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Circannual variation in plasma vitellogenin and gonadotropin II levels in relation to annual ovarian cycle in female mrigal, *Cirrhinus mrigala*

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Abstract

Two forms (HA I and HA II) of vitellogenin (Vg), the yolk-precursor protein, were purified from the plasma of estradiol-17β (E_2)-treated Indian major carp, mrigal (*Cirrhinus mrigala*), by gel filtration on Ultrogel AcA 34 followed by adsorption chromatography on Hydroxylapatite (HA)-Ultrogel. Native HA I and HA II had molecular weights of 500 kDa and 550 kDa, respectively. The apparent masses of purified HA I and HA II after SDS-PAGE under reducing condition were 75 kDa and 85 kDa, respectively. HA I was found to be lipid rich whereas HA II was phosphorous rich. The co-purified Vg containing both HA I and HA II was used to raise polyclonal antisera (a-Vg) and its specificity was assessed by Western blot analysis. In a double immunodiffusion test, the plasma of vitellogenic females and E_2-treated males as well as crude egg yolk protein (CYP), cross-reacted with a-Vg giving two precipitin lines in each case, thereby indicating the presence of two forms of Vg. HA I and HA II each gave single precipitin lines. No cross-reaction was observed with control male plasma. A competitive enzyme-linked immunosorbent assay (ELISA) was developed using a-Vg and HA I. The detection limit of the assay was 6.25 ng/ml, and the intra- and inter-assay variations were 4.48 and 7.57%, respectively. Displacement curves parallel to the standard (HA I) were obtained with plasma samples from vitellogenic female and E_2-treated male mrigal. The assay was validated by estimating Vg in plasma samples from adult female mrigal captured in the field throughout the year and compared with ovarian development. Annual profiles of plasma Vg and gonadotropin (GTH II: estimated by common carp GTH II ELISA) levels presented a good correlation with gonadosomatic index (GSI) and mean number of vitellogenic oocytes during different reproductive phases, i.e., preparatory (Feb–Apr), pre-spawning (May–Jun), spawning (Jul–Aug) and post-spawning (Sep–Jan).

Keywords: Vitellogenin; Gonadotropin; ELISA; Mrigal; *Cirrhinus mrigala*

1. Introduction

In fish, as in other oviparous vertebrates, the synthesis of the yolk-precursor protein vitellogenin (Vg) is the prerequisite for oocyte growth during oogenesis (vitellogenesis) and thus contributes vitally to egg quality and reproductive success. Pituitary gonadotropin (GTH) and

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ovarian steroid hormone (estradiol-17β, E_2) regulate vitellogenesis in fish (Nagahama, 2000). Two gonadotropins, GTH I (similar to follicle stimulating hormone, FSH) and GTH II (similar to luteinizing hormone, LH) have been purified from the pituitaries of many fish species (Swanson, 1991; Van Der Kraak et al., 1992; Tanaka et al., 1993). Studies made in salmonid fish revealed that GTH I is primarily involved in vitellogenesis and spermatogenesis whereas GTH II triggers maturation and ovulation and spermiation. However, both GTHs are equally potent in stimulating E_2 production (see Swanson et al., 1991; Gen et al., 2000). Van Der Kraak et al. (1992) reported that no clear functional differences exist between GTH I and GTH II of common carp and salmon when tested in both *in vitro* and *in vivo* bioassays (Suzuki et al., 1988a; Swanson et al., 1991). These findings suggest that both GTHs are involved in fish reproduction. In response to GTH-induced ovarian estrogen release, hepatocytes synthesize and release Vg into the bloodstream, from where it is taken up and incorporated into growing oocytes via receptor-mediated endocytosis (Sawaguchi et al., 2006 and references therein). Vg(s) undergo limited proteolytic cleavage to form lipovitellin (Lv), phosvitin (Pv) and β′-component (β′-c) prior to deposition as egg yolk proteins in the ooplasm (reviewed in Specker and Sullivan, 1994; Nath, 1999; Hiramatsu et al., 2002a). Vg is considered as a female specific protein but similar proteins have been identified in males of many fish species (Ding et al., 1989; Kishida and Specker, 1993). However, the exact function of Vg in male is yet to be established.

In teleosts, Vg is a high molecular weight (300–600 kDa) glycolipophosphoprotein that circulates as a dimer (Specker and Sullivan, 1994). Although the number of molecular forms of Vg is not confirmed, two forms have been identified in various teleost species including tilapia (Ding et al., 1989; Kishida and Specker, 1993; Buerano et al., 1995), mummichog (LaFleur et al., 1995a), zebra fish (Wang et al., 2000), rainbow trout (Trichet et al., 2000), haddock (Reith et al., 2001) and medaka (Shimizu et al., 2002) and three molecular forms in white perch (Hiramatsu et al., 2002b), mosquito fish (Sawaguchi et al., 2005) and red sea bream (Sawaguchi et al., 2006). The isolation, characterization and specific assay development for different forms of Vg have become more important to understand better the evolution of multiple piscine Vgs and their physiological significance in reproduction (Sawaguchi et al., 2005).

In female fish significant levels of Vg are present in circulation during vitellogenesis and Vg synthesis can be induced by E_2 in both males and immature females. Thus, measurement of plasma Vg is widely practiced to monitor the reproductive status of females. Sensitive assays like radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA) and single radial immunodiffusion have been adopted for quantification of fish Vgs, of which ELISA is believed to be the safest option for routine work (reviewed in Specker and Sullivan, 1994).

The Indian major carp, mrigal, *Cirrhinus mrigala*, is one of the most common carps in the Indian subcontinent and is a very important candidate for freshwater aquaculture. Previously we had reported the role of two different mrigal Vgs in the process of synthesis and incorporation of Vg in Indian catfish, *Clarias batrachus*, through a series of *in vivo* experiments (see Nath and Maitra, 2001). However, circannual variations in plasma Vg and GTH levels in relation to ovarian growth are lacking in this species. Therefore, the major objectives of the present investigation were (i) to purify and partially characterize Vg from the plasma of E_2-treated mrigal, (ii) development of an ELISA for mrigal Vg and (iii) to determine the circannual variations in plasma Vg and GTH II levels in relation to different phases of ovarian growth in female mrigal.

2. Materials and methods

2.1. Collection and care of fish

Sexually mature specimens of *C. mrigala* (body weight range: 0.5–0.75 kg) were collected around Santiniketan (Lat. 23°41′30″ N and Long. 87°30′47″ E) and maintained in the laboratory in cement tanks (240 cm × 150 cm × 120 cm) under natural photoperiod and temperature. Fish were fed *ad libitum* with laboratory-made fish food, containing rice bran, oil cake, fortified with vitamin C and B-complex and rabbit food pellets [Lipton, India]. Water in the tanks was replenished daily and was circulated with the help of motor pumps for aeration at regular time intervals. Fortified procaine penicillin (Allembic Chemical Works, Vadodora, India) was added to tank water (1:1000) occasionally as a prophylactic against skin infection. Fish were acclimated to laboratory conditions for 7 days prior to their use in experiments.

2.2. Preparation of mrigal Vg

2.2.1. Estradiol-17β (E_2) injection for vitellogenin synthesis

E_2 (Sigma, USA) was dissolved in ethanol (10 mg in 0.2 ml) and at the time of injection a suspension was