CHAPTER I

INTRODUCTION

Environmental concern especially in xenoestrogen contamination in water has gained more public attention. Xenoestrogen are described as synthetic chemicals and natural plant or animal compounds that may affect the endocrine system of various organisms. Xenoestrogen include widely distributed organic pollutants like pesticides, drugs, plastics, fuels and heavy metal. Many of the effects caused by these substances have been associated with developmental, reproductive and other health problems. The main effect of xenoestrogen to aquatic organisms in polluted areas is the induction of vitellogenin (Vtg) and choriogenin (Chg) synthesis in adult male and juvenile fish. Vtg and Chg are precursors of egg yolk and eggshell proteins, respectively, which are normally produced in females. In oviparous animals, Vtg is produced in the female liver under the control of estrogen or 17β-estradiol (E_2), which is synthesized in the ovarian follicle cells in response to pituitary gonadotropin hormones (Ng and Idler, 1983; Wallace, 1985). Estrogen receptor (ER) in hepatocyte bound with E_2 and formed ER-E_2 complex that translocated into nucleus and bound with cis-regulatory elements named estrogen responsive element (ERE) on 5'upstream region of *vtg* and *chg* genes and stimulated transcription of *vtg* and *chg* genes. Vtg and Chg are secreted into bloodstream, transported to ovary, incorporated to developing oocyte, and processed to yolk protein named lipovitelline, phosvitin and zona radiata protein, respectively. Generally, Vtg and Chg syntheses occur only in female fish but absent in male and juvenile fish. However, the presence of Vtg and Chg in the plasma of male and juvenile fish can be observed when the fish are exposed to xenoestrogen. Therefore, Vtg and Chg concentration in the plasma and their mRNA expression levels in liver of in male and juvenile fish can be used as biomarker for detecting xenoestrogen contamination in water.

Heavy metals, drugs, pesticides and various numbers of industrial chemical substances have been clarified as xenoestrogen. Many of these chemicals do not share any obvious structural similarity to the endogenous ligand for the ER or E_2, which makes identification based solely on molecular structure difficult (Katzenellenbogen, 1995). Because of the diversity in chemical structure and synergistic effect of xenoestrogen, identification and detection of xenoestrogen by chemical method

cannot provide enough detail on the biochemical effects of the exposed organisms. Xenoestrogen can accumulate in organism more than in aquatic environment. Additionally, synergistic effects of more than 1 species of xenoestrogen have been demonstrated in many organisms.

Various numbers of *in vivo* and *in vitro* biological methods for estrogenicity assay have been developed. For *in vivo* methods, Vtg and Chg concentration in plasma of male and juvenile fish can be determined using ELISA. mRNA expression levels of *vtg* and *chg* in liver of male and juvenile fish can be determined using semi-quantitative RT-PCR and northern blotting as well as reporter gene assay in estrogen sensitive transgenic fish. For *in vitro* methods such as measure Vtg concentration and *vtg* mRNA expression level in fish hepatocyte culture, reporter gene assay using yeast system for determining estrogenicity in estrogen-sensitive human cell, animal cell line and transgenic plant has been demonstrated.

According to the study on estrogenic response in 3 species of Thai local fish (greenback mullet, *Liza subviridis*, milkfish, *Chanos chanos* and giant sea perch, *Lates calcarifer*) by measuring the induction levels of plasma Vtg, Chg in juvenile fish exposed to exogenous estrogen, the result revealed that greenback mullet was the most sensitive fish. Currently, tool for the detection of xenoestrogen contamination in sea water has not been developed in Thailand, so objectives of this research is to develop biomarker of xenoestrogen based on semiquantitative RT-PCR of *chg* and *vtg* genes in *L. subviridis*

1.1 Objectives of the project

1. Cloning and characterization of ER, *chg*, *vtg* and *tif2* genes from greenback mullet, *Liza subviridis*.

2. Study estrogenic response of *chg* and *vtg* genes in liver of greenback mullet, *Liza subviridis* by semiquantitative RT-PCR for applies to biomarker for xenoestrogen contamination in seawater.