PARTIAL NUCLEIC ACID SEQUENCE OF RENAL CARBONIC ANHYDRASE FROM THE EURYHALINE AMERICAN EEL, ANGUILLA ROSTRATA

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In the kidney of freshwater-adapted eels, carbonic anhydrase is essential for the reabsorption of bicarbonate which is filtered from the plasma at the glomerulus. The enzyme converts intracellular CO_2 to HCO_3^- and H^+. The HCO_3^- is transported into the plasma via the HCO_3^-/Cl^- transporter, whereas the H^+ is transported to the tubular lumen (via either a Na^+/H^+ exchanger or a H^+ ATPase) where it reacts with filtered HCO_3^-, forming CO_2 and H_2O. The CO_2 diffuses into the cell where it is converted to HCO_3^- and transported into the blood. The net result is the reabsorption of filtered HCO_3^-, and very little HCO_3^- is lost in the urine. Treatment of freshwater-adapted eels with carbonic anhydrase inhibitors results in the excretion of HCO_3^- in the urine, and a consequent acidosis (Swenson et al., Bull. MDIBL 14:127,1974).

In mammals, carbonic anhydrase is found in at least 6 isoforms, presumably as a result of gene duplication during evolution (Hewett-Emmett et al., N.Y. Acad. Sci. 429:338,1984). Although many other carbonic anhydrases have been sequenced, there are no data available for teleost fish. As part of our investigation into the function of renal carbonic anhydrase in euryhaline fish we wanted to determine the sequence of the eel carbonic anhydrase to examine its relatedness to other vertebrate carbonic anhydrases.

Total RNA was isolated from the kidneys of freshwater-adapted eels by the method of Chomczynski and Sacchi (Anal. Biochem. 162:156, 1987), and mRNA was purified using the FastTrack kit (Invitrogen). First strand cDNA was made using an oligo-dT primer, and PCR amplification of the cDNA was carried out using a pair of synthetic primers which corresponded to two highly conserved regions of carbonic anhydrases (as determined by multiple sequence alignment). Electrophoresis of the PCR product revealed several bands, one of which was the size (~300 bp) predicted on the basis of known sequences. This band was eluted from the gel using a GENECLEAN kit (BIO 101, Inc.) and cloned into the pCR-II vector (Invitrogen). Plasmids were purified from several clones, and sequenced using both Sequenase (United States Biochemical) and fmol (Promega) sequencing systems, using SP6 and T7 promoter primers to sequence in both directions. The resulting nucleic acid sequences were used to search the gene database using the BLAST network service (National Center for Biotechnology Information; NCBI). One of the clones had good homology to other carbonic anhydrases (~60% for amino acids, ~50% for DNA), including identical amino acids at all 14 of the highly conserved residues associated with the active site and/or zinc binding sites found in all carbonic anhydrases (Okuyama et al., Proc. Nat. Acad. Sci. 89:1315,1992). We therefore feel confident that this clone represents the eel carbonic anhydrase cDNA.

The eel sequence (Fig.1) has some of the unique and invariant characteristics of each of the well-known carbonic anhydrase isozymes (Hewett-Emmett et al., N.Y. Acad. Sci. 429:338,1984), and it is therefore impossible to assign it to one of the major isozyme groups; however, the eel carbonic anhydrase described here has an alanine at position 29 (which corresponds to position 126 of the full-length mammalian CA I). This residue is present only in CA I, and therefore it seems that the eel sequence is most accurately described as belonging to the CA I family.

Fig.1 Nucleic Acid Sequence and Derived Amino Acid Sequence of a cDNA Coding for a Portion of Anguilla rostrata Carbonic Anhydrase

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GGA GGG AAG GGC TGC CAG GGG TCG GAG CAC ACG GTG GAC GGG AAG   45
Gly Gly Lys Gly Cys Gln Gly Ser Glu His Thr Val Asp Gly Lys   15

ACC TAC GCn TCC GAG CTT CAC CTG GTC CAC TGG AAC GCC GCC AAG   90
Thr Tyr Ala Ser Glu Leu His Leu Val His Trp Asn Ala Ala Lys   30

TAC AAG TCC TTC GGT GAG GCA GCC GCC GCC CCC GAC GGC CTC GCT  135
Tyr Lys Ser Phe Gly Glu Ala Ala Ala Ala Pro Asp Gly Leu Ala   45

GTC CTT GGT GTC TTT TTA GAG ACA GGT AAC GAG CAC AGA GGT TTA  180
Val Leu Gly Val Phe Leu Glu Thr Gly Asn Glu His Arg Gly Leu   60

AGC GTG ATA ACT GAC GCG TTG TAC ATG GTC AAG TTC AAG GGA AGC  225
Ser Val Ile Thr Asp Ala Leu Tyr Met Val Lys Phe Lys Gly Ser   75

AAG GCA GAC TTC CGG GAC TTC AAC CCC TCG TGC CTC CTG CCC AGC  270
Lys Ala Asp Phe Arg Asp Phe Asn Pro Ser Cys Leu Leu Pro Ser   90

AGC CTC AGC TAC TGG ACG   288
Ser Leu Ser Tyr Trp Thr    96
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