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# Characterization of acid phosphatase from the tick *Haemaphysalis longicornis*

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ABSTRACT

The full-length cDNA encoding acid phosphatase (HL-3) from *Haemaphysalis longicornis* was obtained by 5′ rapid amplification of cDNA ends (RACE). The cDNA contained a 1137 bp open reading frame (ORF) coding for 356 amino acids with a predicted theoretical isoelectric point (p*I*) of 6.35 and molecular weight of 41.0 kDa. The recombinant protein was expressed in *Escherichia coli*. The enzyme could hydrolyze para-nitrophenyl phosphate (pNPP) substrate at an optimum pH of 5.0. Real-time RT-PCR analysis showed that the HL-3 transcripts were expressed in various stages of unfed ticks and were significantly induced by blood feeding. Furthermore, the expression of HL-3 in midguts was significantly higher than in other tested tissues of partially fed adult ticks. The transcripts of the HL-3 mRNA in lipopolysaccharide (LPS)-injected ticks were 1.75 times of the PBS-injected control; *Theileria sergenti* infected larvae expressed 3.86 more times than that of uninfected ones. Western blot analysis showed that rabbit antiserum against the recombinant rHL-3 could recognize a native protein of approximately 41.0 kDa in the lysates from different stages of ticks. Vaccination of rabbits with the rHL-3 conferred partial protective immunity against ticks, resulting in 28% mortality and 10.6% reduction in engorgement weight of adult ticks, respectively. These results suggested that the HL-3 was involved in tick innate immunity and could be used as a potential candidate antigen for the development of anti-tick vaccines.



## 1. Introduction

*Haemaphysalis longicornis* is a three-host tick and can severely affect the health of its hosts and even lead them to die (Yin and Luo, 2007). *H. longicornis* and the diseases it transmits have an adverse effect on the livestock industry and cause significant economic losses (Bitam and Raoult, 2009; Chen et al., 2010). Currently the principal method of tick control is the application of acaricides, however, this has many limitations, such as chemical pollution of the food chain as well as the development of resistance against acaricides (Graf et al., 2004). Therefore, it is necessary to search an alternative method of tick control. The commercialization of Bm86 reveals the promise of an immunological method of tick control (Willadsena et al., 1995). This approach, however, mainly depends on the identification of protective antigens in ticks. To this end, studies have been undertaken to identify and characterize the tick-derived bioactive molecules from *H. longicornis* (Tian et al., 2009, 2010; Liu et al., 2009).

Lysosome was firstly named in 1955 (De Duve et al., 1955). It includes many kinds of enzymes, such as acid phosphatase, alkaline phosphatase and ribonuclease. Lysosome is an acidic membrane-delimited organelle that plays a critical role in the cellular digestion of a diverse range of macromolecules including proteins, carbohydrates, nucleic acids, and lipids (Kimball, 2007). It is also involved in the host immune response and digestion of extraneous mate-

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rial and intracorporal metabolite. Acid phosphatase, as one of the phosphatases, can hydrolyze phosphomonoesters at an acidic pH when fused with endosomes. It is synthesized and transported as an integral type I membrane glycoprotein with a large luminal domain containing the active site of the enzyme, a single transmembrane domain, and a short cytoplasmic domain (Gottschalk et al., 1989).

Acid phosphatase has been found in many species. For example, acid phosphatase played an important role in the invasion and release of the trypanosomes in host, which indicated the significant effects in pathogenesis (Lonsdale-Eccles and Grab, 2002). Three types of acid phosphatase were confirmed in *Trypanosoma brucei*, and this research suggested that the enzyme may exist as an isoenzyme (Amlabu et al., 2009). The location and restricted functional analysis of acid phophastase from *Rhipicephalus sanguineus* and *Boophilus microplus* have been reported (Scopinho et al., 2008; Nunes et al., 2006). However, there has been no report on the acid phosphatase of *H. longicornis*.

Acid phosphatase, as the marker of lysosome, is thought to be essential to the biological functions of organism, including the regulation of metabolism, energy conversion, signal transduction and clearance of xenobiotics (Tasaki et al., 2001; Bull et al., 2002). The enzyme also plays an important role in the immune system of organism, while the innate immunity is critical way for ticks to combat microbial invasion. The functions of acid phosphatase have been reported in many species. For example, acid phosphatase in poplar, as one of the defense proteins, was upregulated when they were wounded, and it was thought to be a component of the defense response against leaf-eating herbivores (Veljanovski et al., 2010). Further reports have described the functions of acid phosphatase as a defense protein in animals and plants, such as regulation of the ingestion and digestion of apoptotic cells and body by macrophages, tissue renovation (Huo et al., 2000) and regulation of the host immune response to apoptosis and foreign bodies (Veljanovski et al., 2010; Scopinho et al., 2008). Additionally, its serum level could be used as a diagnostic marker for disease in the corresponding organs, for example, prostate cancer can be detected by the measurement of elevated levels of prostatic acid phosphatase (Becker et al., 2010). Though the location and specific function of acid phosphatase has been found in several ixodid ticks (Scopinho et al., 2008; Nunes et al., 2006), our knowledge of the gene is still very limited in ticks.

In the present study, we cloned and partially characterized the acid phosphatase from the hard tick *H. longicornis*, and expressed it in *Escherichia coli*. In addition, we assessed the biological function and distribution of this enzyme, and concluded that the enzyme may be involved in the innate immunity against *H. longicornis* tick.

## 2. Materials and methods

### 2.1. Ticks, tissue collection, total RNA extraction and cDNA synthesis

Different stages of *H. longicornis* ticks were cultured by feeding on rabbits and sheep for several generations in our laboratory. Infected *H. longicornis* larvae were maintained on cattle infected with *Theileria sergenti*. Samples of salivary glands, ovaries, and midguts were separated from partially engorged adult females (Tian et al., 2010).

Total RNA was extracted from different stages, unfed and partially fed ticks using the TRIzol reagent (TaKaRa) according to the manufacturer's instructions, and was stored at −70 °C until used. The purity of the isolated RNA was determined by measurement of the OD 260/280 nm ratio (2.06). Total RNA (~4 μg) was reverse transcribed to synthesize first strand cDNA with the Oligo $(dT)_{18}$ primer using cDNA Synthesis Kit (Fermentas, EU).

### 2.2. Cloning and sequence analysis of the full-length cDNA of HL-3

To obtain the full-length sequence of HL-3, which was screened from a cDNA library constructed from unfed female *H. longicornis* ticks, the first strand cDNA synthesis of adult female ticks was performed using the 5′ RACE system (TaKaRa) according to the manufacturer's instructions. A gene-specific primer (GSP: GTACGGCTTGGCGATCTC-CTTG) was designed from the known 3′ fragment and used for 5′ RACE to amplify and clone the full length of HL-3 cDNA. The amplified products were ligated into the pGEM-T Easy Vector (TaKaRa).

The positive clones were sequenced with vector-specific primers (T7 and SP6). The sequence and deduced protein were analyzed using the DNASTAR software (DNASTAR, Madison, WI), Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Motif Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan). Putative signal peptide cleavage site was determined with the prediction servers SignalP (http://www.cbs.dtu.dk/services/SignalP). The transmembrane domain was analyzed using Tmap software (http://weblab.cbi.pku.edu.cn/program.inputForm.do?program=tmap(v6.0.1)). A neighbor-joining (NJ) phylogenetic tree was constructed using MEGA software (Koichiro et al., 2007). The deduced primary protein sequence of HL-3 was submitted to the SWISS-Model server to construct the 3D model structure (http://swissmodel.expasy.org/), which is based on the highest similarity and known three-dimensional structure of human prostatic acid phosphatase (PDB ID: 1nd6).

### 2.3. The expression of HL-3 in vitro

The cDNA encoding HL-3 was generated by PCR using the adult tick cDNA as a template and gene-specific primers (sense primer: 5′-GCGGATCCATGAAGCTCGCGTGCCTCT-3′, containing ATG translation start codon and *BamH*I restriction enzyme site; anti-sense primer: 5′-GCCTCGAGTGCAGTGTTATCTGCGGCT-3′, containing *Xho*I restriction enzyme site). Then, it was ligated into the pGEM-T easy vector.

The purified recombinant plasmid was digested with *BamH*I/*Xho*I restriction enzymes to create the HL-3 cDNA insert, which was subsequently ligated into the BamHI/XhoI cloning site of the PGEX-4T-1 expression vector. The obtained positive plasmids were transformed into *E. coli* strain BL21 (DE3) pLysS (TaKa). In order to induce the expression of recombinant plasmid, it was necessary