Purification and characterization of histidine decarboxylase from mouse kidney

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Histidine decarboxylase was purified 800-fold from the kidneys of thyroxine-treated mice. The purification procedure included precipitation of protein from a crude supernatant after heating it to 55 °C at pH 5.5, fractionation with \( \text{(NH}_4 \text{)}_2 \text{SO}_4 \), phosphocellulose column chromatography, chromatofocusing, DEAE-Sepharose column chromatography, gel filtration on Sephacryl S-300 and preparative polyacrylamide-gel electrophoresis. The native enzyme had an estimated \( M_r \) of 113000. The protein was analysed in SDS/10% polyacrylamide gels and formed a single band corresponding to a subunit \( M_r \) of 55000, indicating that it is a dimer. Three forms of the enzyme were resolved on isoelectrofocusing gels, with pI 5.3, 5.5 and 5.7.

INTRODUCTION

Histidine decarboxylase (HDC; EC 4.1.1.22) catalyses the single-step conversion of L-histidine into histamine. Histamine is involved in a wide range of biological processes, including gastric secretion, neurotransmission, the allergic response, rapid growth (including reparative and malignant growth), pregnancy and the control of microcirculatory homeostasis (Beaven, 1978; Rocha e Silva, 1966, 1978; Schwartz et al., 1982). Corresponding to the physiological diversity of histamine, HDC occurs in a wide range of mammalian tissues, including stomach, brain, skin, placenta and foetal tissues, although there are considerable species differences (see below). In the mouse, HDC activity is highest in the kidney. It has long been known that activity of the mouse kidney enzyme varies according to age and sex, and is elevated during pregnancy (Henningsson & Rosengren, 1972, 1975; Rosengren, 1966). More recently the enzyme has been shown to be inducible by thyroxine and oestrogen and repressible by testosterone (Bulfield & Nahum, 1978; Grahn et al., 1973; Henningsson & Rosengren, 1972; Schayer & Reilly, 1975); these hormones have no effect on HDC activity in 13 other tissues (S. A. M. Martin, unpublished work).

A large number of different HDC phenotypes have been discovered among inbred and wild strains of mice, as well as a substantial amount of genetic variation in the structure of the enzyme and its response to hormones (Bulfield & Nahum, 1978; Martin et al., 1984; Martin & Bulfield, 1984a, b). This variation can be used to investigate the control of (i) enzyme structure, (ii) tissue-specific expression, (iii) temporal expression and (iv) induction and repression by hormones. It is necessary to have pure HDC from mouse kidney in order to extend the biochemical-genetical analysis to the molecular level.

There have been few studies on the biochemistry of HDC because its purification has been difficult, largely owing to the lability of the enzyme and its low concentration in most mammalian tissues. More recently, however, HDC has been purified from a few mammalian sources, though there is some discrepancy between different estimates of its molecular size, subunit structure and isoelectric point. Savany & Cronenberger (1978, 1982) purified HDC from rat gastric mucosa and reported that the enzyme is a 95000-Mr monomer with three forms, with pl 5.35, 5.6 and 5.9. Hammar & Hjerten (1980) described HDC purified from mouse mastocytoma as a 2 x 55000-Mr dimer of pl 5.0. Two groups have purified HDC from foetal rat liver. Tan Tran & Snyder (1981) reported that the foetal rat liver enzyme has Mr 210000, with two unequal subunits of Mr 145000 and 66000, whereas Taguchi et al. (1984) report that it is a dimer of subunit Mr 54000 and has a pl of 5.1.

We report the purification of HDC from mouse kidney and find that its specific activity and molecular structure are in good agreement with the values reported by Taguchi et al. (1984) for foetal-rat liver HDC and by Hammar & Hjerten (1980) for mouse mastocytoma HDC. We also report that mouse kidney HDC exists in multiple isoelectric forms.

MATERIALS AND METHODS

Mice and thyroxine treatment

Female C57BL/10ScSn inbred mice were obtained from Bantin and Kingman, Grimston, Hull, U.K., and used at 2–3 months of age. HDC is inducible by thyroxine (Schayer & Reilly, 1975), and therefore kidneys from thyroxine-treated mice were used as a source of enzyme. The hormone was administered as a pellet of 30 mg of thyroxine (Sigma) compacted with 5 mg of cellulose implanted at the nape of the neck and drawn down the back. Kidney HDC was induced 40–50-fold after 10 days of treatment. Kidneys may be stored frozen at \(-70 \, ^\circ\text{C}\) for at least 6 months without loss of HDC activity.

Enzyme assay

HDC activity was determined by the release of \( ^{14}\text{CO}_2 \) from \( \text{DL-[carboxy-}^{14}\text{C]} \text{histidine} \) (Amersham International, Amersham, Bucks., U.K.) as described previously.

Abbreviations used: HDC, histidine decarboxylase; PLP, pyridoxal 5'-phosphate; DTT, dithiothreitol.
(Martin et al., 1984). Enzyme activity is expressed as munits (μmol of histidine utilized/min at 37°C).

**Protein assay**

Protein was determined by the method of Bradford (1976), with the Bio-Rad dye reagent and bovine serum albumin as a standard, or by measuring the $A_{280}$.

**Purification of histidine decarboxylase**

**Preparation of tissue extract.** All enzyme purification steps were carried out at 4°C. Kidneys (22.0 g) from thyroxine-implanted C57BL/10ScSn female mice were homogenized in 5 vol. (v/w) of 0.1 M-sodium acetate buffer, pH 5.5, with 0.1 mM-PLP (cofactor; Sigma), 1 mM-DTT (Sigma) and 0.01% NaN$_3$, and the homogenate was centrifuged at 15000 $g_{av}$ for 30 min. All HDC activity was in the soluble fraction.

**Heat precipitation.** The homogenate supernatant was heated to 55°C while being stirred for 7 min and centrifuged at 15000 $g_{av}$ for 30 min. The supernatant contained all the HDC activity.

**Fractionation with (NH$_4$)$_2$SO$_4$.** The supernatant was made 45% satd by adding satd. (NH$_4$)$_2$SO$_4$/0.1 mM-PLP dropwise over 1 h with continual stirring, and stirring was continued for at least 1 h more. The solution was centrifuged at 15000 $g_{av}$ for 30 min, and the pellet containing HDC was dissolved in 12 ml of 50 mM-potassium phosphate buffer, pH 6.8, containing 0.1 mM-PLP, 1 mM-DTT and 0.01% NaN$_3$ (Buffer A), and dialysed overnight against the same buffer.

**Phosphocellulose column chromatography.** The dialysis residue from the previous step was applied to a phosphocellulose (Whatman P11) column (2.5 cm$^2$ x 30 cm) equilibrated with Buffer A. The column was eluted with the same buffer at 5.1 ml/h per cm$^2$, and 6.5 ml fractions were collected. HDC was not absorbed to the column under these conditions and was recovered in the flow-through. Active fractions were pooled, concentrated to about 2 ml by ultrafiltration with an Amicon PM-30 membrane at 200 kPa (30 lb/in$^2$) pressure and made up to 10 ml with 25 mM-Bistris/1 mM-DTT/0.01% NaN$_3$, pH 6.7.

**Chromatofocusing.** The concentrate was applied to a Polybuffer exchanger PBE 94 (Pharmacia) column (1 cm$^2$ x 21.5 cm) equilibrated with 25 mM-Bistris/1 mM-DTT/0.01% NaN$_3$, pH 6.7. Chromatofocusing was achieved by eluting the column with a 1:8 dilution of Polybuffer 74 (Pharmacia) in water, pH adjusted to 4.0 with HCl, at 32 ml/h per cm$^2$. The eluate comprised a near-linear pH gradient and was collected in 3 ml fractions into tubes containing 3 ml of 100 mM-potassium phosphate/0.2 mM-PLP/1 mM-DTT/0.02% NaN$_3$, pH 6.8. HDC activity was eluted in a symmetrical peak around pH 5.4, with a shoulder of activity around pH 5.1 (Fig. 1). Active fractions were pooled and concentrated by using the PM-30 membrane as above, and made up to 5.7 ml with Buffer A.

**DEAE-Sepharose column chromatography.** The concentrate was applied to a DEAE-Sepharose CL 6B (Pharmacia) ion-exchange column (8 cm$^2$ x 41 cm) equilibrated with Buffer A. The column was developed with a linear gradient produced from 1 litre of Buffer A and 1 litre of 500 mM-potassium phosphate/0.1 mM-PLP/1 mM-DTT/0.01% NaN$_3$, pH 6.8. The flow rate was 12 ml/h per cm$^2$, and 6.5 ml fractions were collected. HDC activity was eluted in a single symmetrical peak around 125 mM-phosphate. Active fractions were pooled and concentrated as above, and made up to 6.2 ml with 100 mM-potassium phosphate/0.01 mM-PLP/1 mM-DTT/0.01% NaN$_3$, pH 6.8 (Buffer B).

**Sephacryl S-300 gel filtration.** The concentrate was applied to a column (4 cm$^2$ x 46 cm) of Sephacryl S-300 (superfine grade; Pharmacia) equilibrated with Buffer B. The column was eluted with the same buffer at a flow rate

![Fig. 1. Chromatofocusing of histidine decarboxylase](image-url)

Active fractions from the phosphocellulose column were pooled, concentrated and applied to a column of Sepharose 6B-Polybuffer exchanger (Pharmacia) which had been equilibrated with 25 mM-Bistris, pH 6.7. The column was eluted with Polybuffer 74 (Pharmacia), pH 4.0, giving the eluate a nearly linear pH gradient. HDC activity was eluted between pH 5.55 and 5.25. , Enzyme activity; ----, protein; ----, pH gradient.
of 3 ml/h per cm², and 4 ml fractions were collected. Fractions containing HDC activity were pooled and concentrated as above to 1.85 ml.

**Calibration of Sephacryl S-300 column for determination of Mₚ**

The elution volumes of Mₚ markers (thyroglobulin, 699000; ferritin, 440000; catalase, 232000; aldolase, 158000; bovine serum albumin, 67000; Pharmacia) were determined, and the fraction of the gel volume that was available for diffusion of each (Kᵥₘ) was calculated. The Mₚ of the HDC holoenzyme was calculated by regression analysis.

**Polyacrylamide-gel electrophoresis under non-denaturing conditions**

Portions (40 µl) of the concentrated sample from the Sephacryl S-300 column were electrophoresed in a 7.5% -polyacrylamide slab gel as described below for SDS/polyacrylamide-gel electrophoresis, except that no SDS was added to the gel, running buffer or sample buffer, and no 2-mercaptoethanol was added to the sample buffer. Some tracks were cut into 5 mm slices. Each slice was macerated in 200 µl of Buffer B, incubated overnight at 4 °C, and assayed for HDC activity. The rest of the gel was stained for protein in 0.1% Coomassie Brilliant Blue R (Sigma) in 25% (v/v) ethanol/8% (v/v) acetic acid for about 30 min at 37 °C, and destained in several changes of the same solvent at 37 °C. The band running at the position of the enzyme activity was cut from native gels and applied to denaturing SDS/polyacrylamide gels.

**SDS/polyacrylamide-gel electrophoresis**

This was performed in 10% -polyacrylamide gels as described by Laemmli (1970), and the gels were stained with Coomassie Brilliant Blue as described above or silver-stained by the method of Merril et al. (1979).

**Isoelectrofocusing**

This was performed on PAG plates (LKB Instruments), pH range 4.0–6.5, and the gels were either stained for 10 min at 60 °C with Coomassie Brilliant Blue and destained at the same temperature, or silver-stained as above. Some tracks were cut into 5 mm slices and assayed for HDC activity as described above.

### RESULTS

**Purification of histidine decarboxylase from mouse kidney**

HDC was purified by the series of nine steps described in the Materials and Methods section (Table 1). The specific activity of the enzyme was increased 800-fold relative to the initial kidney homogenate supernatant, to 166 munits/mg. The purification process was monitored by Coomassie Brilliant Blue staining of an SDS/10% -polyacrylamide slab gel (Fig. 2). The final preparation was a single protein band cut from a polyacrylamide gel run under non-denaturing conditions, which contained all the enzymic activity. When the protein in this band was subjected to electrophoresis under denaturing conditions, a single band was detected by Coomassie Brilliant Blue staining, and the purity of the sample was confirmed by staining with the 50–100 times more sensitive silver stain (Fig. 3); contaminating proteins comprising 1–2% of the sample would have been detected by this method.

Mₚ of the holoenzyme and subunits

Gel filtration on Sephacryl S-300 indicates an Mₚ of 113400 for the HDC holoenzyme. This step does not, however, significantly improve the purification of HDC, and decreases the yield (Fig. 2), and would therefore be better omitted from the purification procedure.

Electrophoresis of the pure enzyme in the presence of 2-mercaptoethanol in a 10% -polyacrylamide gel containing 0.1% SDS gave a single protein band corresponding to an Mₚ of about 55000 (Fig. 3). Thus HDC from mouse kidney behaves as a dimer consisting of two Mₚ 55000 subunits.

**Isoelectric point**

During the purification process, HDC was eluted from the chromatofocusing column at pH 5.4 (Fig. 1). This gives a first estimate of the pI of the enzyme. Portions of the concentrated sample from the Sephacryl S-300 column containing about 20 µg of protein were subjected to isoelectrofocusing in acrylamide gels with a pH gradient of 4.0–6.5. Chromatography on Sephacryl S-300 is the penultimate step in the purification procedure, and the concentrate contains some contaminating proteins (Fig. 2). HDC assayed in slices of isoelectrofocusing gel showed three peaks of activity. The major (central) peak

### Table 1. Purification of histidine decarboxylase from 22.0 g wet wt. of mouse kidneys

Experimental conditions were as described in the Materials and methods section. One munit of activity catalyses the decarboxylation of 1 µmol of histidine/min at 37 °C.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total activity (munits)</th>
<th>Total protein (mg)</th>
<th>Specific activity (munits/mg)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Homogenate</td>
<td>115</td>
<td>3270</td>
<td>0.04</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2. Supernatant</td>
<td>208</td>
<td>1000</td>
<td>0.21</td>
<td>(1.0)</td>
<td>100</td>
</tr>
<tr>
<td>3. Supernatant after 7 min at 55 °C</td>
<td>578</td>
<td>558</td>
<td>1.04</td>
<td>4.95</td>
<td>280</td>
</tr>
<tr>
<td>4. 0–45%-satd.-NH₄HSO₄ precipitate</td>
<td>483</td>
<td>40.6</td>
<td>11.9</td>
<td>56.7</td>
<td>230</td>
</tr>
<tr>
<td>5. Phosphocellulose</td>
<td>333</td>
<td>23.8</td>
<td>14.0</td>
<td>66.7</td>
<td>160</td>
</tr>
<tr>
<td>6. Chromatofocusing</td>
<td>190</td>
<td>2.42</td>
<td>78.8</td>
<td>375</td>
<td>91</td>
</tr>
<tr>
<td>7. DEAE-Sepharose CL 6B</td>
<td>45.8</td>
<td>0.377</td>
<td>122</td>
<td>581</td>
<td>22</td>
</tr>
<tr>
<td>8. Sephacryl S-300</td>
<td>16.7</td>
<td>0.136</td>
<td>124</td>
<td>591</td>
<td>8.0</td>
</tr>
<tr>
<td>9. Polyacrylamide-gel electrophoresis</td>
<td>13.3</td>
<td>0.080</td>
<td>166</td>
<td>791</td>
<td>6.4</td>
</tr>
</tbody>
</table>
corresponds to a pI of $5.50 \pm 0.06$ ($n = 5$ gels), and the pI values for the other two peaks are $5.29 \pm 0.06$ and $5.71 \pm 0.05$ (Fig. 4).

**Catalytic-centre activity of histidine decarboxylase**

The catalytic-centre activity for HDC purified from mouse kidney was calculated to be 0.315/s. Such a low value raises the possibility that HDC might not be the only component of the final preparation. However, such a low value is a characteristic of many decarboxylases (Table 2). The value of 0.315/s agrees closely with that for HDC purified from the livers of foetal rats (0.470/s; estimated from the data of Taguchi et al., 1984). Since the two preparations were purified from different tissues and organisms by different methods, it is unlikely that they contain the same major contaminant. Therefore there is little doubt that this preparation is homogeneous.

**DISCUSSION**

There has long been interest in the many biological roles of histamine, yet HDC, the enzyme responsible for its one-step synthesis from histidine, has only recently been purified and characterized biochemically. There are, however, problems in purifying HDC, mainly owing to the very low amount of it present in mammalian tissues and its lability, especially on purification (Rocha e Silva, 1978).
Table 2. Catalytic-centre activities of decarboxylase enzymes purified from various sources

HDC from *Lactobacillus 30a* differs from the mammalian enzyme in $M_r$ and subunit structure, and does not require PLP as cofactor.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Turnover number (per s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOPA decarboxylase</td>
<td>Hog kidney</td>
<td>16.18</td>
<td>Christenson <em>et al.</em> (1970)</td>
</tr>
<tr>
<td>Glutamate decarboxylase</td>
<td>Catfish brain</td>
<td>7.25</td>
<td>Su <em>et al.</em> (1979)</td>
</tr>
<tr>
<td>Histidine decarboxylase</td>
<td><em>Lactobacillus 30a</em></td>
<td>220.2</td>
<td>Chang &amp; Snell (1968a,b)</td>
</tr>
<tr>
<td>Histidine decarboxylase</td>
<td>Foetal rat liver</td>
<td>0.47 (estimated)</td>
<td>Taguchi <em>et al.</em> (1984)</td>
</tr>
<tr>
<td>Histidine decarboxylase</td>
<td>Mouse kidney</td>
<td>0.315</td>
<td>This paper</td>
</tr>
</tbody>
</table>

Mouse kidney HDC is highly inducible by thyroxine (Schayer & Reilly, 1975). The specific activity of thyroxine-induced HDC kidney homogenate supernatants (0.21 munit/mg) is twice that in similar preparations from mouse mastocytoma (Hammar & Hjerten, 1980) and foetal rat liver (Taguchi *et al.*, 1984), 12 times that in homogenates of whole foetal rats (Watanabe *et al.*, 1979), and more than 20 times that reported in foetal-rat liver supernatants by Tan Tran & Snyder (1981). Thus kidneys from thyroxine-treated mice provide a very useful source of the enzyme.

During the early stages of purification, yields of HDC are good, but there is a characteristic sharp loss of activity as the purity of the enzyme increases in the later stages (Taguchi *et al.*, 1984; Tan Tran & Snyder, 1981; Watanabe *et al.*, 1979). During the purification of mouse kidney HDC, enzyme activity is increased 5.0-fold at steps 1–3 (Table 1). This may be due to the inactivation or loss of an endogenous inhibitor, or to some other ‘activation’ of HDC activity. There is evidence for the destruction of HDC by proteinases in various tissues of the rat (Leinweber & Braun, 1970; Yamada *et al.*, 1980). However, proteolysis does not seem to affect the HDC activity of crude mouse kidney homogenates, since the amount of CO$_2$ evolved is linear with time for 3 h under standard assay conditions. On the other hand, the presence of an inhibitor has been observed in gastric tissue (Levine & Watts, 1966). Dialysis of kidney homogenates leads to an increase in HDC activity, suggesting that a diffusible endogenous inhibitor may be involved. An increase in the activity of homogenates has also been observed after heating (S. A. M. Martin, unpublished work). Therefore the activation of HDC early in its purification seems to be largely a consequence of heating the homogenate supernatant to 55 °C at step 3 (Table 1), and may be due to the inactivation of an inhibitor.

The specific activity of pure HDC from mouse kidney is 166 munits/mg, which is in good agreement with the value estimated by Taguchi *et al.* (1984), and higher than the values obtained for HDC from some other sources (Hammar & Hjerten, 1980; Savany & Cronenberger, 1982; Tan Tran & Snyder, 1981). The final yield, 6.4% (Table 1), was poor, owing to a sharp loss of activity during the final stages of purification, consistent with the observations by other workers (Taguchi *et al.*, 1984; Tan Tran & Snyder, 1981; Watanabe *et al.*, 1979). The penultimate preparation in 100 mM-potassium phosphate buffer, pH 6.8, containing 0.1 mM-PLP, 1.0 mM-DDT, 0.01% NaN$_3$ and 2% glycerol was stable for 2–3 weeks at 4 °C, but once isolated in polyacrylamide gel the enzyme lost 95% of its activity in 48 h.

There is disagreement as to the molecular size and subunit structure of mammalian HDC (Hammar & Hjerten, 1980; Savany & Cronenberger, 1982; Taguchi *et al.*, 1984; Tan Tran & Snyder, 1981), and some of these discrepancies may arise from impurity of the preparations. The $M_r$ of the native mouse kidney enzyme was calculated to be 113000 by gel filtration on Sephacryl S-300, and that of the enzyme in SDS was found to be about 55000 (Fig. 3). These results are in good agreement with those obtained with the mouse mastocytoma enzyme (Hammar & Hjerten, 1980) and those of Taguchi *et al.* (1984) for HDC purified from the livers of foetal rats, but quite different from those of Tan Tran & Snyder (1981) (also purified from foetal rat liver) and those estimated for rat gastric mucosa HDC (Savany & Cronenberger, 1978, 1982). The subunit $M_r$ values suggested by Tan Tran and Snyder (145000 and 66000) are similar to those of HDC isolated from *Lactobacillus* (Snell, 1977), but their final preparation may not have been pure. The specific activity was considerably lower than that reported by others (Hammar & Hjerten, 1980; Savany & Cronenberger, 1982; Taguchi *et al.*, 1984), and bands other than the two major ones are seen on SDS/polyacrylamide-gel electrophoresis (Tan Tran & Snyder, 1981).

The pI of HDC from various mammalian sources is in the range 5.0–6.0, and multiple forms have been reported in some cases (Hammar & Hjerten, 1980; Savany & Cronenberger, 1978, 1982; Taguchi *et al.*, 1984; Yamada *et al.*, 1984). Three forms of HDC in rat gastric mucosa are resolved on refocusing into multiple bands (Savany & Cronenberger, 1982). Partially purified HDC from mouse kidney exists in three forms, of pI 5.3, 5.5 and 5.7 (Fig. 4). These different forms might be conformational isomers, or the products of different structural genes, or the products of post-translational modification of the native enzyme, or might be due to partial proteolysis, deamination or oxidation during handling. A single structural gene has been identified for mouse kidney HDC (Martin & Bullfield, 1984a). On the other hand, there is some evidence that the enzyme is modified post-translationally: phosphorylation by cyclic-AMP-dependent protein kinase inhibits the activity of HDC in crude preparations of mouse mastocytoma (Hammar, 1983). At the present time the basis of the multiple isoelectric forms of the enzyme remains unknown.

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REFERENCES


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