Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Steroid Biochemistry & Molecular Biology 99 (2006) 121–128
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Cloning and characterization of a cDNA encoding cholesterol side-chain cleavage cytochrome P450 (CYP11A1): Tissue-distribution and changes in the transcript abundance in ovarian tissue of Japanese eel, *Anguilla japonica*, during artificially induced sexual development

Yukinori Kazeto *, Shigeho Ijiri, Shinji Adachi, Kohei Yamauchi

Division of Marine Life Sciences, Research Faculty of Fisheries Science, Hokkaido University, 3-1-1 minato-cho, Hakodate, Hokkaido 041-8611, Japan

Received 22 August 2005; accepted 19 December 2005

Abstract

Cholesterol side-chain cleavage cytochrome P450 (CYP11A1: P450scc) is a crucial steroidogenic enzyme that catalyzes an initial step in the production of all classes of steroids. A cDNA encoding Japanese eel P450scc was cloned and characterized. The cDNA putatively encoded 521 amino acid residues with high homology to those of other vertebrate forms. The recombinant P450scc produced in COS-7 cells efficiently catalyzed the conversion of 25-hydroxycholesterol into pregnenolone. By northern blot, a single P450scc transcript of approximately 3.3 kb was detected in both ovary and head kidney. Transcript levels of this enzyme significantly increased throughout ovarian development artificially induced by salmon pituitary homogenate, which suggests that gonadotropic stimuli can induce ovarian expression of the P450scc gene in teleosts, as has been reported in mammals. Furthermore, RT-PCR analysis revealed that gene expression of three steroidogenic enzymes, P450scc, P450c17 and 3β-hydroxysteroid dehydrogenase (3β-HSD) show distinctly different tissue-specific patterns of expression in the Japanese eel. The P450scc gene was expressed in ovary and head kidney while the sole source of the P450c17 transcript was ovary. In contrast, 3β-HSD transcript was detected in all tissues examined, brain, liver, spleen and trunk kidney, etc. These suggest that some steroidogenic enzymes are also expressed in non-endocrine tissues and could potentially regulate the local and/or circulating steroid levels in teleosts, as they do in mammals.

Keywords: P450scc; CYP11A1; Steroidogenic enzyme; Ovary; Head kidney; Japanese eel

1. Introduction

Steroid hormones modulate many physiological events including development, growth, osmoregulation and reproduction in vertebrates. In the teleost ovary, two distinct steroid hormones, estradiol-17β and a maturation-inducing steroid, typically 17α, 20β-dihydroxy-4-pregnen-3-one (17α, 20β-DHP), are produced during reproductive cycles. It has been well documented that estradiol-17β is essential for oocyte growth, mainly hepatic vitellogenin synthesis [1] and that 17α, 20β-DHP plays an important role in final oocyte maturation [2]. Pregnenolone is a sole precursor of all other steroids and is synthesized from cholesterol by the oxidative side-chain cleavage reaction catalyzed by cytochrome P450 side-chain cleavage (P450scc: CYP11A1) [3]. Therefore, P450scc is indispensable in the production both of estradiol-17β and 17α, 20β-DHP in the ovary as well as in the production of other classes of steroid hormones including androgens in the testis and corticosteroids in the adrenal gland of teleosts.

Japanese eels (*Anguilla japonica*) caught from the wild have immature ovaries and further oogenesis is arrested under captive conditions. However, ovarian development can be induced by the administration of salmon pituitary homogenate (SPH) richly including pituitary hormones, such as pituitary glycoprotein hormones, growth hormone, etc. [4].

* Corresponding author. Tel.: +81 138 40 5546; fax: +81 138 40 5546.
E-mail address: kazeto@fish.hokudai.ac.jp (Y. Kazeto).

0960-0760/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2005.12.004

Since gonadotropic effects of exogenous hormones can therefore be readily assessed, the Japanese eel would be a suitable model for the study of reproductive physiology in fish ovarian tissue. In fact, SPH strongly enhances the activities of several steroidogenic enzymes [5], which is attributable in part to up-regulation of the expression of genes encoding these steroidogenic enzymes [6–8]. P450scc is assumed to be induced during artificial induction of maturation since the titers of serum sex steroids increase during hormonal treatment [5].

The cDNA encoding P450scc has been isolated and characterized in several species of mammals and the predominant expression of this gene in both gonadal and adrenal tissues has been well documented [9–12]. Furthermore, it has been well documented in the mammalian ovary that gene expression of P450scc is mainly modulated by gonadotropins [13,14], and insulin-like growth factor-I (IGF-I) [15,16]. However, the gene regulation of P450scc has received little attention in fish so far although the cDNAs encoding P450scc of rainbow trout [17], zebrafish [18] and stingray [19] have been cloned. Furthermore, there are few available reports on extra-gonadal expression of this gene in fish [18,19].

In the present study, the cDNA encoding P450scc was cloned from ovarian tissue and characterized. The expression of P450scc, and two other steroidogenic enzymes, 3β-hydroxysteroid dehydrogenase (3β-HSD) [20] and P450c17 [6], both in ovarian and extra-gonadal tissues were also investigated. In addition, as a first step to investigate hormonal regulation of the gene expression of P450scc in fish, changes in transcript abundance of P450scc were examined in the ovary of Japanese eels during sexual development artificially induced by treatment with gonadotropin-rich salmon pituitary homogenate.

2. Materials and methods

2.1. Animal treatment, tissue collection and RNA extraction

Forty-three eels feminized by estradiol-17β administration [21] were artificially induced with salmon pituitary homogenate (SPH) and ovarian tissues at various developmental stages were collected. Two further eels served as Ringer-injected controls. The developmental stages of ovaries were classified into pre-, early, mid-, or late vitellogenic stage, or migratory nucleus stage. The detailed procedure of hormonal treatment and the criteria defining the developmental stages of the ovary are described elsewhere [6]. Various tissues (i.e. brain, small intestine, heart, liver, spleen, head kidney, posterior kidney and ovary) were also collected from a female eel with a late vitellogenic ovary. At the time of tissue collection, fish were anesthetized with ethyl-*p*-aminobenzoate and sacrificed by decapitation. Tissues were immediately removed, frozen in liquid nitrogen and stored at −80 °C until analysis. Total RNA was extracted using a commercial product, ISOGEN (Nippongene, Tokyo Japan) according to the manufacturer's instructions. The RNA was subsequently enriched in poly(A)$^+$-RNA with Oligotex-dT-30 (Takara, Otsu, Japan). The quantity and quality of RNA were determined by UV absorbance at 260, 280 and 320 nm wavelengths.

2.2. Cloning and sequence of Japanese eel P450scc cDNA, and phylogenetic analysis

Complementary DNA was synthesized from oligo-$(dT)_{12–18}$ primed poly(A)$^+$-RNA from Japanese eel head kidney using Superscript II Reverse Transcriptase (Life technologies Inc., Carlsbad, CA). Eel P450scc cDNA fragments were obtained by polymerase chain reaction (PCR) using a set of primers designed from consensus sequences of other P450scc forms; forward primer: 5′-GG(TC)CC(AC)AT-(ATC)TA(TC)AG(AG)GA(AG)AA-3′, reverse primer: 5′-GGGTG(AG)AG(TC)CT(ATGC)AG(ATGC)GT(TC)TC-3′. The PCR procedure consisted of 30 cycles at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min. The resultant amplicon inserted into the PCR^{TM}II vector (Life technologies Inc.) was sequenced using an ABI 373 DNA sequencer (Perkin–Elmer, Foster City, CA). The amino acid sequence predicted from the amplicon showed high homology to highly conserved regions of other P450scc forms, thus, the product was utilized as a probe to screen a λgt10 cDNA library constructed from oligo(dT)-primed mRNA purified from eel ovary at the migratory nucleus stage, using Amersham's cDNA cloning system.

Approximately 500,000 phage plaques were screened with the ^{32}P-labeled eel P450scc cDNA fragment, using a random-primed DNA labeling kit (New England Nuclear, Boston, MA). Positive clones longer than 1.5 kb in length were subcloned into a pBluescript KS$^+$ vector and bi-directionally sequenced.

A phylogenic tree of P450scc characterized to date was generated by the neighbor-joining method using the Clustal W and njplot programs [22].

2.3. Transient expression of eel P450scc in COS-7 cells

Japanese eel P450scc cDNA containing the entire open reading frame was inserted into pSG5 (Funakoshi, Tokyo, Japan), an in vitro eukaryotic expression vector with the SV40 promoter. COS-7 cells (Riken, Tsukuba, Japan) were dispensed into wells of 6-well tissue culture plates and subsequently transfected with a complex of eel P450scc cDNA and pSG5, using the DOSPER Liposomal Transfection Reagent (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's protocol. Empty pSG5 served as controls. COS-7 cells putatively expressing eel P450scc were incubated for 6 or 12 h in 2 ml of complete growth media including 10 ng ml^{-1} of 25-hydroxycholesterol (Sigma, St. Louis, MO) as a precursor. After incubation, samples were extracted with diethyl ether and the concentra-