Isolation and Characterization of CA XIV, a Novel Membrane-bound Carbonic Anhydrase from Mouse Kidney

Kiyoshi Mori, Yoshihiro Ogawa, Ken Ebihara, Naohisa Tamura, Kei Tashiro, Takashi Kuwahara, Masashi Mukoyama, Akira Sugawara, Shioichi Ozaki, Issei Tanaka, and Kazuwa Nakao

From the Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan; Center for Molecular Biology and Genetics, Kyoto University, 53 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan, and the Department of Nephrology, Saiseikai Nakatsu Hospital, Shibata 2-chome 10-39, Kita-ku, Osaka 530-0012, Japan

Carbonic anhydrase (CA) is involved in various physiological processes such as acid-base balance and transport of carbon dioxide and ions. In this study, we have succeeded in the isolation of a novel CA from the mouse kidney by use of the signal sequence trap method. It is a 337-amino acid polypeptide with a calculated molecular mass of 37.5 kDa, consisting of a putative amino-terminal signal sequence, a CA domain, a transmembrane domain, and a short hydrophilic carboxyl terminus, which we designated CA XIV. The CA domain of CA XIV is highly homologous with those of known CAs, especially extracellular CAs including CA XII, IX, VI, and IV. The expression study of an epitope-tagged protein has suggested that CA XIV is located on the plasma membrane. When expressed in COS-7 cells, CA XIV exhibits CA activity that is predominantly associated with the membrane fraction. By Northern blot analysis, the gene expression of CA XIV is most abundant in the kidney and heart, followed by the skeletal muscle, brain, lung, and liver. In situ hybridization has revealed that, in the kidney, the gene is expressed intensely in the proximal convoluted tubule, which is the major segment for bicarbonate reabsorption and also in the inner border of the inner stripe of the outer medulla. In conclusion, we have cloned a functional cDNA encoding a novel membrane-bound CA. This study will bring new insights into our understanding of carbon dioxide metabolism and acid-base balance.

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EXPERIMENTAL PROCEDURES

Tissue Preparation and RNA Extraction—The whole kidney and other tissues were obtained from 8-week-old male BALB/c mice. Total RNA extraction was carried out as described by Chomczynski and Sacchi (20). Poly(A)+ RNA was purified using PolyATract (Promega, Madison, WI).

Signal Sequence Trap—Signal sequence trap was performed as described (13–16). 2 μg of poly(A)+ RNA from the mouse kidney was reverse transcribed by random hexamer priming using Superscript II reverse transcriptase (Life Technologies, Inc.), and deoxyadenosine (dA) tails were added at the 3′ end of the first strand cDNA. The second strand was synthesized with a specific primer containing polydeoxothyminide (dT) and an EcoRI restriction site and ligated with an SsrI
adapt. The cDNA fragments of 300–700 base pairs in size were isolated by an agarose gel electrophoresis and subcloned into the EcoRI and SacI sites of pcDL-SRα-Tac(3′) vector (21), to generate a fusion protein with interlin-2 receptor α chain (or Tac antigen) lacking its own signal sequence (22). The expression plasmid library thus obtained was transferred into COS-7 cells by the transfection method using Transfectam (Sepracor, Marlborough, MA). The fusion proteins containing artificial amino-terminal signal sequences were sorted to the cell surface, retained on the plasma membrane by a transmembrane domain of Tac antigen, and detected by cell-surface immunostaining with anti-Tac antibody (23). Otherwise, they remained intracellularly and were not recognized by the antibody.

RESULTS AND DISCUSSION

Isolation and Sequence Analysis of Mouse CA XIV—5,000 clones from the mouse kidney cDNA library were screened by the signal sequence trap method, and 25 positives were isolated. A positive clone G31C5 with a 555-base pair insert encoded a novel 94-amino acid polypeptide. To obtain the full-length cDNA, 3′-RACE was performed, and a 1.6-kilobase fragment was obtained and sequenced. The full-length cDNA encoded a novel CA-like protein with 337 amino acid residues, which had a calculated molecular mass of 37.5 kDa (Fig. 1). Hydropathy analysis (32) predicted that the protein consists of a signal sequence, a CA domain, a transmembrane domain, and a short hydrophilic carboxyl terminus, which we designated CA XIV. CA XIV possessed an N-glycosylation motif in its CA domain and several potential phosphorylation sites by protein kinases A and C in the carboxyl terminus (33). When clone G31C5 was expressed in COS-7 cells as an epitope-tagged protein, it was sorted to the cell surface and detected by cell surface immunostaining (see “Experimental Procedures”). These findings suggest that CA XIV is located on the plasma membrane with its CA domain facing extracellular space.

The amino acid sequence of the CA domain of CA XIV was highly homologous with those of other members of the CA family (Fig. 2). The amino acid identities were 43% for human CA IX and XII, 38% for CA VI and VII, 35% for CA I, II, III, and IV, 32% for CA V and VIII, and 31% for RPTP β and γ (3). The homologies were especially high in the putative CA active site residues including 3 conserved zinc-binding histidine residues (Fig. 1) (3, 34). When CA XIV was compared with CA VII, which is considered the common ancestor of the CA family (3), as many as 30 of 36 active site residues were identical. These findings imply that CA XIV possesses CA activity (3).

Homology search using TBLASTN algorithm (35) revealed the presence of partial cDNA fragments of the putative human counterpart for CA XIV in the data bases, which are >80% identical to mouse CA XIV at the amino acid level, e.g., expressed sequence tag clones with GenBank™ accession numbers H82563, R87427, and AA401879. The structural conservation beyond species may suggest the physiological importance of CA XIV.

Phylogenetic Tree Construction—To examine the evolutionary history of CA XIV among the CA family, a phylogenetic tree was constructed (Fig. 2). The tree consisted of three clusters (3). The first cluster included intracellular CAs (CA I-III, V, and VII), and the second cluster contained RPTP β and γ, which had a calculated molecular mass of 37.5 kDa (Fig. 1). The third cluster may be categorized as extracellular CAs. Consistent with their subcellular localization, CA IV, VI, IX, XII, and XIV possessed putative signal sequences in their amino termini (Fig. 2).

Measurement of CA Activity—To examine whether CA XIV has CA activity, the full-length cDNA was expressed in COS-7 cells. The homogenate of mock-transfected cells exhibited low CA activity, which was inactivated by endogenous CAs (Table 1). Transfection with the CA XIV cDNA resulted in an increase in CA activity by 5.2-fold. The membrane fraction of the homogenate possessed 2.1-fold higher activity than the whole homogenate. After the SDS treatment, which inactivates soluble CAs (29), the CA activity decreased only by 27%. These findings indicate that CA XIV is located predominantly in the membrane fraction.
Northern Blot Analysis—Tissue distribution of CA XIV gene expression was investigated by Northern blot analysis (Fig. 3). The CA XIV mRNA was 1.8 kilobases in size and was expressed most abundantly in the kidney and heart, followed by the skeletal muscle, liver, brain, and lung. No CA XIV transcript was detected in the intestine and spleen. The tissue distribution of CA XIV mRNA is very similar to that of CA IV mRNA, which is expressed in the colon, lung, brain, kidney, and heart but not in the intestine and spleen (37).

In Situ Hybridization Analysis—The intrarenal localization of CA XIV was determined by in situ hybridization analysis with the antisense and sense cRNA probes (Fig. 4). At autoradiograph with the antisense probe, strong hybridizing signals were observed in the cortex. At photomicrograph, these signals were confined to the proximal convoluted tubule, which is known as the most important segment for bicarbonate reabsorption in the nephron (10). At autoradiograph, moderate signals were also seen in the outer border of the inner stripe of the outer medulla. In view of shape, position, number, and comparison with previous reports using histochemical methods (38, 39), these signals most likely correspond to the initial portion of long loop of the thin descending limb of Henle. Physiological roles of CAs in the thin descending limb of Henle still remain to be elucidated (10, 40). Identification of specific inhibitors for CA XIV may facilitate our understanding concerning such issues (2, 10). No specific signals were seen in sections hybridized with the sense probe (not shown).

In the kidney, two CA isoenzymes, CA II and CA IV, have been well characterized (10). CA II is a cytosolic isoenzyme localized in the intercalated cells of the distal tubule and collecting duct, and the thin descending limb of Henle (41, 42). On the other hand, CA IV is a membrane-bound isoenzyme localized in the proximal convoluted tubule and the thick ascending limb of Henle (43). Thus, the intrarenal localization of CA XIV is distinct from those of CA II and CA IV, suggesting its unique function in the kidney.

Comparison of CA XIV with CA IV—CA XIV resembles CA IV in that it has a putative signal sequence in its amino terminus, is membrane-bound, and likely locates on the plasma membrane. It is also similar to CA IV in that it is a membrane-bound enzyme, although its exact subcellular localization is not yet known. However, CA XIV differs from CA IV in that it has a different signal peptide, is localized in different segments of the nephron, and may have different physiological roles.

Table I

| CA activity of CA XIV cDNA-expressing COS-7 cells | CA activity |
|--------------------------------|-------------|
| units/mg |
| Mock-transfected | 3.8 |
| CA XIV-transfected | 19.6 |
| SDS-treated | 14.3 |
| Membrane-fraction | 40.4 |

CA XIV was constructed by comparing the amino acid sequences of the CA XIV and CA IV isoenzymes. The amino-terminal signal sequences of CA XIV and CA IV are very similar, but the signal peptidase cleavage site differs. The signal peptide of CA XIV is 24 amino acids long, while that of CA IV is 26 amino acids long. The signal peptide of CA XIV is localized in the cytoplasm, and the signal peptide of CA IV is localized in the endoplasmic reticulum. The mature form of CA XIV is 503 amino acids long, while the mature form of CA IV is 527 amino acids long. The amino acid sequences of the catalytic domains of CA XIV and CA IV are highly conserved, with 90% identity. The active site of CA XIV is located in the catalytic domain, which is a globular structure containing a zinc ion and a histidine residue.
extreme carboxyl terminus of the precursor protein is cleaved off, and the mature protein is bound to the plasma membrane by a GPI anchor (36, 37). The GPI-anchored form of CA IV is released from the membrane by phosphatidylinositol-specific phospholipase C (PI-PLC) treatment (36). Although CA IV was defined as a membrane-bound isoenzyme of CA (2, 10), approximately half of the membrane-bound CA activity in the kidney still remains after PI-PLC treatment (29). One interpretation for this finding is that some part of CA IV exists as a membrane-spanning form, which is PI-PLC insensitive (29), yet it is also possible that other membrane-bound CAs are present in the kidney. A novel CA described here, CA XIV, is one of such candidates.

Conclusion—In this study, we describe the isolation and characterization of a novel CA designated CA XIV. The primary structure and the expression studies suggest that CA XIV is a type I membrane protein localized on the plasma membrane. Because CA XIV mRNA is expressed abundantly in various tissues and CA XIV has enzyme activity, CA XIV may play important roles in carbon dioxide metabolism and acid-base balance in the kidney and other tissues.

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