxylase. The product of the decarboxylation reaction, β-2:5-dihydroxyphenylethylamine, seems to be a substrate of amine oxidase; this suggests that in the living animal it is metabolized as follows:



L-2:5-dihydroxyphenylalanine

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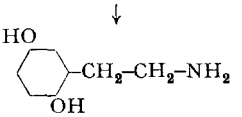

β-2:5-dihydroxyphenylethylamine

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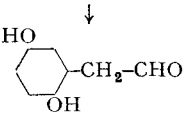

homogentisic aldehyde

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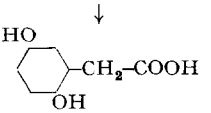

homogentisic acid

It has been shown that the amino-acid gives rise to homogentisic acid in the alcaptonuric subject (NEUBERGER, RIMINGTON, AND WILSON[10]). In normal animals and human subjects, both the amino-acid and the corresponding amine are fully metabolized (NEUBERGER[9]; LEAF AND NEUBERGER[11]). This aspect of our findings has been more fully discussed elsewhere (BLASCHKO *et al.*[2]).

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III. 3:4-DIHYDROXYPHENYLSERINE (NORADRENALINE CARBOXYLIC ACID)

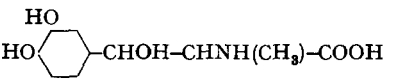
The study of this compound has revealed another difference between the mammalian and the bacterial decarboxylase[1]. On decarboxylation, it yields noradrenaline:

3:4-dihydroxyphenylserine noradrenaline

It was found that the amino-acid was not decarboxylated by extracts of mammalian tissues; it was, however, decarboxylated by the bacterial preparation. The rate of CO_2 formation with dihydroxyphenylserine was much slower than with tyrosine as substrate, but the decarboxylation was almost quantitative; approximately one-half of the racemic substance was decarboxylated. The biological assay on the arterial blood pressure of the spinal cat, together with the measurement of the amount of CO_2 formed, showed that the amine formed was laevo-noradrenaline.

IV. N-METHYLATED AMINO-ACIDS

Ten years ago, the observation was made that the introduction of a N-methyl group abolished the substrate specifity for DOPA decarboxylase (BLASCHKO[12]). Preparations of mammalian liver and kidney which had DOPA decarboxylase activity were found not to act on N-methyl-3:4-dihydroxyphenylalanine:



This observation was made the basis of a scheme of biosynthesis of sympathin and adrenaline. It had often been assumed previously that the formation of adrenaline involved a decarboxylation reaction, but it was now shown that the body was not able to produce a secondary amine by direct decarboxylation of the N-methyl-amino-acid, whereas it was able to produce the corresponding primary amine. Primary amines with sympathicomimetic activity were therefore postulated as intermediary products in adrenaline synthesis. Earlier already, pharmacologists had discussed the possibility of the identity of CANNON's "sympathin E" with noradrenaline (BACQ[13]; STEHLE AND ELLSWORTH[14]). The biochemical findings gave a simple explanation for the occurrence of this substance.

Two amino-acids were studied in 1939: N-methyl-dihydroxyphenylalanine and N-methyl-tyrosine. One important methylamino-acid, however, was not available at that time; this was N-methyl-3:4-dihydroxyphenylserine. Already in 1906, FRIEDMANN[15] had considered this acid as a possible precursor of adrenaline; he suggested that adrenaline was formed in the reaction:

N-methyl-3:4-dihydroxyphenylserine (adrenaline carboxylic acid) adrenaline

This suggestion could not be tested by experiment until the synthesis of adrenaline

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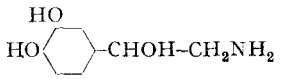
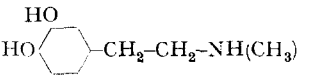
carboxylic acid was achieved by DALGLIESH AND MANN[16]. We have recently examined this compound. It was found not to be decarboxylated by a number of mammalian tissue extracts and, unlike the corresponding amino-acid, dihydroxyphenylserine, it was not a substrate of the bacterial enzyme preparation.

The substrate specificity of DOPA decarboxy .se in connexion with pathways of adrenaline synthesis has recently been reviewed elsewhere (BLASCHKO[17]). Two possible ultimate precursors of adrenaline were discussed: noradrenaline and N-methyl-3:4-dihydroxyphenylethylamine (also known as epinine):



noradrenaline



epinine

The rôle of epinine in the biosynthesis of adrenaline has recently been discussed by DANNEEL[18] and by HOLTZ AND KRONEBERG[19]. The presence of this substance in mammalian tissue has never been demonstrated. Recently, noradrenaline has been found in human tumours of the suprarenal medulla (HOLTON[20]) as well as in the suprarenal gland (SCHÜMANN[21]). Evidence is also accumulating that both adrenaline and noradrenaline are released from the suprarenal medulla (MEIER AND BEIN[22]; BÜLBRING. AND BURN[23]; HOLTZ AND SCHÜMANN[24]).

V. DOPA DECARBOXYLASE AND PYRIDOXINE DEFICIENCY

Like the mammalian enzyme, the bacterial enzyme does not act on N-methyl-tyrosine (EPPS[7]) and N-methyl-dihydroxyphenylserine. This suggests that the inability to act on N-methyl-amino-acids is due to a property common to both enzymes.

It is known that the bacterial codecarboxylase (GALE AND EPPS[25]), the prosthetic group of the bacterial tyrosine decarboxylase, is pyridoxal phosphate (GUNSALUS, BELLAMY AND UMBREIT[26]). GREEN, LELOIR, AND NOCITO[27] achieved a partial purification of DOPA decarboxylase and a reactivation of the apoenzyme by pyridoxal phosphate. It is, however, not generally accepted that DOPA decarboxylase contains pyridoxal phosphate (see MARTIN AND BEILER[28]; WORK AND WORK[29]).

When the DOPA decarboxylase activity was determined in liver extracts of rats reared on a diet deficient in pyridoxine (vitamine B_6), enzymic activity was found to be low, and in a few of the extracts the activity had practically disappeared (BLASCHKO, CARTER, O'BRIEN, AND SLOANE STANLEY[30]; and unpublished observations). Addition of pyridoxal plus ATP *in vitro* brought about a partial restoration of the enzymic activity. More recently, through the kindness of Dr K. FOLKERS, we have been able to test the effect of synthetic codecarboxylase: we have found that it is possible to restore the activity of the extracts from B_6-deficient animals to normal values by the addition *in vitro* of 10 μg of synthetic codecarboxylase to the equivalent of 550 mg of fresh weight of liver. These experiments allow us to conclude that DOPA decarboxylase, like the bacterial tyrosine decarboxylase, contains pyridoxal phosphate.

There is experimental support for a suggestion by SNELL[31] that in transamination the initial reaction between amino-acid and pyridoxal phosphate involves the formation of a $-N = C\langle$ bond. In analogy, it seems likely that the decarboxylation requires a reaction between the amino group of the amino-acid and the aldehyde group of pyridoxal phosphate:

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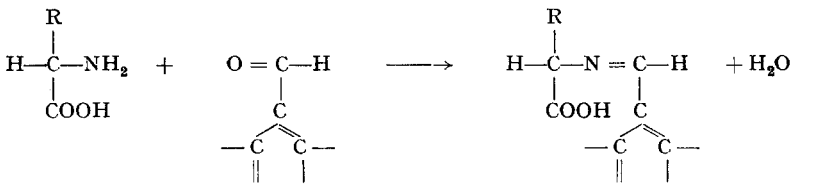


It is clear that this reaction will only occur when the amino group is unsubstituted. We conclude that N-methyl-amino-acids are unable to react with the formation of a $-N = C\langle$ bond. This inability would account for the fact that N-methyl-amino-acids are not substrates of the amino-acid decarboxylases.

VI. THE BASIS OF SUBSTRATE SPECIFICITY

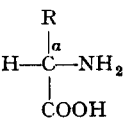
The experiments discussed have shown two different types of substrate specificity. DOPA decarboxylase may serve to demonstrate these:

a. tyrosine is not a substrate of DOPA decarboxylase, because it does not react with the enzyme protein;

b. N-methyl-3:4-dihydroxyphenylalanine is not a substrate of DOPA decarboxylase, because it does not react with the coenzyme.

DOPA decarboxylase, like all the amino-acid decarboxylases, presents a *third* type of substrate specificity: specificity for the members of the L series. HOLTZ, HEISE, AND LÜDTKE[6] suggested already that DOPA decarboxylase was specific for L-dihydroxyphenylalanine; we have confirmed this, using the D isomer which was not decarboxylated (BLASCHKO[32]).

The lack of affinity for the D form is easily understood in the light of the evidence discussed in this review. If we consider the alpha carbon atom of the amino-acid,



we see that three of the groups attached to this atom take part in the decarboxylation reaction:

a. the carboxy group, which loses carbon dioxide,

b. the amino group which reacts with the aldehyde group of pyridoxal, and

c. the group R which reacts with the enzyme protein.

If the decarboxylation requires a fixed relationship of these three groups relative to the enzyme, it is clear that the L and D forms are not equivalent; only one of the stereoisomers can be expected to fulfil the conditions required for decarboxylation. The stereospecificity of other enzymes dealing with amino-acids may have a similar basis (see RYDON[33]), but the conditions of specificity are not so completely known.

It has been pointed out that the presence of a third polar group in R is a common feature of all bacterial amino-acid decarboxylases (GALE[34]). The same is true for the mammalian decarboxylases, not only for DOPA decarboxylase, but also for the L-cysteic decarboxylase of mammalian liver (BLASCHKO[35]).

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Acknowledgement

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SUMMARY

1. The decarboxylation by bacterial and mammalian enzymes of a number of amino-acids structurally related to tyrosine has been studied.

2. The position of the phenolic hydroxyl group in tyrosine and its isomers is shown to determine substrate specificity. This is explained by a reaction between the OH group of the substrate and the enzyme protein.

3. Methylamino-acids are not decarboxylated; this is explained by their inability to react with the aldehyde group in pyridoxal phosphate (codecarboxylase).

4. The stereospecificity of the amino-acid decarboxylases is discussed on the basis of these observations.

RÉSUMÉ

1. La décarboxylation de quelques acides aminés, apparentés à la tyrosine, a été étudiée au moyen de ferments bactériens et animaux.

2. La position des groupes OH dans la tyrosine et ses isomères est déterminante pour la spécificité des décarboxylases. Nous en déduisons que la réaction entre l'apoferment et les acides aminés en question a lieu au niveau du groupe OH.

3. Les acides méthyl-aminés ne sont pas décarboxylés en présence de ces ferments. Ce phénomène s'explique par l'impossibilité du groupe N-méthylique de réagir avec l'aldéhyde du phosphate de pyridoxal (codécarboxylase).

4. Les résultats de ce travail nous permettent de discuter le phénomène de la stéréospécificité des décarboxylases.

ZUSAMMENFASSUNG

1. Die Decarboxylierung einiger dem Tyrosin verwandter Aminosäuren durch tierische und bakterielle Fermente wurde untersucht.

2. Die Position der phenolischen Hydroxylgruppe des Tyrosins und seiner Isomeren ist für die Substratspezifität von Bedeutung. Diese Beobachtung wird erklärt durch die Annahme einer Bindung zwischen der OH-Gruppe des Substrats und dem Apoferment.

3. Methylaminosäuren werden nicht decarboxyliert; dies wird erklärt durch das Ausbleiben der Reaktion mit der Aldehydgruppe des Pyridoxal-Phosphats ("Codecarboxylase").

4. Die Stereospezifität der Aminosäuredecarboxylasen wird im Lichte der gewonnenen Resultate erläutert.

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