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 OD600nm 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 fluorescence 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 fluorescent 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.