Experimental Parasitology 146 (2014) 43–51

Contents lists available at ScienceDirect

Experimental Parasitology

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

Full length article

Cloning, expression and characterization of protein disulfide isomerase of *Schistosoma japonicum*

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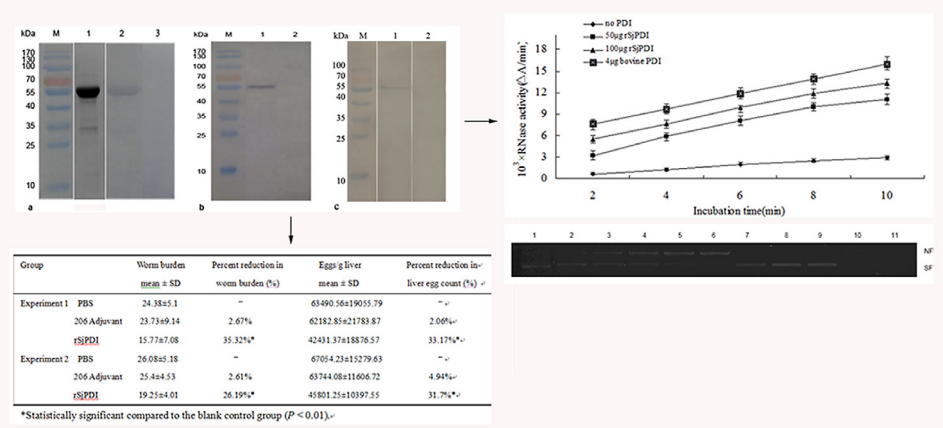
HIGHLIGHTS

- The gene encoding protein disulfide isomerase of *S. japonicum* was characterized.
- The recombinant protein rSjPDI exhibited good antigenicity and enzymatic activity.
- The protein induced significant protection against *S. japonicum* infection in mice.

GRAPHICAL ABSTRACT



Group		Worm burden mean ± SD	Percent reduction in worm burden (%)	Eggs/g liver mean ± SD	Percent reduction in liver egg count (%)
Experiment 1	PBS	24.38±5.1	–	63490.56±19055.79	–
	206 Adjuvant	23.73±9.14	2.67%	62182.85±21783.87	2.06%
	rSjPDI	15.77±7.08	35.32%*	42431.37±18876.57	33.17%*
Experiment 2	PBS	26.08±5.18	–	67054.23±15279.63	–
	206 Adjuvant	25.4±4.53	2.61%	63744.08±11606.72	4.94%
	rSjPDI	19.25±4.01	26.19%*	45801.25±10397.55	31.7%*

*Statistically significant compared to the blank control group ($P < 0.01$).

ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form 9 June 2014
Accepted 17 September 2014
Available online 7 October 2014

Keywords:
Schistosoma japonicum
Protein disulfide isomerase
Cloning
Characterization
Vaccine candidate

ABSTRACT

The excretory/secretory (ES) proteins of schistosomes play important roles in modulating host immune systems and are regarded as potential vaccine candidates and drug targets. Protein disulfide isomerase (PDI) is an essential enzyme that is involved in disulfide bond formation and rearrangement. In the present study, SjPDI, a 52.8 kDa protein previously identified in a proteomics analysis as one of the ES proteins of *Schistosoma japonicum*, was cloned and characterized. Western blot analysis showed that recombinant SjPDI (rSjPDI) was recognized by serum from rabbits vaccinated with schistosome worm antigen. Worm protein extracts and ES protein extracts from *S. japonicum* could react with anti-rSjPDI mouse serum. Real-time PCR analysis indicated that SjPDI was expressed at all developmental stages tested, and a high expression level was detected in 42-day-old male worms. Immunofluorescence analysis revealed that SjPDI was mainly distributed on the tegument and parenchyma of *S. japonicum* worms. An enzyme-linked immunosorbent assay (ELISA) demonstrated that rSjPDI could induce a high level of rSjPDI-specific IgG antibodies. The biological activity of purified rSjPDI was confirmed by isomerization and

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http://dx.doi.org/10.1016/j.exppara.2014.09.004

antioxidative activity assays. The 35.32%, 26.19% reduction in the worm burden and 33.17%, 31.7% lower liver egg count were obtained in mice vaccinated with rSjPDI compared with the blank control group in two independent trials. Our preliminary results suggest that rSjPDI plays an important role in the development of the schistosome and is a potential vaccine candidate for schistosomiasis.

1. Introduction

Schistosomiasis is a widely distributed parasitic disease caused by blood flukes of the genus Schistosoma that affects approximately 200 million people in tropical and subtropical countries (Wang et al., 2008). The isoquinoline-pyrazino derivative praziquantel is the current drug of choice for reducing the intensity of infection and it is effective against almost all species of schistosome; however, a sustained effect has not been achieved and the treatment does not prevent re-infection (Loukas et al., 2007). An effective vaccine against schistosomiasis has not been developed. Therefore, the screening and identification of vaccine candidates or new drug targets are critical to control this disease.

Parasites cause damage to their hosts by competing for vital nutrients, and they have evolved a variety of adaptations to survive including different mechanisms for interfering with the host immune responses, such as the release of excretory/secretory (ES) products (Berasain et al., 2000; Cervi et al., 1996; Jenkins et al., 2005; Lightowlers and Rickard, 1988). Schistosome ES proteins are released or secreted from the tegument as well as other specialized ES organs throughout almost all life stages. Investigation of the ES complex components may facilitate the discovery of vaccines and new therapeutic drug targets for the control of schistosomiasis. Robinson et al. (2009) used an integrated transcriptomics and proteomics approach to profile *Fasciola hepatica* secretory proteins involved in host–pathogen interactions and correlated changes in their expression with the migration of the parasite. A total of 110 ES products of *Clonorchis sinensis* adults were identified including glycometabolic enzymes, detoxification enzymes, and a number of RAB family proteins, which induced hepatic fibrosis in rats (Zheng et al., 2011). Knudsen et al. identified proteins released by *Schistosoma mansoni* cercariae that were experimentally induced to exhibit invasive behavior. The identification of proteins secreted by invasive larvae provides important new information for the validation of models of skin invasion and immune evasion and aids in the development of an anti-schistosome vaccine (Knudsen et al., 2005). The *S. mansoni* egg secretome consists of 188 proteins involved in redox balance, molecular chaperoning and protein folding, development and signaling, scavenging and metabolic pathways, as well as immune response modulation (Cass et al., 2007). Analysis of the ES products of *S. japonicum* adult worms identified 101 proteins including protein disulfide isomerase (SjPDI), providing an insight into host–parasite interactions and resources for the development of diagnostic agents and vaccines for the control of schistosomiasis (Liu et al., 2009). Besides SjPDI, the PDI of *F. hepatica* has also been reported as a component of ES proteins (Salazar-Calderon et al., 2003), and the CsPDI of *C. sinensis*, which was detected in the wall of the intestine by immunohistochemical analysis, was shown to be a component of the ES products by western blot analysis (Hu et al., 2012). Further studies on SjPDI may be useful to better understand the biological function of this parasite product in the definitive host.

In the present study, we describe the cloning, expression, immunolocalization and functional characterization of SjPDI and the evaluation of the protective efficacy against schistosome infection induced by rSjPDI in a murine model.

2. Materials and methods

2.1. Parasites and animals

Male, 6-week-old BALB/c mice were purchased from Shanghai Experimental Animal Centre, Chinese Academy of Sciences. Worms from different developmental stages were obtained by perfusion of New Zealand rabbits artificially infected with schistosome at 7, 14, 21, 28, 35 and 42 days post-infection. Forty-two day-old worms were manually separated into males and females. Study protocols were approved by the Animal Care and Use Committee of the Shanghai Veterinary Research Institute, CAAS.

2.2. Cloning and molecular characterization of S. japonicum PDI

Primers were designed according to the nucleotide sequences of the clone of *S. japonicum* (FN320608.1), and the polymerase chain reaction (PCR) was used to amplify the target gene using a sense primer 5′-CGGAATTCAGTGAGGTTCTCGAACTC-3′ (*Eco*R I, underlined), and a antisense primer 5′-CGGTCGACCTATAGATCACTCTTCTTC-3′ (*Sal* I, underlined). PCR amplification was carried out using the cDNA of 42-day-old adult worms as a template. The amplification conditions involved an initial denaturation step at 94 °C for 5 min, then 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 2 min, and a post-PCR step at 72 °C for 10 min. The purified PCR products were subcloned into the pMD19-T vector (Takara) and sequenced.

2.3. Phylogenetic and sequence analysis

BLAST searches against the NCBI non-redundant protein sequence database were performed using SjPDI as a query and used to identify orthologues of SjPDI. Phylogenetic trees were constructed using the neighbor-joining method and plotted with MEGA (Kumar et al., 2004). The signal peptide was predicted using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/). The molecular weight and isoelectric point (pI) of SjPDI were calculated using the Compute pI/Mw tool (http://web.expasy.org/compute_pi/).

2.4. Expression of recombinant SjPDI

The SjPDI cDNA fragment was digested with *Eco*R I and *Sal* I, purified by agarose gel electrophoresis, and subcloned into the multiple cloning sites present in the pET28a(+) expression vector to produce the recombinant plasmid pET28a(+)-SjPDI. The successful construction of the recombinant plasmid was confirmed by DNA sequencing, and then the recombinant plasmid was transformed into *Escherichia coli* BL21 (DE3) cells. The transformed cells were grown in 500 mL of Luria–Bertani medium at 20 °C until reaching an $OD_{600} = 0.6$, and then induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 6 h at 20 °C. Cells were harvested by centrifugation at 10,000 × g for 15 min. The cell pellets were resuspended with phosphate buffered saline (PBS), sonicated on ice for 10 min, and centrifuged at 10,000 × g for 15 min. The rSjPDI with the His-tag was purified from the supernatant by Ni^{2+} affinity