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*PURIFICATION AND PROPERTIES OF HISTIDINE DECARBOXYLASE FROM LACTOBACILLUS 30a**

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Despite the physiological importance of histamine, the mechanism of its formation by histidine decarboxylase has not been adequately investigated. Three apparently different mammalian enzymes that decarboxylate histidine are known, each of which is reported to require pyridoxal-5′-phosphate (PLP) as coenzyme.[1–3] Even in purified preparations, however, their activities are extremely low. For a source of this enzyme we turned, therefore, to *Lactobacillus* 30a, which produces it in comparatively large amounts.[4, 5] Indirect evidence led to the conclusion that the histidine decarboxylase of this organism[4] and of *Clostridium welchii*,[6] and the histidine and glutamate decarboxylases of *Escherichia coli*[7] did not possess PLP as coenzyme. Purified glutamate decarboxylase has since been shown to contain firmly bound PLP which is necessary for its activity.[8] The nature of the bacterial histidine decarboxylase is not yet clear. Its formation in *Lactobacillus* 30a is dependent upon the presence of vitamin B_6,[5, 9] and some partially purified preparations were activated by PLP and Fe^{+++}.[9] However, these activating effects were subsequently found to be nonspecific, and hence could not be considered evidence for the PLP-dependence of this decarboxylase. We describe herein procedures for preparation of the ap-

parently pure bacterial histidine decarboxylase. No PLP has been detected in the purified enzyme by any of the criteria applied.

Methods.—(1) *Manometric assay of histidine decarboxylase:* The main compartment of the Warburg vessels contained 26 μmoles (5 mg) of histidine monohydrochloride and any other desired addenda in 2.99 ml of 0.167 *M* ammonium acetate buffer, pH 4.8. The enzyme protein in 0.01 ml of buffer was placed in the side arm. After 10 min at 37° the enzyme solution was washed quantitatively into the main compartment, and CO_2 evolution was recorded at 5-min intervals for a period of 15 min. Activity is expressed as Q_{CO_2} (μl CO_2 evolved per hr per mg of protein). Protein was determined by the method of Lowry *et al.*[10] or in purified preparations by absorption at 280 mμ.

(2) *Purification of decarboxylase: Lactobacillus* 30a was grown at 37° from a 5% inoculum in 6 liters of medium C[5] for 18–20 hr. Cells from several such batches were harvested by centrifugation, combined, and dried with acetone (yield: 0.82 gm dry cells per liter). The dry cells can be stored at 4° for months without decrease in activity.

(a) *Preparation of cell extracts:* Acetone-dried cells (25 gm) were shaken with 700 ml of 0.2 *M* ammonium acetate buffer (pH 4.8) at 37° for 5 hr. Cell debris was removed by centrifugation and was almost inactive.

(b) *Ammonium sulfate fractionation:* The supernatant solution from (*a*) was cooled to 0° and finely divided ammonium sulfate added in small portions with stirring to 50% of saturation. The precipitated protein was discarded. Additional ammonium sulfate was then added to 70% of saturation. The active precipitate was collected by centrifugation, dissolved in 45 ml of water, then dialyzed for 3 hr at room temperature against several changes of 0.025 *M* potassium chloride.

(c) *Heat treatment:* The dialyzed protein from (*b*) was heated in 10-ml portions in the water bath to 70°, held for 2 min at this temperature, then cooled to room temperature in tap water. The precipitate was removed by centrifugation and discarded.

(d) *Acetone fractionation:* The solution from (*c*) was cooled to 0°, then mixed rapidly with 0.8 vol of ice-cold acetone. Precipitated material was centrifuged out and discarded. Additional cold acetone (0.5 v/v of supernatant solution) was added to precipitate the enzyme.

(e) *Sephadex filtration:* About 7 gm of Sephadex G-200 was allowed to swell in excess 0.2 *M* ammonium acetate buffer, pH 4.8, for 24 hr. After removing the fines by decanting several times, a 2 × 44-cm column was prepared from the resulting slurry. The active protein fraction from (*d*) was dissolved in 15 ml of the same buffer and passed over the column at room temperature and a flow rate of 9–12 ml per hour. Sixty 3-ml fractions were collected; the decarboxylase appeared in fractions 17 to 31, separated from inactive material which ran ahead and behind the enzyme. These active fractions were pooled and reduced to 20 ml by ultrafiltration at 0° and 740 mm pressure. This fraction was reapplied to the same column; the active fractions (fractions 17–31) were again pooled and reduced in volume by ultrafiltration. This preparation could be stored for over 6 months without loss in activity by passing it through a sterile "Millipore" filter (pore size, 0.65 μ) and storing at 4° in sterile vials each containing about 1.5 ml of solution.

(f) *Crystallization:* Ammonium sulfate was added to fraction (*e*) to 80% of saturation. The precipitated protein was redissolved in water at 4° to give a concentration of 5 mg of protein per ml. Solid ammonium sulfate (0.24 gm per ml of solution) was then added and the pH was adjusted to about 8.0 with aqueous ammonia. If the solution became turbid, it was centrifuged and the insoluble matter discarded. The enzyme was crystallized from the supernatant solution by either (1) adding 0.3 ml of saturated ammonium sulfate solution (pH 8.0) per ml of solution and allowing it to stand overnight, or (2) adding the ammonium sulfate solution dropwise until a faint turbidity developed, then allowing to stand overnight. Figure 1 is a photomicrograph of the crystalline enzyme obtained by the former procedure.

Results.—Purification of histidine decarboxylase: A protocol of the purification procedure described above is given in Table 1. The active material from step (*e*) is essentially homogeneous; its specific activity is not increased by crystallization. Only a single band of protein appeared on starch gel electrophoresis[11] of 300-μg samples under conditions which resolved crystalline serum albumin into five components. Similarly, only traces of impurity were detected by disk electrophoresis[12] at

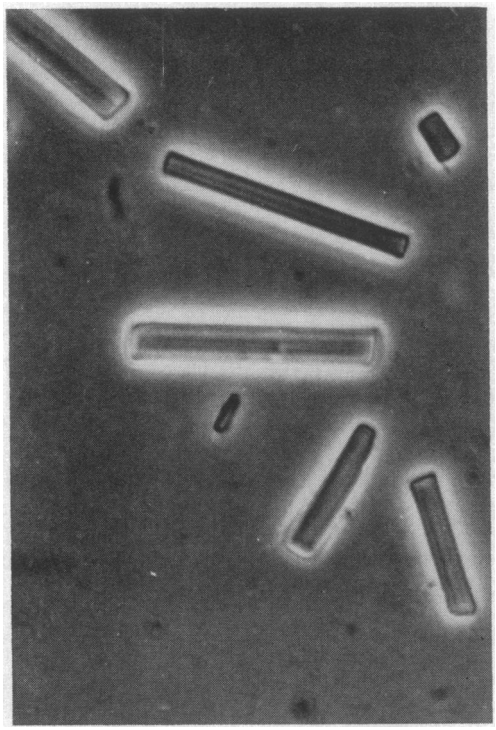

FIG. 1.—Crystalline histidine decarboxylase from *Lactobacillus* 30a (× 2600).

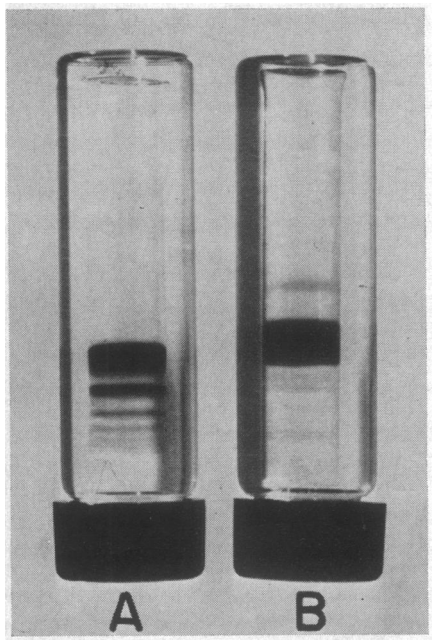

FIG. 2.—Disc electrophoresis patterns of (*A*) crystalline bovine serum albumin (120 μg) and (*B*) histidine decarboxylase (120 μg), both at pH 8.3.

either pH 4.7 or 8.3 (Fig. 2). Following centrifugation in a sucrose gradient, small decreases in the ratio of activity to absorbancy were found at both ends of the protein peak, indicative of quantitatively minor amounts of contaminating proteins at the extremes of the peak (Fig. 3*A*). These amount to less than 5 per cent of the protein peak, and were not resolved from the main peak by sedimentation in the analytical ultracentrifuge, where only a single protein boundary was observed in 0.2 *M* ammonium acetate buffer, pH 4.8.[13] We conclude that the decarboxylase is over 90 per cent pure. By comparison of its sedimentation behavior in sucrose gradients with that of catalase and of yeast alcohol dehydrogenase as separate standards (Fig. 3*B*), values of 9.7 and 9.0*S* (corresponding to a molecular weight of *approximately* 195,000) were calculated[14] for the sedimentation coefficient of the decarboxylase.

Relation to pyridoxal phosphate: Histidine decarboxylase is colorless at all pH values examined and shows the spectrum of a simple protein (Fig. 4). No spectral

TABLE 1

PURIFICATION OF HISTIDINE DECARBOXYLASE FROM *Lactobacillus* 30a

Active fraction	Volume, ml	Protein, mg	Q_{CO_2}	Over-all yield, %
Dried cells, 25 gm	—	—	350*	—
a	630	1764	7,760	(100)
b	65	390	27,160	79
c	65	250	37,200	68
d	15	220	46,000	73
e	6	114	70,500	59

* Expressed in terms of dried cells rather than of protein.

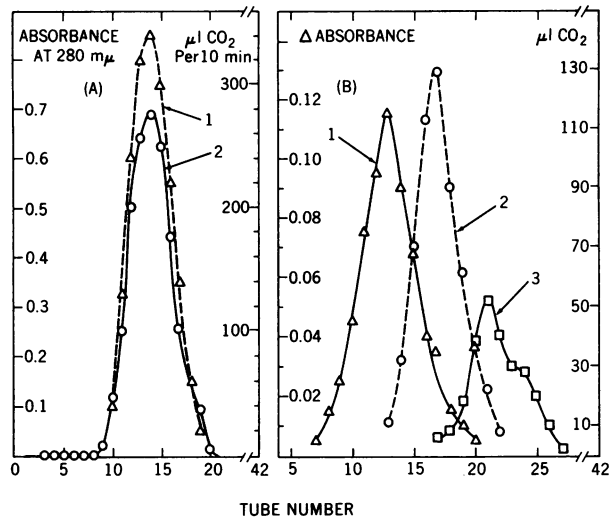

FIG. 3.—(*A*) Distribution of enzymatic activity (*curve 1*) and protein (*curve 2*) following centrifugation of purified histidine decarboxylase (fraction *e*, Table 1) in a sucrose gradient. Protein (0.88 mg) was centrifuged for 13 hr at −22° and 39,000 rpm in the Spinco model L with SW 39 rotor. (*B*) Comparative distribution of catalase, *1*, histidine decarboxylase, *2*, and yeast alcohol dehydrogenase, *3*, in a sucrose gradient. The centrifuge tube contained 0.047, 0.087, and 0.033 mg of the three proteins, respectively, and was centrifuged for 13.67 hr as described in (*A*).

absorption above 300 mμ, similar to that shown by pyridoxal phosphate enzymes,[15] was present. Similarly, no absorption characteristic of pyridoxal phosphate was found in denatured or alkali-treated solutions of the enzyme. Upon addition of pyridoxal phosphate in a molar ratio of 1:1 with protein, its characteristic absorption maximum at 388 mμ was readily detected, thus providing no evidence of interaction with the decarboxylase.[16] In contrast to observations made earlier with impure concentrates of this enzyme,[9] no increase in decarboxylase activity resulted from additions of pyridoxal phosphate or Fe^{+++}, either alone or in combination.[17] Finally, acid hydrolysates of the protein contained no vitamin B_6 as determined by microbiological assay with *Saccharomyces carlsbergensis*.[18] Satisfactory recoveries of vitamin B_6, added to the sample as pyridoxal phosphate prior to hydrolysis, were obtained by this procedure. We conclude that this enzyme, unlike other α-amino acid decarboxylases hitherto examined, is not a pyridoxal phosphate enzyme.

Optimum pH, isoelectric point, and stoichiometry: An isoelectric point of 4.4 was found for histidine decarboxylase by electrophoresis on paper (Fig. 5*A*). The optimum pH range for enzyme activity extends from 4.5 to above 6.0 (Fig. 5*B*); below the isoelectric point enzymatic activity decreases rapidly. Carbon dioxide and histamine (determined fluorometrically[19]) were formed in equimolar amounts, in confirmation of the generally accepted stoichiometry of the decarboxylation reaction.

Substrate specificity and action of inhibitors: Of the compounds tested (Table 2) only L-histidine was decarboxylated under the conditions used. Methylation of the α-amino group, the α-carbon atom, or the N-1 or N-3 positions of the imidazole group of histidine all inactivate the molecule as both substrate and inhibitor. From the inhibitory effectiveness of histidine analogues, it is apparent that the imidazole

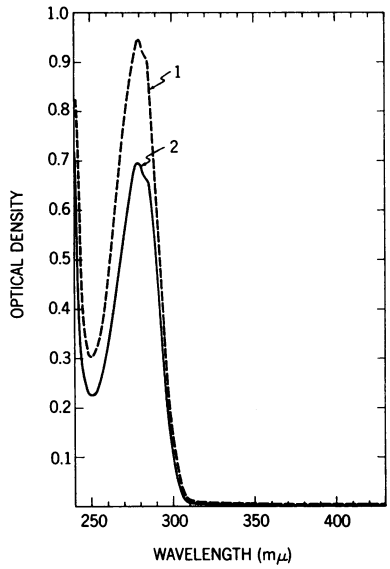

FIG. 4.—Spectrum of crystalline histidine decarboxylase. *Curve 1*, 0.2 *M* Tris buffer, pH 8.50; 0.54 mg protein per ml. *Curve 2*, 0.2 *M* ammonium acetate buffer, pH 4.8; 0.40 mg protein per ml.

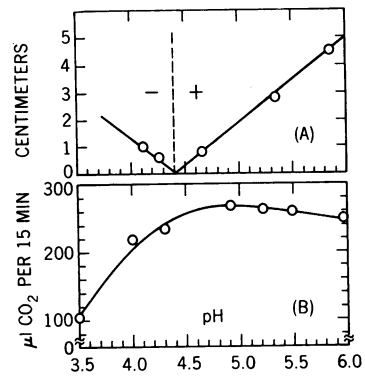

FIG. 5.—Isoelectric point (*A*) and pH optimum (*B*) of histidine decarboxylase. Protein (0.20 mg) was subjected to electrophoresis on Whatman no. 1 paper at variable voltage (to 2500 volts) and 21 milliamps in 0.2 *M* ammonium acetate buffers of the pH indicated. After 25 min protein was located by spraying with p-dimethylaminobenzaldehyde in acid-acetone.

ring is of primary importance in permitting combination with enzyme. The affinity is increased by presence of the 3-carbon side chain of histidine or imidazole-propionate, but any closer approach of the carboxyl group to the imidazole nucleus greatly decreases the affinity of the compound for the enzyme. Cyanide and p-chloromercuribenzoate inhibited the decarboxylase noncompetitively; inhibition by the latter compound was partially reversed by subsequent incubation with 2,3-dimercaptopropanol.

TABLE 2

SUBSTRATE SPECIFICITY AND INHIBITION OF HISTIDINE DECARBOXYLASE

Substrates or inhibitors*	K_m or K_I value, mM	Inactive compounds†
Substrates†		
L-Histidine	0.90	D-Histidine
		N^{α}-Methyl-L-histidine
Inhibitors (competitive)†		
Imidazolepropionic acid	1.8	α-Methylhistidine
Urocanic acid	2.1	1-Methyl-L-histidine
Imidazole acetic acid	130	3-Methyl-L-histidine
Imidazole carboxylic acid	970	2-Thiol-L-histidine
Imidazole	3.2	L-Histidyl-L-histidine
N-Methylimidazole	7.2	2-Thiazole-DL-alanine
		1,2,4-Triazole
Inhibitors (noncompetitive)		
KCN (pH 6.8)	0.8	
p-Chloromercuribenzoate	0.073	

* All compounds appeared pure when checked by paper electrophoresis with ninhydrin or diazotized p-bromoaniline as spray reagents. N^{α}-Methylhistidine[20] and N-methylimidazole[21] were synthesized by cited methods; imidazolepropionic acid was synthesized by hydrogenation of urocanic acid. Other compounds were obtained from commercial sources.

† K_m and K_I values were obtained from Lineweaver-Burk plots of CO_2 evolution after 10 min at pH 4.8 (unless otherwise specified) versus concentration of histidine in the absence or presence of 40, 100, or 400 μmoles of the compounds listed. "Inactive" compounds were neither substrates nor inhibitors under these conditions. At high histidine concentrations certain of the inhibitors were less effective than would be expected from an uncomplicated competitive relationship.

Comparative properties of various decarboxylase preparations: The histidine decarboxylase of *Lactobacillus* 30a described here differs in crystalline form and catalytic efficiency from that obtained from a micrococcus by Mardashev and Seminha.[22] With the possible exception of their enzyme,[23] the histidine decarboxylase described here differs from all other highly purified amino acid decarboxylases so far studied (Table 3) in its lack of dependence on PLP. This coenzyme is apparently required by the specific histidine decarboxylase of mammalian mast cells,[2] indicating the natural occurrence of two mechanistically different routes for histidine decarboxylation. In their substrate affinities the various purified amino acid decarboxylases resemble one another rather closely, but their catalytic efficiencies, in terms of Q_{CO_2}, differ by almost an order of magnitude (Table 3).

TABLE 3

COMPARATIVE PROPERTIES OF VARIOUS AMINO ACID DECARBOXYLASES

Decarboxylase and reference	Source	pH Optimum	K_m value, mM	Pyridoxal phosphate dependent	Q_{CO_2} of best preparation
Homogeneous preparations (bacterial)					
Histidine*	*Lactobacillus* 30a	4.8	0.90	No	70,000
Histidine[22]	*Micrococcus* sp.	(5.6)	—	?	34,000
Arginine[24]	*E. coli* B	5.2	0.65	Yes	314,000
Glutamate[8]	*E. coli* 26	3.8	0.82	Yes	100,000
Partially purified preparations (mammalian)					
Histidine	Mast cells	6.0–7.0	0.5	Yes	20†
Aromatic amino acid[1]	Guinea pig kidney	9.0–9.5	50 (*his*) 0.4 (*DPA*)	Yes	14 (*his*)‡ 7260 (*DPA*)

* This article.
† Calculated from published data[2] for a 200-fold purified enzyme.
‡ Calculated from published data[1] for a 100-fold purified enzyme acting on histidine (*his*, a poor substrate) or dihydroxyphenylalanine (*DPA*, an excellent substrate).

Summary.—The histidine decarboxylase from *Lactobacillus* 30a has been obtained in crystalline and apparently homogeneous form, as judged by ultracentrifugal and electrophoretic criteria. Only L-histidine serves as substrate; several imidazole compounds are effective competitive inhibitors. Cyanide inhibits noncompetitively. The spectrum of the pure enzyme is that of a simple protein; this and other criteria indicate that, unlike other amino acid decarboxylases so far studied, the enzyme is not dependent upon pyridoxal phosphate as a cofactor.

We are indebted to Dr. R. A. Archibald for assistance in developing some of the steps of the purification procedure.

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[15] For references to individual enzymes, see Snell, E. E., in *The Mechanism of Action of Water Soluble Vitamins*, Ciba Foundation Study Group No. 11, ed. A. V. S. de Reuck and M. O'Connor (London: Churchill, 1961), pp. 18–37.

[16] Under the same conditions PLP was observed to interact with serum albumin with appearance of absorption maxima at 332 and 415 mμ, as described by W. B. Dempsey and H. N. Christensen [*J. Biol. Chem.*, **237**, 1113 (1962)].

[17] The activating effects of PLP and Fe^{+++} on these earlier preparations[9] remain unexplained but were partially due to stabilizing effects on the enzyme under the assay conditions then in use. They could be duplicated by Tweens, and hence provide no evidence for a role of PLP in action of the enzyme. Neither crude nor pure preparations of enzyme prepared and assayed by the present procedure show these effects.

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*PHOSPHORYLATION OF NUCLEIC ACID BY AN ENZYME FROM T4 BACTERIOPHAGE-INFECTED ESCHERICHIA COLI**

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In recent years several enzymatic reactions have been identified which result in the modification of nucleic acids at the polynucleotide level. These include the glucosylation of DNA,[1] the methylation of both DNA and RNA,[2, 3] and the addition of single nucleotides to the 3′-hydroxyl termini of RNA[4] and DNA[5, 6] molecules. This paper describes the purification and some properties of an enzyme, polynucleotide kinase, identified in extracts of *Escherichia coli* infected with T4 phage, which catalyzes the transfer of orthophosphate from ATP to the 5′-hydroxyl termini of polynucleotides (Fig. 1). With the purified enzyme the 5′-hydroxyl termini of high-molecular-weight DNA and RNA molecules can be labeled selectively. The ability of this kinase to phosphorylate the 5′-terminus provides a useful reagent for identifying end groups in nucleic acids and for studying the effect of these groups on enzymes involved in nucleic acid metabolism. A preliminary report by Novogrodsky and Hurwitz[7] describes an enzyme with similar properties from T2 phage-infected cells.

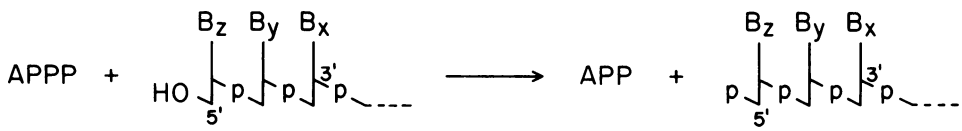

FIG. 1.—The transfer of P_i from ATP to the 5′-hydroxyl terminus of a polynucleotide by polynucleotide kinase.