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## Biochemical characterization of isolated branchial mitochondria-rich cells of *Oreochromis mossambicus* acclimated to fresh water or hyperhaline sea water

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Accepted: 2 June 1993

**Abstract.** Mitochondria-rich cells have been separated from other epithelial cells of tilapia (*Oreochromis mossambicus*) gills by density gradient centrifugation on Percoll. During centrifugation two main bands of cells formed. The viability of the cells in both bands was high (>90%). In one band, 45–74% of the total cell number was mitochondria-rich cells. The other band contained at least 80% pavement cells, representing the majority of other gill epithelial cell types. A comparison of the activities of four enzymes involved in major metabolic and ion regulatory functions was made between these two different fractions of cells. Furthermore, the separation of gill epithelial cells and determination of enzymatic activity was carried out in tilapia after the fish were acclimated to fresh water or hyperhaline sea water (60 mg · ml<sup>-1</sup> S) to gain an indication of the relative contribution of mitochondria-rich cells and pavement cells to both NaCl excretion and absorption. Regardless of acclimation salinity, the activities of Na<sup>+</sup>/K<sup>+</sup>-ATPase, glutamate dehydrogenase and glucose-6-phosphate dehydrogenase were significantly higher in mitochondria-rich cells than in pavement cells. However, tilapia acclimated to hyperhaline sea water possessed significantly lower carbonic anhydrase activity in mitochondria-rich cells than in pavement cells. In contrast, no significant difference of carbonic anhydrase activity was observed between the two cell fractions in tilapia acclimated to fresh water.

**Key words:** Salinity – Gill – Mitochondria-rich cells – Tilapia, *Oreochromis mossambicus*

**Abbreviations:** ATPase, adenosine triphosphatase; CA, carbonic anhydrase; DASPMI, dimethylaminostyrylmethylpyridinium iodine; FW, fresh-water; GDH, glutamate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; HSW, hyperhaline sea water (60 mg · ml<sup>-1</sup>); MR cells, mitochondria-rich cells; S, salinity

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### Introduction

In teleosts the gill is the major site of gas exchange, Na<sup>+</sup> and Cl<sup>-</sup> transport, acid-base regulation and ammonia excretion. Even though the branchial epithelium in these species consists of at least five cell types [pavement cells, MR cells, mucous cells, neuroepithelial cells and undifferentiated cells; Laurent (1985)], the highly differentiated MR cells and pavement cells, which together form the outer epithelial layer, are believed to be the main sites for most of these functions because the other cell types are either undifferentiated or have other specific functions (i.e. mucous secretion or neural signal transduction).

Recently, different types of MR cells have been described of which the chloride cells, responsible for active Cl<sup>-</sup> secretion, may represent only one population (Hootman and Philpott 1980; Pisam et al. 1990; Kültz et al. 1992). In addition, chloride cells themselves may be divided into subpopulations based on developmental state (Wendelaar Bonga and Van der Meij 1989; Wendelaar Bonga et al. 1990). Foskett and Scheffey (1982) provided definitive evidence for the location of active Cl<sup>-</sup> secretion in chloride cells but the cellular localization of NaCl absorption, acid base regulation and ammonia excretion is not well defined (Girard and Payan 1980; Payan et al. 1984; Goss et al. 1992). Pavement cells, which form the major contact surface to the medium, could also be the site of Na<sup>+</sup> and Cl<sup>-</sup> absorption (Girard and Payan 1977, 1980; Goss et al. 1992). However, the mechanism of this active NaCl absorption is not as well understood as the secretion of Cl<sup>-</sup> across chloride cells (Shuttleworth 1989). Due to the evolutionary history of teleost fishes, the gill functions of osmoregulation, acid-base regulation and nitrogen excretion are interdependent (Evans 1984; Matei 1990). Compromises between different gill functions are therefore common in teleosts to ensure the maintenance of all homeostatic processes necessary for survival (Randall and Wright 1989; McDonald et al. 1989; Gonzales and McDonald 1992).

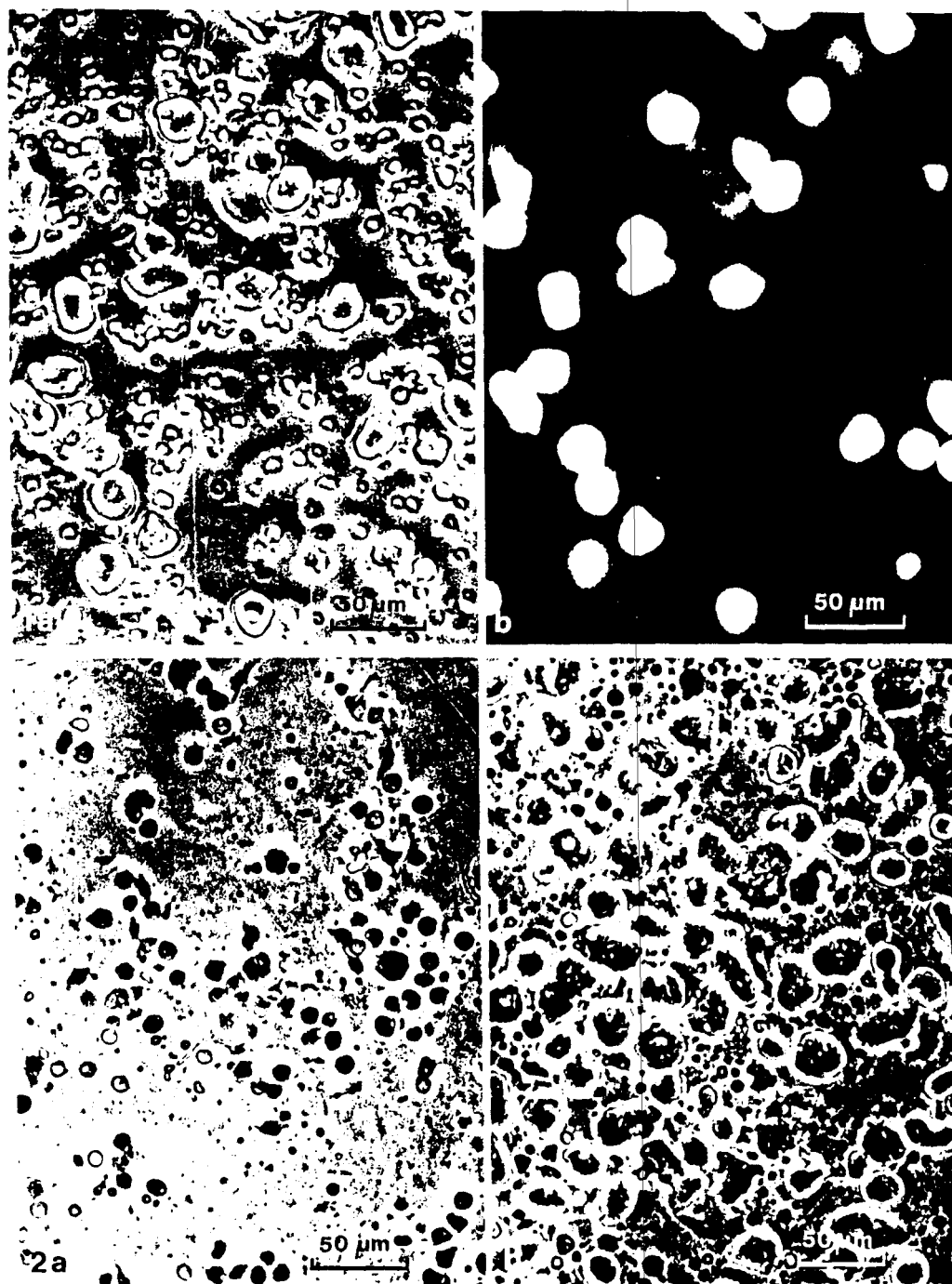


Fig. 1a, b. Comparison of the appearance of MR cells from gills of tilapia acclimated to HSW (a) phase contrast (left) and (b) epifluorescence (right, MR cells are selectively stained with DASPMI). Magnification  $\times 300$ .

Fig. 2a, b. Appearance of cell suspensions from tilapia acclimated to HSW after centrifugation at  $1000g$  for 30 min on a Percoll gradient: (a) band A of the gradient containing predominantly pavement cells (left); (b) band C of the gradient containing predominantly MR cells (right). Magnification  $\times 300$ .

In this study metabolic and transport characteristics of enriched fractions of MR cells and pavement cells were examined by determining the activities of  $\text{Na}^+/\text{K}^+$ -ATPase, GIDH, CA, and G6PDH to gain further insight into the roles of these cells in the function of the gill.

### Materials and methods

**Animals and experimental protocol.** Tilapia (*Oreochromis mossambicus*) were bred and grown from laboratory stock in FW at a temperature of  $24 \pm 4^\circ\text{C}$ . Only sexually mature fish were used. Prior to acclimation all fish were maintained in brackish water ( $10 \text{ mg} \cdot \text{ml}^{-1} \text{ S}$ ) at  $25 \pm 1^\circ\text{C}$  for at least 10 days. This S is isosmotic

with tilapia plasma and was therefore chosen as the starting point. Subsequently, the fish were acclimated to either Rostock tapwater [ $\text{Na}^+$ :  $1.2 \text{ mmol} \cdot \text{l}^{-1}$ ;  $\text{K}^+$ :  $1.5 \text{ mmol} \cdot \text{l}^{-1}$ ;  $\text{Cl}^-$ :  $0.9 \text{ mmol} \cdot \text{l}^{-1}$ ] (Vökler et al. 1987) or HSW. The acclimation to HSW was performed stepwise to 30 and  $45 \text{ mg} \cdot \text{ml}^{-1}$  for 1 week each, before transferring fish into the final S of  $60 \text{ mg} \cdot \text{ml}^{-1}$ . HSW was prepared using artificial sea salt (Tropic Marin, TAGIS) for solutions up to  $35 \text{ mg} \cdot \text{ml}^{-1} \text{ S}$ . The final increase to  $60 \text{ mg} \cdot \text{ml}^{-1} \text{ S}$  was made by adding NaCl. Measurements of S were carried out using a conductometer (WTW LF 196). Tilapia were maintained at  $25 \pm 1^\circ\text{C}$  under a 12:12 L/D photoperiod for at least 5 weeks at the final salinity. They were fed daily with commercial trout pellets equivalent to approximately 2% body mass per day. The water was aerated and filtered continuously through polyamide wool and charcoal.

Fish were killed by excess anaesthesia (0.1% MS 222 neutralized to pH 7.5 with  $\text{NaHCO}_3$ ), weighed (FW:  $50.0 \pm 4.3$  g, HSW:  $47.7 \pm 3.4$  g) and measured (FW:  $14.7 \pm 0.5$  cm, HSW:  $14.9 \pm 0.3$  cm), before being transferred into a dish filled with tilapia Ringer solution ( $\text{mmol} \cdot \text{l}^{-1}$ ): NaCl: 146, KCl: 3,  $\text{NaH}_2\text{PO}_4$ : 1,  $\text{NaHCO}_3$ : 15,  $\text{MgSO}_4$ : 1,  $\text{CaCl}_2$ : 1, TRIS: 5; pH 7.4). The pericardium was opened and the gills were perfused by catheterization of the bulbus arteriosus with tilapia Ringer solution containing  $5000 \text{ U} \cdot \text{ml}^{-1}$  heparin (Sigma). After 5 min of perfusion most of the blood was removed from the gills (less than 5% erythrocytes in all suspensions observed, see Results) and the individual gill arches were dissected and rinsed in tilapia Ringer solution. All procedures were done at  $0-4^\circ\text{C}$ .

**Isolation of gill epithelial cells.** Individual gill arches were blotted free of Ringer solution and the epithelial tissue was scraped off the underlying cartilage using a blunt-tipped scalpel. The tissue was then weighed (FW:  $543 \pm 31$  mg, HSW:  $687 \pm 42$  mg) and incubated for 20 min in  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free tilapia Ringer solution containing  $20 \text{ mmol} \cdot \text{l}^{-1}$  EDTA and  $0.02 \text{ mg} \cdot \text{ml}^{-1}$  elastase (Sigma, Typ I). The use of elastase at this concentration prevented the cells from reaggregating during density gradient centrifugation and had no noticeable effect on the viability of disaggregated cells as determined by trypan blue dye exclusion. After incubation, tissue pieces were filtered through gauze ( $56 \mu\text{m}$ ) and then gently passed through a syringe needle (diameter 1 mm) 15 times. One-fifth of this whole gill cell suspension was collected for morphological examination and for the determination of enzyme activities in the unfractionated gill cells, and was held on ice during further processing of the remaining material.

The remaining suspension (6–8 ml) was layered on top of a continuous density gradient made of Percoll (Sigma; 1.10, 1.08, 1.06, and  $1.04 \text{ g} \cdot \text{ml}^{-1}$ ), prepared from a Percoll stock solution and appropriate volumes of  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free tilapia Ringer solution (Sharpe 1988). After centrifugation at  $1000 g$  for 30 min (Beckmann L-60, SW 28) two main bands formed, one above the  $1.04 \text{ g} \cdot \text{ml}^{-1}$  layer and the other at the boundary between the 1.06 and  $1.08 \text{ g} \cdot \text{ml}^{-1}$  layers. The upper band was designated A and the lower band C. Cells of both bands were collected with a Pasteur pipette and washed twice with  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free tilapia Ringer solution by centrifugation at  $500 g$  for 5 min. Bands A and C and the whole gill cell suspensions were used for the morphological examination and the determination of enzyme activities. Before sonication (UW 60, Bandelin electronic) and freezing at  $-70^\circ\text{C}$  the cells were washed by centrifugation at  $500 g$  for 5 min and resuspended in SEI-buffer (McCormick and Bern 1989).

**Characterization of cell types and viability.** Each cell suspension (whole gill, band A and C) was subdivided into two aliquots for morphological examination and determination of enzyme activities. Morphological examinations were performed using a fluorescence and phase-contrast microscope (Jenalumär, Carl Zeiss). Cells that remained unstained after 5 min in tilapia Ringer solution containing  $0.2 \text{ mg}$  trypan blue were considered viable (Sharpe 1988). MR cells, pavement cells, undifferentiated cells and erythrocytes were easily distinguished microscopically. MR cells are between 10 and  $40 \mu\text{m}$ , and could be selectively stained with the vital mitochondrial-specific fluorescence dye DASPMI [2-(*p*-dimethylaminostyryl)-1-ethylpy-

ridiniumiodine; Aldrich, Milwaukee, USA] (Karnaky et al. 1984). Figure 1 illustrates the specificity of this dye to MR cells as first described by Bereiter-Hahn (1976). Pavement cells appeared dark under phase-contrast with a shape that varied from spherical to ovoid and a diameter of  $5-8 \mu\text{m}$ . Undifferentiated cells appeared light and possessed a regular spherical shape. The size of these cells varied between 4 and  $8 \mu\text{m}$ . Only low numbers of mucous cells were observed. Erythrocytes could be easily distinguished from branchial cells by their characteristic shape and regularly formed nucleus. The percentages of MR cells and pavement cells were determined on micrographs taken of each cell suspension using ORWO NP 20, 80 ASA film.

**Enzyme assays.** The activities of  $\text{Na}^+/\text{K}^+$ -ATPase (EC 3.6.3.1), GDH (EC 1.4.1.2) and G6PDH (EC 1.1.1.49) were measured at  $25^\circ\text{C}$  within 2 weeks after sonication. GDH activity was measured using the UV-test (Schmidt 1974).  $\text{Na}^+/\text{K}^+$ -ATPase activity was determined using the coupled UV-test (Penefsky and Bruist 1984). The detailed procedure and the composition of all solutions are described in Kültz et al. (1992). The reaction was started by the addition and rapid mixing of  $0.25 \mu\text{l}$  sample.  $\text{Na}^+/\text{K}^+$ -ATPase activity was calculated as the difference in the hydrolysis of ATP in the absence and presence of the specific  $\text{Na}^+/\text{K}^+$ -ATPase inhibitor ouabain ( $10^{-4} \text{ mol} \cdot \text{l}^{-1}$ , Merck). The method of Glock (1964) was used for the determination of G6PDH activity.

Samples were stored no longer than 3 weeks before colorimetrically measuring the activity of CA (EC 4.2.1.1) (Maren 1960). Detailed procedures were described previously (Kültz et al. 1992). Total protein content was used to calculate the specific activities of all enzymes. To solvate membrane-bound proteins we slightly modified the method of Lowry et al. (1951) for the determination of total protein (Kültz et al. 1992).

**Statistics.** Statistical analysis of the data was carried out applying the *F*-test followed by Student's *t*-test unless the variances differed significantly, in which case the Welch test was used (Weber 1976). Differences were considered significant when  $P < 0.05$ . All data in Materials and methods and Results sections are means  $\pm$  standard error of mean ( $\bar{x} \pm \text{SE}$ ) and  $n = 8$ .

## Results

The viability and the portion of MR cells and pavement cells in whole gill cell suspensions, as well as of bands A and C of the Percoll gradient, are shown in Table 1. The viability of all cell preparations (before and after Percoll centrifugation) was greater than 90%, indicating a good resistance of branchial cells to the disaggregation procedure. Mucous cells, although present in large numbers on gill filaments and secondary lamellae (Laurent and Hebibi 1989) could only occasionally be detected in cell suspensions, and considerable amounts of cellular debris were visible in all cell suspensions. Thus, not all types of gill epithelial cells seem to withstand this procedure.

**Table 1.** Viability and portion of MR cells and pavement cells in cell suspensions of the whole gill, and bands A and C of the Percoll gradient. Data represent means  $\pm$  SE ( $n = 8$ )

Salinity:	FW			HSW		
	Whole gill	Band A	Band C	Whole gill	Band A	Band C
Unstained (viable) cells (%)	$95 \pm 1.9$	$91 \pm 2.8$	$92 \pm 2.4$	$98 \pm 0.7$	$93 \pm 2.6$	$91 \pm 2.3$
Portion of MR cells (%)	$6 \pm 1.3$	<1	$45 \pm 5.8$	$16 \pm 2.4$	$3 \pm 0.6$	$74 \pm 5.0$
Portion of pavement cells (%)	$72 \pm 4.1$	$88 \pm 2.2$	$27 \pm 4.5$	$61 \pm 5.7$	$80 \pm 2.0$	$11 \pm 3.1$

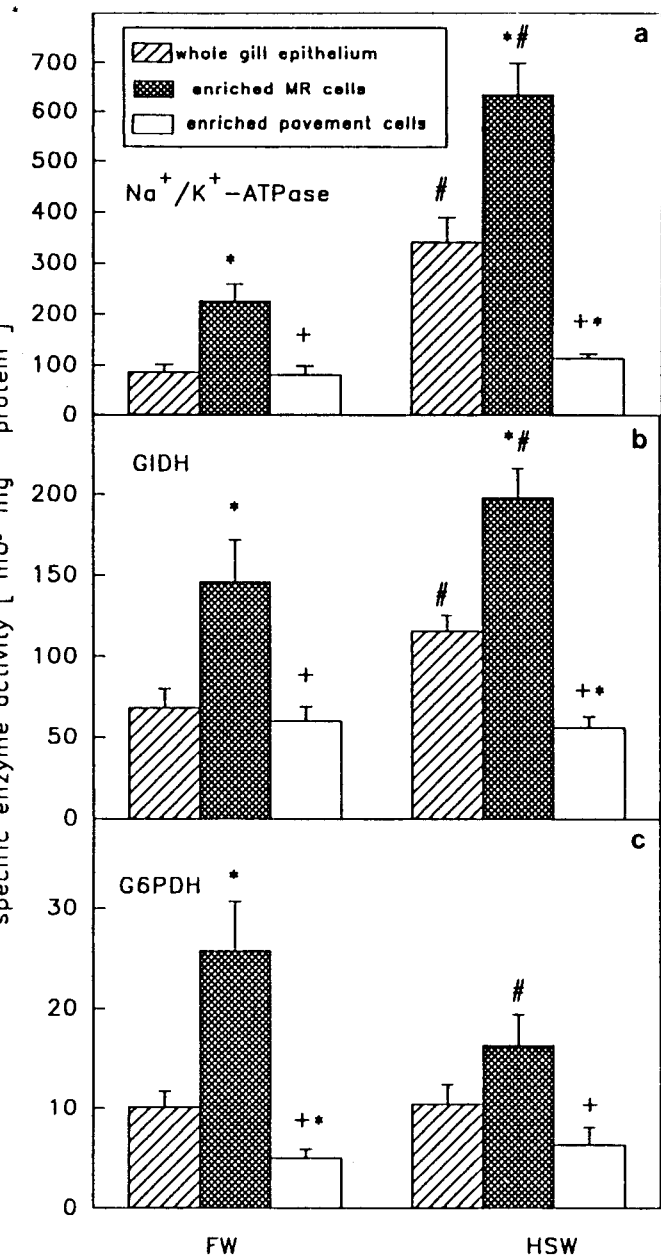


Fig. 3a-c. Specific activities of Na<sup>+</sup>/K<sup>+</sup>-ATPase (a), GIDH (b) and G6PDH (c) in whole gill cell suspensions, enriched MR cells and enriched pavement cells of tilapia acclimated to either FW or HSW; \* significantly different from data shown for whole gill cell suspensions within the same salinity; + significantly different from data for enriched MR cells within the same salinity; # significantly different between FW and HSW for the same cell suspension

Nevertheless, as shown in Table 1, an enrichment of pavement cells (88% in FW tilapia, 80% in HSW tilapia) in band A, and of MR cells (45% in FW tilapia, 74% in HSW tilapia) in band C of the Percoll gradient was achieved. The appearance of cell suspensions collected from band A and C of the Percoll gradient is shown in Fig. 2. MR cells were much larger in HSW fish ( $25.6 \pm 0.6 \mu\text{m}$ ,  $n=8$ ) compared to FW fish ( $12.0 \pm 0.3 \mu\text{m}$ ,  $n=8$ ). This acclimatory response is common in teleosts during adaptation to hyperosmotic environments (Karnaky 1986).

Suspensions enriched in either MR cells or pavement cells were used to determine the activity of enzymes which are important for both ionic transport and cell metabolism. Typical enzymatic properties of pavement cells and MR cells were considered to be represented by cell suspensions collected from bands A and C, respectively.

The activity of the Na<sup>+</sup>/K<sup>+</sup>-ATPase is nearly four-fold in whole gill cell suspensions and threefold in MR cell suspensions of tilapia acclimated to HSW compared to those which had been held in FW. In contrast, the activity of this enzyme is not significantly influenced by salinity in pavement cells (Fig. 3a). Both in tilapia acclimated to HSW and FW, Na<sup>+</sup>/K<sup>+</sup>-ATPase activity is significantly higher in MR cells compared to pavement cells. In the whole gill cell suspension from tilapia acclimated to HSW, the activity of this enzyme is significantly higher than in enriched pavement cells, but this is not so for tilapia held in FW (Fig. 3a).

With respect to either whole gill cell suspensions or enriched MR cells, GIDH activity is about 25-35% higher in tilapia acclimated to HSW compared to those held in FW. The activity of this enzyme in pavement cells was independent of acclimation S. In general, the distribution among cell types of GIDH within the gill resembled the pattern observed for Na<sup>+</sup>/K<sup>+</sup>-ATPase (Fig. 3b). MR cells possessed the highest amount of this enzyme. Regardless of S, GIDH activity in MR cells was significantly higher than in whole gill cell suspensions or pavement cells. The activity of GIDH in pavement cells was significantly lower than that found in whole gill cell suspensions only in tilapia acclimated to HSW.

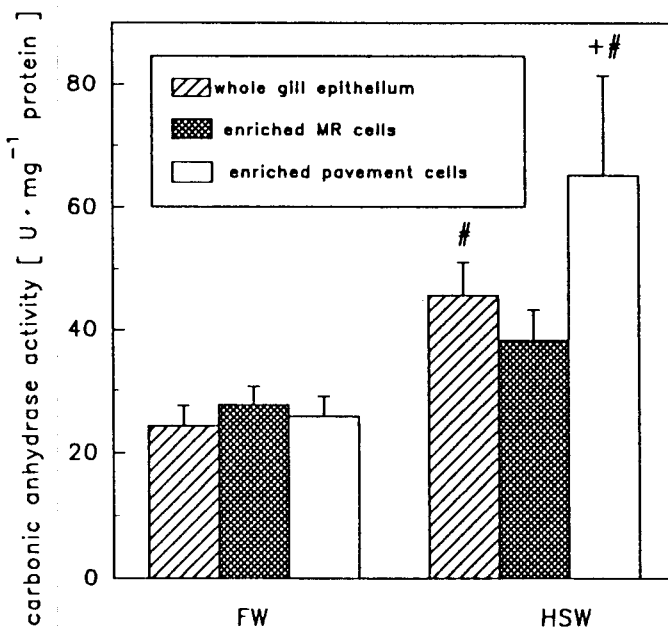


Fig. 4. Specific activity of carbonic anhydrase in whole gill cell suspensions, enriched MR cells and enriched pavement cells of tilapia acclimated to either FW or HSW; \* significantly different from data shown for whole gill cell suspensions within the same salinity; + significantly different from data for enriched MR cells within the same salinity; # significantly different between FW and HSW for the same cell suspensions

The distribution and activity among cell types of G6PDH surprisingly did not vary substantially between FW or HSW acclimated tilapia (Fig. 3c). Compared to the activity of  $\text{Na}^+/\text{K}^+$ -ATPase and GIDH, the activity of this enzyme is very low in the gills. However, our results indicate that most of the enzyme is located within the MR cells. No significant variation of G6PDH activity was detectable between tilapia acclimated to either FW or HSW.

The activity of CA in whole gill cell suspensions, MR cells and pavement cells of tilapia acclimated to FW or HSW are shown in Fig. 4. Since the term "unit" used for the presentation of specific CA activity as defined by Maren (1960) is not identical to  $1/60 \mu\text{Kat}$ , the CA activities shown are not directly comparable to the values determined for the other enzymes. Nevertheless, evaluation of the data reveals that the enzyme is present in substantial amounts in both MR cells and pavement cells. Only in tilapia acclimated to HSW is CA activity significantly higher in pavement cells than in MR cells. When acclimated to FW, no differences in CA activity were found between these two types of gill epithelial cells. The higher values found for tilapia acclimated to HSW compared to those held in FW can be attributed mainly to nearly twofold higher activities in pavement cells.

## Discussion

### Methodology

Kamiya (1972) first separated MR cells from other epithelial cells of the teleost (*Anquilla japonica*) gill using a discontinuous dextran gradient. This method was later improved by other groups by using Ficoll or sucrose gradients and expanded to include other teleost species (Sargent et al. 1975; Hootman and Philpott 1978; Naon and Mayer-Gostan 1983; Battram et al. 1989). However, using these methods, the yield and the condition of MR cells are insufficient to permit metabolic experiments, and cytosolic enzymes may be lost into the medium via damaged plasma membranes (Perry and Walsh 1989). Perry and Walsh (1989) used a continuous Percoll gradient and obtained metabolically viable cells but were unable to achieve the degree of enrichment found earlier.

We further modified the separation procedure for tilapia gills using a discontinuous gradient of Percoll and were able to obtain a high percentage of viable cells (>90%). The degree of enrichment of MR cells, which only cover a small percentage of the branchial epithelium, was comparable to the studies mentioned above (Sargent et al. 1975; Naon and Mayer-Gostan 1983). An important advantage of using Percoll as the separation medium is the very low osmotic activity of these particles (Sharpe 1988). Indeed, our preliminary studies using other media, including Ficoll and sucrose, have shown that the highest specific enzyme activities were found after centrifugation in Percoll (unpublished observations).

Since all procedures during cell separation were performed at 4 °C and all cell suspensions were treated

identically, potential loss of enzymatic activity during cell separation and the freeze-thaw procedure should be comparable. However, suspensions of whole gill epithelial cells used for the determination of enzyme activities and viability were not centrifuged in a Percoll gradient. This might explain the higher percentage of viability of these cells compared to enriched MR cells or pavement cells. Also, it is rather unlikely that different cell types respond identically to the treatment during separation. For this reason the experiments were designed to minimize the separation time. The entire separation procedure can be completed in 2 h.

### Enzyme activities

The activities of the  $\text{Na}^+/\text{K}^+$ -ATPase and GIDH have been studied in isolated MR cells of various teleosts acclimated to FW or euhaline SW (DeRenzis and Bornancin 1984). However, results on the activities of these enzymes in isolated MR cells from teleosts acclimated to HSW have not been published previously. GIDH activity is predominantly located in the mitochondria, and therefore represents a mitochondrial marker, whereas the  $\text{Na}^+/\text{K}^+$ -ATPase has been shown to be located at the basolateral plasma membrane of MR cells using both radioactive labelled ouabain (Karnaky et al. 1976) and histochemical methods (Hootman and Philpott 1979).

The activities of both enzymes have been reported to be significantly higher in MR cells compared to other branchial cells in *Anquilla japonica* (Kamiya 1972), *Anquilla anquilla* (Sargent et al. 1975; Naon and Mayer-Gostan 1983), *Lagodon rhomboides* (Hootman and Philpott 1978) and *Salmo salar* (Langdon and Thorpe 1984), regardless of the salinity these fish were acclimated to. Our results on *Oreochromis mossambicus* are consistent with these findings. The predominant localization of  $\text{Na}^+/\text{K}^+$ -ATPase in MR cells is also indicated by comparable responses of  $\text{Na}^+/\text{K}^+$ -ATPase activity and MR cell number to a variety of influences, e.g. salinity change of the environment (Utida et al. 1971; Thomson and Sargent 1977), salt feeding (Salman and Eddy 1987) or starvation (Kültz and Jürss 1991).

Our results show that the specific activities of  $\text{Na}^+/\text{K}^+$ -ATPase and GIDH are significantly higher in MR cells of tilapia acclimated to HSW compared to those held in FW. This can be attributed at least in part to the lower degree of enrichment of these cells in homogenates from FW fish (45% compared to 74% in HSW fish). Since MR cells are much more frequent in tilapia acclimated to HSW than in those held in FW (Foskett et al. 1981; Kültz and Jürss 1991) it was not possible to achieve comparable degrees of enrichment of MR cells. Therefore, the lower activities of  $\text{Na}^+/\text{K}^+$ -ATPase and GIDH in MR cells from FW- versus HSW-acclimated tilapia do not represent evidence for a lower level of enzymic activity per MR cell in FW tilapia. It is also possible that higher specific activities of these enzymes in whole gill cell suspensions are due to a remarkable increase in cell number only. The degree of enrichment of MR cells is even more significant when the volume rather

than the number of these cells is considered. MR cells are significantly larger in tilapia acclimated to HSW than in those acclimated to FW (Kültz et al. 1992).

Nevertheless, a higher density of mitochondria within MR cells was found in *Rivulus marmoratus* acclimated to 200‰ compared to 1‰ SW (King et al. 1989). Furthermore, the autoradiographic findings of Karnaky et al. (1976) indicate a higher density of  $\text{Na}^+/\text{K}^+$ -ATPase in the basolateral membrane of MR cells of *Fundulus heteroclitus* acclimated to SW compared to FW fish.

In contrast to MR cells, the degree of enrichment of pavement cells is comparable in tilapia acclimated to either FW or HSW. In these cells, the activity levels of  $\text{Na}^+/\text{K}^+$ -ATPase, GIDH and G6PDH are not influenced significantly by salinity. Furthermore, the activity of these three enzymes is independent of the acclimation salinity and is significantly lower in pavement cells than in MR cells.

It is not surprising that the activity of GIDH is significantly higher in MR cells than in pavement cells since this enzyme is located primarily in the mitochondria, which MR cells are richly endowed with (evidenced by DASP-MI staining). High GIDH activities are indicative of high capacities of aerobic metabolism. A high capacity of aerobic metabolism is necessary for active ion transport, and therefore MR cells of the gills likely play a key role in salt transport in tilapia acclimated to both FW and SW environments. Pavement cells may contribute in part to NaCl absorption and utilize an electrochemical gradient built up in MR cells if these two cell types are electrostatically coupled as postulated by Goss et al. (1992). Besides the  $\text{Na}^+/\text{K}^+$ -ATPase, a proton pump, which may be located in either MR cells or pavement cells, is thought to energize active uptake of  $\text{Na}^+$  (Balm et al. 1988; Avella and Bornancin 1989; Lin and Randall 1991).

As far as is known the activities of CA and G6PDH have not been determined previously in isolated MR cells of teleost gills. However, CA activity has been shown to increase with increasing salinity in whole gill homogenates of *Anguilla anguilla* [Istin, cited in Maetz and Bornancin (1975)], *Salmo salar* (Dimberg et al. 1981) and *Oreochromis mossambicus* (Kültz et al. 1992). This increase can be attributed to an enhancement of enzyme activity in the pavement cells. CA is believed to participate in all of the four main functions of the gill. Besides influencing the partial pressure of  $\text{CO}_2$ , it could also facilitate the uptake of  $\text{Na}^+$  and  $\text{Cl}^-$  via apical antiporters by providing counterions for this process [ $\text{H}^+/\text{NH}_4^+$  and  $\text{HCO}_3^-$ ; Kirschner (1991)]. Since pavement cells have large contact areas exposed to the ambient medium they might be involved in acid-base regulation or ammonia excretion. Our results do not support a major participation of pavement cells in energizing active NaCl transport because they have lower aerobic capacities than MR cells and possess lower activities of  $\text{Na}^+/\text{K}^+$ -ATPase. One explanation for the higher activity of CA in pavement cells of gill from tilapia acclimated to HSW compared to FW could be an enhanced demand for acid-base regulation or ammonia excretion in high-salt adapted fish. Indeed, it was shown that in *Oncorhynchus mykiss* the degree of pH compensation increases in parallel to the salinity (Iwama and Heisler 1991). Nonetheless, the role of branchial CA in this process is not well understood.

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G6PDH, as a rate-limiting enzyme of the pentose phosphate pathway, has been shown to occur at rather high activities in the gills of some fishes (Mommensen 1984). The delivery of pentose phosphates for cellular RNA synthesis, a high rate of which would account for a high turnover of RNA and proteins, is controlled by this enzyme. Furthermore, reducing equivalents (NADPH) are produced during the reaction catalyzed by the G6PDH. These reduction equivalents are necessary for lipid synthesis, which clearly is needed at high rates in cells that contain a highly developed membranous system, such as the MR cells of teleost gills. The predominance of G6PDH in MR cells represents further evidence for the location of the major part of active ion transport within these cells. The significantly lower G6PDH activity in tilapia acclimated to HSW compared to those acclimated to FW is surprising. A possible explanation could be that in HSW more apoptosing MR cells (see Wendelaar Bonga and Van der Meij, 1990) with low G6PDH activities occur in tilapia gills. This assumption would suggest that the specific activities of  $\text{Na}^+/\text{K}^+$ -ATPase and GIDH in mature MR cells are even higher than measured in enriched MR cell suspensions from tilapia acclimated to HSW.

In conclusion, the results of this study prove that MR cells of gills are the main site of NaCl transport in tilapia acclimated to hyperosmotic HSW or hyposmotic FW.

*Acknowledgement.* This study was supported in part by a grant to DK of the German Academic Exchange Service (DAAD 5174025293)

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