Research Article

Proteomic Analysis of *Trypanosoma cruzi* Epimastigotes Subjected to Heat Shock

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Trypanosoma cruzi is exposed to sudden temperature changes during its life cycle. Adaptation to these variations is crucial for parasite survival, reproduction, and transmission. Some of these conditions may change the pattern of genetic expression of proteins involved in homeostasis in the course of stress treatment. In the present study, the proteome of *T. cruzi* epimastigotes subjected to heat shock and epimastigotes grow normally was compared by two-dimensional gel electrophoresis followed by mass spectrometry for protein identification. Twenty-four spots differing in abundance were identified. Of the twenty-four changed spots, nineteen showed a greater intensity and five a lower intensity relative to the control. Several functional categories of the identified proteins were determined: metabolism, cell defense, hypothetical proteins, protein fate, protein synthesis, cellular transport, and cell cycle. Proteins involved in the interaction with the cellular environment were also identified, and the implications of these changes are discussed.

1. Introduction

Chagas disease is endemic and is recognized as a major health problem in many Latin American countries, where it is estimated that approximately 10–12 million people are affected by this condition, causing 15,000 deaths per year [1, 2]. This disease is caused by the hemoflagellated parasite *Trypanosoma cruzi*, which has a complex life cycle, alternating its different developmental stages (trypomastigotes, epimastigotes, bloodstream trypomastigotes, and amastigotes) between two different hosts: the insect vector (Hemiptera, family Reduviidae, subfamily Triatominae) and the reservoir (many vertebrates, including man) [3]. Because of the digenetic life cycle of this parasite, it is exposed to growth conditions that differ significantly between the invertebrate and vertebrate hosts, as the physiological characteristics of these two hosts are clearly heterogeneous [4]. These differences are observed in the temperature, pH, nutrient availability, redox potential, and so forth, in which each represent a cellular stress to the parasite [5, 6]. In addition, once the parasite is inside the insect or vertebrate, it is exposed to fluctuations that occur due to the switch from the extracellular to the intracellular environment and the host immune response [6]. In mammalian cells, the parasite is exposed to low pH values, as the parasitophorous vacuole is acidified over time [7], as well as free radicals, which are part of the defense mechanism that the host cell develops [8]. In the digestive tube of the insect vector, the presence of digestive enzymes and a hemolytic factor produce a potentially hostile environment for the parasite [9]. Some of these alterations may lead to cell death, but, like many other parasites, *T. cruzi* has developed mechanisms that allow it to survive and multiply under fluctuating environmental conditions. These adaptations have produced alterations in gene expression modulated by environmental factors [6]. In *T. cruzi*, one of the stresses experienced during the passage

from one host to another is the exposure to sudden changes in temperature, while, inside the insect, the parasites are subjected to an average temperature of 26°C, but, once they enter the mammalian host, they face temperatures of 37°C or more, experiencing a heat shock [10]. Nonlethal heat shock is produced when there is a sudden increase in the environmental temperature; it causes specific changes in the pattern of gene expression and in cellular functions of the organisms, generating a cellular stress response [11].

In trypanosomes, all protein-encoding genes are organized into large polycistronic transcription units (several genes are transcribed by a single mRNA); therefore, gene regulation is controlled by the stability and/or translation of specific mRNAs. Additionally, posttranslational modifications play an important role in modulating protein function [12]. Thus, gene expression in *T. cruzi* is regulated primarily at the posttranscriptional level, and, like other organisms, there has been a poor correlation between mRNA levels and protein expression. Therefore, proteomic analysis of *T. cruzi* is important for the study of changes in global gene expression in specific physiological conditions in this parasite.

Characterizing the gene expression pattern of *T. cruzi* under stress conditions is essential because cellular stress is part of the parasite life cycle. It is important to identify which proteins are modified during this particular phenomenon in order to have a broader understanding of the biology of this pathogen. At this time, there are no safe chemotherapeutic agents or effective preventive vaccines against the parasite. To identify potential therapeutic candidates, methodologies can be used to study a large number of proteins that may be involved in parasite survival after the passage of one host to another.

In this study, a general proteomic analysis of *T. cruzi* parasites exposed to heat shock was performed. The protein pattern of epimastigotes under normal growth conditions was compared to the pattern after heat shock exposure, and, for the first time, twenty-four proteins with a different abundance between the two conditions were identified. Several groups of proteins with important roles were identified, including those localized on the surface.

2. Materials and Methods

2.1. Parasites. T. cruzi epimastigotes of the strain Ninoa (MHOM/MX/1994/Ninoa) [13, 14] were routinely maintained at 28°C in liver infusion tryptose (LIT) media supplemented with 10% fetal calf serum and 25 μg/mL hemin [13].

2.2. Heat Shock Assays. Cultures were grown in 25 cm^2 culture dishes at a concentration of 3×10^6 cells/mL in a final volume of 10 mL fresh LIT medium. After three days of incubation at 28°C, which corresponded to the exponential growth phase, and when the culture had reached a density of $25 \times 10^6 \pm 5 \times 10^6$ cells/mL, the culture was divided into two equal parts. Five mL of the culture was maintained at 28°C for use as a control, and the other

5 mL was subjected to a sudden change in temperature by incubation at 37 or 42°C for 3 h. For each condition, after the incubation period, cultures were harvested by centrifugation at 2,500 ×g for 10 min at 4°C and washed three times with PBS. Finally, the wet weight of the cell button was determined before proceeding with the protein extraction. Before protein extraction, samples of parasites were fixed and Giemsa-stained.

2.3. Protein Extraction. The pellet of parasites was resuspended in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 0.0008 g/mL tris (hydroxymethyl-aminomethane)) with protease inhibitors (12 mM EDTA, 1 mM PMSF, 0.001 mM pepstatin and 0.1 mM leupeptin) in a ratio of 3 mL lysis buffer/g of wet weight. The pellets were stirred vigorously for 3 min, incubated on ice for 10 min and centrifuged at 12,800 ×g for 15 min, and the supernatant was then recovered (protein extract). Protein extracts were kept at −70°C until use, and the protein integrity was assessed by 12% SDS-polyacrylamide gel electrophoresis (PAGE). Protein concentrations were determined using the 2D-Quant kit (Amersham Biosciences), according to manufacturer's instructions.

2.4. Two-Dimensional Gel Electrophoresis (2-DE). For isoelectric focusing (IEF), aliquots of 300 μg of protein in rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2% IPG buffer (pH range 3–10), 8 mg dithiothreitol (DTT), trace bromophenol blue) from control or heat-shock-treated epimastigotes lysates were applied to 17 cm immobilized pH gradient (IPG) gel strips (Bio-Rad) with a nonlinear (NL) pH range of 3–10, by in-gel sample rehydration, overnight. The proteins were then focused in a PROTEAN IEF Cell (Bio-Rad) using the following protocol: 250 V, 20 min, 20°C; 10 000 V, 2.5 h, 20°C; 40 000 V/h, 6–8 h, 20°C. Before the second dimension, proteins were reduced (10 mg/mL DTT) and alkylated (25 mg/mL iodoacetamide) in equilibration buffer (6 M urea, 2% SDS, 0.05 M Tris-HCl pH 8.8, 30% glycerol, trace bromophenol blue) [15]. Proteins were separated in a second dimension on 10% SDS-PAGE gels using a vertical PROTEAN II XL system (Bio-Rad) and standard Tris/glycine/SDS buffer with the following program: 16 mA, 30 min; 24 mA, 6–8 h.

2.5. Gel Staining and Image Analysis. Gels were stained with silver nitrate, according to the following protocol: fixation for 12 h in 50% methanol/10% ethanol; incubation for 1 h in 5% methanol/1% ethanol; three washes of 5 min in deionized water; incubation for 1.5 min in 0.02% sodium thiosulfate; three washes of 30 s in deionized water; staining with 0.2% silver nitrate for 40 min; three washes of 60 s in deionized water; visualization with developing solution (6% sodium carbonate, 0.05% formaldehyde, 0.002% sodium thiosulfate); finally, 6% acetic acid was added [15]. Observed protein pIs were calculated using the chart of Immobiline DryStrip pH 3–10 NL 24 cm, pH as function of distance at 20°C and 8 M urea (GE Healthcare). The molecular mass was calculated based on the protein migration distance

(Rf) calibrated against Silver Stain SDS-PAGE Molecular Weight Standard Mixtures Wide Molecular Weight Standard (Sigma Aldrich). Silver-stained gel images were digitalized using a Fluor-S MultiImager (Bio-Rad). Image analysis was performed using PDQuest 2D analysis software (Bio-Rad). After background subtraction and noise filtering, spots were detected and quantified. The spots were measured by taking the optical density (OD), which is calculated by dividing the incident light intensity by the transmitted light intensity and taking the log (base 10) of the ratio. An OD value was assigned to each pixel, and linear interpolation of each OD was used to express quantification. Spots that showed a change of at least 1-fold in intensity between control and heat-shock-treated samples (mean OD value of control/mean OD value of heat shock treated) from three gel replicates were selected for identification.

2.6. Mass Spectrometry. Protein extracts from heat-shock-treated *T. cruzi* of the strain Ninoa were subjected to 2D-PAGE and were silver stained using the silver SNAP stain for mass spectrometry kit (Pierce), following the manufacturer's instructions. Selected spots were excised manually from the gel and identified by peptide mass fingerprinting (PMF). Briefly, proteins were subjected to in-gel digestion with trypsin (20 ng/μL) at 37°C overnight. After treatment with ammonium bicarbonate, the peptides were extracted with 50% acetonitrile/5% formic acid and concentrated under a vacuum. The peptides generated were incorporated into a metal plate using the acid α-cyano-4-hydroxycinnamico as a matrix. The peptide mixtures were then ionized in a Voyager DE-PRO matrix-assisted laser desorption/ionization/time-of-flight (MALDI-TOF) mass spectrometry system (Applied Biosystems, USA) equipped with a nitrogen laser at 337 nm, and ion/mass (m/z) or peptide fingerprints were obtained. Mass spectra were acquired in reflector mode and externally calibrated with Sequazyme Mass Standards Kit (Applied Biosystems). Proteins were identified by database searching with the peptide fingerprints using the Protein Prospector database (http://prospector.ucsf.edu) and MS-Fit UniProt.2006.03.21 (http://www.pir.uniprot.org/), based on the following search parameters: the *Trypanosoma* species of origin, pI ± 0.1% and MW ± 2% with tolerance of 75 ppm, one tryptic miss-cleavage allowed, variable modifications of methionine (oxidation), cysteine (carbamidomethylation) and pyroglutamate formation at N-terminal glutamine of peptides.

3. Results and Discussion

In *T. cruzi*, the heat shock response has been correlated to heat shock proteins (HSPs) expression [4, 10, 16–18], but the identity of other proteins involved in this process has not been reported. However, due to the physiological changes in cells subjected to heat shock, it is expected that a diverse set of proteins with a wide variety of functions involved in optimizing cellular metabolism will be expressed and that the heat shock response will not be limited to the production of chaperones [19–21]. In this study, we identified, for the

first time, spots showing a different abundance in *T. cruzi* epimastigotes subjected to heat shock compared to control parasites. This means that these spots showed a change in the amount of this particular form of the protein in response to temperature stress, suggesting a potential role in the heat shock response. In 2-DE gel, we can measure the amount of a particular form of a protein in a specific biological situation and experimental setting. The particular form of a protein we observe may result, as has been mentioned by Jungblut et al. in 2008, from transcripts from indistinguishable genes, indistinguishable parts of genes, or posttranslational modifications [22].

Studies on the nonlethal heat shock response in *T. cruzi* epimastigotes have been performed at different growth temperatures ranging from 37 to 43°C [10]. The types of responses observed at different temperatures differ and depend on the strain. In previous work, the Maracay strain exposed to 42°C for 4 h was observed to acquire a round shape [4]. The Silvio X-10/4 strain incubated at the same temperature for 3 h did not show this transformation [10]. de Carvalho et al. [16] determined that the CL strain does not have a classic stress response at 37°C. In another study using *T. cruzi* epimastigotes, a large increase in the expression level of HSP70, one of the key molecules induced in the heat shock response in many organisms, occurred only after a 3 h incubation at 42°C and was not observed after the same incubation time at 37 or 40°C [10]. In Mexico, it has been reported that the vast majority of isolates analyzed belong to the discrete typing unit (DTU) genetic group I, including the Ninoa strain, which we used in this work and for which some of the relevant biological characteristics have been described [13, 14, 23]. As this was the first study performing heat shock assays on the Ninoa strain, the first aim was to determine which temperature was adequate for the production of heat shock. For this, the effect of two nonlethal heat shock temperatures were tested on the mobility of *T. cruzi* epimastigotes, bearing in mind that movement is an indirect parameter of the effect of temperature on parasite homeostasis. Mobility inhibition curves of the *T. cruzi* strain Ninoa incubated at 37 or 42°C were generated. As shown in Figure 1(a), the experimental temperature of 37°C did not have any effect on the mobility of parasites, which remained virtually unchanged compared to the control temperature (28°C). In contrast, when the incubation temperature was elevated to 42°C, a decrease in the parasites' mobility was observed, which was time dependent. Also, parasites at 42°C showed slight morphological changes at 3 h of incubation, some of them began to acquire a round shape (Figure 1(b)). Based on the previous curve and the literature [10, 16], parasites were incubated at 42°C for 3 h to induce heat shock, which led to a mobility rate of about 75% and some morphological changes.

Protein extracts from control and heat-shock-treated parasites were subjected to 2-DE gel electrophoresis. Figure 2 shows 2-DE gels representative of three independent experiments from *T. cruzi* epimastigotes of the strain Ninoa under control (3 h at 28°C) (Figure 2(a)) and heat shock (3 h at 42°C) (Figure 2(b)) conditions, stained with silver nitrate. An average of 506 ± 73 spots were found in gels from
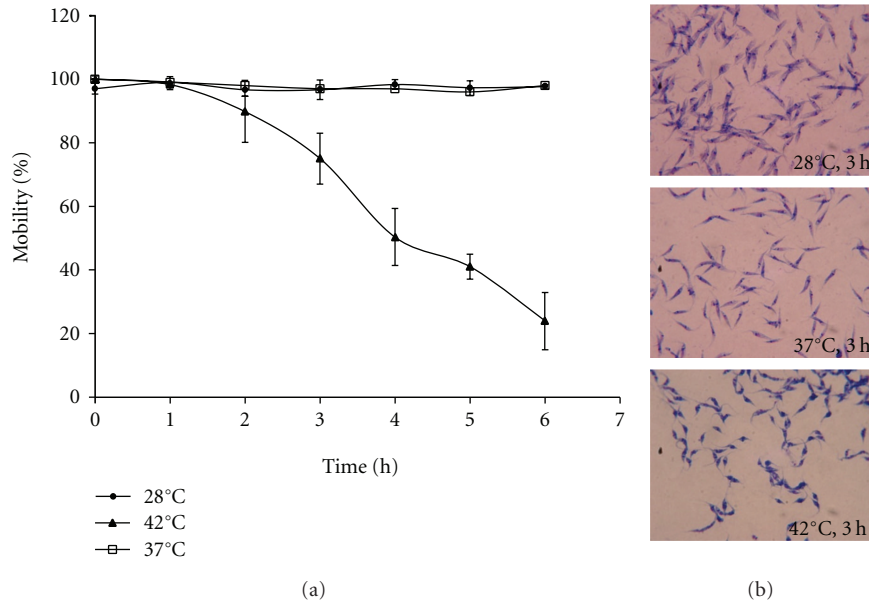
FIGURE 1: *T. cruzi* epimastigotes mobility and morphology after heat shock. (a) *T. cruzi* of the strain Ninoa in log phase growth were incubated at 28, 37, or 42°C for 1 to 6 h. Aliquots of each culture were taken each hour, and the number of mobile and immobile parasites was determined in a hemocytometer by observation in a microscope. The data are representative of three independent experiments and are shown as the mean ± SD. (b) Epimastigotes Giemsa-stained in log phase growth incubated at 28, 37, or 42°C for 3 h are shown.

parasites incubated at 28°C, whereas gels obtained from heat-shock-treated parasites had an average of 521 ± 31 spots. The number of proteins detected was comparable to and even higher than those previously reported by Parodi-Talice et al. in 2004, where an average of 350 spots was found using protein extracts from epimastigotes at the same pH range, run on a 10% polyacrylamide gel, and stained with silver nitrate [24]. An analysis of the distribution of the protein isoelectric point (pI) or the molecular weight (MW) for both conditions was performed. Extracts from parasites growing at either 28°C or 42°C showed a normal distribution of proteins based on established pI or MW ranges (data not shown). In fact, these two conditions had almost identical values for both distributions; most of the proteins had a pI of 5.1–7.0 and a molecular weight of 31–70 kDa, as was previously reported by Parodi-Talice et al. [24]. Additionally, as previously was reported by the same authors, most of the proteins were localized in the acidic region and, consequently, the resolution of the separation of proteins was lower in this region of the gel than in the basic area (Figure 2). In different studies about the *T. cruzi* proteome over pH range of 3–10, there is a constant finding that the acidic region shows a low resolution and that the proteins with molecular weights above 90 kDa are subrepresented [24, 25]. Perhaps the intrinsic characteristics of parasite proteins are involved in this particular distribution.

A master gel from control and heat-shock-treated parasites is shown in Figure 2(c) and Figure 2(d), respectively, which contains all of the spots found in the gels of three independent experiments.

Then, for the selection of proteins identified by MS, we only considered those points that had a differential abundance between control and experimental conditions

(OD relation), were consistently found in three independent experiments, and were in sufficient concentrations for identification by PMF. Ultimately, we obtained peptide mass fingerprints for twenty-four spots. The spots that were analyzed by MS are indicated on the proteome of 42°C (Figure 2(b)), each with a number corresponding to Table 1, where they are listed and their main characteristics are described. Nineteen of the twenty-four spots corresponded to particular forms of proteins in which the value of the average intensity of three independent experiments was higher compared to the control spot, whereas, in the remaining five identified spots, the intensity was lower in the heat-stress experiments with respect to the control spots (Table 1). These spots represent a particular form of the identified protein.

Other parameters included in Table 1 are the MOWSE score, which is a conventional measured validation of peptides identified by MS that reflects the match between the alignment of the theoretical and experimentally determined masses, and, finally, the absolute probability that this match is a random event. Therefore, the protein with the highest score is the most likely to be the protein [26]. The percentage of coverage refers to fragments of the candidate protein sequence that aligned with the ion signals of peptides generated by the mass spectrometer. The accession numbers for each protein correspond to UniProt protein entries. Finally, the abundance (in number of folds) of the particular form of the protein in heat shock compared with the same particular form of the protein in control conditions was calculated.

The twenty-four particular forms of identified proteins showed a variability of functions and belonged to some of the functional groups of proteins that have been described to
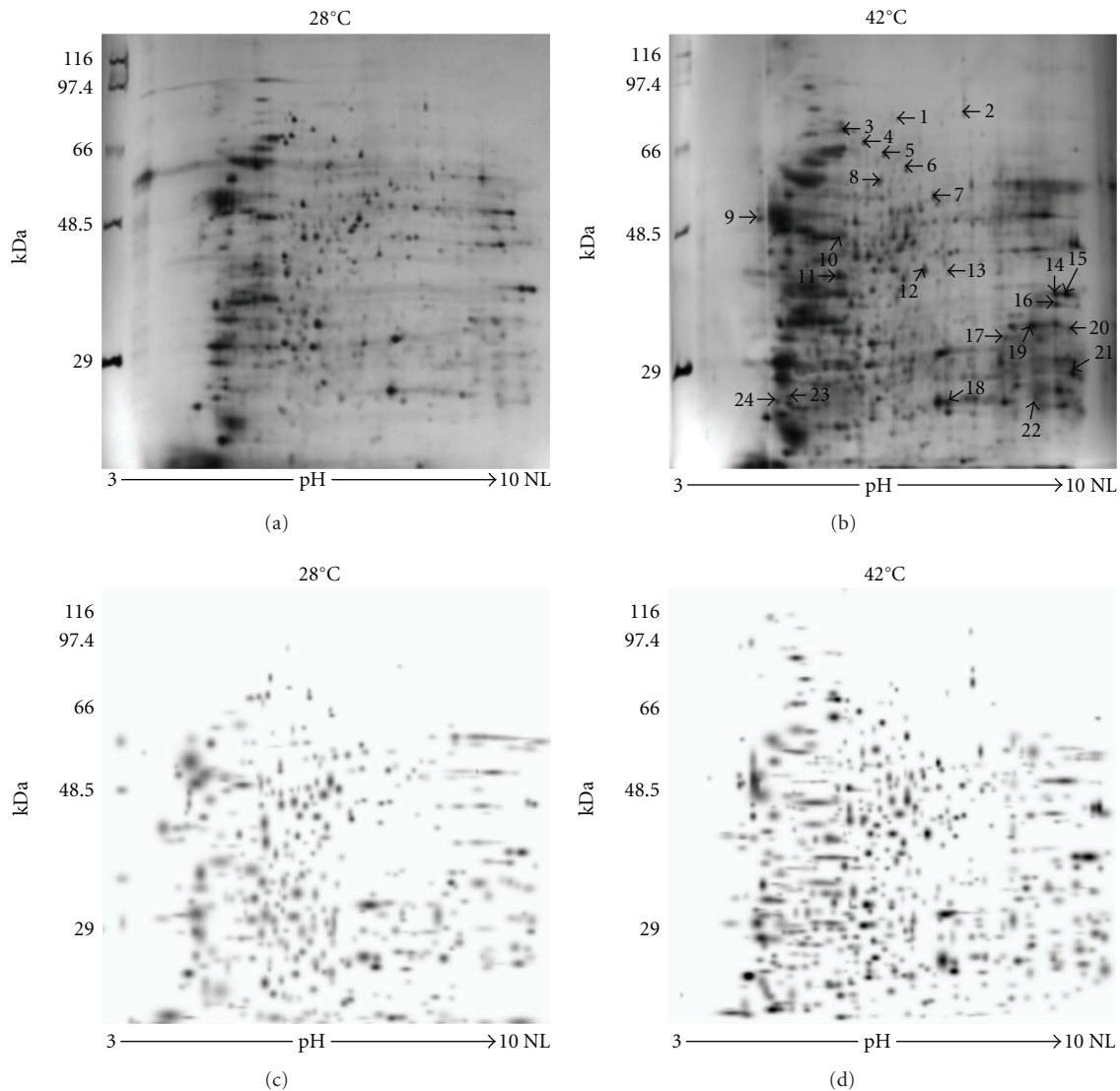
Figure 2: 2-DE polyacrylamide gels of control and heat-treated parasite extracts. (a) and (b) Silver-stained 2-DE gels of soluble proteins (300 μg per gel) from *T. cruzi* epimastigotes at normal growth (28°C, 3 h) (a) or heat shock (42°C, 3 h) temperatures (b). The proteins were firstly separated in a pH range of 3–10 NL and then on 10% SDS-PAGE. An average of 506 ± 73 spots were found in gels from parasites incubated at 28°C, and gels obtained from heat-shocked parasites had an average of 521 ± 31 spots. The molecular standards are indicated in kDa. The gels are representative of three independent experiments. In 2-DE gel from 42°C, the spots indicated by numbers were identified by MS (Table 1). (c) and (d) Master gels of control (c) or heat shock conditions (d). Three gel images obtained from different experiments from each condition (28°C or 42°C) were aligned and matched each other to generate an average master gel using the PDQuest software.

be induced by heat shock in many of the organisms analyzed by proteomic methods. The twenty-four particular forms of proteins identified were grouped into functional categories according to the Functional Catalogue 2004 [27]. Eight functional categories were assigned, and the percentages of particular forms of proteins identified that corresponded to each category were determined: metabolism (25%), cell defense (16.66%), interaction with the cellular environment (16.66%), hypothetical proteins (16.66%), protein fate (12.5%), cellular transport (4.16%), protein synthesis (4.16%), and cell cycle (4.16%). Six of the particular forms of proteins had a MOWSE score lower than 67; therefore, they are not discussed hereafter.

Particular forms of proteins involved in cell metabolism represented the largest group, which included particular forms of proteins involved in energy generation such as ATPase and ATP synthase. Another enzyme was found to be involved in the energy-generating process by means of the catabolism of aromatic amino acids, the hydroxyacyl-alpha-L-aromatic dehydrogenase [28]. Homologous proteins increase in abundance from other organisms like bacteria, and plants under thermal stress have been identified in previous proteomic analyses similar to this study [19–21]. Within the same functional category of proteins, a lower intensity of the NAD/FAD-dependent dehydrogenase, involved in oxidative phosphorylation, was observed after

TABLE 1: Particular form of proteins with a different abundance from *T. cruzi* epimastigotes subjected to heat shock identified by MALDI-TOF.

Particular form of a protein identity	Spot	Accession number	Theoretical pl/MW (kDa)	Experimental pl/MW (kDa)	Sequence coverage (%)	MOWSE score	Abundance (folds relative to control)
Metabolism							
Transitional endoplasmic reticulum ATPase, putative	3	Q4DWB5	5.4/86.1	5.4/77	50.5	$7.47\ e + 6$	2.057
ATP synthase, putative	7	Q4E1T6	6.0/53.7	5.9/57.6	22.8	101	1.876
Glutamate dehydrogenase, putative*	13	Q4CR18	6.0/43.2	6.1/43.4	24.5	16.8	1.317
Calcium translocating P-type ATPase*	16	Q86QH6	8.3/37.3	8.2/37.9	28.2	62.1	1.197
NAD-/FAD-dependent dehydrogenase, putative	11	Q4CVH0	5.3/43.0	5.3/43.4	21.4	72.6	1.072
Aromatic L-α-hydroxy acid dehydrogenase	17	Q9NJT2	6.8/33.7	6.9/34.1	25.3	204	−1.228
Cell defense							
Heat shock protein 70 (HSP70), putative	6	Q4DTM9	5.8/70.9	5.8/63.4	29.7	5611	1.776
Thiol transferase Tc52	10	Q7Z0C8	5.4/48.9	5.5/48.5	50.5	473601	1.544
Tryparedoxin peroxidase, putative	22	Q4CX87	7.6/25.5	7.8/25.6	35.0	124	1.125
Chaperone DNAJ protein, putative	19	Q4E244	7.6/35.3	7.6/34.9	41.7	2349	1.065
Interaction with the cellular environment							
Surface glycoprotein Tc-85/16*	8	Q2VYD0	5.5/66.8	5.6/61.5	27.6	1.81	1.821
Mucin-associated surface protein (MASP), putative	9	Q4CYQ5	4.4/50.0	4.2/53	29.3	692	1.403
Surface protease gp63, putative	5	Q4DTZ3	5.7/78.6	5.6/66	46.1	$3.25\ e + 9$	1.218
Mucin-associated surface protein (MASP), putative*	23	Q4DQ68	4.8/26.5	4.8/26.6	45.3	29.4	−1.514
Hypothetical protein							
Hypothetical protein	1	Q4D9E4	5.6/88.3	5.7/82.3	18.9	1160	1.577
Hypothetical protein	15	Q4CYM6	8.4/39.2	8.5/39.4	41.2	1197	1.569
Hypothetical protein	14	Q4CTH8	8.2/39.5	8.2/39.4	24.3	5713	1.537
Hypothetical protein	20	Q4E5C7	8.7/34.8	8.7/34.6	44.3	3723	−1.114
Protein fate							
Ubiquitin hydrolase, putative	4	Q4E680	5.6/81.1	5.5/71.4	38.6	$1.23\ e + 7$	1.390
Ubiquitin-conjugating enzyme E2, putative	24	Q4DVH2	4.5/25.7	4.5/26	34.8	96.6	−1.293
Proteasome regulatory non-ATPase subunit, putative*	18	Q4CPL0	6.0/25.2	6.1/25.7	41.7	7.13	−1.226
Protein synthesis							
Cysteinyl-tRNA synthetase, putative*	2	Q4CQ34	6.1/89.2	6.2/85.1	13	28.9	1.396
Cellular transport							
Rab6 GTPase-activating protein, putative	12	Q4DHN9	5.8/44.2	5.8/44.3	25.7	4739	1.342
Cell cycle							
Retrotransposon hot-spot (RHS) protein, putative	21	Q4D6R8	8.6/29.0	8.7/28.8	36.1	1456	1.206

*These proteins were not discussed because their MOWSE score is lower than 67.

heat shock. In wheat, a decrease in the expression of ATP synthase was observed after heat shock, and it was suggested that this enzyme might affect energy-dependent processes involved in the resistance to thermal shock [19]. In *T. cruzi*, a decline in energy supply is also a consequence of nutritional stresses of trypomastigotes [29].

There have been few proteomic studies aimed at studying the cellular stress response in pathogenic organisms [30]; however, it has been suggested that the physiology and other biological properties, including pathogenicity, are influenced by environmental parameters [31]. Other particular forms of proteins identified in this study were those involved in the interaction with the cellular environment, including a set of particular forms of proteins found on the parasite surface. Interestingly, among the particular forms of surface proteins that showed an increase in intensity as a result of stress treatment, a mucin-associated surface protein (MASP) was found, which is a *T. cruzi*-specific protein with until lately unknown function [32], but, recently, a MASP has been associated with invasion process ([33] and unpublished data from our laboratory). An increase of abundance of the surface metalloproteinase GP63 was also observed, which has been implicated in virulence, host cell infection, and the release of parasite surface proteins [32]. The attachment to the insect midgut has been described as one of the crucial steps that allow *T. cruzi* epimastigotes to continue their life cycle. For this process, the parasites express different proteins, including the GP82 and GP90 surface glycoproteins, whose appearance on the parasite surface is accompanied by the morphological differentiation from epimastigotes into metacyclic forms [34]. Perhaps the heat shock response triggers a protein expression pattern very similar to that shown during differentiation to trypomastigotes, the infective stage, as suggested by Alcina et al. [35]. For bacteria, it has been suggested that the synthesis of virulence factors is influenced by the availability of nutrients, pH, growth phase, and temperature [30].

Particular forms of proteins involved in cell defense represented 16.6% of the particular forms of proteins identified. HSP70 and the chaperone DnaJ protein (HSP40) increased their intensity after heat shock. The increase in HSP70 (1.7-fold) was similar to what was previously reported in a similar heat stress experiment by Olson et al. [10], where a 2.5-fold increase was observed. This also supports that a true heat shock response was induced in our model. Two other identified proteins, thiol transferase Tc-52 and tryparedoxin peroxidase, are known to be involved in responding to oxidative stress [36, 37]. It has been reported that, after a heat-shock response, oxidative stress is generated, which could occur for several reasons. After heat stress, many proteins are denatured, including antioxidant proteins, which leads to an accumulation of reactive oxygen species, characteristic of aerobic metabolism. It has also been shown that the reactivity of superoxide increases with temperature [36, 38].

Additionally, a small percentage of the particular forms of proteins identified were proteins implicated in protein fate, such as modification and destination. In eukaryotic cells, most of the proteins in the nucleus and cytoplasm are degraded in the 26S proteasome rather than in lysosomes, which is a multisubunit complex located in the cytoplasm and nuclear compartments. The proteasome proteolytic pathway controls a wide variety of cellular functions like the quality control of proteins, which includes the degradation of mutated or damaged proteins [39]. Ubiquitin-conjugating enzyme E2 performs the second step in the ubiquitination reaction that targets a protein for degradation via the proteasome. For *T. cruzi*, an essential role of the proteasome in the transformation to different parasite stages has been reported [40]. An increase in abundance of proteasome proteins was observed in parasites subjected to metacyclogenesis and exposed to Benznidazole [41, 42].

Particular form of protein involved in cellular transport the Rab6 GTPase- (guanosine-triphosphatase-) activating protein, which is involved in the regulation of membrane transport in *T. brucei* [43] was identified in this work. A protein retrotransposon hot spot was also found, which is associated with polymorphic subtelomeric regions [44] and whose role in heat stress could not be determined.

It has been proposed that, although some stress response mechanisms are highly conserved across species, others, like those involved in the optimization of metabolism and cell function or regulation, vary from organism to organism [21]. For this reason, we expected to find proteins with a variety of functions involved in stress resistance. In addition, in the case of *T. cruzi*, which has a large number of proteins with unknown functions, this is more relevant because of the potential that these proteins could be involved in the biology of the parasite. Some of the particular forms of proteins identified in our study have already been found in proteomic studies in which the parasite was subjected to different stresses: nutritional stress, stress by exposure to Benznidazole and metacyclogenesis [41, 42]. Thus, we suggest a convergence of resistance mechanisms to other stresses, including the parasite transformation from one stage to another. Finally, because of the increase in abundance of some surface proteins, it will be interesting to study the infectivity of parasites undergoing heat stress and to determine whether a relationship exists between this response and the infection process.

4. Conclusions

In summary, in this study, the *T. cruzi* epimastigotes proteome subjected to heat shock was described, and particular forms of different proteins with different abundance after heat shock were identified by PMF. To our knowledge, this is the first time a bulk of proteins different from HSPs were found to be differentially abundant during heat shock in *T. cruzi*.

Taken together, these findings offer new insights into the basic physiological processes involved in the *T. cruzi* response to heat shock; this response is vital for parasite survival.

Acknowledgments

References

[1] World Expert Committee, "Control of Chagas disease," Tech. Rep. 905, World Health Organization—Technical Report Series , 2002.

[2] J. Jannin and R. Salvatella, "Quantitative estimates of Chagas disease in the Americas," Tech. Rep. OPS/HDM/CD/425-06, Pan American Health Organization, Washington, DC, USA, 2006.

[3] J. C. P. Dias, "Epidemiology of Chagas disease," in *Chagas Disease (American Trypanosomiasis): Its Impact on Transfusion and Clinical Medicine*, S. Wendel, Z. Brener, M. E. Camargo, and A. Rassi, Eds., ISBT Brazil '92, Sao Paulo, Brazil, 1992.

[4] J. M. Requena, A. Jimenez-Ruiz, M. Soto et al., "Regulation of hsp70 expression in *Trypanosoma cruzi* by temperature and growth phase," *Molecular and Biochemical Parasitology*, vol. 53, no. 1-2, pp. 201–212, 1992.

[5] S. E. B. Graefe, M. Wiesgigl, I. Gaworski, A. Macdonald, and J. Clos, "Inhibition of HSP90 in *Trypanosoma cruzi* induces a stress response but no stage differentiation," *Eukaryotic Cell*, vol. 1, no. 6, pp. 936–943, 2002.

[6] D. Zilberstein and M. Shapira, "The role of pH and temperature in the development of *Leishmania* parasites," *Annual Review of Microbiology*, vol. 48, pp. 449–470, 1994.

[7] B. F. Hall, "*Trypanosoma cruzi*: mechanisms for entry into host cells," *Seminars in Cell Biology*, vol. 4, no. 5, pp. 323–333, 1993.

[8] J. K. Finzi, C. W. M. Chiavegatto, K. F. Corat et al., "*Trypanosoma cruzi* response to the oxidative stress generated by hydrogen peroxide," *Molecular and Biochemical Parasitology*, vol. 133, no. 1, pp. 37–43, 2004.

[9] P. Azambuja, C. B. Mello, and E. S. Garcia, "Immunity to *Rhodnius prolixus*: inducible peptides against bacteria and trypanosomes," in *Host Regulated Developmental Mechanisms in Vector Arthropods*, D. Borovsky and A. Spielman, Eds., Vero Beach, Fla, USA, 1989.

[10] C. L. Olson, K. C. Nadeau, M. A. Sullivan et al., "Molecular and biochemical comparison of the 70-kDa heat shock proteins of *Trypanosoma cruzi*," *Journal of Biological Chemistry*, vol. 269, no. 5, pp. 3868–3874, 1994.

[11] Z. Prohászka and G. Füst, "Immunological aspects of heat-shock proteins—the optimum stress of life," *Molecular Immunology*, vol. 41, no. 1, pp. 29–44, 2004.

[12] C. Clayton and M. Shapira, "Post-transcriptional regulation of gene expression in trypanosomes and leishmanias," *Molecular and Biochemical Parasitology*, vol. 156, no. 2, pp. 93–101, 2007.

[13] V. López-Olmos, N. Pérez-Nasser, D. Piñero, E. Ortega, R. Hernandez, and B. Espinoza, "Biological characterization and genetic diversity of Mexican isolates of *Trypanosoma cruzi*," *Acta Tropica*, vol. 69, no. 3, pp. 239–254, 1998.

[14] M. F. Bosseno, C. Barnabé, E. M. Gastélum et al., "Predominance of *Trypanosoma cruzi* lineage I in Mexico," *Journal of Clinical Microbiology*, vol. 40, no. 2, pp. 627–632, 2002.

[15] T. Berkelman and T. Stenstedt, *2-D Electrophoresis Using Immobilized pH Gradients. Principles and Methods*, Amersham Pharmacia Biotech Inc., 1998.

[16] E. F. de Carvalho, F. T. de Castro, E. Rondinelli, and J. F. Carvalho, "Physiological aspects of *Trypanosoma cruzi* gene regulation during heat-shock," *Biological Research*, vol. 27, no. 3-4, pp. 225–231, 1994.

[17] M. Fernandes, R. Silva, S. C. Rössle, P. M. Bisch, E. Rondinelli, and T. P. Ürményi, "Gene characterization and predicted protein structure of the mitochondrial chaperonin HSP10 of *Trypanosoma cruzi*," *Gene*, vol. 349, pp. 135–142, 2005.

[18] D. Pérez-Morales, P. Ostoa-Saloma, and B. Espinoza, "*Trypanosoma cruzi* SHSP16: characterization of an α-crystallin small heat shock protein," *Experimental Parasitology*, vol. 123, no. 2, pp. 182–189, 2009.

[19] T. Majoul, E. Bancel, E. Triboï, J. B. Hamida, and G. Branlard, "Proteomic analysis of the effect of heat stress on hexaploid wheat grain: characterization of heat-responsive proteins from non-prolamins fraction," *Proteomics*, vol. 4, no. 2, pp. 505–513, 2004.

[20] A. K. Schmid, M. S. Lipton, H. Mottaz, M. E. Monroe, R. D. Smith, and M. E. Lidstrom, "Global whole-cell FTICR mass spectrometric proteomics analysis of the heat shock response in the radioresistant bacterium *Deinococcus radiodurans*," *Journal of Proteome Research*, vol. 4, no. 3, pp. 709–718, 2005.

[21] A. R. Slabas, I. Suzuki, N. Murata, W. J. Simon, and J. J. Hall, "Proteomic analysis of the heat shock response in *Synechocystis* PCC6803 and a thermally tolerant knockout strain lacking the histidine kinase 34 gene," *Proteomics*, vol. 6, no. 3, pp. 845–864, 2006.

[22] P. R. Jungblut, H. G. Holzhütter, R. Apweiler, and H. Schlüter, "The speciation of the proteome," *Chemistry Central Journal*, vol. 2, no. 1, p. 16, 2008.

[23] B. Espinoza, T. Rico, S. Sosa et al., "Mexican *Trypanosoma cruzi* I strains with different degrees of virulence induce diverse humoral and cellular immune responses in a murine experimental infection model," *Journal of Biomedicine and Biotechnology*, vol. 2010, Article ID 890672, 10 pages, 2010.

[24] A. Parodi-Talice, R. Durán, N. Arrambide et al., "Proteome analysis of the causative agent of Chagas disease: *Trypanosoma cruzi*," *International Journal for Parasitology*, vol. 34, no. 8, pp. 881–886, 2004.

[25] J. Paba, J. M. Santana, A. R. L. Teixeira, W. Fontes, M. V. Sousa, and C. A. O. Ricart, "Proteomic analysis of the human pathogen *Trypanosoma cruzi*," *Proteomics*, vol. 4, no. 4, pp. 1052–1059, 2004.

[26] D. J. C. Pappin, P. Hojrup, and A. J. Bleasby, "Rapid identification of proteins by peptide-mass fingerprinting," *Current Biology*, vol. 3, no. 6, pp. 327–332, 1993.

[27] A. Ruepp, A. Zollner, D. Maier et al., "The FunCat, a functional annotation scheme for systematic classification of proteins from whole genomes," *Nucleic Acids Research*, vol. 32, no. 18, pp. 5539–5545, 2004.

[28] C. Nowicki and J. J. Cazzulo, "Aromatic amino acid catabolism in trypanosomatids," *Comparative Biochemistry and Physiology A*, vol. 151, no. 3, pp. 381–390, 2008.

[29] R. M. Martins, C. Covarrubias, R. G. Rojas, A. M. Silber, and N. Yoshida, "Use of L-proline and ATP production by *Trypanosoma cruzi* metacyclic forms as requirements for host cell invasion," *Infection and Immunity*, vol. 77, no. 7, pp. 3023–3032, 2009.

[30] M. R. Lee, S. M. Bae, T. S. Kim, and K. J. Lee, "Proteomic analysis of protein expression in Streptococcus pneumoniae in response to temperature shift," *Journal of Microbiology*, vol. 44, no. 4, pp. 375–382, 2006.

[31] M. E. Konkel and K. Tilly, "Temperature-regulated expression of bacterial virulence genes," *Microbes and Infection*, vol. 2, no. 2, pp. 157–166, 2000.

[32] N. M. El-Sayed, P. J. Myler, D. C. Bartholomeu et al., "The genome sequence of *Trypanosoma cruzi*, etiologic agent of chagas disease," *Science*, vol. 309, no. 5733, pp. 409–435, 2005.

[33] L. M. de Pablos, G. G. González, J. S. Parada et al., "Differential expression and characterization of a member of the mucin-associated surface proteins (MASP) family secreted by *Trypanosoma cruzi*," *Infection and Immunity*, vol. 79, pp. 3993–4001, 2011.

[34] E. M. Cordero, L. G. Gentil, G. Crisante et al., "Expression of GP82 and GP90 surface glycoprotein genes of *Trypanosoma cruzi* during in vivo metacyclogenesis in the insect vector *Rhodnius prolixus*," *Acta Tropica*, vol. 105, no. 1, pp. 87–91, 2008.

[35] A. Alcina, A. Urzainqui, and L. Carrasco, "The heat-shock response in *Trypanosoma cruzi*," *European Journal of Biochemistry*, vol. 172, no. 1, pp. 121–127, 1988.

[36] H. Sies, "Strategies of antioxidant defense," *European Journal of Biochemistry*, vol. 215, no. 2, pp. 213–219, 1993.

[37] R. L. Krauth-Siegel, S. K. Meiering, and H. Schmidt, "The parasite-specific trypanothione metabolism of *Trypanosoma* and *Leishmania*," *Biological Chemistry*, vol. 384, no. 4, pp. 539–549, 2003.

[38] J. F. Davidson, B. Whyte, P. H. Bissinger, and R. H. Schiestl, "Oxidative stress is involved in heat-induced cell death in *Saccharomyces cerevisiae*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 10, pp. 5116–5121, 1996.

[39] J. L. de Diego, J. M. Katz, P. Marshall et al., "The ubiquitin-proteasome pathway plays an essential role in proteolysis during *Trypanosoma cruzi* remodeling," *Biochemistry*, vol. 40, no. 4, pp. 1053–1062, 2001.

[40] J. González, F. J. Ramalho-Pinto, U. Frevert et al., "Proteasome activity is required for the stage-specific transformation of a protozoan parasite," *Journal of Experimental Medicine*, vol. 184, no. 5, pp. 1909–1918, 1996.

[41] A. Parodi-Talice, V. Monteiro-Goes, N. Arrambide et al., "Proteomic analysis of metacyclic trypomastigotes undergoing *Trypanosoma cruzi* metacyclogenesis," *Journal of Mass Spectrometry*, vol. 42, no. 11, pp. 1422–1432, 2007.

[42] H. M. Andrade, S. M. F. Murta, A. Chapeaurouge, J. Perales, P. Nirdé, and A. J. Romanha, "Proteomic analysis of *Trypanosoma cruzi* resistance to benznidazole," *Journal of Proteome Research*, vol. 7, no. 6, pp. 2357–2367, 2008.

[43] M. Berriman, E. Ghedin, C. Hertz-Fowler et al., "The genome of the African trypanosome *Trypanosoma brucei*," *Science*, vol. 309, no. 5733, pp. 416–422, 2005.

[44] F. Bringaud, N. Biteau, S. E. Melville et al., "A new, expressed multigene family containing a hot spot for insertion of retroelements is associated with polymorphic subtelomeric regions of *Trypanosoma brucei*," *Eukaryotic Cell*, vol. 1, no. 1, pp. 137–151, 2002.