Biochemical and Biophysical Research Communications 421 (2012) 51–56

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Identification and characterization of a novel Ribose 5-phosphate isomerase B from *Leishmania donovani*

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ARTICLE INFO

Article history:
Received 14 March 2012
Available online 29 March 2012

Keywords:
Leishmaniasis
Leishmania donovani
Drug target
Pentose phosphate pathway (PPP)
Ribose 5-phosphate isomerase (Rpi)
Ribose 5-phosphate isomerase B (RpiB)

ABSTRACT

Leishmaniasis is a group of tropical diseases caused by protozoan parasites of the genus *Leishmania*. Due to the emergence of resistance to the available antileishmanial drugs there is an immediate need to identify molecular targets on which to base future treatment strategies. Ribose 5-phosphate isomerase (Rpi; EC 5.3.1.6) is a key enzyme of the pentose phosphate pathway (PPP) which catalyses the reversible aldose-ketose isomerization between Ribose 5-phosphate (R5P) and Ribulose 5-phosphate (Ru5P). It exists in two isoforms A and B. These two are completely unrelated enzymes catalyzing the same reaction. Analysis of the *Leishmania infantum* genome revealed that though the RpiB gene is present, RpiA homologs are completely absent. An absence of RpiBs in the genomes of higher animals makes this enzyme a possible target for the chemotherapy of Leishmaniasis. In this paper, we report for the first time the presence of B isoform of the Rpi enzyme in *Leishmania donovani* (*Ld*RpiB) by cloning and molecular characterization of the enzyme. An amplified *L. donovani* RpiB gene is 519 bp and encodes for a putative 172 amino acid protein with a molecular mass of ~19 kDa. An ~19 kDa protein with poly-His tag at the C-terminal end was obtained by heterologous expression of *Ld*RpiB in *Escherichia coli*. The recombinant form of RpiB was obtained in soluble and active form. The *Ld*RpiB exists as a dimer of dimers *i.e.* the tetramer form. The polyclonal antibody against *Trypanosoma cruzi* RpiB could detect a band of ~19 kDa with the purified recombinant RpiB as well as native RpiB from the *L. donovani* promastigotes. Recombinant RpiB obeys the classical Michaelis–Menten kinetics utilizing R5P as the substrate with a K_m value of 2.4 ± 0.6 mM and K_{cat} value of 30 ± 5.2 s^{-1}. Our study confirms the presence of Ribose 5-phosphate isomerase B in *L. donovani* and provides functional characterization of RpiB for further validating it as a potential drug target.

1. Introduction

Leishmaniasis is a major health problem that affects approximately 12 million people worldwide with 2 million new cases diagnosed every year [1]. The causative agents of this disease are parasites of the genus *Leishmania*, which infect and replicate in macrophages of the vertebrate host. Leishmaniasis presents a broad clinical spectrum, ranging from asymptomatic and self-healing infections to those causing significant mortality [2]. There is a dramatic increase in the number of cases of leishmaniasis which has been observed in patients with compromised T-cell function, such as those infected with the human immunodeficiency virus (HIV) [3]. Although, pentavalent antimonials (sodium antimony gluconate [SAG]) are still the first choice among the drugs used for the treatment of visceral leishmaniasis (VL), but due to emerging resistance to SAG has become a major barrier in the treatment of VL [4]. There has been an epidemic of primary resistance to SAG in parts of India, warranting an urgent need to look for more effective drugs and also to identify efficient molecular targets on which the future treatment strategies will be based [5]. An understanding of the biochemical physiology of these parasites will underpin the development of new drugs and likewise, the completion of *Leishmania major* and *Leishmania infantum* genome sequencing is facilitating the identification of new drug targets [6]. The pentose phosphate pathway (PPP) has received relatively little attention in *Leishmania* in spite of it being critical in providing phosphorylated carbohydrate intermediates to the parasites for their growth and NADPH for the defense against oxidative stress [7]. The PPP serves to convert Glucose 6-phosphate (G6P) to Ribose 5-phosphate (R5P) which is used in nucleotide biosynthesis. The PPP can also generate Ribose 5-phosphate (R5P) from glucose [8].

The PPP consists of two branches, the oxidative branch in which G6P is converted to Ribulose 5-phosphate (Ru5P) with the concomitant production of NADPH, and the non-oxidative branch in which various pentose phosphate isomerases lead to the generation of glycolytic intermediates like Fructose 6-phosphate (F6P) and

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0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.03.107

Glyceraldehyde 3-phosphate (GAP) [9]. Ribose 5-phosphate isomerase (Rpi, also named D-ribose 5-phosphate ketol-isomerase, EC 5.3.1.6) is a key enzyme of the PPP which catalyses the reversible aldose-ketose isomerization between R5P and Ru5P. The isoforms of this enzyme, Ribose 5-phosphate isomerase A (RpiA) and Ribose 5-phosphate isomerase B (RpiB) are completely unrelated enzymes however, they catalyze the same reaction [10]. RpiA enzyme is most widely distributed in the three kingdoms of life, including most eukaryotic organisms, fungi and some bacteria. Most RpiBs are found in prokaryotic organisms however, there are few exceptions in lower eukaryotes, such as *Giardia lamblia, Entamoeba histolytica* and some fungi, and in the insect *Anopheles gambiae* [11]. In some organisms such as *Escherichia coli*, both RpiA and RpiB forms of the enzyme are present. One of them, RpiA, is a constitutively expressed 23 kDa protein whereas the other, RpiB, is a 16 kDa protein, whose expression is regulated by a repressor [12–14]. In trypanosomes, RpiA form is absent whereas RpiB form is present which is constitutively expressed in case of *Trypanosoma cruzi* [15]. As in *T. cruzi*, Rpi activity was reported in the different forms of the trypanosomatids *T. brucei*, *L. tropica* and *L. major* [16]. The presence of Rpi activity was first demonstrated in *L. mexicana* promastigotes [17]. In the present study, we describe the overexpression of the *Ld*RpiB in *E. coli* and characterization of the purified recombinant enzyme. Since RpiB is the only Rpi enzyme present in the parasite, and has no homolog in the mammalian host, it opens up the possibility of considering it as a novel drug target for the chemotherapy of Leishmaniasis. This study will form the basis for the future mechanistic and structural studies that might lead to the identification of specific inhibitors of this enzyme.

2. Materials and methods

2.1. Chemicals and reagents

All the restriction enzymes were obtained from Bangalore Genei. R5P, plasmid miniprep kit, GenElute™ PCR Clean-Up kit and alkaline phosphatase conjugated anti-mouse IgG were purchased from Sigma (St. Louis, MO). Oligonucleotide primers were from Sigma and Taq DNA polymerase, dNTPs (deoxynucleotide triphosphates) were from Invitrogen (Carlsbad, CA). Anti-His monoclonal antibody was purchased from Calbiochem. Rabbit polyclonal antibody against *Tc*RpiB was a kind gift from Prof. Juan J. Cazzulo, Instituto de Investigaciones Biotecnologicas-Instituto Tecnologico de Chascomus (IIB-INTECH), Argentina. The other materials used in this study were of analytical grade and commercially available.

2.2. Parasite and culture conditions

Leishmania donovani wild type (WT, MHOM/80/IN/Dd8) promastigotes were cultured at 24 °C in RPMI-1640 HEPES-modified medium supplemented with 0.2% sodium bicarbonate, 100 μg/mL penicillin, 100 μg/mL streptomycin, 100 μg/mL gentamycin and 10% heat inactivated fetal bovine serum (FBS) (Gibco/BRL, Life Technologies, Scotland, UK). Medium was maintained at pH 7.2.

2.3. Cloning of Ribose 5-phosphate isomerase gene from L. donovani

Primers were designed based on the putative sequence of *L. infantum* RpiB. A 519 bp DNA fragment was amplified from *L. donovani* genomic DNA, using a sense primer with a flanking *NdeI* site 5′-GGAATTCCATATGATGCCGAAGCGTGTTGC-3′ which coded for the amino acid sequence MPKRV at position 1–15 and the antisense primer with a flanking *XhoI* site 5′-CCGCTCGAGCT TTCCTTCCTCCTTAAGAC-3′ which encoded for amino acid residues LKEEGK without the stop codon, at position 496–516. Polymerase chain reaction (PCR) was performed in a 50 μl reaction volume containing 100 ng of genomic DNA, 25 pmol each of gene-specific forward and reverse primers, 200 μM of each dNTP, 2 mM $MgCl_2$, 5% DMSO and 5 U Taq DNA polymerase. The PCR conditions were as follows: 95 °C for 5 min, 94 °C for 1 min, 56 °C for 1 min, 72 °C for 30 s, and 30 cycles. The final extension was carried for 10 min at 72 °C. A single 519 bp PCR product was observed on 1% agarose gel and cloned into pET30a vector (Novagen) and subjected to automated sequencing. Sequence analysis was performed by DNAstar whereas comparison with other sequences of the database were performed using the search algorithm BLAST [18]. Multiple sequence alignment of RpiB amino acid sequences was performed using ClustalW program. The recombinant construct was transformed into BL21 (DE3) strain of *E. coli*.

2.4. Expression and purification of recombinant LdRpiB

The BL21 (DE3) cells transformed with RpiB-pET30a construct were plated onto LB agar plates containing kanamycin. A single colony was picked to inoculate LB broth and incubated at 37 °C with overnight shaking at 200 rpm. After 12 h of incubation, the primary culture was used to inoculate 2 L of LB media. The cells were incubated at 37 °C with agitation at 200 rpm. When the cells reached to OD 0.4–0.6, induction was done with 0.1 mM IPTG. Three hours after induction, the cells were harvested by centrifugation at 6000*g* for 10 min and resuspended in 20 mM Tris–HCl, pH 8.0, 0.1% Triton X-100 with 100 μg/mL of lysozyme. The resuspended pellets were kept on rocking platform for 30 min at 4 °C and were further sonicated with 8 short bursts of 10 s followed by intervals of 60 s for cooling on ice. The resultant cell lysate was then centrifuged at 12,000*g* for 30 min at 4 °C and the supernatant was collected and loaded onto a His-Select nickel affinity gel pre-equilibrated with equilibration buffer (20 mM Tris–HCl, 150 mM NaCl, 10 mM Imidazole, pH 7.0, 0.1% Triton X-100). The protein was passed through the column and washed with wash buffer A containing 20 mM Imidazole, followed by wash buffer B containing 50 mM Imidazole. The protein was finally eluted with 300 mM and 500 mM of Imidazole. The eluted protein was subjected to overnight dialysis against 20 mM Tris, pH 7.4.

2.5. Cross-linkage of subunits

Crosslinking of subunits was carried out by glutaraldehyde treatment as described previously [19]. Crosslinked proteins were solubilized by addition of crosslinking buffer (60 mM Tris–HCl (pH 6.8), 2% SDS, 10% glycerol, 5% β-mercaptoethanol and 0.01% bromophenol blue) and resolved on 12.5% SDS–PAGE.

2.6. Preparation of crude lysate of L. donovani for RpiB activity

Late log phase promastigotes of *L. donovani* were harvested at 6000*g* and the pellet was washed with phosphate buffer saline (PBS), pH 7.4. The cell pellet was resuspended in lysis buffer and incubated on ice for 10 min. The cells were lysed by freeze–thaw in liquid nitrogen. The lysate was centrifuged at 12,000*g* for 30 min at 4 °C [20]. Protein estimation was performed by Bicinchoninic acid (BCA) assay using bovine serum albumin (BSA) as standard for RpiB activity [21].

2.7. RpiB assay and determination of kinetic parameters

RpiB activity was determined as described previously [22]. The production of Ru5P was monitored as a change in absorbance at 290 nm in 20 mM sodium phosphate buffer (pH 7.5). K_m value was determined by using R5P concentrations in a range between 1 mM to 10 mM. An absorbance of 0.072 at 290 nm was considered

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Ldonovani       MPKRVALGCDHAVYAAHQEIIGMINACSAVSKVMYMGPSSDSSVDYPDYAAQVCEAILKG 60
Lmajor          MSKRVALGCDHAAYAAHREIMDMISASGAVSKVMYMGPSSDTSVDYPDYAAQVCEAILKG 60
Tcruzi          MTRRVAIGTDHPAFAIHENLILYVKEAGDEFVPVYCGPKTAESVDYPDFASRVAEMVARK 60
Tbrucei         MTRKVAIGADHIGFPIHESIVRYVREAGEEFEPVYIGPHSLERVDYPDYALNVARMVARG 60
Ecoli           -MKKIAFGCDHVGFILKHEIVAHLVERG--VEVIDKGTWSSERTDYPHYASQVALAVAGG 57
                  :::*:* **  :  :.:::  :   .     :  *. :   .***.:* .*.  :

Ldonovani       EADTGILVCGTGIGMSIAANKFRGIRAALCYDHVTARLSRQHNNAHILCIGVRTSGPEII 120
Lmajor          EADAGILVCGTGIGMSIAANKFRGIRAALCYDHVTAQLSRQHNNAHILCIGVRTSGMEII 120
Tcruzi          EVEFGVLACGSGIGMSIAANKVPGVRAALCHDHYTAAMSRIHNDANIVCVGERTTGVEVI 120
Tbrucei         EADVGILVCGSGIGMSIAANKVPGIRAALCFDHYTAVMARQHNDANVVCLGERTTGPAVL 120
Ecoli           EVDGGILICGTGVGISIAANKFAGIRAVVCSEPYSAQLSRQHNDTNVLAFGSRVVGLELA 117
                *.: *:* **:*:*:******. *:**.:* :  :* ::* **:::::..* *. *  :

Ldonovani       RDIIETFLTTEPLEEGRHGSRLDKITVIEEQQMEDKQRWCFSGCGGLKEEGK 172
Lmajor          RDIITTFLTTEPLAEGRHSNRVDKITVIEEEQMKDEQRCCFSGCGGRKEEGK 172
Tcruzi          REIIITFLQTPFSGEERHVRRIEKIRAIEAS---------HAGKKGVQ---- 159
Tbrucei         REIIMTFLQTPYSGEDRHTQRLEKIKAAESN---------TNGC-------- 155
Ecoli           KMIVDAWLGAQYEG-GRHQQRVEAITAIEQR---------RN---------- 149
                : *: ::* :      **  *:: * . *
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Fig. 1. Multiple sequence alignment of complete amino acid sequence of *Ld*RpiB using ClustalW. RpiB sequences from *Leishmania donovani* (AEW46848), *Leishmania major* (CAJ05599.1), *Trypanosoma cruzi* (DQ782334.1), *Trypanosoma brucei* (EAN79850.1) and *Escherichia coli* (BAE78093.1) are shown. The amino acids are numbered to the right of the respective sequences. "∗" Denotes that the residues are identical in all sequences in the alignment. ":" Denotes that conserved substitutions have been observed and "." denotes semi-conserved substitutions.

for 1 mM Ru5P. To determine initial velocities, data was analyzed by using Microsoft excel. The data which was significantly non linear ($r^2 < 0.85$), was eliminated. Lineweaver–Burk plot and error analysis of data was generated using Microsoft excel.

2.8. Western blot analysis

The proteins were resolved on 12.5% SDS–PAGE and transferred onto nitrocellulose membrane at 50 V, 350 mA for 3 h. The membranes were blocked for non-specific binding by incubating in 5% BSA in TBST (10 mM Tris–HCl, 150 mM NaCl, 0.05% Tween-20, pH 8.0). It was further incubated for 1 h with primary anti-His mouse monoclonal antibody (1:1000 dilution), at room temperature followed by incubation with anti-mouse IgG secondary antibody (1:10,000 dilution) for 1 h. The respective protein bands were visualized by incubating with Nitro blue tetrazolium (NBT) and 5-Bromo-4-chloro-3-indolyl phosphate disodium salt (BCIP) as substrates [23]. Anti-*Tc*RpiB antibody (1:500 dilution) for 12 h at 4 °C was used as primary antibody to detect the *Ld*RpiB recombinant protein as well as RpiB in the crude lysate of Leishmania promastigotes.

3. Results

3.1. Sequence analysis

The first question we sought to address was whether the RpiB gene is present in *L. donovani* genome or not. To achieve this, we isolated the *Ld*RpiB gene by designing primers based on *L. infantum* RpiB sequence available in (http://www.ebi.ac.uk/parasites/LGN/). The sense primers were flanked with the *NdeI* restriction site and the antisense primers were flanked with *XhoI* site. The stop codon was removed from the gene sequence to encode the C-terminal His tag. Genomic DNA from *L. donovani* wild type promastigotes was used as a template to amplify a single 519 bp PCR product which was further cloned and sequenced. To find out how much identical the *Ld*RpiB sequence is to RpiB sequence from other organisms, multiple sequence alignment was done using ClustalW (Fig. 1). A single open reading frame consisting of 519 bp of RpiB isolated from *L. donovani* (Strain MHOM/80/IN/Dd8 Ribose 5-phosphate isomerase B gene, GenBank ID: JN882262) showed 91.7% identity to putative *L. major* (GenBank ID: 157872081), 54.4% identity to *T. cruzi* (GenBank ID: 110984573), 56.2% identity to *T. brucei* (GenBank ID: 75758175) and 51.7% identity to *E. coli* (GenBank ID: AP009048).

The ORF encoded for a putative polypeptide of 172 amino acids with an expected molecular weight of ∼19 kDa which is very similar to putative *L. major* RpiB protein. The predicted isoelectric point (pI) of *Ld*RpiB was determined to be 5.85 which is comparable to proteins from *L. major* and *T. brucei* but lower in pI in comparison to *T. cruzi* and *E. coli* (pI ∼6.5).

A phylogenetic tree was constructed using BLOSUM 62 with *Ld*RpiB sequence and RpiB sequence from other organisms. Among the kinetoplastid protozoa, *Ld*RpiB and *Tc*RpiB exhibited close evolutionary relationship (data not shown).

3.2. Overexpression and purification of full length LdRpiB enzyme in E. coli

To characterize the recombinant protein, the encoding *Ld*RpiB was cloned in frame into pET30a vector with its own start codon but without stop codon to enable expression of C-terminal His tag. The protein overexpression was carried out as described under materials and methods. *Ld*RpiB protein with a molecular weight of ∼19 kDa that matched with the estimated molecular weight (according to amino acid composition with 6-His residues) was induced (Fig. 2A). The induced *Ld*RpiB was purified by Nickel affinity chromatography column (Fig. 2B). Yield of the purified protein was found to be 10 mg/L of induced bacterial culture.

3.3. Molecular mass determination

The *Ld*RpiB apparent subunit molecular mass was estimated by SDS–PAGE as shown in Fig. 2A. The active *Ld*RpiB molecular mass was determined by cross linking the homogenous protein with 0.1% of glutaraldehyde prior to electrophoresis (Fig. 2C). The untreated protein showed a band of ∼19 kDa. Band corresponding to dimers *i.e.* ∼38 kDa and a dimer of dimers *i.e.* tetramer of ∼76 kDa was also observed.

3.4. Western blot analysis of LdRpiB

Different concentrations of the purified recombinant *Ld*RpiB protein were blotted with the anti-His antibody to show the

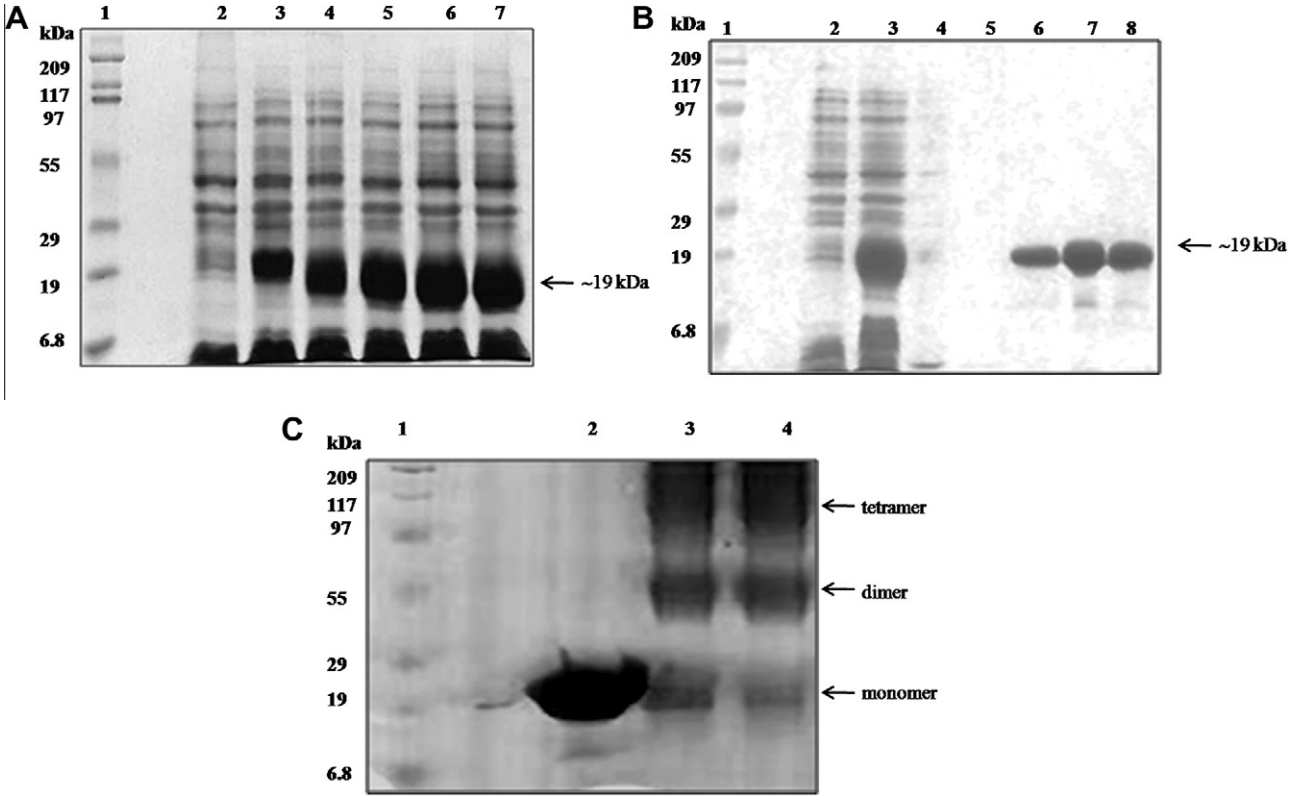

Fig. 2. Overexpression and purification of recombinant *Leishmania donovani* ribose-5-phosphate isomerase (*Ld*RpiB) enzyme. (A) Coomassie blue staining of 12.5% SDS–PAGE showing overexpression of full-length *Ld*RpiB protein in *E. coli*. Lane 1: Prestained broad range SDS–PAGE standards (Bio-Rad), lane 2: RpiB-pET30a bacterial extract before induction and lanes 3–7: RpiB-pET30a bacterial extract after induction at 30 min, 1, 2, 3 and 4 h, respectively with 1 mM IPTG. (B) Purification of *Ld*RpiB protein by Nickel Affinity resin followed by SDS–PAGE and staining with Coomassie brilliant blue. Lane 1: Prestained broad range SDS–PAGE standards (Bio-Rad), lanes 2–5: uninduced crude lysate, induced crude lysate (load), flowthrough, and wash fractions respectively and lanes 6–8: Elute 1, 2 and 3 fractions respectively eluted with 300 mM imidazole. (C) Crosslinking of recombinant RpiB protein with 0.1% glutaraldehyde. Lane1: Prestained broad range SDS–PAGE standards (Bio-Rad), lane 2: untreated recombinant RpiB protein and lanes 3–4: treated RpiB protein with 0.1% glutaraldehyde in PBS (pH 7.5) and 0.1% glutaraldehyde in HEPES buffer (pH 7.5).

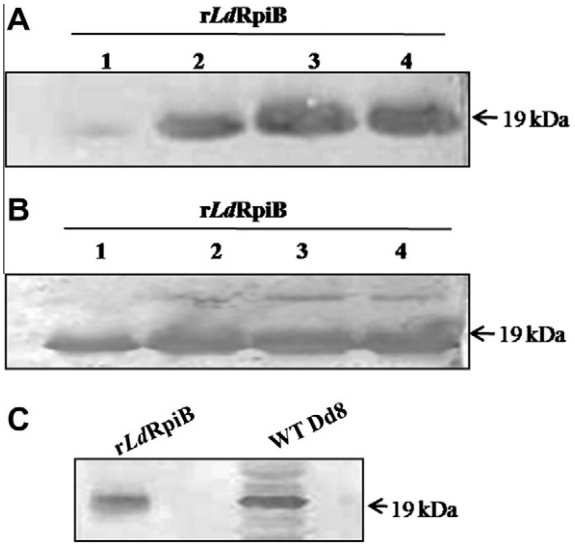

Fig. 3. Western blotting of *Ld*RpiB. Different concentrations of purified recombinant *Ld* RpiB-His tagged protein (lanes 1–4 containing 10 μg, 20 μg, 30 μg, 40 μg respectively blotted with anti-His antibody (A) and anti-*Tc*RpiB antibody (B). Prestained broad range SDS–PAGE standards (Bio-Rad) was used to identify the size of the recombinant protein on the Western blot. (C) *L. donovani* promastigote cell lysate blotted with anti-*Tc*RpiB antibody: Lane 1: 5 μg of purified recombinant *Ld*RpiB protein, lane 2: 150 μg of total cell lysate of *L. donovani* promastigotes.

expression of His tag containing recombinant RpiB. An ~19 kDa band was observed (Fig. 3A). An anti-RpiB antibody from *T. cruzi* was used to detect the recombinant *Ld*RpiB as well as RpiB from the *L. donovani* promastigotes. The antiserum recognized an ~19 kDa fusion protein of recombinant *Ld*RpiB (Fig. 3B). In a Western blot using size-fractionated parasite protein (150 μg), the antiserum could detect a band of *Ld*RpiB of ~19 kDa size in promastigote extracts, which is in agreement with the value calculated from the predicted sequence (Fig. 3C).

3.5. Kinetic analysis of LdRpiB

The kinetic parameters of the recombinant *Ld*RpiB were determined by using R5P as the substrate. Initially, the reading was taken for 15 min but the enzyme reached saturation in 2 min and no further change in absorbance was monitored. Therefore, the initial velocity of the reaction was monitored over a time period of 2 min as there was linear increase in absorbance. Steady state kinetics showed that *Ld*RpiB obeyed classical Michaelis–Menten kinetics as a function of R5P concentration. Double reciprocal plots of 1/*Ld*RpiB activity versus 1/[R5P] was linear and revealed a K_m of 2.45 ± 0.6 mM and turnover value of 30 ± 5.2 s^{-1} (Fig. 4). RpiB activity was detected in lysate of *Leishmania* promastigotes and it exhibited a specific activity of 3200 ± 350 nmoles/min/mg protein when R5P was used as the substrate.

4. Discussion

Rpi catalyses the interconversion of R5P to Ru5P in a branch of pentose phosphate pathway [24]. Two non homologous Rpi's have

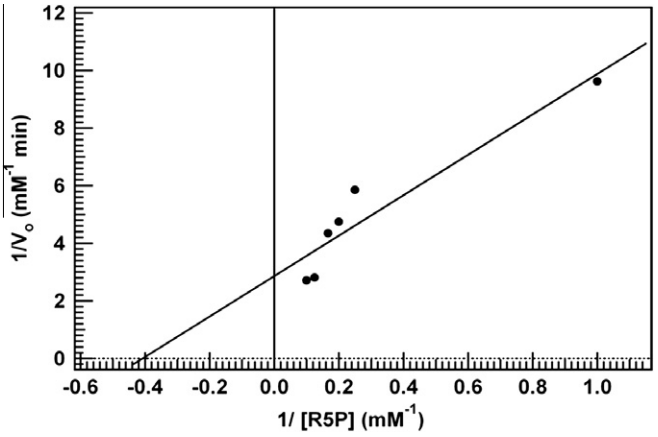

Fig. 4. Lineweaver–Burk plot representation of the *Ld*RpiB kinetics data. Recombinant *Ld* RpiB activity was assayed in the presence of different concentrations of ribose 5-phosphate (R5P). Data is representative of three independent experiments having similar values.

been identified and designated as RpiA and RpiB. RpiA has been sequenced from at least 60 species and cloned from a number of organisms including *E. coli* and spinach [13,25]. On the other hand, till date RpiB has been reported in several species including *E. coli*, *Mycobacterium tuberculosis*, *Clostridium thermicellum*, *T. cruzi* and *Giardia lambia* [11,14,15,26,27]. The Rpi enzyme is essential for cell viability as deletion of the *S. cerevisiae* RpiA gene or of both *E. coli* RpiA and RpiB genes, is lethal [13,28]. However in *E. coli*, the absence of either the RpiA or RpiB is not deleterious [13]. The RpiB however is distinct from RpiA in structure as well in active site which is probably due to independent evolution [27]. In order to characterize the RpiB enzyme in *L. donovani*, the *L. infantum* genome database was screened for the Ribose 5-phosphate isomerase sequence. The sequence of B isoform of the enzyme was retrieved as RpiA sequence was not found in the *L. infantum* database. In this paper, we report for the first time, the molecular cloning and characterization of *Ld*RpiB. The *Ld*RpiB was successfully cloned and sequenced (GenBank ID: JN882262) and it exhibited maximum identity (91.7%) to the putative *L. major* RpiB sequence (GenBank ID: 157872081). Molecular mass determination studies showed the prevalence of *Ld*RpiB as dimer as well as tetramer form. Homodimer form of the enzyme was reported in *T. cruzi* [15]. Although the dimer may form a complete functional unit, tetramer form is found in all available RpiB structures except that of *Mtb*RpiB [27]. Expression of RpiB in *L. donovani* was observed by activity assays and Western blots. Western blot analysis of the whole cell lysate of promastigotes of *L. donovani* with the *Tc*RpiB antibody showed a band of ~19 kDa size and the same antibody recognized the recombinant form of the enzyme as well. The specific activity of the native *Ld*RpiB which was 3200 ± 350 nmoles/min/mg protein was found to be several fold higher in comparison to the reported specific activity value of cell culture trypomastigotes of *T. cruzi i.e.* 40 ± 12 nmoles/min/mg of protein [15]. Earlier Rpi activity was reported in different forms of the trypanosomatids *L. major*, *L. tropica*, *T. brucei* and *T. cruzi* [15,16]. In this paper, we also report the kinetics of recombinant *Ld*RpiB using R5P as the substrate. It obeyed the classical Michaelis–Menten behavior. The K_m of *Ld*RpiB was found to be 2.4 ± 0.6 mM which is in good agreement with the reported K_m values of 4 mM in *T. cruzi* RpiB, 1.23 mM in *E. coli* RpiB and 3.7 mM in *Mtb*RpiB using R5P as the substrate [15,24,27]. The K_{cat} value of *Ld*RpiB is higher in comparison to the reported K_{cat} value of *T. cruzi* RpiB whereas, it is lower in comparison to *E. coli* and *Mtb*RpiB [27].

The presence of B isoform of the Rpi enzyme in *L. donovani* and the absence of the same in humans opens up the possibility of exploring this enzyme as potential drug target. Our report throws light on the functional characteristics of the RpiB enzyme which would facilitate the designing of new and specific RpiB inhibitors especially those that will not interact with the active site of human RpiA. Further work related to overexpression of the enzyme in promastigote form of the parasite is presently going on to elucidate the importance of this enzyme in *L. donovani*.

Acknowledgments

Preet Kamal Kaur, Dinesh Neeradi and Soumya Neelagiri are supported by a grant from NIPER, Mohali, India. We appreciate the Master's student Ms. Ankita Giri for her technical support. We thank Prof. Juan J. Cazzulo, Instituto de Investigaciones Biotecnologicas-Instituto Tecnologico de Chascomus (IIB-INTECH), Argentina for providing the *Tc*RpiB antibody.

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