



Identification and partial characterization of *Olyra longicaudata* (McClelland, 1842) vitellogenins: Seasonal variation in plasma, relative to estradiol-17 β and ovarian growth

Pritha Ghosh^a, Debabrata Das^a, Subir K. Juin^a, Sudip Hajra^a, Akash Kachari^b,
Debangshu N. Das^b, Panchanan Nath^a, Sudipta Maitra^{a,*}

^a Department of Zoology, Visva-Bharati University, Santiniketan, India

^b Department of Zoology, Rajiv Gandhi University, Itanagar, India

ARTICLE INFO

Article history:

Received 9 July 2015

Received in revised form 5 January 2016

Accepted 17 January 2016

Available online 29 January 2016

Keywords:

Vitellogenin

Estradiol-17 β

ELISA

Ovary

Catfish

Olyra longicaudata

ABSTRACT

This study aims at immunochemical characterization of plasma vitellogenin (VTG), development of an heterologous VTG ELISA and to relate seasonal variation in plasma VTG and estradiol-17 β (E2) levels with ovarian growth (gonadosomatic index, GSI) in *Olyra longicaudata* (McClelland, 1842), a rare hill-stream catfish endemic to North East India. On native PAGE, plasma from E2-injected male, vitellogenic as well as gravid females, but not untreated male, resolved into two major protein bands. These two proteins stained positive for carbohydrate, lipid and phosphorous, albeit with differential intensity and cross-reacted well with catfish VTG antiserum (a-VTG) suggesting them as putative VTGs in circulation. Ammonium sulphate (50%) fractionation followed by SDS-PAGE analysis of E2-treated male plasma resolved into four protein bands (150–15 kDa), of which two, with molecular mass of 150 and 130 kDa cross-reacted with a-VTG indicating them as VTG monomers. Immunoprecipitation of E2-induced plasma and immunoblot analysis of crude yolk proteins with a-VTG revealed two proteins in each case indicating two forms of VTG, present in circulation, possibly act as yolk precursors. Competitive antigen-capture ELISA developed earlier for catfish, *Clarias batrachus* VTG (CF-VTG1), revealed parallel binding slopes between dilution curves of plasma from vitellogenic female, E2-treated male and CF-VTG1 standard. Congruent with gradual increase in plasma E2, ovarian weight and appearance of vitellogenic and yolky oocytes, VTG level in circulation increased sharply in May–June, reaching the peak value in July, dropped sharply during August–September and was undetected or negligible in amount during December allowing identification of the ripening, the pre-spawning, the spawning and the quiescent phases respectively.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Olyra longicaudata, McClelland, 1842 (Teleostei: Bagridae: Olyrininae), also known as Asian fighting catfish or banner tail catfish, is endemic to torrential hill streams of Brahmaputra basin in the North East India, Northern Myanmar and Thailand (Talwar and Jhingran, 1991). Distinguished by small size (110 mm standard length), anguilliform body and greatly enlarged upper lobe of the forked caudal fin, this bagrid catfish (Actinopterygii: Siluriformes) prefers fast water current and a place for hiding in the substratum of rocks and sand. While their restricted occurrences in nature

indicate fluctuating reproductive fitness even in the wild, this fish usually spawns during the monsoon months (August–September) when the rainfall is heaviest. However, mature specimens of *O. longicaudata*, particularly the females, rarely attain sexual maturity in captivity. Though popular as an aquarium species, currently no information is available on vitellogenesis or any other reproductive parameters of this hill stream catfish.

In fishes, as in other oviparous vertebrates, the yolk precursor protein, vitellogenin (VTG), is synthesized in the liver under the influence of gonadotropin-induced ovarian estrogen, primarily estradiol-17 β (E2) and is transported via blood to the ovary. Following its uptake through receptor-mediated endocytosis, VTG undergoes limited proteolytic cleavage to form lipovitellin (Lv), phosvitin (Pv), β' -component and C-terminal peptide which ultimately get deposited in the form of yolk granules or platelets in the growing oocytes (Babin et al., 2007; Hiramatsu et al., 2002a;

* Corresponding author. +91 3463261176.

E-mail addresses: sudipta.maitra@visva-bharati.ac.in, smaitra3@gmail.com (S. Maitra).

Matsubara et al., 1999; Mommsen and Walsh, 1988; Sawaguchi et al., 2006; Specker and Sullivan, 1994). Though initially one form of VTG was identified and characterized from the plasma or serum of various teleost fishes; later on two and even three forms of VTGs have been purified rejecting the earlier concept of single VTG model and posing new challenge to explore the functions of individual VTG and their yolk protein derivatives (Ding et al., 1989; Hiramatsu et al., 2002b; Wang et al., 2000). Further, compared to their amphibian and avian counterparts, teleost VTGs display higher variability in their physical and chemical properties such as molecular weight, subunit composition and degree of phosphorylation, lipidation and glycosylation (Mommsen and Walsh, 1988; Specker and Sullivan, 1994).

VTG is usually considered as female-specific protein, largely absent in juveniles and induced by E2 administration in both males and females; however, VTG-like proteins, albeit in low quantity, have been identified in males of few fish (Ding et al., 1989; Kishida and Specker, 1993). Nonetheless, circulatory level of Vg in females undergoes rapid increase during the vitellogenesis and correlates well with gonadal recrudescence and oocyte growth and is generally agreed to be a good indicator of the reproductive fitness and maturity status of the adult females, a useful marker of exposure to endocrine disrupting compounds (EDCs) and for determining the sex in wild and captive fish populations prior to sexual maturity (Bon et al., 1997; Cheek et al., 2004; Heppell et al., 1999; Nunez Rodriguez et al., 1997; Sumpter and Jobling, 1995). Earlier sensitive immunoassays such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), single radial immunodiffusion (SRID) and chemiluminescent immunoassays (CLIA) have been developed for quantification of VTG in the plasma or serum of several teleost species (Babin et al., 2007; Braathen et al., 2009; Nath and Maitra, 2001; Maitra et al., 2007; Takemura and Kim, 2001). Moreover, piscine VTGs from closely related species have been shown to cross-react with heterologous antibodies (Maltais and Roy, 2009; Ndiaye et al., 2006; Watts et al., 2003).

In the Indian sub-continent, a vast majority of the fresh water fishes are seasonal breeders and majority of the catfish species breed during the monsoon season when rainfall is heaviest (Sundararaj and Nath, 1981; Nath and Maitra, 2001; Nath et al., 2007). Currently no information is available on vitellogenesis in *O. longicaudata* and VTG has never been characterized in this species. The major objectives of the present study are partial identification and characterization of VTGs through immunological and biochemical techniques, development and use of a heterologous ELISA to measure VTG in circulation and to find correlation between seasonal variation in plasma VTG, sex steroid (E2) and ovarian growth during vitellogenic months in this rare catfish species.

2. Materials and methods

2.1. Collection and care of fish

Adult specimens of *O. longicaudata* [Standard length (SL): 7.63–9.68 cm] were collected from rivers and streams in the village of Denka (Lat. 27°11' 38.6"N, Long. 93°52'38.7"E), district Papum Pare, Doimukh, India following all indigenous and scientific methods available for catching hill stream fishes (Kachari et al., 2014). Adult males ($n=41$) were transported to the laboratory, maintained under ambient photoperiod and temperature in glass aquaria (capacity: 60 L) with a layer of pebbles (2.5 cm thick). Water (pH 6.0–7.0, temperature $23 \pm 2^\circ\text{C}$) in the tanks was circulated continuously with the help of motor pumps. Fishes were fed *ad libitum* with live blood worms and were acclimatized for at least 7 days prior to their use in experiments.

2.2. E2 induction and collection of plasma samples

All animal experiments were carried out following the approval of Institutional Animal Ethics Committee of Visva-Bharati University. Estradiol-17 β , E2 (Sigma, India) was dissolved in ethanol (stock: 2 mg/ml), diluted with fish saline (0.64% NaCl), mixed thoroughly and the resulting suspension was injected intra-muscularly (im) at the caudal peduncle at the dose level of 50 $\mu\text{g}/100\text{ g}$ body weight, on alternate days for 10 days. Control fish received equal volume ($\sim 25\text{ }\mu\text{l}$) of vehicle only. The day following the last injection, fishes were bled by caudal puncture, blood was collected in heparinized (130 IU/ml blood) tubes (kept on ice) containing protease inhibitor cocktail (P2714, Sigma, India). Plasma was separated by spinning at $1500 \times g$ for 20 min at 4°C , diluted 1:1 with PBS-glycerol (phosphate buffer 10 mM, pH 7.6, NaCl 0.9% glycerol 50%) and was stored in 50 μl aliquots at -80°C for future analysis.

Additionally, a group of five females, captured in their natural habitat during the middle of every month, were anesthetized by immersion in water containing 100 mg tricaine-methane-sulfonate (MS-222, Sigma Chemical Co., India) per liter buffered with sodium bicarbonate (1:1), total length taken (in cm), weighed to the nearest mg, bled by caudal puncture with the help of heparinised tuberculin syringes into tubes containing protease inhibitor cocktail and plasma separated as described above.

2.3. Identification and characterization of VTG in plasma

2.3.1. Native-PAGE

Protein was estimated by Lowry et al. (1951) using bovine serum albumin (BSA) as standard. Plasma samples (50 μg protein/lane) from E2-treated male, untreated male as well as from females in March (non-vitellogenic, resting phase), May (vitellogenic) and July (gravid) were resolved through native PAGE (stacking 4%, resolving 10%) at 4°C using electrophoresis buffer (25 mM Tris, 192 mM glycine, pH 8.3). Gels were stained for protein with Coomassie brilliant blue R-250 (CBB R250), phosphorous (Hegenauer et al., 1977), lipid (Prat et al., 1969) and carbohydrate (Zacharias et al., 1969). Standard high molecular weight marker proteins (Cat. No. 17-0445-01; GE Healthcare), viz. thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), lactate dehydrogenase (140 kDa) and albumin (66 kDa) were run in parallel wells.

2.3.2. Ammonium sulphate fractionation and SDS-PAGE analysis

Pooled plasma from five E2-treated male *O. longicaudata* were subjected to 50% ammonium sulphate fractionation at 4°C overnight, precipitate obtained by spinning at $17,500 \times g$, for 10 min at 4°C , pellet re-suspended in Tris-Cl buffer (pH 8.0) containing 2% KCl, phenylmethylsulphonyl fluoride (PMSF) (0.05%) and aprotinin: 10,000 Kallikrein units/ml, dialyzed extensively against chilled PBS, concentrated in vacuum and protein was estimated (Lowry et al., 1951). Solubilised precipitate thus obtained was subjected to SDS-PAGE (stacking 4%, separating 15%) under reducing condition (Laemmli, 1970) along with pre-stained protein markers (Fermentas, #SM0671).

2.3.3. Immunoprecipitation and immunoblot analysis

To eliminate reactivity to other plasma proteins, anti-catfish Vg antiserum was adsorbed ($3\times$) with hypophysectomized male catfish serum (Hypox. CFS) and the adsorbed antiserum (a-VTG) were used subsequently according to Nath and Maitra (2001). For immunoprecipitation (IP), E2-treated male plasma (100 μg protein) was incubated with a-VTG (5 μl) overnight at 4°C . Around 50 μl of Protein A agarose (Pierce^(R), Thermo Scientific) was added and incubated with gentle agitation for 2 h at room temperature followed by addition of 500 μl of IP buffer (25 mM Tris, 150 mM NaCl; pH 7.2) and centrifugation at $2500 \times g$ for 3 min at 4°C . This

Download English Version:

<https://daneshyari.com/en/article/4438008>

Download Persian Version:

<https://daneshyari.com/article/4438008>

[Daneshyari.com](https://daneshyari.com)