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# PURIFICATION AND PROPERTIES OF L-HISTIDINE DECARBOXYLASE FROM MOUSE STOMACH

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**Abstract**—L-Histidine decarboxylase was purified to electrophoretic homogeneity from mouse stomach according to a procedure described previously [Ohmori E, Fukui T, Imanishi N, Yatsunami K and Ichikawa A, *J Biochem (Tokyo)* **107**: 834–839, 1990]. The purified enzyme exhibited a specific activity of 750 nmol histamine formed per min per mg protein, which constituted a 37,500-fold purification compared to the crude extract, with a 1.6% yield. The molecular mass of the enzyme was estimated to be 54 kDa by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and 100 kDa by gel filtration. The isoelectric point of the enzyme was determined to be pH 5.4. The $K_m$ value for L-histidine was estimated to be 0.29 mM. The single mRNA encoding the amino acid sequence of the mouse stomach enzyme was examined and its size was found to be 2.7 kb. These molecular and catalytic property values of the L-histidine decarboxylase of mouse stomach are quite similar to those of the enzyme from mouse mastocytoma P-815 cells.

L-Histidine decarboxylase (HDC†; EC 4.1.1.22) catalyzes the decarboxylation of L-histidine to form histamine, which plays various significant roles in mammals [1]. HDC has been partially purified from various sources, such as hamster placenta [2], rat brain and stomach [3], and rat gastric mucosa [4], or has been completely purified from fetal rat liver [5], mouse mastocytoma P-815 cells [6], mouse kidney [7] and rat basophilic leukemia cells [8].

In the stomach, histamine is considered to be a physiological stimulant of gastric secretion, and relatively high HDC activity is found in the gastric mucosa of mammals such as monkeys or humans [9]. HDC activity in the stomach is known to vary in response to various stimulants, such as gastrin [10] and hydrocortisone [11, 12]. In addition, HDC activities in stomachs of humans, canines and pigs have been found to be entirely contained in mast cell-like cells, while the enzyme activity in rat stomach has been reported to be localized in enterochromaffin-like cells [13]. However, little is known about the origin of HDC in mouse stomach. In addition, HDC in rat stomach was recognized to exist in two forms which are likely to originate from two cell types, mast cells and non-mast cells [3]. On the other hand, the occurrence of multiple forms of HDC in rat stomach was suspected to be due to the phosphorylation state of HDC [14].

However, these studies revealing the heterogeneity of stomach HDC were carried out using partially purified stomach enzyme preparations. Therefore, it is necessary to purify stomach HDC in order to clarify its molecular properties as well as such heterogeneity of the enzyme. Recently, we succeeded in purifying and characterizing HDC from mouse mastocytoma P-815 cells [6] and have isolated a cDNA clone for its subunit [15]. By applying this purification procedure to the stomach enzyme, we have purified HDC from mouse stomach. Furthermore, we have verified that the contents of HDC mRNA are the highest in stomach among various mouse tissues using the above clone as a probe. The present paper describes the purification of HDC from mouse stomach to electrophoretic homogeneity and some of its properties compared with those of the mastocytoma P-815 cell enzyme.

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† Abbreviations: HDC, histidine decarboxylase; EPD, 1 mM ethyleneglycolbis(aminoethylether)tetra-acetate (EGTA), 0.2 mM phenylmethylsulfonyl fluoride and 0.5 mM dithiothreitol; and PPD, 0.01 mM pyridoxal 5'-phosphate, 2% polyethyene glycol No. 300, and 0.02 mM dithiothreitol.

## MATERIALS AND METHODS

### *Enzyme assay*

Histidine decarboxylase activity was assayed as described previously [6]. Briefly, the assay mixture (1 mL) was comprised of 0.8 μmol L-histidine, 0.2 μmol dithiothreitol, 0.01 μmol pyridoxal 5'-phosphate, 10 mg polyethylene glycol No. 300, 100 μmol potassium phosphate (pH 6.8) and enzyme. The reaction was carried out at 37° and was terminated by adding 0.04 mL of 60% perchloric acid. The histamine formed was isolated on a column of Amberlite CG-50 and then measured by the *o*-phthalaldehyde method [6]. One unit of histidine decarboxylase activity was defined as the formation of 1 nmol histamine/min under the standard assay conditions.

### *Protein assay*

The protein concentration was determined by the method of Lowry *et al.* [16] with bovine serum albumin as a standard. When a more sensitive

Table 1. Purification of histidine decarboxylase from mouse stomach

| Steps | Protein (mg) | Activity (units) | Specific activity (units/mg) | Purification (fold) | Yield (%) |
|---|---|---|---|---|---|
| 1. Cytosol | 5,060 | 101 | 0.020 | 1 | 100 |
| 2. Ammonium sulfate (20–60%) and pH 7.4 dialysis | 2,970 | 86.1 | 0.029 | 1.5 | 85 |
| 3. DEAE-Sepharose CL-6B | 286 | 47.3 | 0.166 | 8.3 | 47 |
| 4. Phenyl-Sepharose CL-4B | 22.7 | 35.6 | 1.57 | 79 | 35 |
| 5. Hydroxylapatite | 4.59 | 22.0 | 4.79 | 239 | 22 |
| 6. Phenyl-Superose (HPLC) | 0.970 | 14.2 | 1.6 | 730 | 14 |
| 7. Mono Q (HPLC) | 0.156 | 9.74 | 62.4 | 3,120 | 9.6 |
| 8. Hydroxylapatite (HPLC) | 0.0190 | 5.9 | 284 | 14,200 | 5.3 |
| 9. Reactive Green 19-Agarose | 0.00211* | 1.58 | 750 | 37,500 | 1.6 |

The purification was carried out with 137 g of stomachs from 1000 mice.
* Protein was estimated by the Bradford method [17] since the amount of protein obtained at this step was very low.

method was required, the Bradford method [17] was also used for the quantification of low levels of protein.

*Enzyme purification*

All purification steps were carried out at 0–4°.

*Step* 1. *Crude extract.* The frozen stomachs (137 g from 1000 mice) of male $BDF_1$ mice (16–20 g) that had been stored at −80° were thawed into 400 mL of 5 mM Tris–HCl (pH 7.4) containing 0.25 M sucrose and EPD (1 mM EGTA, 0.2 mM phenylmethylsulfonyl fluoride and 0.5 mM dithiothreitol), and homogenized using a Polytron homogenizer (30 sec at setting 5, six times; Kinematica, Steinhofhalde, Switzerland). The homogenate was centrifuged at 100,000 *g* for 60 min and the resulting supernatant was used as the crude extract.

*Step* 2. *Ammonium sulfate fractionation and pH 7.4 dialysis.* The crude extract was brought to 60% saturation with solid ammonium sulfate. The solution was stirred for 20 min and then centrifuged at 10,000 *g* for 30 min. More solid ammonium sulfate was added to the supernatant to obtain 60% saturation. After stirring for 20 min, the precipitate was collected by centrifugation at 10,000 *g* for 30 min, dissolved in a minimal volume of 50 mM Tris–HCl (pH 7.4) containing EPD and then dialyzed against two changes of the same buffer for 18 hr. The precipitate formed during the diaylsis was removed by centrifugation at 100,000 *g* for 60 min.

*Step* 3. *DEAE-Sepharose CL-6B chromatography.* The supernatant from step 2 was dialyzed against 20 mM potassium phosphate (pH 6.8) containing PPD (0.01 mM pyridoxal 5′-phosphate, 2% polyethylene glycol No. 300 and 0.2 mM dithiothreitol) for 16 hr and then applied to a column (2.7 × 17.5 cm) of DEAE-Sepharose CL-6B preequilibrated with the same buffer. The column was washed with 340 mL of the same buffer and then the enzyme was eluted with a 500-mL linear gradient of potassium phosphate (pH 6.8, 20 to 400 mM) containing PPD. The active fractions were pooled.

*Step* 4. *Phenyl-Sepharose CL-4B chromatography.* After the potassium phosphate concentration of the enzyme fraction from the previous step had been adjusted to 300 mM, it was applied to a column (2.1 × 11 cm) of Phenyl-Sepharose CL-4B preequilibrated with 300 mM potassium phosphate (pH 6.8) containing PPD. The column was washed with 270 mL of the same buffer and then 340 mL of 80 mM potassium phosphate (pH 6.8) containing PPD, successively, and then the enzyme was eluted with 5 mM potassium phosphate (pH 6.8) containing PPD.

*Step* 5. *Hydroxylapatite chromatography.* The enzyme fraction from step 4 was applied to a column (1.5 × 5.6 cm) of hydroxylapatite preequilibrated with 5 mM potassium phosphate (pH 6.8) containing PPD. After the column had been washed with the same buffer, the enzyme was eluted with a 100-mL linear gradient of potassium phosphate (pH 6.8, 5 to 150 mM) containing PPD.

*Step* 6. *Phenyl-Superose HPLC.* After adjusting the potassium phosphate concentration to 550 mM with 1 M potassium phosphate (pH 6.8) containing PPD, the enzyme fraction from the previous step was applied to a column (0.5 × 5 cm) of Phenyl-Superose HR 5/5 preequilibrated with 550 mM potassium phosphate (pH 6.8) containing PPD, and then the column was washed with the same buffer. Elution of the enzyme was carried out with a 20-mL linear gradient of potassium phosphate (pH 6.8, 550 to 1 mM) containing PPD.

*Step* 7. *Mono Q HPLC.* After being dialyzed against 20 mM potassium phosphate (pH 6.8) containing PPD for 16 hr, the enzyme fraction from step 6 was applied to a column (0.5 × 5 cm) of Mono Q HR 5/5 preequilibrated with the same buffer. The column was washed with 16 mL of the same buffer and then the enzyme was eluted with a 16-mL linear gradient of potassium phosphate (pH 6.8, 20 to 300 mM) containing PPD.

*Step* 8. *Hydroxylapatite HPLC.* The enzyme fraction from the previous step was dialyzed against 5 mM potassium phosphate (pH 6.8) containing PPD for 16 hr and then applied to a column (0.75 × 10 cm) of hydroxylapatite (Pentax SH 0710F) preequilibrated with the same buffer. After the column

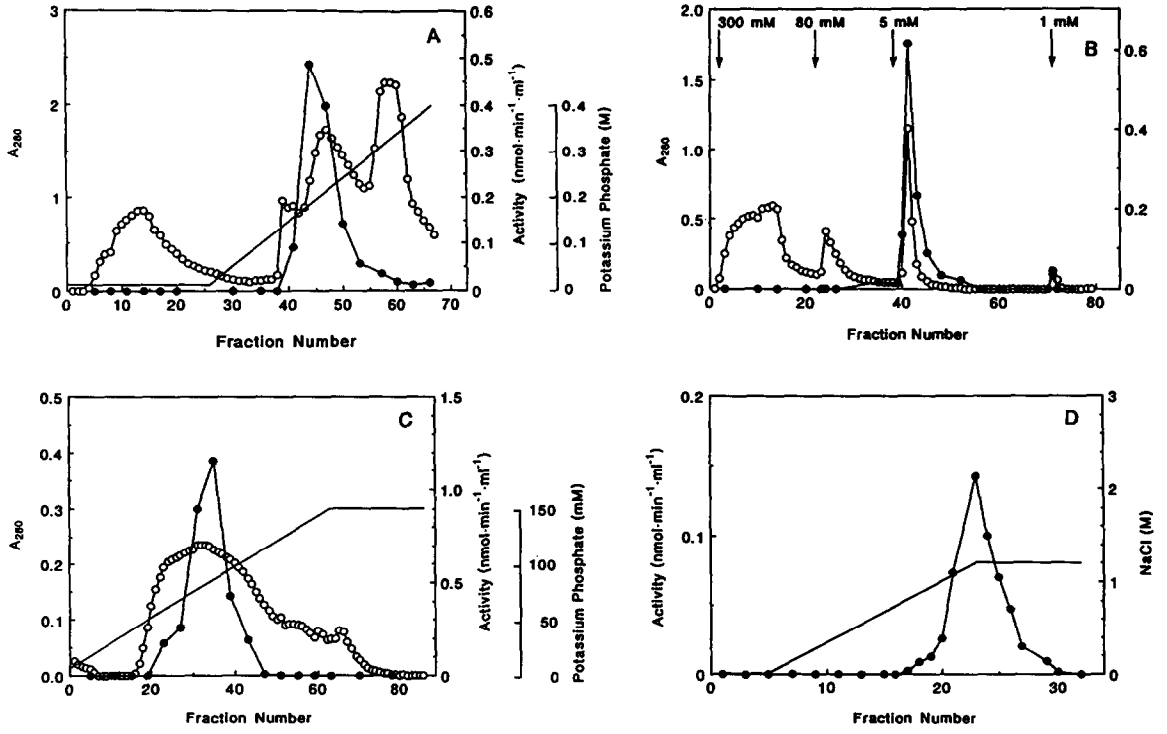


Fig. 1. Elution of histidine decarboxylase from four columns. (A) DEAE-Sepharose CL-6B chromatography. The dialyzed fraction obtained on ammonium sulfate fractionation was applied to a DEAE-Sepharose CL-6B column. The details are given under Materials and Methods. Key: (●) enzyme activity; (○) $A_{280}$; and (—) concentration of potassium phosphate buffer. (B) Phenyl-Sepharose CL-4B chromatography. The concentrations of potassium phosphate buffer used for the elution of the enzyme are indicated with arrows. Key: (●) enzyme activity; and (○) $A_{280}$. (C) Hydroxylapatite chromatography. Key: (●) enzyme activity; (○) $A_{280}$; and (—) concentration of potassium phosphate buffer. (D) Reactive Green 19-Agarose chromatography. Key: (●) enzyme activity; and (—) concentration of NaCl. $A_{280}$ is not shown since the protein concentrations of the fractions were too low to measure the absorbance at 280 nm.

had been washed with 5 mL of the same buffer, the enzyme was eluted with a 15-mL linear gradient of potassium phosphate (pH 6.8, 5 to 200 mM) containing PPD.

*Step 9. Reactive Green 19-Agarose chromatography.* The enzyme fraction from step 8 was dialyzed against 10 mM potassium phosphate (pH 6.0) containing PPD for 16 hr and then applied to a column (1.3 × 7.9 cm) of Reactive Green 19-Agarose preequilibrated with the same buffer. The column was washed with 10 mL of the same buffer and then the enzyme was eluted with a 40-mL linear gradient of NaCl (0 to 1.2 M) in the same buffer, followed by 1.2 M NaCl in the same buffer. The active fractions were pooled and concentrated, using an Ultrafree-PF membrane (10,000 NMWL; Millipore Corp.), and then stored at 0°.

*Electrophoresis and molecular weight determination*

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was carried out as described by Laemmli [18] and the gel was stained with a silver stain kit (Wako Chemicals, Tokyo, Japan). Calibrated gel filtration was performed on a Superose 12 column according to the method of Andrews [19].

*Isoelectric point determination*

The isoelectric point of HDC was determined by chromatofocusing on a Mono P HR 5/20 column. The enzyme was applied to a column (0.5 × 20 cm) preequilibrated with 25 mM bis-tris-iminodiacetic acid (pH 7.1) containing PPD. Elution was carried out with 35 mL of 10% Polybuffer™ 74 containing PPD, of which the pH had been adjusted to 4.0 with 0.1 M iminodiacetic acid.

*RNA preparation and Northern hybridization*

Total RNA was extracted from various mouse tissues by the acid guanidium thiocyanate-phenol-chloroform procedure essentially as described by Chomczynski and Sacchi [20]. Each RNA sample was electrophoresed on a 1.4% agarose gel and then transferred to a Hybond™ nylon membrane (Amersham Corp.). The membrane was hydridized to a $^{32}$P-labeled HDC cDNA insert [15] and then subjected to autoradiography.

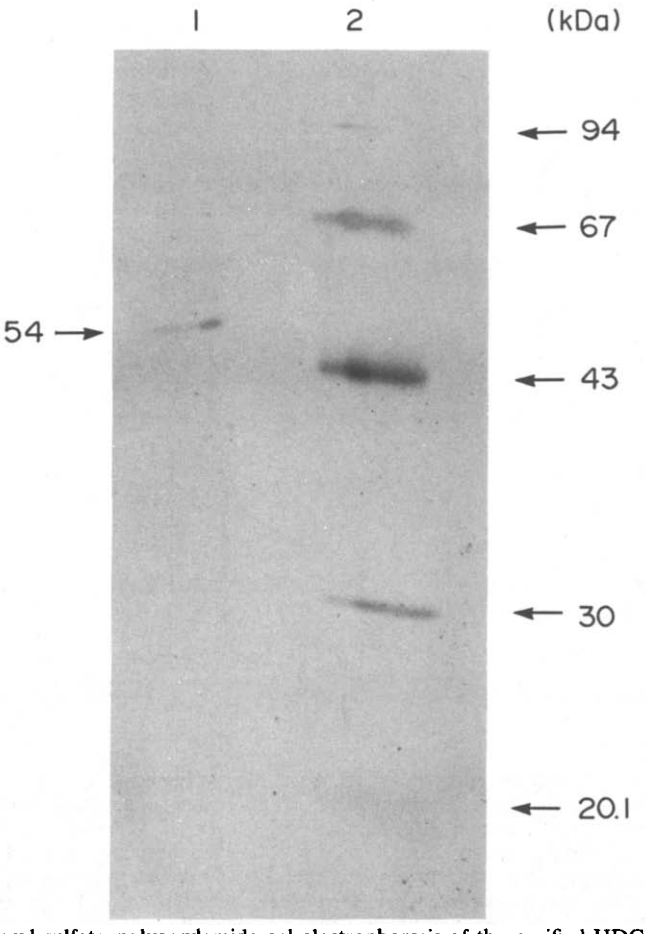


Fig. 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the purified HDC. Lane 1: the active fractions eluted from the Reactive Green 19-Agarose column were pooled, concentrated and then subjected to polyacrylamide gel electrophoresis (10% gel) in the presence of sodium dodecyl sulfate as described in the text. Lane 2: the marker proteins used were: phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa) and soybean trypsin inhibitor (20.1 kDa).

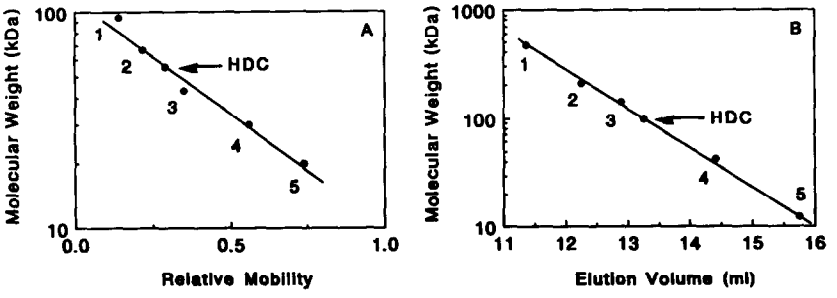


Fig. 3. Determination of the molecular mass and monomer molecular mass of HDC. (A) Polyacrylamide gel electrophoresis of HDC in the presence of sodium dodecyl sulfate. The relative mobilities were calculated from the results in Fig. 2. Key: (1) phosphorylase *b*; (2) bovine serum albumin; (3) ovalbumin; (4) carbonic anhydrase; and (5) soybean trypsin inhibitor. (B) Gel filtration of HDC on a Superose 12 column. The purified HDC was applied to a column (1.0 × 30 cm) of Superose 12 preequilibrated with 150 mM potassium phosphate (pH 7.0) containing 0.01 mM pyridoxal 5'-phosphate, 2% polyethylene glycol No. 300 and 0.2 mM dithiothreitol, and then eluted with the same buffer. The marker proteins used were: (1) ferritin (470 kDa); (2) catalase (210 kDa); (3) lactate dehydrogenase (140 kDa); (4) ovalbumin (43 kDa); and (5) cytochrome *c* (12.4 kDa).

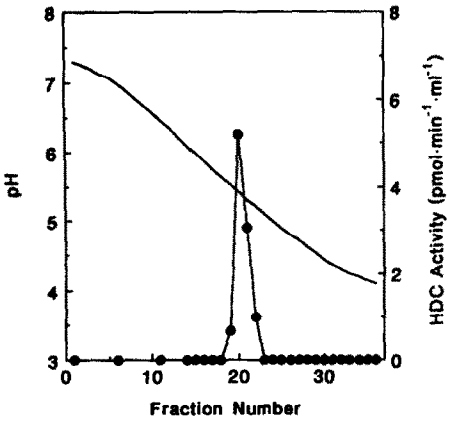


Fig. 4. Isoelectric point of HDC. The partially purified enzyme obtained at step 5 in Table 1 was applied to a Mono P column, as described in the text. Key: (●) enzyme activity; and (—) pH.

*Materials*

The following materials were obtained from the sources indicated: male mice of the $BDF_1$ strain from Shizuoka Experimental Animals and Agricultural Corp. (Hamamatsu, Japan); DEAE-Sepharose CL-6B, Phenyl-Sepharose CL-4B, Phenyl Superose HR 5/5, Mono Q HR 5/5, Superose 12 and an electrophoresis calibration kit from Pharmacia LKB Biotechnology (Uppsala, Sweden); hydroxylapatite from Bio-Rad Laboratories, (Richmond, CA, U.S.A.); a hydoxylapatite HPLC column (SH 0710F) from Asahi Pentax (Tokyo, Japan); and Reactive Green 19-Agarose, cytochrome *c* from horse heart, ovalbumin, lactate dehydrogenase from rabbit muscle, beef liver catalase and ferritin from horse spleen from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other chemicals of reagent grade were obtained commercially.

## RESULTS AND DISCUSSION

*Purification of the enzyme*

Table 1 summarizes the procedure that resulted in an overall 37,500-fold purification of the HDC, with a recovery of 1.6%, and Fig. 1 shows the elution profiles of the enzyme from the DEAE-Sepharose CL-6B (Fig. 1A), Phenyl-Sepharose CL-4B (Fig. 1B), first hydroxylapatite (Fig. 1C) and Reactive green 19-Agarose (Fig. 1D) columns. The final preparation exhibited specific activity of 750 $nmol \cdot min^{-1} \cdot mg^{-1}$, which is practically the same as that of the purified HDC from mastocytoma P-815 cells [6]. Throughout the purification, on several column chromatographies, the enzyme activity was always eluted as a single peak, i.e. without resolution into several active components, and no conspicuous loss of the enzyme activity was observed, except on the final Reactive Green 19-Agarose chromatography (Table 1). The low enzyme recovery at the final step may reflect the low protein concentration (less than 0.4 $\mu g/mL$; at this concentration, the enzyme was easily inactivated) of the enzyme solution from the Reactive Green 19-Agarose column. The dialysis at

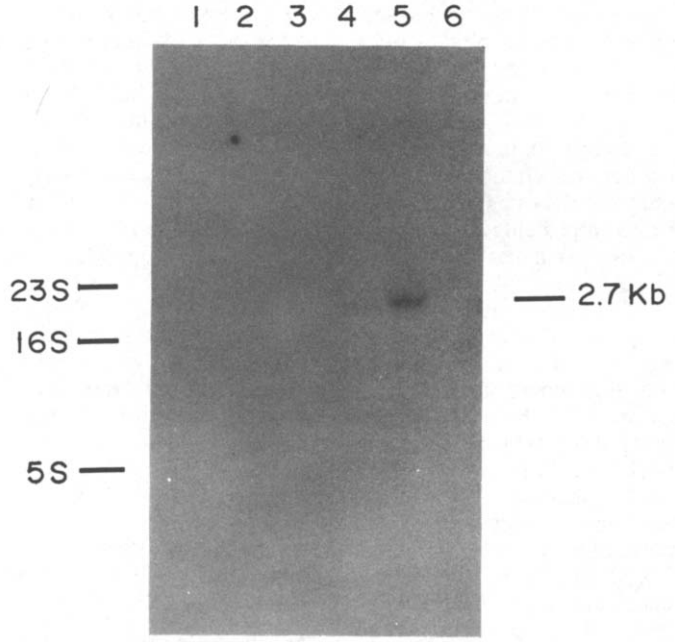


Fig. 5. RNA gel blot analysis of HDC. Twenty micrograms of each total RNA was electrophoresed and then transferred to a membrane. The hybridization was carried out using a $^{32}P$-labeled HDC cDNA insert as a probe and then the filter was subjected to autoradiography, as described in the text. Lane 1, brain; lane 2, spleen; lane 3, liver; lane 4, lung; lane 5, stomach; and lane 6, kidney.

pH 7.4 after the ammonium sulfate fractionation seemed to be essential to remove some impurities, which might have hampered further purification of the enzyme [6]. The final Reactive Green 19-Agarose chromatography was also necessary to obtain an electrophoretically homogeneous enzyme, even though the recovery of the enzyme activity was low.

*Purity*

When the purified enzyme was subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, a single protein band was observed when the gel was silver-stained (Fig. 2).

*Stability*

Although the purified HDC at a low protein concentration was quite unstable, no appreciable loss of enzyme activity was observed for at least 2 weeks when the enzyme solution from the Reactive Green 19-Agarose column was concentrated to more than 4 μg protein/mL, and stored at 0° in 50 mM potassium phosphate (pH 6.8) containing 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate and 2% polyethylene glycol No. 300.

*Molecular mass*

The molecular mass of the purified HDC estimated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was 54 kDa (Fig. 3A). The molecular size of the native enzyme estimated by gel filtration on a calibrated Superose 12 column was 100 kDa (Fig. 3B), suggesting that HDC from mouse stomach is composed of two identical subunits. These values are in agreement with those in the case of mouse mastocytoma cells [6], fetal rat liver [5] and mouse kidney [7].

*Isoelectric point*

Since the purified enzyme or near-pure enzyme preparation lost more than 95% of its initial activity when it was subjected to isoelectric focusing or chromatofocusing, the partially purified enzyme (enzyme solution at step 5 in Table 1) was used for determination of the isoelectric point. As shown in Fig. 4, the enzyme activity was eluted as a single peak from a Mono P chromatofocusing column, the isoelectric point of the enzyme being found to be pH 5.4. This value is the same as that of the enzyme of mastocytoma P-815 cells from the same $BDF_1$ mouse [6]. Yamada *et al.* [3] have suggested that there are two different types of HDC in rat stomach; one is a brain type, originating from non-mast cells, with a pI of 5.4, and the other, a fetal type, originating from mast cells, with a pI of 5.0. Furthermore, Savany and Cronenberger [14] demonstrated three types of HDC (with pI values of 5.9, 5.6 and 5.35) in rat gastric mucosa, and assumed that the enzyme forms having pI values of 5.35 and 5.6 represent the naturally occurring state of phosphorylated HDC, and that the enzyme with a pI of 5.9 is the dephosphorylated form. Very recently we identified two possible sites of phosphorylation by cAMP-dependent protein kinase in the cDNA-derived amino acid sequence of HDC from mouse mastocytoma P-815 cells [15]. However, we were unsuccessful in demonstrating phosphorylated HDC by incubating the purified HDC with the activated catalytic subunit of cAMP-dependent protein kinase A (data not shown). In the present study, we inferred that HDC activity in mouse stomach represented the activity of a single molecular species of HDC, because the enzyme activity throughout the purification, on several column chromatographies, was always recovered as a single peak without any remarkable loss of activity, and the one pI value obtained for HDC in the partially purified preparation indicates a single species, too. However, whether the purified enzyme obtained here is phosphorylated or not remains to be determined.

*pH Optimum*

The optimal pH for the enzyme reaction was about 6.2 under the standard assay conditions with 0.8 mM L-histidine and 100 mM potassium phosphate (data not shown). This value is somewhat lower than that (pH 6.6) of mastocytoma HDC [6].

*Kinetics*

From double-reciprocal plots of histamine formation with various concentrations of L-histidine under the standard assay conditions, the $K_m$ and $V_{max}$ values were calculated to be 0.29 mM and 1.03 $\mu mol \cdot min^{-1} \cdot mg^{-1}$, respectively. These values are quite similar to those obtained for mastocytoma HDC [6].

*Northern blot hybridization analysis of HDC mRNA*

To determine the size and species of the mRNA encoding the amino acid sequence of mouse stomach HDC, in addition to the tissue distribution of HDC mRNA, we carried out Northern hybridization using HDC cDNA from mouse mastocytoma P-815 cells [15] as a probe. As shown in Fig. 5, HDC mRNA was expressed mainly in stomach among the various mouse tissues examined, suggesting the physiological importance of the enzyme in the stomach. A trace amount of HDC mRAN was found in mouse lung. The HDC and mRNA of stomach migrated as a single 2.7-kb species, which is the same in size as that of mouse mastocytoma cell [15]. It is likely that the single form of HDC mRNA induces a single form of HDC subunit in mouse stomach.

In conclusion, mouse stomach contains a single species of HDC, which is quite similar to that of mouse mastocytoma P-815 cells [6] in molecular weight, isoelectric point, kinetic constants and mRNA size. The genetic relationship between mouse stomach HDC and mouse mastocytoma cell HDC is currently under investigation.

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