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Some properties of mouse mastocytoma histidine decarboxylase

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by

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Introduction

A mammalian histidine decarboxylase was first described in 1936 by Werle (64). Since then, enzymes that catalyze the decarboxylation of histidine have been demonstrated in many mammalian species and tissues. It has become clear that all of these enzymes are not identical, that they have different properties in different tissues, and that species differences do exist. A broad classification can be made, however, on the basis of substrate specificity, the pH at which the enzyme exhibits maximal activity, and the effects of various enzyme inhibitors and activators. Two main types emerge: (i) an aromatic L-amino acid decarboxylase (29) which decarboxylates a wide variety of natural and synthetic aromatic amino acids including histidine (29, 62); this enzyme exhibits maximum activity in an alkaline medium (pH > 8), is strongly inhibited by alpha-methyl DOPA (a strong inhibitor of 3,4-dihydroxyphenylalanine (DOPA) decarboxylase), and is activated <u>in vitro</u> by the addition of a small amount of benzene (61). It is probably identical to the DOPA decarboxylase and 5-hydroxytryptophan (5-HTP) decarboxylase of rabbit and guinea pig kidney (29, 45, 62). (ii) a specific L-histidine decarboxylase which seems to decarboxylate only histidine, is most active at slightly acid

or neutral pH, is inhibited only slightly or not at all by alpha-methyl DOPA, and is either unaffected or inhibited *in vitro* by the addition of a small amount of benzene. These properties of histidine decarboxylase from many mammalian sources are summarized in Table 1. In addition, the affinity for the substrate, L-histidine, differs greatly between these two types, specific histidine decarboxylase having a much lower Michaelis-Menten constant (K_m) than aromatic L-amino acid decarboxylase (33, 63).

Both types of enzymes share a requirement for the coenzyme pyridoxal-5-phosphate, and consequently both are inhibited by compounds such as hydroxylamine and semicarbazide which react with pyridoxal phosphate (3, 8, 9, 10, 31, 38, 41, 46, 48, 54). The specific enzyme appears to bind this cofactor more loosely than aromatic amino acid decarboxylase, the former enzyme losing activity much more rapidly with dialysis (46, 48). Although a metal ion requirement has been demonstrated for bacterial histidine decarboxylase (6), this has not been shown for the mammalian enzyme. The addition of EDTA (10^{-2} M) has no effect on the mammalian enzyme activity (63).

In addition to the classification discussed above, which considers only the *in vitro* biochemical behavior of the enzymes, groupings have been made which include functional considerations as well. In all the tissues of

Table 1. Properties of Histidine decarboxylases from various mammalian tissues

Source	pH optimum	Affinity for L-histidine	Inhibition by DOPA or DOPA decarboxylase inhibitors	Effect of benzene	Substrate specificity	Classification	References
Mastocytoma, mouse	6.0; 7.0	high	weak; none	none	?histidine	specific	1, 8, 63
Liver, fetal rat	varies with substrate conc.	high	none	none	histidine	specific	3, 5, 10,24, 56,58
Hepatoma, rat	6.5; 6.8; 6-6.5	high	very weak	none	?histidine	specific	23,31, 33,42, 43,44
Mast cells, rat	6.5-7.6	high	weak	strong inhib.	?histidine	specific	46,52
Bone marrow, rat	varies with substrate conc.	high	none	?	histidine	specific	11,15
Stomach, rat	7.2; 7.0	high	weak	strong inhib.	?histidine	specific	47,52, 57
Fetal mouse	6.5	?	?	none	?	?specific	24
Platelets, rabbit	7.2-7.4	?	?	?	?	?	54
Skin, Brain, rat	7.2	?	?	?	?	?	47

(continued)

Table 1. (continued)

Source	pH optimum	Affinity for L-histidine	Inhibition by DOPA or DOPA decarboxylase inhibitors	Effect of benzene	Substrate specificity	Classification	References
Duodenum, Kidney, rat	7.5	?	?	potentiates	?	?non-specific	57
Fetal guinea pig, rabbit, hamster	8.0	?	?	potentiates	?	?non-specific	24
Fetus and Newborn, man	9	?	?	potentiates	?	?non-specific	36,37
Liver, adult rat	8.0	low	strong	potentiates	?aromatic amino acids	non-specific	41,56, 57,58
Kidney, rabbit	8.0	low	strong	potentiates	aromatic amino acids	non-specific	5, 45, 47
Kidney, guinea pig	8.0; 9.0; 9.0-9.5	low	strong	potentiates	aromatic amino acids	non-specific	28,29, 33,34, 44,47, 63,65

the mouse and rat except blood, Schayer has found histidine decarboxylase activity which has been "induced" in the animals by exposure to stress, such as cold, bacterial endotoxin, catechol amines, trauma and delayed hypersensitivity reactions (49, 50, 51, 52, 53, 59). This "induced" enzyme, according to Schayer, is intimately involved in the local production of unbound histamine which, acting as an antagonist of epinephrine, helps maintain homeostasis of the microcirculation (50). Schayer (49, 51, 52) and others (59) have made extensive investigations concerning the effects of glucocorticoids on the inducible enzyme. The in vitro properties of this enzyme have not been well characterized however. It has a pH optimum ranging from pH 7.4 to 8 (52) but the effects of inhibitors and benzene have not been carefully investigated. Schayer suggests that the inducible enzyme is located in the capillary endothelial cells (51).

Another point of view has been championed by Kahlson, who has found histidine decarboxylase activity with the characteristics of the specific enzyme in tissues characterized by rapid growth, such as fetal rat liver (14, 18, 19, 20, 21), regenerating rat liver (14), rat hepatoma (23), rat bone marrow (15), ascites tumor (16), and healing wounds (13). He suggests that this enzyme activity is related to the general process of growth (12).

However, this view has been challenged by many investigators who have failed to find high enzyme levels in the tissues of other mammalian fetuses (22, 24, 58) and in other rapidly growing tissues (32, 41, 56).

A strong stimulus for the continued investigation of histidine decarboxylase has been the search for a specific inhibitor of the enzyme. The availability of a "histamine-free" animal to investigations of the physiological role(s) of histamine, and the possible therapeutic uses of such an inhibitor are of obvious interest.

That the specific enzyme is the primary mediator of endogenously produced histamine is suggested by its greater affinity for histidine and by the observation that alpha-methyl DOPA, a strong inhibitor of the aromatic amino acid decarboxylase, does not significantly alter urinary histamine excretion or whole mouse histamine levels (63). Studies in the rat also suggest that the specific enzyme is responsible for histamine synthesis <u>in vivo</u> (17, 25). Thus, if inhibition of histamine production is of interest, it is reasonable to consider first the properties of the specific histidine decarboxylase.

An active source of this enzyme is the transplantable mouse mastocytoma (8, 39, 40, 63). Some of the characteristics of this enzyme are included in Table 1. DOPA decarboxylase and 5-HTP decarboxylase activities have also

been described in this tissue (7, 8, 39, 40), but it has not been determined if these activities are the property of one or of several enzymes. Although mastocytoma histidine decarboxylase has been partially purified (2) it's substrate specificity has not been definitely established. The enzyme has been tested with a number of inhibitors (1, 63).

Experiments will be discussed in this paper which suggest that mouse mastocytoma histidine decarboxylase is specific for histidine and that the observed DOPA and 5-HTP decarboxylase activities are the property of another enzyme present in the tissue. In addition, the observed change in pH optimum of the mastocytoma enzyme at different substrate concentrations will be discussed with reference to similar finding previously reported by Hakanson for fetal rat histidine decarboxylase (10).

Materials

Radioactive chemicals: DL-histidine-1-C^{14}·2HCl was obtained from Merck, Sharp and Dohme of Canada, Ltd.; DL-3,4-dihydroxyphenylalanine-1-C^{14} was purchased from the New England Nuclear Corp., Boston; DL-5-hydroxy-tryptophan-1-C^{14} was supplied by the ChemTrac Corp., Cambridge.

DL-histidine·HCl·H_2O, DL-3,4-dihydroxyphenylalanine, DL-5-hydroxytryptophan·H_2O and pyridoxal-5-phosphate were obtained from California Foundation for Biochemical Research.

Methods

Preparation of Enzymes

Mastocytoma. All procedures involving enzymes were carried out at 0 to 3°C. A water extract of the transplantable mouse mastocytoma of Furth, et al. (4) was used as the starting material. It was lyophilized, powdered and stored at -70°C. This powder was suspended in 40 volumes of water, stirred for three hours to redissolve most of it, and then centrifuged at 35,000 x g for 30 minutes. To the supernatant solution 0.05 N HCl was added slowly (>1 hour) to bring the pH to 4.7 and precipitate the bulk of the proteins. The solution was stirred an additional one hour and then centrifuged. The supernatant fluid was discarded and the precipitate was homogenized with sufficient Teorell-Stenhagen universal buffer[1] (diluted 1:1 with water), pH 7, to regain the original volume. The pH of the resulting solution-suspension was 6.2, so a small amount of 0.05 N NaOH was added slowly to bring the pH to 6.8 and allow the precipitate to completely redissolve. To this solution 0.05 N HCl was again added slowly to bring the pH to 5.3. The precipitate which formed was removed by centrifugation and discarded, and the

1. Teorell-Stenhagen universal buffer (60) contains 0.0100 M phosphate, 0.0114 M borate and 0.0067 M citrate.

pH of the supernatant solution was lowered further to pH 4.8. The precipitate which formed was recovered by centrifugation, dissolved in Teorell-Stenhagen buffer (diluted 1:1 with water) and the solution was adjusted to pH 6.8 with 0.05 N NaOH. This solution, which contained the protein fraction which precipitated between pH 5.3 and 4.8, constituted the semipurified histidine decarboxylase used in further studies. It represented a 3 to 4 fold purification of histidine decarboxylase and contained 21% of the initial enzyme activity. The activity of this preparation was stable for several weeks when frozen.

Rat Fetus. Histidine decarboxylase from fetal rats was purified by selective heat denaturation and ammonium sulfate fractionation according to Hakanson (10). However, the protein fraction which precipitated between 25-40% saturation was used as the final preparation and was not fractionated further. It is referred to as purified fetal histidine decarboxylase in this paper.

Assay of Enzyme Activity

Histidine decarboxylase. The enzyme preparation, pyridoxal phosphate and Teorell-Stenhagen buffer, pH 6.8, were mixed together, incubated at 37° for 20 minutes to allow interaction between apo- and coenzyme, and cooled

again to 0°. Substrate was then added and the pH carefully adjusted to 6.8 with a small amount of 0.05 N HCl or NaOH. Three 0.5 ml aliquots of this mixture were then taken and incubated in rubber-stoppered 5-ml Erlenmeyer flasks for 30 minutes at 37° in a shaking incubator. Unless otherwise stated, each aliquot contained 0.1 ml of the enzyme preparation, 2.0×10^{-5} M pyridoxal phosphate, 1.0×10^{-3} M DL-histidine-1-C^{14} (100,000 counts/minute), and enough buffer to bring the volume to 0.5 ml. When incubations were carried out at other than pH 6.8, Teorell-Stenhagen buffer was used at a pH which required as little final adjustment as possible after addition of the substrate. Following incubation the samples were cooled to 0° and made alkaline by the injection of 0.1 ml of 0.1 N NaOH through the rubber stopper. This prevented loss of $C^{14}O_2$ when the stoppers were removed. Ampoules containing 2.0 ml of phenylethylamine-POPOP-PPO solution were attached as described by Aures and Clark (1). The subsequent absorption and liquid scintillation counting of $C^{14}O_2$ was performed as described by these authors. Blanks were identical except that enzyme was either omitted or denatured by boiling for ten minutes.

DOPA decarboxylase. An attempt was made to measure DOPA decarboxylase activity in an identical manner to that used for histidine decarboxylase, using DL-DOPA-1-C^{14} as

substrate. However, it was found that under these conditions a non-enzymatic breakdown of the C^{14}-labeled substrate occurred, often yielding very high and unreproducible blank values. This non-enzymatic breakdown was accelerated in an oxygen atmosphere, depressed slightly by incubation in a nitrogen atmosphere, and unaffected by the addition of EDTA (10^{-3} M) or serum protein (2 mg/ml). The addition, however, of certain fetal rat tissue extracts, glutathione (10^{-3} M) or ascorbic acid (10^{-3} M) completely suppressed the non-enzymatic breakdown. To show that the blank was due to the decomposition of DOPA-1-C^{14} and not of a radioactive contaminant, the radiopurity of the compound was confirmed by paper chromatography. DOPA was eluted from the paper and shown to undergo the same non-enzymatic decomposition as the unchromatographed compound. Because of these findings the DOPA decarboxylase assays were performed with the addition of glutathione to the incubation mixture. Unless otherwise stated, each incubate contained 0.1 ml of the enzyme preparation, 2.0×10^{-5} M pyridoxal phosphate, 4.4×10^{-4} M DL-DOPA-1-C^{14} (40,000 counts/minute), 1×10^{-3} M glutathione and enough Teorell-Stenhagen buffer to bring the volume to 0.5 ml. The pH was adjusted to 6.8 following addition of the substrate and incubation time was 10 minutes at 37°. A 20 minute incubation before the addition of substrate and all other

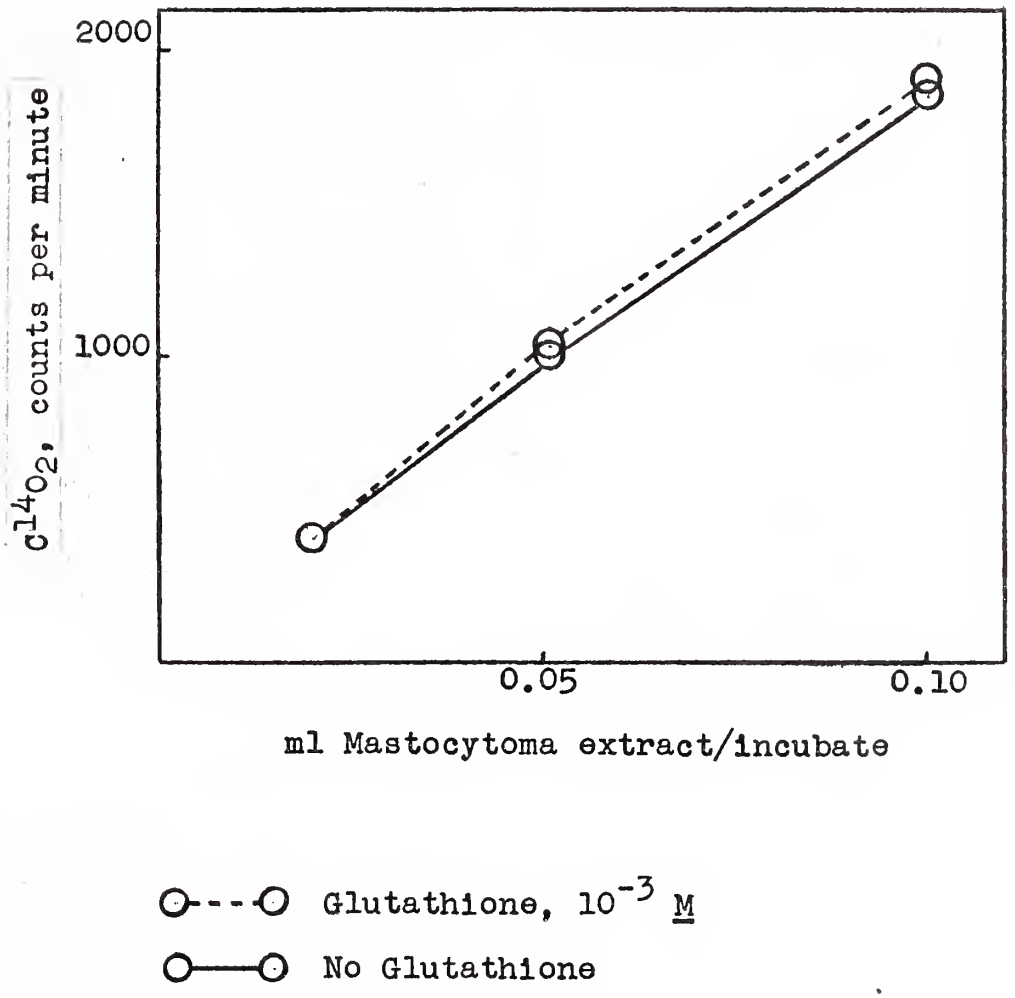
aspects of the assay were identical with the histidine decarboxylase assay. Figure 1 illustrates that the addition of 10^{-3} M glutathione did not significantly affect the DOPA decarboxylase activity of mastocytoma extract, indicating that this inhibitor of the non-enzymatic decomposition of DOPA had no effect on the enzymatic decarboxylation of the compound.

5-HTP decarboxylase. This assay was identical to the histidine decarboxylase assay except that incubations were done at pH 8.0 with a substrate concentration of 4.4×10^{-4} M DL-5-HTP-1-C^{14} (110,000 counts/minute).

Protein

Protein concentration was determined by the method of Lowry, et al. (30).

Figure 1. Lack of effect of Glutathione on the DOPA decarboxylase activity of Mastocytoma extract



Results

Mastocytoma

Table 2 shows the activity of mastocytoma extract and of the semipurified enzyme preparation with different substrates. While purification increased the specific activity of histidine decarboxylase 3.5 fold, the DOPA decarboxylase and 5-HTP decarboxylase activities decreased markedly. This suggests that (i) the histidine decarboxylase is a different enzyme from DOPA and 5-HTP decarboxylase, (ii) a single enzyme responsible for decarboxylation of all three amino acids had undergone alteration, or (iii) the fractionation procedure had removed or altered some other substance(s) present in the original tissue extract that variously affected the three decarboxylase activities of a single enzyme. That the first possibility is probably correct is supported by the findings demonstrated in Figure 2. This shows that when carboxyl-labeled histidine and carboxyl-labeled DOPA were incubated together with crude mastocytoma extract, the amount of $C^{14}O_2$ formed was far greater than when either substrate was present alone and approached the sum of the amounts of $C^{14}O_2$ formed when each substrate was present alone. This finding is characteristic of two independent sites of decarboxylation rather than of a single site where competition of the two substrates would

Table 2. Changes in absolute and relative decarboxylase activities of Mastocytoma extract after purification

Substrate	mμMoles CO_2/30 min./mg protein - Crude extract	Semipurified	Purification factor
Histidine	9.6	34	3.5
DOPA	23.7[1]	4.2[1]	0.18
5-HTP	5.8[2]	0.2[2]	0.04

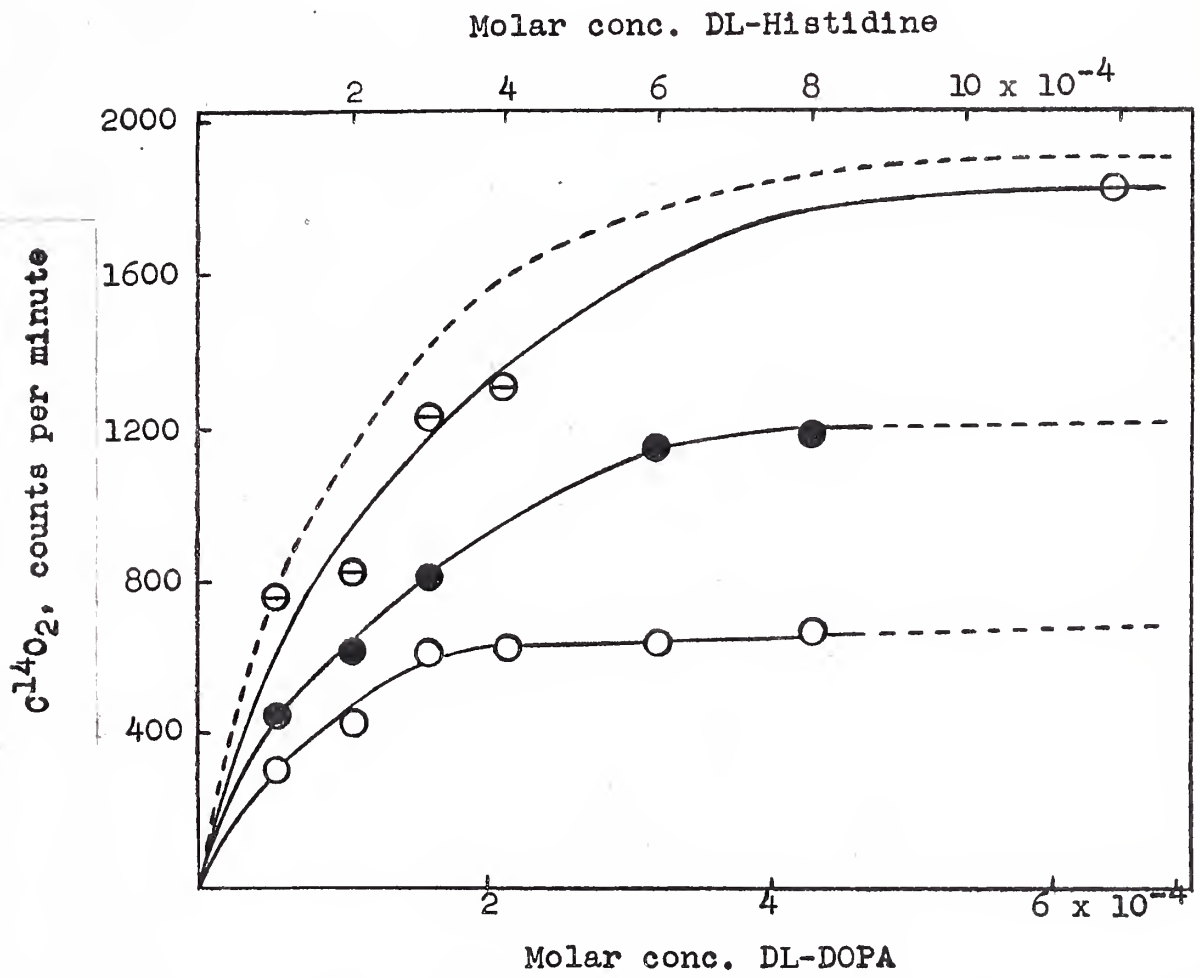
1. Extrapolated from values obtained at 10 min. incubation.
2. Data obtained from another enzyme preparation in which the histidine decarboxylase purification factor was 2.3.

Table 3. Lack of Inhibition of Mastocytoma Histidine decarboxylase by DOPA

Molar conc. DL-DOPA	Per cent Activity Crude extract	Semipurified
None	100	100
1×10^{-5}	96	98
1×10^{-4}	103	100
1×10^{-3}	98	96
1×10^{-2}	89	85

DL-Histidine, 1×10^{-3} M, was used as substrate.

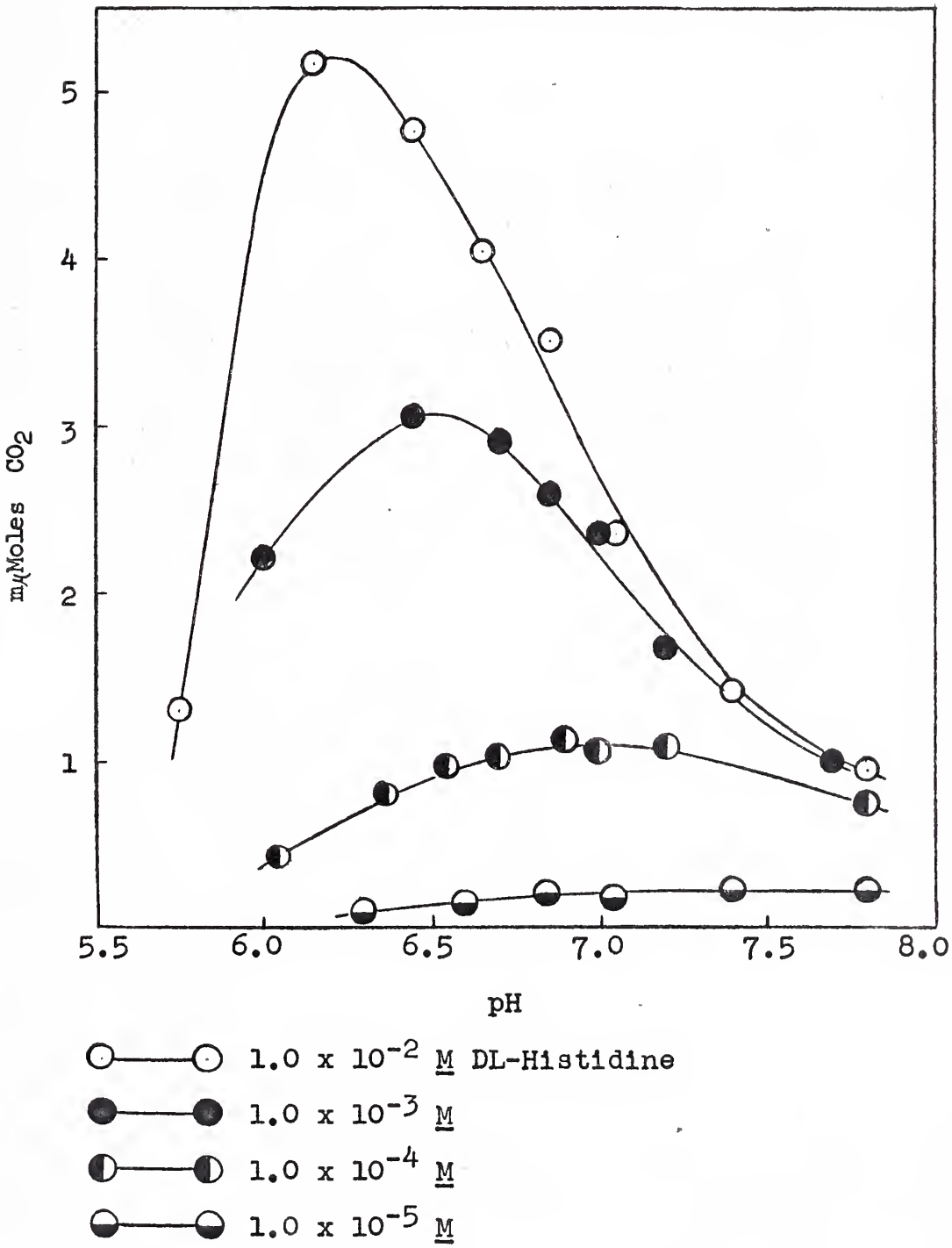
Figure 2. Histidine decarboxylase and DOPA decarboxylase activities of mastocytoma extract



Incubation time was 30 minutes. No glutathione was added.

O—O Curve 1. DOPA alone.
●—● Curve 2. Histidine alone.
Θ—Θ Curve 3. DOPA plus Histidine.
----- Curve 4. Sum of Curves 1 and 2.

Figure 3. Change in pH optimum with different substrate concentrations



take place. Additional evidence is provided in Table 3 which shows that the histidine decarboxylase activity of both the mastocytoma extract and semipurified preparation was not inhibited to a significant degree by the addition of unlabeled DOPA.

With the semipurified preparation, the pH at which the histidine decarboxylase exhibited maximal activity was found to vary with substrate concentration (Figure 3). This is in agreement with Hakanson's findings with the fetal rat (10) and rat bone marrow (11) enzymes.

Fetal rat

The purified enzyme preparation had a histidine decarboxylase activity of 11 mμMoles CO_2/mg protein/30 min. This compares with an activity of 22 mμMoles CO_2/mg protein/30 min. reported by Hakanson (calculated from figures in his text) with a more purified preparation.

No DOPA decarboxylase activity could be demonstrated in the purified preparation. There was abundant DOPA decarboxylase activity in the tissue homogenate, but this was totally lost during the heat denaturation step of the purification procedure.

Comparison of histidine decarboxylase activities of homogenates of fetal liver and the remaining fetal carcass showed the liver to have 20 times the activity of the remaining tissue. This is in agreement with the findings of Burkhalter (3).

Discussion

The semipurified mastocytoma histidine decarboxylase preparation investigated in this paper was not specific for histidine as it still contained some DOPA decarboxylase and 5-HTP decarboxylase activity. The evidence presented suggests strongly that this was due to contamination of a specific histidine decarboxylase by small amounts of another enzyme, possibly aromatic L-amino acid decarboxylase, rather than a property of the histidine decarboxylase itself. The report by Aures and Clark (1) that histidine decarboxylase prepared from this same tissue was inhibited 50% by 4.0×10^{-3} M L-DOPA and by 1.0×10^{-3} M D-DOPA is not confirmed by the data presented here. It is well known that DOPA and pyridoxal-5-phosphate react rapidly to form a cyclic compound (35, 55). Furthermore, it has been shown that high concentrations of DOPA will inhibit DOPA decarboxylase and that the inhibition can be reversed by the addition of more pyridoxal phosphate (55). It is highly likely, therefore, that the inhibition observed by these authors was due to sequestration of the coenzyme away from the apoenzyme by the added DOPA. In the studies reported here the apo- and coenzyme were allowed to react together for 20 minutes before substrate or inhibitor was added, thereby minimizing the possibility of coenzyme deficiency

due to interaction with the inhibitor. The otherwise curious result of these authors that D-DOPA should be as good an inhibitor as L-DOPA is explained if the inhibition is considered as due to interaction with the coenzyme, since both isomers react equally well with pyridoxal phosphate.

Unfortunately it was not possible to separate the histidine decarboxylase and DOPA decarboxylase activities by the simple fractionation procedures employed. All fractions were tested for both enzyme activities and none convincingly showed an increase of DOPA decarboxylase activity relative to the histidine decarboxylase present. It is possible that all the fractionation served to do was to progressively denature the aromatic L-amino acid decarboxylase present in the initial extract.

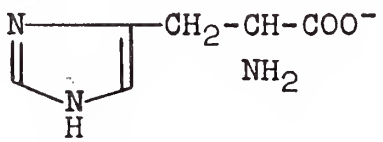
The pH optimum of the mastocytoma histidine decarboxylase varied with the substrate concentration (Figure 3). Hakanson has reported similar results with purified histidine decarboxylase from rat fetus (10) and rat bone marrow (11). He has also shown that the K_m of fetal rat histidine decarboxylase decreases as the pH increases. He analyzed this phenomenon by considering which ionic species of histidine is the actual substrate of the enzyme. Thus, when the K_m values were recalculated using the concentration of the anionic form of histidine (see Table 4 for further explanation) as the substrate concentration, the K_m values

were almost the same at all hydrogen ion concentrations, averaging 6 x 10^{-7} M (range 4.3-6.9 x 10^{-7} M). When the other ionic forms of histidine were considered as the substrate, the K_m values at different hydrogen ion concentrations did not agree nearly so well. He concluded that the anionic form of histidine was the actual substrate of the enzyme. When this analysis is applied to the findings reported in this paper for mastocytoma histidine decarboxylase, the result is as shown in Table 4. It can be seen that whereas the K_m values calculated to total histidine concentration vary by a factor of 37, those obtained by considering the anionic form of histidine as the substrate vary only by a factor of 2. Mackay, Riley and Shepherd (33), working with the specific histidine decarboxylase of the transplantable rat hepatoma have also reported a wide range in the K_m at different pH values (1.4 x 10^{-4} M at pH 7.8; 6.8 x 10^{-3} M at pH 5.8), suggesting that this may be a general property of the specific histidine decarboxylase of many tissues and that the anionic form of histidine is the true substrate of these enzymes. This would be an important consideration in the search for a specific inhibitor of histidine decarboxylase.

Table 4. The influence of pH on K_m

pH	K_m(total Hd)[1]	Per cent of Histidine in anionic form[2]	K_m(anionic form)[3]
6.0	7.1×10^{-4}	0.035	2.5×10^{-7}
6.4	2.7×10^{-4}	0.13	3.5×10^{-7}
6.8	1.2×10^{-4}	0.37	4.4×10^{-7}
7.2	0.44×10^{-4}	1.1	4.8×10^{-7}
7.6	0.19×10^{-4}	2.8	5.2×10^{-7}

1. K_m(total Hd) was estimated from the data presented in Figure 3 by use of Lineweaver-Burk plots (27) and was calculated using total L-Histidine concentration.
2. Per cent of Histidine in anionic form represents that fraction of L-Histidine present in the following form:



This fraction was calculated using published (26) dissociation constants of histidine.
3. K_m(anionic Hd) is obtained when K_m is calculated using the anionic form of histidine as substrate.

Summary

Mouse mastocytoma histidine decarboxylase was partially purified by fractional precipitation of protein with increasing hydrogen ion concentration. Studies suggested that the semipurified preparation contained a specific histidine decarboxylase; the small amount of DOPA and 5-HTP decarboxylase activity which remained was probably due to another enzyme(s). The pH optimum of the mastocytoma histidine decarboxylase was found to vary inversely with the substrate concentration. The possible significance of this observation is discussed with reference to the nature of the ionic species of histidine utilized as substrate by the enzyme.

In addition, it was confirmed that fetal rat histidine decarboxylase had no DOPA decarboxylase activity.

References

1. Aures, D., and Clark, W. G., A Rotating Diffusion Chamber for $C^{14}O_2$ Determination as Applied to Inhibitor Studies on Mouse Mast Cell Tumor Histidine Decarboxylase, Anal. Biochem. 9, 35-47 (1964)

2. Aures, D., Hartman, W. J., and Clark, W. G., Purification and Properties of Mast Cell L-Histidine Decarboxylase, Proc. Intern. Congr. Physiol. Sci., 22nd Congr., Leiden, 2, 694 (1962)

3. Burkhalter, A., The Formation of Histamine by Fetal Rat Liver Specific Histidine Decarboxylase, Biochem. Pharmacol. 11, 315 (1962)

4. Furth, J., Hagen, P., and Hirsch, E., Transplantable Mastocytoma in the Mouse Containing Histamine, Heparin, 5-Hydroxytryptamine, Proc. Soc. Exptl. Biol. Med. 95, 824-828 (1957)

5. Ganrot, P. O., Rosengren, A. M., and Rosengren, E., On the Presence of Different Histidine Decarboxylating Enzymes in Mammalian Tissues, Experientia 17, 263-264 (1961)

6. Guirard, B. M., and Snell, E. E., Pyridoxal Phosphate and metal ions as cofactors for histidine decarboxylase, J. Am. Chem. Soc. 76, 4745-4746 (1954)

7. Hagen, P., and Lee, F. L., Amino acid decarboxylases of mouse mast cells, J. Physiol. 143, 7P-8P (1958)

8. Hagen, P., Weiner, N., Ono, S., and Lee, F. L., Amino Acid Decarboxylases of Mouse Mastocytoma Tissue, J. Pharmacol. Exptl. Therap. 130, 9-12 (1960)

9. Hakanson, R., Formation of Histamine in Transplants from a Rat Mammary Carcinoma, Experientia 17, 402 (1961)

10. Hakanson, R., Histidine Decarboxylase in the Fetal Rat, Biochem. Pharmacol. 12, 1289-1296 (1963)

11. Hakanson, R., Histidine Decarboxylase in the Bone Marrow of the Rat, Experientia 20, 205-207 (1964)

12. Kahlson, G., A Place for Histamine in Normal Physiology, Lancet I, 67-71 (1960)

13. Kahlson, G., Nilsson, K., Rosengren, E., and Zederfeldt, B. Wound Healing as Dependent on Rate of Histamine Formation, Lancet II, 230-234 (1960)

14. Kahlson, G., and Rosengren, E., Prevention of Foetal Development by Enzyme Inhibition, Nature 184, 1238-1239 (1959)

15. Kahlson, G., and Rosengren, E., Histamine Formation in Bone Marrow, Experientia 19, 182 (1963)

16. Kahlson,G., Rosengren, E., and Steinhardt, C., Activation of Histidine Decarboxylase in Tumour Cells in Mice, Nature 194, 380 (1962)

17. Kahlson, G., Rosengren, E., and Svenson, S. E., Inhibition of Histamine Formation in vivo, Nature 194, 876 (1962)

18. Kahlson, G., Rosengren, E., and Westling, H., Increased Formation of Histamine in the Pregnant Rat, J. Physiol. 143, 91-103 (1958)

19. Kahlson, G., Rosengren, E., Westling, H., and White, T., The Site of Increased Formation of Histamine in the Pregnant Rat, J. Physiol. 144, 337-348 (1958)

20. Kahlson, G., Rosengren, E., and White, T., Formation of Histamine by the Foetus in the Rat and Man, J. Physiol. 145, 30P-31P (1959)

21. Kahlson, G., Rosengren, E., and White, T., The Formation of Histamine in the Rat Foetus, J. Physiol. 151, 131-138 (1960)

22. Kameswaran, L., Telford, J. M., and West, G. B., Histamine Formation in the Rat, J. Physiol. 157, 23P-24P (1961)

23. Kameswaran, L., and West, G.B., Histidine Decarboxylase in Rat Hepatoma, J. Pharm. Pharmacol. 13, 191-192 (1961)

24. Kameswaran, L., and West, G.B., The Formation of Histamine in Mammals, J. Physiol. 160, 564-571 (1962)

25. Levine, R. J., Sato, T. L., and Sjoerdsma, A., Inhibition of Histamine Synthesis in the Rat by alpha-Hydrazino Analog of Histidine and 4-Bromo-3-hydroxy Benzyloxyamine, Biochem. Pharmacol. 14, 139-149 (1965)

26. Levy, M., A Note on the Titration Constants of Imidazole Derivatives, J. Biol. Chem. 109, 361-381 (1935)

27. Lineweaver, H., and Burk, D., The Determination of Enzyme Dissociation Constants, J. Am. Chem. Soc. 56, 658-666 (1934)

28. Lovenberg, W., Barchas, J., Weissbach, H., and Udenfriend, S., Characteristics of the Inhibition of Aromatic L-Amino Acid Decarboxylase by alpha-Methylamino Acids, Arch, Biochem. Biophys. 103, 9-14 (1963)

29. Lovenberg, W., Weissbach, H., and Udenfriend, S., Aromatic L-Amino Acid Decarboxylase, J. Biol. Chem. 237, 89-93 (1962)

30. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., Protein Measurement with the Folin Phenol Reagent, J. Biol. Chem. 193, 265-275 (1951)

31. Mackay, D., Marshall, P. B., and Riley, J. F., Histidine Decarboxylase Activity in a Malignant Rat Hepatoma, J. Physiol. 153, 31P (1960)

32. Mackay, D., Reid, J. D., and Shepherd, D. M., Histidine Decarboxylase and Growth, Nature 191, 1311-1312 (1961)

33. Mackay, D., Riley, J. F., and Shepherd, D. M., Amino-acid Decarboxylase Activities in Rat Hepatoma, J. Pharm. Pharmacol. 13, 257-261 (1961)

34. Mackay D., and Shepherd, D. M., A Study of Potential Histidine Decarboxylase Inhibitors, Brit. J. Pharmacol. 15, 552 (1960)

35. Mackay, D., and Shepherd, D. M., Inhibition of Histidine Decarboxylases and Interaction of the Inhibitors with Pyridoxal 5'-Phosphate, Biochim. Biophys. Acta 59, 553-561 (1962)

36. Mitchell, R. G., Histidine Decarboxylase Activity in the Human Infant, J. Physiol. 164, 20P-21P (1962)

37. Mitchell, R. G., Histidine Decarboxylase in the Newborn Human Infant, J. Physiol. 166, 136-144 (1963)

38. Ono, S., and Hagen, P., Pyridoxal Phosphate: A Coenzyme for Histidine Decarboxylase, Nature, 184, 1143-1144 (1959)

39. Ono, S., Hagen, P., and Furth, J., Transplantable mastocytomas in mice, Chem. Abstr. 59, 9177c (1963)

40. Ono, S., Hirota, Y., Ito, Y., Watanabe, T., Kunii, A., Mastocytoma and cancerostatic agent, Chem. Abstr. 59, 9222c (1963)

41. Reid, J. D., Riley, J. F., and Shepherd, D. M., Histological and Enzymatic Changes in the Livers of Rats Fed the Hepatic Carcinogen Diethylnitrosoamine, Biochem. Pharmacol. 12, 1151-1156 (1963)

42. Reid, J. D., and Shepherd, D. M., Inhibition of Histidine Decarboxylases, Life Sci. 2, 5-8 (1963)

43. Robinson, B., and Shepherd, D. M., Inhibition of Histidine Decarboxylases, Biochim. Biophys. Acta 53, 431-433 (1961)

44. Robinson, B., and Shepherd, D. M., Inhibition of the L-Histidine Decarboxylases of Guinea-pig Kidney and Rat Hepatoma, J. Pharm. Pharmacol. 14, 9-15 (1962)

45. Rosengren, E., Are Dihydroxyphenylalanine Decarboxylase and 5-Hydroxytryptophan Decarboxylase Individual Enzymes?, Acta Physiol. Scand. 49, 364-369 (1960)

46. Rothschild, A. M., and Schayer, R. W., Characterization of Histidine Decarboxylase from Rat Peritoneal Fluid Mast Cells, Biochim. Biophys. Acta 34, 392-398 (1959)

47. Schayer, R. W., Histidine Decarboxylase of Rat Stomach and Other Mammalian Tissues, Amer. J. Physiol. 189, 533-536 (1957)

48. Schayer, R. W., Further Evidence that Pyridoxal Phosphate is the Coenzyme of Mammalian Histidine Decarboxylase, Fed. Proc. 18, 317 (1959)

49. Schayer, R. W., Relationship of Induced Histidine Decarboxylase Activity and Histamine Synthesis to Shock from Stress and from Endotoxin, Am. J. Physiol. 198, 1187-1192 (1960)

50. Schayer, R. W., Relationship of Stress-Induced Histidine Decarboxylase to Circulatory Hemostasis and Shock, Science 131, 226-227 (1960)

51. Schayer, R. W., Evidence that Induced Histamine is an Intrinsic Regulator of the Microcirculatory System, Am. J. Physiol. 202, 66-72 (1962)

52. Schayer, R., Histidine Decarboxylase in Mast Cells. Ann. N. Y. Acad. Sci. 103, 164-178 (1963)

53. Schayer, R. W., and Ganley, O. H., Adaptive Increase in Mammalian Histidine Decarboxylase Activity in Response to Nonspecific Stress, Am. J. Physiol. 197, 721-724 (1959)

54. Schayer, R. W., and Kobayashi, Y., Histidine Decarboxylase and Histamine Binding in Rabbit Platelets, Proc. Soc. Exptl. Biol. Med. 92, 653-655 (1956)

55. Schott, H. F., and Clark, W. G., DOPA Decarboxylase Inhibition through the Interaction of Coenzyme and Substrate, J. Biol. Chem. 196, 449 (1952)

56. Telford, J. M., and West, G. B., Studies with Histidine Decarboxylase, J. Physiol. 155, 16P (1960)

57. Telford, J. M., and West, G. B., The Formation of Histamine in the Rat, J. Pharm. Pharmacol. 13, 75-82 (1961)

58. Telford, J. M., and West, G. B., The Effect of Age on the Formation of Histamine in the Rat, J. Physiol. 157, 306-314 (1961)

59. Telford, J. M., and West, G. B., Some Effects of Corticosteroids on the Metabolism of Histamine and 5-Hydroxytryptamine in the Rat, Brit. J. Pharmacol. 16, 360-368 (1961)

60. Teorell, T., and Stenhagen, E., Ein Universalpuffer fur den pH-Bereich 2,0 bis 12,0, Biochem. Z. 299, 416-419 (1938)

61. Waton, N. G., The Effect of Organic Solvents on Mammalian Histidine Decarboxylase, Biochem. J. 64, 318-322 (1956)

62. Weissbach, H., Lovenberg, W., and Udenfriend, S., Enzymatic Decarboxylation of alpha-Methyl Amino Acids, Biochem. Biophys. Res. Comm. 3, 225-227 (1960)

63. Weissbach, H., Lovenberg, W., and Udenfriend, S., Characteristics of Mammalian Histidine Decarboxylating Enzymes, Biochim. Biophys. Acta, 50, 177-179 (1961)

64. Werle, E., Uber die Bildung von Histamin aus Histidin durch tierisches Gewebe, Biochem. Z. 288, 292-293 (1936)

65. Werle, E., and Koch, W., Zur Kenntnis der Aminosaure-Decarboxylasen und ihres Wirkungsmechanismus, Biochem. Z. 319, 305-322 (1949)

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