MOLECULAR BIOLOGY OF THE ELECTROGENIC SODIUM/HYDROGEN ANTIPORTER IN GILLS OF THE GREEN SHORE CRAB CARCINUS MAENAS

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The Na^+/H^+ antiporter in crustacean gill epithelia is uniquely electrogenic, apparently exchanging 2 Na^+ for 1 H^+, unlike the electroneutral 1 Na^+/1 H^+ antiporter in vertebrate tissues (Shetlar, Alexander and Towle, Bull. MDIBL 27:59, 1988; Shetlar and Towle, Am. J. Physiol. 257:R924, 1989). The 2:1 stoichiometry has been confirmed in other crustacean as well as echinoderm tissues (Ahearn and Clay, Am. J. Physiol. 257:R484, 1989; Ahearn and Franco, J. Exp. Biol. 158:495, 1991). The vertebrate "housekeeping" Na^+/H^+ antiporter has recently been cloned and sequenced from mammalian tissues (Sardet, Franchi and Pouysségur, Cell 56:271, 1989; Tse et al., EMBO J. 10:1957, 1991). Due to the generosity of Dr. Jacques Pouysségur who provided a cDNA fragment containing 80% of the human Na^+/H^+ antiporter sequence, we have been able to investigate whether discernible sequence homology exists between the vertebrate and crustacean antiporter genes.

Genomic DNA and total RNA were isolated from male gonad and gill respectively of the green shore crab Carcinus maenas according to the methods of Miller, Dykes and Polesky (Nucl. Acids Res. 16:1215, 1988) and Chomczynski and Sacchi (Anal. Biochem. 162:156, 1987). Aliquots of crab DNA and RNA were applied to nylon membrane using a slot-blot apparatus (BioRad Laboratories). The membranes were then hybridized overnight with the human Na^+/H^+ antiporter cDNA probe which had been biotinylated using the Flash™ labelling and detection system provided by Stratagene Cloning Systems. Under conditions of moderate stringency, hybrids could be detected between crustacean and human nucleotide sequences, indicating partial sequence homology of the antiporter genes.

Several oligonucleotide primers were synthesized based on putative transmembrane and intracellular regions of the human Na^+/H^+ antiporter. Using crab genomic DNA as template, primers representing transmembrane sequences supported polymerase chain reaction amplification of the DNA, producing discrete bands discernible upon agarose gel electrophoresis. However, primers representing intracellular regions did not support amplification, suggesting that only transmembrane sequences of the Na^+/H^+ antiporter may exhibit partial homology between human and crab.

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