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# Human mitochondrial carbonic anhydrase: cDNA cloning, expression, subcellular localization, and mapping to chromosome 16

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ABSTRACT **A full-length cDNA clone encoding human mitochondrial carbonic anhydrase (CA), CA V, was isolated from a human liver cDNA library. The 1123-bp cDNA includes a 55-bp 5′ untranslated region, a 915-bp open reading frame, and a 153-bp 3′ untranslated region. Expression of the cDNA in COS cells produced active enzyme. The 34-kDa precursor and 30-kDa mature form of CA V were identified on Western blots of COS-cell homogenates by a CA V-specific antibody raised to a synthetic peptide corresponding to the C-terminal 17 aa of CA V. Both 34-kDa and 30-kDa bands were also present in mitochondria isolated from transfected COS cells, whereas only the 30-kDa band was present in mitochondria isolated from normal human liver. The N-terminal sequence determined directly on the 30-kDa soluble CA purified from transfected COS cells indicated that processing of the precursor to mature human CA V involves removal of a 38-aa mitochondrial leader sequence. The 267-aa sequence deduced for mature human CA V shows 30–49% similarity to amino acid sequences of previously characterized human CAs (CA I–CA VII) and 76% similarity to the corresponding amino acid sequence deduced from the mouse cDNA. PCR analysis of DNAs from human–rodent somatic cell hybrids localized the gene for CA V to human chromosome 16, the same chromosome to which CA VII has previously been mapped.**

Seven carbonic anhydrases (CAs), CA I–CA VII, have been identified in mammals (1). They differ in their physicochemical and enzymatic properties and in their subcellular localizations. CA I, CA II, CA III, and CA VII are cytosolic (1, 2). CA IV is anchored to the extracellular surface of the plasma membranes in certain differentiated cells (3–5), CA V is mitochondrial (6), and CA VI is secreted in saliva (7–9). The physiological importance of CA II became clear from the findings that a deficiency of CA II was responsible for the inherited syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification (10, 11).

The presence of CA in mitochondria was recognized as early as 1959 (12). Later, acetazolamide was reported to inhibit $HCO_3^-$-linked $Ca^{2+}$ uptake by mitochondria (13). In 1980, Dodgson *et al.* (14) characterized a CA in mitochondria isolated from guinea pig liver. The physiological significance of mitochondrial CA was suggested by observations from several groups that synthesis of glucose and urea in alligators and chameleons (15) and in isolated rat hepatocytes (16) was decreased by CA inhibitors. Inhibition of citrulline synthesis in intact isolated guinea pig mitochondria was also reported (17).

Mitochondrial CA has been purified from guinea pig liver using inhibitor affinity chromatography. The N-terminal amino acid sequence was determined and was found to contain homology with other CAs (18). Immunochemical studies were recently interpreted to indicate that CA V is present in most rat tissues (19). However, a mouse cDNA was isolated (20) that appeared to encode a mitochondrial CA (21), but Northern blot analysis indicated expression only in mouse liver (20). We made the assumption that this mouse cDNA, which was provisionally named CA Y (21), is the cDNA for the mouse mitochondrial CA (CA V) and used it as a probe to isolate the cDNA for human CA V. Here we describe the isolation, sequence, and expression of the human CA V cDNA,† the N-terminal sequence determined directly on the mature CA V expressed in COS cells, the demonstration of the enzyme immunochemically in mitochondria isolated from COS cells and human liver, and the chromosome localization of the gene specifying human CA V.

## MATERIALS AND METHODS

**Isolation and Characterization of cDNA Clones.** A mouse CA V cDNA was prepared from C57/BL total mouse liver RNA by the method of reverse transcriptase–polymerase chain reaction using PCR primers 5′-GACAGATCTACCTAACTCTGAGGTGGC-3′ and 5′-GTCCTCGAGTGGCTGTCCTGGAACTCA-3′ as forward and reverse primers, respectively (20). A human liver cDNA library in the Uni-ZAP XR vector (Stratagene) was screened using the mouse CA V cDNA as probe (22). The DNA insert in pBluescript was excised from the Uni-ZAP XR clone and the double-strand DNA insert was sequenced by the dideoxynucleotide chain-termination method of Sanger using $^{35}S$-labeled dATP (23).

**Transfection of COS-7 Cells.** The cDNA insert from the Uni-ZAP XR clone was subcloned into the pCAGGS vector originally described by Miyazaki *et al.* (24) and utilized by Yoshida *et al.* (25). The pCAGGS human CA II cDNA was from Peiyi Hu of this laboratory. COS-7 cells in 60-mm dishes were transfected with 15 μg of DNA per dish using the DEAE-dextran procedure (26) and chloroquine treatment 12 h after transfection (27). Transfected cells were harvested by scraping into phosphate-buffered saline (PBS) 84 h after transfection and homogenized in 25 mM Tris·$H_2SO_4$, pH 7.2/1 mM benzamidine/0.05% Triton X-100.

**CA Assay.** CA activity was assayed in cell homogenates as described (28, 29). The protein concentration was determined (30) using bovine serum albumin as standard.

Metabolic labeling of transfected COS cells was carried out in 60-mm dishes transfected with 10 μg of plasmid as described (46). Sixty hours after transfection, cells were labeled with [$^{35}S$]methionine at 100 μCi per 60-mm dish (1 Ci = 37 GBq) for 3 h, followed by a 4-h chase with unlabeled methionine. Cells were harvested in PBS containing 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM benzamidine, and 5 mM iodoacetamide.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L19297).

**Purification of Human CA V.** The COS-7 cells transfected with CA V cDNA were homogenized in 10 mM Tris·$H_2SO_4$ (pH 9) containing 1 mM benzamidine, 2 mM PMSF, 5 mM iodoacetamide, and 0.1% Triton X-100. The clear supernatant after centrifugation at 40,000 × *g* for 1 h was applied to the benzenesulfonamide affinity column. The unbound proteins were removed by washing, and the bound enzyme was eluted in 100 mM sodium acetate (pH 5.5) containing 0.5 M sodium perchlorate (5).

**Immunochemical Methods.** Antiserum against the synthetic C-terminal 17-aa peptide was produced by injecting the C-terminal peptide conjugated to porcine thyroglobulin into rabbits in complete Freund's adjuvant and giving the animals a booster injection 4 weeks later with the same antigen in incomplete Freund's adjuvant (5). The titer and specificity of the antiserum from rabbits were determined by Western blot analysis.

**Western Blot Analysis and N-Terminal Amino Acid Sequencing.** SDS/PAGE was performed under reducing conditions according to Laemmli (31). The polypeptides were electrophoretically transferred to poly(vinylidene difluoride) (PVDF) membranes as described (5). The PVDF membranes were incubated with rabbit antiserum to the CA V C-terminal peptide at a 1:250 dilution followed with a goat anti-rabbit IgG peroxidase conjugate at a 1:500 dilution. The immunoblots were developed as described (32). For N-terminal sequencing, the PVDF membrane was stained with Coomassie blue. After destaining, a piece of membrane with the polypeptide was analyzed in an Applied Biosystems model A77 automatic protein sequencer (5).

**Chromosome Localization.** DNAs from the human–rodent reduced somatic cell hybrid panel for chromosome localization was purchased from the Human Genetic Mutant Cell Repository, Camden, NJ (panel 2). By assuming that intron–exon junctions for exons 2–7 were conserved in CA V, as in the other CAs, PCR primers spanning the putative intron 4 were synthesized. The forward primer from exon 4 was 5′-GAGAGAATGGTTTGGCTGTG-3′ and the reverse primer from exon 5 was 5′-CCTCTGCAGCGTCTGATGAT-3′. PCR was carried out under standard conditions with DNA from each of the samples in the hybrid panel including the human positive and rodent negative controls. PCR products were analyzed after electrophoresis on 0.8% agarose gels and staining with ethidium bromide. The amplified fragment from the hybrid DNA containing chromosome 16 was isolated, subcloned in pBluescript, and sequenced.

**Isolation of Mitochondria.** Mitochondria were isolated from Dounce homogenates of transfected COS cells and human liver in 1 mM PMSF/1 mM benzamidine/5 mM iodoacetamide on a discontinuous sucrose gradient as described (33). Cytochrome oxidase (34) and glycerol-3-phosphate dehydrogenase (35) were measured as markers for mitochondria and cytosolic fractions, respectively.

## RESULTS

**Isolation of Full-Length cDNA for Human CA V.** We used PCR to produce a murine cDNA for CA V from primers designed from the published sequence of the murine cDNA (20) and mRNA from livers of C57/BL mice. After verifying the sequence of the murine cDNA, we used this cDNA as a probe to screen a commercial human liver cDNA library (Stratagene). Four positive clones were identified in $1 \times 10^6$ plaques. The first clone, selected arbitrarily, was subcloned, sequenced, and found to represent a full-length cDNA.

**Nucleotide Sequence and Deduced Amino Acid Sequence.** Fig. 1 presents the nucleotide sequence determined for the 1123-bp cDNA and the deduced amino acid sequence encoded by the 915-bp open reading frame, beginning with the

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                                                                Met Leu Gly Arg Asn Thr  -33
AGACAGCAGGGAACATCACCCTCTTCAGACTGGAGTCAGTGGGAACAGACCCAAG ATG TTG GGG AGG AAC ACT

Trp Lys Thr Ser Ala Phe Ser Phe Leu Val Glu Gln Met Trp Ala Pro Leu Trp Ser Arg  -13
TGG AAG ACC TCA GCT TTC TCC TTC TTG GTT GAG CAG ATG TGG GCC CCT CTC TGG AGT CGT

Ser Met Arg Pro Gly Arg Trp Cys Ser Gln Arg Ser▼Cys Ala Trp Gln Thr Ser Asn Asn   +8
TCG ATG AGG CCA GGG CGA TGG TGT TCT CAG CGT TCC TGT GCA TGG CAA ACC AGC AAT AAC

Thr Leu His Pro Leu Trp Thr▽Val Pro Val Ser Val Pro Gly Gly Thr Arg Gln Ser Pro   28
ACT TTG CAC CCA CTC TGG ACG GTC CCG GTC TCC GTG CCA GGG GGC ACC CGG CAG TCT CCT

Ile Asn Ile Gln Trp Arg Asp Ser Val Thr Asp Pro Gln Leu Lys Pro Leu Arg Val Ser   48
ATT AAC ATC CAG TGG AGG GAC AGC GTC TAT GAC CCC CAG CTG AAG CCA CTC AGG GTC TCC

Tyr Glu Ala Ala Ser Cys Leu Tyr Ile Trp Asn Thr Gly Tyr Leu Phe Gln Val Glu Phe   68
TAT GAA GCG GCA TCC TGC CTG TAC ATC TGG AAC ACT GGC TAC CTC TTC CAG GTG GAA TTT

Asp Asp Ala Thr Glu Ala Ser Gly Ile Ser Gly Gly Pro Leu Glu Asn His Tyr Arg Leu   88
GAC GAT GCC ACC GAG GCA TCA GGA ATT AGT GGT GGG CCC TTG GAA AAC CAC TAC AGA CTG

          *       *
Lys Gln Phe His Phe His Trp Gly Ala Val Asn Glu Gly Gly Ser Glu His Thr Val Asp  108
AAG CAA TTT CAC TTC CAC TGG GGA GCA GTG AAC GAG GGG GGC TCA GAG CAC ACA GTG GAC

                  *
Gly His Ala Tyr Pro Ala Glu Leu His Leu Val His Trp Asn Ser Val Lys Thy Gln Asn  128
GGC CAC GCG TAC CCC GCA GAG CTG CAT TTA GTT CAC TGG AAT TCT GTG AAA TAC CAA AAT

Tyr Lys Glu Ala Val Val Gly Glu Asn Gly Leu Ala Val Ile Gly Val Phe Leu Lys Leu  148
TAC AAG GAA GCT GTC GTG GGA GAG AAT GGT TTG GCT GTG ATA GGC GTG TTT TTA AAG CTC

Gly Ala His His Gln Thr Leu Gln Arg Leu Val Asp Ile Leu Pro Glu Ile Lys His Lys  168
GGG GCC CAT CAT CAG ACG CTG CAG AGG CTG GTG GAC ATC TTG CCG GAA ATA AAA CAT AAG

Asp Ala Arg Ala Ala Met Arg Pro Phe Asp Pro Ser Thr Leu Leu Pro Thr Cys Trp Asp  188
GAC GCG CGG GCG GCC ATG CGC CCC TTC GAC CCC TCC ACT CTG CTG CCC ACC TGC TGG GAT

Tyr Trp Thr Tyr Ala Gly Ser Leu Thr Thr Pro Pro Leu Thr Glu Ser Val Thr Trp Ile  208
TAC TGG ACC TAC GCG GGC TCG CTC ACC ACC CCG CCG CTG ACC GAG TCG GTC ACC TGG ATC

Ile Gln Lys Glu Pro Val Glu Val Ala Pro Ser Gln Leu Ser Ala Phe Arg Thr Leu Leu  228
ATC CAG AAG GAG CCC GTT GAA GTG GCC CCA AGC CAG CTC TCT GCA TTT CGT ACT CTC CTG

Phe Ser Ala Leu Gly Glu Glu Glu Lys Met Met Val Asn Asn Tyr Arg Pro Leu Gln Pro  248
TTT TCT GCA CTT GGT GAA GAG GAG AAG ATG ATG GTG AAC AAC TAT CGC CCA CTT CAA CCC

Leu Met Asn Arg Lys Val Trp Ala Ser Phe Gln Ala Thr Asn Glu Gly Thr Arg Ser ***
TTG ATG AAC CGG AAG GTC TGG GCG TCC TTC CAG GCC ACT AAT GAG GGC ACA AGG TCC TAG

AGAGACATTAGGTCCACATGAATAGCAGAACTGACTTTGAAGGAAGGAAGCGTTGTTTCCCAAGTTTCACAATGTGATT
GTACATGACTTCTGAAAATTAAAAAGAGAGCATGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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FIG. 1. Nucleotide and deduced amino acid sequence of human CA V cDNA. The amino acid sequence is shown above the nucleotide sequence. The putative leader sequence is aa −38 to −1. The solid triangle preceding aa +1 indicates the cleavage site preceding the N-terminal amino acid found in the mature enzyme. The open triangle preceding aa +16 indicates the N-terminal residue found in proteolytically nicked CA V extracted in the absence of protease inhibitors. Amino acid residues showing similarity to those reported for the N terminus of purified guinea pig liver CA V are underlined (aa +20 to +49). Sixteen of the 17 aa residues that are thought to be near the "active sites" and are common to nearly all CAs are boxed. Three potential zinc-binding histidines are indicated by asterisks. The C-terminal polypeptide (aa +251 to +267) used for producing antibodies is indicated by the double underline. The putative polyadenylylation signal in the 3′ noncoding region is underlined.

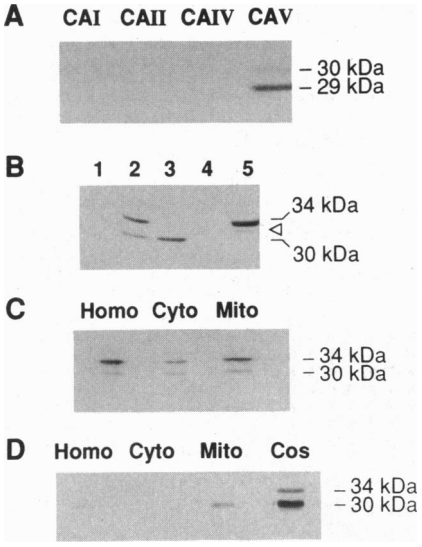


FIG. 2. (*A*) Characterization of monospecific CA V antibodies. Purified human CAs I, II, IV, and V (each at 200 ng per lane) were subjected to SDS/PAGE followed by immunoblot analysis. (*B*) Immunochemical detection of CA V. Homogenates of COS-7 cells transfected with vector only (lane 1) and cDNA for human CA V (lane 2) and soluble CA V fractions from an affinity column (lanes 3 and 4) and membranes sedimented from transfected COS-cell homogenates (lane 5) were subjected to SDS/PAGE and analyzed on an immunoblot. The apparent molecular masses of the polypeptides are indicated. The arrowhead shows a 33-kDa polypeptide that may be a degraded product of CA V associated with membrane. (*C*) Fluorogram of immunoprecipitates of $^{35}$S-radiolabeled CA V. COS cells were transfected with human CA V metabolically labeled with [$^{35}$S]methionine for 3 h and chased for 5 h before Dounce homogenization and fractionation. Immunoprecipitates were analyzed by SDS/PAGE followed by fluorography. (*D*) Western blot of homogenate and fractions from human liver. Protein (150 $\mu$g) from total homogenate, cytosol, and purified mitochondria was analyzed by SDS/PAGE followed by a Western blot. Protein (30 $\mu$g) from a transfected COS-cell homogenate was included in the analysis. (*C* and *D*) Homo, total homogenate; Cyto, cytosol; Mito, purified mitochondria; Cos, transfected COS-cell homogenate.

initiator ATG. The cDNA contains 55 bp of 5′ untranslated sequence and 153 bp of 3′ untranslated sequence, which contains a polyadenylylation cleavage signal 17 bp upstream from the poly(A) tail. The 305-aa sequence for the human CA V mitochondrial precursor predicted from the open reading frame shows 71% similarity to the amino acid sequence deduced from the mouse cDNA (20). The site of proteolytic processing at which the 38-aa mitochondrial leader peptide is cleaved to produce the mature protein is shown in Fig. 1. Another site of presumed proteolytic cleavage, which we infer from direct N-terminal sequencing of the CA V that was expressed in COS cells and isolated in the absence of the protease inhibitors PMSF and iodoacetamide, is also indicated (Fig. 2*A*). The direct sequence result was VPVXVPG-GTXQXPI. The 30-aa sequence from Val-20 to Tyr-49 aligns with the N-terminal sequence reported for the CA purified from guinea pig liver mitochondria (18).

Table 1. CA activities in COS-cell homogenates expressing human CA V cDNA

| Enzyme | CA activity, units per mg of cell protein |
|---|---|
| Vector only | 0.05 ± 0.05 (0.00–0.15) |
| HCA V | 0.32 ± 0.20 (0.11–0.71) |
| HCA II | 312.9 ± 66.1 (234.76–417.38) |

CA activities were measured on four transfections. Numbers in parentheses are the range of CA activities. HCA, human CA.

**Expression and Mitochondrial Localization of CA V.** Table 1 summarizes the CA activity in homogenates of COS cells transfected with an expression vector without, or with, a cDNA insert corresponding to the cDNA for human CA V or human CA II. Extracts of COS cells expressing the CA V cDNA had 6 times as much CA activity as COS cells transfected with vector only. Thus, expression of the CA V cDNA produced an active enzyme. However, extracts of COS cells expressing the CA II cDNA produced 1000 times as much catalytic activity as cells expressing the CA V cDNA, suggesting that CA V is a low-activity isozyme compared to CA II.

To identify the expressed proteins immunologically, we developed a CA V-specific antibody by raising a rabbit antibody to a peptide corresponding to the 17-aa C-terminal sequence predicted by the cDNA sequence for human CA V (Fig. 1). Fig. 2*A* shows the reactivity of this antiserum on Western blots to 200 ng of CA V, affinity-purified from transfected COS cells. Reactivity was nondetectable with comparable amounts of other purified human CAs. Although the 29-kDa band was predominant in this preparation of CA V, the 30-kDa band was predominant in subsequent preparations from homogenates of transfected COS cells to which iodoacetamide (5 mM) and PMSF (2 mM) were added as protease inhibitors in addition to 1 mM benzamidine.

Fig. 2*B* shows the immunoreactivity for CA V in homogenates of COS cells transfected with CA V cDNA (lane 2). Immunoreactive polypeptides of 34 kDa and 30 kDa were identified. No reactivity was seen in homogenates of COS cells transfected with vector only (lane 1). Lanes 3 and 4 show the immunoreactive bands from fractions eluted from an affinity column to which the soluble supernatant from the transfected COS cell homogenate had been applied. Most of the 30-kDa soluble enzyme was eluted in the first fraction (lane 3). Lane 5 shows the 34-kDa immunoreactive band associated with the membranes sedimented from the transfected COS cell homogenate at 40,000 × *g* for 1 h.

Fig. 2*C* shows a fluorogram of the radiolabeled 34- and 30-kDa bands immunoprecipitated from total homogenate of transfected COS cells, the cytosol fraction (9000 × *g*, 15 min, supernatant), and purified mitochondria isolated on a discontinuous sucrose gradient. More than 80% of the immunoprecipitable radioactivity was recovered in the purified mitochondria. Fig. 2*D* shows a Western blot of a total homogenate, cytosol and mitochondrial fractions from normal human liver, and a total homogenate from transfected COS cells. Only the 30-kDa mature form was detected in human liver.

Direct amino acid sequencing of the 30-kDa polypeptide isolated by affinity chromatography from homogenates of transfected COS cells (Fig. 2*B*, lane 3) indicated an N-terminal sequence of XAWQTSNNTLH. From this result, we infer that the 30-kDa protein corresponds to the mature CA V and that it is produced by a proteolytic processing step that cuts between the serine at position −1 and the cysteine at position +1 (Fig. 1) and removes a 38-aa mitochondrial leader sequence. The 34-kDa membrane-associated form of CA V (Fig. 2*B*, lane 5, *C*, and *D*) is presumed to be the CA V precursor.

**Comparison of the Amino Acid Sequence of CA V with Those of Other Human CAs.** The deduced amino acid sequence of mature CA V is aligned with those of the other six isozymes in Fig. 3. Forty residues (15% of the total amino acids in human CA V) are identical in all seven isozymes. Comparison of the amino acid residues of the mature form of human CA V with residues at the same positions of the other aligned CAs reveals the following percent identities with mature CA V: CA I, 47%; CA II, 49%; CA III, 44%; CA IV, 30%; CA VI, 35%; CA VII, 48%. Sixteen of the highly conserved amino acids present in nearly all CAs are also present in CA V (Fig. 1). The exception is one highly conserved tyrosine (Tyr-7 in CA I), which is replaced by threonine (Thr-5 in CA V).

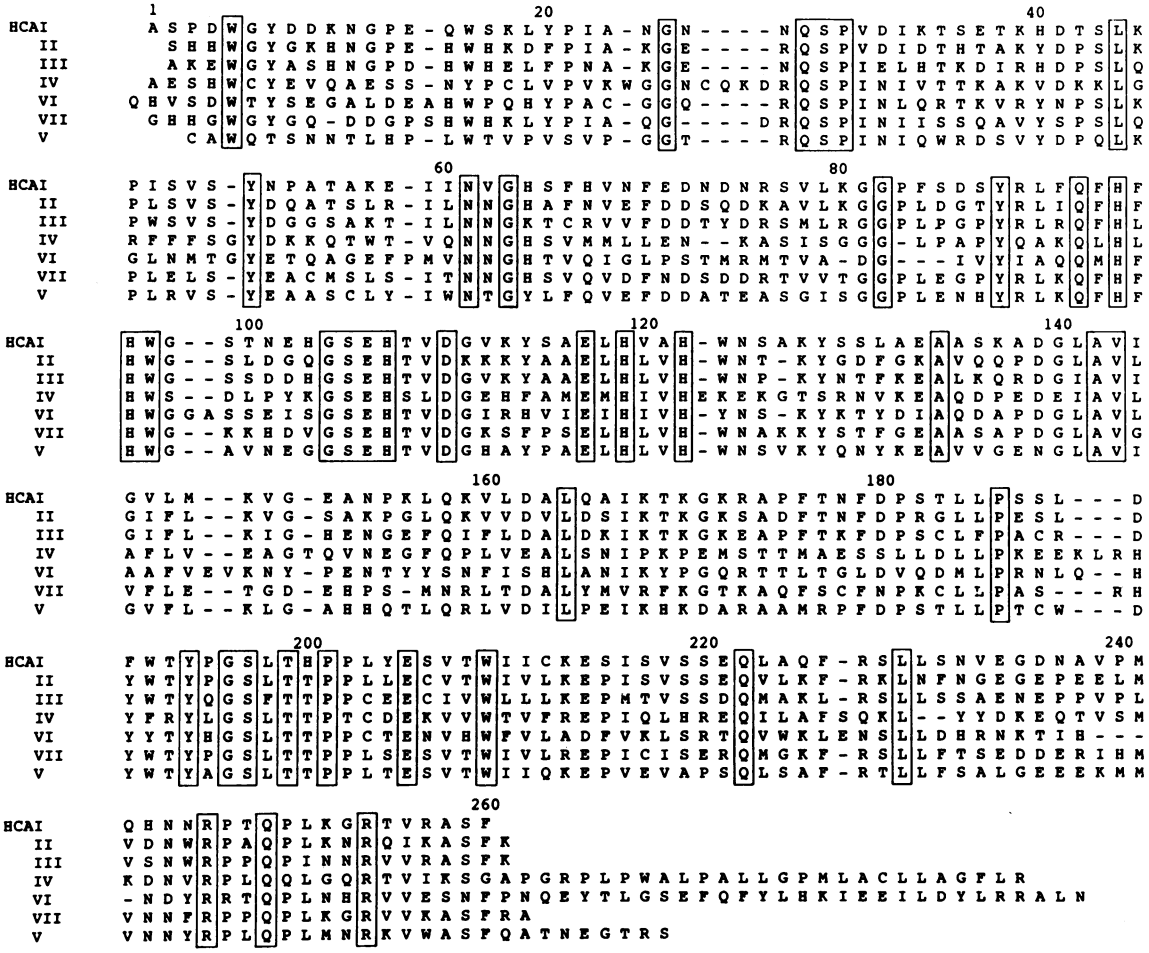

FIG. 3. Comparison of amino acid sequences of human CAs I–VII. Homologous residues in all seven isozymes are boxed. Gaps were introduced to optimize homology. Numbering system used is based on that of human CA I and sequence data for other CAs are from ref. 36.

His-64, a highly conserved histidine that is important for the catalytic activity of CA II (37) and is replaced by lysine in CA III, is replaced by a tyrosine in CA V. Three potential zinc-binding histidine residues are present in human CA V.

**Chromosome Localization of the Human CA V Gene.** The intron–exon boundaries are generally conserved in mammalian CAs, at least for exons 2–7 (38, 39), we postulated that we might use one or more pairs of forward and reverse PCR primers from adjacent exons to amplify a human-specific PCR product from DNA containing the human chromosome that contains the CA V gene. Fig. 4, lanes Hu, M, and Ha, shows that amplification from PCR primers on either side of putative intron 4 produces a PCR product with human DNA but not with mouse or hamster DNAs. PCR of DNAs from the hybrid panel of reduced human–rodent somatic cell hybrids, each of which contains human DNA only from the chromosome indicated, in addition to rodent DNA, produced the band of expected size band only on PCR of DNA from the hybrid containing human chromosome 16. The PCR product was gel-isolated, cloned into pBluescript, sequenced, and found to contain the expected exon boundaries and intervening intronic sequences. These results indicate that CA V is encoded on human chromosome 16.

## DISCUSSION

The evidence presented here leaves no doubt that the cDNA reported is that of authentic human mitochondrial carbonic anhydrase. The subcellular localization of the precursor and mature forms of CA V expressed in COS cells is predominantly mitochondrial. The mature form is the only form seen in human liver, where it is also predominantly mitochondrial. The excess of precursor in the overexpressing COS cells that is not seen in adult liver suggests that production of the mitochondrial precursors exceeds the capacity of the mitochondria in COS cells to import and process the newly synthesized CA V.

The deduced amino acid sequence has several features expected of a mitochondrial CA. (*i*) A classical 38-aa mitochondrial leader sequence (40) includes only one acidic residue, five arginines, six serines, three leucines, and a hydrophobic sequence (aa −30 to −23). (*ii*) The mitochondrial cleavage site deduced from the N-terminal sequence of the mature enzyme is preceded by an arginine at position −2, which was found in 74% of matrix protease cleavage sites (41). (*iii*) A portion of the mature sequence aligns with the limited amount of N-terminal sequence reported for guinea pig mitochondrial CA (42). Only 5 of the 23 residues reported for guinea pig mitochondrial CA are mismatches for human CA V, whereas there are 11–14 mismatches with other human CAs at these 23 sites.

The N-terminal sequence determined on the mature CA V purified from transfected COS cells begins 19 aa upstream of the N-terminal sequences reported for the mitochondrial CAs from guinea pig (18) and rat (43) liver. The differences in N-terminal sequences could reflect species differences in mitochondrial processing. Alternatively, they might indicate that the guinea pig and rat enzymes undergo further processing from the initial mature form in mitochondria, or, more likely, partial proteolytic degradation during isolation. In this regard, we observed a 29-kDa species to be the predominant

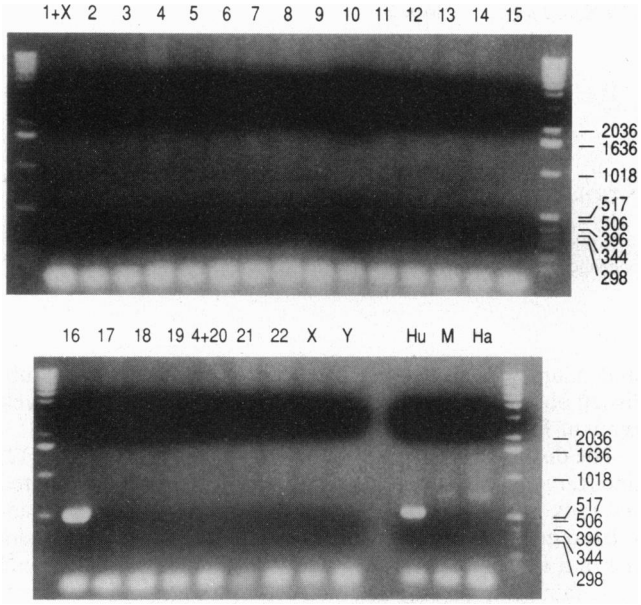


FIG. 4. PCR products containing sequences spanning the putative intron 4 of the CA V gene from genomic DNAs of a human–rodent somatic cell hybrid panel. Genomic DNAs from a human–rodent somatic cell hybrid panel, each of which contained only the human chromosome(s) indicated, were amplified using a forward primer from exon 4 and a reverse primer from exon 5. The PCR products were analyzed by electrophoresis in a 0.8% agarose gel. Only the hybrid panel containing human chromosome 16 gave the specific band expected. The products from control human (lane Hu), mouse (lane M), and hamster (lane Ha) are also shown.

enzyme form isolated by affinity chromatography when we failed to add sufficient protease inhibitors before isolating the mature CA V from transfected cells (see Figs. 1*A* and 2*A*). Direct sequencing of the 29-kDa species showed it to be 15 aa shorter than the mature CA V.

Human CA V appears to be a "low-activity" isozyme, like that isolated from guinea pig mitochondria (6, 18). Two amino acid differences between CA V and CA II could possibly explain why CA V might be a low-activity isozyme. (*i*) His-64 is known to be a crucial residue for the high activity of CA II (37), and it is replaced by tyrosine in CA V. Replacement of this residue by lysine in CA III at least partially explains its low activity (44). (*ii*) Tyr-7 is replaced by threonine in CA V. Whether this residue is important for activity is unclear, but it is conserved in nearly every CA so far characterized (21).

CA V is the second human CA to be assigned to chromosome 16. The other is CA VII, which was mapped to 16q21–23 (45). CAs I–III map very closely on 8q22 and are thought to have arisen relatively recently from gene duplications (47). Other chromosome localizations include chromosome 17q23 for CA IV (39) and chromosome 1p36 for CA VI (48).

Based on the effects of CA inhibitors, CA V may play an important role in ureagenesis (providing $HCO_3^-$ for carbamoyl phosphate synthetase) and for gluconeogenesis (providing $HCO_3^-$ for pyruvate carboxylase) (18). Human diseases that might result from deficiency of CA V might, therefore, have hyperammonemia and fasting hypoglycemia as manifestations. The availability of the cDNA for human CA V should make it possible to evaluate DNA from patients with these manifestations for mutations in the CA V gene.

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