cDNA-derived amino acid sequence of L-histidine decarboxylase from mouse mastocytoma P-815 cells

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The primary structure of L-histidine decarboxylase (HDC: L-histidine carboxy-lyase, EC 4.1.1.22) from mouse mastocytoma P-815 cells has been determined by parallel analysis of the amino acid sequence of the protein and the nucleotide sequence of the corresponding cDNA. HDC contains 662 amino acid residues with a molecular mass of 74 017, which is larger by about 21 000 Da than that of the previously purified HDC subunit (53 kDa), suggesting that HDC might be posttranslationally processed. The HDC cDNA hybridized to a 2.7 kilobase mRNA of mastocytoma cells. Homology was found between the sequences of mouse mastocytoma HDC and fetal rat liver HDC.

Histidine decarboxylase; Mastocytoma P-815 cell; Amino acid sequence; cDNA sequence; Dopa decarboxylase; Polymerase chain reaction

1. INTRODUCTION

In the central nervous system and various peripheral organs, histamine acts as a neurotransmitter or a modulator of inflammatory reactions, gastric acid secretion and other processes [1]. L-Histidine decarboxylase (HDC; L-histidine carboxy-lyase, EC 4.1.1.22) catalyzes the formation of histamine from its precursor, histidine, in a single step. Thus, clarification of the control mechanism for HDC activity and of its synthesis is necessary not only from biochemical but also clinical aspects.

Most recently, the cDNA-derived amino acid sequence of HDC in fetal rat liver with a molecular mass of 73 450 (655 amino acid residues) was reported, which was determined without purification of the enzyme during studies on the genes for androgen-binding proteins [2]. However, no information is available as to which cells the HDC originated from.

Mouse mastocytoma cells are the proper cell type for elucidating the mechanism underlying histamine formation in mast cells, because they synthesize HDC in response to various stimuli. For instance, we have previously shown that glucocorticoids stimulate histamine synthesis by inducing HDC in mouse neoplastic mast cells (mastocytoma P-815 cells) [3], and in rat stomach [4]. In addition, preliminary experiments have shown that HDC is induced through protein kinases A and/or C [5].

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We have recently succeeded in purifying and characterizing HDC from mouse mastocytoma P-815 cells [6], and have begun to isolate cDNA clones for the HDC subunit in the cells in order to determine its primary structure and the genetic relationship between HDC and other decarboxylases, and also to clarify the regulatory mechanism for histamine synthesis.

2. MATERIALS AND METHODS

HDC was purified from mastocytoma P-815 cells as previously reported [6], and was digested with *Achromobacter* protease I(AP-I) at 37°C for 2 h in the presence of dithiothreitol. The resultant peptides were separated on a reverse-phase HPLC column (YMC A-302) and the peptide fragments were analyzed using a gas-phase sequencer model 477A equipped with an on-line PTH analyzer model 120A (Applied Biosystems).

The sense and antisense primers corresponding to the peptide sequences, Met-Leu-Gly-Leu-Pro-Glu-Tyr-Phe (residues 1-8 of peptide 12) and His-Gly-Thr-Glu-Met-Ala-Lys (residues 8-14 of peptide 1), respectively, were synthesized. They were 5′-ATGCTGGGGCT-CCCTGARTAYTT-3′ and 5′-TTGGCCATCTCTGTRCCRTG-3′, respectively (where R is A or G; and Y is C or T).

Single-stranded cDNA was synthesized from 5 μg of poly(A)$^+$RNA of P-815 cells and used as a template for the synthesis of a cDNA probe by means of the PCR protocol. The reaction conditions were as follows: PCR buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.3) and 0.01% gelatin); sense and antisense primers (1 μM each); 2.6 μg of template DNA; 1.5 mM $MgCl_2$; dATP, dCTP, dGTP and dTTP (200 μM each); and 2.5 units of Taq DNA polymerase were mixed, the total reaction volume being 100 μl. The reaction was allowed to proceed for 30 cycles of denaturation (40 sec at 94°C), annealing (1 min at 46°C) and polymerization (2 min at 72°C), followed by a single 10 min extension at 72°C using an automated heating/cooling block (Zymoreactor, Atto Corp.)

Total RNA was extracted from P-815 cells by the acid guanidium thiocyanate-phenol-chloroform procedure essentially as described by Chomczynski and Sacchi [7].

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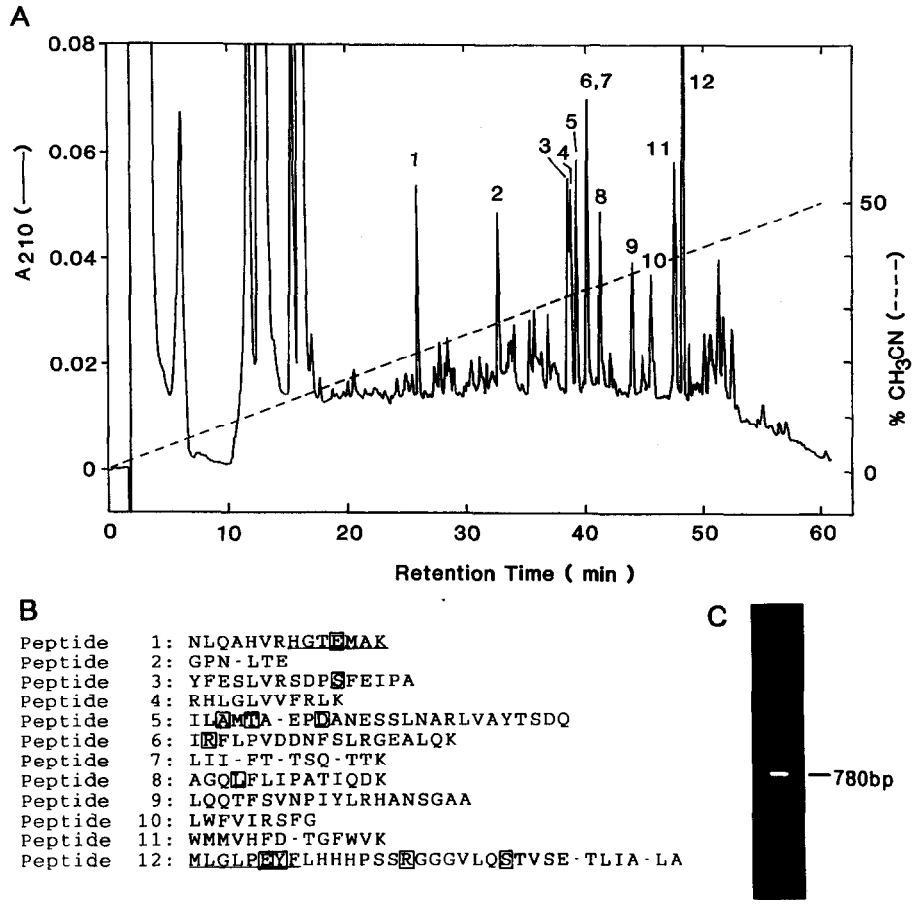


Fig. 1. Synthesis of a cDNA probe for HDC from P-815 cells. (A) Separation of AP-I-digests by reverse-phase HPLC. (B) Partial amino acid sequences of peptides (1–12). Boxes indicate differences in amino acid residues between HDC of P-815 cells and that of rat liver. The sequences used for the synthesis of primers are underlined. (–); not detected. (C) Visualization of the cDNA probe separated on a 1.0% agarose gel.

Double-stranded cDNAs were prepared from total poly(A)$^+$RNA according to Gubler and Hoffman [8], and size-fractionated cDNAs (>500 bp) were inserted into the unique *Eco*RI site of λgt11 [9]. Recombinant phages were screened using the ^{32}P-labeled HDC cDNA probe.

3. RESULTS

The purified HDC was digested with AP-I, and the peptidic fragments thus obtained were separated (Fig. 1A). Fig. 1B shows the twelve partial amino acid sequences that were determined by the automated Edman degradation method. Of these sequences, the two for peptides 1 and 12 were selected to generate primers for the preparation of a cDNA probe as described in section 2. Fig. 1C shows the visualized fragment of the amplified cDNA product with a size of about 780 bp, which is in good agreement with the size of 782 bp expected from the sequence of rat HDC [2].

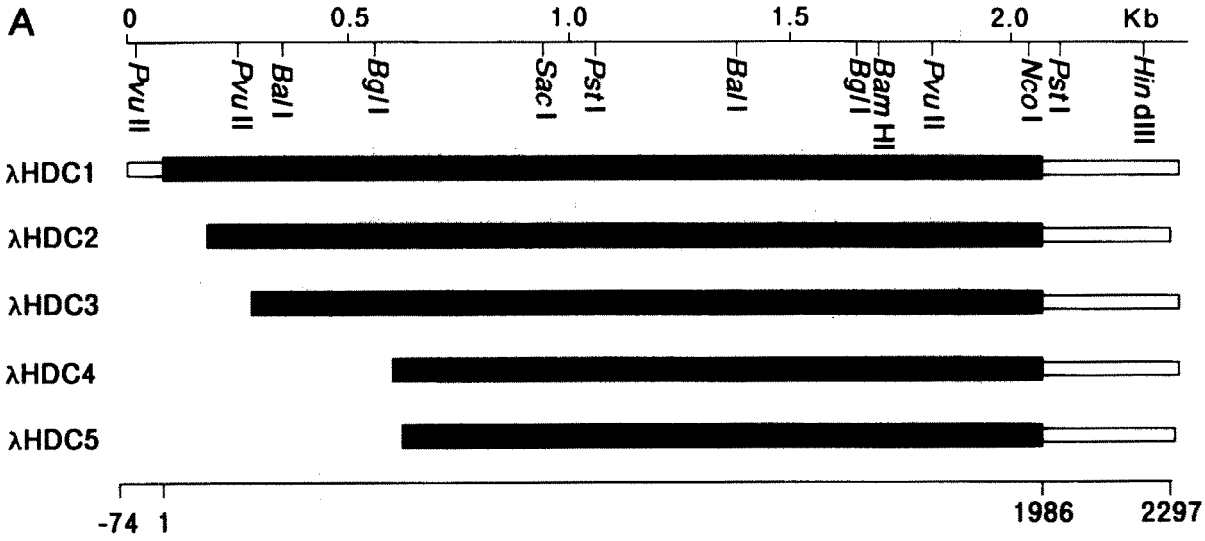
During the primary screening of the cDNA library, 20 phage plaques out of 80000 recombinants gave positive signals, 7 of which were chosen and purified. All of the latter were ultimately proved by nucleotide sequence analysis to contain coding sequences for the partial amino acid sequences determined for HDC (Fig. 1A) in the same reading frame. λHDC1 (2.4 kb) contained the complete coding sequence for HDC including the 3′-terminal poly(A) tract.

Fig. 2A shows the restriction maps and the results of sequence analysis of the cDNA clones. Fig. 2B shows the 2371-nucleotide sequence of the cDNA encoding HDC in P-815 cells. Thus, there is a stretch of 74 nucleotides in the 5′ non-coding region, and the coding sequence for HDC begins at an initiator codon (nucleotides 1–3) and ends with a TGA terminator codon (nucleotides 1987–1989). There is an additional sequence of 311 nucleotides downstream from the terminator codon. A presumed recognition site for polyadenylylation, A-A-T-A-A-A, was found at positions 2263–2268 in the 3′ non-coding region, 15 bp upstream of the poly(A) tract. When the HDC cDNA was subcloned into the eukaryotic expression vector pcDNA-I, a modified pCMV plasmid, and transfected into COS-1 cells, the enzymatic activity of HDC was detected (data not shown).

Total RNA prepared from P-815 cells was hybridized to ^{32}P-labeled HDC cDNA at 65°C for 15 h. As shown in Fig. 3, a single band corresponding to 2.7 kilobases was observed.

4. DISCUSSION

The cDNA-derived amino acid sequence of HDC from mouse mastocytoma P-815 cells comprises 662



B

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                                                -74  CGACTAAGAAGCCCTGTTGACAGCTGCCTTCCAGCCTCCTCTGTCTGTCTGCCAGGAGGAGCAATCCAAGGGAG   -1

ATGATGGAGCCCTGTGAATACCGTGAATACCGTGAATACTACCGAGCTAGAGGGAAAGAGATGGTGGATTACATCTCCCAGTACCTGAGCACTGTGCGGGAGAGGCAGGTGACTCCAAAT  120
MetMetGluProCysGluTyrArgGluTyrArgGluTyrTyrArgAlaArgGlyLysGluMetValAspTyrIleSerGlnTyrLeuSerThrValArgGluArgGlnValThrProAsn   40

GTGCAGCCTGGATACCTGCGAGCCCAGCTACCTGCGAGTGCTCCCGAGGAACCCGACAGCTGGGACAGCATCTTTGGGGACATTGAACGAGTCATCATGCCTGGGGTGGTTCACTGGCAG  240
ValGlnProGlyTyrLeuArgAlaGlnLeuProAlaSerAlaProGluGluProAspSerTrpAspSerIlePheGlyAspIleGluArgValIleMetProGlyValValHisTrpGln   80

AGCCCCCACATGCACGCCTACTATCCTGCTCTTACCTCATGGCCATCCCTGCTAGGAGACATGCTGGCCGATGCCATTAACTGCTTAGGATTCACGTGGGCATCTAGCCCAGCTTGCACA  360
SerProHisMetHisAlaTyrTyrProAlaLeuThrSerTrpProSerLeuLeuGlyAspMetLeuAlaAspAlaIleAsnCysLeuGlyPheThrTrpAlaSerSerProAlaCysThr  120

GAGCTGGAGATGAACATCATGGACTGGCTGGCGAAGATGCTGGGGCTCCCGGAGTACTTCCTGCACCACCATCCCAGCAGCCGAGGGGGAGGTGTCTTACAGAGCACTGTCAGCGAATCC  480
GluLeuGluMetAsnIleMetAspTrpLeuAlaLysMetLeuGlyLeuProGluTyrPheLeuHisHisHisProSerSerArgGlyGlyGlyValLeuGlnSerThrValSerGluSer  160

ACTTTAATTGCCCTGCTGGCAGCAAGAAAGAACAAAATCCTAGCAATGACAGCGTGCGAGCCCGATGCTAACGAGTCCTCCCTGAACGCTCGTCTCGTTGCCTACACCTCTGATCAGGCT  600
ThrLeuIleAlaLeuLeuAlaAlaArgLysAsnLysIleLeuAlaMetThrAlaCysGluProAspAlaAsnGluSerSerLeuAsnAlaArgLeuValAlaTyrThrSerAspGlnAla  200

CACTCTTCAGTGGAGAAGGCTGGCTTGATTTCCCTTGTGAAGATCAGATTTCTACCTGTGGACGACAACTTCTCACTCCGAGGAGAAGCTCTCCAGAAAGCTATTGAGGAAGACAAGCAA  720
HisSerSerValGluLysAlaGlyLeuIleSerLeuValLysIleArgPheLeuProValAspAspAsnPheSerLeuArgGlyGluAlaLeuGlnLysAlaIleGluGluAspLysGln  240

CAGGGCTTGGTGCCTGTGTTTGTCTGTGCAACGTTAGGGACTACTGGAGTCTGTGCATTTGACAGGCTGTCAGAACTGGGGCCCATCTGTGCCAGTGAGGGACTGTGGCTCCACGTCGAT  840
GlnGlyLeuValProValPheValCysAlaThrLeuGlyThrThrGlyValCysAlaPheAspArgLeuSerGluLeuGlyProIleCysAlaSerGluGlyLeuTrpLeuHisValAsp  280

GCTGCTTATGCAGGCACGGCCTTTCTGTGCCCTGAGCTCCGAGGGTTCCTGGAGGGCATCGAGTACGCCGACTCCTTCACCTTTAACCCTTCCAAGTGGATGATGGTACACTTTGACTGT  960
AlaAlaTyrAlaGlyThrAlaPheLeuCysProGluLeuArgGlyPheLeuGluGlyIleGluTyrAlaAspSerPheThrPheAsnProSerLysTrpMetMetValHisPheAspCys  320

ACTGGATTCTGGGTCAAGGACAAGTACAAGCTGCAGCAGACCTTTAGTGTGAACCCCATCTACCTCCGACATGCCAACTCTGGTGCAGCCACGGACTTCATGCATTGGCAGATCCCCTTG 1080
ThrGlyPheTrpValLysAspLysTyrLysLeuGlnGlnThrPheSerValAsnProIleTyrLeuArgHisAlaAsnSerGlyAlaAlaThrAspPheMetHisTrpGlnIleProLeu  360

AGCCGGCGCTTTCGCTCCATTAAGCTGTGGTTTGTGATTCGGTCCTTCGGGGTGAAGAATCTTCAAGCACATGTCAGACACGGCACAGAAATGGCTAAATACTTTGAATCTCTGGTCAGA 1200
SerArgArgPheArgSerIleLysLeuTrpPheValIleArgSerPheGlyValLysAsnLeuGlnAlaHisValArgHisGlyThrGluMetAlaLysTyrPheGluSerLeuValArg  400

AGCGACCCTTCCTTCGAAATTCCTGCTAAGAGGCACCTTGGTTTGGTGGTTTTCCGTCTGAAGGGTCCTAATTGTCTCACAGAAAGTGTGTTAAAGGAAATAGCCAAAGCTGGCCAGCTC 1320
SerAspProSerPheGluIleProAlaLysArgHisLeuGlyLeuValValPheArgLeuLysGlyProAsnCysLeuThrGluSerValLeuLysGluIleAlaLysAlaGlyGlnLeu  440

TTTCTCATCCCGGCTACTATCCAAGACAAGCTGATCATCCGTTTCACTGTGACGTCCCAGTTTACCACCAAGGAGGACATCCTGAGAGATTGGCACCTCATCCAAGAGGCTGCTAACCTT 1440
PheLeuIleProAlaThrIleGlnAspLysLeuIleIleArgPheThrValThrSerGlnPheThrThrLysGluAspIleLeuArgAspTrpHisLeuIleGlnGluAlaAlaAsnLeu  480

GTCCTGAGCCAGCACTGCACTTCCCAGCCGAGCCCTCGGGCCAAGAACGTCATCCCGCCACCGCCAGGGACCAGAGGGCTATCCCTGGAGTCAGTCAGCGAGGGAGGAGATGACCCAGCA 1560
ValLeuSerGlnHisCysThrSerGlnProSerProArgAlaLysAsnValIleProProProProGlyThrArgGlyLeuSerLeuGluSerValSerGluGlyGlyAspAspProAla  520

CAGGCCCGGAAGATCATCAAGCAGCCAGGAGCCAGTCTGGCGAGAAGGGAAGGCGGCTCTGATCTGGAAACGATGCCGGATCCCTTTGATGATTGCTTCTCTGAAGAGGCCCCCAACACC 1680
GlnAlaArgLysIleIleLysGlnProGlyAlaSerLeuAlaArgArgGluGlyGlySerAspLeuGluThrMetProAspProPheAspAspCysPheSerGluGluAlaProAsnThr  560

ACCAAGCACAAGCTGTCATCCTTTCTGTTCAGTTACTTGTCGGTCCAGAACAGGAGGAAGACAACGCGGTCCCTCAGCTGCAACAGTGTGCCTATGAGTGCCCAGAAGTCACTCCCCGCA 1800
ThrLysHisLysLeuSerSerPheLeuPheSerTyrLeuSerValGlnAsnArgArgLysThrThrArgSerLeuSerCysAsnSerValProMetSerAlaGlnLysSerLeuProAla  600

GACGCTTCACTGAAGAATGGGGGCTCCTTCCGGGCCAGAATCTTTTCCGGGTTCCCAGAACAAATGATGATGATGAAGAAAGGTGCCTTCAAAAAGCTGATCAAGTTCTACAGCGTCCCC 1920
AspAlaSerLeuLysAsnGlyGlySerPheArgAlaArgIlePheSerGlyPheProGluGlnMetMetMetMetLysLysGlyAlaPheLysLysLeuIleLysPheTyrSerValPro  640

AGCTTTCCTGAATGCAGTTCTCAGTGTGCTCGCCAGCTACCGTGCTGCCCCCTGGAGGCCATGGTGTAGAGTCCTCAATCAGAATGCAAGGGTGCCTGTGCTTCAGGGAGTTGGGGAACC 2040
SerPheProGluCysSerSerGlnCysAlaArgGlnLeuProCysCysProLeuGluAlaMetVal***                                                        662

CTCGAAATTGCCTGCAGTTTGTGTGCTTATTATGTGTGTGTGCATCTTGAGGGAAGCAAGCCCATTATTTTGATCATAACCTCACAGGGTCTTCGTGATCCACAACAGATTGTAACTGGG 2160
AAGTTTAAGCGGGCATGCTCCAGAGGTTGCAGGCGCTTGTGTGATAGAAGGGGCTGAGACGGTGGCATGCTGTTAAGCTTGTAATGTGAAAAACAACTTAGAAATAAATTGTGCTTATAT 2280
CTAAAAAAAAAAAAAAA                                                                                                          2297
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Fig. 2. Sequence analysis of HDC cDNA clones. (A) Restriction maps of 5 cDNA clones. The amino acid coding region is shown as a bold line. (B) Sequences of cDNA and amino acids of HDC. The partial amino acid sequences determined by amino acid sequence analysis (Fig. 1B) are underlined. A consensus poly(A) signal is boxed. (...) and (~) indicate potential sites of N-glycosylation and of phosphorylation by cAMP-dependent protein kinase, respectively. The nucleotide sequence was determined by the dideoxynucleotide chain-termination method of Sanger et al. [10].

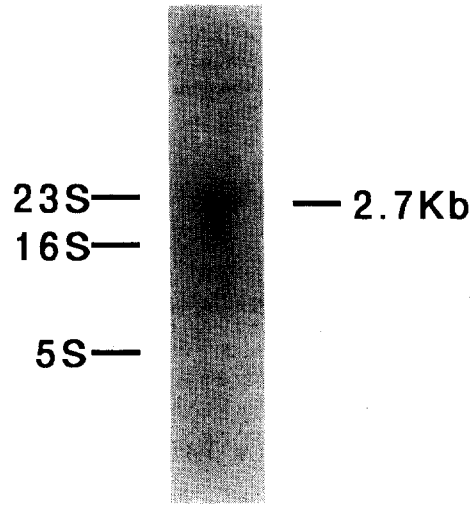

Fig. 3. Sizing of the mRNA for HDC. Total RNA (10 μg) was denatured by treatment with glyoxal and then separated on a 1.0% agarose gel.

amino acids. The calculated M_r is 74 017, which is larger than that of the purified HDC subunit (53 kDa) [6]. One possible explanation for this discrepancy is that HDC from P-815 cells may be posttranslationally processed. That no amino acid sequences of AP-I-digested peptidic fragments were obtained for the long stretches of the N- and C-terminus of the enzyme strongly suggests that the N- and/or C-terminal regions of the translated product might be processed.

Although the attempt at determining the N-terminal amino acid sequence of the purified HDC was unsuccessful, the ATG codon at nucleotides 1 to 3, which is located downstream from the in-frame stop codon at nucleotides −57 to −55, is concluded to be the initiator codon. This assignment is supported by the fact that the nucleotide at −3 position of the ATG codon is purine, guanine, as is the rule with eukaryotic mRNAs [11].

As shown in Fig. 3, the mRNA encoding the amino acid sequence of HDC is 2.7 kb in size. Although we cannot rule out the possibility at this time that minor HDC mRNA species exist, as was described in rat fetal liver [2], we tentatively conclude that the single form of HDC mRNA specifies the premature HDC subunit (74 kDa), which can be posttranslationally processed to yield the mature HDC subunit (53 kDa), and that the native HDC enzyme is composed of two of the latter subunits as previously described [6].

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mHDC                    MMEPCEYREYREYYRARGKEMVDYISQYLSTVRERQVTPNVQPGYLRAQLPASAPEEPDSWDSIFGDIER   70
rHDC                    ****S**H**Q----**********C**************K******I*S****************Q   66
rDDC                          MDS**-F*R*********AD**DGIEG*P*Y*D*E******LI*TT**Q**ETYED*IR***K   62
dDDC    MSIGFRYRANNYARLITKYFCIHIKIDM*AP*-FKDFA*T***F*AE**ENI***R*L*E*K****KPLI*DA***K*EKWQDVMQ****   89
 TDC          MGSIDSTNVAMSNSPVGEFKPL*AE*-F*KQAHR***F*AD*YKN*ETYP*LSE*E*****KRI*ET**YL*EPL*D*MK**QK   83

mHDC    VIMPGVVHWQSPHMHAYYPALTSWPSLLGDMLADAINCLGFTWASSPACTELEMNIMDWLAKMLGLPEYFLHHHPSSRGGGVLQSTVSES 160
rHDC    I**********************************STAW*SRGL-PA********************DF********Q******R***** 155
rDDC    I*****T**H**YFF**F*TAS*Y*AM*A***CG**G*I**S**A********TVM****G***E***A**AGR-AGE****I*GSA**A 151
dDDC    ******T**H**KF***F*TAN*Y*AYVA***SG**A*I****IA********VVM****G***E**AE**ACS-GGK****I*G*A*** 178
 TDC    D*I**MTN*M**NFY*FF**TV*SAAF**E**ST*L*SV****V****A*****IV*****QI*K**KS*MF----*GT****I*N*T*** 170

mHDC    TLIALLAARKNKILAMTACEPDA-NESSLNARLVAYTSDQAHSSVEK----AGLISLVKIRFLP--VDDNFSLRGEALQKAIEEDKQQGL 243
rHDC    **************E*K*H**N*-D**********A**********----**********K*****-************************ 238
rDDC    **V******TKM*RQLQ*AS*EL-TQAA*MEK**************R----****GG***KAI*--S*G*Y*M*AA**RE*L*R**AA** 234
dDDC    **V*SAGSQGQEVEGGEGAPSGVGL*HTILGK**G*C*********R----***LGG**L*SVQ--SE-*HRM**A**E****Q*VAE** 261
 TDC    I*CTII***-ERA*EKLG--**-----*-I-GK**C*G***T*TMFP*TCKL**IYPNN-**LI*TT*ETD*GISPQV*R*MV*D*VAA*Y 250

mHDC    VPVFVCATLGTTGVCAFDRLSELGPICASEGLWLHVDAAYAGTAFLCPELRGFLEGIEYADSFTFNPSKWMMVHFDCTGFWVKDKYKLQQ 333
rHDC    ******************K*********R****************R******K*********************************** 328
rDDC    I*F**VV*****SC*S**N*L*V****NQ**V***I******S**I***F*YL*N*V*F****N***H**LL*N***SAM***KRTD*TE 324
dDDC    I*FYAVV*****NS****Y*D*C**VGNKHN**I********S**I***Y*HLMK***S****N***H**-*L*N***SAM*L**PSWVVN 351
 TDC    **L*L*******STT*T*PVDS*SE*ANEFI**I********S*CI***F*HY*D***RV**LSLS*H**LLAYL***CL***QPHL*LR 340

mHDC    TFSVNPIYLRH-AN-SGAATDFMHWQIPLSRRFRSIKLWFVIRSFGVKNLQAHVRHGTEMAKYFESLVRSDPSFEIPAKRHLGLVVFRLK 421
rHDC    **********-**-**V**************************************D************V*****E********** 416
rDDC    A*NMD*V****SHQD**LI**YR******G*****L*M***F*MY***G***YI*KHVKLSHE******Q**R***CTEVI****C**** 414
dDDC    A*N*D*L**K*DMQ--*S*P*YR******G****AL*****L*LY**E*****I*RHCNF**Q*GD*CVA*SR**LA*EINM***S**** 439
 TDC    ALTT**E**KNKQSDLDKVV**KN***ATG*K***L***LIL**Y**V***S*I*SDVA*G*M**EW****SR***VVP*NFS**C**** 430

mHDC    GPNC-LT-ESVLK----EIAKAGQLFLIPATIQDKLIIRFTVTSQFTTKEDILRDWHLIQEAANLVLSQHCTSQPSPRAKNVIPPP--PG 503
rHDC    ****-**-*****----****T**V**************************D******N**R********************L****VTRD 500
rDDC    *S*Q-*N-*TL*Q----R*NS*KKIH*V*CRLR**FVL**A*C*RTVESAHVQLA*EH*RDL*SS**RAEKE 480
dDDC    *S*E-RN-*AL**----R*NGR*HIH*V**K*K*VYGL*MAIC*R**QS**MEYS*KEVSA**DEMEQEQ 503
 TDC    PDVSS*HV*E*N*KLLDMLNST*RVYMTHTIVGGIYML*LA*G*SL*EEHHVR*V*D***KLTDDLLKEA 500

mHDC    TR----GLSLESVSEGGDDPAQARKIIKQPGASLARREGGSDLETMPDPFDDCFSEEAPNTTKHKLSSFLFSYLSVQNRRKTTRSLSCNS 589
rHDC    SKDLTN*******N******V*V***FRL**D**----*--T---**-***********SD*****************KK**M******* 581

mHDC    VPMSAQKSLPADASLKNGGSFRARIFSGFPEQMMMMKKGAFKKLIKFYSVPSFPECSSQCAR-QLPCCPLEAMV 662
rHDC    M*******P*P***V*H**F**********E*******G*******************GTL*******Q*** 655
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Fig. 4. Homology between the amino acid sequences of HDC of P-815 cells and other decarboxylases. (*) indicates an identical residue. The putative PLP-binding domain of HDC is boxed. mHDC, mouse mastocytoma HDC; rHDC, rat liver HDC; rDDC, rat liver DDC; dDDC, *Drosophila* DDC; TDC, periwinkle tryptophan decarboxylase.

By screening the protein sequence database of the Protein Research Foundation, we found significant homology between the sequence of HDC from P-815 cells and that of fetal rat liver HDC [2]. As shown in Fig. 4, the amino acid sequence for P-815 cells shares 86.1% homology with that for rat liver. HDC from P-815 cells also shows significant homology with dopa decarboxylases (DDC) from fetal rat liver [12] and *Drosophila* [13], and with periwinkle tryptophan decarboxylase (TDC) [14], the homology being 53.3, 48.3 and 41.6%, respectively. The putative seven-amino acid pyridoxal phosphate (PLP)-binding domain (amino acids 307–313) and the PLP-binding site (Lys-312) are completely identical in both the cDNA and amino acid sequences, respectively, in P-815 cells and rat liver. Interestingly, the threonine-307 and serine-311 residues of HDC are replaced by asparagine and histidine residues, respectively, in rat [12] and *Drosophila* DDC [13]. Although the significance of these differences in this domain is unclear, it is possible that the amino acid sequence surrounding the PLP-binding site (lysine) may be substrate-specific.

Four potential N-glycosylation sites [Asn-Xaa-(Thr or Ser)] (amino acids 184–186, 223–225, 309–311 and 559–561) were found in the HDC sequence in P-815 cells. Of these sites, two (residues 223–225 and 309–311) are conserved in the rat HDC sequence. Two possible sites of phosphorylation by cAMP-dependent protein kinase were also observed at amino acids 343–347 and 362–366. It is likely that these sites are involved in cAMP-dependent modulation of HDC activity. In fact, dibutyryl cyclic AMP increased HDC activity in cultured P-815 cells [5].

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