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Molecular Cloning of Carp Vitellogenin

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Introduction

Vitellogenin (Vg) is a precursor of egg yolk proteins, which is produced by the liver in response to circulating estradiol-17β (E2) released into the bloodstream, and taken up by developing oocyte in teleosts. Male and immature fish can synthesize Vg when exposed to exogenous estrogen or to substances in the environment that mimic estrogens. Therefore, Vg is used as a biomarker for the assessment of mimic estrogens in aquatic areas. In Japan, carp (*Cyprinus carpio*) is used as a test specie of fish for the monitoring of mimic estrogens. But the nucleotide sequence of carp Vg cDNA has not been determined. For the further investigations of endocrine disrupting chemicals using carp, carp Vg cDNA will become a useful tool. Thus, we tried to get the carp Vg cDNA and then confirm the induction of Vg by E2 and phytoestrogens.

Materials and Methods.

The first-strand cDNA was synthesized from hepatopancreas of E2-treareed carp. The PCR was performed using degenerate primers which were designed from conserved region of other teleost Vgs. For the observation of Vg-induction, carp were injected two times (0 and 7th days) with E2 and phytoestrogens (Daidzein Genistein, Coumestrol, and Biochanin A) at a dose of 200 μg /fish. 2 weeks after injection, blood and hepatopancreas were collected. The serum Vg levels were measured by chemiluminescent immunoassay (CLIA) for carp Vg. And the detection of carp Vg mRNA was done by RT-RCR using specific Primer for carp Vg.

Results and Discussion

A 2.2 kbp band was obtained by RT-RCR and the nucleotide sequence was analyzed. The nucleotide sequence of the band had about 85 % homology to fathead minnow Vg and it was also similar to other teleost Vgs (rainbow trout, Mummichog etc.). In Northern blotting using the band as a probe, a single band was observed in only E2-treated fish at the position of about a 6 k base. From these results, the band was identified as a fragment of the carp Vg of cDNA. In the experiment of Vg-induction, Vg was detected in E2-, biochanin A- and coumestrol- treated fish by CLIA and RT-RCR. The results of the fish in which Vg was detected were almost coinciding with the results of both (CLIA and RT-PCR). It was confirmed that the carp Vg cDNA could be used for the monitoring of mimic estrogens.