

Molecular cloning, genomic structure, polymorphism and expression analysis of major histocompatibility complex class IIA gene of swamp eel *Monopterus albus*

Wei LI¹, Wen-xiu SUN^{1*}, Liang MENG² & Deng-wei HONG¹

¹ Department of Biotechnology, College of Life Science, Yangtze University, Jingmi 266, Jingzhou 434025, People's Republic of China; e-mail: wenxiusun@163.com

² Key Lab for Sustainable Utilization of Marine Fisheries Resources, Ministry of Agriculture, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, People's Republic of China

Abstract: Major histocompatibility complex (MHC) class II molecules play an important role in the immune response of vertebrates. In this paper, full-length MHC IIA cDNA was isolated from swamp eel (*Monopterus albus*) by rapid amplification of cDNA ends PCR. The genomic structure, molecular polymorphism, tissue distribution, and immune response of the MHC IIA gene to bacterial challenge were investigated. The full-length cDNA (GenBank accession No.: KC616308) is 1,509 bp in length including an 83 bp-long 5' untranslated region (UTR) and a 709 bp-long 3' UTR, which encoded a 238 amino acids protein. In the 2,339 bp-long MHC IIA genomic DNA, four exons and three introns were identified. Sequence comparison exhibited that the deduced amino acid sequence shared 27.1–66.3% identity with those of other species. Seven alleles were identified from five healthy individuals. Number of alleles per individual diversified from two to five. Five different 5' UTR sequences and two different 3' UTR sequences from one individual may infer the existence of five loci at least. Real-time quantitative PCR demonstrated that swamp eel MHC IIA transcripts were ubiquitously expressed in ten tissues, but the expression level was distinctly different. Significant changes were observed in liver, spleen, kidney and intestine after challenged with pathogenic bacteria *Aeromonas hydrophila*.

Key words: swamp eel; *Monopterus albus*; MHC IIA; gene cloning and expression.

Abbreviations: MHC, major histocompatibility complex; RACE, rapid amplification of cDNA ends; UTR, untranslated region.

Introduction

Major histocompatibility complex (MHC) loci encode cellular glycoproteins responsible for presenting self or non-self antigens to T cell receptors, and thereby initiate immune responses in invertebrates (Srisapoomee et al. 2004; Croisetiére et al. 2008). MHC molecules, in general, are divided into two main subgroups according to their chemical structures and molecular functions (Rothbard & Geftér 1991). MHC class I molecules are highly expressed on the surface of almost all nucleated cells except sperm cells and some neurons. They are involved in presenting foreign peptide products, produced by the degradation of intracellular pathogens, to cytotoxic CD8⁺ T cells (Piértney & Oliver 2006).

MHC class II genes, on the other hand, are expressed in antigen-presenting cells such as B cells, macrophages, monocytes and dendritic cells. The predominant function of these genes is to present foreign peptides derived from extracellular pathogens to helper CD4⁺ T cells (Srisapoomee et al. 2004). MHC class II proteins are heterodimers, composed of two peptides

(α and β chains). The α -1 and β -1 domains of the two chains form the peptide-binding region, which facilitates the T lymphocyte-mediated immune recognition of pathogens (Evans et al. 2009). Unlike mammals and other vertebrates, MHC class I and class II genes have been found to reside on different linkage groups in teleosts (Sato et al. 2000).

All MHC molecules are characterized by an extremely high degree of polymorphism in terms of both the large amount of alleles exist within populations, and the high sequence variation between alleles (Grimholt et al. 2003; Kjoeglum et al. 2006). The high polymorphism seen in MHC class I genes is commonly confined to exon 2 and exon 3, whereas the polymorphism found in class II genes is centralized in exon 2 (Zhang & Chen 2006a; Xu et al. 2011). These regions also contain a number of putative peptide-binding region sites. Major changes in the amino acid sequences of the peptide-binding regions alter the peptide binding capabilities of the MHC locus and also affect pathogen recognition (Brown et al. 1993; Sommer 2005). Thus, high degrees of MHC polymorphism are closely related to resis-

* Corresponding author

tance or susceptibility to disease in mammals (Medina & North 1998; Paterson et al. 1998; Hill et al. 1991; Tang et al. 2012), poultry (Briles et al. 1983; Nikolich-Zugich et al. 2004; Banat et al. 2012) and teleosts (Zhang et al. 2006b; Wynne et al. 2007; Rakus et al. 2009; Du et al. 2011).

Since the first fish MHC was cloned from carp in 1990 (Hashimoto et al. 1990), lots of MHC genes have been isolated from different teleosts, including rainbow trout (Ristow et al. 1999), half-smooth tongue sole (Xu et al. 2009), Nile tilapia (Zhou et al. 2013; Pang et al. 2013), zebrafish (Sültmann et al. 1994), and so on. There are many other reports on allelic polymorphism and expression of the MHC class II genes in various tissues (Chen et al. 2006; Xu et al. 2009; Li et al. 2011; Pang et al. 2013; Zhou et al. 2013). However, the MHC II expression in tissues following the pathogenic infection remains unclear in swamp eel.

Swamp eel, *Monopterus albus*, is an important economical freshwater fish in China and other Asian countries because of its medicinal and food value (Zhou et al. 2002). *Aeromonas hydrophila* is one kind of important pathogen of bacterial hemorrhagic septicemia of swamp eel, which could cause great loss of eel production (Chen & Lu 1991). The swamp eel cultivation has been seriously threatened by *A. hydrophila* bacteria infection in recent years (Hu et al. 2012; Xu et al. 2013). The use of antibiotics has partially solved the problem of bacterial disease, but has raised concerns regarding antibiotic residues in fish, environmental pollution, and antibiotic resistance development. The better choices include the use of vaccines or breeding disease-resistant stock. Hence, it is a potential approach to culture strains of fish with enhanced resistance to bacterial disease via marker-assisted selection. Thus, cloning and polymorphism analysis of MHC genes of swamp eel are very important.

In this study, we report the cloning and structural analysis of the swamp eel MHC class IIA gene. We also describe its molecular polymorphism and expression in tissues in response to infection with pathogenic bacteria. The objective of the study was to facilitate better understanding of vertebrate immunity and thereby help to formulate disease management strategies for farming swamp eels.

Material and methods

Fish and sampling

Two hundred healthy swamp eels (weighing 60–70 g) were purchased from the Taihu Fishery Farm (Jingzhou, China). The fish were raised in tanks at 20°C for 1 week before genetic analysis. Ten different tissues (heart, liver, spleen, stomach, kidney, blood cells, intestine, skin, muscle, and brain) were removed and immediately immersed in liquid nitrogen at –80°C until RNA extraction.

Challenge of swamp eel with bacteria

Challenge of swamp eel with pathogenic bacteria was performed as reported previously (Zhou et al. 2013) but with

minor modifications. Briefly, the pathogenic bacteria (*A. hydrophila*) were cultured at 28°C to mid-logarithmic growth in Luria-Bertani medium. The bacteria were then collected by centrifugation at 4,500 rpm for 5 min, and re-suspended at approximately 2.2×10^7 CFU/mL in phosphate-buffered saline.

The challenge concentration used in the experiments was below the LD₅₀ (10⁸ CFU/mL) determined in pre-challenge experiments. The fish were anesthetized by immersion in MS222 and injected intraperitoneally with 20 µL of bacterial suspension or with the same volume of phosphate-buffered saline (controls). Infected and control fish were sacrificed 4, 12, 24, 48 and 72 h after injection. Four tissues (kidney, liver, spleen and intestine) were collected and kept at –80°C for RNA extraction.

DNA isolation and cDNA synthesis

Genomic DNA was isolated from the liver of swamp eels using a DNA isolation kit (Solarbio, Shanghai, China). Forty mg of various tissues were dissected from three healthy fishes and subjected to total RNA extraction. Total RNA was extracted from the tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA samples were treated with PrimeScript[®] RT reagent kit with gDNA eraser (Takara, Dalian, China) to eliminate contamination of genomic DNA prior to reverse transcription. cDNA synthesis was carried out according to manufacturer's instructions. The synthesized cDNA was kept at –80°C until further use.

Primer design, amplification of MHC class IIA fragment and RACE-PCR amplification

A pair of degenerate primers, seMHC-N1 and seMHC-C1 (Table 1), were designed according to the conserved sequences of MHC IIA genes from other species, such as large yellow croaker (GenBank: ABV48905), red sea bream (AAW21980), turbot (AAZ06134), rainbow trout (CAB96452) and striped sea bass (ACU46019). Rapid amplification of cDNA ends (RACE) was performed to isolate the full-length cDNA of MHC class IIA gene. Gene-specific primers (seMHC-GSP5 and seMHC-GSP3) (Table 1) were synthesized based on the cDNA fragment obtained. seMHC-GSP5 was used as the 5'-RACE primer and seMHC-GSP3 was used for 3'-RACE primer. 5'-RACE and 3'-RACE-PCR amplification of the MHC-DAA gene were performed using a Smart Race cDNA amplification kit (Clontech, Mountain View, CA, USA) according to the manufacturer's instructions.

Touchdown PCR was used for RACE-PCR. The sequence was: 94°C for 3 min, 94°C for 1 min, 70°C for 45 s, 72°C for 2 min, for 5 cycles. This was followed by 94°C for 1 min, 65°C for 50 s, 72°C for 1 min, for 30 cycles; and 72°C for 10 min for elongation. The PCR products were purified using the Zymoclean Gel DNA Recovery kit (ZYMO, Irvine, CA, USA). The purified cDNA fragments were ligated to pEASY-T1 (Transgen, Beijing, China) vectors and cloned into DH5α competent cells. At least three clones were sequenced using the ABI3730 automated sequencer with M13F primer.

Genomic sequences of swamp eel MHC class IIA gene

Additional primers were synthesized to amplify the introns in order to further characterize swamp eel MHC class IIA (Table 1). Intron 1 was amplified with the primer pair DAA-in1-F and DAA-in1-R. Intron 2 was amplified with the primer pair DAA-in2-F and DAA-in2-R. Intron 3 was amplified with the primer pair DAA-in3-F and DAA-in3-R. The

Table 1. Primers used in this study.

Primer name	Primers sequences (5'-3') ^a	Utility in this study
β -Actin1	GCTGTGCTGTCCCTGTA	Expression of β -actin
β -Actin2	GAGTAGCCACGCTCTGTC	Expression of β -actin
seMHC-N1	TGGYTG ^Y TCAGM ^M CTCTGATGG	cDNA fragment amplification
seMHC-C1	ATGAGGAAGAAGGTTCC ^Y GCAGC	cDNA fragment amplification
NUP	CTAATACGACTCACTATAGGG	RACE amplification
seMHC-GSP5	GGTTCTGTCTGCACTGTTCCCGC	5' RACE amplification
seMHC-GSP3	AGACATCTACAGCTGTACAGTG	3' RACE amplification
DAA-in1-F	CTGCTCCTCGTCCTCTTCT	To amplify intron 1
DAA-in1-R	AGTCCAAACATTTCTTCTGC	To amplify intron 1
DAA-in2-F	GCGGGAACAGTGCAGACAG	To amplify intron 2
DAA-in2-R	AGTTCCACATCATCTCTGG	To amplify intron 2
DAA-in3-F	ACAGCTGTACAGTGGAAACA	To amplify intron 3
DAA-in3-R	GCTGGTAACAGCGGAGAACA	To amplify intron 3
DAA-orf-F	TGGTAATGAAGATGAAGGTG	To amplify full ORF
DAA-orf-R	TCAGCGGCACTCGATTCC	To amplify full ORF
DAA-rt-F	CAACCACTGACCAGGATCTG	RT-PCR expression
DAA-rt-R	GCGGCACTCGATTCTTTGA	RT-PCR expression

^a Notes: degeneracy bases in primers, Y = C or T; M = A or C (emphasized in gray).

conditions of the PCR were as follows: pre-denaturalization at 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 50–60°C for 35 s and 72°C for 1 min 30 s, followed by one extension cycle at 72°C for 10 min. Exon-intron junctions were deduced according to the known MHC IIB genes.

Molecular polymorphism analysis of the swamp eel MHC class IIA

Five randomly selected healthy individuals were used to analyze the molecular polymorphism of the swamp eel MHC class IIA gene. With primers DAA-orf-F and DAA-orf-R, PCR was performed on cDNA to amplify the complete ORF sequences. The alleles were named according to the principle published by Klein et al. (1990), and there had to be at least three identical clones for each allele, identified either in two separate PCRs from the same individual or from PCRs from at least two different individuals (Kennedy et al. 2002). Peptide-binding region sites were determined using previously published methods (Brown et al. 1993).

Sequence alignment and phylogenetic tree construction

The alignment of deduced amino acid sequences of MHC class IIA was performed using the Clustal-W procedure in the MEGA software version 4.0 (Tamura et al. 2007). A phylogenetic tree was constructed using deduced amino acid sequences from MHC class IIA genes using the neighbour-joining method (Saitou & Nei 1987). Bootstrap tests were replicated 10,000 times to derive the confidence values for the phylogeny analysis.

Expression analysis of swamp eel MHC class IIA

Real-time quantitative reverse transcriptase (RT) PCR was performed to determine the expression profiles of the MHC IIA mRNA in normal tissues and to investigate the response of the MHC IIA following bacterial challenge. Total RNA was extracted from ten normal tissues and three infected tissues. cDNAs were synthesized using the PrimeScript[®] RT reagent kit (Takara, Dalian, China) according to the manufacturer's instructions. Primers, DAA-rt-F1 and DAA-rt-R1 (Table 1), were used to amplify the gene fragments. Expression of β -actin was used as internal control.

RT-PCR was performed on a 7500 Real-time PCR system (Applied Biosystems, USA). The 20 μ L reaction system contained 1 μ L cDNA template, 10 μ L SYBR Premix Ex

Taq[™] (Takara), 0.4 μ L ROX reference dye II, 0.4 μ L of each primer and 7.8 μ L of sterile H₂O. Reactions carried out without the template were used as a blank control.

PCR was performed in triplicate wells, three samples per treatment, using the following conditions: 30 s at 95°C, followed by 40 cycles consisting of 5 s at 95°C, 25 s at 54°C and 1 min at 72°C. Dissociation curve analysis was performed after each assay to determine target specificity.

Results

cDNA and genomic sequences of swamp eel MHC class IIA

The full-length cDNA of MHC class IIA gene (GenBank: KC616308) that was designated as moal-DAA*0101 is 1,509 bp, including 83 bp 5' terminal UTR, 717 bp encoding region, 694 bp 3' terminal UTR with a canonical polyadenylation signal (AATAAA) and a 15 bp poly (A) tail. The 717 bp encoding region was found to code a protein with 238 amino acid residues (Fig. 1a).

The secondary structure of the deduced protein was analyzed. All the characteristic domains present in the MHC IIA protein of other species could be found in the swamp eel MHC IIA sequence, including a leader peptide, two extracellular domains (α -1 and α -2), a connecting peptide, a transmembrane region, and a cytoplasmic domain (Fig. 2). In addition, one N-glycosylation site (N-X-S/T) was found in the α -2 domain region. One protein kinase C phosphorylation site (S/T-X-R/K), three casein kinase II phosphorylation sites (S/T-X-X-D/E) and four N-myristoylation sites (G-E/D/R/K/H/P/F/Y/W-X-X-S/T/A/G/C/N-G/I/T) were identified in the α -1, α -2, and transmembrane regions. One immunoglobulin and MHC protein signature (F/Y-X-C-X-V/A-X-H) were also observed in the α -2 domain region. Four conserved cysteine residues were found in the α -1 and α -2 domains. The transmembrane region of the swamp eel MHC class IIA molecule contains a GXXXGXXXG motif, which is believed to be im-

(a) 1 ACATGGGGAGTGTCTCACAGATTCACGCTGATCCAGACTCACATAGATCTGTTGCTGCTGGTAAT
 61 GAAGATGACAGCTGGTAATGAAGATGAAGGTGTCAGAGCTGCTCCTCGTCTCTTCTGTGCCCTCT
 1 M K M K V S E L L L V L F C A L
 133 GTATTTGTGCTGATGgtaagtgactctctttaagctgtgaactgtttactgacagtcgatgactgaa
 17 C I C A D
 199 caacacgtggcagtaaaacaaaaaacagtgcgtaaacagggttaatgaggttgacttctgtttcctc
 265 ttcagTTGTCATGAAGACATTAAGTTTGGTGGTTGTTTCAGACTCTGATGCAGAAGAAATGTTTGG
 22 V V H E D I K F G (G C S D S D) A E E M F G
 331 ACTGGAGGTTGAAGAGCAGTGGTACGCAGACTTCGTCACCAACAGAGGAGTGGACGCTCTACCTGA
 43 L E G E E Q W Y A D F V N N R G V D A L P D
 397 CTTTGTAGATCATTTCCTTCCCAGGACTTTATGAATTCGCTTTGAGTGAGCGGGAACAGTGCAG
 65 F V D H F T F P G L Y E F A L [S E R] E Q C R
 463 ACAGAACCTGAAAAAATCCGTGCAGCCTACAAGGACCTCCCATTGGAACCTTGgttaggatattaac
 87 Q N L K K L R A A Y K D L P L E L
 529 gctaatacctaatacttctgtccatttaactcatgttttgagttaaagaaaccactttaacacagtaag
 595 gtaaatgtaataaaaggctgtgattttaagtttctgtattttgcatcatagatgttactgtcaa
 661 catcatttactataatcacagtcctaaaaatggccaattgcattactgcattaatacccccacactg
 727 actgagaaccataaaaaaccataaagtggttaataaataacccaggtcactgcagtggtgaaacacacac
 793 cttaaagtgtgtgtgagtagaaccagcaaaacaaagtgtcagcttaactggtaaaactcagtgaaa
 859 gcaagaaatgagtgcttaagtcatacagaacagctgtctgacaacaggacaacattgggacgaaga
 925 ttctttatggctgaaccacaagacaagtgtacaaaaacaaaaacaataacaaaaacaaaccagaaa
 991 acaagaaatgttagtcagctctcaagaacaccccaggtgtagtaacaaaaacaaacacacacac
 1057 aaacaaaagcaaatagataatgagtgtagtagtattgatgtatgctgtgctgttctacagATACTC
 104 D T
 1123 CTTCTGGTCCAGTTCGCTACCCACAGAGATGATGTGGAACCTGGGAGAGAAGAACACACTGACCTGCT
 106 P S G P V V Y P R D D V E L G E K N T L T C
 1189 ACGTGACTGGTTTTCTATCCTGCTCCTGTCAAAGTCTACTGGACCAGGAATGGAGACAATGTCACTG
 128 Y V T G F Y P A P V K V Y W T R N G D N V T
 1255 AAGGAATGTACAACAATGTCCCTTTCATGAACAAAGATGGCACCTATCACCAGATCTCCAGACTGG
 150 E (G M Y N N V) P F M N K D G T Y H Q I S R L
 1321 AGTTCACCCACAGCTGGGAGACATCTACAGTGTACAGTGGAAACATCCAGCCTGAGCCAACCAC
 172 E F T P Q L G D I { Y S C T V E } H P A L S Q P
 1387 TGACCAGGATCTGGGgtgagacaccttgatttacagagacagctgaccagaccacagctgattggt
 194 L T R I W
 1453 cagataactgacgtaatgtctctgtctccagatgtggagccctgggttaacgtctctgttttctgtc
 1519 tccagATTTTGAGGTGCAGCAGCCAGTGTGGACCGGCCGTGTTCTGTGGACTGGGCCTGACTGT
 199 D F E V Q Q P S V G P A V F C (G L G L T V)
 1585 CGGTCTGCTCGGTGTGGCAGCTGGAACCTTCTTCTCATCAAAGGAATCGAGTCCGCTGATTGGT
 220 G L L (G V A A G T) F F L I K G I E C R *
 1651 CAAGGCTGATGATGTATAACTCCTTGTGATCTAGTCATGTAAAGAAGCATCATGTTCAGTGGCGTC
 1717 TCATTTTTAAATTAATCATAATGTGCTTTATTGCTTGTAAAGGGCTGTTCTCCGCTGTTACCAGC
 1783 TTTTCTAATGACAAACTAAGACTCAGAATAAACAAAGGAAAATGCTTTAATCTCTCAAATTAATTT
 1849 AGTTAATTTCCATTAATCCTAATGTTTCAGTCAGAAACCTACTGTACATCTCTGCATGTTTTACTC
 1915 TTAGATTTCCAGCTAAAGAGAATTCCACTTCACTGTTTTTACACTTTTCTACATTTTTAATTAATGTC
 1981 TGTTTTATCTGATTATAGTTAGAAAAATCTTTGATTGCAAGTTTGTGACTTCTAACGAGGAATTAC
 2047 CAGAATATGGAAAGTCATGGAGTGTGGGTTAAGGCTTCTTTTACACAGCTCCCAACAATAAAGTC
 2113 CAAAATCTTGTTCAGAGCAGGCACATGAATTATTGGGTGGATTGTGAATAAGAGGAACAATCACT
 2179 GAGAACCTGTATCATGTTTCATGTTTACATAAACACCTGGTTGCTGTTGTAAGACTTTGAAAACCT
 2245 GCATGATTTTTACAGTTTAATGTTCAAAATGTTTTATGTTTCATGTAATAAACAGCAACATCACTTTA
 2311 ACATTTTAATAAAGGCTCATTCTCCGGTAAAAA

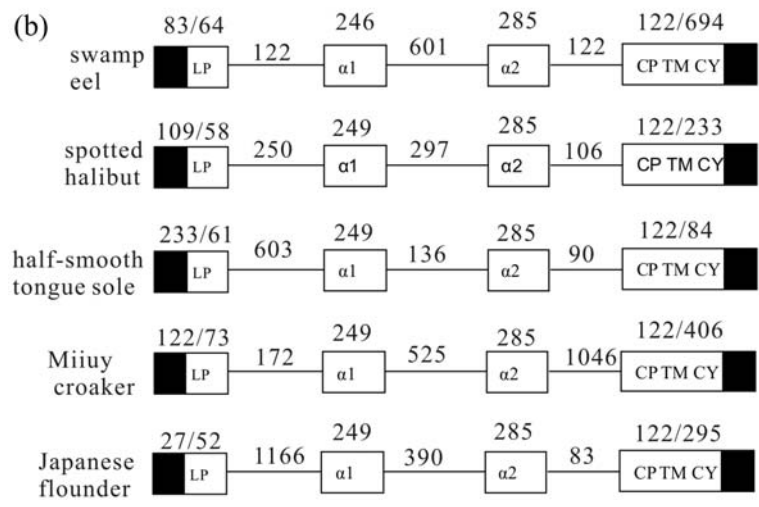


Fig. 1. cDNA and genomic sequence (a) and the schematic illustration (b) of swamp eel MHC IIA gene. Exons are in uppercase and introns are in lowercase. The stop codon is indicated by an asterisk. N-linked glycosylation site is shadowed with dark gray; protein kinase C phosphorylation site is in the normal frame. Casein kinase II phosphorylation and N-myristoylation sites, as well as immunoglobulin and MHC protein signatures are underlined.

Table 2. MHC class II A amino acid identity determined by Clustal W.

Species	Abbreviation	GenBank Acc. No.	Identity (%)
Orange-spotted Grouper	<i>E. coioides</i>	ACU46019	66.3
Large yellow croaker	<i>L. crocea</i>	ABV48905	65.3
Mi-iuy croaker	<i>M. miuy</i>	ADF59029	64.5
Striped sea-bass	<i>M. saxatilis</i>	AAB67861	64.4
Red seabream	<i>P. major</i>	AAW21980	62.6
Turbot	<i>S. maximus</i>	AAZ06134	61.9
Japanese flounder	<i>P. olivaceus</i>	AAY18782	61.5
Nile tilapia	<i>O. niloticus</i>	XP_003459300	61.2
Spotted halibut	<i>V. variegates</i>	ADB43565	59.0
Stone flounder	<i>K. bicoloratus</i>	AFY98544	58.6
Tongue sole	<i>C. semilaevis</i>	ACI88825	58.2
Fugu rubripes	<i>T. rubripes</i>	BAH30162	57.3
Atlantic salmon	<i>S. salar</i>	AAL40122	56.7
Rainbow trout	<i>O. mykiss</i>	CAB96452	53.1
Zebrafish	<i>D. rerio</i>	CAD60677	50.6
Common carp	<i>C. carpio</i>	CAA64707	46.4
House mouse	<i>M. musculus</i>	BAE42123	29.4
Human	<i>H. sapiens</i>	AAC41950	27.1

clones of 3' UTR and 5' UTR sequences from the same individual were sequenced. Five different 5' UTR sequences and two different 3' UTR sequences were identified, which further suggested that swamp eel seems to express up to five class IIA loci at least. All the five 5' UTR sequences contain the same 8-bp leader fragment "ACATGGGG" (Fig. 3a). In addition, compared to the first type 3' UTR sequence, a total of 70-bp deletion was found existing in the second type 3'UTR sequence (Fig. 3b). Further analysis showed that the proportion of variable sites for nucleotide and amino acid sequences across the seven alleles were 21.5% (154/717) and 16.4% (39/238), respectively.

Sequence alignment and phylogenetic analysis

Alignments of the deduced amino acid sequence of swamp eel MHC IIA with that of other vertebrates showed that it shared 26.4–74.7% similarity with other species (Table 2). To evaluate the molecular evolutionary relationship of swamp eel MHC class IIA against other MHC class IIA genes, a phylogenetic tree was constructed based on the protein sequences using the neighbour-joining method. The phylogenetic tree exhibited a close relationship with other teleosts. All the swamp eel MHC class IIA sequences clustered together with those from teleosts and then formed a group independent from the mammalian MHC IIA genes (Fig. 4).

Expression analysis of swamp eel MHC class IIA gene

Quantitative real-time RT-PCR showed that MHC transcripts were ubiquitously expressed in ten tissues, but the expression level was distinctly different (Fig. 5). The high levels of MHC IIA transcripts were detected in stomach, skin, and spleen; moderate expression in liver, blood cell, brain, intestine and kidney, and low or negligible expression in heart and muscle (Fig. 5).

Challenge of swamp eel with the pathogenic bacteria, *A. hydrophila*, resulted in marked changes in the pattern of expression levels of MHC IIA. In liver, the expression level of MHC IIA gene sustained decreased

from infection starting time to 72 h after challenge; the lowest expression level was checked at 48 h. In intestine, the expression level of MHC IIA transcripts was initially up-regulated from 0 h to 4 h. It then decreased acutely between 4 and 48 h, following by a recovery to normal level after 24 and 48 h, then by a marked increase at 72 h. A similar expression profile in spleen and kidney was detected, expression levels of MHC genes were found first up-regulated and then down-regulated (Fig. 6).

Discussion

MHC is a large genomic region consisting of closely linked and highly polymorphic loci that play a central role in the immune system of vertebrates (Croisetiere et al. 2008). There is a high degree of allelic polymorphism and a significant association with disease resistance and susceptibility (Zhang et al. 2006b; Wynne et al. 2007; Rakus et al. 2009; Du et al. 2011; Banat et al. 2012; Tang et al. 2012). For this reason, MHC molecules are considered to be ideal candidates for studying mechanisms that underlie molecular adaptation in vertebrates (Grimholt et al. 2003).

Identification and characterization of genes including MHC molecules involved in immune responses provide a basis for the elucidation of immune defence mechanisms and disease control. In view of the good outcomes using MHC in marker-assisted selection breeding programs, a range of studies has been undertaken to better define the isolation and polymorphism of MHC molecules. Fish MHC genes have been cloned and identified, including those of zebrafish (Sültmann et al. 1994), channel catfish (Godwin et al. 1997), cichlid (Málaga-Trillo et al. 1998), rainbow trout (Ristow et al. 1999), flounder (Srisapoome et al. 2004), turbot (Zhang & Chen 2006a), half-smooth tongue sole (Xu et al. 2009), yellow croaker (Yu et al. 2010), spotted halibut (Li et al. 2011), Mi-iuy croaker (Xu et al. 2011), Chinese long snout catfish (Shen et al. 2011) and Nile

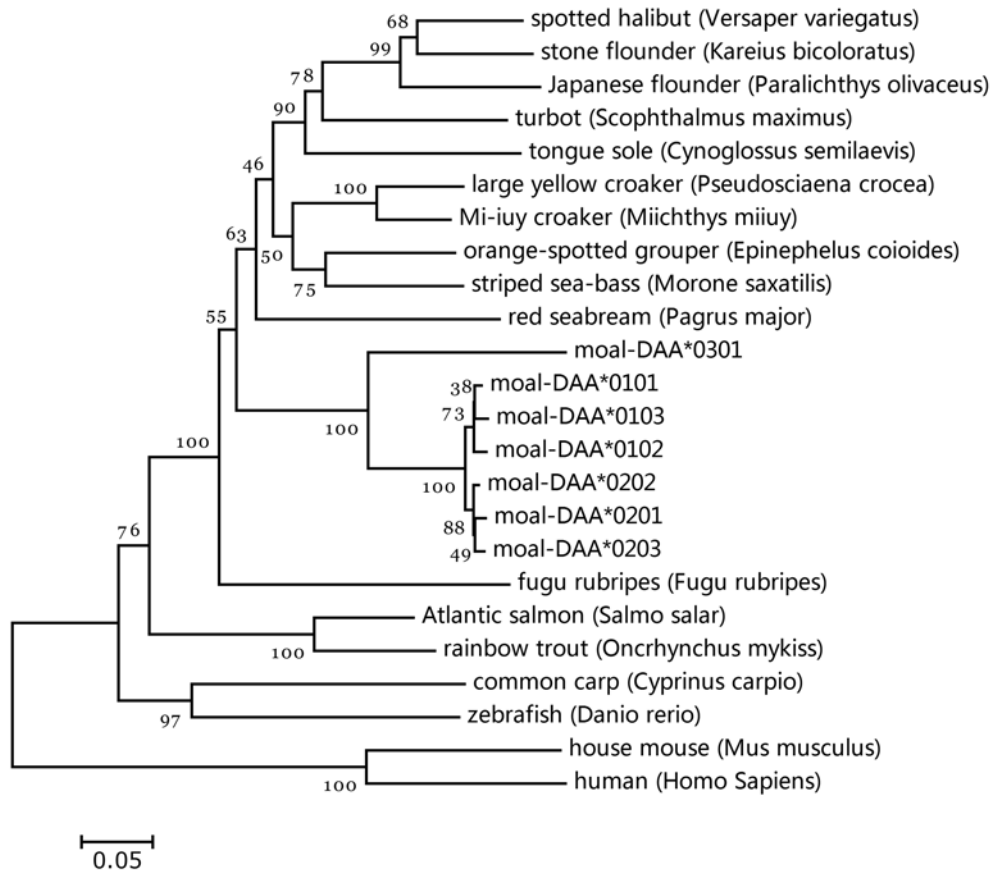


Fig. 4. Phylogenetic tree of MHC IIA gene from swamp eel and other vertebrates.

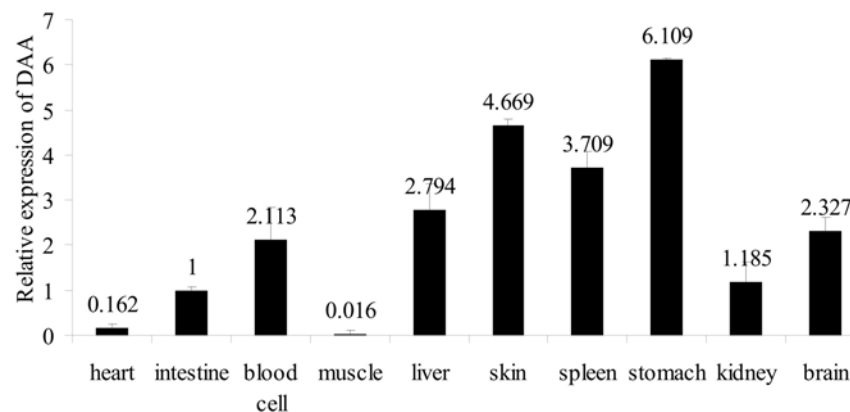


Fig. 5. Expression analysis of swamp eel MHC-DAA gene in various tissues. MHC gene mRNA levels were expressed as a ratio relative to β -actin levels in the same samples after real-time PCR.

tilapia (Pang et al. 2013; Zhou et al. 2013).

In this study, we cloned the full-length cDNA sequence of MHC IIA from swamp eel by RACE technology. Furthermore, the complete genomic structure, molecular polymorphism, genomic structure, expression profiles and immune response of MHC to bacterial stimulation were also investigated.

We showed that the complete genomic sequence of swamp eel MHC IIA consists of four exons and three introns, and is similar to that of the turbot (Zhang & Chen 2006a), spotted halibut (Li et al. 2011), Mi-iuy croaker (Xu et al. 2011) and Nile tilapia (Pang et al.

2013). Alignment analysis showed that the MHC IIA gene shares high degree of similarity to that of other vertebrates. In addition, many specific structural features, such as one N-linked glycosylation site, one protein kinase C phosphorylation site, and three casein kinase II phosphorylation sites were well conserved in the swamp eel MHC IIA and other vertebrates (Srisapoome et al. 2004; Xu et al. 2009; Pang et al. 2013; Zhou et al. 2013). The presence of four conserved cysteine residues and a 'GXXXGXXGXXXG' sequence are also consistent with previous findings in other teleosts and humans. All these results strongly suggest that swamp eel

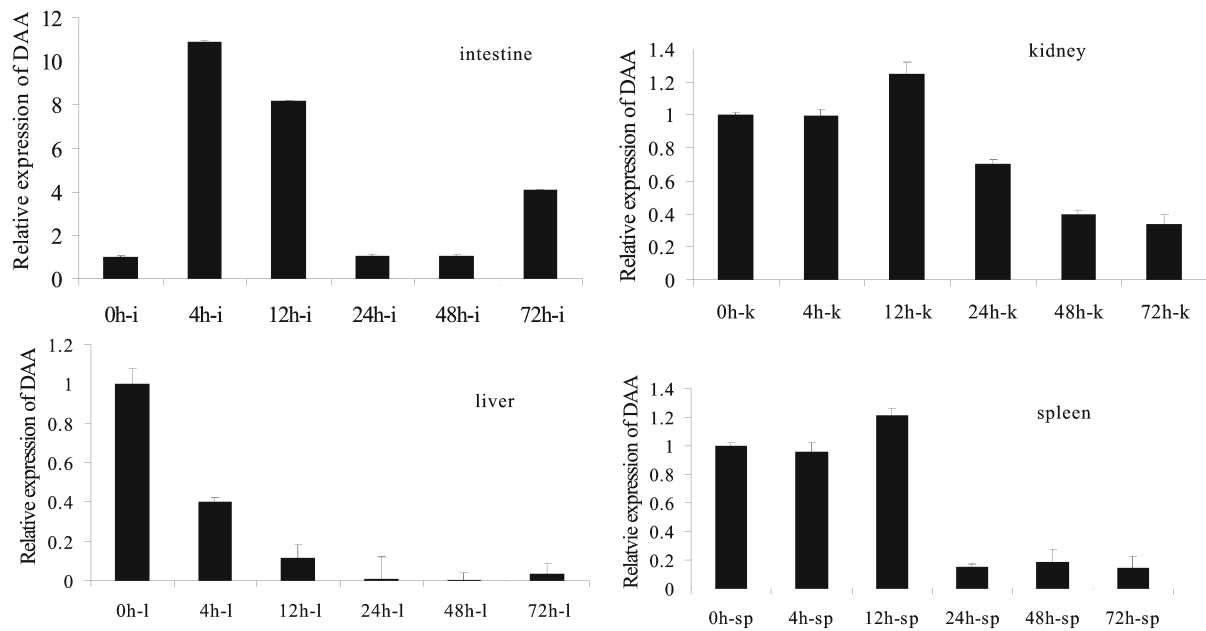


Fig. 6. Expression swamp eel MHC-DAA gene in four tissues after challenge with pathogenic bacteria *Aeromonas hydrophila*. Abbreviations: i, intestine; k, kidney; l, liver; sp, spleen. MHC gene mRNA levels were expressed as a ratio relative to β -actin levels in the same samples after real-time PCR.

MHC class IIA gene was a novel member of the MHC superfamily.

Animals with a high degree of polymorphism tend to have high disease resistance (Zhou et al. 2013). According to the previous studies (Chen et al. 2006; Xu et al. 2009; Li et al. 2011; Pang et al. 2013; Zhou et al. 2013) MHC genes have been characterized by the high degree of polymorphism in vertebrates. In the present study, seven alleles were isolated from five swamp eels. Numbers of alleles per individual varied from 2 to 5. Further analysis showed that five different 5' UTR sequences and two different 3' UTR sequences could also be found from a single individual, implying the existence of five loci at least. Different class IIA numbers have been also found in other fish species, such as two loci have been identified in both half-smooth tongue sole (Xu et al. 2009) and spotted halibut (Li et al. 2011). More than four MHC IIA alleles were identified in a single individual suggested the existence of at least four loci in Nile tilapia (Pang et al. 2013).

Generally, MHC class II genes are mainly expressed in professional antigen-presenting cells, such as B cells, dendritic cells and macrophages in mammals (Pierny & Oliver 2006). In this paper, the real-time PCR showed that MHC class IIA transcripts were ubiquitously expressed in ten tissues, but the expression levels were distinctly different. The most abundant expression of MHC IIA was detected in stomach and spleen. This result was consistent with the previous reports in flounder (Srisapoome et al. 2004) and half-smooth tongue (Xu et al. 2009) and Nile tilapia (Pang et al. 2013). However, the expression level in blood cells was relatively lower, which is similar to what had been reported in turbot (Zhang & Chen 2006a), half-smooth tongue (Xu et al. 2009) and spotted halibut (Li et al. 2011).

The high expression in stomach and spleen implied that the fish immune system responded through these organs when pathogenic antigens entered (Srisapoome et al. 2004). Relatively low or negligible expression of MHC class IIA in muscle seen in swamp eels has also been found in half-smooth tongue sole (Xu et al. 2009), large yellow croaker (Yu et al. 2010), spotted halibut (Li et al. 2011) and Nile tilapia (Pang et al. 2013). These results imply that low levels of expression of MHC class IIA in muscle may be universal in teleosts. The presence of MHC class II mRNA in all tissues could be a result of contamination from leukocytes because it can be difficult to eliminate contaminated leukocytes of the blood in tissues (Srisapoome et al. 2004). The similar result of expression of MHC class II gene has been reported in other teleosts (Chen et al. 2006; Xu et al. 2009; Li et al. 2011; Pang et al. 2013; Zhou et al. 2013). This suggests that MHC class II gene expression in all investigated fish tissues is possible a result of the lymphoid/myeloid cell content of the tissues (Srisapoome et al. 2004).

Previous studies have shown that expression levels of MHC class IIA mRNA were markedly affected when challenged with pathogenic bacteria (Xu et al. 2009; Li et al. 2011; Pang et al. 2013). Challenge with *Streptococcus agalactiae* resulted in up-regulation of the MHC IIA gene in various tissues after infection (Pang et al. 2013). In the same way, challenge with *Vibrio anguillarum* resulted in significant enhancement of the spotted halibut MHC IIA genes in blood and liver (Li et al. 2011). Xu et al. (2009) reported that expression level of tongue sole MHC IIA was stable from 0 to 72 h in spleen, sharply decreased in 96 h after challenged with *V. anguillarum*; however, the expression level decreased after an early increase in liver and intestines, following by a recovery to normal level in intestines after 96 h.

Similarly, pathogenic bacteria *Aeromonas hydrophila* can also influence the expression level of MHC II genes. Zhou et al. (2013) found that the mRNA expression levels of Nile tilapia MHC IIB was significantly regulated after *A. hydrophila* challenge. The results showed that the mRNA levels seemed unchanged in the intestine and spleen after 96 h post-induction. However, challenge with *A. hydrophila* resulted in a down-regulation in 48 h after infection in the liver and gill, followed by a recovery. In the kidney, there was a significant decrease from 24 h to 72 h after infection.

In the present study, challenge of swamp eel with *A. hydrophila* resulted in marked changes in the expression levels of MHC IIA. In liver, the expression level of MHC IIA gene sustained decreased from infection starting time to 72 h after challenge. In intestine, the expression level of MHC IIA transcripts was initially up-regulated and then decreased acutely, following by a recovery to normal level and a marked increase. However, in spleen and kidney, expression levels of MHC genes were found first up-regulated and then down-regulated. These results imply that the MHC IIA genes may be related to the immune response to the bacterial stimulation in swamp eels. However, further studies are needed to elucidate the precise role and mechanism of MHC II in defence response in fish.

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