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Characterization of cDNA clones encoding major histocompatibility class II receptors from walleye (*Sander vitreus*)☆

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ABSTRACT

The teleost major histocompatibility (MH) class II receptor presents peptides from exogenous sources to $CD4^+$ T cells, leading to the initiation of the adaptive immune response. The genes encoding MH class II have been identified in a number of teleost species, but not in walleye, an important recreational fish and commercial fishery in North America. In this study, we cloned and characterized the sequences encoding walleye MH class II α and β chains. These sequences contained all of the domains typical for functional MH class II α and β chain proteins, and aligned with other teleost sequences of MH class II. The walleye MH class II α amino acid sequence, along with other members of the Supraorder Percomorpharia, contains a high concentration of methionine residues in the beginning of the leader peptide. Southern blotting indicated that there is more than one gene copy for both MH class II α and β, while northern blotting analysis of both genes showed that expression of these genes is greatest in lymphoid tissues and at potential entry points for pathogens. These results help to further the understanding of MH class II receptors in teleosts, and could prove useful in the study of disease issues in walleye such as dermal sarcoma virus.

1. Introduction

In mammals, major histocompatibility complex (MHC) class II receptors present peptides derived from exogenous proteins to $CD4^+$ T cells, leading to the initiation of antigen-specific responses against pathogens (Holling et al., 2004). The receptor is composed of α and β chains that are non-covalently attached, and have molecular weights of 33–35 kDa and 25–30 kDa respectively (ten Broeke et al., 2013). Each chain is a transmembrane glycoprotein that contains a short cytoplasmic domain, and an extracellular membrane-proximal immunoglobulin domain. Peptides produced from exogenous proteins via lysosomal proteolysis are bound in the peptide-binding groove of the receptor, which is formed by the N-terminal regions of the two chains. Constitutive MHC class II expression is restricted to professional antigen-presenting cells, including dendritic cells, macrophages, and B cells (Blum et al., 2013; Neefjes et al., 2011; Roche and Furuta, 2015).

While the structure of class II receptors in fish is similar to their mammalian counterparts, at the genetic level teleost MHC class II genes exhibit some differences. The genes are highly polymorphic in both mammals and fish, with a corresponding variation in amino acid sequence that is concentrated in the area that comprises the peptide-binding groove (Gomez et al., 2010). However, unlike in all other vertebrates where the genes for MHC class I and II form a genetic complex on a single chromosome, the MHC class I and II genes are unlinked in teleost fish, and are therefore referred to as only major histocompatibility (MH) genes (Stet et al., 2003). This phenomenon was first observed in zebrafish, but has since been reported in all other teleosts studied to date, ranging phylogenetically from medaka to rainbow trout (Bingulac-Popovic et al., 1997; Hansen et al., 1999; Naruse et al., 2000). Most of the teleost MH class II genes lack defined synteny across species, leading to the idea that class II genes translocated out of an ancestral teleost MHC in a species-independent manner (Dijkstra et al., 2013; Kuroda et al., 2002). In cartilaginous fish, such as the nurse shark and the banded hound shark, and non-teleost ray-finned fish, such as the gar the MHC class I and class II genes are closely linked, suggesting that the lack of linkage of the genes in teleosts is a derived trait rather than an ancestral condition (Dijkstra et al., 2013; Ohta et al., 2002).

☆ The nucleotide sequences reported in this paper have been submitted to the Genbank database under the following accession numbers: AY158837–AY158884.

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MH class II genes have been identified in a number of species from the Supraorder Percomorpharia, including the sea bass, three-spined stickleback, large yellow croaker, miiuy croaker, and mangrove red snapper, but have not been completely characterized in many cases (Buonocore et al., 2007; Reusch et al., 2004; Silva et al., 2007; Wang et al., 2015; Xu et al., 2011; Yu et al., 2010). For example, gene copy number has only been determined for the three-spined stickleback, which has two MH class II α and β genes (Reusch et al., 2004). Additionally, MH class II protein levels were found to be greater in lymphoid tissues, such as the spleen and head kidney, and in the gills, a potential site for entry of pathogens, in the three-spined stickleback (Reusch et al., 2004). This pattern was also observed at the genetic level in the European sea bass, although there seems to be variable expression levels in the head kidney (Buonocore et al., 2007; Silva et al., 2007). On the whole, these receptors are still relatively uncharacterized, and have not been identified to date in another member of the Percomorpharia, the walleye.

Walleye (*Sander vitreus;* previously *Stizostedion vitreum*) is a freshwater member of the Order Perciformes, and Supraorder Percomorpharia, whose native range encompasses all of North America east of the Rocky Mountains with the exception of Atlantic Canada and Florida (Bingulac-Popovic et al., 1997; Hartman, 2009). They are the top predators in many ecosystems, resorting to cannibalism when prey fish species are not abundant (Hartman, 2009). Walleye were the most caught recreational fish in Canada in 2010, representing 23% of the total catch that year in an industry that was estimated to be worth approximately 8.3 billion dollars (FAO, 2010) and contributed the most value to the world's largest freshwater commercial fishery, valued at $305 million for the Ontario Great Lakes region in 2014 (Hill, 2015). In this study, we cloned the previously unidentified walleye MH class II α and β genes and characterized their copy number and tissue expression *in vivo* to better understand the role of these receptors in this economically important species.

2. Materials and methods

2.1. Library construction and screening

Head kidney tissue from a freshly sacrificed walleye was collected and stored in RNAlater (Ambion) at 4 °C until use. Construction of a unidirectional cDNA library from the collected tissue was performed using the ZAP Express XR Library Construction Kit (Stratagene) according to the manufacturers instructions. Insert sizes were checked by PCR using the M13 forward and reverse primers.

Probes for screening were amplified from the cDNA library fragments using degenerate primers designed from conserved regions in the nucleotide sequences of aligned teleost MH class II genes (perMHIIA and perMHIIB in Table 1). Appropriately sized fragments (211 bp) were cloned into the pCR2.1 vector using the TA Cloning Kit (Invitrogen) and sequenced at the York University Core Molecular Biology Facility.

Recombinant phage vectors from the cDNA library were plated at a density of 3.5×10^4 PFU/plate, transferred to Magnacharge membranes (Osmonics Inc.), and screened by two rounds of hybridization with the generated probe. Positive plaques were subjected to a third screen by PCR using the primers described above. Insert size was confirmed by PCR with M13 and T3/T7 primers using PCR SuperMix High Fidelity (Gibco) in plaques that were positive after the three rounds of screening. Clones were then sequenced on an ABI377 automated sequencer with standard and specific primers at the York University Core Molecular Biology Facility.

2.2. Allele sequencing and phylogenetic analyses

Predicted amino acid sequences from walleye MH class II α and β genes were aligned with orthologous sequences from other species in Geneious using MUSCLE. N-linked glycosylation sites were identified by searching for N-X-T/S motifs in the aligned sequences (Gavel and von Heijne, 1990).

A phylogenetic tree was generated in MEGA7 from walleye MH class II α and β gene coding sequences and orthologous sequences from other vertebrates (Kumar et al., 2016). The tree was constructed by the neighbour joining method and used the Jukes and Cantor model for distance estimation (Jukes and Cantor, 1969; Saitou and Nei, 1987). Branching order stability was confirmed by performing 1000 bootstrap replicates.

2.3. Southern blotting

Genomic DNA was isolated from walleye whole blood according to Sambrook et al., and 10 mg of the isolated DNA was digested to completion at 37 °C for 12 h with 20U of either *Bam*HI, *Eco*RI, *Eco*RV, *Hind*III, or *Pst*I (Sambrook et al., 1989). Digests were then separated on a 1% agarose gel, transferred to a Magnacharge membrane (Osmonics Inc.), and UV cross-linked for 1 min. Membranes were pre-hybridized for 2 h at 42 °C in a pre-hybridization solution containing 50% formamide, 7% SDS, 0.1% sodium *N*-lauroylsarcosinate, 50 mM sodium phosphate buffer (pH 7.0), 5 × SSC, and 2% DIG blocking reagent, and then hybridized at 42 °C for 16 h with DIG-labelled DNA probes of 445 bp for MH class II α, and 290 bp for MH class II β. Probes were prepared by PCR amplification of the genomic DNA using clone c2a2 as a template and the SaviMHIIA F (nt 674-693) and SaviMHIIA R (nt 933-914) primers for MH class II α, and the SaviMHIIB F (nt 505-525) and SaviMHIIB R (nt 602-621) primers with clone c2b1 as a template for MH class II β (see Table 1), and DIG-labelled using the PCR DIG Probe Synthesis Kit (Roche). Membranes were washed twice at 65 °C for 30 min in 0.1 × SSC containing 0.1% SDS, and hybridized DNA

Table 1
Primers used for PCR.

Gene	Primers	Sequence (5'-3')	Application
MH class II α	perMHIIA F	CTGACGWTCTTCATGAGGAC	Probe Amplification
	perMHIIA R	AGGTTCTGTCTGCAGATCTG	Probe Amplification
	SaviMHIIA F	GTCCTTCATGCCTATACGGG	Probe Amplification
	SaviMHIIA R	CCCAGTCAGTGATCAGAGG	Probe Amplification
	SaviMHIIA F2	TCTCCGGTCTCCTCTGTGTC	Probe Amplification
	SaviMHIIA R2	GCAGCCACACCGAGCAGACCC	Probe Amplification
MH class II β	perMHIIB F	CCATGTTGGTCTGCAGCGTC	Probe Amplification
	perMHIIB R	GGGYGTGTACTCCAGGTGWG	Probe Amplification
	SaviMHIIB F	CTCCCTGCTCTTCATCAG	Probe Amplification
	SaviMHIIB R	CCCAGTCAGTGATCAGAGG	Probe Amplification
	SaviMHIIB R2	CCCAGTCAGTGATCAGAGG	Probe Amplification
S11	OnmyS11 F	AGCAGCCAACCATCTTCCAG	Internal Control Probe Amplification
	OnmyS11 R	ACTCTCCGACGGTAACAATG	Internal Control Probe Amplification