BIOCHEMICAL AND BIOLOGICAL STUDIES WITH RECOMBINANT *TRYPANOSOMA CRUZI* APYRASE (NTPDASE I)

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An ecto-apyrase activity was characterized on the surface of *T. cruzi* and a cDNA encoding a full-length NTPDase was cloned (Fietto et al., 2004). Trypomastigotes were shown to have a 2:1 ATP/ADP hydrolysis ratio, while epimastigotes presented a 1:1 ratio, suggesting a possible role for the NTPDase in the parasite's virulence mechanism. To further characterize NTPDaseI 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 NTPDaseI as a soluble exported protein. Using this information we designed a strategy to express the soluble NTPDaseI. Full-length NTPDaseI 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. Soluble and insoluble recombinant apyrases were purified using Ni-NTA-agarose and showed specific activity for ATP hydrolysis between 2-17 nmol.mg protein^{-1}.h^{-1}. Substrate specificity assays showed preference for tri-phosphate nucleotides. Activity was higher at pHs between 6.5 – 7.5. We concluded that rNTPDaseI was produced in an active form that should be suitable to start crystallization tests and to evaluate its potential as new target in specific drug design tests.