Introduction

Walking catfish, *Clarias macrocephalus* Günther is one of the commercially important freshwater cultured fish species in Thailand. *C. macrocephalus* is popular among Thai consumers and has a high market value because of its good flesh quality. The production of walking catfish, however, has been limited due to its slow growth and disease susceptibility. In 1988, hybrid catfish was produced by artificial cross-breeding between male *C. gariepinus* and female *C. macrocephalus*. Because of its fast growth rate and high disease resistance, the hybrid becomes a dominant catfish species widely cultured throughout the country. Culture of *C. macrocephalus* remains importance for providing female broodstock used to generate the hybrid catfish, but due to its low disease resistance, *C. macrocephalus* has always been infected by bacteria and parasites resulting in shortage of female broodfish. Knowledge of fish immunity is therefore necessary to establish strategies for prophylaxis and control of disease in the culture. Up to present, studies on immunity of *Clarias* sp. are restricted, particularly in responses of immunity at the molecular level. Recently, express sequence tag (EST) from liver and muscle of *C. macrocephalus* was characterized and two important immune related genes of the complement system, including the third complement component (C3) and the gamma subunit of the eighth complement component (C8γ), were discovered (Panprommin *et al.*, 2007).

Complement system is a powerful host defense mechanism that contributes to both innate and acquired immunity (Song *et al.*, 2000; Carroll. 2004; Dunkelberger and Song, 2010). This system comprises of about 35 proteins acting as a cascade reaction and is activated through one of the following three pathways: the classical pathway, the alternative pathway, and the lectin pathway. The classical pathway is initiated by interaction between antigen–antibody complex and C1 component, while the alternative pathway activation occurs when C3 component binds to various microbial cell surfaces. The lectin pathway requires the interaction of mannose binding lectin with carbohydrate structures on microorganisms. Activation of any of these pathways results in cleavage of the C3 component that leads to formation of the membrane attack complex (MAC) inducing lysis of invading microorganism. Furthermore, the activation of the complement system generates biological products that enhance phogocytosis, inflammatory reaction, and antibody production (Kirschfink, 1997; Boshra *et al.*, 2006).

C3 is one of the most important components of the complement system, which plays the central role in all three activation pathways inducing the generation of MAC. It belongs to the acute phase proteins whose synthesis increases in response to inflammation (Bayne and Gerwick, 2001) and is a major opsonin of the complement system coating microorganisms for phagocytosis. As in mammals, teleost fish C3 has been characterized as a glycoprotein composed of two disulfide linked chains (α and β chain), containing an intrachain thioester bond in the α-chain (Abelseth *et al.*, 2003; Lange *et al.*, 2003). In contrast to mammals, teleost fish have been found to possess multiple forms of C3, which show different binding efficiencies to complement activating surfaces (Zarkadis *et al.*, 2001; Nakao *et al.*, 2003). Expression studies of C3 isoforms in rainbow trout revealed a different regulation of C3 isoforms after stimulation with LPS and β-glucan, which support hypothesis that functional diversity of complement components in teleosts provides a mechanism for recognition of diverse microorganism to extend their innate immunity (Lϕvoll *et al.*, 2007, Nakao *et al.*, 2003). Although C3 is mainly synthesized in liver hepatocytes but it has also been shown to be produced by other cell types and tissues such as skeleton muscle cell, osteocyte, epithelial cell of skin, brain, gill, gut and head kidney. (Andrews *et al.*, 1995; Morgan and Gasque, 1997; Lang *et al.*, 2004; Magnadottir *et al.*, 2005; Lang *et al.*, 2006; Lϕvoll *et al.*, 2007). Furthermore, extrahepatic synthesis of C3 has been found at different stages of Atlantic halibut and Atlantic cod larval development indicating that C3 complement may play a role in the formation and generation of organ (Lang *et al.*, 2005, 2006).

C8γ is a subunit of the eighth component of complement (C8), which is one of the five components (C5b, C6, C7, C8 and C9) that assemble on the surface of pathogenic organism to form MAC inducing cell lysis (Bubeck *et al.*, 2011). In human, C8 contains three genetically distinct subunits (C8α, C8β, C8γ) arranged as a disulfide-liked C8α-γ dimmer that is noncovalently associated with C8β (Steckel *et al.*, 1980; Kaufman *et al.*, 1989). C8α and C8β are homologous to each other and to C6, C7 and C9. They all contain a variable number of N- and C-terminal modules and a central membrane attack complex/perforin (MACPF) domain (Hobart *et al.*, 1995). By contrast, C8γ is unrelated to any protein in the complement system and is a member of the lipocalin family of proteins that have common ability to bind small hydrophobic molecules (Chiswell *et al.*, 2007). Several studies have shown that MAC formed with C8 composed of C8α + C8β has only ~ 15 % of hemolytic and bacterial activity of MAC formed with intact C8 indicating the effect of C8γ

on C8 function (Schreck *et al.*, 1998; Parker and Sodetz, 2002). Moreover, crystal structure study of human C8α MACPF domain in complex with C8γ (αMACPF- γ) reveals that C8γ makes contact with core β-strands 1-1' and 2 and the I-helix in αMACPF inducing conformation change in αMACPF. This may either facilitate unfolding of C8α to allow edge sharing and more efficient MAC formation or affect the C9 binding site by increasing the binding affinity for C9 to enhance formation of a fully functional MAC (Slade *et al.*, 2008). Therefore, C8γ has a strong positive effect on C8 activity by enhancing the formation of MAC. Among fish species, only trout C8γ has been cloned and characterized (Papanastasiou and Zarkadis, 2006a). It shows sequence similarity to the human C8γ and other known lipocalins. Trout C8γ also has a conserved lipocalin fold in its structure and is produced by a separate gene different from C8α and C8β (Kazantzi *et al.*, 2003; Papanastasiou and Zarkadis, 2006b).

In order to understand the walking catfish immunity at the molecular level, the cDNA encoding for the third complement component (C3) and the gamma subunit of the eighth complement component (C8γ) were initially cloned and characterized. The expression of the complement components during larval development and in different tissues of mature walking catfish were also examined by RT-PCR. *In situ* hybridization was used to localize the expression of the complement components in the larval tissue. Moreover, the expression of the complement components in fingerlings supplemented with β-glucan was determined by semi-quantitative RT-PCR.