The Decarboxylation of *o*-Hydroxyphenylalanine

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Two new substrates of the mammalian enzyme DOPA decarboxylase have recently been described, *L-m*-hydroxyphenylalanine and *L-2:5*-dihydroxyphenylalanine (Blaschko & Sloane Stanley, 1948). Both these amino-acids have, like *L-3:4*-dihydroxyphenylalanine, the phenolic hydroxyl group in the meta position relative to the side chain, and it was, therefore, concluded that it is the presence of this group in these three substances which enables them to be substrates.

Tyrosine is not a substrate of the enzyme, but it is not known if the presence of a phenolic hydroxyl group in the ortho position is of importance for substrate specificity. In the experiments described in this paper the decarboxylation of *o*-hydroxyphenylalanine by mammalian tissue extracts has, therefore, been studied. At the same time, a few observations on the action of amino-acid oxidases on *o*-hydroxyphenylalanine are reported.

MATERIAL AND METHODS

The observations were made on a sample of *DL-o*-hydroxyphenylalanine for which I am grateful to Dr A. Neuberger.

For the experiments with decarboxylase, extracts were freshly prepared by grinding the organs with sand and adding 1 ml. of 0.067 M-sodium phosphate buffer (pH 7.4)/g. of tissue. The supernatant fluid after 5 min. centrifuging was used. It was assumed that 1 ml. of extract contained the activity of 0.5 g. of tissue. The rate of CO₂ formation was measured manometrically as previously described (Blaschko, 1942); all the data given are corrected for CO₂ retention.

For the study of amino-acid oxidases two preparations were used. D-Amino-acid oxidase was obtained from an acetone-dried powder of pig kidney. The powder (400 mg.) was left to stand at room temperature for 1 hr. with 4 ml. of 0.067 M-sodium phosphate buffer (pH 7.4); of the supernatant fluid after centrifuging 0.6 ml. was used/flask. The second preparation was a sample of cobra venom which contained a powerful L-amino-acid oxidase described by Zeller & Maritz (1944).

RESULTS

(a) Decarboxylation of *o*-hydroxyphenylalanine

Extracts from guinea pig kidney and rat liver were used. These organs contain DOPA decarboxylase. A solution of *DL-o*-hydroxyphenylalanine, when incubated with these extracts under anaerobic

conditions at 37.5°, gave rise to CO₂, the rate of formation of which was measured. The total amount of CO₂ formed suggested that only one stereoisomer was decarboxylated. For instance, in one experiment with 0.8 g. of guinea pig kidney the reaction had come to a standstill after 45.5 ml. of CO₂ had been formed from 0.4 ml. of 0.01 M-*DL-o*-hydroxyphenylalanine added. This corresponds to a formation of 0.51 mol. CO₂/1.0 mol. *DL*-amino-acid. In view of what is known of the stereospecificity of DOPA decarboxylase (Holtz, Heise & Lüdtke, 1938; Blaschko, 1942; Blaschko, Holton & Sloane Stanley, 1949) it seems safe to assume that it is the *L*-isomer of *o*-hydroxyphenylalanine which is decarboxylated.

The rate of decarboxylation of *o*-hydroxyphenylalanine is a little slower than that of 3:4-dihydroxyphenylalanine. The rates of formation of CO₂ from *o*-hydroxyphenylalanine, 3:4-dihydroxyphenylalanine and *m*-hydroxyphenylalanine were compared; in one experiment guinea pig kidney extract was used, in the second rat liver extract. Each flask contained 0.4 ml. of 0.02 M-*DL*-amino-acid; the total volume was 2 ml. During the first experiment in which each flask contained extract from 0.15 g. of guinea pig kidney, the amounts of CO₂ formed in the first 6 min. were:

	μl.
With 3:4-dihydroxyphenylalanine	43
With <i>o</i> -hydroxyphenylalanine	32
With <i>m</i> -hydroxyphenylalanine	35

In the second experiment, with extract from 0.8 g. of rat liver, the corresponding figures for CO₂ formation were:

	μl.
With 3:4-dihydroxyphenylalanine	52
With <i>o</i> -hydroxyphenylalanine	40
With <i>m</i> -hydroxyphenylalanine	50

In different experiments the relative rates of decarboxylation have differed slightly, but the rate with 3:4-dihydroxyphenylalanine as substrate was always slightly greater than with either *o*- or *m*-hydroxyphenylalanine.

DOPA decarboxylase is the catalyst responsible for the decarboxylation of *m*-hydroxyphenylalanine (Blaschko *et al.* 1949); the experiments described above suggested that *o*-hydroxyphenylalanine was another substrate of the same enzyme.

This was confirmed in a substrate competition experiment: the initial rate of CO₂ formation in the presence of both *o*- and *m*-hydroxyphenylalanine was about the same as with either substrate alone. With the extract from 0.15 g. of guinea pig kidney the amounts of CO₂ formed in the first 9 min. were:

	μl.
With 0.008 M- <i>m</i> -hydroxyphenylalanine	28.5
With 0.008 M- <i>o</i> -hydroxyphenylalanine	35
With both amino-acids	31

(b) *Enzymic oxidation of o-hydroxyphenylalanine*

An extract of pig kidney powder consumed O₂ in the presence of DL-*o*-hydroxyphenylalanine. This is taken as an indication that D-*o*-hydroxyphenylalanine is a substrate of D-amino-acid oxidase. The rate of oxidation of *o*-hydroxyphenylalanine was compared with those of *m*-hydroxyphenylalanine and methionine; the initial concentrations of the amino-acids were 0.03 M. The amounts of O₂ consumed in the first 12 min. were:

	μl.
With DL- <i>o</i> -hydroxyphenylalanine	8
With DL- <i>m</i> -hydroxyphenylalanine	16
With DL-methionine	198

o-Hydroxyphenylalanine is also a substrate of the L-amino-acid oxidase of cobra venom. The rates of O₂ uptake with the same three amino-acids have been compared in an experiment in which each flask contained 1.2 mg. of cobra venom; the initial amino-acid concentrations were 0.028 M. The O₂ uptakes during the first 15 min. of the reaction were:

	μl.
With DL- <i>o</i> -hydroxyphenylalanine	10
With DL- <i>m</i> -hydroxyphenylalanine	54
With DL-methionine	90

DISCUSSION

Metabolism of o-hydroxyphenylalanine

DL-*o*-Hydroxyphenylalanine was first synthesized by Blum (1908), who showed that, like *m*-hydroxyphenylalanine, it did not give rise to homogentisic acid in human alcaptonuria. He found that in a normal subject about one third of a dose of 5 g. was excreted as *o*-hydroxyphenylacetic acid in the urine. *o*-Hydroxyphenylacetic acid was also found in the urine of rabbits after feeding either *o*-hydroxyphenylalanine or *o*-hydroxyphenylpyruvic acid (Flatow, 1910). Subsequently the experiments of Blum (1908) and Flatow (1910) were often quoted as proof for Neubauer's scheme of degradation of amino-acids by oxidative deamination. The results described in the experimental part of this paper can be considered as giving support to this scheme. The

two stereoisomers of *o*-hydroxyphenylalanine were found to be substrates of amino-acid oxidases, and it therefore seems likely that *o*-hydroxyphenylacetic acid can arise from the DL-amino-acid via *o*-hydroxyphenylpyruvic acid.

The present findings make it necessary, however, to consider another possible pathway of degradation of *o*-hydroxyphenylalanine, which would also lead to *o*-hydroxyphenylacetic acid. This pathway leads from L-*o*-hydroxyphenylalanine via *o*-hydroxyphenylethylamine and *o*-hydroxyphenylacetaldehyde to *o*-hydroxyphenylacetic acid. The first of these steps is the decarboxylation reaction described in the first part of this paper; the second step is the oxidation of *o*-hydroxyphenylethylamine by amine oxidase, a reaction described by Randall (1946). The oxidation of the aldehyde formed in the oxidative deamination of *o*-hydroxyphenylethylamine to the corresponding acid—*o*-hydroxyphenylacetic acid—is parallel to the breakdown of closely related substances. The fate of *o*-hydroxyphenylethylamine considered in this scheme is in fact similar to that of tyramine. Ewins & Laidlaw (1910) have shown that tyramine is excreted as *p*-hydroxyphenylacetic acid in the dog, and that the same end product is found when the livers of cats and rabbits are perfused with tyramine. These observations found their explanation in the discovery of amine oxidase (Hare, 1928; Bernheim, 1931).

It is not known to what extent these two pathways contribute to the formation of *o*-hydroxyphenylacetic acid from *o*-hydroxyphenylalanine. The same is true for the breakdown of *m*-hydroxyphenylalanine: *m*-hydroxyphenylacetic acid is found in the urine when *m*-hydroxyphenylalanine is fed (Blum, 1908; Flatow, 1910); *m*-hydroxyphenylalanine is also a substrate of amino-acid oxidases as well as of DOPA decarboxylase (Blaschko *et al.* 1949).

Substrate specificity of DOPA decarboxylase

The substrate specificity of DOPA decarboxylase can now be more precisely defined. It has been shown that the presence of two phenolic hydroxyl groups is not required; the presence of one such group is essential. However, the position of this group in relation to the side chain is of importance; a hydroxyl group in the para position to the side chain is not sufficient, but the presence of a hydroxyl group in either the meta or in the ortho position is sufficient.

A similar study of substrate specificity has been carried out in this laboratory for the L-tyrosine decarboxylase of *Streptococcus faecalis* R (Sloane Stanley, 1949). This enzyme has a completely different pattern of affinities; it has a marked

preference for tyrosine, it acts less readily on *m*-hydroxyphenylalanine and has practically no affinity for *o*-hydroxyphenylalanine.

The bacterial and the mammalian enzyme are closely related; both are pyridoxalphosphate-proteins (Gunsalus, Bellamy & Umbreit, 1944; Gale, 1946; Green, Leloir & Nocito, 1945; Blaschko, Carter, O'Brien & Sloane Stanley, 1948). Their different affinities for tyrosine and its two isomers must, therefore, be due to structural differences of the enzyme proteins. The observations reported here show that the position of the phenolic hydroxyl group is one of the factors which govern the enzyme-substrate relationships of the two decarboxylases.

SUMMARY

1. Extracts of rat liver and guinea pig kidney form carbon dioxide when incubated anaerobically with *o*-hydroxyphenylalanine; it is suggested that *L*-*o*-hydroxyphenylalanine is a substrate of DOPA decarboxylase.

2. *o*-Hydroxyphenylalanine is a substrate of the *D*-amino-acid oxidase of pig kidney.

3. *o*-Hydroxyphenylalanine is a substrate of the *L*-amino-acid oxidase of cobra venom.

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REFERENCES

- Bernheim, M. L. C. (1931). *J. biol. Chem.* **93**, 299.
 Blaschko, H. (1942). *J. Physiol.* **101**, 337.
 Blaschko, H., Carter, C. W., O'Brien, J. R. P. & Stanley, G. H. Sloane (1948). *J. Physiol.* **107**, 18 P.
 Blaschko, H., Holton, P. & Stanley, G. H. Sloane (1949). *J. Physiol.* (In the Press.)
 Blaschko, H. & Stanley, G. H. Sloane (1948). *Biochem. J.* **42**, iii.
 Blum, L. (1908). *Arch. exp. Path. Pharmacol.* **59**, 269.
 Ewins, A. J. & Laidlaw, P. P. (1910). *J. Physiol.* **41**, 78.
 Flatow, L. (1910). *Hoppe-Seyl. Z.* **64**, 367.
 Gale, E. F. (1946). *Advances in Enzymology*, **6**, 1.
 Green, D. E., Leloir, L. F. & Nocito, V. (1945). *J. biol. Chem.* **161**, 559.
 Gunsalus, I. C., Bellamy, W. D. & Umbreit, W. W. (1944). *J. biol. Chem.* **155**, 685.
 Hare, M. L. C. (1928). *Biochem. J.* **22**, 968.
 Holtz, P., Heise, R. & Lüttke, K. (1938). *Arch. exp. Path. Pharmacol.* **138**, 580.
 Randall, L. O. (1946). *J. Pharmacol.* **88**, 216.
 Stanley, G. H. Sloane (1949). *Biochem. J.* **44**. (In the Press.)
 Zeller, E. A. & Maritz, A. (1944). *Helv. chim. Acta*, **27**, 1888.

Reducing-group Production from Starch by the Action of α - and β -Amylases of Barley Malt. Activity of α - and β -Amylases

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Recent proposals for the determination of α -amylase of barley malt (Graesser & Dax, 1946; Preece, 1947, 1948) involve differential heat inactivation at 70° in the presence of calcium ions, followed by standardized conversion of a soluble starch substrate (American Society of Brewing Chemists, 1944; Institute of Brewing, 1933), with determination of reducing groups by ferricyanide titration or by Fehling's titration respectively. Thus, β -amylase is heat-inactivated and the observed saccharification of the starch is brought about by α -amylase alone (cf. Kneen, Sandstedt & Hollenbeck, 1943). Total saccharification by the joint action of the α - and β -amylases present in the malt being readily determined as a measure of diastatic power by a similar method with the heat inactivation omitted, saccharification by β -amylase should be obtainable by difference.

These proposals involve a number of assumptions. Thus, it is necessary to be able to work under such conditions for starch conversion that the production of reducing groups shows a linear relationship with enzyme concentration and with duration of action, and also to know that the reactions of the two components are, in fact, truly additive in the reaction range used. The published data relating to these points have been developed under a wide variety of experimental conditions. Thus, of the more recent work, Kneen & Sandstedt (1941), using 2% (w/v) soluble starch solution for hydrolysis at 30°, find linearity between enzyme concentration and reducing-group production (ferricyanide method) up to 40% hydrolysis for the joint action of the two enzymes, and up to 30% for β -amylase alone; for α -amylase they found no region of linearity. Further, it was shown that, under the experimental condi-