DIFFERENTIAL EXPRESSION OF VITELLOGENIN GENES (VGA AND VGB) BY HEPATOCYTES OF THE INDIAN FRESHWATER MURREL, *CHANNA PUNCTATUS*, ON EXPOSURE TO ESTRADIOL

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Introduction:

The egg-yolk of fishes is derived from a blood-borne, female-specific precursor, vitellogenin (Vg), which is synthesized in the liver under estrogenic influence during periods of reproductive activity. This protein is modified in the liver, secreted into blood and transported to the ovary where it is incorporated by the growing oocytes via specific receptor-mediated endocytosis. More than one form of vitellogenin (VgA, VgB and VgC) has been reported in teleosts. We have reported earlier that murrel Vg exists as three charge isomers in the blood [1]. In the present study we have shown the existence of VgA and VgB in this fish comparable to other teleosts and confirm the presence of both the proteins at gene level. Further VgA and VgB genes are expressed in liver when fishes are exposed to estrogen under *in vivo* condition whereas only Vg B is expressed by cultured hepatocytes.

Methods:

Fish were injected with E_2, plasma was collected in the presence of proteolytic inhibitors and processed for isolating Vg by gel filtration chromatography on Ultrogel AcA-34 followed by FPLC on a strong anion exchanger (UNO Q). One of the FPLC purified Vg isomer was subjected to LCMS/MS for molecular identification. Peptide sequences were matched with fish Vg sequences database. Amino acid sequences and nucleotide sequences were aligned using Clustal W software. cDNA was amplified using primers for VgA and VgB, and the amplicons were sequenced which were subjected to bioinformatical analysis. Estradiol-treated fish were bled and Vg was estimated by ELISA; total RNA was extracted from liver, gill, kidney and ovary, and processed for amplification of Vg gene by RT-PCR. Hepatocytes were isolated and cultured. Monolayer of cultured hepatocytes was exposed to estradiol; Vg was quantified in the culture medium, total RNA was extracted from all the treated hepatocytes and processed for amplification of Vg gene.

Results and Discussion:

In order to assess the purity of Vg and ti isolate charge isomers of Vg fractionated by gel filtration chromatography on Ultrogel AcA 34, specific fractions were further subjected to FPLC on a strong anion exchanger, which separated the proteins on charge differences. The Vg fraction resolved into two distinct peaks eluting at 0.3 M and 0.32 M NaCl suggesting that variants of vitellogenin have different charge composition. The fraction eluting at 0.3 M NaCl was electrophoresed under denaturing conditions, digested with trypsin and processed for LC-MS/MS. The sequences of peptide fragments of 'β' isomer of Vg matched significantly with amino acid sequences of VgB and Vg precursor derived from Melanogrammus aegelefinus, Oryzias latipes, Cyprinus carpio, Oreochromis aureus, Oncorhynchus mykiss, Pimphales promelas, Sillago japonica and Fundulus heteroclitus. The presence of VgA was also indicated with peptide fragment of VgA from *Melanogrammus aegelefinus, Oncorhynchus mykiss* and *Sillago japonica*. VgA derived yolk proteins are cleaved into free amino acids and play distinct roles with respect to oocyte maturation and embryonic nutrition. Free amino acids appear to increase the buoyancy and support early embryonic development whereas a major part of VgB remained as a large peptide and is probably used as an important nutrient source at later stages of embryonic development [2]. *Channa punctatus* lays pelagic eggs the precondition for which is the presence of VgA.

First strand cDNA was synthesized by reverse transcriptase. Primers were designed from the aligned sequences for VgA and VgB (Table 1). These primers were used to amplify single strand cDNA (Fig. 1) to study expression patterns of VgA and VgB genes when murrel hepatocytes were exposed to estradiol in vivo or in vitro conditions. This study suggests that E_2 up-regulates levels of Vg in plasma or in culture medium in a dose dependent manner. The VgA and VgB mRNA levels in the liver of E_2 treated murrel was analyzed by RT-PCR showed a

Table 1: Primer sequences for amplification of Vitellogenin A and Vitellogenin B

mRNA	Primer	Primer sequence
VgA	Forward primer	5' TGAGGAACATTGCAAAGAAGG 3'
	Reverse primer	5' ATTCCCTCAGTTCTCACTCC 3'
VgB	Forward primer	5' AGTGAGAACAGAGGGAATCC 3'
	Reverse primer	5' TGTTCACGCCCATGACAGC 3'
	Forward primer	5' ATGTCCAAGCTACTGTGACG 3'
	Reverse primer	5' TCGCCAACTTGAATCTCAACC 3'

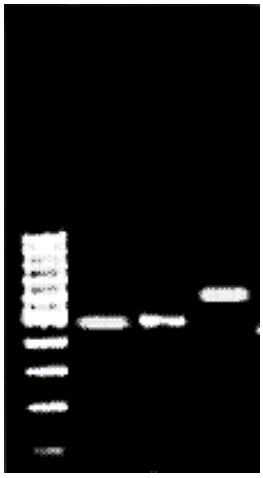

Fig. 1: Agarose (1%) gel electrophoresis of purified PCR products of FA1RA1 primers for Vg A, FB1RB1 and FB2RB2 primers for Vg B.

Lane 1: DNA ruler

Lane 2: FA1RA1 PCR product

Lane 3: FB1RB1 PCR product

Lane 4: FB2RB2 PCR product

pattern of expression which was also dose dependent. Cultured hepatocytes expresses only VgB gene.

Conclusion:

These results suggest that most of the peptide sequences of purified isomer of murrel matches with VgB while a few sequences also match with VgA of fishes. We conclude that both VgA and VgB genes are present in the genome of *Channa punctatus*. The investigation also suggests that *in vitro* system cannot be entirely translated into *in vivo* system.

References:

[1]SEHGAL, N., GOSWAMI S.V. 2005. Vitellogenin exists as charge isomers in the Indian freshwater murrel, *Channa punctatus* (Bloch). Gen. Comp. Endocrinol., 141: 12-21.

[2]HIRAMATSU, N., MATSUBARA, T., FUJITA, T., SULLIVAN, C.V., HARA, A. 2006. Multiple piscine vitellogenins: Biomarkers of fish exposure to estrogenic endocrine disruptors in aquatic environments. Mar. Biol., 149: 35-47.