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Short communication

Molecular cloning and expression analysis of major histocompatibility complex class I, IIA and IIB genes of blunt snout bream (*Megalobrama amblycephala*)

Wei Luo [a,b], Jie Zhang [a], Jiu-fu Wen [a], Hong Liu [a,b], Wei-min Wang [a], Ze-xia Gao [a,b,*]

[a] College of Fisheries, Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education/Key Lab of Freshwater Animal Breeding, Ministry of Agriculture, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[b] Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Wuhan 430070, China

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ABSTRACT

Major histocompatibility complex (MHC) plays an important role in the immune response of vertebrates. In this study, we isolated MHC class IIA and IIB genes from blunt snout bream (*Megalobrama amblycephala*) by rapid amplification of cDNA ends polymerase chain reaction (RACE-PCR). In order to study the function of the MHC genes in *M. amblycephala*, tissue distribution and immune response of the MHC IIB gene to bacterial challenge were analyzed. All the characteristic features of MHC class II chain structure could be identified in the deduced amino sequences of MHC IIA and IIB, including the leader peptide, α1/β1 and α2/β2 domains, connecting peptide and transmembrane and cytoplasmic regions, as well as conserved cysteines and N-glycosylation site. The deduced amino acid sequence of the MHC IIA and IIB molecule shared from 48% to 88% and from 65% to 77% similarity with those of other teleosts, respectively. Quantitative real-time PCR (qRT-PCR) demonstrated that MHC I and II genes were ubiquitously expressed in ten tissues, with high level in immune related tissues, including kidney, intestine, gill and spleen. Challenge of *M. amblycephala* with the extracellular pathogen, *Aeromonas hydrophila*, resulted in a significant increase in the expression of MHC I, MHC IIA and IIB mRNA within 72 h after infection in gill, kidney, intestine and liver, followed by a recovery to normal level after 120 h. The changes of expression levels for MHC IIA and IIB in most tissues were significantly higher than that of MHC I in the corresponding tissues at most time points ($P < 0.05$). These results demonstrated the MHC genes played an important role in response to bacterial infection in *M. amblycephala*; however, MHC class I and II genes showed different functional activity, which need be further investigated in teleost.

1. Introduction

The major histocompatibility complex (MHC) is a large genomic region that encodes highly polymorphic polypeptides, which serve the immune system as peptide receptors (Mona et al., 2008). Based on the chemical structure and molecular function, MHC genes in fish were divided into two categories, termed class I and class II. The MHC class I molecule consisting of one alpha chain and β2-microglobulin presents foreign peptide product by the degradation of intracellular pathogens to cytotoxic $CD8^+$ T cells (Srisapoome et al., 2004). The MHC class II molecules are heterodimers consisting of α and β chains and are mainly expressed on antigen-presenting cells. The α1 and β1 domains form the peptide binding region (PBR), in which peptides are bound and recognized by $CD4^+$ helper T-cell receptors (Evans and Neff, 2009). As MHC genes have been recognized as the significant elements of the adaptive immunity in vertebrate (Edwards and Hedrick, 1998; Lohm et al., 2002; Barribeau et al., 2008), many MHC genes have been isolated and characterized in various fish species (Ristow et al., 1999; Srisapoome et al., 2004; Pinto et al., 2013).

Blunt snout bream (*Megalobrama amblycephala* Yih, 1955) is one of the main aquaculture species in the freshwater polyculture system since 1960s in China. Unfortunately, diseases of the cultured fish have occurred frequently and losses due to infectious disease limit the profitability and the development of aquaculture. Until now, the cloning of MHC I cDNA and the tissue expression of *M. amblycephala* has been studied by Ma et al. (2011), while report of MHC II genes in *M. amblycephala* is not available. In the present study, we mainly aimed to clone the full-length cDNA of MHC IIA and IIB from *M. amblycephala*, and determine their structures. Moreover, to understand the fish immune system, we examined the expression of MHC class I, IIA and IIB genes in various tissues

* Corresponding author. Address: College of Fisheries, Huazhong Agricultural University, Wuhan, 430070 Hubei, China. Tel.: +86 27 87282113; fax: +86 27 87282114.
E-mail address: gaozexia@hotmail.com (Z.-x. Gao).

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and investigated the immune response of MHC genes to the bacterial challenge of *Aeromonas hydrophila* in *M. amblycephala*.

2. Materials and methods

2.1. Fish sampling

Healthy *M. amblycephala* of about 1 year- (body weight: 150–200 g) and 2 years-old (body weight: 400–500 g) was collected from the fish base of Huazhong Agricultural University (Wuhan, China). The fish were acclimated in the tanks at 20 ± 2 °C for 7 days. Subsequently, six 2 years-old mature individuals were anesthetized by 1.5% tricaine methanesulfonate (MS-222, Sigma, USA) and dissected, and 10 types of tissues, including blood, brain, spleen, kidney, heart, gill, liver, muscle, intestine and gonad, were removed and kept at −80 °C until use. The 1 year-old fish were used for the bacterial challenge.

2.2. Challenge experiment

For the *A. hydrophila* challenge experiment in *M. amblycephala*, all the tested fishes (1 year-old) were inoculated by intraperitoneal injection. In order to determine the median lethal concentration (LD50), three concentrations (5×10^7, 1×10^7, 1×10^6 CFU/mL) of *A. hydrophila* were tested in a pre-challenge experiment on fish prior to the challenge trial and finally the concentration 1×10^7 CFU/mL was determined as LD50. In the challenge experiment, 240 individuals were injected with 100 μL (1×10^7 colony forming units (CFU)/mL) bacterial suspension per individual in the treatment groups, while 240 individuals were injected with the same volume of phosphate-buffered saline (PBS, pH 7.2) as the control group. After the treatment, the fish were returned to tanks, and 30 individuals were anesthetized by MS222 and decapitated at 4, 24, 72 and 120 h post-infection (hpi), respectively. Tissues, including liver, gill, intestine, spleen and kidney, were removed after bleeding and kept at −80 °C until use.

2.3. Cloning of full-length cDNA by rapid amplification of cDNA ends (RACE)

Total RNA was extracted from tissues using Trizol reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. Frist strand of cDNA was synthesized using PrimeScript® RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China) according to the manufacturer's instructions. The partial cDNA sequences of *M. amblycephala* MHC IIA (GenBank accession No. KF193863) and MHC IIB (GenBank accession No. KF193865) were obtained by our previous study on *M. amblycephala* transcriptome data using 454 pyrosequencing technology (Gao et al., 2012). The 5′ and 3′ end sequences of the MHC IIA and MHC IIB cDNA were amplified using the 5′-/3′- Full RACE kit (TaKaRa, Dalian, China) following manufacturer's instructions (special amplified primers were shown in Table 1 of supporting information). The 5′-RACE and 3′-RACE products were ligated into pGEM-T Easy vector (TaKaRa, Dalian, China) for sequencing. The full-length cDNA sequences of MHC IIA and MHC IIB were assembled by the SeqMan software.

2.4. Quantitative real-time PCR analysis

Expression patterns of three genes (MHC I, MHC IIA and IIB) were analyzed as templates using cDNA from different adults' tissues and different stages after bacterial challenge of *M. amblycephala* based on quantitative real-time PCR (qRT-PCR). Three reference genes, *ACTB* (b-actin), *EF1α* (elongation factor 1, alpha) and 18S rRNA, were selected based on expression stability, and all primer sequences were described in Supplementary Table 1. The qRT-PCR assay was performed using SYBR® Premix Ex Taq™ (TaKaRa, Dalian, China) on a Roche Light Cycler 480 machine (Roche, Sussex, UK). Real-time PCR conditions were as follows: pre-denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, annealing at 57 °C (MHC I)/55 °C (MHC IIA)/57 °C (MHC IIB) for 20 s, and elongation at 72 °C for 15 s. The relative quantification of the target and reference genes was evaluated according to standard curves. Each experiment was repeated in triplicate. To select the reference genes with the most stable expression, the relative stability measure (*M*) of the reference genes was calculated by GeNorm (http://medgen.ugent.be/genorm/) as described in our previous studies (Zhang et al., 2013). The value *M* represents an average pairwise variation of a reference gene with all other reference genes and a lower *M* value corresponds to the higher expression stability. According to this rule, the most stable gene was 18s in analysis samples (data not shown).

For statistical analysis, data from qRT-PCR were presented as the mean ± SE. Statistical differences were determined by one-way ANOVA followed by Duncan's multiple range test. All statistics were performed using IBM SPSS Statistics 19.0 (SPSS, Chicago, IL, USA).

2.5. Sequence alignment and phylogenetic tree

The DNASTAR multiple program package (DNASTAR Inc., USA) was used to find the open reading frame (ORF), the calculated molecular weight and theoretical isoelectric point. Protein structure analysis was performed using ExPASy online tools (http://us.expasy.org/tools). The transmembrane helices were predicted according to TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/). Alignment of the nucleotide sequences and putative amino acid sequences of *M. amblycephala* and other known vertebrates was carried out by the neighbor-joining method using MEGA 5.0 program (Tamura et al., 2011), and the reliability of the estimated tree was evaluated by the bootstrap method with 1000 pseudo-replications.

3. Results and discussion

3.1. Character of M. amblycephala MHC IIA and IIB cDNAs

The complete sequence of MHC IIA cDNA (GenBank accession No. KF193864) was 1549 bp, consisting of a 5′-UTR of 54 bp, a 3′-UTR of 790 bp, and an ORF of 705 bp encoding a polypeptide of 234 amino acids, with a predicted molecular weight of 26.10 kDa and a theoretical isoelectric point of 4.62. The full-length cDNA of the MHC IIB (GenBank accession No. KF193866) was 1196 bp, including a 61 bp 5′-UTR, a 376 bp 3′-UTR and an ORF of 759 bp encoding polypeptide of 252 amino acids, with a predicted molecular weight of 28.26 kDa and a theoretical isoelectric point of 6.70. In addition, one N-glycosylation site was observed in the MHC IIA α2 domain region (NHT) and MHC IIB β1 domain region (NST), respectively; two predicted disulfide bonds (cysteine pairs) were in MHC IIA α1 domain and the α2 domains and in MHC IIB β1 and the β2 domains (in Supplementary Fig. 1), respectively. The structures of MHC II genes of *M. amblycephala* are consistent with characteristic features of the MHC gene family of teleost fishes (Srisapoome et al., 2004; Xu et al., 2009; Zhang and Chen, 2006). Blast search showed that the complete sequence of MHC IIA and IIB cDNA from *M. amblycephala* are highly homologous to MHC class IIA and IIB genes sequences in other fish species, and its homology goes from 88% with zebrafish (*Danio rerio*) to 48% with medaka (*Oryzias latipes*) and from 77% with common crap (*Cyprinus carpio*) to 65% with rainbow trout (*Oncorhynchus mykiss*), respectively.

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