ELECTROGENIC Na^+/H^+ EXCHANGE IN MEMBRANE VESICLES FROM CRAB (CARCINUS MAENAS) GILL

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The Na^+/H^+ exchanger has been implicated in a variety of roles in the epithelial cell. These include intracellular pH regulation (Doppler, Maly and Grunicke, J Memb Biol 91:147-155, 1986; Montrose, Friedrich and Murer, J Memb Biol 97:63-78, 1987) and cell volume regulation (Grinstein, Cohen, Goetz, Rothstein, Mellors and Gelfland, Curr Tops Memb Trans 26:115-134). In the mammalian systems that have been studied the exchanger has been shown to be an electro-neutral carrier exchanging one Na^+ for one H^+ (Aronson and Igarashi, Curr Tops Memb Trans 26:57-75, 1986). In the gills of Carcinus maenas, the exchanger may participate in bringing Na^+ into the epithelial cell from the medium, permitting an osmoregulatory response to reduced salinities (Shetlar and Towle, Bull MDIBL 26:125-128, 1986; Siebers, Leweck, Markus and Winkler, Marine Biol 69:37-43, 1982). The present work was undertaken to determine the kinetic characteristics of the Na^+/H^+ exchanger from crab gill, relative to the characteristics of the exchanger from vertebrate tissues.

Green shore crabs (Carcinus maenas) were collected from the intertidal and subtidal areas adjacent to MDIBL. The animals were maintained as previously described (Shetlar and Towle, 1986) in 10 ppt seawater for at least one week prior to use in experiments. Membrane vesicles were prepared by sucrose density gradient centrifugation (Towle and Hølleland, Amer J Physiol 21:R479-R489, 1987). The membranes were loaded with the desired medium (150 mM sodium gluconate, 20 mM HEPES, 56 mM sucrose, pH 6.8 with Tris) by dilution, centrifugation and resuspension of the membrane fraction (Shetlar and Towle, 1986).

Na^+/H^+ exchange assays were carried out using the acridine orange fluorescence method (Sabolic and Burckhardt, Biochim Biophys Acta 734:210-220, 1983; Warnock, Reenstra and Yee, Amer J Physiol 242:F733-F739, 1982; Shetlar and Towle, 1986). Briefly, 10 ul of Na^+-loaded membrane vesicles were injected into 2 ml of a Na^+-free incubation medium containing the dye acridine orange (10 uM), tetramethylammonium (TMA) gluconate (150 mM), sucrose (36 mM), HEPES (20 mM), pH 6.8 with Tris. Fluorescence changes were continuously monitored using an Aminco-Bowman spectrofluorometer attached to a strip chart recorder (excitation at 493 nm, emission at 525 nm). The assay mixture was stirred throughout the experiment. Adding Na^+-loaded vesicles to a Na^+-free medium caused a pH gradient to develop across the membrane due to the action of the exchanger. This pH gradient was then collapsed by addition of sodium gluconate to the cuvette. In order to obtain kinetic data the concentration of sodium gluconate was varied from 7.5 mM to 75 mM and rates were determined by drawing tangents to the curves of fluorescence recovery.

Experiments with the potential-sensitive fluorescent dye DiS-C_3-(5) were done to determine if the observed Na^+/H^+ exchange was electrogenic (Stieger, Burckhardt and Murer, Pfluegers Arch 400:178-182, 1984; Bashford and Smith, Meth Enzymol 54:569-586, 1979; Cabrini and Verkman, J Memb Biol 92:171-182, 1986). Sodium-loaded membrane vesicles (10 ul) were injected into an incubation medium containing an equimolar concentration of sodium gluconate ($Na+_i$ = $Na+_o$) or TMA gluconate (Na^+_i > Na^+_o) and the fluorescent dye DiS-C_3-(5) (2 uM). The remaining components of the incubation medium were as described for the Na^+/H^+ exchange assay. Fluorescence changes were monitored as described above (excitation at 620 nm, emission at 669 nm).

The results of the kinetic experiments indicate a Na^+:H^+ stoichiometry different than the 1:1 ratio which has been reported for the Na^+/H^+ exchanger in vertebrate systems (Murer, Hopfer and Kinne, Biochem J 154:597-604, 1976; Aronson and Igarashi, 1986). The rate of fluorescence recovery showed a sigmoid relationship to Na^+ concentration, giving an apparent $K_{0.5}$ of about 34 mM Na+ (Fig. 1). The sigmoidicity of the curve suggested a coupling ratio greater than one. When the data were plotted according to the Hill equation (Fig. 2), the slope of the resulting line indicated that the coupling ratio was two sodium ions for one proton (slope = 2.1).

The results of the potential-sensitive dye experiments confirmed that under the assay conditions employed the exchange is electrogenic, unlike the antiporter of vertebrate cells. The dye DiS-C_3-(5) is a large organic cation which distributes across the membrane according to the potential difference. In the control situation, there was no Na+ gradient ($Na+_i$ = $Na+_o$) and any decrease in fluorescence signal should be due to binding interactions between the dye molecules and the membrane exterior. In the experimental situation, the incubation medium contained no Na+ and any decrease in fluorescence signal greater than

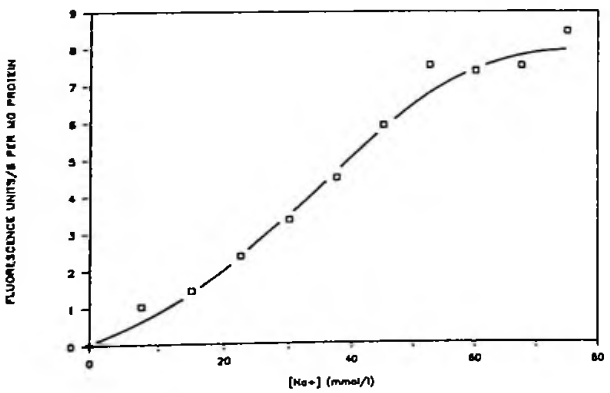

Figure 1. A kinetic plot of fluorescence recovery vs. extravesicular Na^+ concentration. Sodium-loaded vesicles were injected into Na+-free incubation medium. The pH gradient that developed was then collapsed by addition of sodium gluconate to the cuvette. The rate of fluorescence recovery was determined as a tangent to the curve two seconds after Na+ addition. Each point is the mean of three trials.

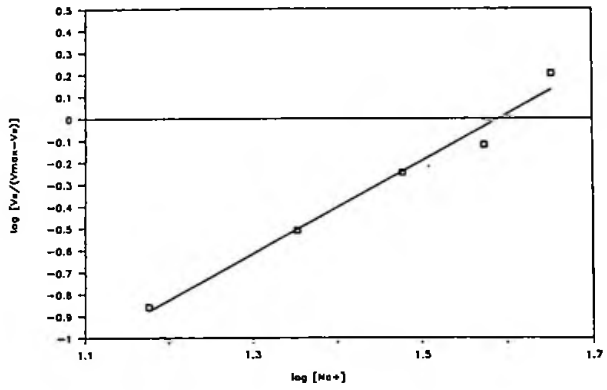

Figure 2. A Hill plot of the kinetic data from the previous figure. The slope is equal to the coupling ratio, in this case = 2.1.

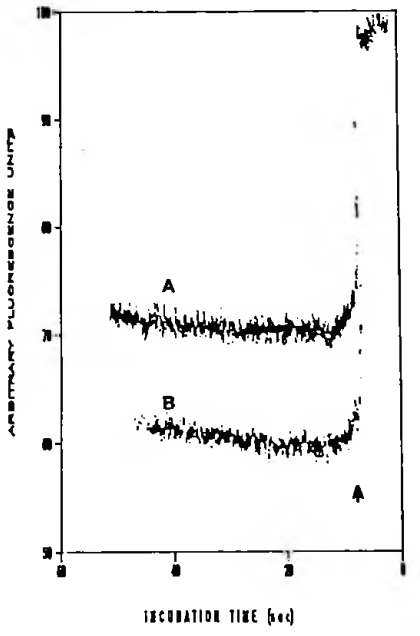

Figure 3. A representative experiment with the potential sensitive dye DiS-C3-(5). Vesicles (10 ul) were injected at the arrow into incubation medium containing 2 uM DiS-C3-(5). For the curve labelled A, $Na^+_i = Na^+_o$ and no inside negative diffusion potential is expected, thus the observed dye quench is due to dye binding to the membrane surface. For the curve labelled B, $Na^+_i > Na^+_o$ and the greater quench is due to an inside negative diffusion potential resulting from electrogenic 2 Na^+/1 H^+ exchange.

that observed in the absence of a Na^+ gradient would be due to a potential difference. In this case a further decrease in fluorescence signal was observed (Fig. 3). Our results therefore indicate that the Na^+/H^+ exchanger in Carcinus gill is an electrogenic transporter with an apparent stoichiometry of two sodium ions for one proton.

Acknowledgements

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