Technical report

Channel catfish (*Ictalurus punctatus* Rafinesque, 1818) CD156a (ADAM metallopeptidase domain 8): cDNA clone, characterization and expression in tissues

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**ABSTRACT**

CD156a, also known as a disintegrin and metalloprotease domain 8 (ADAM-8), is a type 1 transmembrane glycoprotein of the ADAM family. This protein plays important roles in immune and other physiological functions. In this communication, the channel catfish CD156a cDNA was characterized and its expression in various tissues was determined. The full-length of channel catfish CD156a cDNA had 3035 nucleotides, including an open reading frame which appears to encode an 850 amino acid peptide with a calculated molecular mass of 94.6 kDa. The peptide had three potential N-glycosylation sites. By comparison with other species, the degree of homology of the CD156a amino acid sequences ranged from 31.6% (vs. chicken CD156a) to 59.5% (vs. zebrafish CD156a). The channel catfish CD156a peptide could be structurally divided into nine domains. Several canonical features for CD156a functions were conserved in channel catfish. The CD156a transcript was detected by two-step RT-PCR in anterior kidney and gill, suggesting that CD156a may be involved in the innate immune response in channel catfish. Reagents for further elucidating the immune functions of channel catfish CD156a are under development.

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brain cancer (Vildeboer et al., 2006), pancreatic cancer (Valkovskaya et al., 2007), renal cell carcinoma (Roemer et al., 2004) and prostate cancer (Fritzsche et al., 2006). In allergic disorders, a study showed that the cleavage of the low affinity, membrane-bound IgE receptor, CD23, on B cell surface by CD156a leads to production of IgE and many inflammatory cytokines (Fourie et al., 2003; Matsuno et al., 2008). Further, studies found that CD156a molecules are involved in experimentally induced asthma in mice (King et al., 2004; Matsuno et al., 2006). Given these observations, the CD156a may be a clinically important target for therapeutic intervention (Hall et al., 2008; Matsuno et al., 2006, 2008; Valkovskaya et al., 2007).

Channel catfish (Ictalurus punctatus) production is the most important aquacultural industry in the southeastern United States, having sales over 410 million dollars in 2008 (USDA, 2009). In the course of studying Edwardsiella ictaluri pathogenesis in channel catfish, we observed up-regulation of the CD156a expressed sequence tags after infection with the bacterium (Yeh and Klesius, unpublished data). In this communication, we report the cloning, characterization and expression analysis of the channel catfish CD156a transcript.

Channel catfish (NWAC103 strain) were maintained and acclimated for two weeks before use in experiments as described previously (Jenkins and Klesius, 1998). The protocol of fish use in the experiments was approved by the Institutional Animal Care and Use Committee, Aquatic Animal Health Research Unit, Agricultural Research Service, U.S. Department of Agriculture. Prior to tissue excision, fish were euthanized by immersion in 200 mg/l of tricaine methanesulfonate (MS222) per the Guidelines for the Use of Fishes in Research (Nickum et al., 2004).

Total RNA was isolated by using Tri Reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer’s instructions. The rapid amplification of cDNA end (RACE) libraries were constructed by using a GeneRacer kit (Invitrogen, Carlsbad, CA) or SMART™ RACE cDNA Amplification kit (Clontech Laboratories, Inc., Mountain View, CA) as per the manufacturers’ instructions. Primers for PCR amplification of channel catfish CD156a are following: GeneRacer 5′Primer (Invitrogen), 5′-GACTG-GAGCACAGAGCACCCT-G-3′; GeneRacer 3′Primer (Invitrogen), 5′-GCTGTAACGATGCTAGCTGAG-3′; UPM Primer (Clontech), 5′-CTAATACGACTCTATATTGCTGAG-3′; Clontech, 5′-CCTCTAGACGCCTGAG-3′; ADAM8-8F, 5′-CTAATACGACTCTATATTGCTGAG-3′; ADAM8-272F, 5′-CTAATACGACTCTATATTGCTGAG-3′; ADAM8-302F, 5′-CTAATACGACTCTATATTGCTGAG-3′; ADAM8-32R, 5′-CTAATACGACTCTATATTGCTGAG-3′; ADAM8-257R, 5′-CTAATACGACTCTATATTGCTGAG-3′; ADAM8-335R, 5′-CTAATACGACTCTATATTGCTGAG-3′.

DNA sequencing reactions were performed, and chromatograms were edited, trimmed and analyzed at the USDA ARS MidSouth Genomic Laboratory (Stoneville, MS) as described previously (Yeh and Klesius, 2007a,b, 2008a,b,c). The amino acid sequence of channel catfish CD156a was translated from nucleic acid sequence by using Transeq (Rice et al., 2000), and aligned with other CD156a amino acid sequences deposited in GenBank by using ClustalW2 (Larkin et al., 2007).

Two-step RT-PCR assays were used to profile CD156a gene transcript in various channel catfish tissues as described previously (Yeh and Klesius, 2007a,b, 2008a,b,c). β-Actin was used as an internal control. The amplified products were analyzed in 2% agarose gel electrophoresis, and stained with ethidium bromide. Images were recorded by a KODAK Gel Logic 440 Imaging System (Eastman Kodak, Rochester, NY), and processed with Imagej software (version 1.41o) (Abramoff et al., 2004).

Previously, we identified three expressed sequence tags (EST) of channel catfish CD156a by subtractive suppression hybridization (unpublished data). Based on these EST, we designed primers to determine the complete CD156a transcript. The full-length of channel catfish CD156a cDNA consisted of 3035 nucleotides, including a 5′-untranslated region (UTR), an open reading frame and a 3′-UTR (GenBank accession no. FJ594762). In the 5′-UTR, the sequence had a Kozak sequence (A/G NNATG) (Kozak, 1987). The 3′-UTR had 451 nucleotides in length and contained three canonical features of mRNA: (1) an mRNA instability motif (attta), (2) a polyadenylation signal sequence (aataaa), and (3) a 28-nucleotide polyadenylation tail. The open reading frame of the channel catfish CD156a transcript appears to encode an 850 amino acid residue peptide with a calculated molecular mass of 94.6 kDa and pl of 7.96 at pH 7.0. The peptide had three potential N-glycosylation sites-Asn82, Asn115 and Asn170 residues (numbering after the channel catfish CD156a peptide; Fig. 1). No cysteine switch sequence (Cys-Gly-Val) was found in the deduced channel catfish CD156a cDNA amino acid sequence.

When the deduced channel catfish CD156a amino acid sequence was compared with those from other species deposited in GenBank, we found that the length of CD156a varied from 726 amino acids (chicken) to 850 amino acids (channel catfish), and the degree of conservation ranged from 31.6% (vs. chicken CD156a) to 59.5% (vs. zebrafish CD156a) (Table 1). Like human and mouse CD156a (Hall et al., 2008; Schlomann et al., 2000; Yamamoto et al., 1999), the channel catfish CD156a peptide could structurally be divided into nine domains: (1) signal peptide, (2) pro-metalloprotease domain, (3) metalloprotease catalytic domain, (4) disintegrin domain, (5) cysteine rich domain, (6) epidermal growth factor-like domain, (7) pre-transmembrane domain, (8) transmembrane domain, and (9) intracellular domain (Fig. 1). Among the domains, the disintegrin, cysteine-rich and epidermal growth factor-like domains are able to interact with integrins or other cell adhesion molecules (Bridges and Bowditch, 2005). Further, several important features for CD156a functions were conserved in channel catfish. First, the histidine triad motif
Fig. 1. Alignment of deduced channel catfish CD156a amino acid sequence with other CD156a sequences deposited in GenBank. Gaps were introduced in the sequences indicated by hyphens (-). Identity of amino acids are denoted by (*). The structural domains are indicated above the sequences. The conserved cysteine (C) residues are indicated in bold blue. The His-triad, SH3 and Abl SH3 motifs are highlighted and indicated. Accession numbers of each species are shown in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
in the metalloprotease catalytic domain, HEXCHNLGMXH, that coordinates active zinc binding and catalysis (Yamamoto et al., 1999; Hall et al., 2008) is highly conserved between teleosts and mammals (Fig. 1). Second, the channel catfish CD156a peptide had 50 cysteine residues, mainly in the disintegrin and cysteine-rich domains. More striking is that the numbers (40 out of 50) and positions of cysteine residues were conserved among teleost fish and mammals, indicating that the tertiary structure of CD156a is conserved via disulfide bonds during the evolutionary
process (Fletcher et al., 1994; Rushmere et al., 1994) (Fig. 1). In our previous studies, we found that conservation of disulfide linkages exist in many channel catfish peptides, such as CD59, cathepsins and CD81 (Yeh and Klesius, 2007b, 2008c, 2009a,b). Third, the intracellular domain of human and mouse CD156a has SH3 and Abl SH3 consensus sequences RPPPAPP and PXXXPPXPP, respectively, indicating that CD156a is involved in signal transduction (Yamamoto et al., 1999; Yoshiyama et al., 1997). In the Abl SH3 sequence, the first proline residue was substituted by a lysine residue in the channel catfish CD156a. This substitution is also found in zebrafish. In addition, the
Table 1

Channel catfish CD156a amino acid sequence identity with those from other species.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of amino acids</th>
<th>Molecular mass (kDa)</th>
<th>% identity</th>
<th>Accession no.</th>
</tr>
</thead>
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<td>94.6</td>
<td>59.5</td>
<td>ACM61987</td>
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<td>Human</td>
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<td>88.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Species No. of amino acids Molecular mass (kDa) % identity Accession no.

Channel catfish 850 94.6 59.5 ACM61987
Zebrafish A 843 93.5 41.4 NP_956931
Zebrafish B 784 87.3 41.6 XP_001344600
Chicken 726 79.5 41.3 XP_421552
Rat 825 89.7 40.8 NP_031429
Mouse 826 90.0 39.1 AA15405
Human 824 88.7 39.1

Molecular mass of each CD156a and percentage of identity were calculated by the Pepstats and the Blosum 62 matrix of the Needle softwares, respectively, via [http://www.ebi.ac.uk](http://www.ebi.ac.uk).

Fig. 2. Tissue distribution of the channel catfish CD156a gene transcript (n = 3). Two-step RT-PCR assays were performed as described previously (Yeh and Klesius, 2007a,b, 2008a,b,c). The sizes of the PCR amplified fragments for CD156a (upper band) and β-actin (lower band) transcripts were 841 and 203 nucleotides, respectively. Spleen, lanes A, G, and M; anterior kidney, lanes B, H, and N; liver, lanes C, I, and O; intestine, lanes D, J and P; skin, lanes E, K, and Q; and gill, lanes F, L and R. Lanes S, no RT template control, and T, λ DNA/HindIII molecular size markers (Promega Corp., Madison, WI).


