SEQUENCE ANALYSIS OF PUTATIVE Na^+/H^+ ANTIPORTER cDNA FROM THE SHORE CRAB CARCINUS MAENAS

David W. Towle[1], Marek Kordylewski[2], and Susann W. Bowring[1]
[1]Department of Biology, Lake Forest College, Lake Forest, IL 60045
[2]Harvard University, Cambridge, MA 02138

An electrogenic Na^+/H^+ antiporter, apparently exchanging 2 Na^+ for 1 H^+, has been found in membrane vesicle preparations from crab gill (Shetlar and Towle, Am. J. Physiol. 257:R924-R931, 1989) and other crustacean tissues (Ahearn and Clay, Am. J. Physiol. 257:R484-R493, 1989; Ahearn and Franco, Am. J. Physiol. 259:F758-F767, 1990). The question investigated here is whether the crab Na^+/H^+ antiporter is structurally similar to the electroneutral Na^+/H^+ antiporter of vertebrate tissues. We based our studies on the possibility that DNA sequence information obtained for a vertebrate antiporter might be used to identify and sequence the crab antiporter. The published cDNA sequence for the human "housekeeping" Na^+/H^+ antiporter suggests a protein of 91 kDa containing ten putative transmembrane segments and an extensive intracellular hydrophilic region (Sardet, Franchi, and Pouysségur, Cell 56:271-280, 1989; Sardet, Counillon, Franchi, and Pouysségur, Science 247:723-726, 1990). Oligonucleotide primers based on likely conserved regions of the sequence were synthesized by Dr. Alison Morrison-Shetlar (Max-Planck-Institut für Systemphysiologie) and employed in the following studies.

Polyadenylated mRNA was isolated from posterior gills of Carcinus maenas acclimated to 10 o/oo salinity, using the Micro-FastTrack procedure (Invitrogen). Synthesis of cDNA was accomplished using oligo-dT as a primer according to the cDNA Cycle Kit from Invitrogen. Polymerase chain reaction amplification of the putative crab Na^+/H^+ antiporter cDNA was attempted using several primer pairs which would define sequences coding for different transmembrane regions of the human antiporter. A primer pair encompassing the tenth transmembrane segment yielded PCR products which exhibited a prominent discrete band at 0.58 Kb. A sample of the PCR product was ligated directly into the 3.0 Kb TA cloning vector pCR™1000 (Invitrogen) which was then used to transform competent INVαF' E. coli. Plasmids were prepared from overnight cultures of positive colonies and were used for sequencing from the T7 priming site (Sequenase kit, USB), extending premature terminations with terminal deoxynucleotidyl transferase (Kho and Zarbl, Biotechniques 12:228-230, 1992).

Sequences were analyzed using DNASIS software (Hitachi). DNA translation identified several potential coding sequences. The resulting hypothetical amino acid sequences were compared with sequences available in protein databases via computation performed at the National Center for Biotechnology Information using the BLAST network service (Altschul et al., J. Mol. Biol. 215:403-410, 1990). According to this analysis, none of the crab sequences we determined compared favorably with any vertebrate Na^+/H^+ antiporter. However, one of the translated crab sequences contains adjacent hydrophobic regions which would be characteristic of a membrane-spanning protein (Fig. 1 and 2). A full-length sequence will be sought which can be injected into Xenopus oocytes for functional tests (Towle et al., J. Exp. Biol. 159:359-369, 1991). Newly published sequence information for trout Na^+/H^+ antiporter (Borgese et al., Proc. Natl. Acad. Sci. USA 89:6765-6769, 1992) and a possibly related sequence from Caenorhabditis elegans (Marra et al., Mol. Gen. Genet., in press, 1992) will assist in designing more specific primers for future work.

Supported by National Science Foundation (Research at Undergraduate Institutions Grant Number IBN-9024293, Research Experiences for Undergraduates Supplement, and EPSCoR).

```
  1  GAT TTA GTG AGA TGT TCT ACC TCA ATC ACT TTA TCA CCT AAA CGT GGA   48
  1  Asp Leu Val Arg Cys Ser Thr Ser Ile Thr Leu Ser Pro Lys Arg Gly   16

 49  CCA GGT GGG ATA AAG AGT TCG TTA GTC TCG TTA CGT TTG GTA TCG CCA   96
 17  Pro Gly Gly Ile Lys Ser Ser Leu Val Ser Leu Arg Leu Val Ser Pro   32

 97  GAG TTC ACT CAT CAA CGC GCC ATA CTG CTT TGC TTT TCG CTT GCG GCC  144
 33  Glu Phe Thr His Gln Arg Ala Ile Leu Leu Cys Phe Ser Leu Ala Ala   48

145  TTA GAT GTT GAC GAC CAC TCT ATC TTC ATC GTT TGC GTG CTG TCT GTC  192
 49  Leu Asp Val Asp Asp His Ser Ile Phe Ile Val Cys Val Leu Ser Val   64

193  TAC GTC TCT TGC TCT ACA GAG GTG TGC TCG TCT C                    226
 65  Tyr Val Ser Cys Ser Thr Glu Val Cys Ser Ser                      75
```

Figure 1. Translation of a crab cDNA sequence obtained using primers defining the tenth putative transmembrane domain of the human Na^+/H^+ antiporter.

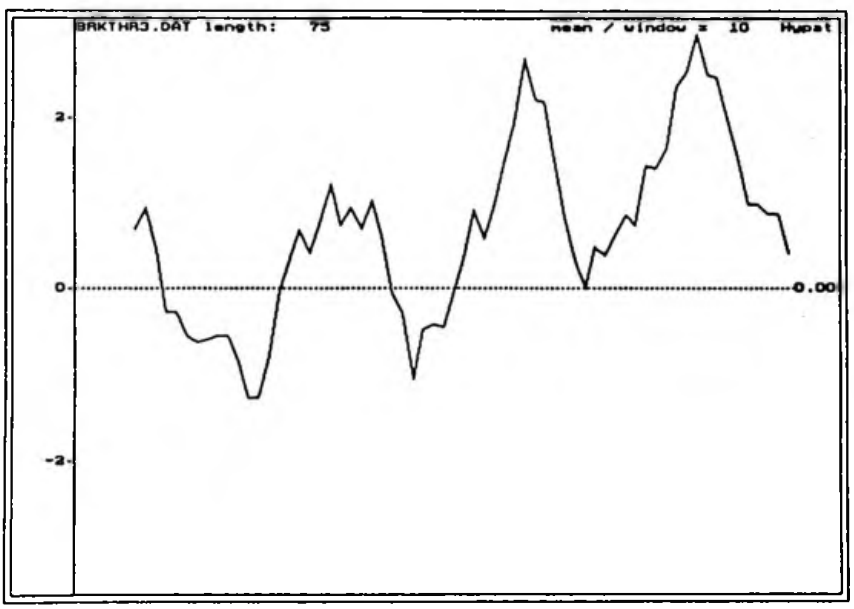

Figure 2. Hydropathy plot of amino acid sequence indicated in Fig. 1 (Kyte and Doolittle, J. Mol. Biol. 157:112-122, 1982), analyzed by PROFILEGRAPH software (K.O. Hofmann).