

Sequence of a novel carbonic anhydrase-related polypeptide and its exclusive presence in Purkinje cells

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I isolated a mouse cDNA clone encoding a novel polypeptide which has strong homology with carbonic anhydrase. Unlike the other carbonic anhydrases, it has an additional N-terminal domain with a glutamic acid stretch and an arginine substitutes one of the three histidine residues which bind zinc ion. In the central nervous system, carbonic anhydrase is known to be expressed only in glia cells, but this gene is expressed in neuron, but only in Purkinje cells.

Carbonic anhydrase; Purkinje cell; cDNA cloning

1. INTRODUCTION

The zinc metalloenzyme carbonic anhydrase (CA; carbonate dehydratase, EC 4.2.1.1) catalyzes the reversible hydration of CO₂. Seven isozyme forms (CA I–VII) and several related genes have been identified in mammals [1], and their primary structures show that they belong to a large multiple gene family. The three-dimensional structures of several carbonic anhydrases are known [2,3] and the amino acid residues located in the active site are strongly conserved among different members of the family [1].

In the central nervous system, carbonic anhydrase is expressed only in glia cells, and has been used as a glia cell marker [4]. In particular, extensive developmental studies have been done in retina using it as a marker for Müller cells [5]. Based on immunological, electrophoretic and kinetic criteria, the enzyme in glia cells is a CA II type isozyme [6], and distinct from carbonic anhydrases of the other tissues because over 50% of it is membrane-bound [7].

Mouse cDNA clones have been collected with specific expression patterns in mouse brain [8]. DNA sequence analysis reveals that one of them has strong homology with carbonic anhydrases. However, unlike other carbonic anhydrases, the sequence has an additional N-terminal domain with a glutamic acid stretch. Amino acid residues within the active site are well-conserved, but one histidine residue which binds zinc ion is substituted. Interestingly, this gene is expressed unique in Purkinje neurons of the cerebellum.

2. MATERIALS AND METHODS

Total RNA was extracted by the guanidinium-CsCl method from mouse brain and poly(A)⁺ RNA was purified by oligo(dT) cellulose column chromatography [9]. A λ gt10 mouse brain cDNA library was constructed using the method of Gubler and Hoffman [10]. To obtain full-length copies, the cDNA was fractionated on a Sepharose CL-4B column and the top 5% was used for the library. The probe used is H30 [8]. Among several positive clones, the longest one was selected and sequenced by the dideoxy chain termination method using the shot-gun strategy [11].

Poly(A)⁺ RNA (1 μ g per lane) was electrophoresed in 1% (w/v) formaldehyde agarose gel, blotted onto a nylon membrane (GeneScreenPlus, Du Pont). Northern hybridization was done by a standard condition [8]. The probe was the same as that used for the library screening.

For in situ hybridization, mouse brain sections were mounted on organosilane-treated microscope slides and kept at –20°C until use. Following fixation and pronase digestion, the sections were prehybridized for 2 h at 37°C in 50% formamide, 5 \times SSC, 10 mM Na-PO₄ (pH 7.0), 25 mM DTT, 2 \times Denhardt's and 0.1% SDS. Hybridization was performed at 42°C for 30–40 h in the above solution containing 250 μ g/ml denatured salmon sperm DNA and ³⁵S-labelled DNA probe (1–1.5 \times 10⁶ cpm/ml). The probe used was single stranded DNA derived from an M13 clone containing the H30 insert. The sections were washed in three changes of 2 \times SSC at room temperature, and then in 50% formamide, 0.5 M NaCl, 10 mM Tris-HCl and 1 mM EDTA (pH 7.4) at 37°C for about 6 h. They were dehydrated in 70% ethanol, and were dipped into Ilford K-5 emulsion and kept at 4°C for 3 weeks. After developing and fixation, sections were stained with Cresyl violet and mounted.

3. RESULTS AND DISCUSSION

In a previous experiment, 35 cDNA clones with specific expression patterns in mouse brain were selected from 950 cDNA clones by Northern and in situ hybridization using the cDNA inserts as probes [8]. One clone, H30, is expressed only in Purkinje cells. The DNA sequence of a full-length clone was determined on both strands. Its longest open reading frame encodes

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1	TAATACGACTCACTATAGGCGGAATTGGAGCTCCACCGCGGTGGCGCCGCGCTCCCGC	60
-6	M A D L S F I E D A V A F	13
61	ACCCGTGTCGAGAGCTGCCATGGCTGACCTGAGCTTCATTGAGGATGCAGTCGCCTTC	120
14	P E K E E D E E E E E E G V E W G Y E	33
121	CCCGAGAAGGAGGAGACGAGGAAGAGGAGGAGGAGGCGGTGGAGTGGGGTTACGAG	180
34	E G V E W G L V F P D A N G E Y Q S P I	53
181	GAAGTGTGAGTGGCGCTTAGTGTCTTCTGATGCTAATGGGAGTACAGTCACCTATC	240
54	N L N S R E A R Y D P S L L D V R L S P	73
241	AACCTAACTCCAGAGAAGCCAGGTATGACCCCTCACTGCTGGATGTCGCGCTGTCTCCA	300
74	N Y V V C R D C E V T N D G H T I Q V I	93
301	AATTATGTGGTGTGCCGGGACTGTGAAGTCACCAACGATGGACACACCATTCAGTCATC	360
94	L K S K S V L S G G P L P Q G Q E F E L	113
361	CTGAAGTCAAAATCAGTCTTGTCAGGAGGACCACTGCCTCAGGACAGGAGTTTSGCTG	420
114	Y E V R F H W G R E N Q R G S E H T V N	133
421	TATGAAGTTAGATTCTACTGGGGAAGAGAAACAGCGTGGTCTGAGCACACGGTCAAT	480
134	F K A F P M E L H L I H W N S T L F G S	153
481	TTCAAAGCCTTTCCATGGAGCTCCACCTGATCCACTGGAATCCACACTGTTTGGCAGC	540
154	I D E A V G K P H G I A I I A L F V Q I	173
541	ATCGATGAGCGGTTGGGAAACCCCATGGTATCGCCATCATTGCCTATTGTTTCAGATC	600
174	G K E H V G L K A V T E I L Q D I Q Y K	193
601	GGGAAGGAACATGTTGGCTTGAAGGCTGTGACTGAGATCCTCCAGGATATTCATTAAG	660
194	G K S K T I P C F N P N T L L P D P L L	213
661	GGAAATCCAAACAATTCATGCTTTAATCCTAACACTTTATTACCAGACCCCTCTCTG	720
214	R D Y W V Y E G S L T I P P C S E G V T	233
721	CGGGATTACTGGGTCTATGAAGGATCTCTTACTATCCACCTTGCAGCGAAGGAGTTACC	780
234	W I L F R Y P L T I S Q M Q I E E F R R	253
781	TGGATATTATCCGATACCCCTTTAATATATCCAGATGCGATAGAAGAATTCGAAGG	840
254	L R T H V K G V E L V E G C D G I L G D	273
841	CTGAGGACACATGTCAGGGGGTAGAAGTGGTGGAGGCTGCGATGGGATTTGGGAGAC	900
274	N F R P T Q P L S D R V I R A A F S S Q	293
901	AATTTCCGACCTACCCAGCCCTGAGTGATAGAGTCATCCGAGCGGCTTCAGTAGCCAG	960
294	R D R E Q T H L H Q *	313
961	AGAGACCGGGAACAAACCCATCTGCATCAGTGAAGAAGACAGTGGTCCACCGGAGTCCCT	1020
1021	TGATGAGGAGTGGAACTCCTGAGTGGCAGCTTTGTTTAAACCTCAAGCCTGCAGATAG	1080
1081	AAGAATTCGAAGGCTGAGGACACATGTCAGGGGGCAGAATGGTGGAGGGCTGCGATG	1140
1141	GGATTTTGGGAGACAAATTCGACCTACCCAGCCCTGAGTGATAGAGTCATCCGAGCGG	1200
1201	CCCTTCAGTAGCCAGAGACCGGGGAACAAACCCATCTGCATCAGTGAAGAAGACAGTGG	1260
1261	TCCCACGGAGTCCCTTGTGATGAGGAGTGGAACTCCTGAGTGGCAGCTTTGTTTAAACCT	1320
1321	CAAGCCTGCATTATCTTCAGTTACTGCGATGTTGATGGATATCTGTGACCATTTGCTGTA	1380
1381	TACATGCTGTATGAAATATTAGAAGTGGCTGTTTCATTTTAAAGAAACACAGTGTGTGT	1440
1441	TGCACACACCACAGCTTCTGGAATTCATACTTTTGCACATACTGCTTGTGCTTATGTGT	1500
1501	AGAAAGAATGAAGTAGTTTTCAGACATACTAATATGACTGATTATATATCAATATGAG	1560
1561	ATAAGAAAAGCACATTCAGAGAGCCAGTCATTATTCTGCTCTGATATAGAAAATATG	1620
1621	CTCAGTAGTGTATTCTTGATAATAAGCAAGATGTTTGATCTACACAGCTTAGAACAT	1680
1681	CAAAATACAAATATGAAGGAGTCATAAAAATTATCTCGTGTGTTTGTGCAATATTGCAC	1740
1741	CAAAATTAATGTCAAACCTGGTTTGTGATGGAAAAATTTTGAAGTTAATGCAAGATATT	1800
1801	TTATATATAATTTTAAAAAGAATTTAATTGAATCTAATATACCTTATGGCATATCTGAAT	1860
1861	GTATGGCTCTAAGGAGTTAAGATAGCATAAAAATAGATAGGAGGAAAGA*GGGGACAG	1920
1921	CATACCAAGTAATAGCTTTGTTTCCATATAGAAATAAAATCCACTAGTATACTATGTTAA	1980
1981	TTT 1983	

Fig. 1. Nucleotide and predicted amino-acid sequence of a cDNA encoding the carbonic anhydrase-related polypeptide (CARP). The amino acid sequence of the predicted polypeptide is shown as the single letter code above the nucleotide sequence.

303 amino acids (Fig. 1). Comparison of the predicted amino acid sequence with the existing data base reveals that H30 is closely related to carbonic anhydrase. The overall similarity is 37.7% with mouse CA I, 40.7% with mouse CA II, 37.3% with rat CA III and 32.9% with sheep CA VI. This polypeptide is therefore designated as carbonic anhydrase-related polypeptide (CARP). The deduced amino acid sequence and comparison with other carbonic anhydrases are shown in Fig. 2.

It is possible to compare the active site residues of the different CA isozymes. Of 36 such residues at homologous positions, 17 are invariant among all animal sequences [1]. Those of CARP are also well-conserved, but histidine at the position 124 in Fig. 2 and glutamine at the position 122 are substituted by arginine and glutamic acid, respectively. Histidine 124

is one of the three histidine residues which binds zinc ion, and the glutamine residue participates in forming hydrogen-bond network to zinc-bound solvent molecule [1].

Northern hybridization shows that CARP is expressed only in the cerebellum, and is absent from the other parts of the brain (Fig. 3, left). There are two band sizes, of 2.1 and 1.6 kb respectively, possibly reflecting two different polyadenylation sites. In situ hybridization experiment reveals that CARP is localized in Purkinje cells (Fig. 3, right). This makes a clear contrast with carbonic anhydrase, because it is expressed only in glia cells.

The function of CARP is not at all clear, and it is therefore uncertain that CARP even possesses a carbonic anhydrase activity. Because several activities other than the reversible hydration of CO₂ have been

Fig. 2. Comparison of the amino acid sequence of CARP and carbonic anhydrases. Identical residues are indicated by asterisks and conserved residues by dots. The three histidine residues which bind zinc ion are indicated by arrowheads.

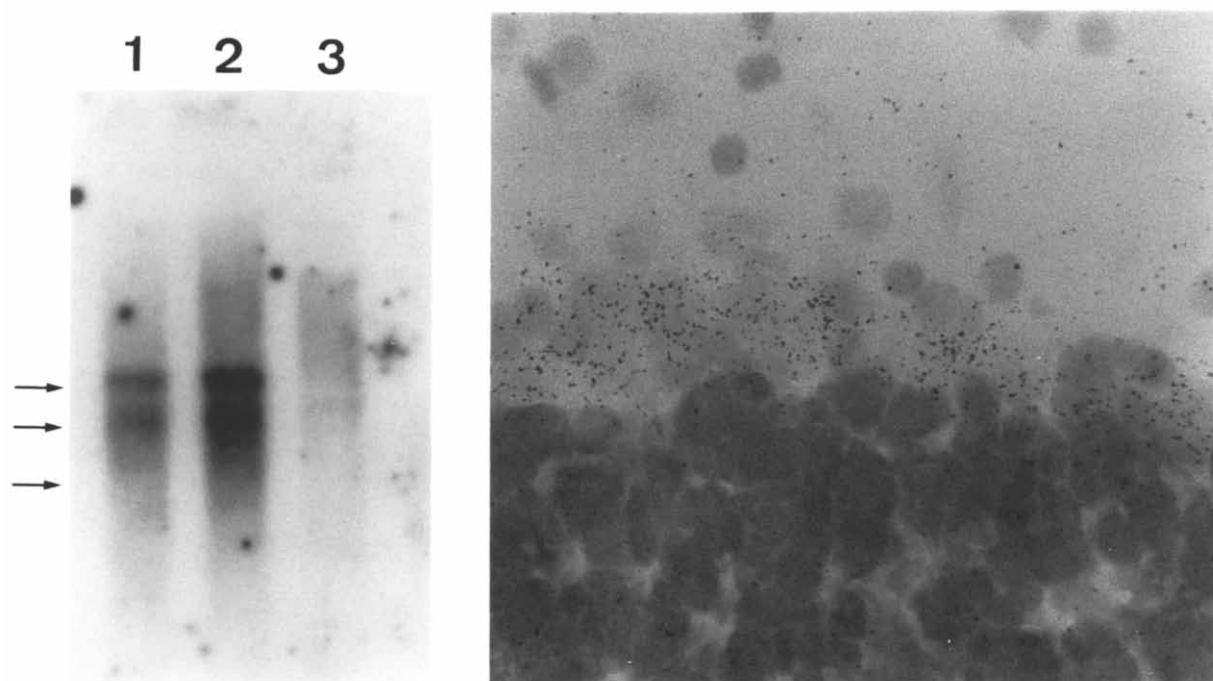


Fig. 3. (Left) Northern blot analysis of CARP. The sources of poly(A)⁺-RNA are as follows: lane 1, 12-day-old mouse cerebellum; lane 2, 22-day-old mouse cerebellum; lane 3, 22-day-old mouse whole brain minus cerebellum. Left arrows indicate size markers: from top, 1.9, 1.5 and 1.1 kb, respectively. (Right) In situ hybridization analysis of CARP in the mouse cerebellum. Note that silver grains are scattered over only the Purkinje cell layer. The probe was the same as that used for the library screening.

	20	40	60
CARP (mouse)	ADLSFIEDAVAFPEKEDEEEEEEGVEWGY--EE-GVE-WGLVFPDANGEYQSPINLNS		
CAI (mouse)	AS-----ADWGYGSEN-GPDQWSKLYPIANGNNQSPIDIKT		
CAII (mouse)	SH-----HWGYSKHN-GPENWHKDFPIANGDRQSPVDIDT		
CAIII (rat)	AK-----EWGYGSHN-GPEHWHELYPIAKGDNQSPIELHT		
CAVI (sheep)	GH-----GVEWTYSEGMLDEAHWPLEYFKCGGRQSPIDLQM		
	. * . * . * . * . * . *		
	80	100	120
CARP (mouse)	REARYDPSLLDVRLSPNYVV-CRDCEVTNDGHTIQVILK---SKSVLSGGPLPQGGQEFEL		
CAI (mouse)	SEANHDSSL--KPLSISYNF-ATAKEIVNVGHSFHVIFDDSSNQSVLKG-PLADS--YRL		
CAII (mouse)	ATAHHDPAL--QPLLSYDK-AASKSIVNNGHSFNVEFDDSQDNAVLKGGPLSDS--YRL		
CAIII (rat)	KDIRHDFSL--QFWSVSYDP-GSAKTI LNNGKTCRVVFDFTDRSMLRGGPLSGP--YRL		
CAVI (sheep)	KKVQYNPSLRALNLT-GYGLWHGEFFVTNNGHTVQISLPSTMSMTT-----SDGTQYLA		
 * * * *		
	140	160	180
CARP (mouse)	YEVRFHWGRENQ--RGSEHTVNFKAFFMELHLIHWNSTLFGSIDEAVGKPHGIAITIAFV		
CAI (mouse)	TQFHFHWGNSND-HGSEHTVDGTRYSGELHLVHWNSAKYSSASEAISKADGLAILGVLM		
CAII (mouse)	IQPFHFWG--SSDG-QGSEHTVNKKYAAELHLVHWNT-KYQDFGKAVQPPDGLAVLGYFL		
CAIII (rat)	RQFHLHWG--SSDD-HGSEHTVDGVRYAAELHLVHWNP-KYNTSEELKQPDGIAVVGIFL		
CAVI (sheep)	KQMHFWGCGASSEISGSEHTVDGMRVYIEIHVVHYNK-KYNSYEEAQKEPDGLAVLAALV		
 * * * *		
	200	220	240
CARP (mouse)	QIGK--EHVGLKAVTEILQDIQYKGKSKTIPCFNPNTLLPDLRDYVWYEGSLTIPFCS		
CAI (mouse)	KVGP--ANPSLQKVLDALNSVKTGKRAPPNTNFDPSLLPSSL--DYWTYFGSLTHPPLH		
CAII (mouse)	KIGP--ASQGLQKVLEALHSIKTKGKRAAFANFDPCSLLPGNL--DYWTYFGSLTTPPL		
CAIII (rat)	KIGR--EKGEFQILLDALDKIKTKGKEAPFNHFDPSCLFPACR--DYWTYHGSFTTTPCE		
CAVI (sheep)	EVKDYTENAYYSKFISHLEDIRYAGQSTVLRGLDIEDMLPGD-LRYYYSYLGSITTPPCT		
 * * * *		
	260	280	300
CARP (mouse)	EGVTWILFRYPLTISQMQIEEFRRLRTHVKGVELVEGCDGILGDNFRPTQPLSDRVIRAA		
CAI (mouse)	ESVTWVICKDSISLSPEQLAQLRGLLSSAEG-----ESAVPVLNHRPPQPLKGRTRVRS		
CAII (mouse)	ECVTWIVLREPITVSSEQMSHFRTLNFNEEG-----DAEEAMVDNWRPAQPLKNNRIKAS		
CAIII (rat)	ECIVWLLKPEMTVSSDQMANVRSLFASAEN-----EPPVPLVGNWRPPQPLKGRVVRAS		
CAVI (sheep)	ENVHFWFVADTVKLSKTQVEKLEN-----SLLNHQNKTIQNDYRRTQPLNHRVVEAN		
	* * * * * *		
	320	340	
CARP (mouse)	FSSQRDRE---QTHLH-----Q		
CAI (mouse)	F-----		
CAII (mouse)	F-----K		
CAIII (rat)	F-----K		
CAVI (sheep)	FMSRPHQEYTLASKLHFYLNNDQITLEYLRRFIEQKIPKRRKQENWP		
	*		

reported with CA isozymes [12], the main function of CARP might be different from the reversible hydration of CO₂. Destruction of the CARP gene using some transgenic procedure may be useful to analyze its function [13].

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