TISSUE DISTRIBUTION OF A NOVEL AQUAPORIN IN A TELEOST FISH (*Sparus auratus*)

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Introduction

The aquaporin family consists of several transmembrane channel proteins which function as channels for non-ionic compounds. In vertebrates two principal groups have been identified, the CHIP (CHannel forming Integral Protein) and GLP (GLycerol intrinsic Protein) proteins (2,4,6,7). Members of the CHIP cluster function exclusively as water channels (aquaporins), while members of the GLP group are considered to be primarily glycerol transport proteins (aquaglyceroporins) but are also permeable to water and other small solutes.

Relatively few reports about AQP proteins in non-mammalian vertebrates exist. In fish a CHIP protein (AQP-0) has been identified *Fundulus heteroclitus* (8) and recently an AQP-3 was reported in eel, *Anguilla anguilla* (1) and *Danio rerio* (Ac.No.BC044188).

Fish are an interesting group in which to study aquaporin as they inhabit diverse aquatic environments and face a constant osmotic and ionic challenge which varies according to the water they inhabit (fresh water <0.1 mOsm/kg; seawater aprox. 1000 mOsm/kg).

A sea bream AQP cDNA encoding a protein of 298 amino acids was isolated from a kidney cDNA library. Functional studies showed that this protein is permeable to water and also to glycerol and urea, placing it in the GLP group.

Comparison of sea bream AQP with other GLP members, including the recently isolated AQP-10 (3,5) indicated it was most similar to human AQP-3 and AQP-10, 49 and 48% identity, respectively.

The aim of this work was to characterise the tissue and cellular distribution of the sea bream aquaglyceroporin (sbAQP) in this marine teleost.

Methods (I)

1) Animals and tissues

Adult sea bream (≈ 350 g); in seawater, at 17 ± 2ºC; winter (Algarve)

Tissues: liver, kidney, GIT, gills, head kidney and gonads

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Fixed in 4% PFA, 4ºC, o/n

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Dehydrated and embedded in wax

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serial sections (8 μm) mounted in poly-L-lysine coated slides

2) Riboprobe synthesis

Sea bream AQP cDNA

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cloned in pBluescript vector

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Digestion with BamHI

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Purified linearised vector

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In vitro Transcription (T7 RNA Polymerase)

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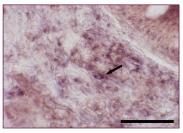
Antisense probe labeled with Digoxigenin

Methods (II)

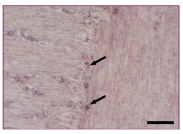
3) *In situ* Hybridisation

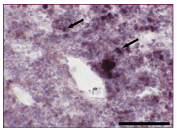
- Sections dewaxed and rehydrated
- Pre-hybridisation - 54ºC, 4 hours
- Hybridisation - 54ºC, o/n, 15μL probe/mL of Hyb mix
- High stringency washes - 2xSSC, 54ºC, 3 x 5'
- Probe detection - Anti-digoxigenin-AP Fab Fragments
- Color development - NBT/BCIP; 30ºC, 2-17 hours
- Sections analysed using a microscope coupled to a digital camera

Tissue distribution of sbAQP

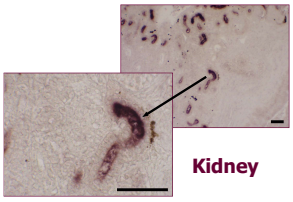


Hind gut

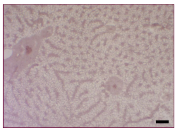




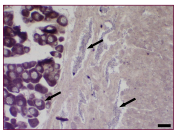
Head kidney



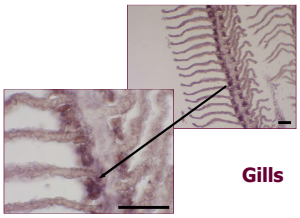
Kidney



Liver



Gonad

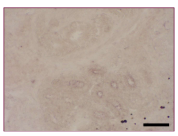


Gills

Expression of the sbAQP mRNA is very intense in hind gut, kidney, head kidney, gills and gonad. No signal was detected in the liver.

Sections of kidney were used as negative controls. In these sections *in situ hybridisation* was done without the AQP riboprobe or applying the riboprobe after a RNase treatment of the tissue. No signal was detected in these sections.

Size bars in the photos corresponde to 50 μm.



Kidney - Negative control

Results and conclusions

Results showed the following cellular distribution of sea bream aquaporin in tissues:

Tissue	Localization	Intensity
Hind gut	*Lamina propria* and interface of muscle layers	Intense
Mid gut	Similar to hind gut	Weak
Duodenum	*Serosa*	Very weak
Kidney Head Kidney	Tubules Sporadic cells	Intense Intense
Gills	Primary filaments (Chloride cells)	Intense
Gonad Ovary Testis	Larger oocytes Head of sperm Sporadic cells	Intense Intense
Liver	-	No signal

We can conclude that the tissue distribution in the gut and kidney suggests that the sea bream aquaporin may be involved in water balance at these sites.

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