Ticks and Tick-borne Diseases 4 (2013) 138–144

Contents lists available at SciVerse ScienceDirect

Ticks and Tick-borne Diseases

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

Original article

Identification and partial characterization of a gut *Rhipicephalus appendiculatus* cystatin

Saiki Imamura[a], Satoru Konnai[a,*], Shinji Yamada[a], Luís F. Parizi[b], Naftaly Githaka[a], Itabajara da S. Vaz Jr.[b], Shiro Murata[a], Kazuhiko Ohashi[a]

[a] *Department of Infectious Diseases, Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Sapporo 060-0818, Hokkaido, Japan*
[b] *Centro de Biotecnologia do Estado do Rio Grande do Sul and Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, C.P. 15005, 91501-970 Porto Alegre, RS, Brazil*

ARTICLE INFO

Article history:
Received 24 September 2012
Received in revised form 8 November 2012
Accepted 8 November 2012

Keywords:
Anti-tick vaccine
Cystatin
Rhipicephalus appendiculatus

ABSTRACT

Vaccines are among the alternative tick control methods expected to replace at least in part the volumes of chemical acaricides currently used worldwide. However, a vaccination approach depends on a host immune response against proteins that are essential to tick physiology. The cystatin family is a protein class recently investigated to compose an effective antigen in a tick vaccine. In this study, a cDNA from *Rhipicephalus appendiculatus* with high sequence similarity to cystatins type 2 was identified by random sequencing analysis and called *R. appendiculatus* cystatin 1 (*Ra-cyst-1*). DNA sequence analysis showed that the cloned *Ra-cyst-1* has a 423-bp open reading frame and codified to a 140-amino acid polypeptide. The putative mature protein consists of 115 amino acid residues with a deduced molecular weight of 12.8 kDa. The highly conserved G (P-I), QxVxG (P-II), and PW (P-III) type 2 cystatins motifs are present in *Ra-cyst-1* cDNA. RT-PCR analysis showed that the *Ra-cyst-1* gene is expressed in nymph, male, and female midgut following blood feeding, but not in the salivary glands of fed females. In addition, Western blot revealed that recombinant *Ra-cyst-1* was not recognized by sera derived from rabbits infested with ticks, suggesting that this cystatin is not secreted into the host during infestation. We hypothesize that Ra-cyst-1 may play a role in the tick feeding process and could be a concealed antigen candidate in further anti-tick vaccination trials.

© 2012 Elsevier GmbH. All rights reserved.

Introduction

Ticks have been recognized as harmful ectoparasites to animals and humans since ancient times (Sonenshine, 1993). Although ticks cause a variety of adverse effects in cattle, such as reduced growth and low milk production, they are mostly known for their role as vectors of pathogens that cause severe diseases (Sonenshine, 1993). Conservative estimates indicate that ticks affect approximately 800 million cattle (Sutherst et al., 1982), with economic losses to the cattle industry nearing US$ 7 billion (Sutherst et al., 1982; de Castro, 1997). The economic losses, public importance of ticks and tick-borne diseases and the growing difficulties around current tick control methods reveal the need for new alternative multi-faceted vector control programs, including anti-tick vaccines (Parizi et al., 2012). *Rhipicephalus appendiculatus*, the brown ear tick, infests both domestic and wildlife hosts that transmits numerous pathogens, like *Theileria parva*, an intracellular protozoan parasite that causes East Coast fever and other related theileriosis (Norval et al., 1991) as well as several viruses, such as the Thogoto virus (Nuttall, 1998) and the Nairobi sheep disease virus (Holzer et al., 2011). Chemical acaricides have prevailed as the most effective and commonly used method to control *R. appendiculatus*. However, growing public concern over the presence of acaricide residues in milk and meat (Nolan, 1990) is a powerful incentive to vaccine development strategies directed towards preventing tick parasitism. However, the development of tick vaccines is a lengthy process, which includes antigen identification and characterization of the immune response induced by vaccination (Maritz-Olivier et al., 2012).

Cystatins are a large family of inhibitors that interact reversibly with papain-like cysteine proteases and legumains (Abrahamson et al., 2003). As regulators of the activity of cysteine proteases, cystatins modulate physiological processes such as antigen processing and presentation, phagocytosis, and expression of cytokines (Zavasnik-Bergant, 2008). Moreover, cystatins protect the cells/tissues against lysosomal cysteine proteases released from apoptotic cells, phagocyte degradation, proliferating tumor cells, and invading pathogens (Abrahamson et al., 1991). In recent years, tick cystatins have been identified in several hard and soft tick

* Corresponding author at: Graduate School of Veterinary Medicine, Hokkaido University, Kita 18, Nishi 9, Sapporo 060-0818, Hokkaido, Japan.
Tel.: +81 11 706 5215; fax: +81 11 706 5217.
E-mail address: konnai@vetmed.hokudai.ac.jp (S. Konnai).

1877-959X/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.ttbdis.2012.11.001

species (reviewed by Schwarz et al., 2012). In ticks, these proteins are involved in processes such as hemoglobin digestion (Yamaji et al., 2010), blood feeding (Kotsyfakis et al., 2007, 2008; Yamaji et al., 2009; Salát et al., 2010), immune system regulation (Karim et al., 2005; Kotsyfakis et al., 2006, 2007; Salát et al., 2010), and pathogen transmission (Kotsyfakis et al., 2010). Due to their importance in tick physiological processes and immunogenicity analysis, the potential application of cystatins as antigens in the development of an anti-tick vaccine has been the object of research (Kotsyfakis et al., 2007, 2008; Salát et al., 2010). In this study, we report the molecular identification and partial characterization of a new tick cystatin (*Ra-cyst-1*) from *R. appendiculatus*.

Materials and methods

Animals and ticks

R. appendiculatus ticks used in this study were originally collected from vegetation by dragging with a cotton flannel in the region of Shybuyunji, central Zambia. A laboratory colony of *R. appendiculatus* was established using 4-week-old Japanese white rabbits as host (Branagan, 1973).

RNA extraction

Tick tissues were dissected in ice-cold Medium-199 (Sigma) with Hanks' salts and 20 mM MOPS buffer (pH 7.0), 0.1 g/L streptomycin sulfate, and 0.03 g/L penicillin. After removal, tissues were washed in the same ice-cold buffer. Dissected tissues were immediately stored in RNA later (Ambion, Austin, TX, USA) until use. Total RNA was extracted from salivary glands (SG), midguts (MG), and carcasses (C) (tick remnant after removal of SG, MG, and ovary) (Chalaire et al., 2011) of fed and unfed female ticks and from whole male and nymphal ticks using trizol reagent (Invitrogen, CA, USA), according to the manufacturer's instructions.

cDNA library construction and random sequencing

cDNA library was constructed from total RNA extracted from adult *R. appendiculatus* female ticks using the Creator SMART cDNA library Construction Kit (Becton, Dickinson and Company, USA), according to the manufacturer's instructions. Nucleotide sequencing was performed in an automated sequencer (Beckman Coulter, CA, USA) with vector-specific primers packaged in the Creator SMART cDNA library Construction Kit. DNA sequence analysis was carried out using a software package, GENETYX-WIN version 4.04 (Software development Co. Ltd., Tokyo, Japan), and sequence fragments were compared with those of known proteins on the Swiss-Prot database.

In silico analyses

Phylogenies were constructed according to the Neighbor-Joining method using MEGA5 (Tamura et al., 2011). Bootstrap support values were estimated using 1000 bootstrap replicates. The accession numbers for sequences used in the phylogenic analysis are as follows: *Amblyomma variegatum*, BK007289; *Dermacentor silvarum*, HQ904072; *Haemaphysalis longicornis*, EU426545, EU019714, AB510962; *Ixodes scapularis*, XM002400180, XM002408543, XM002403952, XM002412212, XM002434396, DQ066048, AF483724; *Ornithodoros moubata*, AY521024, AY547735; *Ornithodoros parkeri*, EF633981; *R. appendiculatus*, JX082307.

The antigenic index analysis of Ra-cyst-1 and other tick proteins was calculated using the Jameson–Wolf algorithm in the software LASERGENE, version 7.0.0, to predict antigenic determinants by combining existing methods for protein structural predictions (Jameson and Wolf, 1988). The species used in the analysis were: *A. variegatum*, *D. silvarum*, *H. longicornis*, *I. scapularis*, *O. moubata*, and *R. appendiculatus*.

RT-PCR expression analysis

To determine expression profile of *Ra-cyst-1*, the oligo-dT primed first strand cDNA was synthesized in 10 μL of a standard reaction mixture (Takara Bio Inc., Shiga, Japan) using 5 μg of total RNA from female SG, MG, and C, whole male and nymphal ticks. RT-PCR product aliquots of 1 μL were used as templates in 50 μL of a standard PCR reaction mixture with gene specific primers (sense primer: 5′-ATGGCTCCTTTGAGAATCGCCAC-3′, anti-sense primer: 5′-TTAGGTGGATGTGGTACTT-3′). Tick β-actin primers (sense primer: 5′-TGTGACGACGAGGTGGACAATG-3′, anti-sense primer: 5′-GAAGCACTTGAGGTGGACAATG-3′) were used as RNA quality control (Vaz et al., 2005). The recombinant plasmid bearing a cloned *Ra-cyst-1* was used as a PCR template for positive control, while filtered distilled water was used as a negative control. Template without reverse transcription was used as negative control for contamination with genomic DNA. Ten μL of the PCR products were analyzed on a 2% agarose gel containing 1 μg/mL ethidium bromide.

Expression of recombinant Ra-cyst-1 (rRa-cyst-1)

The coding sequences were initially generated by PCR using the cloned full-length cDNA of *Ra-cyst-1* as a template, using gene-specific primers bearing the *Eco*R V/*Sac* I (New England Biolabs Inc. MA, USA) restriction enzyme sites to the directional cloning. The resulting plasmid was transformed and propagated in *Escherichia coli* strain DH5α (Promega), and subsequently purified by QIAprep Spin Miniprep Kit (Qiagen, Hilden, Germany). The purified plasmid was digested with appropriate restriction enzymes to create the *Ra-cyst-1* cDNA insert, which was subsequently ligated into corresponding cloning sites of the pET-43a expression vector. The recombinant Ra-cyst-1 was expressed in *E. coli* strain AD494 (DE3) pLysS (Novagen, WI, USA) following induction with IPTG 0.9 mM. The bacteria culture was incubated at 37 °C for 6 h post-induction, and the recombinant Ra-cyst-1 recovered in insoluble form in fusion with Nus-tagged thioredoxin (Nus). The Nus-rRa-cyst-1 insoluble protein was refolded using the protein refolding kit (Novagen, USA) according to the manufacturer's instructions. The refolded rRa-cyst-1 was affinity-purified on nickel-charged columns under denaturing conditions following manufacturer's instructions (Novagen). The recombinant protein RIM36 (rRIM36) expressed previously was used as a serological positive control, since it is an immunodominant protein presents in saliva (Imamura et al., 2008).

Generation of anti-tick saliva rabbit sera

Rabbit sera against feeding ticks (larva, nymphs, and adults) were generated as described previously (Mulenga et al., 1999). Briefly, 2 rabbits were repeatedly infested with each stage of ticks 4 times (10 adult pairs, 40 nymphal, and 100 larval ticks per rabbit ear) to induce antibodies against antigens present in tick saliva during feeding. Western blot analyses were performed using pooled sera of infested rabbits to confirm the presence of antibodies in the sera against tick saliva proteins.

Download English Version:

https://daneshyari.com/en/article/2474242

Download Persian Version:

https://daneshyari.com/article/2474242

Daneshyari.com