# IN VITRO REFOLDING OF RECOMBINANT TSA-1 ANTIGEN OF *TRYPANOSOMA CRUZI*

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**Introduction.** Chagas disease is an important neglected tropical disease caused by the protozoan parasite *Trypanosoma cruzi.* The treatments available to control this infection lack of efficiency and are expensive. An alternative to improve the control of this infection is a therapeutic vaccine (1). Recent DNA vaccine studies have shown TSA-1 antigen as a potential vaccine candidate (2). However, DNA vaccines have poor immunotherapeutic history and regulatory limitations; therefore, the long term goal is to develop a therapeutic vaccine against Chagas disease by the immunization with the properly folded recombinant protein counterpart (1).
The aim of this work was to refold the rTSA-1 expressed in *E. coli* as inclusion bodies.

**Methods.** *E. coli* BL21 (DE3) cells harboring the *pET41a-TSA-1* construct were grown in Fernbach flask containing 1 L of LB medium at 30 °C and 200 rpm. The recombinant TSA-1 (rTSA-1) was induced by adding 1 mM IPTG at $OD_{600nm}$ of 0.6. Cells were collected by centrifugation and inclusion bodies were isolated and washed from insoluble fraction (3). The inclusion bodies were solubilized in buffer A (20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 8 M urea, 1 mM 2-Mercaptoethanol and 20 mM imidazole). Denature rTSA-1 (0.4 mg/ml) was refolded by a two-step dialysis; first from 8 to 2 M urea and then from 2-0 M urea. A similar strategy was used to refold rTSA-1 by size-exclusion chromatography, denature protein was loaded into a PD-10 desalting column (GE, Healthcare) equilibrated with buffer B (buffer A with 4 M urea and without imidazole). The eluted protein was loaded into a second PD-10 column equilibrated with buffer C (buffer B without urea and with 20% glycerol). Also, the denature protein was loaded into a nickel affinity column (GE Healthcare) and rTSA-1 was refolded by using a gradient with buffer A and buffer A without urea and 2-Mercaptoethanol. Then rTSA-1 was eluted with 500 mM imidazole. The refolded protein was stored in buffer C at 4 °C until intrinsic fluorescence and circular dichroism spectra were determined.

**Results.** rTSA-1 was present only in the insoluble fraction (Fig. 1A, lane 1). The intrinsic fluoresce and circular dichroism spectra of the refolded protein by dialysis, size-exclusion and affinity chromatography suggest that rTSA-1 is properly folded (Fig 1B). Interestingly, no protein aggregation was observed after one month of storage of the refolded rTSA-1 at 4°C.

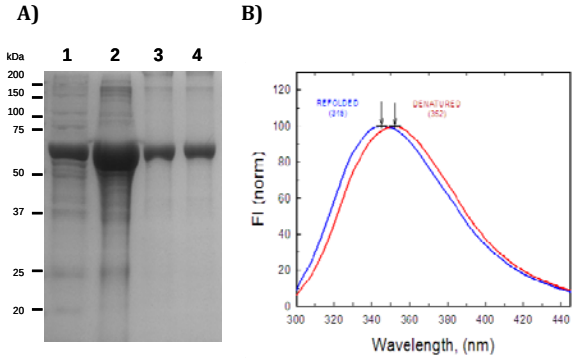


**Fig.1** Purification and refolding by affinity chromatographic of TSA-1. A) SDS-PAGE analysis of the recombinant TSA-1. Total protein (lane 1), inclusion bodies (lane 2), purified and refolded protein before (lane 3) and after removing the imidazole (lane 4). B) Intrinsic fluorescence of refolded and denatured TSA-1.

**Conclusions.** TSA-1 expressed in *E. coli* as inclusion bodies can be refolded by dialysis, size-exclusion or affinity chromatography.

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**References.**

1. Dumonteil E, Botazzi Maria, *et. Al.*(2012). *Expert rev. vaccines.*11 (9):1043-1055
2. Quijano I. and Dumonteil E. (2011). *Hum vaccines.* vol. 7(11):1184-1191.
3. Coligan J., Dunn B., Speicher D., *et. Al.* (2007). *Current Protocols in Protein Science.* Taylor G. John Wiley and Sons, USA. 6.3.1 - 6.3.15.