PC-6

Molecular cloning and analysis Gonadotrophin-I and II subunits from flounder (*Paralichthys olivaceus*)

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Introduction

The vertebrate pituitary produces a family of structurally related glycoproteins including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH). They are structurally related and consist of two non-identical, non-covalently linked subunits, alpha and beta. The alpha-subunit is common to all GTHs; while the beta-subunit is hormone specific (Pierce and Parsons, 1981), and is divergent during the evolution of vertebrate GTH (Chang *et al.*, 1992). Gonadotrophic hormones have been identified in most tetrapod species (reptiles, amphibians and birds). Their structure and function are related to the mammalian FSH and LH. Unlike the mammalian glycoprotein hormones, in teleosts, two chemically distinct gonadotrophins, referred to as GTH-I and GTH-II, were first identified in the chum salmon *Oncorhynchus keta* (Walbaum) (Sekine *et al.*, 1989).

Materials and Methods

Isolation of total RNA and poly A+ RNA

Total RNA was isolated using TRIzol reagent (Gibco).

Construction of flounder pituitary cDNA library

A pituitary cDNA library was constructed from pituitaries that were obtained from male and female flounder at all reproductive stages. cDNA library was constructed as described in the Manufacture's instruction (Stratagene, cDNA library construction kit).

Screening of the flounder pituitary library

The PCR probe was labeled with Dig(Digoxigenin)-11-dUTP. The rest hybridization methods followed the procedure of ZAP-cDNA Synthesis Kit (Stratagene) manual.

Purification of plasmid DNA and Sequence analysis

The clone obtained from screening of library was purified using Wizard Plus SV minipreps DNA purification system (Promega) and checked for the size of the inserted cDNA. The nucleotide sequences of cloned cDNAs were determined by a chain-termination method using BigDye terminator premix kit (Perkin Elmer). Sequencing was carried out using T3 and T7 Universal primers. The fluorescence-labeled nucleotides were analyzed on an ABI PRISMTM 310 automatic sequencer (Perkin Elmer).

Results and Conclusion

We report the isolation and sequencing of the cDNAs encoding the alpha- and beta-subunits of two distinct flounder gonadotrophins (GTH-I and GTH-II). The amino acid sequence deduced from the flounder pituitary glycoprotein alpha-subunit cDNA is identical to ten amino acids determined by N-terminal sequencing of the native protein. Flounder GTH-I and GTH-II share a sequence identity of 49% at the nucleic acid level, and 23 % at the amino acid level. Alignment of the amino acid sequence deduced from the flounder GTH-I with those obtained for other species shows 12 half-cystines and the N-linked glycosylation site at position 12 are conserved. Flounder GTH-II was well conserved cysteine positions when aligned with other members of the glycoprotein family. Especially, flounder GTH-II had different N-linked glycosylation site, other members had at 10 position but flounder GTH-II had at 93 position.

References

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