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Sequence similarity between dopamine β-hydroxylase and peptide α-amidating enzyme: evidence for a conserved catalytic domain

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A comparison of human dopamine β-hydroxylase (EC 1.14.17.1) with bovine peptide C-terminal α-amidating enzyme (EC 1.14.17.3), revealed a 28% identity extending throughout a common catalytic domain of approximately 270 residues. The shared biochemical properties of these two enzymes from neurosecretory granules suggests that the sequence similarity reflects a genuine homology and provides a structural basis for a new family of copper type II, ascorbate-dependent monooxygenases.

Dopamine β-hydroxylase; Peptide α-amidating enzyme; Sequence similarity; Copper type II monooxygenase

1. INTRODUCTION

Dopamine β-hydroxylase (DBH; EC 1.14.17.1) catalyses the conversion of dopamine to the neurotransmitter norepinephrine (for review see [1]). The possible role of DBH as a control point in catecholamine biosynthesis, mechanistic questions regarding the involvement of copper in the hydroxylation reaction, and interactions with other proteins and cofactors within the adrenal chromaffin granule, have generated intense research towards understanding the structure and function of this protein. The recent availability of cDNA-derived protein sequences [2,3] and a complete gene sequence for human DBH [3] are important advances but have not provided direct answers to these questions.

In the course of our active-site mapping studies of bovine DBH we have located attachment sites for two mechanism-based inhibitors [4,5] and have determined approximately 85% of the primary structure (unpublished results). The attachment site for β-ethinyltyramine at His 398 was isolated as a 25 residue tryptic peptide that included a putative copper binding site [5]. A search of the protein sequence data banks with this peptide revealed, in addition to a match with the corresponding section from human DBH, a similarity with peptide C-terminal α-amidating enzyme (PAM; EC 1.14.17.3) from *Xenopus laevis* [6]. Because both enzymes are classified as copper type II, ascorbate-dependent monooxygenases, we decided to investigate possible similarities in primary structure between these two proteins using sequence comparison methods on the DBH and PAM sequences available in the databases.

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2. EXPERIMENTAL

Computer analyses were performed on a VAX/VMS system using the suite of programs purchased from the University of Wisconsin Genetics Computer Group (UWGCG) [7]. Initial searches were done using the FASTA program with the current Dayhoff library of 20,535 protein sequence entries. Human DBH, frog PAM and other copper binding proteins were found in the current SwissProt protein database (release 10.0 3/89). Other protein sequences including bovine PAM were obtained by using TRANSLATE on the appropriate cDNA sequences retrieved from the GenBank database. Dotplot comparisons were done with the COMPARE program and alignments produced with the GAP program.

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3. RESULTS

Our initial search of the protein databases with the tryptic peptide containing the attachment site of β-ethinyltyramine gave a score of 96% identity over 25 amino acids with human DBH and 47% over 17 amino acids with the peptide α-amidating enzyme from the frog *Xenopus laevis* [6]. When the FASTA search was repeated using the entire human DBH sequence the only match with a significant score over an extended length of polypeptide chain was with frog PAM.

After our initial finding of similarity between human DBH and frog PAM we carried out further intra-vertebrate sequence comparisons between human DBH and bovine PAM. The full-length bovine PAM cDNA encodes a 976 residue protein [8]. This contains a 25 residue signal sequence followed by a putative 10 residue propeptide, an 831 residue catalytic/intragranular domain, and a 25 residue transmembrane domain with an 86 residue cytoplasmic tail. A dotplot comparison of PAM and DBH showed a clear diagonal section characteristic of sequence similarity over a section approximately 280 residues long corresponding to the centre of DBH and the N-terminal region of PAM (fig.1). The relationship between the two proteins is more clearly shown in the expanded plot of the diagonal section in fig.2.

An alignment of the two sequences obtained with the GAP program is shown in fig.3. The small deviations of the matching sections from the true diagonal in fig.2 indicate that a good alignment could be achieved using a gap weight favouring small insertions and deletions over large ones. Based on the slightly larger DBH section of 295 residues aligned with 270 residues from PAM, the number of identical positions was 27%. The similarity score calculated by GAP from probability of amino acid replacements during evolution was 52% indicating structural conservation by the exchange of similar residues in positions of non-

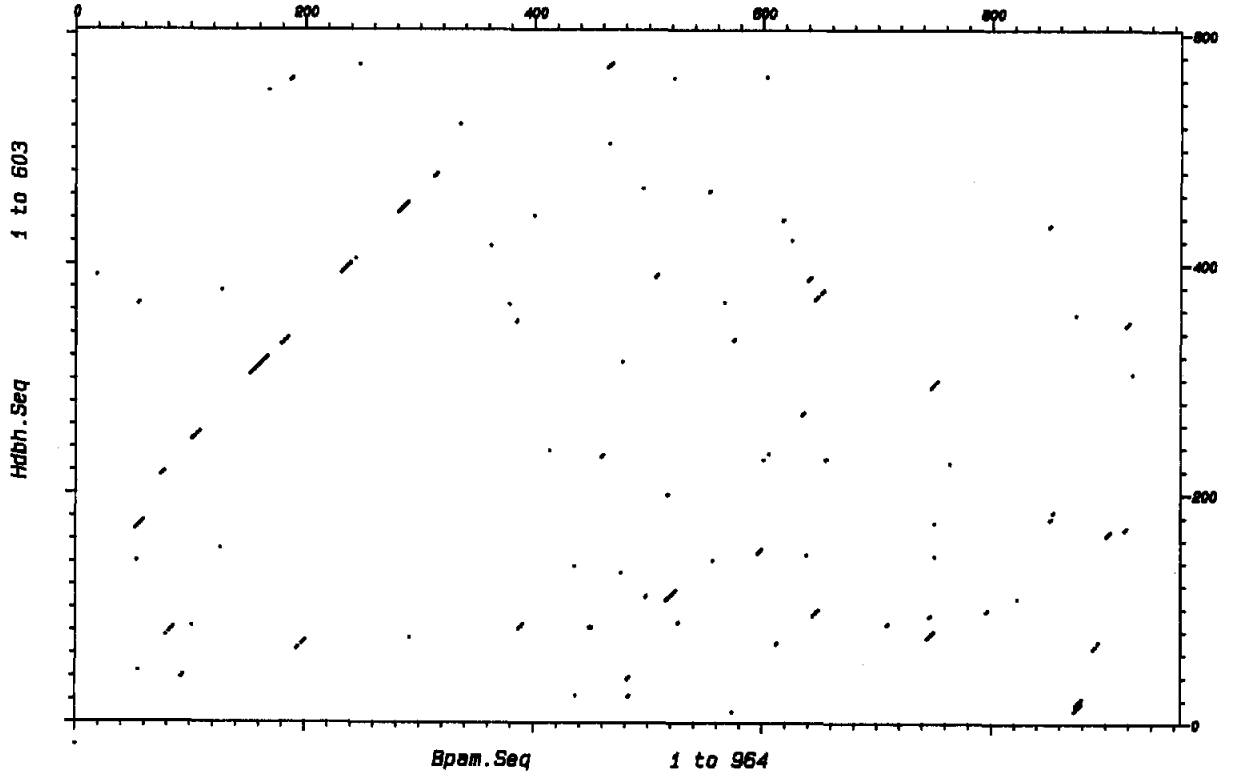

Fig.1. A dotplot comparison using the program COMPARE, of bovine PAM against human DBH. A window size of 15 and stringency of 10.0 were used to filter the plot to 253 points.

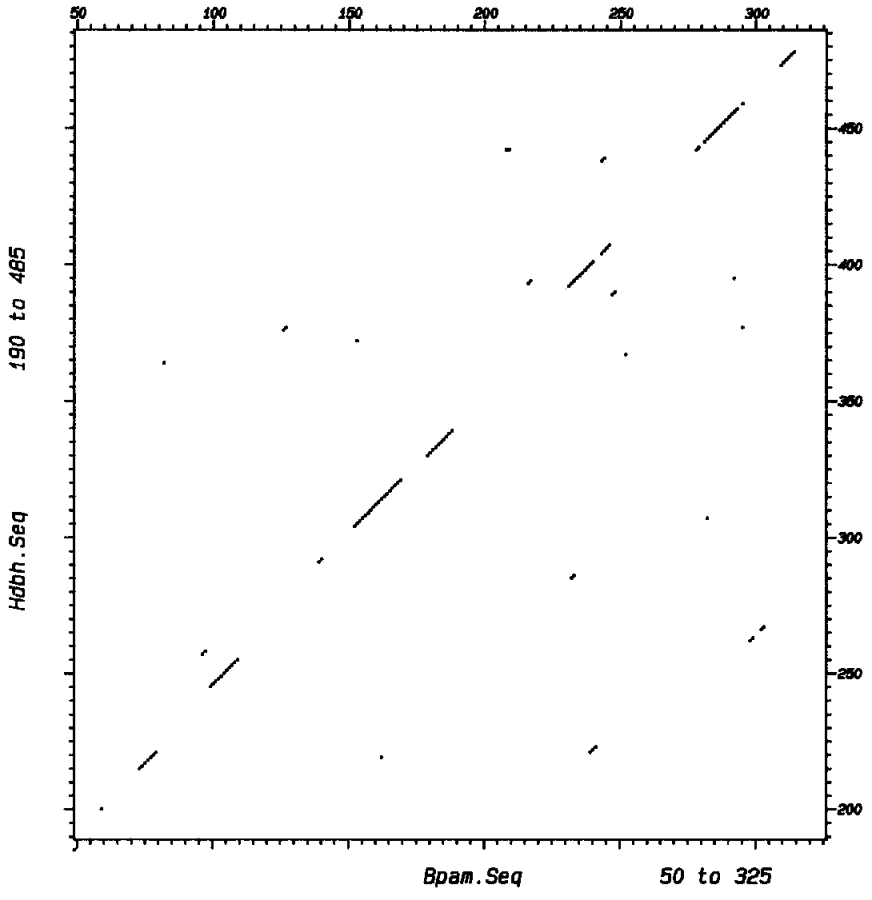

Fig.2. An expanded dotplot of the similarity diagonal from fig.1. A window size of 15 and stringency of 9.0 filtered the plot to 113 points.

identity. However, it should be noted that comparison algorithms of this type can produce a number of alternative alignments with similar scores.

4. DISCUSSION

The finding of sequence similarity throughout an extended section of primary structure, with only limited gapping, suggests a genuine homology in the sense that the two proteins may have evolved from a common precursor. The possible biological significance is supported by previous proposals of similarity between these two enzymes based on biochemical evidence alone [1,9]. PAM enzymes catalyse the conversion of carboxy-terminal glycine extended peptides to an α-amidated product. This occurs concomitantly with secretion and is essential for full biological potency of some pro-hormones (for reviews see [9,10]). Biochemical similarities between DBH and PAM include the following. Both enzymes are type II copper-containing monooxygenases from secretory granules, requiring ascorbate and molecular oxygen. The ascorbate is recycled in both cases via a mitochondrial semidehydroascorbate reductase and cytochrome b561 [11,12]. They have similar kinetic mechanisms, both enzymes can use catechol or ferrocyanide as single-electron reductants. They also both show significant deuterium isotope effects in V_{max}, although the value for PAM is significantly larger [13].

Several lines of evidence indicate that the section

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DBH 190 EPELPSDACTMEVQAPNIQIPSQETTYWCYIKELPKGFSRHHIIKYEPIV 239
PAM  50 .IPIDSSDFALDIRMPGV.TPKQSDTYFCMSVRLPMD.EEAFVIDFKP.. 94

240 TKGNEALVHHMEVFQCAPEMDSVPHFSGPCDSKMKPDRLNYCRHVLAAWA 289
 95 .RASMDTVHHMLLFGCNMPASTGNYWF..CDEGTCTDKAN....ILYAWA 137

290 LGAKAFYYPEEAGLAFGGPGSSRYLRLEVHYHNPLVI.EGRNDSSGIRLY 338
138 RNAPPTRLPKGVGFRVGGETGSKYFVLQVHYGDISAFRDNHKDCSGVSLH 187

339 YTAKLRRFNAGIMELGLVYTPVMAIPPRETAFILTGYCTDKCTQLALPPS 388
188 LTRLPQPLIAGMYLMMSVDT...VIPP..GGKVVNSDISCHYKKYPM... 229

389 GIHIFASQLHTHLTGRKVVTVLVRDGREWEIVNQDNHYSPHF.QEIRMLK 437
230 ..HVFAYRVHTHHLGKVVSGYRVRNG.QWTLIGRQ...SPQLPQAFYPVE 273

438 KVVSVHPGDVLITSCTYNTEDR.ELATVGGFGILEEMCVNYVHYYPQTQ 485
274 HPVDVSFGDILAARCVFTGEGRTEVTHIGGTSS.DEMCNLYIMYYMEA. 320
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Fig.3. An optimised alignment of the similarity region between human DBH (top) and bovine PAM using the GAP program with a gap setting of 2 and length weight of 0.3. The positions of gaps are marked with dots. Identical residues are indicated with vertical lines. Two histidine clusters are underlined and the position of conserved Cys residues is marked with an asterisk.

of similarity between these two enzymes represents a common catalytic domain. Between rat, bovine and frog PAMs this section is highly conserved and tissue-specific truncated forms of active PAM of between 300 and 350 residues can be produced in vivo by alternative mRNA splicing and/or endoproteolytic processing [8]. Residues 38 to 381 of frog PAM expressed in *E. coli* show amidating activity in vitro [6]. Independent evidence for an extended catalytic domain in DBH comes from attachment sites of mechanism-based inhibitors. Three of these have recently been identified, Tyr 216 [4] and Tyr 357 for *p*-cresol (unpublished results) and His 398 for β-ethinyltyramine [5]. The chemistry of inactivation for these compounds indicates that covalent incorporation should be restricted to the vicinity of the active site. This idea is supported by the location of all 3 modified residues within the putative catalytic domain described above. Characterisation of the attachment site peptides [4,5] together with extensive partial sequence data (unpublished observations) indicate approximately 95% sequence identity between human and bovine DBH throughout this domain.

Several consensus features in this section of the PAM sequences from 3 species are also present in DBH (fig.3). Out of 7 Cys residues in PAM, 5 are, allowing for small gaps, in identical or similar positions in DBH. This implies some conservation of the pattern of intra-chain disulphide formation. Two histidine clusters are also conserved at positions 250 and 398 in human DBH. Recent spectroscopic studies have been carried out to examine the local environment of the copper sites in bovine DBH [14–18]. There is a general agreement of a coordination sphere of 3–4 histidyl imidazoles for each Cu^{2+} although none of the studies suggest if

additional ligands are necessary in changing from an occupancy of 1 to 2 copper atoms per subunit. Direct evidence for a ligand motif of the type H-X-H in copper type II proteins comes from the 3-dimensional structure of the copper binding site in superoxide dismutase [19]. Two candidates for ligand clusters of this type, conserved between 3 PAM species and human DBH, would be -H-X-M- and H-X-H- as indicated in fig.3. The proximity of Met as a ligand is supported by the data of Scott et al. [14] suggesting Cu-S coordination to the reduced, Cu^+ form of DBH. It has recently been proposed that the catalytically competent form of DBH contains two reduced coppers per subunit in functionally non-equivalent sites separated by more than 4 Å [18]. This clearly requires more ligands than the two pairs indicated above. Although the copper binding stoichiometry of PAM has not yet been established, both enzymes contain additional potential ligand clusters but in different positions. These would include HYH at position 319–321 in DBH and an unusual Met/His cluster conserved at position 345 in PAM.

To extend our search for possible copper binding domains we compared DBH to some of the other major types of copper proteins for which sequences were available in the databases, including other copper type II enzymes and those containing type I and type III copper sites amongst which evolutionary relationships have already been detected [20,21]. Some of these were only available from GenBank entries and would therefore not have been accessible to our initial search of the Dayhoff library. Dotplots of human DBH against human tyrosinase, tyrosine-3-hydroxylase, ceroplasmin, cytochrome oxidase, superoxide dismutase, *Neurospora* laccase, and lobster haemocyanin, were made at a similar level of signal filtration to that used in fig.1 but no extended diagonals were seen. Although these results do not preclude the existence of distant relationships between DBH and these proteins they support our proposal that on the basis of both primary structure similarities and biochemical evidence DBH and PAM are members of a new protein family of type II copper monooxygenases.

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