Cloning and characterization of the gene encoding the heat shock protein HSP83 from *Trypanosoma cruzi*

Clonación y caracterización del gen codificante de la proteína de choque térmico HSP83 de Trypanosoma cruzi

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Abstract

[Objective] *Tryapanosoma cruzi*, causal agent of Chagas' disease, is a parasite that presents morphological alternation between an invertebrate (triatomine) and a vertebrate (mammal) host. Several studies have shown that in *T. cruzi*, HSP83 (homolog of HSP90) is essential for cell division and control of the response to thermal stress. The objetive of the present work was the cloning, bioinformatics characterization and expression of the heat shock protein HSP83 *T. cruzi* gene. **[Methodology]** RNA extraction was performed from epimastigotes using a commercial kit. The cDNA encoding HSP83 was obtained by RT-PCR, from the extracted mRNA, for which the primers were designed based on the HSP83 sequence of *T. cruzi* strain CL Brener. Cloning was performed on pGEM®T-Easy, and subcloned into the expression vector pQE30. Sequence and bioinformatics characterization was performed. The gene was expressed and the recombinant protein was purified by affinity chromatography and identify by immunoblotting. **[Results]** Sequence analysis showed similarity to the gene encoding HSP83 from *Trypanosoma cruzi* and HSP domains were observed, as well as B epitopes in the sequence. After 3 hours of induction with IPTG a recombinant protein with an approximate weight of 83 kDa was obtained. The immunoblotting reaction with hyperimmune anti-*T. cruzi* epimastigote serum allowed the detection a single band with a molecular weight of approximately 83 kDa **[Conclusions]**. All results indicate that the cloning and characterization of HSP83 from *Trypanosoma cruzi* was achieved.

Keywords: *Trypanosoma cruzi*; heat shock protein; HSP83; cloning; expression

Resumen

[Objetivo] *Tryapanosoma cruzi*, agente causal de la enfermedad de Chagas, es un parásito que presenta alternancia morfológica entre un hospedador invertebrado (triatomino) y vertebrado (mamífero). Varios estudios han demostrado que en *T. cruzi*, HSP83 (homólogo de HSP90) es esencial para la división celular y el control de la respuesta al estrés térmico. El objetivo del presente trabajo fue la clonación, caracterización bioinformática y expresión del gen de la proteína de choque térmico HSP83 de *T. cruzi*. **[Metodología]** La extracción de ARN se realizó a partir de epimastigotes utilizando un kit comercial. El ADNc que codifica HSP83 se obtuvo por RT-PCR, a partir del ARNm

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extraído, para lo cual se diseñaron los cebadores con base en la secuencia HSP83 de *T. cruzi*. La clonación se realizó en pGEM®T-Easy y se subclonó en el vector pQE30. Se realizó la caracterización bioinformática de la secuencia. El gen se expresó y la proteína recombinante se purificó por cromatografía de afinidad y se identificó por inmunotransferencia. **[Resultados]** El análisis de secuencia mostró similitud con el gen HSP83 de *Trypanosoma cruzi* y se observaron dominios HSP, así como epítopos B en la secuencia. Después de la inducción se obtuvo una proteína recombinante con un peso aproximado de 83 kDa. La reacción de inmunotransferencia con suero hiperinmune. anti- epimastigote de *T. cruzi* permitió la detección de una sola banda con un peso molecular de aproximadamente 83 kDa **[Conclusiones]**. Todos los resultados indican que se logró la clonación y caracterización de HSP83 de *Trypanosoma cruzi*.

Keywords: *Trypanosoma cruzi*; proteína de choque térmico; HSP83; clonación; expresion

Introduction

Tryapanosoma cruzi, causal agent of Chagas' disease, is a parasite that presents morphological alternation between an invertebrate (triatomine) and vertebrate (mammal) host. Individuals with Chagas' disease may present symptoms cardiological, digestive and neurological and might decease. Currently, among 6 and 7 million persons be affected by the disease and 25 million have the possibility of infection (WHO, 2023) In Venezuela, increases in prevalence and oral transmission outbreaks, have been described in several states (Alarcón de Noya et al., 2015; Añez et al., 2020).

The parasite response is essential for viability in the heat and osmotic stress during transition from the insect to the mammalian host, and when invading mammalian cells. The contribution of heat shock proteins in morphological and functional changes during stress situations has been implicated. This class of proteins is divided in several conserved families according to molecular weight and broad function, among which the five majors are: HSP104, HSP90, HSP70, chaperonins, and small HSPs (Folgueira and Requena 2007; Urményi et al., 2014). They participate in protein folding and cell signaling processes, and play an important role in the host's immune response and have been identified as dominant antigens in diseases caused by protozoa (De Andrade et al., 1992; Requena et al., 1993; Angel et al 1996; Menezes-Zouza et al., 2014 and helminths (Rothstein et al., 1989; Moser et al., 1990; Colebrook and Lightowlers, 1997; Ferrer et al., 2005).

There is evidence that indicates that in *T. cruzi*, Hsp are constitutively expressed and are induced by different types of stress. In particular, 90 kDa heat shock proteins (HSP90) are involved in numerous intracellular processes in eukaryotic cells. Studies have shown that in *T. cruzi* HSP83 (homolog of HSP90) is essential for cell division and control of the response to thermal stress (Jhonson, 2012). The overexpression and the participation of HSP83 in the differentiation processes of *T. cruzi* has been suggested and the inhibition of HSP83 of *T. cruzi* produces arrest in proliferation, demonstrating the importance in the control of the cell cycle (Graeffe et al., 2002).

To date there are only two old reports about the cloning of this molecule (Dragon et al., 1987; Nadeau at al., 1992) and only its genomic structure and ATPase activity have been studied. The objective of the present study was to carry out the cloning, bioinformatics characterization and expression of the heat shock protein HSP83 of *T. cruzi* coding gene, which would allow subsequent studies of the adaptation processes of the parasite to different

microenvironments and the identification of new therapeutic or diagnostic targets and the study of host-parasite interaction in Chagas disease.

Methodology

Parasite material

T. cruzi epimastigotes (EPm6 clon, MHOM/VE/2007/Elpidio Padrón 6c) were cultivated at 27 ºC in liver infusion tryptose broth (LITB) (Contreras et al., 1994). Parasites were counted employing a Neubauer chamber and phase contrast microscope (Nikkon, Optiphot) and were adjusted to 1.5 x 10^9 epimastigotes/mL. Subsequently, the parasites were pelleted by centrifugation at 8,000 rpm for 15 minutes at 4 ° C (IEC Centrifuge, B22M) and washed twice in 0.15 M phosphate saline buffer (PBS) pH 7.2 (57 mM Na2HP04, 18 mM KH2P04, 76.9 mMNaCl) in order to eliminate the remains of the culture medium, and were stored at -80 ºC until its use.

RNA extraction and the complementary DNA (cDNA) obtaining

RNA extraction was carried out through the FavorPrep ™ Viral Nucleic Acid Extration Kit (Favorgene), where the parasite masses were previously treated with lysis buffer supplemented with Proteinase K (0.45 mg/mL) and RNase (80 U/µL), then continue with the instructions described on the commercial kit. Obtaining the complementary DNA (cDNA) was carried out using the Reverse Transcription-Polymerase Chain Reaction technique), with the use of the Access RT-PCR System (Promega) commercial kit. Specific primers were designed manually using BioEdit® Software v7.1.11 (Hall, 2013) and as template sequence the hsp83of *T. cruzi* of 2115 bp (*Trypanosoma cruzi* strain CL Brener hsp83) obtained from GenBank (Accession No. XM_809799) strain used in the T. cruzi genome project (El-Sayed et al., 2005). The primers used was TcHSP83-D (5'-ATGACCGAGACATTCGCA -3') and TcHSP83-R (5'- CTAGTCAACCTGCTCCAT -3'). The reaction mixture consisted of AMV/Tfl reaction buffer, deoxynucleotide triphosphates (0.2 mM), forward and reverse primers (1 µM) each, MgSO4 (1 mM), AMV Reverse Transcriptase (0.1 U /µL), Thermus flavus (Tfl) DNA polymerase (0.1 U / µL), 10 µL of the extracted RNA sample, resulting in a final reaction volume of 50 µL. The amplification reaction was carried out in an automatic C1000 ™ Thermal Cycler (BioRad), using the amplification program: Reverse Transcription 45 °C x 45 min, initial denaturation 94 ° C x 2 minutes, 30 cycles (denaturation 94 ° C x 1 min, hybridization 60 °C x 1 min, extension 68 °C x 2 min) and final extension 68 °C x 7 min.

Electrophoresis

The amplification products of RT-PCR reactions were detected by electrophoresis (2% agarose gel, stained with 0.5 µg/mL ethidium bromide). DNA migration was carried out in a horizontal electrophoresis chamber (Minicell * EC 370M), with constant voltage 60-100 V and TAE buffer (40 mM Tris-acetic acid, 0.5 M EDTA, pH 8). The DNA bands were visualized on a photodocumentation system, Gel Doc 1000 (Bio-Rad®) using the Multi-Analyst Program (Bio-Rad®), the size of the amplification bands was compared with a molecular marker of 1 kb DNA Ladder (Bioneer).

cDNA purification and cloning

The cDNA samples to be purified were fractionated on agarose gels prepared in the TAE buffer and the bands were visualized on the ultraviolet light transilluminator. The fraction of interest was cut with a sterile scalpel and the DNA was eluted with the commercial Wizard®SV Gel and PCR Clean-Up System kit (Promega), according to the manufacturer's instructions. For the ligation reaction of the purified PCR products and the commercial vector pGEM®-T Easy, 1 U of the T4 DNA ligase enzyme was used. A plasmid-insert molar ratio 1: 5 was used, in [30 mM Tris-HCl (pH 7.8), 10 mM MgCl2, 10 mM DTT, 1 mM ATP] buffer and incubated at 4 °C for 12 h. After confirm the hsp83 into pGEM®-T Easy, this recombinant molecule was digested and the purified cDNA was subcloned in the expression plasmid pQE30 (Qiagen, Valencia, CA).

cDNA sequencing and Bioinformatic analysis

Sequencing of cloned cDNA was achieved at the Sequencing Service of the National Center for Microbiology, Instituto de Salud Carlos III, Madrid, Spain, using 373A system, Model 377, Applied Biosystem, following the protocol BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI-PRISM, PE Biosystems). The cDNA sequence obtained was compared with the sequences included in the databases (nucleic acid and proteins) (GenBank, EMBL) and analysed by bioinformatics programs. Multiple alignments were done using the Clustal W. The alignment was revised using BioEdit Sequence Alignment Editor version 7.0.5.3 (Hall, 2013). Nucleotide and amino acid sequences were analyzed by the EditSeq program of DNAstar (Lasergene®, Madison, USA). Identity and similarities were look for in the databases (protein and nucleic acid) (EMBL, GenBank) by BLAST (Boratyn et al., 2019). Others analysis were performed using CDD-Search of the NCBI (National Center for Biotechnology Information) (Lu et al., 2020), Interpro of the EBI (European Bioinformatics Institute) (Mitchell et al., 2019), Motif scan and ExPASy (Expert Protein Analysis System) of the SIB (Swiss Institute of Bioinformatics) (Proteomics Server) (Artimo et al., 2012). Epitopes B prediction was performed using the Protean program from DNAstar (Lasergene®, Madison, USA). In addition, the program BcePred (Prediction of continuous B-cell epitope in antigenic sequences using physico-chemical properties) (Saha et al., 2005) was employed.

cDNA expression

Recombinant His-fusion proteins were expressed in Escherichia coli M15 (Qiagen, Valencia, CA), by induction with isopropyl-β-D-thiogalactopyranoside (IPTG, 1 mM) (Sigma, Madrid, Spain). The cultures were lysed using sonication and examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the recombinant proteins were purified by affinity chromatography with niquel agarose as described by the manufactured (Qiagen, Valencia, CA). The protein concentration was determined by Bradford technique (Bradford, 1976). The purity of the proteins was analysed by SDS polyacrylamide gel electrophoresis (SDS-PAGE) done under denaturing conditions according to Laemmli (1970) on 10% separation gel and 4.5% packing gel at 15 mA. After separating the sample, the gel was stained with Comassie Blue-Silver (De Moreno et al., 1985) for 45 min, then placed in the bleaching solution until the sharp bands were visible.

The molecular weight (MW) of the expressed proteins was compared with the MW marker Broad Range Protein Molecular Weigh Markers (Promega).

Western blot (immunoblotting)

After the proteins were separated by electrophoresis, they were transferred to a nitrocellulose membrane according to Towbin et al., (1979), using a wet system and the Mini Trans-Blot® Cell (BioRad) equipment. For this, two sponges and two filter papers the size of the gel, impregnated in transfer buffer and the nitrocellulose membrane, were placed and the gel was placed on the membrane. Then two filter papers and a sponge equally impregnated with the buffer were placed in the same way and transferred at 100 mA for one hour at 4 ° C. After the transfer had elapsed, the nitrocellulose membrane was incubated in blocking solution (0.15M PBS pH 7.2, 7.5% skim milk) overnight at 4 °C. Subsequently, it was incubated with anti-Epi rabbit hyperimmune serum (against *T. cruzi* epimastigotes) in a 1/1500 dilution in reaction solution (0.15 M PBS pH 7.2, 150 mM NaCl, 10 mM Tris-HCl pH 7.4) for 90 min at 37 °C, then eight washes of 5 min each were performed with the wash solution (0.15 M PBS pH 7.2, 0.1% Tween 20) and subsequently incubated with the conjugate, anti-rabbit IgG coupled to peroxidase at a dilution of 1/3000 in reaction solution (PBS 0.15 M pH 7.2, NaCl 150 mM, Tris-HCl 10 mM pH 7.4) and under the same conditions described above, then the washes were repeated. The visualization of the immune complexes was carried out by chemiluminescence incubating the membrane with the mixture provided by the commercial Super Signal® West pico kit (Pierce) for one minute in the dark and developing using a photographic plate according to the manufacturer's instructions with an exposure time of three minutes.

Analysis and results

By RT-PCR, the cDNA was obtained with an approximate size of 2000 bp which corresponds approximate to the expected size of the hsp83 molecule of *T. cruzi*. Figure 1A shows the bands obtained by RT-PCR from the RNA samples extracted with the different commercial kits. For gene expression, M15 transformed cells were induced with IPTG, control aliquots were taken from uninduced cells and post-1, 2 and 3 hour of induction, and analyzed on Coomassie-Silver stained acrylamide gel. The expected size of the recombinant protein according to the deduced amino acid sequence is 80.7 kDa + 1 kDa of the histidine tail, so a molecular weight of almost 83 kDa would be expected. Figure 1 B shows the discrete expression of a protein of approximately 83 kDa in sample two, at the second hour of incubation and slightly higher at the third hour. Additionally, bands are observed that are not in the uninduced control and that appear at the second and third hour post-induction (Figure 1B), perhaps they are truncated forms of the protein, which the bacteria tend to eliminate.

The recombinant protein was purified by affinity chromatography, from the cultures induced at 3 hours with IPTG. Each of the fractions obtained by chromatography, as well as the sediment, and the supernatant before and after passing through the Ni-NTA nickel column were analyzed by means of a polyacrylamide gel. For the identification of the recombinant protein, the fractions obtained from the purification of the recombinant protein HSP83 from *T. cruzi* were electrophoresis (SDS-PAGE 10%) and subsequently electroblotting on a

nitrocellulose membrane. The nitrocellulose membrane was incubated with hyperimmune rabbit serum anti-Epimastigotes of *T. cruzi*, after development with luminol a specific reaction with the recombinant protein is evidenced (Figure 1C).

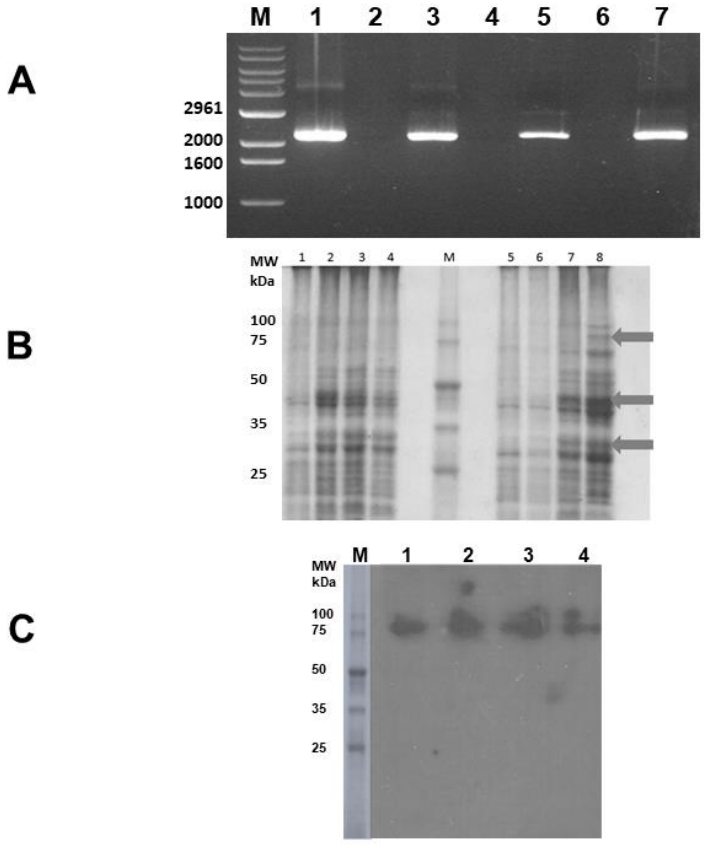

Figure 1. *Cloning and expression of HSP83 of T. cruzi. A) bands obtained by RT-PCR from the RNA samples. B) transformed M15 cells induced with IPTG, (1,5) control aliquots taken from uninduced cells and post-1, 2 and 3 hours of induction (2,3,4 and 6,7,8, respectively) and analyzed on Coomassie-Silver stained acrylamide gel. C) Immunoblotting using hyperimmune rabbit serum anti-Epimastigotes of T. cruzi, to detect purified recombinant protein.*

The complete sequence of hsp83 cDNA was a fragment of 2115 bp corresponding to the open reading frame (ORF) that coded for a peptide of 704 amino acids, with a molecular mass of approximately 83 kDa, and an isoelectric point of 5.0. The deduced amino acid sequence showed a amidation site (10-13), four possible N-glycosylation sites (48-51, 116-119, 151-154, 452-455), 3 cAMP and cGMP-dependent protein kinase phosphorylation sites (246-249, 273-276, 329-332), 11 Casein kinase II phosphorylation sites (104-107, 155-158, 164-167, 291-294, 296-299, 306-309, 360-363, 525-528, 531-534, 609-612, 621-624), 4 N-myristoylation sites (160-164, 190-195, 232-237,442-447), 9 Protein kinase C

phosphorylation sites (104-106, 291-293, 332-334, 380-382, 410-412, 531-533, 553-555, 644-646, 685-687), 4 Tyrosine kinase phosphorylation sites (57-65, 218-225, 273-281, 616-623). Several domains and motif were found, such as, a Chaperone protein htpG [htpG] motif (80-704), a Heat shock hsp90 proteins family signature (103-112), a TonB-dependent receptor proteins signature 1 (1-40), 2 Bipartite nuclear localization signals (400-414, 554-571), a Glutamic acid-rich region profile (257-327), a HATPase_cHistidine kinase-, DNA gyrase B-, and HSP90-like ATPase domain (105-258), a HSP90 protein domain (261-704) (InterPro IPR001404 pFam PF00183). Regarding its possible immunogenicity, five B epitopes could be predicted in the molecule ($_{226}$TKNEE$_{230}$, $_{244}$EEGEKKKKT$_{252}$, $_{327}$FEPSKK$_{332}$, $_{470}$TGDSKK$_{475}$ and $_{527}$EETEEEKKQR$_{536}$) (Figure 2).

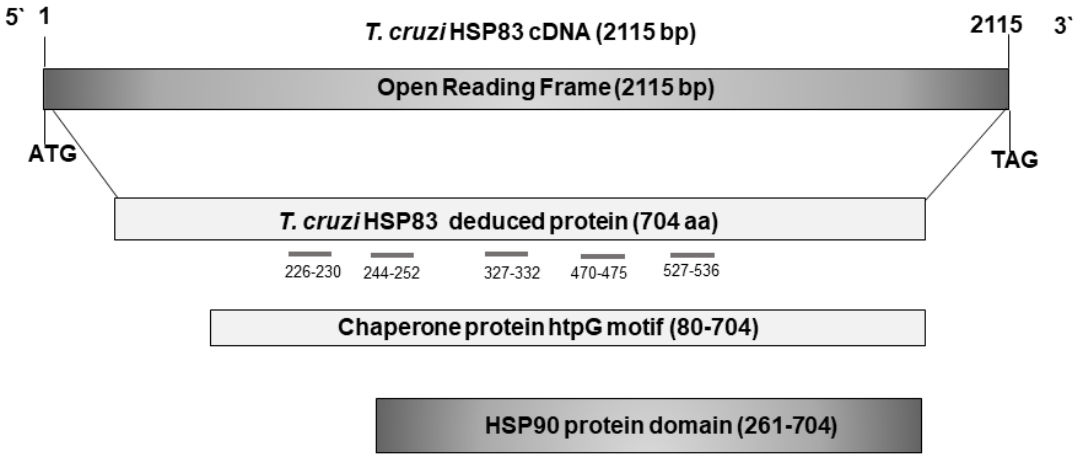

Figure 2. *Schematic representation of the cloned cDNAs analyzed. The bars below deduced proteins represent the domains predicted in the sequence. The short grey lines with below numbers represent the positions of the B epitopes. Base par (bp), amino acid (aa).*

This sequence showed high identity with heat shock protein 85 [*Trypanosoma cruzi* Dm28c, Venezuela] (ESS64949.1), with Heat shock-like 85 kDa protein [*Trypanosoma cruzi* Dracon, Perú] (P06660.1) and lower identity with heat shock protein 85, putative [*Trypanosoma cruzi* CL Brener El Sayed, Brazil] (XP_811791.1) (Figure 3).

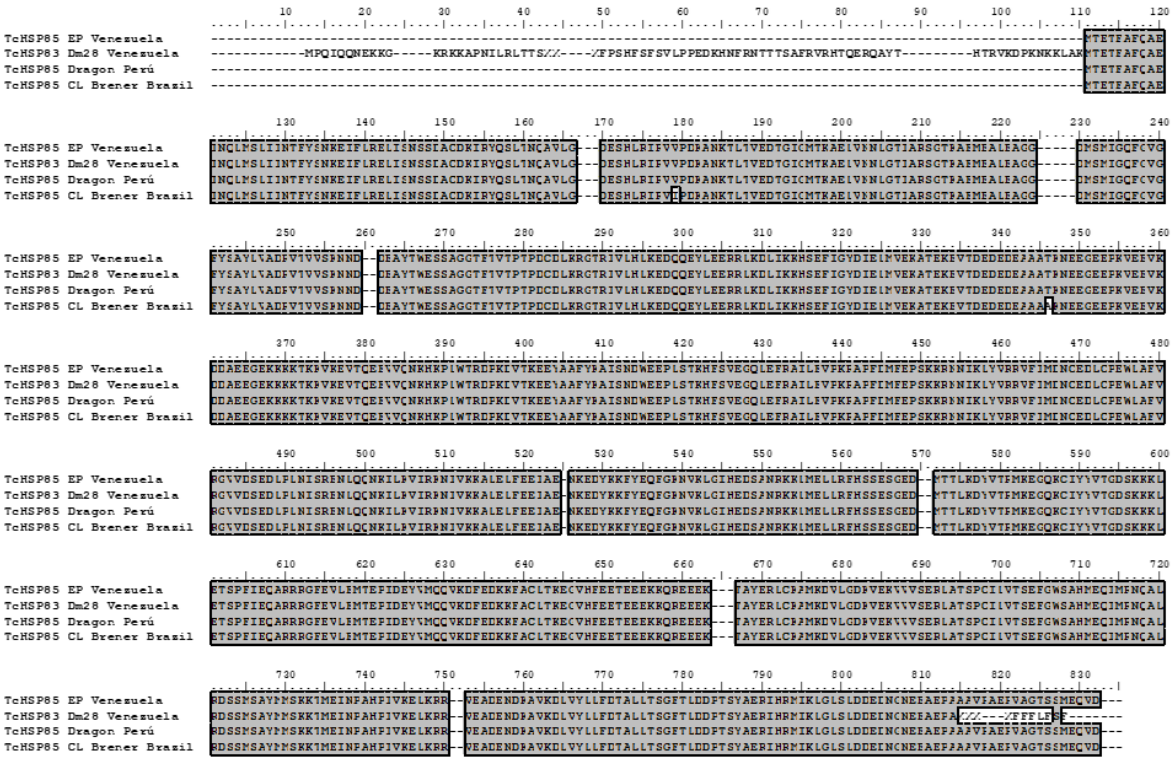

Figure 3. *Clustal Alignment with heat shock protein 85 [Trypanosoma cruzi Dm28c, Venezuela] (ESS64949.1), Heat shock-like 85 kDa protein [Trypanosoma cruzi Dracon, Perú] (P06660.1) and heat shock protein 85, putative [Trypanosoma cruzi CL Brener El Sayed, Brazil] (XP_811791.1).*

Discussion

The cloning of genes and their expression as recombinant proteins allows the study of both these genes and the proteins they encode and their use as tools in tests that allow elucidating the cellular mechanisms in which they are involved. In the present work, it was possible to clone and express the gene encoding the heat shock protein HSP83 from *T. cruzi*. To date there are only two old reports about the cloning of this molecule (Dragon et al., 1987; Nadeau at al 1992) and only its genomic structure and ATPase activity have been studied. In addition, cloning and expression of the gene encoding HSP83 has been obtained in others tripanosomatid, such as; *Trypanosoma brucei* (Mottram et al., 1989), Hsp83 from *Leishmania mexicana amazonensis* (Shapira and Pinelli, 1989), Hsp83 of *Leishmania infantum* (Angel et al., 1996) and Hsp83 from *Leishmania braziliensis* (Silva et al., 2013; Menezes-Souza et al., 2014).

In view of the fact that HSP83 has been reported to be a protein highly expressed by *T. cruzi*, even under basal conditions (Dragon et al., 1987), and given its importance in the mechanisms of cellular metabolism of the parasite and with the need to expand studies in this

area, it was decided to address the cloning strategy using the mRNA as a starting sample and the amplification of the coding region by means of the RT-PCR technique. Thanks to the information from the *T. cruzi* Genome project, this sequence was made available to design the cloning strategy.

In all cases, a band with a size of 2139 bp was amplified, the expected size according to the GenBank sequence. The verification of the cloning by colony PCR, using the primers for the coding region of HSP83, allowed the amplification of a band of 2139 bp in 8 of the 19 colonies analyzed.

For the expression of the cDNA, it was subcloned into different expression vectors. However, although in the recent subcloning it was possible to verify the presence of the insert, when trying to express it could be observed that in some cases the HSP83 sequence or the complete plasmid disappeared. This suggests that the HSP83-plasmid construction could be unstable in these complex plasmids and bacteria could degrade the plasmid (with the loss of the HSP83 sequence) or even eliminate it (Maizels et al., 1991).

It was decided to carry out the subcloning process in the vector pQE30, which is a simpler vector and was used for the cloning and expression of HSP83 from *Leishmania infantum* (Angel et al., 1996). However, the same thing happened, in principle subcloning was achieved and then it was lost. This reinforces the hypothesis that the recombinant plasmid is expelled from the bacterium, perhaps because the HSP83 sequence, being evolutionarily conserved, could interfere with the cell's metabolism and the latter tends to eliminate it (Huynh et al., 1985).

In view of the fact that a high similarity has been reported between the sequences of Hsp83 from *T. cruzi* with Hsp83 from *T. brucei* and Hsp83 from *L. m. amazonensis*, being 95 and 85%, respectively (Rondinelli, 1994), and the expression of *L. infantum* Hsp83 has been reported in cells transformed with pQE30 (Angel et al., 1996), and thinking that the problem may be the residence time in the cell, direct cloning in this last vector was decided, without waiting for the processes to verify the presence of the insert, that is, a "blind" strategy. In this way, the residence time in the bacterium would be reduced, which could prevent its expulsion before the respective expression. In this case, it was possible to obtain positive results, with the appearance of a protein of approximately 83 kDa in the fractions eluted by chromatography of the supernatants of the lysates of competent cells transformed E. coli M15

The review of published articles regarding the cloning of genes encoding Hsp90 heat shock proteins in different parasitic models, either from genomic DNA or from mRNA indicate that the expression of proteins of the Hsp90 family of Trypanosomatids was possible. by cloning in expression vectors, such as the Hsp83 from *L. braziliensis* with the vector pET28a-TEV (Menezes-Souza et al., 2014), Hsp90 from *T. evansi* with the vector pRSETA (Pallavi et al., 2010), Hsp83 from *Leishmania braziliensis* from pET28a (Silva et al., 2013) and Hsp83 from *Leishmania infantum* with pQE30 (Angel et al., 1996). Of the expression vectors used in these works, only pRSETA is more complex and was used in the present work, but without obtaining satisfactory results, perhaps because the time lapses between subcloning in the expression vectors and expression were of several weeks, the recombinant plasmid may have been shed by bacteria during that time due to its instability. Perhaps using the direct "blind" subcloning strategy would have obtained good results in these vectors. Or, that it is a particular characteristic of the Hsp83 of *T. cruzi*, since it is strange that such an

important molecule, from a parasite that causes such a serious public health problem, has not had more cloning and expression studies, perhaps it is due to the difficulty of its expression in prokaryotic systems.

In this sense, it is striking that in the review of the cloning and expression procedure of *Plasmodium falciparum* Hsp90, it was verified that they cloned directly into the expression vector pET-23a and that they obtained the recombinant protein by using competent cells E. coli BL21 (Kumar et al., 2003). Even though the authors do not mention anything in this regard, it is possible that they have experienced circumstances similar to those presented in this work.

Regarding the expression of the recombinant protein, it could be observed that it is low and that some smaller bands are also observed, which could be truncated forms of it, possibly due to the fact that the bacterium tries to degrade the recombinant protein, which perhaps due to sequence similarity may interfere with its metabolism (Huynh et al., 1985). This could be the reason why so little of the recombinant protein is observed.

The expected size of the recombinant protein according to the deduced amino acid sequence is 80.7 kDa + 1 kDa of the histidine tail, so a molecular weight of almost 82 kDa would be expected, which was observed in the results. It is important to note that HSP83 or HSP85 was so named since the native protein has 83-85 kDa. The small difference in size of approximately 3 kDa may be due to the native protein being glycosylated, as the prediction analysis of possible post-translational modifications of the sequence by Motif Scan of ISB-ISREC (Pagni et al., 2001), evidenced 4 potential N-glycosylation sites. One of these sites can be thought of as functional, since it is estimated that an N-glycosylation contributes an additional 3 kDa to the molecular weight of the protein (Obregon-Henao, et al., 2001).

As expected in the purification of the protein, there were also problems due to the fact that it is a large molecule of approximately 83 kDa and that due to its same weight it is difficult to bind to the resin of the column only by a 6 histidine tail. It may be convenient to use another purification protocol or to subclone the cDNA into a vector that allows the binding of a larger fusion molecule that serves as a support and can then be eliminated, for example GST or MBP.

In view of the fact that the expression of hsp 83 was poor and in order to confirm that the band of approximately 83 kDa whose expression increased with induction time corresponded to the HSP83 protein of the parasite, a rabbits hyperimmune serum against *T. cruzi* epimastigotes (from the same isolate used in the present work) was used. This serum has been used in several experimental protocols, with recognition of antigens with approximate molecular weights of 85, 80, 65, 62, 52, 38, 35, 34, 30 and 27 kDa in epimastigotes of the EP strain (Contreras et al., 1998; Graterol et al, 2013).

Immunobloting analysis of the fractions obtained by nickel Ni-NTA column chromatography using hyperimmune anti-epimastigote serum allowed the identification of a single band with an approximate molecular weight of 83 kDa, indicating in this case a reaction with the recombinant protein obtained during the present work. Due to the low concentration of the recombinant protein used in the immunobloting technique, it was decided to use a highly sensitive development system such as luminography, in particular with the Super Signal® West pico system, which allows the detection of immune complexes in the order of the picograms. This explains the strong signal of the band obtained on the X-ray film used as a record of the reaction.

Heat shock proteins have been shown to be immunogenic in many parasitic models (Polla, 1991) and most are important antigens recognized by patient sera. In addition, it has been reported that the immune response is directed to the less conserved areas of the protein, ensuring the specificity of the response (Skeiky et al., 1995; Angel et al., 1996). For example, specific anti-HSP90 antibodies have been described in schistosomiasis (Johnson et al.,, 1989), leishmaniasis (de Andrade et al.,, 1992) and malaria (Zhang et al.,, 2001), it has also been reported as an immunodominant HSP70 antigen in patients with hydatidosis (Colebrook et al.,, 1997), schistosomiasis (Moser et al.,, 1990), onchocerciasis (Rothstein et al.,, 1989), leishmaniasis (de Andrade et al., 1992; Wallace et al., 1992; Skeiky et al., 1995; Quijada et al., 1996, 1998), Chagas disease (Engman et al., 1989; Requena et al., 1993) and malaria (Kumar et al., 2003) and HSP60 in the case of leishmaniasis (Rey-Ladino et al., 1997). Regarding low molecular weight HSPs, their diagnostic value in schistosomiasis and cysticercosis has been studied and confirmed (Nene et al., 1986, Ferrer et al., 2005).

It is important to note that no reactivity of the *T. cruzi* anti-epimastigote hyperimmune serum was observed with any other band, of any size, only the 83 kDa, which would indicate that the possible truncated molecules lack the epitopes recognized by these antibodies. It would be interesting to use the complete protein to immunize rabbits and obtain hyperimmune sera that allow epitope mapping assays.

This recombinant protein HSP83 from *T. cruzi*, even at low concentrations, can be used for the elaboration of a monospecific polyclonal antibody, which constitutes a very valuable tool to study the participation of this stress protein in the differentiation processes of the parasite. such as epimastigogenesis, metacyclogenesis, amastigogenesis, and trypomastigogenesis.

Conclusions

Despite the difficulties in the expression and purification of the recombinant protein, it could be expressed and identified with the hyperimmune serum, therefore this cloning and expression strategy is recommended for molecules with difficult cloning and expression.

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Conflict of Interest

-The authors declare no competing interests.

Author contribution statement

All the authors declare that the final version of this paper was read and approved.

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Data availability statement

Data sharing is not applicable, since no new data was created or analyzed in this study.

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