CHLORIDE TRANSPORT BY MEMBRANE VESICLES FROM GILLS OF THE GREEN SHORE CRAB CARCINUS MAENAS

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In the gills of euryhaline crabs, chloride uptake from dilute salinities is independent of external Na^+ (reviewed by Towle, Comparative Aspects of Sodium Cotransport Systems, R.K.H. Kinne, ed., Karger, pp. 241-263, 1990). Experiments with perfused gills suggest that a Cl^-/HCO_3^- antiporter is responsible for apical uptake of Cl^- (Lucu, Comp. Biochem. Physiol. 92A:415-420, 1989). Chloride channels have been implicated in the basolateral transfer of Cl^- from cytosol to blood, also in studies of perfused gills (Bianchini et al., Comp. Biochem. Physiol. 90A:315-319, 1988). An anion-sensitive ATPase was described in membrane preparations of crab gill (DePew and Towle, Mar. Biol. Lett. 1:59-67, 1979; Lee, Biochim. Biophys. Acta 689:143-154, 1982) but its role in transepithelial chloride transport has never been established. This study was designed to explore the mechanisms of chloride transport in plasma membrane vesicles isolated from posterior gills of the crab Carcinus maenas.

Crabs were obtained from intertidal areas of Frenchman Bay and were maintained at 13-15°C in filtered circulating sea water diluted to 10 o/oo salinity. Membrane vesicles enriched in Na^++K^+-ATPase activity were prepared from posterior gills by sucrose density gradient centrifugation (Towle and Hølleland, Am. J. Physiol. 252:R479-R489, 1987). Vesicles were loaded either with standard loading buffer (250 mM sucrose, 0.5 mM EDTA, 1 mM dithiothreitol [DTT], 20 mMTris-HEPES, pH 7.8) or with bicarbonate loading buffer (50 mM potassium bicarbonate, 150 mM sucrose, 0.5 mM EDTA, 1 mM DTT, 20 mM Tris-HEPES, pH 7.8). Transport assays were conducted by incubating 5 µl of vesicle suspension (15-40 µg protein) in 100 µl of uptake medium containing 0.1 µCi $^{36}Cl^-$ in 235 mM sucrose, 5 mM magnesium gluconate, 20 mM Tris-HEPES (pH 7.8), and 1 mM NaCl. In some transport experiments, ATP was added at a final concentration of 5 mM. Following incubation for 0-300 secs, uptake was terminated by adding 2 ml of cold wash buffer (250 mM sucrose, 2 mM DTT, 10 µM DIDS, and 20 mM Tris-HEPES, pH 7.8), filtering through a Schleicher and Schuell sub-micron filter, and washing twice with 2 ml wash buffer. The filters were immersed in 5 ml Ecolume scintillation medium for detection of radioactivity. "Zero-time" uptake represented 5-20% of equilibrium uptake.

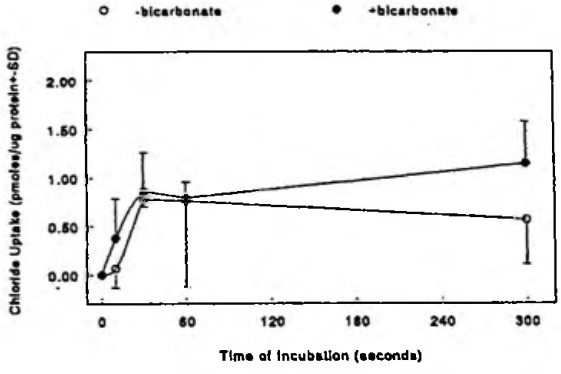

Figure 1. Effect of intravesicular bicarbonate on Cl^- uptake by membrane vesicles from crab gill. Zero time values were subtracted from uptake measurements.

Preliminary results indicated that loading vesicles with bicarbonate did not enhance uptake of Cl^- (Fig. 1). This finding is in contrast to those of previous investigators (Lee and Pritchard, Am. J. Physiol. 249:R544-R550, 1985), who reported a strong effect of intravesicular bicarbonate in stimulating Cl^- uptake into vesicles prepared from gills of the blue crab Callinectes sapidus. In our preparation, the initial outwardly-oriented bicarbonate gradient may collapse very rapidly during chloride uptake measurements, reducing or eliminating a possible bicarbonate effect. Alternatively, membrane vesicles from Carcinus maenas gill may lack a Cl^-/HCO_3^- antiporter.

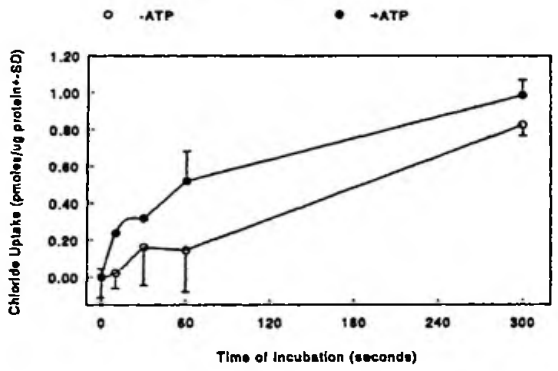

Figure 2. Effect of extravesicular ATP on Cl^- uptake by membrane vesicles from crab gill.

Addition of extravesicular ATP appeared to enhance uptake of Cl^-, suggesting that an ATP-energized anion pump may be operational in this system (Fig. 2). Thus a transport role for the previously characterized anion-stimulated ATPase may exist. Alternatively, ATP-dependent phosphorylation or activation of channels and/or antiporters may be implicated in the transport process. Additional studies will be needed to reduce the observed scatter in the data and to further characterize chloride transport mechanisms by examining the effects of increasing chloride concentration and the effects of such inhibitors as SITS, bumetanide, vanadate, thiocyanate, and the Cl^- channel blocker diphenylamine-2-carboxylate.

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