LOCALIZATION OF Na^++K^+-ATPase IN BASOLATERAL MEMBRANES OF CRAB (CARCINUS MAENAS) GILL EPITHELIUM

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Recent models for the mechanism of Na^+ absorption by gills of teleosts and crustaceans have assumed that Na^++K^+-ATPase is restricted to the basolateral membrane of these epithelial cells (Kirschner, Amer. J. Physiol. 244:R429-R443, 1983; Towle, Amer. Zool. 24:177-185, 1984). For teleosts, this assumption is based on localization of ^{3}H-ouabain-binding sites (Karnaky, Kinter, Kinter and Stirling, J. Cell Biol. 70:157-177, 1976), but for crustaceans no direct studies have been reported. Indeed, two pieces of evidence suggest that the expected polarity of Na^++K^+-ATPase distribution may not apply to crustacean epithelia. First, perfused ouabain was reported to be ineffectual in blocking $^{22}Na^+$ uptake by isolated gills of the Chinese crab (Pequeux and Gilles, J. Exp. Biol. 92:173-186, 1981). Second, cytochemical studies of cells isolated from salt gland of larval brine shrimp indicated that K^+-dependent p-nitrophenylphosphatase was found not only in basolateral membranes but also in apical membranes and associated with mitochondria as well (Conte, Internatl. Rev. Cytol. 91:45-106, 1984). The present study was undertaken to determine the subcellular location of Na^++K^+-ATPase in the gill epithelium of the green crab, Carcinus maenas.

Crab gills are lamellar, with a single layer of epithelial cells lining a cuticular envelope. The circulatory pattern within a gill is simple, via a single afferent vessel through lamellae and a single efferent vessel. Two major cell types exist in gills of portunid crabs, mitochondria-rich "thick" cells with extensive infoldings of both basolateral and apical membranes, and "thin" cells with limited infolding of basolateral membranes parallel to the plane of the epithelium (Copeland and Fitzjarrell, Z. Zellforsch. 92:1-22, 1968). The latter cell type is believed to participate primarily in gas exchange and is found throughout anterior gills. Mitochondria-rich cells occur in lamellae of posterior gills in dark-colored patches, visible to the naked eye. These homogeneous populations of cells lie adjacent to the afferent blood vessel and are surrounded by thin respiratory-type cells. In the blue crab, Callinectes sapidus, mitochondria-rich cells contain a large proportion of posterior gill Na^++K^+-ATPase activity (Neufeld, Holliday and Pritchard, J. Exp. Zool. 211:215-224, 1980). In C. maenas, posterior gills contain 4-5 times the specific Na^++K^+-ATPase activity of anterior gills, and demonstrate increased Na^++K^+-ATPase activity upon acclimation to reduced salinity (Siebers, Leweck, Markus and Winkler, Mar. Biol. 69:37-43, 1982).

Cytochemical localization of Na^++K^+-ATPase in posterior gills of C. maenas was accomplished by incubating gills in a medium designed to detect ouabain-sensitive, K^+-dependent p-nitrophenylphosphatase (p-NPPase) activity (Mayahara, Fujimoto, Ando and Ogawa, Histochemistry 67:125-138, 1980). The procedure was adapted to suit the osmotic and structural requirements of crab gill, in the following way. Gills 7 and 8 (counting anterior -> posterior) of crabs acclimated for at least one week to 10 o/oo salinity were perfused with about 10 ml of the various media at about 2 ml/min via polyethylene tubing, simultaneously immersing the gill in the same medium. Initial fixation at 0-4°C for 1 hr was accomplished with 2% formaldehyde plus 0.5% glutaraldehyde in buffer A (=0.5 M sucrose plus 0.1 M cacodylate, pH 7.2 with

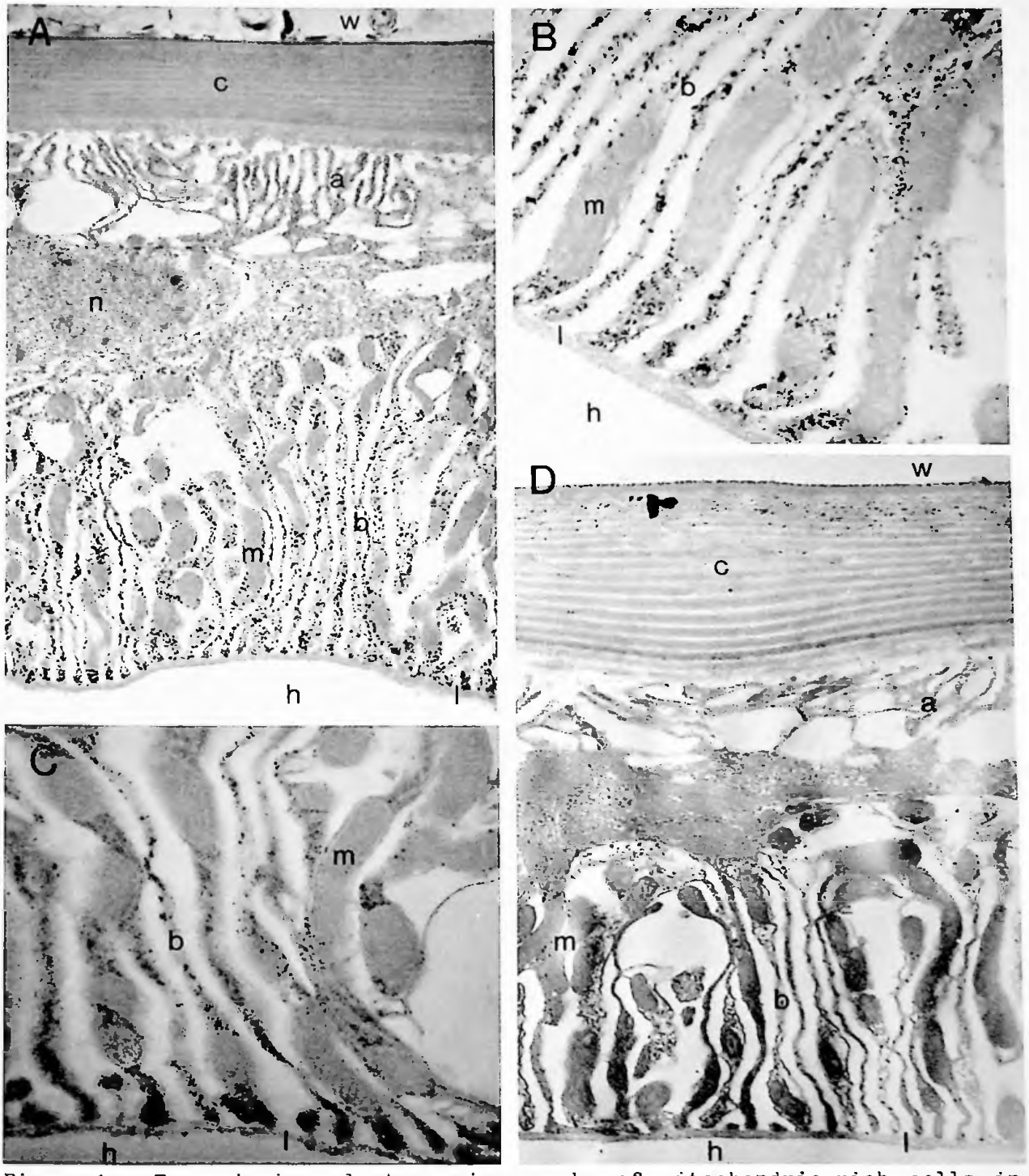

Figure 1. Transmission electron micrographs of mitochondria-rich cells in gill epithelium of Carcinus maenas. A: Incubated in complete medium for detection of ouabain-sensitive, K^+-dependent p-nitrophenylphosphatase (13,950 x). B: Basolateral region, incubated in complete medium (44,250 x). C: Basolateral region, incubated in medium containing 10 mM ouabain (35,500 x). D: Incubated in medium lacking K^+, replaced by Na^+ (18,400 x). w = water space, c = cuticle, a = apical membrane folds, n = nucleus, m = mitochondria, b = basolateral membrane folds, l = basal lamina, h = hemolymph space.

HCl). Each gill was perfused with 0.59 M sucrose, 0.01 M cacodylate, pH 7.2, plus 10% dimethylsulfoxide (DMSO) and stored overnight at 4°C in fresh medium of the same composition. The gill was then perfused with and incubated in the cytochemical reaction medium for a total of 10 min at room temperature. The reaction medium contained 0.5 M glycine, 0.07 M KOH, 4 mM lead citrate, 25% DMSO, 10 mM p-nitrophenylphosphate (Mg salt), and 2.5 mM levamisole, final pH 8.8. Control incubations were of four types: (1) replacing p-NPP with 10 mM $MgCl_2$, (2) replacing KOH with NaOH, (3) omitting levamisole, and (4) incubating with 10 mM ouabain. In the latter case, gills were perfused with 10 mM ouabain in 0.5 M glycine and 0.05 M KOH (pH 9.0) and incubated in the same medium at 0-4°C for 30 min prior to incubation in the reaction medium, to which 10 mM ouabain was added. Following incubation in reaction medium, gills were perfused with cold buffer A, postfixed with 1% osmium tetroxide in buffer A for 1 hr at 0-4°C, and finally rinsed by perfusion with buffer A. Following dehydration in ethanol and propylene oxide, individual lamellae were embedded in Epon and sectioned with a diamond knife, identifying regions of thick epithelium by their darker color. Sections were poststained with 1% uranyl acetate in 50% methanol and examined in a JEOL JEM-100CXII transmission electron microscope at 80 kV.

Thick epithelium of C. maenas gill is ultrastructurally similar to that of C. sapidus (Copeland and Fitzjarrell, 1968) and is characterized by infoldings of basolateral and apical membranes arranged approximately perpendicular to the cuticle, which separates apical membrane from the external environment. A thin basal lamina is interposed between basolateral membrane termini and the hemolymph space, and numerous mitochondria lie within the basolateral membrane folds (Fig. 1A). Upon incubation with the medium to detect K^+-dependent p-NPPase, cytochemical reaction product was found predominantly in the basolateral region of the cell. Very little reaction product was found in the apical region, and the precipitate granules that were present were much smaller than those in the basolateral region (Fig. 1A).

The slightly hyperosmotic buffers used in fixation and reaction media induced sufficient cellular shrinkage to permit clear delineation between cytoplasmic and extracellular surfaces of the basolateral membrane, although the image of the membrane itself remained somewhat diffuse. Cytochemical reaction product was restricted to the cytoplasmic surface of the membrane, and was not found in sections of mitochondria (Fig. 1B). This distribution of reaction product suggests that it is the result of enzymatic activity within the basolateral membrane, the catalytic site for substrate hydrolysis lying at the cytoplasmic surface, properties that are consistent with those of Na^++K^+-ATPase. Incubation without substrate (p-NPP) produced electron micrographs lacking any characteristic electron-opaque reaction products. Omission of levamisole, an inhibitor of alkaline phosphatase, had no noticeable effect on formation of reaction product. However, treatment with 10 mM ouabain reduced the formation of reaction product slightly (Fig. 1C), and substituting Na^+ for K^+ in the reaction medium dramatically reduced formation of precipitate (Fig. 1D).

The results indicate that K^+-dependent p-NPPase, representing part of the Na^++K^+-ATPase reaction mechanism, is restricted primarily to the basolateral membrane of mitochondria-rich cells in gill epithelium of the green crab. The weak inhibition by ouabain may be characteristic of crustacean Na^++K^+-ATPases, in light of the remarkably high K_i values reported

(reviewed by Towle, 1984), and may help to explain the lack of Na^+ uptake inhibition by ouabain noted earlier in perfused crab gill (Pequeux and Gilles, 1981). Potassium dependence of the formation of reaction product clearly demonstrates the presence of Na^++K^+-ATPase in the basolateral membrane. Here, the enzyme is poised to serve as the driving force for transepithelial Na^+ absorption.

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