Influence of steroid hormones on plasma proteins in freshwater tilapia

*Oreochromis mossambicus*

Francis Sunny¹, K G Mohan², & Oommen V Oommen³*

¹Department of Zoology, University College, Trivandrum 695 034, India
²Department of Zoology, Christian College, Kattakada, Trivandrum 695 572, India
³Department of Zoology, University of Kerala, Kariavattom, Trivandrum 695 581, India

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The effect of administration of cortisol, corticosterone, testosterone, progesterone and a synthetic estrogen, diethylstilbestrol on plasma proteins of tilapia (*Oreochromis mossambicus*) was investigated. SDS-PAGE clearly revealed the appearance of several new bands of protein, which were not present in the control plasma and were comparable to the known bands of the molecular markers. Of the different bands appeared in the steroids treated plasma, the most important ones were the presumed vitellogenin and corticotrophin binding globulin with a molecular weight of 180 and 17 kDa, respectively. Increase in protein bands in the steroid treated plasma of *O. mossambicus* confirmed the anabolic role of steroids in teleost.

Measurement of plasma vitellogenin is a commonly used method to determine the effect of exposure to estrogen and estrogenic substances in fish¹⁻³. The importance of estrogen on vitellogenin synthesis in the liver of teleosts has been well documented⁴⁻⁶. Besides estrogen, vitellogenin production in fish is also induced by androgens, progesterone, cortisol, and estrogenic compounds like diethylstilbestrol (DES)⁷⁻¹⁰. Studies have shown that some steroid hormones produce protein anabolic effect¹¹. Therefore, in the present experiment an attempt has been made to confirm the protein anabolic effect of steroid hormones in tilapia, *Oreochromis mossambicus* by studying the changes in the plasma proteins using SDS-PAGE. It is assumed that the presence of protein in the blood of fish can serve as a useful tool for assessing protein synthesis following injection of specific hormones.

The tilapia obtained locally were kept in large cement tanks with continuously flowing dechlorinated water at 26±2°C under natural photoperiod for a month for acclimatization. Two weeks prior to experiment, adult healthy females of 50±2 g body weight were transferred to aerated aquarium tanks (24" x 12" x 12"), maintained under conditions identical to those of stock tanks. These fish were grouped in 6 aquarium tanks containing 4 fish each. The fish were fed every day with laboratory prepared feed comprising rice bran, tapioca, fish meal, ground nut oil cake with adequate amount of vitamins. The fish were starved for 24 hr before sacrifice for getting optimum experimental conditions. Fish in 5 groups received ip injections of 0.2µg/g body weight cortisol, corticosterone, DES, progesterone and testosterone respectively between 0630 and 0700 hrs. The fish in the sixth tank were treated as control and received alkaline saline instead of hormone. The total dose of hormones received by each fish was 10µg.

After 24hr of injections, all fish were anesthetized in MS 222. Blood taken directly from the heart in a heparinized hypodermic syringe by lifting the gill lamellae was transferred into separately labeled micro centrifuge tubes. The samples were centrifuged in a micro centrifuge (Universal, 16R) for 10 min at 10000 rpm. The plasma collected was diluted to four times with sample buffer (62.5 mM Tris-HCl, pH6.8; 1% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.1% bromophenol blue) and boiled at 100°C for 3-5 min and immediately used for electrophoresis.

Plasma samples were analyzed using a 7 well gel. Each well was loaded with 20 µl plasma. Electrophoresis was performed under denaturing and discontinuous conditions on 12% sodium dodecyl sulfate (SDS) polyacrylamide gel (PAGE) using a mini vertical gel unit (Genei, India, Bangalore) as per Laemmli¹². Known molecular weight proteins ranging from 250-15 kDa were used as molecular markers. The plasma samples loaded in the wells were electrophoresed at a constant current of 60V for stacking and 120V for

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¹Correspondent author:
Phone: +91-471-418906
Fax: +91-471-307158
E-mail: oommen@md2.vsnl.net.in
running gel at 16°C in an electrophoresis buffer consisting of 0.1% SDS, 0.05M Tris and 0.384 M glycine buffer pH 8.3 for 3hr. The gels were stained with 0.25% Coomassie brilliant blue R 250 in a mixture of 50% methanol and 10% acetic acid overnight, destained in a solution of 10% methanol and acetic acid to get the best quality protein bands. Stained gels were fixed in 7.5% acetic acid at 4°C. The approximate molecular weights of resolved proteins were determined by comparison with known standards.

The results are presented in Fig. 1.

Comparison of different bands appeared in the electropherogram revealed variations in the plasma proteins following treatment with steroid hormones. Several new protein bands, which were not present in the control plasma, were detected at different levels of hormones’ treated plasma and were comparable to the known protein bands in the molecular markers (Fig. 1 lanes 1 to 6). The most apparent ones were those detected at the levels of 180 kDa regions (black mark) in all steroid hormones’ treated plasma. These bands were more concentrated in sex hormones treated plasma particularly, in DES (Fig. 1, lane 2) than glucocorticoids treated ones. Since the yolk precursor protein vitellogenin normally ranging between 145 and 200 kDa is the major estrogen induced protein in the blood of most teleosts, it was assumed that the 180 kDa protein is vitellogenin. Similarly a unique concentrated band was detected towards the bottom of the gel, nearly having a molecular weight of 17 kDa only in cortisol and corticosterone treated plasma (Fig. 1 lanes 2 and 5, green mark) and presumed to be corticosteroid binding globulin (CBG), the carrier protein for glucocorticoids since it appeared only in the glucocorticoids treated plasma. In addition to these, a number of protein bands were detected having approximate molecular weights 158, 30, 27, 20 and 19 in all the hormones treated plasma, while protein bands having approximate molecular weights 44 and 39 were more intensified in steroids treated plasma. The approximate molecular weights of resolved proteins were determined by comparison with known standard molecular markers.

The electropherogram clearly revealed the appearance of some new plasma proteins and the disappearance of one band following steroid hormones treatment in this freshwater fish. The role of estrogen and estrogenic compounds, androgen, progesterone and cortisol on vitellogenin expression in the plasma has been reported in teleosts7-10. The presence of vitellogenin is now considered as biomarker for the detection of estrogen and estrogenic compounds in aquatic medium3. Vitellogenesis presents a versatile model for the study of hormone-induced gene expression. In the present study, the intensified protein band having an approximate molecular weight of 180 kDa appeared in steroids treated plasma was presumed to be vitellogenin. The vitellogenin identified in the plasma of fish had molecular weight usually ranging from 100-200 kDa2,13. Hence from the present results, it is evident that cortisol, corticosterone, testosterone, progesterone and DES control vitellogenin synthesis. This observation is consistent with the induction of vitellogenesis by steroid hormones in other teleosts5,13,14.

Estrogen-induced protein (presumed vitellogenin) in fish serum was identified by a similar electrophoretic method in a surface water fish, Leuciscus idus13. Under flow-through conditions, 7 days of exposure to 6 and 4 ng/L estrogen significantly induced serum vitellogenin in juvenile fish L. idus. Exposure to estrogen stimulated vitellogenin gene expression in the liver of Oreochromis aureus4, sheepshead minnow Cyprinodon variegates1, and vitellogenin synthesis in male marine fish Sparus aurata3. Administration of 17β estradiol and DES upregulated the characteristic expression pattern of genes for vitelline envelope proteins and vitellogenin16 and vitellogenin synthesis5 in C. variegates. Physiological concentration of progesterone and testosterone induced vitellogenin mRNA in the primary culture of immature rainbow trout hepatocytes5. Cortisol also triggered rapid but transient transcription of the silent vitellogenin gene in
male *O. aureus*. These aforesaid data strongly substantiate the findings of the present study in *O. mossambicus*.

The molecular weight of presumed vitellogenin in the present study appeared to be 180 kDa. Almost same molecular weight (180 kDa) vitellogenin was detected in the plasma of a marine fish, *S. aurata* by gel electrophoresis following estrogen treatment. Equivalent vitellogenin having molecular weights 145 and 150 kDa were detected in the plasma of catfish, *Amieturus nebulosus* and surface water fish, *L. indicus* respectively. A monomeric vitellogenin having molecular weight of 200 kDa was determined by SDS-PAGE in a lizard *Podarcis sicula Raf*. These data further confirmed the vitellogenin band identified in this freshwater fish after steroids' administration.

Steroid hormones are only sparingly soluble in water, and most are transported to their target tissues by plasma proteins within the plasma. Cortisol is reversibly bound to two proteins, transcortin or corticosteroid binding globulin (CBG) and to a lesser extent to progesterone, progesterone and DES treated plasma. Cortisol is reversibly bound to two proteins, transcortin or corticosteroid binding globulin, as it was not detected in testosterone, progesterone and DES treated plasma.

In conclusion, it is suggested that protein anabolic role of steroids as reported by Sunny et al. is further confirmed by the appearance of presumed vitellogenin, CBG and a number of other proteins in the plasma of steroid hormone-treated fish as reported by Berduco et al. where glucocorticoid administration increased fetal plasma CBG levels in sheep.

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References


