

Identification and functional analysis of a novel mitochondria-localized 2-Cys peroxiredoxin, BbTPx-2, from *Babesia bovis*

Tatsunori Masatani¹ · Masahito Asada² · Hassan Hakimi² · Kei Hayashi^{3,4} · Junya Yamagishi⁵ · Shin-ichiro Kawazu⁶ · Xuenan Xuan⁶

Received: 31 March 2016 / Accepted: 14 April 2016 / Published online: 19 April 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract Cysteine-based peroxidases, known as peroxiredoxins (Prx) or thioredoxin peroxidases (TPx), are important antioxidant enzymes that prevent oxidative damage caused by reactive oxygen species (ROS). In this study, we identified a novel mitochondrial 2-Cys Prx, BbTPx-2, from a bovine *Babesia* parasite, *B. bovis*. BbTPx-2 complementary DNA (cDNA) encodes a polypeptide of 254 amino acid residues. This protein has a mitochondrial targeting peptide at the N-terminus and two conserved cysteine residues of the typical 2-Cys Prx. By using a thiol mixed-function oxidation assay, the antioxidant activity of recombinant BbTPx-2 was revealed, and its antioxidant activity was comparable to that of a cytosolic 2-Cys Prx from *B. bovis*, BbTPx-1. Notably, we

confirmed that BbTPx-2 was expressed in the mitochondrion of *B. bovis* merozoites. Taken together, the results suggest that the mitochondrial BbTPx-2 is an antioxidative enzyme for scavenging ROS in *B. bovis*.

Keywords Antioxidant activity · *Babesia bovis* · Mitochondria · Peroxiredoxin

Introduction

Babesia parasites are tick-borne intra-erythrocytic protozoa in the phylum Apicomplexa. They infect a wide range of mammals, such as cattle, sheep, and horses, and the majority of them are responsible for serious economic losses in the livestock industry (Brown and Palmer 1999; Dewaal 2000; Schnittger et al. 2012). Similar to other parasites of this phylum such as *Plasmodium*, *Babesia* parasites undergo a complex life cycle involving both tick and mammalian hosts. *Babesia* parasites initiate infection in mammalian hosts by sporozoites, which are transmitted through the bites of infected ticks; subsequently, the merozoites invade and replicate within the infected erythrocytes, eventually leading to babesiosis (Hunfeld et al. 2008; Schnittger et al. 2012).

Babesia bovis is one of the most important species because of its impact on the cattle industry. *B. bovis* causes bovine babesiosis with clinical features such as anemia, fever, renal failure and, in severe cases, cerebral babesiosis, which is characterized by sequestration of infected erythrocytes in the microvasculature of the brain (Homer et al. 2000). Cattle that have recovered from acute infection become asymptomatic carriers, in which the parasites persist in blood for many years and recrudescence of parasitemia can occur at unfixed intervals (Bock et al. 2004). Although bovine babesiosis can be controlled by treatment with antiparasitic drugs, many drugs

✉ Tatsunori Masatani
tatsunorimasatani@gmail.com; masatani@vet.kagoshima-u.ac.jp

✉ Xuenan Xuan
gen@obihiro.ac.jp

¹ Transboundary Animal Diseases Research Center, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan

² Department of Protozoology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

³ Laboratory of Veterinary Parasitology, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka 020-8550, Japan

⁴ Department of Pathogenetic Veterinary Science, United Graduate School of Veterinary Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

⁵ Research Center for Zoonosis Control, Hokkaido University, North 20, West 10 Kita-ku, Sapporo 001-0020, Japan

⁶ National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

have been withdrawn from the market due to safety issues or emergence of resistance (Bork et al. 2005a; Vial and Gorenflot 2006). Therefore, to develop new therapeutic strategies for bovine babesiosis, more detailed analysis of mechanisms that are essential for survival of *Babesia* parasites in the host is important.

Since *Babesia* parasites proliferate in their mammalian hosts and the tick vectors under oxygen-rich environments, the parasite is likely to be subject to the toxic effects of reactive oxygen species (ROS) that could cause damage to membrane lipids, nucleic acid, and proteins (Robinson et al. 2010). Redox balance control is thought to be an important biological property for parasites living in host erythrocytes (Müller et al. 2003; Becker et al. 2004; Bosch et al. 2015). A major source of ROS in the parasite cell is heme, which is produced as a byproduct of hemoglobin digestion. Moreover, the parasites have a mitochondrion with a functional electron transport chain that may produce ROS. To protect themselves from damage caused by ROS, malaria parasites are equipped with antioxidant enzymes, such as peroxiredoxins (Prxs) (Becker et al. 2004; Kawazu et al. 2008). Prxs are known collectively as thioredoxin peroxidase (TPx) and are widely distributed among both eukaryotes and prokaryotes (Rhee et al. 2005). The family is classified into three groups based on the number and position of highly conserved active cysteine residues: namely, 1-Cys, typical 2-Cys, and atypical 2-Cys types (Vaca-Paniagua et al. 2009; Wood et al. 2003). In recent years, several TPxs of malaria parasites were characterized, and the structural and functional properties of the enzymes have been determined as key factors for the development of new drugs (Kawazu et al. 2000, 2008; Richard et al. 2011; Hakimi et al. 2012, 2014, 2015; Usui et al. 2013, 2015; Masuda-Suganuma et al. 2012; Jortzik and Becker 2012). In *Plasmodium falciparum*, two typical 2-Cys Prxs, PfTPx-1 and PfTPx-2, have been characterized in detail (Kawazu et al. 2008; Yano et al. 2005; Boucher et al. 2006). While PfTPx-1 is expressed in the cytoplasm of the parasite, PfTPx-2 is localized to the mitochondrion. It is thought that the presence of PfTPx-2 in the mitochondrion is imperative for the malaria parasites to maintain the integrity of the organelle (Boucher et al. 2006).

In *Babesia*, the presence of some antioxidant proteins, including superoxide dismutase (SOD), catalase, glutathione peroxidase (Gpx), thioredoxin (Trx), and thioredoxin reductase (TrxR) has been reported (Becuwe et al. 1992; Clarebout et al. 1998; Regner et al. 2014). Moreover, BbTPx-1 and BgTPx-1, cytoplasmic 2-Cys Prxs of *B. bovis* and *B. gibsoni*, respectively, have recently been identified and their antioxidant activities have been revealed (Tanaka et al. 2009; Masatani et al. 2014). In this study, we identified a novel 2-Cys peroxiredoxin from *B. bovis*, BbTPx-2, that is localized to the mitochondrion of the parasite. To our knowledge, this is the first report on mitochondrial peroxiredoxin in *Babesia* parasites.

Materials and methods

Parasites

The Texas strain of *B. bovis* (Hines et al. 1992) was maintained in purified bovine red blood cells with GIT medium (Wako, Osaka, Japan) by a microaerophilic stationary-phase culture system (Asada et al. 2012; Bork et al. 2005b).

Multiple sequence alignment analysis

Multiple sequence alignment of BbTPx-2 (GenBank accession no. XM_001609049) with BbTPx-1 (XP_001610019), 2-Cys Prxs from *Theileria parva* (TpTPx: XM_760611), *B. gibsoni* TPx-1 (BgTPx-1: AB829722), *P. falciparum* TPx-1 and TPx-2 (PfTPx-1: BAA97121 and PfTPx-2: XM_001350518) was performed using GENETYX ver. 10 (Genetyx Co., Tokyo, Japan).

Cloning of genes coding for TPxs of *B. bovis*

The RNA of *B. bovis* was prepared from cattle erythrocytes infected with *B. bovis* by using TRI reagent (Sigma, St. Louis, MO, USA). Parasite complementary DNA (cDNA) was synthesized from the extracted RNA by using a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Basel, Switzerland). The sequences of the DNA encoding *B. bovis* BbTPx-1 and BbTPx-2 were amplified by PCR using the PrimeSTAR MAX enzyme (Takara Bio Inc., Otsu, Japan) with the following sets of primers: forward primer (5'-CGT GCT CGA GAA TTG CTG TTG GTC AAC CTG CAC-3') and reverse primer (5'-GCT CGA ATT CTT ATG AGT GCT TGC TAG TAA GG-3') for BbTPx-1, and forward primer (5'-CGT GCT CGA GAA ACG GTG TGT TGC GTC TAC C-3') and reverse primer (5'-GCT CGA ATT CTT AAG AAA AGG TCT TGA AAA GG-3') for BbTPx-2. *Xho*I and *Eco*RI sites are underlined. The PCR products were digested with *Xho*I and *Eco*RI and then ligated to the pRSET-B vector (Invitrogen, Carlsbad, CA, USA). The plasmids were designated as pRSET-BbTPx-1 and pRSET-BbTPx-2, respectively, and the nucleotide sequences were analyzed with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA).

Expression and purification of recombinant proteins

The plasmids pRSET-BbTPx-1 and pRSET-BbTPx-2 were transformed into *Escherichia coli* strain BL21 (DE3). Recombinant BbTPx-1 and BbTPx-2 (rBbTPx-1 and rBbTPx-2, respectively) were expressed as histidine-tagged fusion proteins in *E. coli* and purified using HisTrap (GE Healthcare, Piscataway, NJ, USA). To express recombinant glutathione-S-transferase (rGST), pGEX-6P1 (GE

Healthcare) was transformed into *E. coli* strain BL21 (DE3). Expressed rGST was purified using Glutathione-Sepharose 4B beads (GE Healthcare). After dialysis in phosphate buffered saline (PBS), protein concentrations were measured using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA). The expression and purification of the recombinant proteins were confirmed by performing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 12 % gel and standard coomassie brilliant blue staining.

Antioxidant activity assay

The antioxidant activity of rBbTPx-2 was evaluated by a mixed-function oxidation (MFO) assay (Hakimi et al. 2012; Sauri et al. 1995; Masatani et al., 2014). The reaction mixture containing 40 μM FeCl_3 , 10 mM dithiothreitol (DTT), 20 mM EDTA, and 25 mM HEPES was pre-incubated with or without rBbTPx-1, rBbTPx-2 protein (200, 100, 50, and 25 $\mu\text{g}/\text{ml}$), or rGST (200 $\mu\text{g}/\text{ml}$) at 37 °C for 1 h. We used rGST as a negative control because its molecular weight (26 kDa) is similar to that of rBbTPx-2. After the pre-incubation period, 0.5 μg of pBluescript SK (+) plasmid DNA was added and the reaction mixture was incubated for another 3 h. Nicking of the supercoiled plasmids was evaluated by 1 % agarose gel electrophoresis and ethidium bromide staining.

Production of mouse anti-BbTPx-2 serum

Ten-week-old female ICR mice (Clea Japan, Tokyo, Japan) were used in this study for production of antisera against BbTPx-2. One hundred micrograms of the rBbTPx-2 was mixed with TiterMax Gold (TiterMax USA Inc., Norcross, GA, USA) and subcutaneously injected into mice. On day 14 after the first injection, boost immunization was performed. The mouse sera were collected 20 days after the second immunization. The mice 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: 24–118).

Western blotting

B. bovis-infected RBCs were pelleted by centrifugation and hemolyzed with 0.05 % saponin after the supernatant had been discarded. After centrifugation, the pellets were washed three times with PBS and resuspended in RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5 % sodium deoxycholate, 0.1 % sodium dodecyl sulfate, and 1 % NP-40). The solubilized parasite pellet was mixed with an equal volume of SDS-PAGE loading buffer (0.1 M Tris-HCl pH 6.8, 5 % SDS, 15 % glycerol, 4.5 % DTT, and 10 % 2-mercaptoethanol) and

heated at 100 °C for 5 min. Parasite proteins were separated by SDS-PAGE (15 % gel) and subsequently transferred onto a polyvinylidene difluoride membrane (Amersham Hybond PVDF Blotting Membrane; GE Healthcare). The membrane was blocked with a blocking solution (Blocking One; Nacal Tesque) and reacted with anti-BbTPx-2 mouse serum at 1:200. Then, the membrane was washed with PBS containing 0.05 % Tween 20 (PBS-T) and reacted with horseradish-peroxidase-conjugated goat anti-mouse IgG (W402B; Promega, Madison, WI, USA) at 1:25,000. The signals were developed with Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA) and detected by an LAS-4000 mini luminescent imaging analyzer (Fujifilm, Tokyo, Japan).

Indirect immunofluorescent antibody test

B. bovis-infected RBCs (IRBCs) were incubated with 250 nM of Mitotracker Red CM-H₂XRos (Invitrogen) for 20 min at 37 °C and washed three times with PBS. A thin smear was prepared from the IRBCs and fixed with 4 % paraformaldehyde-containing 0.0075 % glutaraldehyde. The smear was blocked with 10 % normal goat serum (Life Technologies, Rochester, NY, USA) in PBS and incubated with anti-BbTPx-2 mouse serum at 1:200. After washing with PBS-T, Alexa-Fluor 488-conjugated goat anti-mouse IgG (Invitrogen) was used at 1:1000. Finally, nuclei were stained by Hoechst 33342 at 0.3 $\mu\text{g}/\text{ml}$, and fluorescence was detected using a laser scanning confocal microscope (Nikon A1R, Nikon, Tokyo, Japan) equipped with a $\times 60$ objective lens (Nikon).

Results and discussion

The BbTPx-2 gene (762 bp) codes for a protein comprised of 253 amino acid residues with the predicted molecular weight and theoretical isoelectric point of 28.02 kDa and 9.06, respectively. The multiple sequence alignment of BbTPx-2 with 2-Cys Prxs from other Apicomplexan parasites revealed that two VCP motifs of the 2-Cys Prx active sites (Cys 107 and Cys 227) were conserved (Fig. 1). Amino acid sequence analysis by using SignalP 4.1 server (<http://www.cbs.dtu.dk/services/SignalP/>) showed that the protein had no signal peptide. However, notably, both PSORT II server (<http://psort.hgc.jp/cgi-bin/runpsort.pl>) and TargetP 1.1 server (<http://www.cbs.dtu.dk/services/TargetP/>) showed that the expected values for mitochondrial localization of this protein are 60.9 % and 0.816 (maximum values being 100 % in PSORT II and 1 in TargetP 1.1). Moreover, TargetP 1.1 predicted that this protein has a mitochondrial targeting peptide at the N-terminus (aa 1 to 54) (Fig. 1). Thus, it is expected that the mature BbTPx-2 protein is comprised of

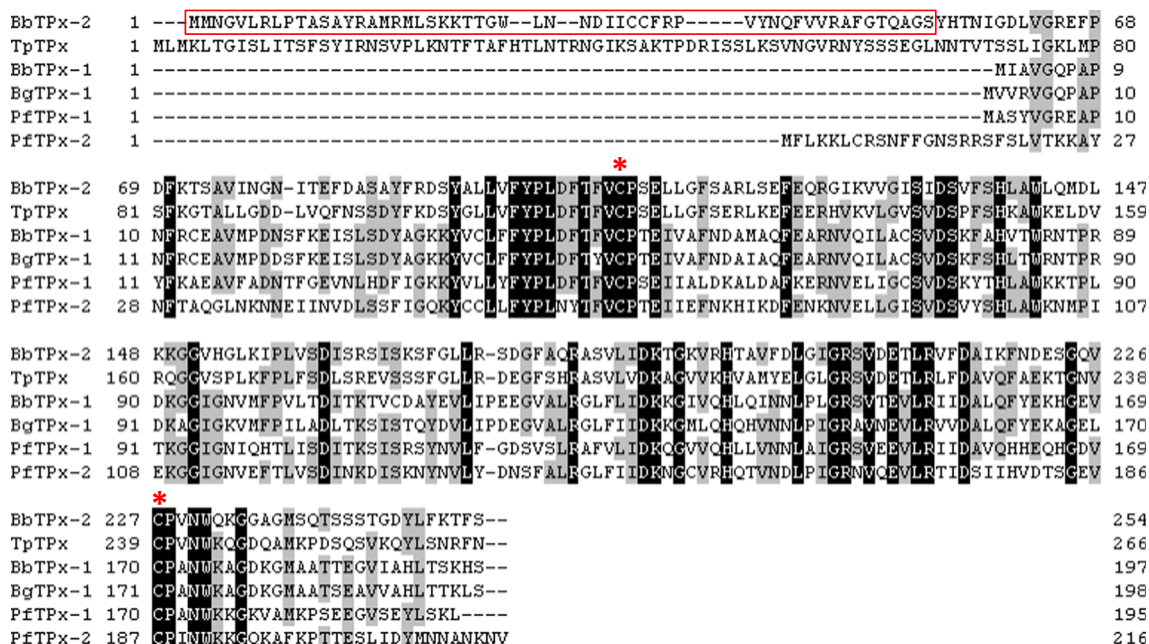


Fig. 1 Multiple sequence alignment of the deduced amino acid sequences of BbTPx-2 with the sequences of other 2-Cys Prxs from *T. parva* (TpTPx), *B. bovis* (BbTPx-1), *B. gibsoni* (BgTPx-1), and *P. falciparum* (PfTPx-1 and PfTPx-2). Black boxes with white letters show identical residues among all of the sequences, and gray boxes

with black letters show common residues in many of the sequences. The dashes indicate gaps introduced between the sequences. The red box shows a predicted mitochondrial targeting peptide of BbTPx-2. Two conserved cysteine residues are marked with asterisks

199 amino acid residues with the predicted molecular weight and theoretical isoelectric point of 21.8 kDa and 6.29, respectively.

In order to demonstrate the enzymatic activity of this BbTPx-2, the coding sequence of the BbTPx-2 gene was amplified by RT-PCR (Fig. 2a) and cloned into the prokaryotic expression vector pRSET-B. Then, recombinant rBbTPx-2 was expressed in *E. coli* as a soluble protein. The purified

rBbTPx-2 had an apparent molecular weight of approximately 28 kDa, as determined by SDS-PAGE (Fig. 2b). Then, the antioxidant activity of rBbTPx-2 was evaluated by an MFO assay (Fig. 3). In this assay, the hydroxyl radicals that are generated by FeCl₃ and DTT damage DNA (Sauri et al. 1995). In the reaction mixture containing both FeCl₃ and DTT but not rBbTPx-1 or rBbTPx-2, hydroxyl radicals nick the supercoiled plasmid DNA, and thereby the apparent size of the plasmid was increased (Fig. 3, lane 4). However, the presence of 100 and 200 µg/ml of rBbTPx-2 in the reaction mixtures prevented nicking of the supercoiled plasmid DNA (Fig. 3, lanes 11 and 12) and this antioxidant activity was comparable to that of rBbTPx-1 (Fig. 3, lanes 7 and 8). These results strongly suggested that BbTPx-2 has antioxidant activity similar to that of BbTPx-1 and acts as an antioxidant enzyme catalyzing hydrogen peroxide in the antioxidant system of *B. bovis*.

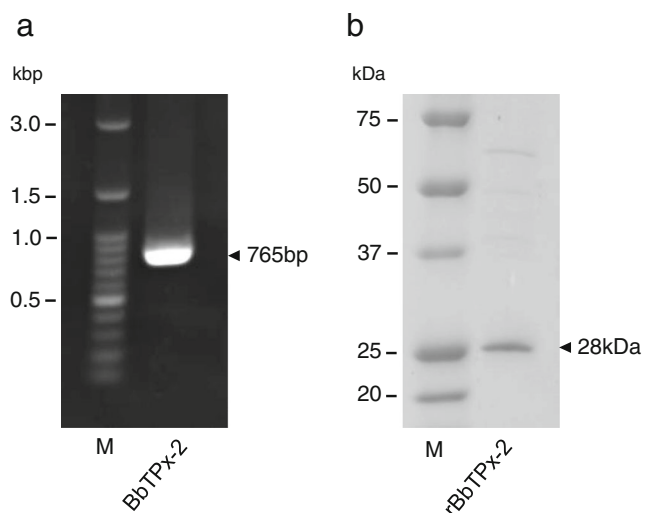


Fig. 2 Amplification of BbTPx-2 gene and expression of BbTPx-2 in *E. coli*. **a** Agarose gel electrophoresis images of the RT-PCR-amplified BbTPx-2 gene (765 bp). *M* 100-bp DNA ladder marker. **b** SDS-PAGE images of recombinant BbTPx-2 (*rBbTPx-2*) (28 kDa). *M* protein marker

Next, we produced an antiserum against rBbTPx-2 and analyzed the expression of BbTPx-2 in *B. bovis* by Western blotting and indirect immunofluorescent antibody test (IFAT) (Fig. 4). Western blotting analysis showed that an antiserum against rBbTPx-2 reacted to a protein of the expected monomeric size of about 24 kDa in extracts of *B. bovis*-infected RBCs (Fig. 4a). This size, which is smaller than the size predicted from its amino acid sequence, suggests that most BbTPx-2 proteins in the parasite cells are present as mature proteins without a mitochondrial targeting peptide. The absence of any additional bands in the Western blotting

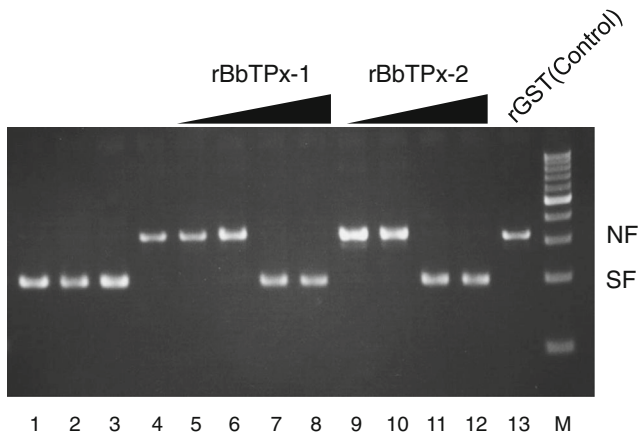


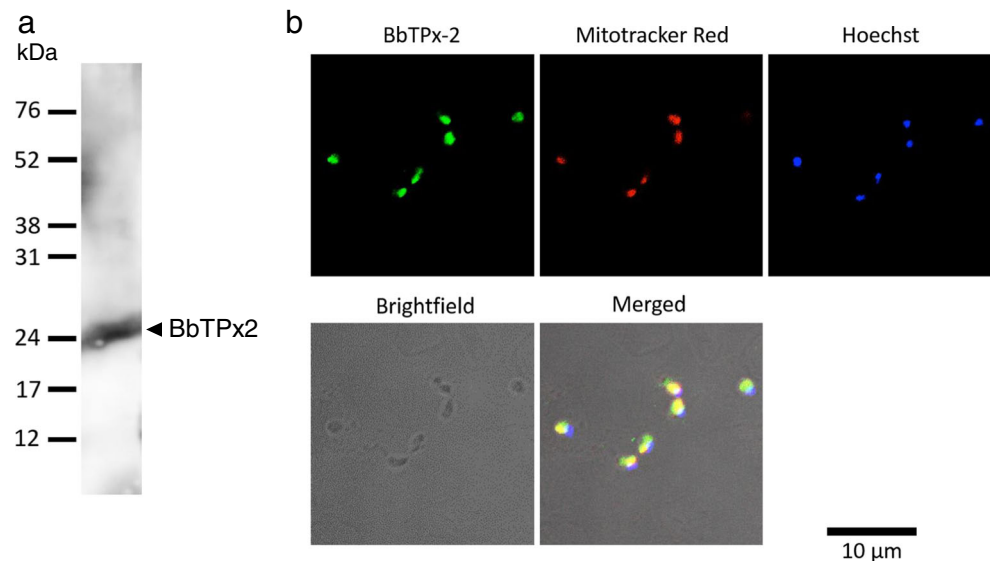
Fig. 3 MFO assay to evaluate rBbTPx-2 antioxidant activity. Nicking of the supercoiled plasmids by the MFO was evaluated on 1.0 % agarose gels stained with ethidium bromide. The nicked form (NF) and supercoiled form (SF) of the plasmid are indicated on the right. *M* 100-bp DNA ladder marker. *Lane 1*, pBluescript plasmid DNA only; *lane 2*, pBluescript plasmid DNA and FeCl₃; *lane 3*, pBluescript plasmid DNA and DTT; *lane 4*, pBluescript plasmid DNA, FeCl₃, and DTT; *lanes 5–8*, pBluescript plasmid DNA, FeCl₃, DTT, and 25, 50, 100, and 200 µg/ml of rBbTPx-1 protein, respectively; *lanes 9–12*, pBluescript plasmid DNA, FeCl₃, DTT, and 25, 50, 100, and 200 µg/ml of rBbTPx-2 protein, respectively; *lane 13*, pBluescript plasmid DNA, FeCl₃, DTT, and 200 µg/ml of recombinant glutathione-*S*-transferase (rGST) from *Schistosoma japonicum* as a negative control. *Triangles* show the increasing concentration of the recombinant protein

experiment shows the specificity of the mouse polyclonal antiserum in detecting only the target protein. Collectively, the data indicate that BbTPx-2 is expressed in *B. bovis* merozoites. IFAT with antiserum against rBbTPx-2 (Fig. 4b) showed dot-like strong fluorescence with some diffused signals in the cytoplasm of the parasites. Notably, co-localization of anti-BbTPx-2 signal (green) with Mitotracker (red) indicates that most of BbTPx-2 was present in the mitochondrion of parasites as predicted by bioinformatics analysis (Fig. 4b, Merged). Taken together, our results indicate that BbTPx-2 is expressed

in the mitochondrion during the asexual stage of *B. bovis*. In *P. falciparum*, a causative agent of human malaria, while cytoplasmic TPx (PFTPx-1) is expressed throughout the erythrocytic stage of *P. falciparum*, mitochondrial PFTPx-2 is expressed during the trophozoite and schizont stages in parasites (Yano et al. 2005). In contrast, both BbTPx-1 and BbTPx-2 are expressed in merozoite stage of *B. bovis* (Tanaka et al. 2009 and Fig. 4b). Although culture systems for *B. bovis* to evaluate other stages (e.g., sporozoite, trophozoite and tick stages) have not been established at this time, it is interesting to evaluate expression levels of these TPx proteins on other stages in the future.

In conclusion, we have characterized a typical 2-Cys Prx, BbTPx-2, in *B. bovis* mitochondria. Since BbTPx-2 has antioxidant activity, we believe that it plays a vital role in the reduction of ROS produced in mitochondria. On the other hand, *Babesia* parasites have other antioxidant proteins, such as SOD, catalase, Gpx and another 2-Cys Prx, BbTPx-1 (Becuwe et al. 1992; Clarebout et al. 1998; Tanaka et al. 2009). Thus, it would be interesting to determine the correlations between BbTPx-2 and these antioxidant proteins. Further experiments to determine the precise roles of BbTPx-2 using a knock-out system (Asada et al. 2012, 2015) will lead to a better understanding of the antioxidant system of the parasite. It was recently reported that the expression of a mitochondrial Prx in *Leishmania donovani* protects the parasites against hydrogen peroxide-induced cell death (Harder et al. 2006). Other study showed that mitochondrial 2-Cys Prx of *L. infantum* is essential for the establishment of a successful infection in mammals (Castro et al. 2011). Moreover, recently, Teixeira et al. (2015) revealed that mitochondrial Prx of *L. infantum* functions as a chaperon reservoir that allows parasites to deal with protein unfolding conditions during the transition from the insect stage to mammalian stage. Thus, it would be interesting to reveal other functions

Fig. 4 Molecular characterization of native BbTPx-2 in parasites. **a** Western blot analysis of native BbTPx-2. The positions of molecular mass standards are indicated on the left. **b** Indirect immunofluorescence microscopy to determine the cellular localization of BbTPx-2 in the parasite cells. Brightfield and fluorescent images (green, BbTPx-2; red, mitochondria; blue, nucleus) are merged on one panel (Merged)



of mitochondrial BbTPx-2 in future studies. Our data may provide important information on BbTPx-2 that can be used as a base for future studies to investigate the precise role of BbTPx-2 in the parasite and its potential as a drug target against bovine babesiosis.

Acknowledgments This work was partially supported by a grant from the Global COE program (Grant No. J02), Sasakawa Scientific Research Grant from The Japan Science Society (Grant No. 24–404), and Grant-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan (Grant Nos. 25850199 and 15 K18783).

References

- Asada M, Tanaka M, Goto Y, Yokoyama N, Inoue N, Kawazu S (2012) Stable expression of green fluorescent protein and targeted disruption of thioredoxin peroxidase-1 gene in *Babesia bovis* with the WR99210/dhfr selection system. *Mol Biochem Parasitol* 181:162–170
- Asada M, Yahata K, Hakimi H, Yokoyama N, Igarashi I, Kaneko O, Suarez CE, Kawazu S (2015) Transfection of *Babesia bovis* by double selection with WR99210 and blasticidin-S and its application for functional analysis of thioredoxin peroxidase-1. *PLoS ONE* 10:e0125993
- Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, Ginsburg H (2004) Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. *Int J Parasitol* 34:163–189
- Becuwe P, Slomianny C, Valentin A, Schrevel J, Camus D, Dive D (1992) Endogenous superoxide dismutase activity in two *Babesia* species. *Parasitology* 105:177–182
- Bock R, Jackson L, de Vos A, Jorgensen W (2004) Babesiosis of cattle. *Parasitology* 129:S247–269
- Bork S, Yokoyama N, Igarashi I (2005a) Recent advances in the chemotherapy of babesiosis by Asian scientists: toxoplasmosis and babesiosis in Asia. *Asian Parasitol* 4:233–242
- Bork S, Okamura M, Matsuo T, Kumar S, Yokoyama N, Igarashi I (2005b) Host serum modifies the drug susceptibility of *Babesia bovis* *in vitro*. *Parasitology* 130:489–492
- Bosch SS, Kronenberger T, Meissner KA, Zimbres FM, Stegehake D, Izui NM, Schettert I, Liebau E, Wrenger C (2015) Oxidative stress control by apicomplexan parasites. *Biomed Res Int* 2015:351289
- Boucher IW, McMillan PJ, Gabrielsen M, Akerman SE, Brannigan JA, Schnick C, Brzozowski AM, Wilkinson AJ, Muller S (2006) Structural and biochemical characterization of a mitochondrial peroxiredoxin from *Plasmodium falciparum*. *Mol Microbiol* 61:948–959
- Brown WC, Palmer GH (1999) Designing blood-stage vaccines against *Babesia bovis* and *B. bigemina*. *Parasitol Today* 15:275–281
- Castro H, Teixeira F, Romao S, Santos M, Cruz T, Flório M, Appelberg R, Oliveira P, Ferreira-da-Silva F, Tomás AM (2011) *Leishmania* mitochondrial peroxiredoxin plays a crucial peroxidase-unrelated role during infection: insight into its novel chaperone activity. *PLoS Pathog* 7:e1002325
- Clarebout G, Gamain B, Precigout E, Gorenflot A, Slomianny C, Camus D, Dive D (1998) *Babesia hylomysci* and *B. divergens*: presence of antioxidant enzymes destroying hydrogen peroxide. *Parasitol Res* 84:75–77
- Dewaal DT (2000) Global important of piroplasmosis. *J Protozool Res* 10:106–127
- Hakimi H, Asada M, Angeles JM, Inoue N, Kawazu S (2012) Cloning and characterization of *Plasmodium vivax* thioredoxin peroxidase-1. *Parasitol Res* 111:525–529
- Hakimi H, Suganuma K, Usui M, Masuda-Suganuma H, Angeles JM, Asada M, Kawai S, Inoue N, Kawazu S (2014) *Plasmodium knowlesi* thioredoxin peroxidase 1 binds to nucleic acids and has RNA chaperone activity. *Parasitol Res* 113:3957–3962
- Hakimi H, Goto Y, Suganuma K, Angeles JM, Kawai S, Inoue N, Kawazu S (2015) Development of monoclonal antibodies against *Plasmodium falciparum* thioredoxin peroxidase 1 and its possible application for malaria diagnosis. *Exp Parasitol* 154:62–66
- Harder S, Bente M, Isermann K, Bruchhaus I (2006) Expression of mitochondrial peroxiredoxin prevents programmed cell death in *Leishmania donovani*. *Eucaryot Cell* 5:861–870
- Hines SA, Palmer GH, Jasmer DP, McGuire TC, McElwain TF (1992) Neutralization-sensitive merozoite surface antigens of *Babesia bovis* encoded by members of a polymorphic gene family. *Mol Biochem Parasitol* 55:85–94
- Homer MJ, Aguilar-Delfin I, Telford SR 3rd, Krause PJ, Persing DH (2000) Babesiosis. *Clin Microbiol Rev* 13:451–469
- Hunfeld KP, Hildebrandt A, Gray JS (2008) Babesiosis: recent insights into an ancient disease. *Int J Parasitol* 38:1219–1237
- Jortzik E, Becker K (2012) Thioredoxin and glutathione systems in *Plasmodium falciparum*. *Int J Med Microbiol* 302:187–194
- Kawazu S, Tsuji N, Hatabu T, Kawai S, Matsumoto Y, Kano S (2000) Molecular cloning and characterization of a peroxiredoxin from the human malaria parasite *Plasmodium falciparum*. *Mol Biochem Parasitol* 109:165–169
- Kawazu S, Komaki-Yasuda K, Oku H, Kano S (2008) Peroxiredoxins in malaria parasites: parasitologic aspects. *Parasitol Int* 57:1–7
- Masatani T, Asada M, Ichikawa-Seki M, Usui M, Terkawi MA, Hayashi K, Kawazu S, Xuan X (2014) Cloning and characterization of a 2-Cys peroxiredoxin from *Babesia gibsoni*. *J Vet Med Sci* 76:139–143
- Masuda-Suganuma H, Usui M, Fukumoto S, Inoue N, Kawazu S (2012) Mitochondrial peroxidase TPx-2 is not essential in the blood and insect stages of *Plasmodium berghei*. *Parasit Vectors* 5:252
- Müller S, Walter RD, Krauth-Siegel RL (2003) Thiol-based redox metabolism of protozoan parasite. *Trends Parasitol* 19:320–328
- Regner EL, Thompson CS, Iglesias AA, Guerrero SA, Arias DG (2014) Biochemical characterization of thioredoxin reductase from *Babesia bovis*. *Biochemie* 99:44–53
- Rhee SG, Chae HZ, Kim K (2005) Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic Biol Med* 38:1543–1552
- Richard D, Bartfai R, Volz J, Ralph SA, Muller S, Stunnenberg HG, Cowman AF (2011) A genome-wide chromatin-associated nuclear peroxiredoxin from the malaria parasite *Plasmodium falciparum*. *J Biol Chem* 286:11746–11755
- Robinson MW, Hutchinson AT, Dalton JP, Donnelly S (2010) Peroxiredoxin: a central player in immune modulation. *Parasite Immunol* 32:305–313
- Sauri H, Butterfield L, Kim A, Shau H (1995) Antioxidant function of recombinant natural killer enhancing factor. *Biochem Biophys Res Commun* 208:964–969
- Schnittger L, Rodriguez AE, Florin-Christensen M, Morrison DA (2012) *Babesia*: a world emerging. *Infect Genet Evol* 12:1788–1809
- Tanaka M, Sakurai T, Yokoyama N, Inoue N, Kawazu S (2009) Cloning and characterization of peroxiredoxin in *Babesia bovis*. *Parasitol Res* 105:1473–1477
- Teixeira F, Castro H, Cruz T, Tse E, Koldewey P, Southworth DR, Tomás AM, Jakob U (2015) Mitochondrial peroxiredoxin functions as crucial chaperone reservoir in *Leishmania infantum*. *Proc Natl Acad Sci U S A* 112:E616–624
- Usui M, Masuda-Suganuma H, Fukumoto S, Angeles JM, Inoue N, Kawazu S (2013) Expression profiles of peroxiredoxins in liver

- stage of the rodent malaria parasite *Plasmodium berghei*. Parasitol Int 62:337–340
- Usui M, Masuda-Suganuma H, Fukumoto S, Angeles JM, Hakimi H, Inoue N, Kawazu S (2015) Effect of thioredoxin peroxidase-1 gene disruption on the liver stages of the rodent malaria parasite *Plasmodium berghei*. Parasitol Int 64:290–294
- Vaca-Paniagua F, Parra-Unda R, Landa A (2009) Characterization of one typical 2-Cys peroxiredoxin gene of *Taenia solium* and *Taenia crassiceps*. Parasitol Res 105:781–787
- Vial HJ, Gorenflot A (2006) Chemotherapy against babesiosis. Vet Parasitol 138:147–160
- Wood ZA, Schroder E, Robin-Harris J, Poole LB (2003) Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci 28:32–40
- Yano K, Komaki-Yasuda K, Kobayashi T, Takemae H, Kita K, Kano S, Kawazu S (2005) Expression of mRNAs and proteins for peroxiredoxins in *Plasmodium falciparum* erythrocytic stage. Parasitol Int 54:35–41