Characterization of CA XIII, a Novel Member of the Carbonic Anhydrase Isozyme Family*

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The carbonic anhydrase (CA) gene family has been reported to consist of at least 11 enzymatically active members and a few inactive homologous proteins. Recent analyses of human and mouse databases provided evidence that human and mouse genomes contain genes for still another novel CA isozyme hereby named CA XIII. In the present study, we modeled the structure of human CA XIII. This model revealed a globular molecule with high structural similarity to cytosolic isozymes, CA I, II, and III. Recombinant mouse CA XIII showed catalytic activity similar to those of mitochondrial CA V and cytosolic CA I, with $k_{cat}/K_m$ of $4.3 \times 10^3$ M$^{-1}$ s$^{-1}$ and $k_{cat}$ of $3.2 \times 10^8$ s$^{-1}$. It is very susceptible to inhibition by sulfonamide and anionic inhibitors, with inhibition constants of 17 nM for acetazolamide, a clinically used sulfonylurea, and 0.25 $\mu$M, for cyanate, respectively. Using panels of cDNAs we evaluated human and mouse CA13 gene expression in a number of different tissues. In human tissues, positive signals were identified in the thymus, small intestine, spleen, prostate, ovary, colon, and testis. In mouse, positive tissues included the spleen, lung, kidney, heart, brain, skeletal muscle, and testis. We also investigated the cellular and subcellular localization of CA XIII in human and mouse tissues using an antibody raised against a polypeptide of 14 amino acids common for both human and mouse orthologues. Immunohistochemical staining showed a unique and widespread distribution pattern for CA XIII compared with the other cytosolic CA isozymes. In conclusion, the predicted amino acid sequence, structural model, distribution, and activity data suggest that CA XIII represents a novel enzyme, which may play important physiological roles in several organs.

Carbonic anhydrases (CAs) are zinc-containing metalloenzymes that catalyze reversible hydration of carbon dioxide in a reaction $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$. CAs are produced in a variety of tissues where they participate in several important biological processes such as acid-base balance, respiration, carbon dioxide and ion transport, bone resorption, ureagenesis, gluconeogenesis, body fluid generation, and lipogenesis (1, 2). The CA isozymes differ in their kinetic properties, their tissue distribution, and subcellular localization. The mammalian α-CA gene family has been reported to include at least eleven enzymatically active isoforms with different structural and catalytic properties (1, 3). Four of the active CA isozymes are cytosolic (CA I, II, III, and VII), four are membrane-associated (CA IV, IX, XII, and XIV), two are mitochondrial (CA VA and -VB), and one is a secretory form (CA VI). In addition to these isozymes with measurable CA activity, there are several proteins with homologous “CA-like” domains. These include e.g. carbonic anhydrase-related proteins (CA-RP)-VIII, CA-RP-X, CA-RP-XI, and receptor-type protein-tyrosine phosphatases (RPTP) β and γ (3–8). A common feature for these CA-like proteins is that some of the critical histidine residues in the active site are missing, and the zinc ion, which is necessary for the enzymatic activity, is not bound to the molecule.

Recent exploitation of mammalian genomic data has allowed researchers to search for novel proteins including new isoforms of the existing protein families. The mouse CA13 cDNA sequence had been submitted to the National Center for Biotechnology Information (NCBI) database by the Dr. Hewett-Emmett group in 2000. By comparing sequences, we also identified a human cDNA sequence, which appeared to encode human CA XIII protein. Based on the predicted amino acid sequence of human CA XIII and the known tertiary structure of CA I (9), we designed a computer-based model for the structure of CA XIII. We also studied the distribution of CA XIII gene and protein expression in a number of human and mouse tissues. Mouse CA XIII was produced in Escherichia coli, and the kinetic and inhibition data for the recombinant enzyme were measured using a colorimetric method.

EXPERIMENTAL PROCEDURES

Phylogenetic Analysis—The sequence alignment of CAs was performed with ClustalW (10). The phylogenetic analysis was done with PAUP* 4.0.11 (11). The majority rule consensus tree was obtained by three
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bovine brain with different random seeds. The dataset was bootstrapped 1000 times for each run.

*Structure Modeling*—The model of human CA XIII was based on the structure of the human CA I at 2.0 Å resolution (9) (PDB entry 1CZM). The model was built with the program InsightII (Accelrys Inc., San Diego, CA). Amino acid substitutions were built using a side chain rotamer library. The initial model was refined with Discover™ in a stepwise manner by energy minimization using the Amber™ force field. First, the newly built loops were refined with 500 steps of minimization with a fixed and a free backbone, respectively. Then, all side chains with a constrained backbone were minimized for 500 steps, followed by another 1000 steps of minimization for the whole model.

*Kinetic and Inhibition Measurements*—Initial rates of CO₂ hydration were measured in a KinJournal™ stopped-flow instrument (Oxford, UK) by the changing pH indicator method (12). Phenol red (at a concentration of 0.2 mM) was used as an indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5), 10 mM Tris, and 0.1 M Na₂SO₄, following the CA-catalyzed CO₂ hydration reaction for a period of 10–100 s. Saturated CO₂ solutions in water at 25 °C were used as substrate (12). Stock solutions of inhibitors (1 mM) were prepared in distilled-deionized water with 10–20% (v/v) Me₂SO (which is not inhibitory at these concentrations), and dilutions up to 0.1 mM were made thereafter with distilled-deionized water. Inhibitor and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the enzyme-inhibitor complex. Triplet experiments were done for each inhibitor concentration, and the values reported throughout the study are the mean of such results. Inhibition constants were calculated as described earlier (13).

**Antibodies**—The anti-mouse CA XIII serum was raised by Innovagen AB (Lund, Sweden) in a rabbit against a synthetic peptide (AC-) DGGQSPIEIKTKEC (-COOH), which was designed based on the predicted amino acid sequence of mouse CA XIII. This peptide was found to be identical in the predicted human CA XIII sequence (Fig. 1), and thus, the same antibody was also used to detect the human orthologue. Both mouse and human CA13 cDNA sequences are available in GenBank™ (accession numbers AF231123 and AK095314). Rabbit antisera against human and mouse CA13 were produced by Innovagen AB (Lund, Sweden). Rabbit anti-CA XI antibody was raised against mouse CA XI produced in E. coli. The specificity of the antibody was confirmed using blood lysates from both normal and CA XI-deficient (Car-2−/−) mice (data not shown).

**Western Blotting**—30 µg of protein from human colon, human liver, and mouse colon total homogenates or 20 µl of purified fraction from ProBond™ or inhibitor affinity chromatography were analyzed by SDS-PAGE under reducing conditions according to Laemmli (13). The separated proteins were stained by Colloidal Coomassie Blue (Invitrogen, Carlsbad, CA) or transferred electrochemically from the gel to a polyvinylidene fluoride membrane (Invitrogen) in a Novex Xcell II blot module. The electroblots were incubated first with 20% (v/v) Me₂SO, then with horseradish peroxidase-conjugated goat anti-rabbit IgG (Zymed Laboratories Inc.) (diluted 1:1000). The sections were washed three times for 10 min in PBS after incubation steps b and c and four times for 5 min after incubation step d. All of the incubations and washings were carried out at room temperature. Preimmune serum and anti-mouse CA II serum were used for control purposes.

**Mouse tissue sections** were also stained by the immunofluorescence method and analyzed by confocal laser scanning microscopy. The steps in the immunofluorescence staining were as follows: (a) pretreatment of the sections with undiluted cow colostral whey (Biotop) for 40 min and rinsing in PBS; (b) incubation for 1 h with the primary antisemur or preimmune serum diluted 1:100 in 1% BSA-PBS; (c) incubation for 1 h with the secondary horseradish peroxidase-conjugated antibody diluted 1:100 in PBS (Zymed Laboratories Inc.); (d) incubation for 30 min with peroxidase-conjugated streptavidin diluted 1:750 in PBS (Zymed Laboratories Inc.); (e) incubation for 1 min with DAB solution (9 mg of DAB in 15 ml of PBS plus 5 µl of H₂O₂). The sections were washed three times for 10 min in PBS after incubation steps b and c and four times for 5 min after incubation step d. All of the incubations and washings were carried out at room temperature. Preimmune serum and anti-mouse CA II serum were used for control purposes.

**PCR Methods**—The expression of human and mouse CA XIII mRNAs was examined using cDNA kits purchased from BD Biosciences (Palo Alto, CA), and the expression of human and mouse CA XIII proteins was examined using cDNA clones purchased from BD Biosciences. Total RNA was isolated from a number of different tissues.

Two primers for amplifying human CA13 cDNA were chosen based on the human cDNA sequence (accession number AK095314), which appeared to encode human CA XIII; forward 5'-ACACGCGTCTCATTCGACT-3' (nucleotides 103–100) and reverse 5'-CAGGTTAGTGTCAGTGTAG-3' (nucleotides 623–640), which generated a 557-bp product. The primers were produced by Sigma Genosys.

To amplify mouse CA13 cDNA, three primers (Sigma Genosys) were chosen based on the mouse cDNA sequence published in GenBank™ (AF231123); forward (F1) 5'-GTCCTGCGCAAGCGCTC-3' (nucleotides 3–20) and reverse (R1) 5'-TGCAAAGGCTCCGGA-3' (nucleotides 712–729), which generated a 730-bp product. Forward primer F1 together with reverse primer R2 5'-ATACATGGGGACAAATC-3' (nucleotides 993–1010) generated a full-length 1008-bp amplification product. Primers for glycerolaldehyde-3-phosphate dehydrogenase (G3PDH, BD Biosciences) were used to monitor the quality of the cDNA samples.

5 ng of total cDNA was used as a template for PCR. All the reagents for PCR were from BD Biosciences. The PCR reactions were amplified on the thermal cycler (Gene Amp PCR system 9700, Applied Biosys-

**Immunohistochemistry**—Immunoperoxidase staining for human CA XIII was performed using the biotin-streptavidin complex method. In the mouse tissues, the localization of CA XIII was examined by both immunoperoxidase and immunofluorescence methods.

Human and mouse tissue samples fixed in Carnoy’s fluid and embedded in paraffin were cut at 5 µm sections and placed on microscope slides. The biotin-streptavidin complex method included the following steps: (a) pretreatment of the sections with undiluted cow colostral whey (Biotop) for 40 min and rinsing in phosphate-buffered saline (PBS); (b) incubation for 1 h with the primary antisemur or preimmune serum diluted 1:100 in 0.1% bovine serum albumin-PBS solution (BSA-PBS); (c) incubation for 10 min with biotinylated second antibody (Histostain-plus, Zymed Laboratories Inc., South San Francisco, CA); (d) incubation for 10 min with peroxidase-conjugated streptavidin (Histostain-plus, Zymed Laboratories Inc.); (e) incubation for 3 min with 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution (Liquid DAB substrate kit, Zymed Laboratories Inc.). The sections were washed three times for 5 min in PBS after incubation steps b and c and four times for 5 min after incubation step d. All of the incubations and washings were carried out at room temperature. Anti-human CA II serum was used as a positive control. Additional control staining was performed in the presence of the corresponding peptide (1:100 diluted anti-CA XIII serum plus 100 µg of peptide/100 µl of BSA-PBS). The sections were finally mounted in Neo-Mount (Merck, Darmstadt, Germany). The stained sections were examined and photographed with a Zeiss Axioskop 40 microscope (Carl Zeiss, Göttingen, Germany).
ethidium bromide with DNA standards (100 bp DNA Ladder, New England Biolabs, Beverly, MA).

**Sequencing the PCR Products**—The PCR products from the human thymus, mouse spleen, and mouse 17-day-embryo were purified from agarose gel using GFX™ PCR DNA and Gel Band Purification kit (Amersham Biosciences). The sequencing was performed using ABI PRISM™ Big Dye Terminator Cycle Sequencing Ready Reactions Kit, version 2.0 (Applied Biosystems) following the protocol of the manufacturer. The bound enzyme was eluted using 0.1 M imidazole, pH 8.0. The inhibitor affinity chromatography was performed using the carboxymethyl (CM) Bio-Gel A (Bio-Rad Laboratories, Richmond, CA) coupled to p-aminomethylbenzenesulfonamide. The gel was washed using 10 mM HEPES buffer (pH 7.5) containing 150 mM NaCl and the bound enzyme was eluted using 0.1 M sodium acetate, pH 5.6, containing 0.5 M sodium perchlorate. The sequencing was performed using ABI PRISM™ Big Dye Terminator Cycle Sequencing Ready Reactions Kit, version 2.0 (Applied Biosystems) following the protocol of the manufacturer. The sequencing was performed on the ABI PRISM™ 3100 Genetic Analyser instrument (Applied Biosystems).

**Expression and Purification of the His-tagged CA XIII**—The full-length PCR product from mouse 17-day-embryo was cloned into the E. coli TOP 10 bacterium using pTrcHis TOPO TA expression kit (Invitrogen) following the manufacturer’s instructions. First, the PCR product was cloned into the TOPO vector and then the recombinant vector was transformed into E. coli. The transformed bacteria were grown in 1 liter of LB medium containing 50 μg/ml ampicillin and 0.5% glucose. The colonies were picked for analysis of positive clones. First, the colonies were cultured overnight in LB medium containing 50 μg/ml ampicillin and 0.5% glucose at 37 °C. Then the plasmids were isolated using QIAprep Spin Miniprep kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. The isolated plasmids were analyzed by restriction analysis and by sequencing. The restriction analysis was performed using two restriction enzymes, BamHI and HindIII (New England Biolabs), according to the manufacturer’s protocol. The sequencing was performed using ABI PRISM™ Big Dye Terminator Cycle Sequencing Ready Reactions Kit, version 2.0 (Applied Biosystems) following the protocol of the manufacturer. The sequencing primers, Xpress Forward primer and pTrcHis Reverse primer were from Invitrogen.

E. coli TOP 10 strain transformed with pTrcHis plasmid containing mouse CA XIII cDNA was grown at 37 °C in 50 ml of LB medium containing 50 μg/ml ampicillin and 0.5% glucose to an O.D of 0.6 at 600 nm. The inducer, isopropyl-1-thio-β-p-galactopyranoside, was then added to a final concentration of 1 mM and then the culture was incubated at 37 °C. The cells were harvested at 5 h by centrifugation, and the pellet was frozen at −80 °C for later use. For large scale expression the bacteria were grown in 1 liter of LB medium.

The bacterial cell pellet was resuspended in 100 ml of lyzing buffer (50 mM NaHPO₄, 0.5 M NaCl, pH 8.0) and 160 mg lysozyme (Sigma) was added. The resuspended cells were incubated on ice 30 min and lysed by pipetting. Bacterial cell components were removed by centrifugation (13,000 rpm, 15 min, +4 °C). The supernatant was directly applied onto either Invitrogen’s ProBond™-purification system or conventional CA-inhibitor affinity chromatography purification.

The ProBond™ is a nickel-charged agarose resin that has been designed to purify recombinant proteins containing a histidine sequence. The ProBond™ purification was performed under native conditions according to Invitrogen’s protocol. The bound enzyme was eluted in 8 M urea, imidazole, pH 8.0. The inhibitor affinity chromatography was performed using the carbamoxethyl (CM) Bio-Gel A (Bio-Rad Laboratories, Richmond, CA) coupled to p-aminomethylbenzenesulfonamide. The gel was washed using 10 mM HEPES buffer (pH 7.5) containing 150 mM NaCl and the bound enzyme was eluted using 0.1 M sodium acetate, pH 5.6, containing 0.5 M sodium perchlorate. The purified His-tagged CA XIII was visualized by two methods: Coomassie Blue protein stain (Invitrogen) and Western blotting.

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**Phylogenesis and Structural Model of CA XIII**—Fig. 1 shows the amino acid sequence alignment of human CAs including the novel isozyme, CA XIII. All three histidine residues (His-94, -96, and -119) known to be critical for CA enzymatic activity are conserved in the predicted human and mouse (data not shown) CA XIII sequences. The phylogenetic analysis (Fig. 2) showed that CAs form three distinct clusters. CA I, II, III, V, and VII are clustered together as cytosolic/intracellular CAs. The second cluster includes extracellular and membrane-bound isozymes CA IV, VI, IX, XIII, and XIV. The CA-RP VIII, X, and XI belong to the third cluster. The novel CA XIII is most closely related to CA I, II, and III with about 60% sequence identity, and thus, is clustered as cytosolic/intracellular isozyme.

In Fig. 3, the surfaces of three CAs (human CA I (1CZM), human CA II (1CNX), and human CA XIII) are colored according to the hydrophobic/hydrophilic properties of amino acids. The superimposition of these structures indicates that the location of the inhibitor binding pocket is conserved. The sulfonamide-type inhibitors are located inside the cavity and are bound to the zinc and the hydrophobic patch of the active site.

The sequence similarity between the template (human CA I) and the target (human CA XIII) was 77%. A sequence identity above 50% generally guarantees that the modeled protein structure is correct at a level comparable to that of an x-ray crystal structure (16). The sequence alignment showed that the three Zn⁡⁺² coordinating histidine residues are conserved in CA XIII molecule. Therefore, the Zn⁺² ion was directly merged into the structural model. The quality of the final model was checked using the three-dimensional profiles verification (17) and PROCHECK (18). The three-dimensional profiles verification method showed that the three-dimensional/one-dimensional scores of our model were always positive, which are within the range of scores for x-ray structure determinations. Additionally, PROCHECK confirmed that the model of human CA XIII had good geometric structural parameters.

** Catalytic Activity and Inhibition by Sulfonamides and Antions**—Table I shows the kinetic parameters for the CO₂ hydration reaction catalyzed by several α-CAs including recombinant mouse CA XIII. Inhibition data with acetazolamide, a clinically used CA inhibitor of the sulfonamide type, and cyanate, an anion inhibitor, are also presented.

**CA13 Gene Expression**—CA13 gene expression was investigated by PCR amplification of commercially available sets of cDNAs produced for selected human and mouse tissues. Human CA XIII mRNA was expressed in the thymus and small intestine, testis, spleen, prostate, ovary, and colon (Fig. 4). No CA XIII transcript was detected in the human leukocytes. In the mouse tissues, the positive signals were detected in the spleen, lung, kidney, heart, brain, skeletal muscle, testis, 7-day-old embryo, 11-day-old embryo, 15-day-old embryo, and 17-day-old embryo (Fig. 5). No CA XIII transcript was detected in the mouse liver.

**Sequencing the PCR Amplification Products**—Automated sequencing of the PCR products confirmed that correct CA13 sequences were amplified from the cDNAs of human thymus, mouse spleen, and 17-day-old mouse embryo. The primers for mouse CA13 were designed to obtain a full-length cDNA, while the corresponding human PCR product represented only a partial one. For sequencing the PCR product of human CA13 cDNA was amplified forward from the 5'-end, while mouse CA13 was amplified from both the 5'- and 3'-ends covering the whole coding sequence. The results clearly indicated that the PCR fragments represented CA13 cDNAs in both species.

**Localization of CA XIII in Human and Mouse Tissues**—Immunoreactivity of anti-CA XIII serum was confirmed by Western blotting. Single, 30-kDa polypeptides were detected in the human and mouse colon, while the signal in the human liver was barely apparent (Fig. 6). The control experiments using preimmune serum or peptide blocking were negative.

The immunolocalization studies for CA XIII revealed three major features on this novel enzyme: First, CA XIII showed an intracellular staining pattern, typical for cytosolic proteins.
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**Fig. 1.** Amino acid sequences of human CA XIII and other catalytic human CAs. Numbers above the aligned sequences correspond to numbering of amino acids in CA I. The conserved residues are bold-faced. Arrows above His-94, His-96, and His-119 indicate Zn binding active site residues. The **underlined** polypeptide was used to raise anti-CA XIII antibody.
Second, there were distinct differences in the distribution of CA XIII between human and mouse tissues. Third, CA XIII was found in a number of different tissues in both species. Although the distribution of CA XIII resembles that of CA II in several tissues, we found some differences that make CA XIII unique with respect to its localization.

In the human alimentary tract, CA XIII was found in a number of tissues from the salivary glands to the large intestine. In the submandibular gland, CA XIII was expressed in serous acinar cells and duct epithelial cells (Fig. 7). Compared with CA II, CA XIII showed stronger signal in the duct epithelium. In the gastric mucosa, CA XIII showed only very weak staining in the surface epithelial cells of the body and antrum segments (Fig. 8). By contrast, CA II showed strong positive staining in the surface epithelial cells and parietal cells, which is in line with the fact that it is the major isozyme of the human gastric mucosa (2). Fig. 9 demonstrates the distribution of CA XIII in different segments of the human gut, and the immunostaining results of CA II are shown for comparison. CA XIII was found in enterocytes in every segment, although the variation in the staining intensity was notable between different parts of the gut. CA II showed stronger staining in the duodenum and large intestine, while CA XIII was somewhat more prominent in the jejunum and ileum. Immunostaining revealed no positive signal for CA XIII in the human liver (data not shown). Human pancreas also showed much weaker staining for CA XIII compared with CA II (data not shown). Only faint reactions were observed in the duct epithelium and acini.

CA XIII was highly expressed in the human testis, and in a pattern quite distinct from that of CA II (Fig. 10). Positive immunoreactions were observed in all stages of the developing sperm cells. In contrast, CA II showed strong signal only in the mature sperm cells limited to the most luminal face of the seminiferous tubules. CA XIII is also an abundant isozyme in the female reproductive tract. Based on histochemical demonstration of CA activity, it had been reported that the human endometrium expresses some CA isozyme (19). Recently, Karhumaa et al. (20) demonstrated that CA XII is present at the basolateral plasma membrane of the human endometrial epithelium. However, the presence of CA XII could not explain the cytosolic staining pattern that had been observed in early histochemical staining. Fig. 11 shows that CA XIII is, indeed, highly expressed in the uterine cervix and some endometrial glands were also positive. In contrast, CA II immunoreactivity was limited to the blood capillaries. The cervical and endome-
trial glands were negative for CA II.

Kidney was one of the human tissues we found positive for CA XIII. In both renal cortex and medulla, strongest immunoreactions were located to the collecting ducts (Fig. 12). Positive, albeit usually weak, immunoreactions were seen in the glomerulus. For comparison, CA II showed similar staining pattern in the collecting ducts in addition to its high expression in the nephron.

CA XIII expression was also investigated in mouse tissues using immunohistochemistry. Fig. 13 shows some representative examples of immunostaining for CA XIII and CA II. In the mouse brain (cerebrum and cerebellum) these isoforms were both expressed in the oligodendrocytes and positive signal was also associated with the nerve fiber bundles. The latter finding conceivably indicates their location in myelin, which is known to contain CA II (21). In the mouse kidney, positive staining for CA XIII was detected in the cortical and medullary collecting ducts. Comparison of immunostaining reactions for CA XIII and -II revealed that CA XIII is most probably expressed in the intercalated cells like CA II. From the various gastrointestinal tissues, the strongest immunoreaction for CA XIII was observed in the colon. The more proximal segments of the gastrointestinal canal showed none or only a faint signal. No CA XIII-specific staining was detected in the mouse testis, whereas CA II was present in the mature spermatozoa locating in the seminiferous tubules. Epithelial cells of the mouse uterus contained CA XIII and CA II, the latter of which has been previously located to those cells (22). Expression for CA XIII was

![Fig. 3. Structural model of human CA XIII (A). Structures of human CA I and II are shown for comparison (B and C). The surfaces are colored according to the hydropathy (blue, hydrophilic; red, hydrophobic). Green color in panel A indicates the location of the zinc ion. Green molecules in panels B and C demonstrate the binding site of sulfonamide CA inhibitors.](image)

![Fig. 4. PCR analysis of human CA XIII mRNA expression.](image)
also detected in the mouse lung, where the staining was most abundant in rounded cells of the alveolar wall, most probably representing the type II pneumocytes, which are known to contain CA II (23).

**Cloning and Expression of the Mouse CA XIII in E. coli**—The coding region of the mouse CA13 gene was amplified from cDNA and inserted into the bacterial expression vector, pTrcHis, for expression of recombinant CA XIII in E. coli strain TOP 10. CA XIII protein was expressed as a fusion protein containing a His tag at its N-terminal end. The successful cloning was confirmed by double digestion of the pTrcHis vector and automated sequencing.

The fusion protein was purified using two methods, the CA inhibitor affinity chromatography and ProBond™ purification system. The purified fusion protein was visualized by Colloidal Coomassie Blue staining and Western blotting (Fig. 14). ProBond™ purification method was more efficient, but the purified

Fig. 6. Western blotting of the human and mouse colon and human liver using anti-CA XIII serum. Negative control experiments were performed using preimmune serum or anti-CA XIII antibody in the presence of the blocking peptide. Positive controls were visualized using a mixture of anti-human CA I and II and anti-mouse CA II antibodies.

Fig. 7. Immunohistochemical staining of CA XIII (A and B) and II (C) in the human submandibular gland. Positive staining for CA XIII is located to the serous acinar and duct epithelial cells. CA II is highly expressed in the acinar cells, while it shows only weak staining in the duct cells. The control staining (D) using preimmune serum is negative. Original magnifications: A, C, and D ×200; B ×400.

Fig. 8. Immunolocalization of CA XIII (A and B) and II (C and D) in the human gastric mucosa. Panels A and C represent sections from the body of the stomach and panels B and D are from the pyloric antrum. Only very faint staining is detected for CA XIII (arrows), whereas both segments of the gastric mucosa are strongly positive for CA II. Original magnifications: A, B, and C ×200; D and inset in B ×400.

Fig. 9. Localization of CA XIII (A–E) and -II (F–J) in different segments of the human gut. CA XIII is seen in enterocytes in every segment of the gut, although the variation in the staining intensity is notable between different parts. Panels A and F, duodenum; B and G, jejunum; C and H, ileum; D, E, I, and J, colon. Original magnification: A–D and F–I ×200; E and J ×400.

Fig. 10. Immunohistochemical staining of CA XIII (A and B) and CA II (C and D) in the human testis. CA XIII is expressed in all stages of the developing sperm cells (bar in panel B), while CA II is confined to the mature spermatozoa locating in the middle of the seminiferous tubules (bar in panel D). Control staining using preimmune serum is negative (E). Original magnifications: A, C, and E ×200; B and D ×630.
fraction contained small amounts of contaminant proteins in addition to a 35-kDa polypeptide representing His-tagged CA XIII. The CA inhibitor affinity chromatography method showed higher specificity in that the purified fraction showed only a single polypeptide of 35 kDa.

**DISCUSSION**

The present results indicated that CA XIII belongs to the cluster of cytosolic CA isozymes. Compared with human CA I and -II, the environment of the sulfonamide inhibitor binding pocket in human CA XIII is more hydrophilic due to the amino acid residue Arg-91 (which corresponds to Phe-91 in the struc-
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The present findings in mind it would be interesting to have either a CA XIII-specific inhibitor or CA XIII-deficient animal model to determine the role of this novel enzyme on spermatogenesis and fertilization capacity. Based on the distribution of CA XIII, other major target tissues in physiological studies should include at least the brain, salivary glands, intestine, kidney, and lymphoid organs. Being such a widely expressed isozyme, CA XIII could compensate other CAs, and thus, needs to be considered when any CA-deficient animal model is tested in phenotypic analyses. It is also notable that positive signals for CA13 gene expression were detected in the samples obtained from mouse embryos. This finding suggests that CA XIII may play a role in embryogenesis and perturbation of its function by genetic modification could potentially lead to developmental abnormalities.

Keeping the present findings in mind it would be interesting to have either a CA XIII-specific inhibitor or CA XIII-deficient animal model to determine the role of this novel enzyme on spermatogenesis and fertilization capacity. Based on the distribution of CA XIII, other major target tissues in physiological studies should include at least the brain, salivary glands, intestine, kidney, and lymphoid organs. Being such a widely expressed isozyme, CA XIII could compensate other CAs, and thus, needs to be considered when any CA-deficient animal model is tested in phenotypic analyses. It is also notable that positive signals for CA13 gene expression were detected in the samples obtained from mouse embryos. This finding suggests that CA XIII may play a role in embryogenesis and perturbation of its function by genetic modification could potentially lead to developmental abnormalities.