Cloning and Characterization of a 2-Cys Peroxiredoxin from Babesia gibsoni

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ABSTRACT. Peroxiredoxins (Prxs) are a family of antioxidant enzymes. Here, we cloned a 2-Cys Prx, BgTPx-1, from the canine Babesia parasite B. gibsoni. Sequence identity between BgTPx-1 and 2-Cys Prx of B. bovis was 81% at the amino acid level. Enzyme activity assay by using recombinant BgTPx-1 (rbGTPx-1) indicated that BgTPx-1 has antioxidant activity. Antiserum from a mouse immunized with rbGTPx-1 reacted with parasite lysates and detect a protein with a monomeric size of 22 kDa and also a 44 kDa protein, which might be an inefficiently reduced dimer. BgTPx-1 was expressed in the cytoplasm of B. gibsoni merozoites. These results suggest that the BgTPx-1 may play a role to control redox balance in the cytoplasm of B. gibsoni.

KEY WORDS: antioxidant activity, Babesia gibsoni, canine, peroxiredoxin.

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Babesia gibsoni is a tick-borne intraerythrocytic apicomplexan parasite that causes piroplasmosis in dogs [5, 10]. The disease is characterized by remittent fever, progressive anemia, marked splenomegaly and hepatomegaly and sometimes causes death [9]. B. gibsoni infection is endemic in many regions of Asia, Africa, Europe and Americas [18]. Generally, B. gibsoni infection is characterized by recurrent infections even after treatment with anti-babesia drugs. To establish a method for effective treatment of canine babesiosis, more detailed analysis of mechanisms essential for survival of Babesia parasites in the host is important. However, the biological properties and life cycle of this pathogenic parasite remain poorly understood.

Since aerobic parasites live in an oxygen-rich environment in their host bodies, the parasites are likely to be subjected to the toxic effects of reactive oxygen species (ROS) that could cause damage to membrane lipids, nucleic acid and proteins [22]. For those parasites, redox balance control is considered to be an important biological property. To protect biological molecules from the effect of ROS, aerobes have evolved efficient defense systems of enzymatic antioxidants [24]. The 4 major cellular antioxidant enzymes are superoxide dismutase (SOD), glutathione peroxidase (Gpx), catalase and peroxiredoxin (Prx). Prxs constitute a family of proteins structurally homologous to the antioxidant of yeast [6] and have been identified in all living organisms from bacteria to humans [7, 20, 29]. Prxs reduce and detoxify hydrogen peroxides through the action of the highly conserved redox-active cysteine [29]. The family is classified into 3 groups based on the number of active cysteine residues: 1-Cys, typical 2-Cys and atypical 2-Cys types [28, 29]. Since 2-Cys Prxs use electrons provided by the small protein thioredoxin, these enzymes are also called thioredoxin peroxidases (TPx) [15, 19]. In recent years, several Prxs of malaria parasites have been characterized, and the structural and functional properties of the enzymes have been determined as key factors for development of new drugs [3, 11, 13, 16, 21]. Recently, a Prx from the bovine Babesia parasite B. bovis (BbTPx-1) was identified, and its antioxidant activity was demonstrated [27]. However, Prx in B. gibsoni has not yet been characterized.

In this study, we found a predicted 2-Cys Prx gene, BgTPx-1, from an expressed sequence tag (EST) database of B. gibsoni [1]. The size of the open reading frame (ORF) of the BgTPx-1 gene was 597 bp, and the gene coded for a protein comprised of 198 amino acid residues with a predicted molecular weight and theoretical isoelectric point of 21.95 kDa and 6.42, respectively (ExPASy Compute pl/Mw; http://web.expasy.org/). Amino acid sequence analysis using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/) and TargetP 1.1 server (http://www.cbs.dtu.dk/services/TargetP/) showed that the protein had no signal peptide. Multiple sequence alignment of BgTPx-1 with 2-Cys Prxs from other apicomplexan parasites revealed that BgTPx-1 had 81% sequence similarity with B. bovis BbTPx-1 [27], 57% with Toxoplasma gondii TgPrx [25], 59% with Cryptosporidium parvum CpTPx [14] and 52% with P. falciparum PfTPx-1 [17] (Fig. 1A). The presence of two conserved cysteine
residues in BgTPx-1 (Cys50 and Cys171), corresponding to Cys47 and Cys170 of the yeast Prx [6], suggested that it is a typical 2-Cys type Prx. The full sequence of the BgTPx-1 gene was deposited in the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) under accession number AB829722.

To demonstrate the enzymatic activity of this BgTPx-1, we produced a recombinant BgTPx-1 protein (rBgTPx-1) in Escherichia coli. Total RNA of B. gibsoni was prepared from dog erythrocytes infected with B. gibsoni Oita strain maintained in vitro as previously described [26] by using TRI reagent (Sigma-Aldrich, St. Louis, MO, U.S.A.). The dogs were housed, fed and given clean drinking water in accordance with the stipulated rules for the care and use of research animals promulgated by Obihiro University of Agriculture and Veterinary Medicine, Japan (approval number: 24–117). Parasite cDNA was synthesized from the total RNA using a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Basel, Switzerland). The BgTPx-1 gene ORF was amplified by reverse transcription-polymerase chain reaction (RT-PCR) using primer sets: forward primer (5′- CCC GAA TTC GTA GTT CGC GTA GGA CAG CCT GC −3′) and reverse primer (5′- CCC TTC GAG TTA AGA GAG TTT AGT GGT GAG GTG G −3′) (underlined sequences containing the EcoRI site and Xhol site, respectively). The PCR product was digested with EcoRI and Xhol and then ligated to the pGEX-6P1 vector containing an ORF encoding a glutathione S-transferase (GST)-fusion protein (GE Healthcare, Piscataway, NJ, U.S.A.). rBgTPx-1 was expressed as a GST-fusion protein in E. coli and purified using Glutathione-Sepharose 4B beads and PreScission protease. An SDS-PAGE image of rBgTPx-1 protein is shown. M, protein marker.
functional, typical 2-Cys Prx antioxidant, BgTPx-1, from B. gibsoni. Since BgTPx-1 has antioxidant activity, we assume that BgTPx-1 plays a role in the reduction of ROS.

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Fig. 3. Localization of native BgTPx-1 in B. gibsoni merozoites. (A) Western blot analysis of native BgTPx-1 using mouse anti-rBgTPx-1 serum. We lysed $10^9$ of B. gibsoni-infected canine erythrocytes (iRBC, parasitemia of 5%) or the same amount of normal erythrocytes (nRBC, negative control) as reported previously [32]. Samples were dissolved in 2 × SDS-PAGE sample buffer and heated at 96°C for 5 min. Then, 10 µl of lysates containing 5 × 10^6 RBC were separated by SDS-PAGE. The positions of molecular mass standards are indicated on the left. (B) Indirect immunofluorescence microscopy to determine cellular localization of BgTPx-1 in the parasite cells. For nuclear staining, Hoechst 33342 (Lonzans) was used. BF: bright field.
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