The Production of 53–55-kDa Isoforms Is Not Required for Rat l-Histidine Decarboxylase Activity*

John V. Fleming and Timothy C. Wang‡
From the University of Massachusetts Medical School, Worcester, Massachusetts 01655

Post-translational processing of the histamine-producing enzyme, l-histidine decarboxylase (HDC), leads to the formation of multiple carboxyl-truncated isoforms. Nevertheless, it has been widely reported that the mature catalytically active dimer is dependent specifically on the production of carboxyl-truncated 53–55-kDa monomers. Here we use transiently transfected COS-7 cells to study the properties of carboxyl-truncated rat HDC isoforms in the 52–58-kDa size range. Amino acid sequences important for the production of a 55-kDa HDC isoform were identified by successive truncations through amino acids 502, 503, and 504. Mutating this sequence in the full-length protein prevented the production of 55-kDa HDC but did not affect enzymatic activity. Further truncations to amino acid 472 generated an inactive 53-kDa HDC isoform that was degraded by the proteasome pathway. These results suggested that processed isoforms, apart from 53–55-kDa ones, contribute toward histamine biosynthesis in vivo. This was confirmed in physiological studies where regulated increases in HDC activity were associated with the expression of isoforms that were greater than 55 kDa in size. We provide evidence to show that regulation of HDC expression can be achieved by the differential production or differential stabilization of multiple enzyme isoforms.

Histamine is generated by the catalytic decarboxylation of l-histidine. In mammals the enzyme that performs this reaction, l-histidine decarboxylase (HDC); 4.1.1.22), is initially translated as a 73–74-kDa protein. Studies to purify the enzyme from native sources, however, led to the isolation of 100–110-kDa HDC complexes (fetal rat liver (1), mouse stomach (2), mouse mastocytoma cells (3–5), mouse kidney (6)). These complexes resolved to give 53–55-kDa protein bands on 100–110-kDa HDC complexes (fetal rat liver (1), mouse stomach (2), mouse mastocytoma cells (3–5), mouse kidney (6)).

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‡ Supported by National Institutes of Health Grant DK48077. To whom correspondence should be addressed: Division of Digestive Diseases and Nutrition, Department of Medicine, 364 Plantation St., Worcester, MA 01603. Tel.: 508-856-4778; Fax: 508-856-4770; E-mail: timothy.wang@umassmed.edu.

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of rats. We describe the properties of carboxyl-truncated HDC isoforms ranging in size from 51 to 58 kDa and identify sequences that constitute part of the 55-kDa processing site. Mutation of this cleavage site prevented production of the 55-kDa isoform but did not affect enzymatic activity. A carboxyl-truncated 53-kDa isoform of the rat HDC protein was unstable and had no enzymatic activity.

EXPERIMENTAL PROCEDURES

Plasmid DNA Constructs—Unless otherwise stated, all expression constructs were generated by PCR amplification using Pfu DNA polymerase (Stratagene) on a thermocycler (PerkinElmer Life Sciences 9700) and using the CMV-HDC18 vector as template (15). The pEP-HA vector backbone used in this study was generated by cloning double stranded oligonucleotides, for which the sense strand was 5′-gatgcgt-aataacgatcactaggctgcacgatcgactgtagctgacgtggtgcag-3′ and the antisense primer, 5′-gatgcgt-3′, which was the I site of the pEP-empty vector, which has already been described (14). The pEP-HDC1/471HA, pEP-HDC1/486HA, pEP-HDC1/505HA, pEP-HDC1/516HA, and pEP-HDC1/656HA constructs were generated using the common sense primer, 5′-ggagactcgagcagac-agacagtcccagg, and the following antisense primers, 5′-ccggc-3′, which was the I site of pEP7-HA empty vector. This cloning strategy meant that the HA tag was in-frame at the carboxyl terminus of expressed proteins.

Mutations were generated using the QuikChange site-directed mutagenesis protocol (Stratagene). The sense primers used for SKD502/503/504PNS (5′-ggagactcgagcagac-agacagtcccagg) and the antisense primer used for K308G (5′-ccggc-3′) mutation was 5′-gctctcatcttctttgctctggagctgtattg-3′. Mutations were generated from the pEP-HDC1/516HA or pEP-HDC1/656HA vectors as described in the lower panel.

Cell Culture—COS-7 cells were maintained in complete medium, which consisted of Dulbecco’s modified Eagle’s medium (BioWhittaker) containing 10% fetal bovine serum and 1% penicillin/streptomycin solution (Invitrogen). Cells were cultured in a 5% CO2 humidified incubator at 37°C. For transient transfection experiments, cells were seeded at a density of 1 × 104 per 100-mm dishes. After 24 h, cells were transfected for 3 h with 15 μg of test or empty vector (−ve) plasmids and were supplemented with radiolabeled (for gel fractionation) or cold (for enzymatic assays) methionine or as described by the manufacturer (Qiagen). When appropriate 10 μM lactacystin (BioMol), 100 μM forskolin (Sigma), or 10 μM tunicamycin (BioMol) were added after transfection. Unless otherwise stated, transfected cells were harvested 48 h after transfection in 200 μl of 0.1 M sodium phosphate buffer, pH 7.4, supplemented with complete protease inhibitors (Roche Molecular Biochemicals) and sonicated. Protein concentration was determined using the method of Bradford.

Coupled Transcription/Translation Reactions—In vitro transcription/translation reactions were performed using rabbit reticulocyte lysates with 1 μg of test or empty vector (−ve) plasmids and were supplemented with radiolabeled (for gel fractionation) or cold (for enzymatic assays) methionine or as described by the manufacturer (Qiagen). Northern blots were probed for HDC and G3PDH mRNA expression as stated elsewhere (14).

Recovery of Tissue from Rats—Two groups of three male Sprague-Dawley rats (~450 g) were fasted. 24 h later standard dietary nats were given to one group of animals, and the rats were allowed to feed ad libitum for 2 h. Whole stomachs were isolated and cleaned in PBS. Whole livers were harvested from rat fetuses at day 20 of gestation. Rat tissues were successively homogenized and then sonicated in 0.1 M sodium phosphate buffer, pH 7.4, supplemented with complete protease inhibitors. Protein concentration was determined using the method of Bradford. Rat experiments were performed in accordance with local animal welfare regulations.

53–55-kDa Isoforms Are Not Required for Rat HDC Activity

Assay of Histidine Decarboxylase Activity—HDC activity in 40 μl of total cell or tissue lysates (200 μg) was determined from the linear range of the activity curve as described elsewhere (13). Unless otherwise stated, enzymatic activities are shown above fractionated protein samples from the same experiment (mean ± S.D.) and are representative of three independent experiments.

Immunoblotting Analysis—Whole cell or tissue lysates (100 μg) were diluted in 2× sample buffer and electrophoresed on denaturing SDS-polyacrylamide (8%) or native (6%) polyacrylamide gels. Fractionated proteins were transferred to a polyvinylidene difluoride membrane, and membranes were immunoblotted by standard procedures using an anti-HD antibody (diluted 1:1000 in 2% non-fat dried milk; Accurate Chemical and Scientific) or an anti-HA antibody (diluted 1:2000 in 2% non-fat dried milk; Santa Cruz Biotechnology). Immunoreactive proteins were detected using the Renaissance kit (PerkinElmer Life Sciences) and Biomax MS autoradiographic film with intensifying screens (Eastman Kodak Co.). All immunoblots were shown representative of at least three independent experiments.

Immunoprecipitation of HA-tagged Protein—COS-7 cells were seeded at a density of 1 × 105 cells per 100-mm dish and transfected with 15 μg of pEP7-HDC1/473HA, pEP7-HDC1/486HA, or pEP7-HDC1/516HA as described. 12 h after transfection the cells were washed twice with PBS and incubated in Cys−Met− medium, which consisted of cysteine- and methionine-free Dulbecco’s modified Eagle’s medium (BioWhittaker) supplemented with 10% dialyzed fetal bovine serum (Invitrogen), 2 mM l-glutamine, and 1% penicillin/streptomycin solution (Invitrogen). After 1 h the medium was replaced with Cys−Met− medium supplemented with 200 μCi of Easytag express [35S]methionine/cysteine mix (1175 mCi/mmol [35S]methionine; PerkinElmer Life Sciences). After a 2-h pulse, cells were washed twice with PBS, and ultraculture medium was added. When required the medium was supplemented with 0.1 μM phenol 12-myristate 13-acetate or 10 μM lactacystin. Cells were harvested at appropriate time points in 750 μl of radioimmune precipitation assay buffer (150 mM NaCl, 20 mM Tris-HCl, pH 7.5, 2 mM EDTA, 0.1% SDS, 0.25% deoxycholate, 1% Triton X-100) supplemented with protease inhibitors (Roche Molecular Biochemicals). HA-tagged proteins were immunoprecipitated using an anti-HA antibody (Santa Cruz Biotechnology) and 30 μl of protein A-Sepharose CL-4B (30 mg/ml in PBS, 0.02% sodium azide; Amersham Biosciences) as described elsewhere (14).

RESULTS

Carboxyl-terminal Processing of the ~74-kDa Primary Translation Product Is Required for Rat HDC Activity—Studies with reticulocyte lysates unambiguously demonstrated that the full-length rat HDC1/656HA protein is inactive in the absence of physiological processing (Fig. 1A, top panel, lane 3). The expression of a carboxyl-truncated HDC1/516HA isoform (~58 kDa), on the other hand, gave significant enzymatic activity (Fig. 1A, top panel, lane 2).

These studies demonstrated that physiological processing of the primary translation product is necessary for activity, and in living cells expression of the full-length rat HDC protein was associated with physiological processing that resulted in the production of a number of different sized isoforms and histidine decarboxylation activity. This is shown in Fig. 1B, where HDC expression was compared between extracts of fetal rat liver (lane 1) and transiently transfected COS-7 cells expressing full-length rat HDC1/656HA protein (lane 2). COS-7 cells, which do not express endogenous HDC, were capable of processing the primary translation product, and in both cases significant enzymatic activity (upper panel) and multiple processed isoforms (lower panel) could be detected. Although the ratio of the post-translationally processed isoforms clearly differed between the two cell models, the sizes of the major processed isoforms were similar, including the main 63-, 58-, 55-, and 36-kDa isoforms (large arrows). Other minor isoforms were also detected (small arrows).

~74-kDa HDC Undergoes Carboxyl-terminal Processing in Transfected COS-7 Cells to Generate the ~55-kDa Isoform—On account of the fact that so many different isoforms were being detected in our immunoblots, we did not think it was possible to attribute activity to one specific isoform. Furthermore, while...
our results with reticulocyte cell lysates indicated that carboxyl-terminal processing is essential for activity, we had no evidence to prove that the processed isoforms specifically detected in transfected COS-7 cells were carboxyl-terminally truncated. Nevertheless, numerous studies have suggested that carboxyl-truncated 53–55-kDa isoforms are responsible for HDC catalysis in vivo (7–9). Accordingly, we were particularly interested in the major 55-kDa isoform generated by COS-7 cells and wanted to determine whether its expression was a result of carboxyl-terminal processing.

COS-7 cells were transiently transfected with pEP7-HDC1/656HA, pEP7-HDC1/516HA, or pEP7-HDC1/486HA as indicated. −ve refers to cell transfected with the pEP7-HA empty vector. A, lysates were fractionated by denaturing SDS-PAGE and analyzed for expression of HDC isoforms by immunoblotting with an anti-HDC antibody. B, total RNA was extracted and probed for expression of HDC (upper panel) and G3PDH (lower panel). The immunoblot (A) and Northern blot (B) results shown were derived from the same experiment and are representative of three independent experiments.

Fig. 2. COS-7 cells were transiently transfected with pEP7-HDC1/656HA and pEP7-HDC1/516HA expression vectors were used as templates in coupled transcription translation reactions. Cold expression reaction products were analyzed for enzymatic activity (top panel, mean ± S.D., n = 3). [35S]Radiolabelled reaction products were fractionated on denaturing SDS-polyacrylamide gels (lower panel). B, lysates of fetal rat liver or transfected COS-7 cells were compared for enzymatic activity (upper panel, mean ± S.D.) or by denaturing SDS-PAGE (lower panel) as indicated. Large and small arrows are described in the text.

Fig. 1. A, the pEP7-HDC1/656HA and pEP7-HDC1/516HA expression vectors were used as templates in coupled transcription translation reactions. Cold expression reaction products were analyzed for enzymatic activity (top panel, mean ± S.D., n = 3). [35S]Radiolabelled reaction products were fractionated on denaturing SDS-polyacrylamide gels (lower panel). B, lysates of fetal rat liver or transfected COS-7 cells were compared for enzymatic activity (upper panel, mean ± S.D.) or by denaturing SDS-PAGE (lower panel) as indicated. Large and small arrows are described in the text.
Mutating Amino Acids 502, 503, and 504 Prevents Formation of the 55-kDa Isoform—These data indicated that the ~55-kDa processed isoform was generated by carboxyl-terminal truncations. Accordingly, we sought to determine the catalytic contribution of such an isoform specifically when processed from the 74-kDa primary translation product and determine, as has been suggested in previous reports, whether HDC activity is dependent on the production of such a carboxyl-truncated isoform. As a first step, we generated additional constructs that express carboxyl-truncated HDC1/498HA and HDC1/505HA proteins, which more accurately encompass the putative cleavage site between 486 and 516. The expression constructs were transiently transfected into COS-7 cells where it was demonstrated that each transfectant had very similar levels of HDC activity (Fig. 3A, upper panel).

From immunoblots with the anti-HDC antibody (Fig. 3A, lower panel) it was apparent that the primary translation products of the HDC1/516HA and HDC1/505HA proteins (lanes 5 and 4, respectively) were still being processed to generate the smaller 55- and 36-kDa isoforms (arrows). In both cases the 55-kDa isoform was visible just under the primary translation product. Additional carboxyl-terminal truncations to produce the HDC1/498HA and HDC1/486HA proteins, on the other hand, seemed to abolish the ~55-kDa cleavage site (lanes 3 and 2, respectively). These truncated proteins were unable to produce the endogenous ~55-kDa isoform but were just as catalytically active as isoforms that underwent processing. Indeed, on native polyacrylamide gels, where HDC monomers could not be detected, the HDC1/486HA protein was as capable of forming presumptive dimers as the HDC1/516HA isoform (Fig. 3B, upper panel).

These data suggested that amino acid sequences located specifically between amino acids 498 and 505 are important for 55-kDa HDC processing but that the specific production of the ~55-kDa isoform is not essential for catalysis. To test this hypothesis we generated a number of mutations in the HDC1/516HA protein just upstream from 505. The first mutant, which carried mutations in amino acids 502, 503, and 504 (HDC1/516HA Δ502/3/4), was transiently expressed into COS-7 cells and lysates analyzed for enzymatic activity and production of the 55-kDa isoform (Fig. 4A). As shown in the upper panel, this mutation had no effect on enzymatic activity. Nevertheless it was apparent in anti-HDC immunoblots that the SKD502/503/504PNS mutation compromised the processing of HDC1/516HA, because the 55-kDa isoforms was no longer being detected (Fig. 4A, lower panel). A second protein, HDC1/516HA Δ492/3/4, carrying the mutation LIPΔ492/493/494HAS, had no affect on HDC processing or enzymatic activity (data not shown).

Similar results were obtained when the full-length HDC1/656HA was mutated at amino acids 502, 503, and 504 (HDC1/656HA Δ502/3/4). In Fig. 4B, lower panel, it is apparent that the major 55-kDa HDC isoform was no longer detected (lane 3). This mutation had no significant effect on enzymatic activity (Fig. 4B, upper panel). Interestingly, these mutations had little effect on formation of the 36-kDa carboxyl-truncated isoform, indicating that formation of this isoform is not sequentially dependent on formation of the ~55-kDa isoform.

Differential Expression of HDC Isoforms Allows for the Physiological Regulation of HDC Activity—Our results indicated that the ~74-kDa primary translation product generates a 55-kDa carboxyl-truncated isoform that is enzymatically active. Nevertheless, HDC activity is not solely dependent on production of this isoform, or at least not to the extent that has previously been proposed. Instead, multiple active isoforms appear to be generated. We were interested in seeing whether this might be important in the physiological regulation of histamine biosynthesis. Little is known about the regulation of HDC activity in our previous in vivo model, the fetal liver. Instead, histamine biosynthesis by enterochromaffin-like cells of the gastric mucosa is known to represent a major regulatory step in the control of gastric acid secretion (19–21). Furthermore, it has been demonstrated that regulation of HDC activity in gastric enterochromaffin-like cells can occur independently of mRNA expression (22). To determine whether the production of multiple active HDC isoforms is important in gastric histamine production, rats that had been fasted for 24 h were re-fed.

![Figure 3](http://www.jbc.org/)
This led to a significant increase in HDC activity (Fig. 5, top panel, mean ± S.D., n = three pairs). When whole stomach lysates were fractionated for anti-HDC immunoblotting it was apparent that this treatment was increasing the expression of only isoforms that were greater than ~55 kDa in size (Fig. 5, lower panel, see arrows). The expression and regulation of multiple HDC isoforms appears to be important therefore for the physiological regulation of HDC activity.

**Activation of Protein Kinase C and Protein Kinase A Pathways, Respectively, Allows for the Regulated Stabilization or Regulated Processing of HDC Isoforms**—Our data from rat stomach extracts provided supportive evidence that at least one of the higher molecular weight HDC isoforms is capable of contributing toward activity in a whole animal model and indicated that the ratio of expressed isoforms is not fixed within a particular organ. Instead, the physiological expression of different isoform could be differentially regulated. We wished to explore the molecular basis for this pattern of expression, and it was immediately apparent from our re-feeding experiments that the differentially regulated isoforms were, based on a carboxyl-terminal patterns of processing, all expected to contain parts of the degradation-promoting PEST domain. This suggested regulation at the level of protein stabilization; however it has never been specifically demonstrated that PEST domain-containing isoforms could be regulated differently from carboxyl-truncated PEST-deficient ones. To determine whether this is the case, and to characterize the cellular signaling pathways that might mediate such a pattern of regulation, COS-7 cells were transiently transfected to express HDC1/656HA, which contains the full PEST domain, and HDC1/486HA, which does not. Cells were metabolically labeled for 2 h and chased for 8 h in the presence or absence of 0.1 μM phorbol 12-myristate 13-acetate, 100 μM forskolin, or 1 μM thapsigargin, which, respectively, activate protein kinase C, protein kinase A, and intracellular calcium release. Of these compounds only activation of protein kinase C pathways resulted in the stabilization of the PEST domain-containing HDC1/656HA isoform (Fig. 6A, upper panel). This stabilization could be mimicked by the addition of the proteasome inhibitor lactacystin.
The PEST-deficient HDC1/486HA isoform was not stabilized by either of these two treatments (Fig. 6A, lower panel).

These are the first studies to definitively demonstrate that PEST-containing HDC isoforms can be regulated differently from PEST-deficient ones. Furthermore, they indicated that isoform stabilization was specific for activation of protein kinase C pathways; it was not observed when the cells were treated with forskolin or thapsigargin (data not shown). Instead, it was noted that forskolin was capable of suppressing the production of the ~63- and ~55-kDa isoforms although the exact molecular mechanisms involved remain unclear (Fig. 6B, see arrows on right hand side). A slight decrease in enzyme activity was consistently noted but was not significant over three independent experiments (11.7 ± 1.1 nmol/mg/h versus 9.5 ± 0.9 nmol/mg/h, mean ± S.D., n = 3).

The HDC protein contains two putative N-glycosylation sites located at amino acids Asn-219 and Asn-305. We wished to determine therefore whether isoform expression could be influenced by post-translational glycosylation. Culturing cells in the presence of 0.1 μM tunicamycin, which inhibits N-glycosylation, had no effect on patterns of isoform expression, suggesting that HDC is not regulated in this way (data not shown).

Isoforms Less Than 53.7 kDa in Size (i.e. Carboxy-terminal Truncations Beyond Amino Acid 477) Are Inactive—Thus far our results indicated that protein processing is essential for enzyme activity and that multiple isoforms, 55 kDa and greater, contribute toward catalysis. However other shorter isoforms, such as the carboxyl-truncated ~36-kDa isoform, were also generated. Although we found no evidence in these studies for the regulation of these isoforms, we nevertheless wished to determine their contribution to overall levels of HDC catalysis. To study the functional significance of generating these smaller HDC isoforms, a series of constructs were generated that express proteins where the carboxyl terminus was further truncated. The isoforms HDC1/477HA, HDC1/472HA, and HDC1/460HA were developed to express carboxyl-truncated proteins that were 53.7, 53.2, and 51.7 kDa in size.

Constructs expressing these proteins, along with that expressing HDC1/486HA, were transfected into COS-7 cells. Lysates were used in enzyme assays and indicated that truncations to amino acids 472 and 460 was inactivating the enzyme (Fig. 7A, top panel). Identical results were obtained when the constructs were expressed in reticulocyte cell lysate reactions or when COS-7 cells were transfected to express constructs that completely lacked the HA tag (data not shown). Carboxyl-truncated ~53-kDa rat HDC isoforms were inactive therefore, both in vitro and in vivo. Further carboxyl-truncations to generate proteins HDC1/339HA (~37 kDa) and HDC1/242HA (~27 kDa) were also inactive (data not shown).

Lysates were subsequently fractionated on denaturing SDS-PAGE (Fig. 7A, lower panel) and immunoblotted with an anti-HDC antibody. These experiments initially suggested that the removal of amino acids between 477 and 472 was leading to the loss of the 36-kDa isoform, in parallel with loss of enzymatic activity. One explanation for these data is that the inactive 36-kDa HDC isoform is derived from larger active isoforms after the release of histamine and might therefore not be produced by inactive isoforms such as HDC1/460HA and HDC1/472HA. To test this hypothesis, COS-7 cells were transfected to express either HDC1/516HA or a second protein carrying a mutation in lysine residue 308 that binds the essential pyridoxal phosphate co-factor (18, 23). Lysates were analyzed for enzymatic activity and demonstrated that the mutant HDC1/516HA Δ308 was indeed inactive (Fig. 7B, upper panel). In anti-HDC immunoblots, however, it was apparent that the mutant protein was still capable of generating the 36-kDa isoform (Fig. 7B, lower panel).

**Enzyme Inactivation by Truncation Beyond Amino Acid 477 Is Associated with Increased Degradation of HDC Isoforms**—Our results suggested that inactivation of the enzyme by truncation between amino acids 477 and 472 might also be associated with a decrease in the steady state levels of the HDC1/472HA and HDC1/460HA proteins (Fig. 7A, lanes 3 and 2, respectively). Indeed, increased degradation of the HDC1/460HA and HDC1/472 isoforms could explain why it was not possible to detect the processed 36-kDa isoform in cell transfected with these proteins. Pulse-chase experiments (Fig. 8A) and incubation of HDC1/472HA-expressing cells with the proteasome inhibitor lactacystin (Fig. 8B), indicated that inactivation and decreased steady state protein levels was associated with increased degradation of HDC1/472HA by the proteasome.

**DISCUSSION**

Within the wider family of mammalian L-amino acid decarboxylases the histamine-producing decarboxylase, HDC, exhibits a unique pattern of post-translational processing. Specifically, it is translated as an unstable protein that is proposed to undergo carboxyl-terminal processing post-translationally. Therefore, whereas enzymes such as ornithine decarboxylase, DOPA decarboxylase, and glutamic acid decarboxylase are all translated as active enzymes, it has been widely reported that the active unit of cellular HDC activity is a 100–110-kDa dimer consisting of two carboxyl-truncated ~53–55-kDa monomers.
Recently, however, a number of additional processed isoforms have been described. If the cell is capable of generating multiple isoforms, what evidence then exists that a 53–55-kDa one is the preferred cellular isoform for histamine biosynthesis, as is suggested from other studies. Much emphasis has been placed on studies that isolated active 100–110-kDa HDC complexes, but these purification procedures were essentially performed on soluble protein fractions (2, 4). Although this is standard practice in many purification protocols it has only become apparent in recent years that HDC isoforms are differentially localized within the cell (7–9). Under such circumstances where the first step of an isolation procedure specifically selects against some isoforms, it is questionable whether the purified isoform accurately reflects the active form in the cell. Because carboxyl-truncated HDC isoforms are now known to be more stable (14), it is hardly surprising that the most stable and soluble isoform turned out to be the isoform that was eventually isolated.

Emphasis has also been placed on results obtained for one particular antiserum that has without doubt contributed significantly to our current understanding of HDC. However, in rat basophilic RBL-2H3 cells this antiserum recognized only 74- and 53–55-kDa isoforms and not 63-, 58-, or 36-kDa isoforms (9). In contrast, Fajardo et al. (24), using the RBL-2H3 cell line but the same antibody used in this study, were recently able to observe expression of the 63/4-kDa isoform. Other studies that claimed to detect the 63/4-kDa isoform in fetal rat liver were initially described as flawed, yet here we show that such an isoform is clearly expressed. Considerable confusion exists therefore, with regard to the processing of the primary translation product, as well as the relative activities of processed isoforms.

Here we demonstrate definitively that post-translational processing of 74-kDa HDC is required for catalytic activity. However, while an active 55-kDa isoform is certainly generated by carboxyl-terminal processing, HDC catalysis is not solely dependent on this isoform, as would be suggested from previous studies (5, 6). Instead, other processed isoforms greater than −55 kDa in size can contribute toward histamine produc-
tion. This conclusion has wide ranging implications with respect to our overall understanding of HDC catalysis, as it suggests that the enzyme is quite flexible. Therefore, while there is likely to be a core domain in HDC whose structure is essential for catalysis, amino acid sequences outside this domain are less important for function. Instead, our studies here showed that the non-essential carboxyl-terminal domain contains regulatory elements that allow for the differential expression of HDC isoforms. This is important in the rat stomach, where feeding triggers the release of histamine from enterochromaffin-like cells in the gastric mucosa and leads to histamine H2 receptor mediated stimulation of gastric acid secretion (25). In physiological terms therefore the pattern of regulated HDC isoform expression observed here would allow for a transient spike in activity and histamine biosynthesis to occur. This would mean that there is a constant background synthesis of histamine but that production could be boosted and rapidly replenished in anticipation of subsequent feeding. This unique pattern of post-translational regulation could explain in a physiological setting why it is so important to generate multiple active isoforms.

We also showed that the expression of different isoforms can be suppressed by the activation of protein kinase A pathways and demonstrated for the first time that HDC isoform expression can be regulated in this way. Although the exact physiological benefit of this is not yet clear, there have been reports of tissue-specific decreases in HDC activity by cAMP, although the molecular basis for this regulation was never identified (26,27). A range of physiological conditions therefore are likely to determine the ratio of PEST-containing and PEST-deficient isoforms and consequently, the duration of histamine synthetic pulses.

The fact that HDC catalysis involves multiple active isoforms might in some respects have been anticipated from previous studies where carboxyl-truncated 64-kDa HDC isoforms were transiently expressed in COS-7 or infected SF9 cells (7,14). However, in these earlier studies, no steps were taken to prevent the formation of other isoforms, including the 55-kDa one, and the activity levels could have been due in part to processing of 64-kDa HDC. Here, however, not only do we provide the first definitive evidence that processed isoforms apart from the 55-kDa one contribute to catalysis, but we additionally identified the TRDSKDL domain that is important for 55-kDa processing. Human β-galactosidase is post-translationally processed by cleavage at a similar RDS motif. The protease that performs this maturation is known as “protective protein” and post-proteolytically acts to stabilize β-galactosidase and regulate its intralysosomal degradation (28,29). These are the first data that implicate a specific cellular protease with HDC processing in vivo, and it is interesting that this protease is linked to the lysosome. Is it possible therefore that the 55-kDa isoform is only being produced so as to be chaperoned until degradation?

In support of other studies we found evidence for a minor 1–2-kDa amino-terminal truncation (14). An example of this is shown in Fig. 7A where the minor band runs just underneath the primary translation product. Future studies will need to address the functional significance of such a step, particularly because amino truncation of the ~55-kDa isoform would generate a ~53-kDa one, and might help explain some of the inconsistencies observed in the literature. This is highlighted in, but by no means limited to, studies on mouse mastocyteoma cells. Hammar and Hjerten (5) described production of a ~55-kDa isoform, whereas Yamamoto et al. (T,29) described the production of a carboxyl-truncated ~53-kDa isoform from the same cell type. Although we cannot completely rule out slight variations in the exact size estimations performed in other studies, our results on this is clear, the ~53.7-kDa HDC1/477 isoform is the minimal unit of carboxyl-truncated rat HDC that is capable of retaining enzymatic activity. Carboxyl-truncated 53-kDa isoforms are inactive, both in vitro and in vivo. A carboxyl-truncated 36-kDa isoform is also inactive, which is of interest given recent speculation concerning the functional importance of smaller HDC isoforms (30), but leads to the question of why inactive HDC isoforms are generated as part of normal in vivo HDC protein processing. It was not apparent from our study why the removal of amino acids 472 to 477 was inactivating the enzyme. There are no cysteine residues in this region that could be critical for structure. Furthermore, inactivity was not a gradual event as might be expected if the enzyme was gradually unfolding. Instead, inactivation was immediate and absolute, suggesting a more direct role for amino acids 472–477 in catalysis. Future studies will address why these AANLV (472–477) residues are so important for catalysis and stability.

Data presented here certainly do not discount the possibility that the 55-kDa isoform is the preferred isoform for histamine biosynthesis. However, our data support the proposition that activity does not depend solely on this isoform, and we conclude that current evidence does not allow for cellular HDC activity to be attributed to any specific isoform. Indeed, we highlight physiological conditions where it would be advantageous to express and regulate multiple active isoforms. We feel that it is inappropriate, therefore, in light of evidence and arguments presented here, to continue referring to the 55-kDa isoform as the active or mature isoform. The challenge remains to devise experimental approaches to specifically address which of the isoforms is responsible for histamine biosynthesis in vivo. Our study suggests that multiple isoforms are likely to be involved.

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