Manuscript title

Identification and characterisation of the gene encoding an amastine-like surface protein (ALSP) in the *Leishmania donovani* genome: a putative anti-leishmanial drug target

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ABSTRACT

The host-parasite interaction is a complex molecular cross-talk between the host and its parasites, where the parasite employs a repertoire of surface and secretory effector molecules for host cell manipulation and successful parasitism. Identification and functional analysis of such parasite specific effectors is an efficient approach to understand the molecular basis of host-parasite interaction which provides us a potential drug target against the parasitic diseases. The current study recognizes an Amastine like Surface Protein (ALSP), present and expressed in abundant amount in intracellular amastigotes form of Leishmania donovani, the causative agent of visceral leishmaniasis. In-silico analysis indicates that this protein is localized in the cytosol of the amastigotes. Characterization of the protein is performed through Indirect ELISA, Immunoprecipitation, MALDI-TOF, MALDI-TOF-MS-MS analysis and subcellular localization. MALDI-TOF MS analysis reveals that the molecular mass of the protein is 10.147 kDa. Following digestion with trypsin, MALDI-TOF-MS-MS analysis using peptide fragments of the purified native protein in amastigotes shows 100% identity with the leishmanial ALSP. Prediction of its presence and expression was done by localization and transcript level studies. Based on in-silico study, ALSP is has been hypothesized to serve as a source of energy for the intracellular parasite during parasitism by conversion of triglycerides into glycerol and fatty acid, assigning a role in virulence through triglyceride lipase activity. The intracellular survival of the parasite with the intervention of the target protein may help in harnessing the candidate

molecule, as an appealing target for design of novel chemotherapeutics against visceral leishmaniasis in future.

INTRODUCTION

Leishmaniasis is a vector-borne disease, which is spread by protozoan parasites of the genus *Leishmania*. The causative agent *Leishmania* exists in 20 species all over the globe, which are transmitted to the human by the bites of ~30 different species of infected female phlebotomine sandflies and of *Lutzomyia* in the Old World and in the New World respectively (1). Clinical manifestation of *Leishmania* typically has four forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis, visceral leishmaniasis, or kala-azar (VL) and post-kala-azar dermal leishmaniasis (PKDL) (2,3). The most fatal form of the disease, if left untreated, is visceral leishmaniasis, which is caused by *Leishmania donovani* in East Africa and the Indian subcontinent and *Leishmania infantum* in North Africa, Latin America and Europe (4,5). The *Leishmania*-affected cells' morphology consists of two distinct forms: an extracellular flagellated promastigotes form found in the midgut of female phlebotomine sandflies and an obligate intracellular non-flagellated amastigotes form in the mammalian host (6,7). They comprise of the ninth-most inherited diseases among the 13 parasitic and bacterial neglected tropical diseases worldwide (8,9). 98 countries and three territories across five continents are affected by these diseases (10). Based on the World Health Organization (WHO) reports, 12 million people suffer from leishmaniasis worldwide, among which 20,000–40,000 people succumb to death every year (10,11). Few drugs such as antimonials, amphotericin B, and miletefosine, are used to treat VL but they are not much effective due to their lack of efficacy, adverse effects, increasing drug resistance, and high cost.

Moreover there are no available vaccines against Leishmaniasis and chemotherapeutic treatment of infected people is one of the main strategies to control the disease. The development of a novel chemotherapeutic to control Leishmaniasis therefore of utmost importance. However lack of well-validated molecular targets in *L. donovani* has hindered the development of future chemotherapeutics. Hence identification and functional validation of novel drug targets in the trypanosomatid parasite is urgently required and to this end, a much better understanding of parasite biology is needed.

In our previous comparative genomics analysis between *L. donovani* and other *Leishmania species*, a repertoire of fifty seven species-specific genes in *L. donovani* was identified, which

indicates their essentiality in virulence and/or pathogenesis in VL leishmaniasis (12). Among them, the putative functions of thirty eight genes have been assigned through *in-silico* analysis (12). In the present study, we have studied the expression and functional implication of amastin like surface protein (ALSP) (Gene ID: LdBPK_301490.1) in *L. donovani* parasitism through cell biology, proteomics and bioinformatics approaches. Amastin is a transmembrane glycoprotein found on cell surfaces of leishmanial species (12,13). Our previous studies have documented the presence of the amastin-encoding genes at multiple chromosomal locations in leishmania-causing parasites, of which one amastin gene copy on chromosome 34, specific for *L. donovani*, was considered responsible for visceralization of the pathogen (12). Rochette et al suggest that amastin genes are largely expressed in *L. donovani* amastigotes from patients of visceral leishmaniasis (14). We observed that ALSP was expressed only in amastigotes but absent in promastigotes form of the parasite. ALSP was present in the cytoplasm of host cells and is involved in the parasitism through triglyceride lipase activity. The study supports that *Leishmania* needs energy to stay alive in the host cells during parasitism and ALSP provides the necessary energy to the amastigotes for their intracellular survival through the production of glycerol from triglycerides.

MATERIAL AND METHODS

Cell and parasite culture

L. donovani (AG83) parasites were maintained as promastigotes at 23°C in M199 media supplemented with 10% FBS, penicillin (50 U/mL) and streptomycin (50 µg/mL) (Gibco, US). Promastigotes in their late log phase (10 million promastigotes/mL) were harvested by centrifugation at 1000*g* for 10 min and washed with phosphate buffer saline (PBS) pH 7 (15). Human monocytic cell line THP1 was maintained in RPMI (Gibco, US) media similarly supplemented with FBS, penicillin and streptomycin (16). The promastigotes were transformed into amastigotes by infecting THP1 cells with *L. donovani* promastigotes at a ratio of 1:10 (cell: parasite). The amastigotes were isolated following the method described with some modifications (17).

Accession numbers

The IDs and annotations of the protein sequence are as follows:

(CBZ37742.1 LDBPK_342650), GeneDB[i] (LdBPK_301490.1.1: pep), Gene ID:

13392833.

Production of customized antibody and ELISA

The production of customized antisera against the ALSP (Amastine like surface protein) was done by using the best probable peptide epitopic domain from that protein (SSPFSSTRSSSSSRS –C, the addition of a Cystine residue at C terminal end required for Keyhole limpet hemocyanin (KLH) conjugation) by employing bioinformatics tool outsourced from BioBharati LifeScience Pvt. Ltd. (Kolkata, India). A New Zealand (NZ) White Rabbit model was immunized with the help of a KLH conjugation solution containing KLH used for coupling with the peptide epitopic domain. The serum was collected from both batches of the NZ White Rabbit model before (pre bleed) and after giving the 7th immunogenic injections (6th Booster dosage). It took 2 months 15 days to collect the final antisera (Buffer et al. 2013).

Determinations of antibody titer and affinity purification of antisera were done by Indirect ELISA. In both cases, 500 pg of purified ALSP per well have been used to check their titer of various dilutions of antisera containing the specific antibody of both the batches (1:500, 1:1000, 1:2000, 1:5000, 1:10000, 1:20000, 1:400000 dilutions). The equal dilution of preimmune sera taken from the same rabbit was also used to check the specificity of the antibody.

Oriented affinity based immunoprecipitation assay for purification of the native protein

To detect the molecular weight of the native ALSP, 10 million promastigotes and amastigotes of *L.donovani* were resuspended in 1x PBS (pH 7.4) buffer and incubated at 4^0c for 10 min. Rapid freeze-thawing was done in the presence of liquid nitrogen and the insoluble part removed by the centrifugation at 200g for promastigotes and 100g for amastigotes forms. Finally supernatants were taken for immunoprecipitation along with the preimmune sera and antisera of the NZ white rabbit. The immunoprecipitation was carried out with Protein A-Sepharose bead which were added to the preimmune sera and antisera in a 1:2 volume ratio and incubated at 4^0c overnight with gentle inversion rotation. The unbound preimmune sera and antisera were washed with PBS (pH 7.4) at room temperature thrice by centrifugation at 200g for promastigotes and 100g for amastigotes forms. The soluble supernatants of both forms were added to the specific antibody loaded Protein A- sepharose column and incubated overnight at 4^0 c with gentle inversion rotation. The unbound protein was removed similarly

like before. Hence the bound protein with antisera and preimmune sera through Protein A-sepharose bead in both forms was eluted by boiling with SDS-PAGE loading buffer for 15 min at 100^0 c and collected by centrifugation (same parameters).The elutes were analyzed through immunoblotting after probing with Anti-ALSP polyclonal antibody (18).

MALDI-TOF

The dialysate of the native protein was observed in MALDI-TOF to detect its purity and molecular weight. The dissolved protein was spotted on a target MALDI plate using α-cyano-hydroxycinnamic acid (CHCA) as a matrix and analyzed using a MALDI-TOF mass spectrometer (Applied Biosystems, USA). Spectra were calibrated using the matrix. This study was done at CSIR-IICB Kolkata using the central instrumentation facility for mass spectrometry (MALDI) (19).

Two-step reverse transcription PCR

The presence of ALSP transcripts in promastigotes and amastigotes forms of *L. donovani* was checked using isolated whole-cell RNA from both structural forms of *L. donovani* with TRI Reagent (Sigma T9424). Then, the whole-cell RNA was used to synthesize cDNA using reverse transcriptase enzyme at 42 °C for 60 min with the gene-specific ALSP-F and ALSP-R primers (Thermo Scientific RevertAid First Strand cDNA Synthesis Kit). Subsequently, PCR was employed to check the expression level of the native ALSP, maintaining a Tm value of 55°C and by adding 5 µl of cDNA (20).

Fluorescence microscopy

L.donovani promastigotes and amastigotes were harvested by centrifugation (4000g for promastigotes and 100g for amastigotes) and the pellets were washed with PBS two times by centrifugation. Cells were fixed in chilled methanol for 2 min at 4^0c and washed with PBS. Following permeabilisation with permeabilisation buffer for 30 sec-1min at 4^0c, cell pellets were washed with PBS and incubated with preimmune sera and immune sera against the native ALSP protein (1:25 dilution) in the presence of 3% bovine serum albumin for 45 min. Unbound proteins were removed by washing three times in PBS and probed with fluorescein isothiocyanate (FITC)-conjugated goat-derived IgG (Thermo Fisher SCINETIFIC, Waltham)

(1:500 dilution) for 30 min. Further cells were mounted with mounting media along with DAPI (16) and its presence observed under a fluorescence microscope (Zeiss, UK) at 10× magnification and oil immersion. The parasites and infected cells were stained with the pre-immune sera as negative controls and similar optical filters were used for DAPI (λ = 461 nm) and FITC (λ = 591 nm).

Determination of native protein conformation through sequencing

The MALDI-TOF-MS/MS analysis was done using trypsin digested native ALSP sample to check its sequence identity based on MS/MS Ions Search on the MASCOT database server (http://www.matrixscience.com/search_form_select.html). Parameters considered were: carbamidomethyl (C) for fixed modification, oxidation (M) for variable modification, trypsin as enzyme, peptide mass tolerance (100 ppm), and fragment mass tolerance (+0.2).

This study was carried out at CSIR-IICB Kolkata using the central instrumentation facility for mass spectrometry (MALDI-TOF-MS/MS).

Protein 3D structure and function prediction through I-TASSER server

The amino acid sequence of native ALSP was uploaded to the I-TASSER server (https://zhanglab.ccmb.med.umich.edu/I-TASSER/). For each target, I-TASSER simulations generate a large ensemble of structural conformations, called decoys. To select the final models, I-TASSER uses the SPICKER program to cluster all the decoys based on pair-wise structure similarity and reports up to five models which correspond to the five largest structure clusters. The confidence of each model is quantitatively measured by the C-score that is calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score is typically in the range of [-5, 2], where a C-score of a higher value signifies a model with a higher confidence and vice-versa. TM-score and RMSD are estimated based on C-score and protein length following the correlation observed between these qualities. Since the top 5 models are ranked by their cluster sizes, it is possible that the lower-rank models have a higher C-score in rare cases. Although the first model has a better quality in most cases, it is also possible that the lower-rank models have a better quality than the higher-rank models as seen in our benchmark tests.

The function of the ALSP has been annotated using COFACTOR and COACH based on the I-TASSER structure prediction. While COFACTOR deduces protein functions (ligand-binding sites, EC and GO) using structure comparison and protein-protein networks, COACH is a meta-server approach that combines multiple function annotation results (on ligand-binding sites) from the COFACTOR, TM-SITE and S-SITE programs J (Yang J, Zhang Y; Zhang C, Freddolino PL, Zhang Y).

RESULTS

Production of antiserum against the best probable epitopic ALSP domain

The best probable antigenic domain from the ALSP with KLH conjugation was used for immunisation of NZ White Rabbit model. Determination of antibody titers was done and affinity purification of antisera was performed through indirect ELISA. 500 pg of purified ALSP per well was taken to check the titers of different antisera dilutions while the same dilutions of pre immune sera were taken from the same rabbit to check the specificity of the antibody. Both the batches showed a decrease of titer value along with the increase of dilution, though the titer of pre-immune sera remains very less in comparison with the immunized batches which implies that the raised antisera is specific against the antigen. In comparison with two batches, the Batch 1 showed more titer than Batch 2 (Fig. 1a). Therefore Batch 1 has been selected to purify ALSP-antibody through true affinity purification (Fig. 1b) and used for further analysis.

Specificity check of immunized antibody

The specificity was checked of raised antibody against the most probable peptide epitopic domain from ALSP through Indirect ELISA (Fig.1a and 1b) and IP (Supplementary data).

Immunolocalization of the native protein in *Leishmania donovani*

Sub-cellular localization of the native protein in the promastigotes and amastigotes was detected by fluorescence microscopy. Fluorescence microscopy with the fixed *L. donovani* promastigotes and amastigotes, probed with antisera, demonstrated the presence of the native protein in the cytoplasm of amastigotes through release of a fluorescent signal, while it was

absent in promastigotes. The ALSP protein was probed with FITC-tagged IgG and the parasites were observed through semiconfocal microscopy. Through *in-silico* analysis the exact location of the protein in cytosol was addressed, with both structural forms of the protein stained with pre immune sera as negative control (Fig.2).

Stage-specific expression and characterization of the native protein in *L. donovani*

The stage-specific expressions of ALSP gene in the promastigotes and amastigotes forms of *L. donovani* were analysed by reverse transcription PCR, using cDNA obtained from the whole-cell RNA from both its structural forms. PCR with the specific primers for ALSP gene showed a clear band near 255 bp (Fig. 3.I, L3) for the amastigotes whereas no bands were found for the promastigotes (Fig. 3. I, L2). This clearly showed that the expression of ALSP mRNA was absent in the promastigotes while amastigotes have abundant amount of expressed mRNA for the native protein.

The presence of the native protein with its both structural stages in *L.donovani* was also checked through fluorescence microscopy. The protein was found only in the amastigote stage (Fig. 3. II. b) while promastigotes showed no presence of the same (Fig. 3. II. a). The analysis of sub-cellular localization of native ALSP by immunofluroscence, coupled with validation of its gene expression by RT PCR, revealed a selective presence of ALSP at intracellular amastigote stage (absent in extracellular promastigotes), indicating its potential role in infection and parasitism.

Purification and sequence analysis of the native protein from *L. donovani* amastigotes

Physical characterization of the native protein from the amastigote stage was done through its purification from the intracellular extract while the crude extract from promastigotes was used as negative control (since expression of ALSP was not observed in promastigotes) . The antisera with sufficient titre of antibody against ALSP was used to purify the native protein from the amastigote lysate (intracellular lysate). The antisera and preimmune sera were cross-linked with protein A-Sepharose bead (Invitrogen-101041) to avoid the contamination of heavy and light chains of the antibody with the bound antigen, viz our target native protein, while being eluted. The purification was done in the presence of another negative control: albumin like protein in amastigote form of *L.donovani* (21). Both the negative controls exhibited an

absence of the native protein, whereas the lysate of amastigotes exhibited a sharp band near 10 kDa (Fig. 4.a) after incubation with protein-A-sepharose bead cross-linked with the antisera. The precise molecular weight of the native protein obtained through MALDI-TOF MS analysis was 10.147 kDA (Fig. 4.b). Few extra peaks are present in the MALDI-TOF MS analysis data due to the impurity of the sample. Further MALDI-TOF-MS-MS analysis supported its presence through sequence study employing the peptide fragments of the native protein by trypsin digestion in amastigotes form (Fig. 4.c).

3D structure and function prediction from the sequence of the native protein

The 3D structure of native ALSP was predicted via the I-TASSER online tool through homology modeling by utilising its amino acid sequence. The top five models were predicted by I-TASSER using the SPICKER program based on the structural conformations. The confidence of each model was measured by the C-score, calculated based on the significance of the threading template alignments and the convergence parameters of the structure assembly simulations. The range of the C-score is [-5, 2], wherein a C-score of a higher value signifies a model with greater confidence and vice-versa. C-scores of top 5 models were estimated as: -3.58 for model 1, -4.36 for model 2, -4.77 for model 3, -4.56 for model 4 and -4.95 for model 5. Owing to its higher C-score, the structure of Model 1 is depicted here (Fig. 5).

The function and cellular component of the native ALSP was also predicted by COFACTOR and COACH based on the I-TASSER structure prediction. The predictable function of the protein is triglyceride lipase activity (GO: 0004806) wherein the biological process involves lipid catabolism (GO: 0016042) in the cellular compartment of cytosol (GO: 0005829).

DISCUSSION

Our study provides evidence for presence of a hypothetical species-specific gene encoding an amastin like protein in *L.donovani,* which is responsible for pathogenesis of visceral leishmaniasis. To characterize the gene, a polyclonal antibody was raised against the most antigenic site of the leishmanial native protein, which is present only in the amastigote stage of *L.donovani*. Immunofluroscence analysis indicates that the amastin like surface protein is present in the cytosolic compartment of amastigotes but not of promastigotes, which is supported by studies at the transcript level. This indicates that the protein is selectively expressed in the intracellular amastigote form of *L.donovani*.

Further characterization of the native protein was carried out by purifying the whole cell lysate of amastigotes through immunoprecipitation. The antisera which was raised against the best possible epitopic site of the native leishmanial protein was cross-linked with protein A-Sepharose bead. Loading of the amastigote lysate in the protein-A-antibody column prevented antibody co-elution and led to localization of a protein band with molecular weight of 10.147 kDa after MALDI-TOF MS analysis. Further MALDI-TOF-MS-MS analysis was carried out with the peptide fragments of that protein band after trypsin digestion, following which an amino acid sequence was detected that exhibited 100% identity with the leishmanial ALSP (CBZ37742.1).

Subsequently the 3D structure, sub-cellular localisation and function of the native ALSP was predicted by homology modelling using its amino acid sequence via the I-TASSER online tool. The predicted function of the protein is triglyceride lipase activity (GO: 0004806) and it is localised in the cytosol (GO: 0005829). Based on *in-silico* study, ALSP is predicted to serve as a source of energy for the intracellular parasite during parasitism by conversion of triglycerides into glycerol and fatty acid. Phospholipids and sphingolipids form important constituents of leishmanial cell membranes which are essential for virulence and viability of the pathogen (22,23). A plausible explanation of the role of ALSP might be that the metabolism of lipid moieties leads to membrane remodelling in *L. donovani*, which in turn plays a role in entry of the extracellular promastigote form of the pathogen into the host cell and helps in its subsequent transformation into the intracellular non-flagellated amastigote form. Previous investigations suggest that amastins help in survival of the *L. donovani* parasites during their differentiation from the promastigote to the amastigote stage inside the acidic environment of the phagolysosome. This is reportedly achieved by alteration of pH via transport of water-soluble protons and ions across the cell membrane (14,24).

The complex molecular processes underlying the interaction between *L. donovani* and the host cell, followed by endocytosis of the pathogen by the host cell deploy a range of receptors and surface proteins which help trigger signalling pathways required for prolonged survival and host-to-host transmission of the pathogen. This host-parasite dialogue can thus potentially affect the outcome of parasitic infection. Hence such parasite specific molecular target deployed by the parasite to manipulate the host behaviour may serve as an attractive future drug target against parasitic diseases. To this end, investigation of the precise molecular conformation in which the amastin protein exists, its functional interactions with other proteins in the parasite genome and its binding dynamics with potent drug molecules might help

researchers identify new drug candidates. Understanding the mechanistic basis of stage specific expression of ALSP in obligatory intracellular amastigote form of *L. donovani*, together with its putative role as triglyceride lipase to generate glycerol from triglycerides and generation of necessary energy for intracellular survival may help in harnessing this protein as an appealing target for design of novel chemotherapeutics against visceral leishmaniasis in future.

AVAILABILITY

I-TASSER (Iterative Threading ASSEmbly Refinement) is a hierarchical approach to protein structure and function prediction (https://zhanglab.ccmb.med.umich.edu/I-TASSER/)

COFACTOR is a structure, sequence, and protein-protein interaction (PPI) based method for biological function annotation of protein molecules (https://zhanglab.ccmb.med.umich.edu/COFACTOR/)

COACH is a meta-server approach to protein-ligand binding site prediction (https://zhanglab.ccmb.med.umich.edu/COACH/)

SPICKER is a clustering algorithm to identify the near-native models from a pool of protein structure decoys (https://zhanglab.ccmb.med.umich.edu/SPICKER/)

SUPPLEMENTARY DATA

Supplementary Figure:1. I. ALSP-GST protein purification on 15% SDS-PAGE in respect of 1kb Pre-stained Protein Marker (Lane 1). Lane 2: Purified BL21 cells with empty vector, Lane 3: Purified BL2 1 cells without Construct; Lane 4, 5 and 6: 1st, 2nd, and 3rd elution of Purified ALSP-GST fusion protein. II. Illustrate the conformation of the purified ALSP-GST fusion protein (about 35kDa) in induced (Lane 2) and uninduced form (Lane 3) by Western Blotting with Anti-GST-tagged antibody in respect of 1kb Pre-stained Protein Ladder (Lane 1). III. Immunoprecipitation (in Classical method) of the ALSP-GST fusion protein. The purified induced and uninduced ALSP-GST fusion protein pulled down with Antisera and Preimmune sera of an NZ White Rabbit by probing customized anti -ALSP antibody and anti-Rabbit-HRP conjugate Secondary antibody. Lane 2 and 3: Preimmune Sera and antisera with the purified fusion protein; Lane 4 and 5: Preimmune Sera and antisera with the uninduced fusion protein; and Lane 1: 1kb Pre-stained Protein Marker.

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CONFLICT OF INTEREST

The authors do not declare any conflict of interest.

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FIGURE LEGENDS:

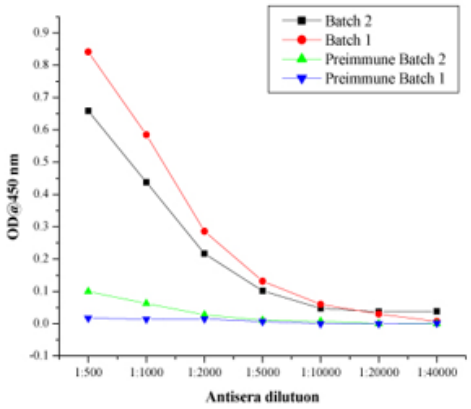
Fig. 1 Characterisation of polyclonal antibody against L.donovani ALSP protein. (A) Binding capacities of polyclonal antibodies raised against both Batch 1 and Batch 2 before and after immunisation of an NZ White Rabbit model, measured by indirect ELISA method. The titre of pre immune serum with different dilutions is indicated by the green and blue curves while the binding capacities of antisera in batch 1 and batch 2 are indicated by red and black curves respectively. (B) True affinity purification of ALSP-Ab from batch 1 of the NZ white rabbit model with varying dilutions.

Fig. 2 Sub-cellular localisation of the native ALSP in both structural forms of L.donovani. (A) Panels i and ii represent visualization of the protein probed with antisera and FITC conjugate goat- derived anti-rabbit IgG secondary antibody through semiconfocal fluorescence microscopy using filters at 461 nm for DAPI and 591 nm for FITC. (B) Panels iii and iv respectively represent L.donovani promastigotes and amastigotes probed with pre immune sera as negative controls.

Fig. 3 Stage-specific expression of native ALSP in L.donovani promastigotes and amastigotes. (A) Reverse transcription-based amplification of ALSP-encoding gene transcripts using whole-cell RNA obtained from promastigote and amastigote forms of L.donovani. Lane 1, 1 kb DNA marker; Lane 2 and 3, RT-PCR products of whole-cell RNA from promastigotes and amastigotes respectively. Lane 3 contains a sharp band near 255 bp; no detection of bands in Lane 2. (B) Immunocytochemical localisation by probing with antisera and FITC conjugated goat-derived anti-rabbit IgG secondary antibody using fluorescence microscopy. For detection, filters were used at 461 nm for DAPI and at 591 nm for FITC.

Fig. 4 MALDI-TOF based analysis of protein expression level and determination of molecular weight of the native purified protein in L.donovani amastigotes. Confirmation by sequencing performed via MALDI-TOF-MS-MS technique. (A) Purification of native ALSP from cell lysate of promastigotes and amastigotes using antisera cross-linked with protein A-sepharose bead. Immunoblotting using antiserum and anti-rabbit HRP conjugated secondary antibody. Lane 1, protein marker (10-250 kDa); Lane 2, purified native protein expressed in promastigotes, taken as negative control; Lane 3, native ALSP band detected near 10 kDa; Lane 4, purified albumin-like protein expressed in amastigotes, taken as negative control. (B) Determination of molecular weight of purified native ALSP. Exact molecular weight determined after being singly (M) charged with the lesser. The m peak at 10.147 kDa represents the accurate molecular mass of the purified native protein. (C) Sequencing of the native protein by MALDI-TOF-MS-MS analysis after digestion with trypsin. Peptide of the protein exhibited 100% identity with Amastine like surface protein (ALSP).

Fig. 5 Prediction of three-dimensional structure of the native ALSP by homology modelling based on amino acid sequence. C-score=-3.58; estimated TM-score = 0.32Â±0.11 and estimated RMSD =11.6Â±4.5Ã.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0.2
0.1
0.0
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1:1000
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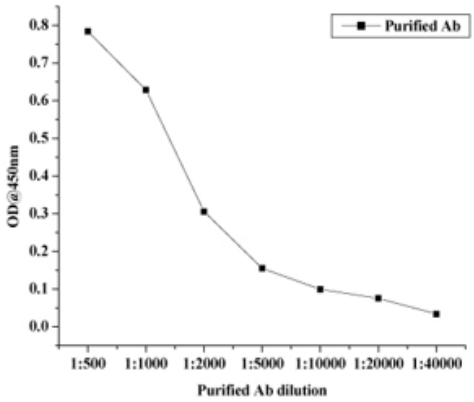
1:40000
Antisera dilutuon

a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1:40000
Purified Ab dilution

b

Fig. 1

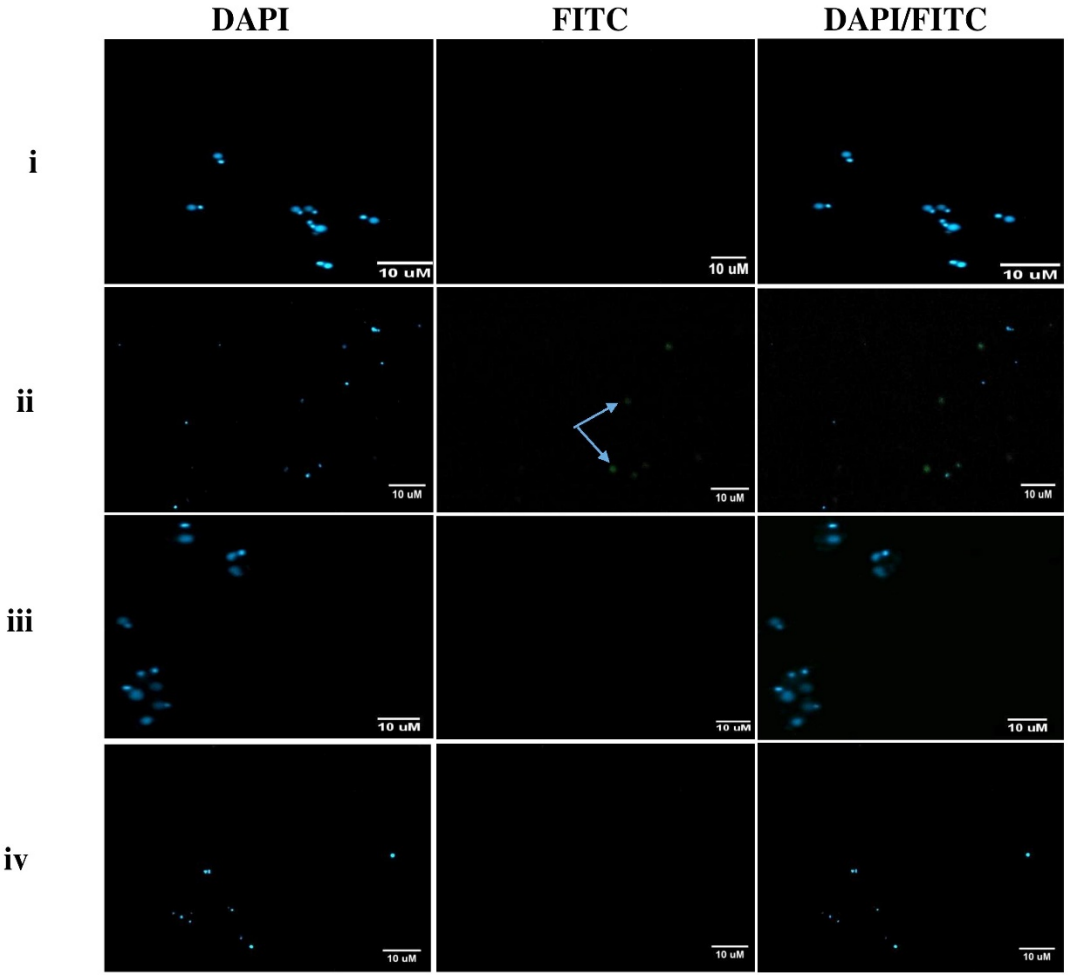
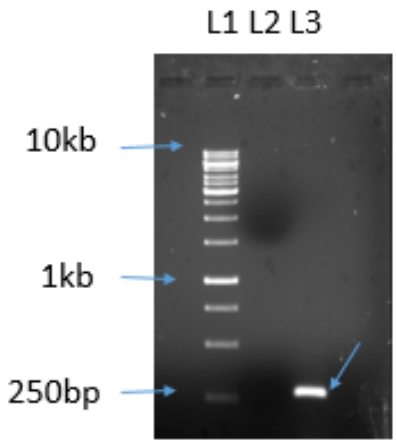
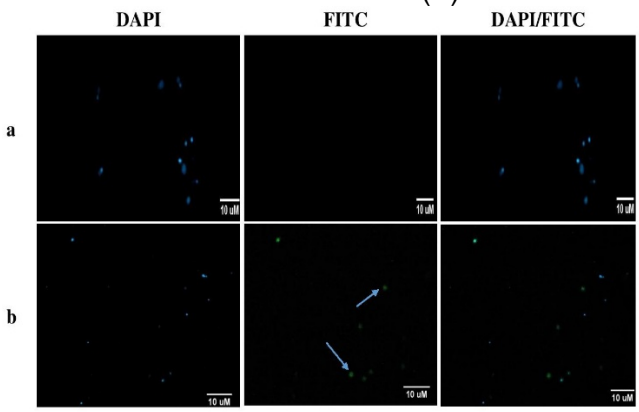


Fig. 2

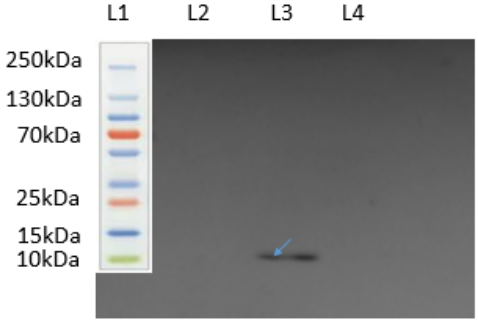


(A)



(B)

Fig. 3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L3
L4
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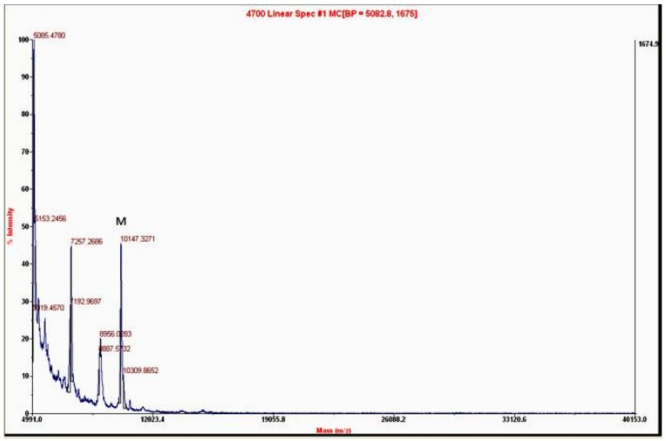
70kDa
25kDa
15kDa
10kDa

(A)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M

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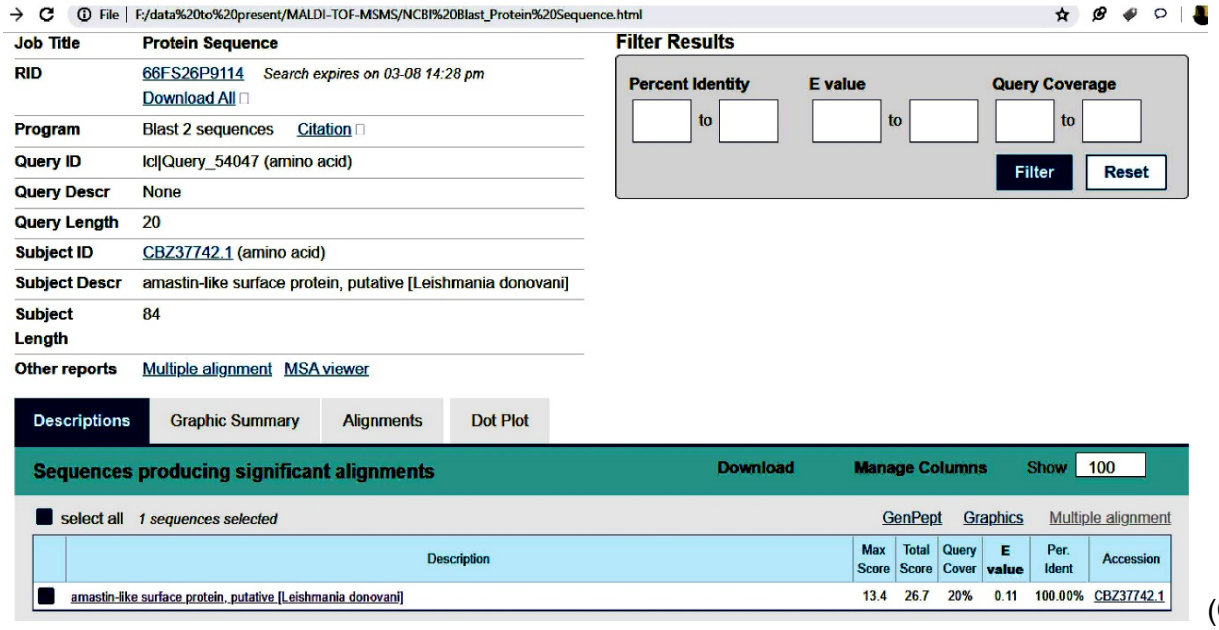
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Citation
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Subject Descr
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Subject Length
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Other reports
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MSA viewer
Filter Results
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to
Filter
Reset
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Graphic Summary
Alignments
Dot Plot
Sequences producing significant alignments
Download
Manage Columns
Show
100
select all
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GenPept
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Query Cover
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Per. Ident
Accession
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0.11
100.00%
CBZ37742.1

(C)

Fig.4

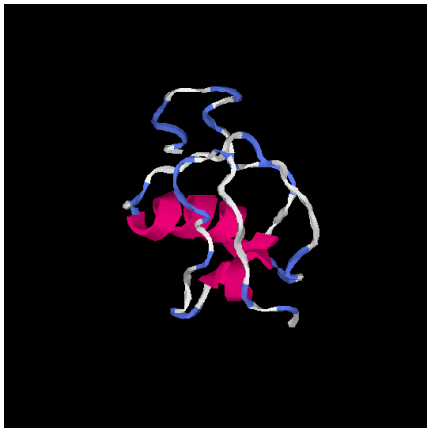

Fig. 5