ISOLATION, PURIFICATION AND *IN VITRO* TRANSLATION OF mRNA FROM GREEN SHORE CRAB *(CARCINUS MAENAS)* GILLS

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In salinities less than 25 o/oo salt, the green shore crab *(Carcinus maenas)* maintains hyperosmotic hemolymph via independent sodium and chloride transport systems in posterior gills. Sodium uptake from dilute seawater depends on basolateral Na^++K^+-ATPase, which mediates Na^+/NH_4^+ as well as Na^+/K^+ exchange (Towle, Bull. MDIBL 23:10-12, 1983; Towle, Bull. MDIBL 25:80-83, 1985). Sodium uptake by perfused gills is amiloride-sensitive (Lucu and Siebers, J. Exp. Biol. 122:25-35, 1986) and an amiloride-inhibitable Na^+/H^+ antiporter has been characterized in vesicle preparations (Shetlar, Alexander and Towle, Bull MDIBL 27:59-61, 1987). Unlike the vertebrate Na^+/H^+ antiporter, the crustacean antiporter is uniquely electrogenic, apparently exchanging two Na^+ for one H^+. To further describe the molecular nature of the crustacean Na^+/H^+ exchange system, we have initiated experiments designed to clone and sequence the antiporter gene.

Total RNA was isolated from the three posterior gills of green crabs by the guanidine isothiocyanate method (Ullrich *et al.*, Science 196:1313-1319, 1977). RNA concentrations were measured spectrophotometrically at 260 nm. Agarose gel electrophoresis revealed a smear of material with two distinct bands corresponding to the ribosomal RNAs of these cells. In addition to the rRNA bands, three bands representing mRNAs which exist in high copy numbers were noted. These bands may represent the mRNA for proteins which are particularly important in the function of these cells. The mRNA was separated from total RNA by affinity chromatography using an oligo-dT cellulose column. Subsequent gel electrophoresis revealed a smear free from the ribosomal components (but still containing the other three bands mentioned above) and thus representing a purified preparation of mRNA.

Rabbit reticulocyte lysate *in vitro* translation system from Amersham was used to translate proteins using the purified mRNA as template in a reaction mixture containing ^{35}S-methionine. The radiolabeled proteins were then subjected to polyacrylamide gel electrophoresis followed by autoradiography. The optimal conditions for efficient translation were found to be 1 ug/ml mRNA with Mg^{+2} and K^+ concentrations of 132 mM and 1.3 mM respectively. The non-specific proteins synthesized in control incubations without gill mRNA were 87 kDa and 47 kDa in size. Eleven other bands present on the gel were the result of translation of the crab gill mRNA. These varied in molecular weight from 185 kDa to 19 kDa. Work is in progress to fractionate and further characterize the purified mRNA by injection into *Xenopus* oocytes.

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