Biochemical Characterization and Immunolocalization of Recombinant *Trypanosoma cruzi* Apyrase (NTPDase1)

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An ecto-apyrase activity was characterized on the surface of *T. cruzi* and a 2282 bp cDNA encoding a full-length NTPDase1 was cloned (Fietto et al., 2004). Trypomastigotes were shown to have higher ATP/ADP hydrolysis that seems to be involved with parasites infectivity and virulence (Santos et al., 2009). To further characterize *T. cruzi* NTPDase1 we performed heterologous expression of active recombinant enzyme. *In silico* analyses of the sequence predicts a possible cleavage signal peptide at amino acid position 36, immediately following an amino-terminal predicted transmembrane segment suggesting that NTPDase1 could be produced as a soluble exported protein. Using this information we designed a strategy to express the soluble NTPDase1. Full-length NTPDase1 cloned in pGEM vector was used as template to amplify a 1700 bp DNA fragment that was transferred to pET21b vector. This construction was used to transform *E. coli* BL21 cells. Recombinant protein was expressed after 1 hour of induction. Recombinant NTPDase1 was purified using Ni-NTA-agarose and showed specific activity for ATP hydrolysis between 2-17 nmol.mg $protein^{-1}.h^{-1}$. The ATPase activity was higher at alkaline pH and in the presence of Mg^{2+} rather than Mn^{2+} and Ca^{2+}**.** This enzyme presents a Km for ATP = 189.6 uM. The use of apyrase inhibitors (Suramin, Gadolinio and ARL) showed a partial ATPase and ADPase inhibition. Immunolocalization experiments using policlonal antiserum anti-NTPDase1 display a strong signal at the parasite surface. We concluded that NTPDase1 seems to be present in *T. cruzi* surface and that the recombinant rNTPDase1 was produced in an active form that should be suitable to start crystallization tests and to evaluate its potential as new target in rational drug design.

Keywords: Apyrase, NTPDase1, *Trypanosoma cruzi,*