Steroids 77 (2012) 1133–1140

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Cloning and expression of 3β-hydroxysteroid dehydrogenase during gonadal recrudescence and after hCG induction in the air-breathing catfish, *Clarias gariepinus*

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ARTICLE INFO

Article history:
Received 19 March 2012
Received in revised form 6 June 2012
Accepted 26 June 2012
Available online 11 July 2012

Keywords:
3β-hsd
Differential gene expression
Spermatogenesis
Oogenesis
Steroidogenesis
Catfish

ABSTRACT

3β-hydroxysteroid dehydrogenase (*3β-hsd*) plays an important role in biosynthesis of both androgens and estrogens during steroidogenesis. In this study, we report the cloning of a full-length cDNA of *3β-hsd* from gonads of the air-breathing catfish, *Clarias gariepinus* a seasonally reproducing teleost fish. We studied the expression pattern of *3β-hsd* during gonadal ontogeny and recrudescence (flanking two years of reproductive cycle) using real-time PCR. We also examined the influence of gonadotropin on *3β-hsd* expression in gonads of catfish by human chorionic gonadotropin (hCG) induction. The real-time PCR results revealed that *3β-hsd* transcript was detectable much earlier in undifferentiated gonads i.e. before the sex differentiation and later on its expression was seen in both male and female gonads throughout the development. The expression analysis during subsequent seasonal reproductive cycle in catfish (older than one year) showed that in adult males, the transcripts were significantly high during prespawning phase (spermatogenesis) and declined during spermiation. In adult females, the transcripts were abundantly expressed in the ovarian follicles both at prespawning and spawning phases. Furthermore, the *3β-hsd* mRNA levels in different follicular stages were markedly high in vitellogenic follicles (maturing oocytes; stage III) compared to other stages. Treatment of hCG in recrudescing female fish, *in vivo* as well as in testicular slices, *in vitro* resulted in the up-regulation of gonadal *3β-hsd* mRNA indicating that it is under the regulation of gonadotropins. These results together suggest that *3β-hsd* gene plays an important role during spermatogenesis and oogenesis as well as in the gonadal recrudescence of catfish.

1. Introduction

Steroid hormones are involved in various physiological functions like development, growth and reproduction of vertebrates. Biosynthesis of steroid hormones from cholesterol involves a cascade of steroidogenic enzymes. Among these, 3β-hydroxysteroid dehydrogenase/Δ^5–Δ^4 isomerase (3β-HSD; EC1.1.1.145) catalyzes the dehydrogenation and isomerization of Δ^5 steroids to Δ^4 steroids including pregnenolone, 17α-hydroxypregnenolone and dihydroepiandrosterone to progesterone, 17α-hydroxyprogesterone and androstenedione, respectively which is an important step in the biosynthesis of sex steroids [1,2]. The two-step reaction of the 3β-HSD/isomerase involves the reduction of NAD^+ to NADH by the rate-limiting HSD activity and the requirement of this NADH for the activation of the isomerase on the same enzyme [2]. This bifunctional enzyme is essential for the biosynthesis of all classes of steroid hormones namely, glucocorticoids, mineralocorticoids, progesterone, androgens and estrogens. In addition, enzymes of the 3β-HSD family catalyzes the formation and/or degradation of 5α-androstanes and 5α-pregnanes, such as dihydrotestosterone and dihydroprogesterone [3,4]. Therefore, it controls critical steroidogenic reactions in the adrenal cortex, gonads, placenta and a variety of peripheral target tissues [5].

The *3β-hsd* gene belongs to short-chain dehydrogenase/reductase (SDR) family which has characteristic steroid (substrate) binding domain and cofactor binding domain (Rossmann fold: GXGXXG) which are common for all HSDs [6]. The coenzyme-binding site TGXXXGXG is present on the N-terminus and the putative substrate-binding site YXXXK is on the C-terminus [7]. The onset and regulation of *3β-hsd* expression exhibits a wide variation among different species [8].

Multiple isoforms of *3β-HSD* exist in human, rat and mouse tissues [2,9]. In humans, expression of the type I isoenzyme accounts for the *3β-HSD* activity found in placenta and peripheral tissues like brain, skin and kidney, whereas the type II 3β-HSD isoenzyme is predominantly expressed in the adrenal gland, ovary, and testis,

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http://dx.doi.org/10.1016/j.steroids.2012.06.002

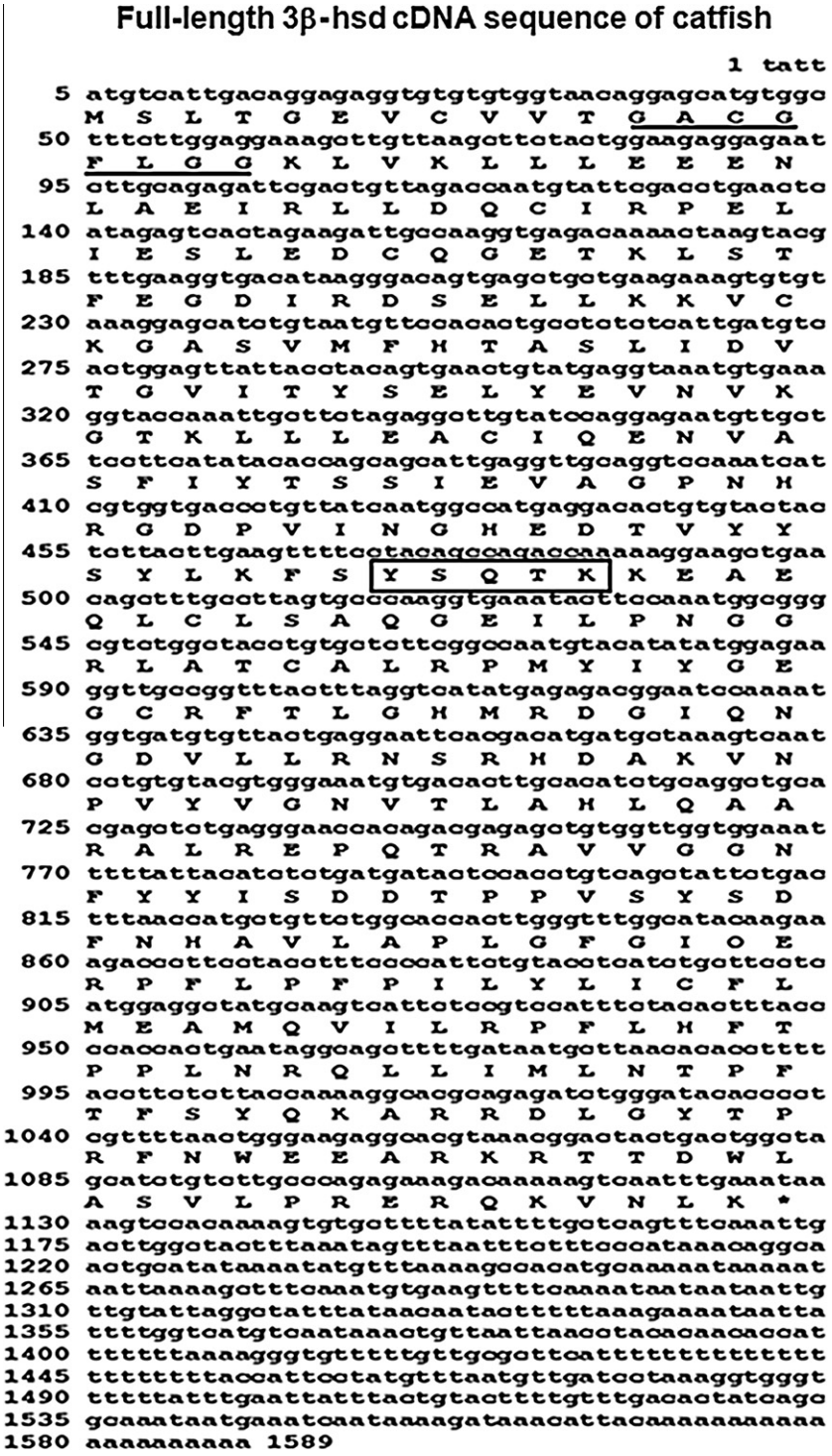

Fig. 1. Nucleotide sequence of catfish full-length *3β-hsd* cDNA and its deduced amino acid sequence. Nucleotides are numbered to the left. The Rossmann fold is indicated by the under line and the putative substrate binding domain (active site) is designated by the box. *Indicates stop-codon.

and its deficiency is responsible for a rare form of congenital adrenal hyperplasia [3]. Rat *3β-hsd* type I and II were exclusively expressed in gonadal and adrenal tissues whereas rat type III isoform was expressed in liver where it acts as specific ketoreductase to inactivate steroids [10,11]. In the mouse, six tissue-specific isoforms have been identified which showed temporal expression pattern [12]. The complexity of *3β-hsd* expressions through multiple signaling pathways acting on a multigene family of enzymes may contribute to the diverse patterns and locations of steroid hormone biosynthesis.

Attempts for cDNA cloning of *3β-hsd* were done in teleostean species such as zebrafish, eel and trout [13–15]. Recently, studies from our laboratory in collaboration with others revealed two novel forms of *3β-hsd* (type I and II) in the Nile tilapia [16]. In the males, only *3β-hsd* type I expression was seen in mature testis of tilapia. Stable expression of *3β-hsd* type I and abundant levels of *3β-hsd* type II in vitellogenic and mature follicles indicates that both forms are important for ovarian steroidogenesis in tilapia while *3β-hsd* type-II has a potential role in steroidogenic shift vis-à-vis final oocyte maturation [16]. In accordance to this, in