Barrier function of teleost and elasmobranch gill apical membranes

Warren G. Hill[1,2], John C. Mathai[1,2], Rebekah H. Gensure[1,2], Joshua D. Zeidel[1], James P. Saenz[2], Evamaria Kinne-Saffran[2,3,4], Rolf Kinne[2,3] and Mark L. Zeidel[1,2].

[1]Laboratory of Epithelial Cell Biology, Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.
[2]Mount Desert Island Biological Laboratory, Salisbury Cove, ME 06472.
[3]MPI fur molekulare Physiologie, Dortmund, Germany
[4]Deceased on December 6th 2002

Teleosts and elasmobranchs deal with the severe osmotic challenge of life in seawater (950 mOsm/kg) in different ways. Teleosts maintain a tissue osmolality of approximately 340 mOsm/kg by continuous drinking and gut absorption of seawater coupled to excretion of excess salt via the gills and operculum. By contrast, elasmobranchs maintain tissue osmolalities close to 1000 mOsm/kg by keeping high tissue concentrations of urea, which therefore balance the osmolality of the seawater.

The gills enhance gas exchange by presenting a large surface area over which blood and seawater come into close contact. While effecting gas exchange, the gills must maintain a tight barrier to the efflux of water down its osmotic gradient in teleosts, and urea down its concentration gradient in elasmobranchs. Moreover, the gills are known to play an active role in ammonium/ammonia excretion. Prior measurements in intact animals and in freshly excised and perfused gill preparations have shown exceptionally low permeabilities to water and urea. If the major barrier to water and urea flux resides in the cell membrane, particularly the apical cell membrane, then isolated apical membrane vesicles would be expected to exhibit exceptionally low permeabilities. Analysis of the lipid structure of these membranes would yield important insights into how such low permeability membranes could be formed.

We isolated apical membranes vesicles from the gills of *Pleuronectus americanus* (winter flounder) and *Squalus acanthias* (dogfish shark) in the presence of 2 mM carboxyfluorescein using the method of Booth and Kenny[1]. Membranes demonstrated approximately 6-fold enrichment of the apical marker, ADPase compared to homogenate and no enrichment for the basolateral marker, Na,K-ATPase. We also isolated basolateral membranes from shark gill epithelium (enriched 2.3-fold for Na,K-ATPase compared to homogenate) and using stopped-flow fluorometry measured the membrane permeabilities to water, urea and NH_3 using well established techniques[4]. Apical membrane water permeabilities were similar between species and quite low ($7.4 \pm 0.7 \times 10^{-4}$ cm/s and $6.6 \pm 0.8 \times 10^{-4}$ cm/s for shark and flounder respectively), while shark basolateral membranes showed 2-fold higher water permeability ($14 \pm 2 \times 10^{-4}$ cm/s). Permeabilities to urea and NH_3 were also low in apical membranes (for flounder and shark respectively; urea permeability averaged $5.9 \pm 0.5 \times 10^{-7}$ cm/sec and $4.3 \pm 1.7 \times 10^{-7}$ cm/sec, while ammonia permeabilities averaged $1.9 \pm 0.3 \times 10^{-2}$ cm/sec and $1.4 \pm 0.1 \times 10^{-2}$ cm/sec).

Because flux of water through aquaporin water channels has a low activation energy we measured and calculated the activation energies for water flux across each preparation. The activation energies for flounder and shark apical membranes and shark gill basolateral membranes were 12.0, 9.2 and 14.2 kcal/mol respectively, well above the values of 2 – 4 kCal/mole reported for water channel containing membranes.

Due to the much lower apical surface area compared to basolateral membrane surface area (which is highly infolded) we conclude that the apical membrane represents a somewhat effective barrier to water and urea. However, the values we obtained were not low enough to account for low water loss (in teleost) and urea loss (in elasmobranch) measured *in vivo* by others. Prior measurements of gill water permeability *in vivo* and in intact *ex vivo* preparations have yielded apparent permeability values far lower than ours. Measurements in rainbow trout conditioned to fresh water averaged 1.56×10^{-5} cm/sec, while those for freshwater-adapted eel, averaged 1.46×10^{-5} cm/sec[5]. Measurements of dogfish gill place the apparent water permeability at $6.6 - 7.6 \times 10^{-6}$ cm/sec, values about half of those observed in the teleost preparations[2,3]. These values are ~100 times lower than the values we obtained in freshly isolated vesicle preparations.

In conclusion, we have measured the water, urea and ammonia permeabilities of apical membranes from the gill epithelia of a teleost and an elasmobranch as well as those of the basolateral membrane of the elasmobranch. The apical membrane clearly serves as the best barrier membrane in the epithelium. Although the apical membrane permeabilities we have measured place the gill apical membranes well into the range of other barrier apical membranes that have been studied, the permeabilities are not low enough to account for gill barrier function *in vivo*. It appears highly likely that ventilation/perfusion mismatches, diffusion barriers such as the mucin layer and regulation of chemical gradients across the apical membrane play a prominent role, along with the barrier apical membrane in determining the efficacy of the gill permeability barrier. We conclude that in addition to the low permeability of the lipid bilayer there are other mechanisms utilized by both species to permit gill epithelia to serve as effective barriers to equilibration between seawater and plasma.

Supported by NIH grant DK43955 and an MDIBL new investigator award (MLZ).

1 Booth AG, and Kenny AJ. A rapid method for the preparation of microvilli from rabbit kidney. *Biochem J* 142: 575-581, 1974.

2 Fines GA, Ballantyne JS, and Wright PA. Active urea transport and an unusual basolateral membrane composition in the gills of a marine elasmobranch. *Am J Physiol* 280: R16-R24, 2001.

3 Part P, Wright PA, and Wood CM. Urea and water permeability in dogfish (*Squalus acanthias*) gills. *Comp Biochem Physiol* 119: 117-123, 1998.

4 Rivers R, Blanchard A, Eladari D, Leviel F, Paillard M, Podevin RA, and Zeidel ML. Water and solute permeabilities of medullary thick ascending limb apical and basolateral membranes. *Am J Physiol* 274: F453-F462, 1998.

5 Steen JB, and Stray-Pedersen S. The permeability of fish gills with comments on the osmotic behavior of cellular membranes. *Acta Physiol Scand* 95: 6-20, 1975.