Fish & Shellfish Immunology 41 (2014) 466–472

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

HdhCTL1 is a novel C-type lectin of abalone *Haliotis discus hannai* that agglutinates Gram-negative bacterial pathogens

Jian Zhang [a, c, 1], Reng Qiu [a, b, 1], Yong-hua Hu [a, *]

[a] Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[b] China-UK-NYNU-RRes Joint Laboratory of Insect Biology, Nanyang Normal University, Nanyang, 473061 Henan, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 30 May 2014
Received in revised form
16 September 2014
Accepted 29 September 2014
Available online 7 October 2014

Keywords:
Haliotis discus hannai
C-type lectin
Agglutination

ABSTRACT

C-type lectins (CTLs) are Ca^{2+}-dependent carbohydrate recognition proteins, which play important roles in the innate immunity of both vertebrates and invertebrates. In this study, we identified and characterized a C-type lectin (named HdhCTL1) from Pacific abalone, *Haliotis discus hannai*. HdhCTL1 is composed of 176 amino acid residues and shares low (23.9%) identity with the known CTL of abalone. HdhCTL1 possesses a putative signal peptide and a carbohydrate-recognition domain (CRD) typical of CTLs. The CRD of HdhCTL1 contains four disulfide bond-forming cysteine residues that are highly conserved in CTLs. HdhCTL1 mRNA was detected in a wide range of tissues and expressed abundantly in the digestive gland. Experimental infection with the bacterial pathogen *Vibrio anguillarum* significantly upregulated HdhCTL1 expression in a time-dependent manner. Recombinant HdhCTL1 (rHdhCTL1) purified from *Escherichia coli* was able to agglutinate Gram-negative bacterial pathogens. The agglutinating ability of rHdhCTL1 was abolished in the presence of mannose. These results suggest that HdhCTL1 is a novel CTL which is likely to be involved in host defense against bacterial infection.

1. Introduction

The innate immune system is a universal and ancient form of defense against microbial infection. Invertebrates, which lack an adaptive immune system, mainly rely on innate immune reactions for their defense [1]. The first step of innate immune response is to recognize the conserved molecular patterns that are essential products of microorganisms, such as lipopolysaccharides (LPS), lipoteichoic acids, lipoproteins, peptidoglycan (PGN) and (1 → 3) β-D-glucans. These invariant structures are referred to as pathogen-associated molecular patterns (PAMPs). These PAMPs are recognized by receptors of the innate immune system called pattern recognition receptors (PRRs) [2]. There are at least six subgroups of PRPs in invertebrate, i.e. peptidoglycan recognition protein (PGRP), thioester-containing protein (TEP), gram-negative binding protein (GNBP), multi-domain scavenger receptor (SCR), C-type lectin (CTL) and galectin (GALE) [3].

CTLs are a superfamily of Ca^{2+}-dependent carbohydrate recognition proteins. The main characteristic of these proteins is the presence of a carbohydrate-recognition domain (CRD), which consists of approximately 115–130 amino acids with several conserved motifs. It is structurally characterized by double-loops composed of four cysteines, which form two highly conserved disulfide bridges located at the bases of the loops. Different CTLs can have one or more CRDs in their multi-domain structures [4].

CTLs play important roles in the innate immunity of both vertebrates and invertebrates, including phagocytosis and cell–cell adhesion and recognition [5–7]. In recent years, there are more and more C-type lectins identified from invertebrates, especially from arthropods such as insects [8–11] and crustaceans [12–14]. Compared to arthropods and crustaceans, much less research has been performed with mollusk. The documented mollusk lectins are mainly from scallops, clams and oysters, and most of them are believed to participate in immune defense [15–18]. To date only one C-type lectin gene from abalone (*Haliotis discus discus*) was cloned and analyzed [19].

Pacific abalone *H. discus hannai* is a commercially valuable mollusk species cultured in China. Various diseases, commonly caused by bacterial pathogens belonging to the *Vibrio* genus [20–23], have threatened the sustainable development of the

* Corresponding author. Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China. Tel.: +86 532 82898779.
E-mail address: huyonghua@qdio.ac.cn (Y.-h. Hu).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.fsi.2014.09.032
1050-4648/

abalone industry. In this report, in order to facilitate the understanding of the immune system of abalone, we cloned the full-length cDNA of a C-type lectin from Pacific abalone *H. discus hannai* and studied its expression and activity.

2. Materials and methods

2.1. Animals

Clinically healthy Pacific abalone, *H. discus hannai*, averaging 63 ± 4.5 mm in shell length, were kept in aerated sea water (20 °C). The animals were acclimatized in the laboratory for two weeks before experimental manipulation. Before experiment, abalone (8% of the stock) were randomly sampled for the examination of potential existence of bacteria in hemocytes. For this purpose, hemocytes were taken under aseptic conditions and plated in Luria–Bertani broth agar plates. The plates were incubated at 28 °C for 48–72 h and examined for bacterial appearance. No bacteria were detected from the sampled animals.

2.2. Bacterial strains and culture conditions

Edwardsiella tarda TX1, *Vibrio/Listonella anguillarum* C312, and *Streptococcus iniae* SF1 have been reported previously [24–26]. *Escherichia coli* BL21(DE3) was purchased from TransGen Biotech Beijing, China. All strains were cultured in Luriae-Bertani broth (LB) medium at 28 °C except for *E. coli*, which was cultured at 37 °C.

2.3. Cloning of HdhCTL1

Total RNA was isolated from hemocytes using the HP Total RNA kit (Omega Bio-tek, USA). The mRNA was purified and used for the construction of cDNA library with the Super SMART PCR cDNA Synthesis Kit (Clontech, USA) according to manufacturer's instructions. One thousand and five hundred clones of the library were randomly selected and subjected to DNA sequence analysis; one clone was discovered to contain the full-length cDNA of *HdhCTL1* with 5′- and 3′-untranslated regions (UTRs). The nucleotide sequence of *HdhCTL1* has been deposited in GenBank database under the accession number KJ865914.

2.4. Sequence analysis

Nucleotide and amino acid sequences were analyzed with the BLAST program at the National Center for Biotechnology Information (NCBI). Domain architecture analysis was performed using the simple modular architecture research tool (SMART) version 4.0 and the conserved domain database (CDD) of NCBI. The molecular mass and theoretical isoelectric point (pI) calculations were carried out with EditSeq in DNASTAR software package (DNASTAR Inc. Madison, WI, USA). Multiple sequence alignment was implemented with the ClustalX program. Phylogenetic analysis was performed using MEGA 4.0 with Neighbor-joining (NJ) algorithm.

2.5. Quantitative real time reverse transcription-PCR (qRT-PCR)

For *HdhCTL1* expression under normal physiological conditions, digestive gland, foot muscle, hemocytes, mantle, gill and adductor muscle were taken aseptically from four abalone and used for total RNA extraction with the HP Total RNA kit (Omega Bio-tek, USA). The RNA was treated with DNase with the kit of RNase-Free DNase Set (Omega Bio-tek, Doraville, GA, USA). One microgram of total RNA was used for cDNA synthesis with the Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Quantitative real time reverse transcriptase-PCR (qRT-PCR) was carried out in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) using the SYBR ExScript qRT-PCR Kit (Takara, Dalian, China) as described previously [27,28]. The expression level of *HdhCTL1* was analyzed using comparative threshold cycle method ($2^{-\Delta\Delta CT}$) with elongation factor-1-α (EF1A) as the internal control as reported previously [29]. The PCR primers for *HdhCTL1* are RTF1 (5′-AGGACTGCGTGCTGATGAAG-3′) and RTR1 (5′-TGGTTACGGGTGCTGTTGTG-3′). Melting curve analysis was operated at the end of each PCR to verify that only one amplicon was amplified. All data are given in terms of mRNA levels relative to that of EF1A. For *HdhCTL1* expression during bacterial infection, *Vibrio anguillarum* was cultured in LB medium and resuspended in PBS to 2×10^8 colony forming units (CFU)/mL. Abalone were divided randomly into two groups (24 abalone/group) and injected intramuscularly with 100 μl of bacteria or PBS (control). At 1.5 h, 3 h, 6 h, 12 h, 24 h and 48 h post-infection, hemocytes and gill were taken from the abalone (four/per time point) in each group and used for qRT-PCR as described above. The internal qRT-PCR references for hemocytes and gill were α-tubulin and β-actin

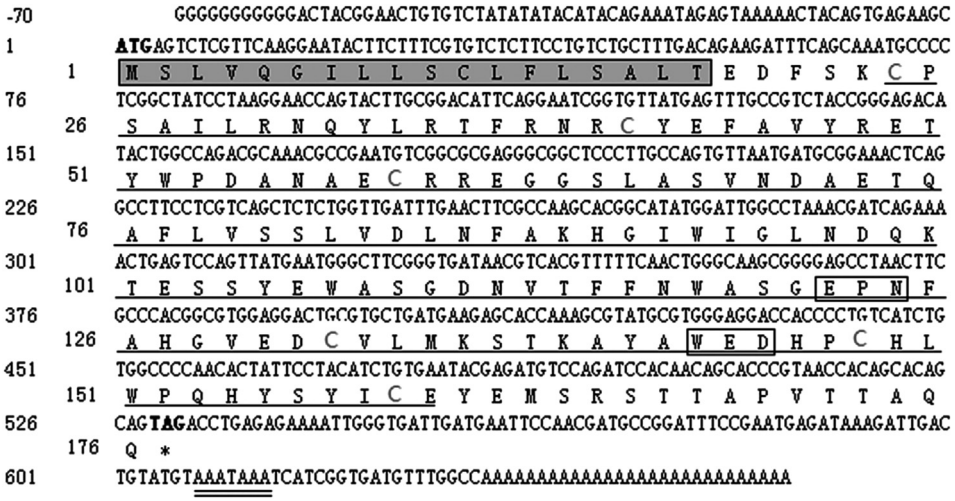

Fig. 1. The nucleotide and deduced amino acid sequences of HdhCTL1. The nucleotides and amino acids are numbered along the left margin. In the nucleotide sequence, the translation start and stop codons are marked in bold and the polyadenylation signal is doubly underlined. In the amino acid sequence, the putative signal peptide sequence is shaded and the CRD is underlined. In CRD, the conserved cysteine residues are in red, and the EPN and WED motifs are boxed.