Available online at www.sciencedirect.com
ScienceDirect
Insect Biochemistry and Molecular Biology 36 (2006) 943–953
Insect Biochemistry and Molecular Biology
www.elsevier.com/locate/ibmb

New member of the protein disulfide isomerase (PDI) family identified in *Amblyomma variegatum* tick

Petra Knizetova[a,1], Iveta Vancova[a], Paulina Kocakova[a], Mirko Slovak[c], Paul Proost[b], Juraj Kopacek[a,*]

[a]*Centre of Molecular Medicine, Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava 845 05, Slovakia*
[b]*Laboratory of Molecular Immunology, Rega Institute, K.U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*
[c]*Institute of Zoology, Slovak Academy of Sciences Dúbravská cesta 9, Bratislava 845 06, Slovakia*

Received 6 February 2006; received in revised form 15 August 2006; accepted 12 September 2006

Abstract

Ticks belonging to arthropoda are blood feeding, geographically widespread ectoparasites of mammals, reptiles and birds. Their saliva contains active substances that protect them from host immune attack and allow for transmission of various pathogens during the feeding process. Characterization of tick saliva components can therefore contribute to the development of effective methods for the control of tick-borne diseases.

Here we describe the identification and basic characterization of a gene encoding a 55 kDa protein found in the salivary glands (SG) of *Amblyomma variegatum* tick. Based on the primary structure and homology to the family of protein disulfide isomerases (PDI; EC 5.3.4.1) the gene was named *AvPDI.* The 1461 nt long AvPDI open reading frame codes for a 487 amino acid protein. *In vitro* expressed AvPDI was exclusively localized in the endoplasmic reticulum. RT-PCR and Western blot analysis revealed that AvPDI expression is not restricted to the SG of the tick. More detailed analysis on tissue slides from SG detected an AvPDI specific signal in granular cells of the acini type II and III. Finally, reductase activity of AvPDI was confirmed in an insulin assay. The structural and functional characteristics suggest that AvPDI is another member of the PDI protein family and represents the first more closely characterized PDI in the ticks.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: *Amblyomma variegatum*; Gene expression; Protein disulfide isomerase; Endoplasmic reticulum

1. Introduction

Correct posttranslational modification of proteins during the maturation process is essential for proper functioning of the cell. Folding of nascent polypeptides, including secretory proteins is associated with disulfide bond formation in the endoplasmic reticulum (ER). Disulfide formation is initially error prone, so that false cysteines are connected, or the correct cysteines are paired in temporal order that inhibits proper folding (Creighton, 1991). To minimize mistakes in disulfide bond formation, the cell disposes with a wide range of proteins (as folding assistants), which are involved in the processes of formation, reduction and rearrangement of disulfide bonds. PDI is one of the most abundant ER-resident proteins participating in these processes. Moreover, independently of its catalytic activity, PDI exhibits chaperone-like activity by inhibiting the aggregation of unfolded proteins (Puig and Gilbert, 1994). The slow spontaneous folding of disulfide-containing proteins (hours to days) *in vitro* is incompatible with the time scale of secretion (~30–60 min) *in vivo*. In eukaryotic cells, this discrepancy is solved by the special redox conditions in the ER which create a more oxidizing environment than in the cytosol to facilitate disulfide bridge formation and rearrangement (Fassio and Sitia, 2002). PDI and other folding catalysts accelerate slow chemical steps that accompany folding. PDIs were isolated from a broad range of different species, including plants

*Corresponding author. Tel.: +421 2 59302 404; fax: +421 2 54774 284.
E-mail address: virukopa@savba.sk (J. Kopacek).

[1]Present adrress: Laboratory of Molecular Pathology and Institute of Pathology, Palacky University, Hnevotinska 3, Olomouc CZ-775 15, Czech Republic.

0965-1748/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibmb.2006.09.005

but the structure and function of PDI have been only intensively studied in mammals.

In general, PDI is a dimer consisting of two identical subunits of about 57 kDa. The main structural feature of PDIs is the presence of two independent thioredoxin domains comprising two active sites that contain the consensus sequence WCGHC (Vuori et al., 1992; Lyles and Gilbert, 1994) and an ER retention signal (K/HDEL) present in the carboxyl terminus (Denecke et al., 1992). Several PDI-like proteins with different primary structures and expression patterns were also identified (Ferrari and Söling, 1999).

To facilitate blood feeding, ticks have developed special host immunity-modulation mechanisms, in which bioactive molecules present in salivary glands (SG) play important role. Tick saliva contains cement-like material for tick's attachment to the host, prostaglandins, anticoagulants, immunosuppressive and anti-inflammatory protein molecules that help to overcome host defense responses (Sauer et al., 1979, 1995; Binnington and Kemp, 1980; Jaworski et al., 1992; Ribeiro et al., 1992; Bowman et al., 1997). The goal of the majority of studies is to characterize tick SG proteins that influence pathogen–host–vector relationships. Such an understanding could lead to the establishment of new means of control and prevention against tick-dependent pathogen transmission. The multifunctional character and in particular the ubiquitous expression of PDI highlights the question on the importance of its activity for tick's feeding and survival.

In the present study, we identified *Amblyomma variegatum* (*A. variegatum*) gene encoding a protein with homology to PDI. We focused on the characterization of the primary structure, the cellular localization and the basal functional characterization of this novel member of the PDI family.

2. Materials and methods

2.1. Dissection of SG

A. variegatum male and female individuals were allowed to feed on California White rabbits. At first, male ticks were placed on the host, followed by the female ticks 5 days (d) later. Female and male ticks were gently taken from the rabbit on day 5 and 9, respectively, of the blood-feeding cycle. The SG from both male and female ticks were promptly taken out. Dissected organs (free of surrounding tissues) from 20 separately fed ticks were pooled and washed twice in ice-cold 0.2 mM NaCl solution, immediately frozen on dry ice and stored at −80 °C until use.

2.2. 2-D gel electrophoresis

Lyophilized samples of SG protein extract (SGE) from different partially fed ticks *A. variegatum* were solubilized in isoelectric focussing (IEF) buffer (8 M urea and 4% Triton X-100) containing 2% β-mercaptoethanol and 2% ampholines and incubated for 2 h at RT. After centrifugation at 50,000*g* for 2 h (Biofuge 28 RS, Heraeus), 200 μg of sample was loaded per IEF gel prepared with 2% ampholines of pH range 3–10 (Sigma) and focussed at 700 V overnight. For the second dimension, a linear gradient of 7.5–12.5% acrylamide was used. After IEF, gels were equilibrated for 10 min in a buffer containing 62.5 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol and applied on the acrylamide gels. The gels were run at a constant current of 10 mA in a 3% stacking gel and at 80 mA in the resolving gradient gel. After electrophoresis, the gels were incubated overnight in a fixation solution and stained with silver according to Damerval et al. (1987).

2.3. Total RNA isolation

Total RNA from dissected organs of adult ticks that were fed for 5 d was isolated using the RNA Instapure^{TM}-LS System (Eurogentec) according to the manufacturer's instructions. Homogenization of dissected organs was improved using a special homogenizer (MOTOR, CORDLESS, Sigma). RNA concentration was quantified spectrophotometrically (GENEQUANTpro/Amersham Biosciences) and stored at −80 °C until use.

2.4. 3′ race RT-PCR

The first strand cDNA was reverse transcribed in a final reaction volume of 25 μl containing: 3 μg of total RNA; 10 μM of primer 5′-AGTACTAGTCGACGCGTGGCC-T_{20}-3′ and DNase and RNase-free (DEPC)-water up to 15 μl. The mixture was heated for 10 min at 70 °C and quickly chilled on ice. Then the master mix was added: 2.5 μl of 10 × M-MuLV Reaction Buffer (FINNZYMES, Finnzymes Oy, Finland); 1.25 μl of dNTP Mix (10 mM each, FINNZYMES) and 5.25 μl of DEPC-water. Mixture was gently shaken and incubated for 2 min at 42 °C, then 1 μl of M-MuLV RT (RNase H^-) (200 U/μl) was added. Following incubation for 50 min at 42 °C, the reaction was stopped by heating for 10 min at 70 °C. The primary PCR reaction was performed in a total volume of 25 μl. The reaction mixture contained: 2.5 μl of 10 × DyNAzyme EXT buffer (FINNZYMES); 2 μl of dNTP Mix; 1 μl of 10 μM 3′RACE primer 5′- AGTACTAGTCGACGCGTGGCC -3′; 1 μl of 10 μM AMB primer 5′-GTTCTCGATTACTCTGGATCCGAT-3′; 5 μl of cDNA; 1 μl of DyNAzyme EXT DNA polymerase (1 U/μl) (FINNZYMES) and 5 μl of sterile water. After the initial denaturation step at 95 °C for 3 min, the amplification was accomplished in 35 cycles each at 95 °C for 30 s; with a gradient of annealing temperatures: 50–55–60–65 °C for 30 s; 72 °C for 30 s and a final extension at 72 °C for 5 min. The second PCR reaction was performed in a total volume of 25 μl. The reaction mixture contained: 2.5 μl of 10 × DyNAzyme EXT buffer (FINNZYMES); 2 μl of dNTP Mix (10 mM each); 1 μl of

Download English Version:

https://daneshyari.com/en/article/1983127

Download Persian Version:

https://daneshyari.com/article/1983127

Daneshyari.com