Isolation and properties of multiple forms of histidine decarboxylase from rat gastric mucosa

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(Received 4 March 1982/Accepted 30 April 1982)

The heterogeneity of histidine decarboxylase from rat gastric mucosa was studied. The partially purified enzyme was fractionated by preparative isoelectric focusing on a flat-gel bed by using narrow-pH-range carrier ampholytes and a short focusing time. The activity was resolved, with about 95% recovery, into three forms, designated I, II and III, with pI values of 5.90, 5.60 and 5.35 respectively. These three forms exhibited similar molecular weights, indicating that the forms were not the result of different degrees of polymerization. By preparative refocusing each form refocused as a single peak of enzyme activity with reproducible pI, but a high loss of activity occurred with repeated focusing. Forms I, II and III were purified by the combined use of preparative isoelectric focusing and gel chromatography and other fractionation methods. The active forms could be distinguished by electrophoresis and isoelectric focusing on polyacrylamide gels and displayed protein heterogeneity. These forms were found in the crude extract and in the partially purified preparations in the presence or absence of proteinase inhibitors. Form II had the highest specific activity, but all three forms had the same optimum pH and K_m value for histidine.

Histidine decarboxylase (EC 4.1.1.22) is the specific enzyme responsible for the formation of histamine in tissues (Beaven, 1978). A high activity is found in the gastric mucosa of many mammalian species (Håkanson, 1970; Noll & Levine, 1970), and the enzyme appears to play an important role in the gastric functions. Most studies have been concerned with the physiological and pharmacological aspects of enzyme activity; for example, previous work indicates that the gastric histidine decarboxylase activity is related to the concentration of circulating gastrin (Håkanson *et al.*, 1974). However, since the work of Håkanson (1970) and Aurès & Håkanson (1971) on partially purified extracts, very few attempts have been made to purify further and characterize this enzyme, related in part to the lability of enriched enzyme preparations.

We previously obtained the specific gastric histidine decarboxylase with a high specific activity (Savany & Cronenberger, 1978); the enzyme consists of several forms that can be distinguished by analytical thin-layer isoelectric focusing on polyacrylamide gel, as they have characteristic isoelectric points. A large number of enzymes are found to exist as multiple molecular forms; several forms of other mammalian decarboxylases, e.g. of ornithine decarboxylase from rat liver (Obenrader & Prouty,

1977), have been reported. The multiplicity might be due to genetically independent proteins, or to post-translational modification of a native enzyme, or to conformational isomers (Hayes & Wellner, 1969; Wilkinson, 1965; Gockel & Lebherz, 1981). In order to elucidate the basis of the observed histidine decarboxylase heterogeneity, the individual forms must be isolated. Isoelectric focusing is a high-resolution technique (Righetti & Drysdale, 1974; Vesterberg, 1975), and thin-layer preparative isoelectric focusing in a granulated gel has revealed itself to be a powerful tool for protein fractionation and purification (Radola, 1973, 1974). The present study provides evidence that the enzyme occurs in several forms and, at the same time, reports the application of thin-layer preparative isoelectric focusing for the isolation and purification of individual forms, so that further characterization of their physical and kinetic properties could be accomplished.

Experimental

Chemicals

L-Histidine, pyridoxal 5'-phosphate, α -methyl-L-dihydroxyphenylalanine and 2-mercaptoethanol were obtained from Fluka, Buchs, Switzerland; DL-[carboxy- 14 C]histidine (50 Ci/mol) was from The Radiochemical Centre, Amersham, Bucks., U.K. All

Sephadex gels were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Ampholine carrier ampholytes, Ampholine polyacrylamide-gel plates and Ultrogel AcA 44 were supplied by LKB Produkter, Bromma, Sweden. Coomassie Brilliant Blue R-250 and chemicals for electrophoresis were from Merck, Darmstadt, Germany. Pepstatin A, ϵ -amino-*n*-hexanoic acid and bovine serum albumin were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Enzyme purification

Specific histidine decarboxylase was partially purified from the mucosa covering the glandular part of the stomach of adult Sprague–Dawley male rats (Savany & Cronenberger, 1978). All steps were carried out at 4°C. Homogenization was performed in 6 vol. (v/w) of 100 mM-sodium acetate buffer, pH 5.5, containing 10 μ M-pyridoxal phosphate, 1 mM-2-mercaptoethanol and 10 mM-EDTA. The supernatant after centrifugation at 50 000 *g* for 1 h was adjusted to 43% saturation with $(\text{NH}_4)_2\text{SO}_4$ (263.4 g/l). The suspension was centrifuged at 30 000 *g* for 20 min and the precipitated proteins were dissolved in 50 mM-potassium phosphate buffer, pH 6.8, containing 10 μ M-pyridoxal phosphate, 1 mM-mercaptoethanol and 10 mM-EDTA. The 27–40%-satd. $(\text{NH}_4)_2\text{SO}_4$ fraction (80.6 g/l) was prepared and the resulting pellet was dissolved in 50 mM-potassium phosphate buffer, pH 6.8, and applied to a DEAE-Sephadex A-50 column (2.5 cm $\times$ 18 cm), equilibrated with this buffer, and eluted at pH 6.8 with 330 ml of a linear potassium phosphate buffer gradient (50–500 mM). The pooled enzyme fractions, concentrated by $(\text{NH}_4)_2\text{SO}_4$ precipitation, were dialysed overnight against 2 mM-potassium phosphate buffer, pH 6.8, containing 10 μ M-pyridoxal phosphate, then concentrated to 3 ml by ultrafiltration.

Enzyme assay

Histidine decarboxylase activity was assayed by measuring $^{14}\text{CO}_2$ released from DL-[carboxy- ^{14}C]-histidine at 37°C in a reaction mixture of 1.2 ml containing 20 μ M-pyridoxal phosphate, 50 μ M-L-histidine and 50 mM-potassium phosphate buffer, pH 6.8, as described by Savany & Cronenberger (1978). Studies with $\text{Na}_2^{14}\text{CO}_3$ showed that the CO_2 recovery was 95%. With the crude extract as well as with the separated forms, the enzyme activity was linear with time throughout the duration of the assay. Ampholines did not affect enzyme activity. One unit of enzyme activity was defined as the amount of enzyme that produced 1 pmol of product/min under the assay conditions. Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Kinetic properties

The dependence of activity on pH was determined by incubating each form with 0.4 mM-histidine in 0.1 M-potassium phosphate buffer in the pH range 5.7–8. The kinetic parameters for the various forms were determined in 50 mM-potassium phosphate buffer, pH 6.8, with various concentrations of unlabelled substrate (33.3–300 μ M) and a constant amount of radioactivity. Estimations of K_m and V were performed by means of an iterative non-linear fitting program, based on the Marquardt (1963) algorithm, by using a Michaelian model. The square of the multiple correlation coefficient was always higher than 97%.

Preparative isoelectric focusing

Preparative isoelectric-focusing procedures were performed as described by Radola (1974) and Winter (1977) on a flat-bed system (LKB Multiphor) with some modifications. The layer of Sephadex G-75 (superfine grade), 23 cm $\times$ 11 cm $\times$ 0.3 cm, contained 2% (w/v) of Ampholines pH 5–8, giving a pH gradient of 5–7.5. The sample (3 ml) was applied halfway towards the electrodes by mixing a slice of gel with the sample and repouring. The migration was along the large dimension. Focusing was run at 4°C and 500 V for 1.5 h, followed by 900 V for 1 h; the voltage was then raised to 1000 V for an additional 5 h. At the end of the focusing run, the gel was sliced into 7 mm-wide bands. The pH was determined at 4°C on gel samples mixed with 1 ml of water (Righetti & Caravaggio, 1976). The proteins were eluted from each gel band with 6 ml of 50 mM-potassium phosphate buffer, pH 6.8, containing 10 μ M-pyridoxal phosphate, and 15 min centrifugation at 1500 *g*; then the pH was adjusted to 6.8 for activity measurement.

Gel chromatography

Pooled active fractions from isoelectric focusing were concentrated by ultrafiltration and applied to an Ultrogel AcA 44 calibrated column (89 cm $\times$ 2.5 cm). The column, equilibrated at 4°C with 50 mM-potassium phosphate buffer, pH 6.8, containing 10 μ M-pyridoxal phosphate, was eluted with the same buffer (flow rate 50 ml/h). The void volume ($V_0 = 177$ ml) was determined with Blue Dextran 2000.

Analytical isoelectric focusing

Analytical isoelectric focusing was performed on LKB polyacrylamide-gel plates pH 3.5–9.5 and 4–6.5 (Davies, 1975). Samples were applied to polyacrylamide gel with Whatman 3 MM papers 1 cm from the cathode. Gels were run at 4°C for 2 h with a maximum voltage of 1000 V. With pH 4–6.5 gradient plates, samples were applied 1 cm from the

anode, and gels were run at 4°C for 2.5 h. The gels were sliced into 2 mm-wide bands, the pH of which was measured; these bands were ground in 50 mM-potassium phosphate buffer, pH 6.8, and measured for enzyme activity. Gels were stained for proteins with Coomassie Brilliant Blue R-250 as specified in LKB instructions and by Vesterberg *et al.* (1977).

Electrophoresis

Disc electrophoresis was performed in 7.5% polyacrylamide gel with 25 mM-Tris/200 mM-glycine buffer, pH 8.5 (Ornstein, 1964). The separations were run at 4°C and the current was kept constant at 2 mA/gel; Bromophenol Blue was used as the tracking dye. Gels were stained for proteins with Coomassie Brilliant Blue R-250.

Results

Preparative isoelectric focusing

Analytical isoelectric focusing on polyacrylamide gel revealed that the partially purified histidine decarboxylase preparation from rat gastric mucosa contained several active forms within a pH range of 5–6.1. For the isolation of individual forms, preparative isoelectric focusing on Sephadex gel was used in narrow-pH-range (5–7.5) carrier ampholytes; Fig. 1 illustrates the results obtained when a partially purified histidine decarboxylase preparation was subjected to isoelectric focusing for 7.5 h. After this time interval focusing was complete, since a constant pattern developed that did not change when focusing was continued, no active material remained at the point of application and there was no protein precipitation regardless of the amount of protein applied. The enzyme activity mainly focused between pH 5 and 6.1, and three distinct peaks were apparent. The major peak of enzyme activity appeared at pI about 5.60. The second peak, more anodic and with a lower activity, focused at pI 5.35. A third peak, the most cathodic, and with a lower activity than the other two, had pI 5.90. The pH gradient showed minor deviations between experiments, and sometimes this third peak appeared as a shoulder of the adjacent peak. The sum of the activities of individual gel bands (Fig. 1) gave a recovery of about 95% of the applied histidine decarboxylase activity. Similar focusing patterns were obtained when extracts were prepared from fresh or frozen gastric mucosa. As compared with the polyacrylamide-gel isoelectric-focusing technique, there was a small variation in the estimated pI values (about 0.1 pH unit) related to the ionic parameters of the pH gradient, or this might be due to differences in temperature during the separation.

Isolation and purification of the multiple forms

The enzyme-activity profile shown in Fig. 1 was

Fig. 1. Preparative isoelectric focusing of histidine decarboxylase

The focusing was performed in a pH gradient of 5–7.5 on plates of 23 cm × 11 cm, the thickness of the Sephadex G-75 (superfine grade) layer being 0.3 cm. Partially purified enzyme preparation (80 mg of protein; 3 ml) was applied 10 cm from the cathode and focusing was run for 7.5 h at 4°C. O, pH gradient; ●, enzyme activity.

divided into three fractions, designated forms I, II and III according to their pI. The forms contained 12.1, 43.4 and 18.7% respectively of the initial activity. An additional purification of the separated forms was performed by gel filtration on Ultrogel AcA 44 (Table 1). As shown in Fig. 2 for form II, a single symmetrical peak of enzyme activity was obtained for each form, with an activity recovery from each column of about 80%. The resulting elution volumes were close together. After calibration of the gel column with standard proteins, the molecular weights of these three forms were found to be about 95 000.

Preparative refocusing was performed with granulated gel in the pH range 5–7.5 after concentration of each form by ultrafiltration (activity recovery was 75–80%). Typical refocusing separations of forms I, II and III are presented in Fig. 3. Each form refocused as a single peak of enzyme activity, without significant contamination by the adjacent forms, at the same pI value as that observed after the initial focusing. At the maximum activity peak the pI values were 5.90, 5.60 and 5.30 for forms I, II and III respectively. The minor pI deviations observed presumably came from small variations in the volume of gel samples scraped from the plate for pH measurement. Pooled active gel bands from each form I, II and III gave a histidine decarboxylase activity recovery of about 25, 23 and 21% respectively of the applied enzyme activity (Table 1).

After preparative refocusing, the removal of carrier ampholytes and an additional purification of each form were performed by gel filtration. Similar elution characteristics as for the above chromatography were obtained, but enzyme-activity recoveries ranged only from 40 to 65%. The results of the isolation procedure of each form are summarized in Table 1. The preparations were obtained

Fig. 2. Gel chromatography of histidine decarboxylase on Ultrogel AcA 44

Form II (from Fig. 1) was concentrated to 7 ml and applied to an Ultrogel AcA 44 column (89 cm $\times$ 2.5 cm), eluted with 50 mM-potassium phosphate buffer, pH 6.8, containing 10 μ M-pyridoxal phosphate (flow rate 50 ml/h; fraction volume 2.5 ml). Void volume was 177 ml (fraction 71). —, A_{280} ; ●, histidine decarboxylase activity.

with activity recoveries of about 6–10% of the separated forms. Form II exhibited the highest specific activity, increased about 13-fold as compared with the 'DEAE-Sephadex extract dialysed' referred to as starting material.

Polyacrylamide-gel electrophoresis and isoelectric focusing

The enzyme forms separated as described above were analysed by polyacrylamide-gel electrophoresis (Fig. 4). The insufficient amount of form I did not give rise to any detectable protein band. Active form II exhibited two major protein-staining bands: the most cathodic band, highly intense, was wide and rather diffuse and with a tendency to divide into two separate bands, as revealed by a shoulder of the densitometric scan at 580 nm; the second protein band, less intense, was wide and migrated faster towards the anode. Form III appeared as a major protein band with an electrophoretic mobility slightly faster than that of the most anodic band of form II, and a minor protein band, more cathodic.

Analytical refocusing patterns of the isolated enzymic forms are shown in Fig. 5. Both forms II and III displayed several protein-staining components in varying proportions, as if proteins separated by disc electrophoresis underwent further subdivision. A complex pattern was obtained with form II; a densitometric analysis of focused material revealed as many as 18 protein bands. Form III was also resolved into multiple components which focused, mainly as a group, in a more-acidic zone

Fig. 3. Preparative refocusing of each histidine decarboxylase form

The focusing was performed in a pH gradient of 5–7.5 on 23 cm $\times$ 11 cm $\times$ 0.3 cm Sephadex G-75 (superfine grade) plates. Each form I, II and III (isolated as in Fig. 1), from Ultrogel AcA 44 chromatography, was concentrated to 3 ml and applied 10 cm from the cathode. Focusing was run for 7.5 h at 4°C. ▽, pH gradient; ●, enzyme activity. The arrows indicate the position of the maximum activity peak of other forms. (a), form I; (b), form II; (c), form III.

Table 1. *Isolation and purification of several forms of histidine decarboxylase*

The three enzyme forms, from 66 g of rat gastric mucosa, were purified together up to the step 'DEAE-Sephadex extract dialysed' and concentrated to 3 ml as described in the Experimental section. Subsequently this enzyme preparation was referred to as starting material; it represented the initial 50000 g supernatant extract (2.58 units/mg) purified 21-fold with an activity yield of 57%. The values in parentheses are referred to the assumed 100% recovery of each of the separated forms I, II and III.

Step	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Yield (%)	Purification (fold)
DEAE-Sephadex extract dialysed	4415	80.1	55.1	100	1
Isoelectric focusing*					
Form I	535			12.1 (100)	
Form II	1915			43.4 (100)	
Form III	828			18.7 (100)	
Ultrogel AcA 44					
Form I	463	1.31	353.4	10.5 (86.5)	6.4
Form II	1488	4.11	362.0	33.7 (77.7)	6.6
Form III	653	3.16	206.6	14.8 (78.9)	3.7
Second isoelectric focusing*					
Form I	88			2.0 (16.5)	
Form II	278			6.3 (14.5)	
Form III	110			2.5 (13.3)	
Second Ultrogel AcA 44					
Form I	35	0.08	437.5	0.8 (6.5)	7.9
Form II	177	0.25	708.0	4.0 (9.2)	12.8
Form III	60	0.23	260.9	1.4 (7.2)	4.7

* Enzyme activity was measured in the presence of Ampholines.

Fig. 4. *Polyacrylamide-gel electrophoresis of separated histidine decarboxylase forms*

Acrylamide gels (7.5%) were prepared and electrophoresis of form II (60 µg) and form III (40 µg) enzymes was performed at 2 mA/gel for 2 h and 4°C. The cathode was at the top. The gels were stained with Coomassie Brilliant Blue R-250.

Kinetic properties

Kinetic parameters were studied by using the graphic Lineweaver-Burk method and a computer-aided curve-fitting procedure. The effect of substrate concentration on the enzyme activity indicated that in all three cases Michaelis-Menten kinetics were evident. The usual statistical tests that were appropriate in the linear model case were not appropriate when the model was non-linear, and Table 2 shows that forms I, II and III had similar K_m values for histidine. They were in good agreement with the K_m of 0.19 mM obtained with a partially purified unresolved enzyme preparation containing the three forms (A. Savany & L. Cronenberger, unpublished work). The apparent maximum velocities for forms I and III were similar, and different (1.7-fold lower) from that calculated for form II.

Forms I and II appeared to be more stable than form III when stored in 50 mM-potassium phosphate buffer, pH 6.8, containing 10 µM-pyridoxal phosphate. Both forms I and II could be stored at -20°C for at least 6 weeks with no significant loss of activity, whereas the activity of form III declined steadily.

The pH-activity profiles for the three enzyme forms were similar, and forms I, II and III had a pH optimum of 6.8 in 0.1 M-potassium phosphate buffer and with 0.4 mM-histidine.

than those of form II. Seven major protein components, one of them being predominant, were detected by densitometric scan.

Table 2. *Properties of histidine decarboxylase forms*

K_m and V were derived from computer curve-fitting to a Michaelian model. Results are given $\pm$ s.d. for the numbers of observations in parentheses. All experimental details are indicated in the text.

Enzyme	pI	pH optimum	K_m (μ M)	V (pmol/min per mg)
Form I	5.90	6.8	154 ± 15 (11)	682 ± 35 (11)
Form II	5.60	6.8	181 ± 29 (12)	1198 ± 99 (12)
Form III	5.35	6.7–6.8	213 ± 38 (12)	708 ± 69 (12)

Fig. 5. *Analytical isoelectric focusing of separated histidine decarboxylase forms*

Analytical isoelectric focusing was performed on LKB polyacrylamide-gel plates pH 3.5–9.5. Samples (50 μ g) were applied 1 cm from the cathode. Runs were performed at 4°C for 2 h. Proteins were stained with Coomassie Brilliant Blue R-250. II = form II; III = form III.

Multiple forms in crude enzyme preparation

When the 50000 g supernatant from the gastric-mucosa homogenate prepared in the presence of 1 mM-2-mercaptoethanol was analysed by polyacrylamide-gel isoelectric focusing in ampholytes in the pH range 4–6.5, three distinct peaks of enzymic activity were observed. The pI values of these peaks corresponded to those obtained with the partially purified enzyme subjected to isoelectric focusing. The enzyme activity was measured in the presence of 1 mM- α -methyl-L-dihydroxyphenylalanine in the standard assay mixture. On the other hand, enzyme purification was performed under conditions chosen to minimize proteolysis and in sterile medium. Proteinase inhibitors used were not inhibitory for the enzyme under the conditions tested. Gastric mucosa, quickly scraped off, was homogenized in 5 μ M-pepstatin and 5 mM- ϵ -aminohexanoic acid in the

standard buffer; the 50000 g supernatant was filtered through a 0.22 μ m Millipore filter. After immediate analytical isoelectric focusing in pH 4–6.5 ampholytes, the crude enzyme-activity profile, measured in the presence of 1 mM- α -methyl-L-dihydroxyphenylalanine, displayed the same three peaks as those obtained with the crude enzyme prepared without inhibitor. The presence of these inhibitors throughout the typical purification procedure did not alter focusing and chromatographic profiles, nor enzyme specific activity; the final enzyme preparation showed the same heterogeneity as the enzyme prepared without inhibitor.

Discussion

Thin-layer isoelectric focusing on a granulated gel provides a new approach for the isolation of several histidine decarboxylase forms displaying different charges. The shape of the thin-layer gel and the efficient cooling are favourable for heat dissipation, thus permitting the use of high voltage. Isoelectric focusing performed by the method described here is complete in a relatively short time; this is an advantage over the preparative method on a sucrose-gradient column. The recovery of the enzyme activity is better with such a short focusing time, and the cathodic drift of the resulting pH gradient is decreased. Focusing patterns are independent of protein loading (within the load capacity; Radola, 1974) and of the position of sample application on the gel plate. In the narrow-pH-range (5–7.5) ampholytes, a heterogeneous pattern of enzyme activity is demonstrated: histidine decarboxylase exhibits three active forms, with pI values of about 5.35, 5.60 and 5.90. At least 50% of the recovered enzyme activity is located in the peak focusing at pI 5.60; activities at pI 5.90 and 5.35 represent about 30 and 50% respectively of the activity of the predominant form. Preparative isoelectric focusing used for this purification step appears to be a mild technique, since the recovery of the total activity is nearly complete.

Investigations were made to elucidate the basis of the observed enzyme heterogeneity. The multiplicity of forms may arise in several ways: isoenzymes reflect genetically determined differences giving rise

to distinct physico-chemical properties; multiple forms may also be attributed to post-synthetic modifications of a native protein and modifications also arising from separation and purification processes. Heterogeneity may result from focusing artifacts, and a pattern exhibiting multiple forms does not necessarily represent inherent heterogeneity (Hare *et al.*, 1978). In several cases, binding of carrier ampholytes to proteins or interactions between macromolecules is given as an explanation for the observed complex isoelectric-focusing patterns (Wallevick, 1973; Guengerich, 1979). Multiple forms differing in surface charge may also arise from the chemical alteration of the covalent structure during focusing, e.g. deamidation or anodic oxidation (Williamson, 1973). Thus isolated histidine decarboxylase forms were refocused to see if they appear as single active entities. Preparative refocusing in the narrow-pH-range (5–7.5) Ampholines resulted in a good fractionation; each form refocused as a single peak of enzyme activity with pI values similar to those observed in the original experiment. This indicates that the three active forms display charge differences and that they do not redistribute when separated and refocused.

Differences in molecular aggregation states may be responsible for enzyme multiplicity (Hammar & Hjerten, 1980). Each of the three isolated forms can be further purified by gel chromatography, yielding single peaks of activity and being characterized by the same elution volume. This indicates that the molecular weights of the enzyme forms are similar, and that a form is not the polymer of another; at the same time, these results show that molecular sieving on a focusing gel bed does not contribute to the isoelectric-focusing patterns obtained.

A striking point is the important loss of histidine decarboxylase activity on preparative refocusing. Thus prolonged exposure to unfavourable pH may result in a destabilization of the active conformation of the enzyme. Similar findings with carboxypeptidase N are related to removal of sialic acid residues, and the existence of several isoelectric forms is attributed to differences in their carbohydrate content (Koheil & Forstner, 1978).

The isolation procedures used for histidine decarboxylase are very mild conditions. The three forms are not an artifact of the purification process, since they are observed in the crude extract, always prepared in the presence of a reducing agent, as well as in the final enzyme preparation from fresh or frozen tissues. It is therefore concluded that the multiple enzyme forms pre-exist in the gastric-mucosa homogenate.

On the other hand, proteolytic modification of the native protein (Yamada *et al.*, 1980) can induce enzyme multiplicity. However, it seems that the three forms are not formed by proteolytic cleavages

during isolation. This is suggested by the fact that all the three forms have similar molecular weights, but differ significantly in their net surface charge, and that they are found in crude and partially purified enzyme preparations carried out in the presence of proteinase inhibitors, such as pepstatin, a very potent inhibitor of acid proteinases (e.g. pepsin and gastricsin). Moreover, the mucosa covering the glandular part of the stomach, used as enzyme source, is carefully scraped off in order to avoid contamination with surrounding tissues. Nevertheless, the possibility that enzyme damage during preparation may be involved in the generation of multiple forms cannot be excluded without knowing their exact chemical differences. These differences do not appear to affect significantly the catalytic process of histamine formation: the kinetic properties and the Michaelis constants for histidine are similar for all the enzyme forms.

We have developed a procedure for isolation and purification of the gastric histidine decarboxylase forms by the combination of isoelectric focusing with some other fractionation techniques. Preparative isoelectric focusing as a first step of fractionation does not permit a good purification. More effective results need an initial fractionation by classic methods. Afterwards, isoelectric focusing in layers of granulated gels combined with gel chromatography and refocusing gives rise to the isolation of each form I, II and III. In the final purification steps, the increase in specific activity is restricted by the enzyme lability, mainly on refocusing but partly by dilution of the enzyme solutions, rendered free from Ampholines, by gel filtration.

Although each isolated form appears homogeneous as regards its enzyme activity, evidence for protein microheterogeneity comes from electrophoretic and analytical-refocusing studies. Because of the high sensitivity of analytical isoelectric focusing, with superior resolving power to that of electrophoresis, heterogeneity is revealed as more complex: each active form consists of several protein bands. So the exact number of forms is not clear. The occurrence of at least three major forms has been evinced, but the possibility that there are several species of histidine decarboxylase in each major peak cannot be excluded, and enzyme activity may be associated with several of the observed protein bands. However, contaminating proteins, displaying similar molecular weights and pI to those of the forms I, II and III, may still remain in the final enzyme preparations.

Isoelectric focusing on preparative or analytical scale can provide a useful tool for the investigation of multiple enzyme forms occurring in tissues and their physiological role in histamine biosynthesis and regulation, as well as of their behaviour in normal and pathological states.

This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (INSERM, U 205) and by the Centre National de la Recherche Scientifique (CNRS, ERA no. 560). We are indebted to Dr. J. F. Pageaux for computer analysis of the kinetic data.

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