HETEROLOGOUS EXPRESSION OF *Trypanosoma cruzi* APYRASE (NTPDase-I) WITH AND WITHOUT SIGNAL PEPTIDE

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An ecto-NTP diphosphohydrolase (NTPDase-I) was previously characterized on the surface of live *T. cruzi* parasites and a 2282 bp cDNA encoding a full-length NTPDase-I (~70.8 KDa) was cloned. In order to further characterize *T. cruzi* NTPDase-I we performed the heterologous expression of recombinant NTPDase-I. *In silico* analysis using Signal-P program predicts a possible cleavage signal peptide at amino acid position 36, immediately following an amino-terminal predicted transmembrane segment, thus suggesting that NTPDase-I could be produced as a soluble exported protein (~66.6 kDa). Using this information we design strategies to express full NTPDase-I and putative secreted protein. Full NTPDase-I was used as template to amplify DNA fragments that were transferred to pET21b bacterial expression vector (that codes for Hexa-HIS tag at the carboxy terminal of the recombinant fusion protein). These constructions (full-NTPDase-I-pET21b and partial-NTPDase-I-pET21b) were used to transform *E. coli* BL21 cells. Recombinant proteins were expressed after 4 hours of 0.1mM IPTG induction. Soluble and insoluble recombinant proteins were purified using Ni or Co-agarose affinity chromatography. Our results showed that recombinant proteins with and without signal peptide were eluted with 80mM and 160mM of imidazol, respectively. Western blot analysis showed that anti-NTPDase-I polyclonal antiserum was able to recognize recombinant proteins and evidenced that signal peptide was recognized by the bacterial system. We intend evaluated biochemical properties of these proteins and applied then in Chagas disease immune diagnosis assays.