

Cloning, Expression and Purification of Phosphomevalonate Kinase from
Trypanosoma cruzi

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Chagas disease continues to be an important public health problem in Americas. The etiological agent of the disease is the hemoflagellate protozoan *Trypanosoma cruzi*. There are some drugs that are being used to treat this disease but they are active only against the acute phase. Then, it is essential importance the development of new effective drugs against this parasite, principally to chronic phase. We used AnEnp, a computational tool that possibilities the detection of analogous enzymes, new possible drug targets, based on the structural differences of enzymes shared by humans and *T. cruzi*. In other words, they have similar functions but differences on the 3D structure. One of these enzymes is that catalyzes an important step of mevalonate pathway. The objective of this study is the molecular and biochemical characterization, and the cellular localization of the phosphomevalonate kinase (FMK), a putative analogue enzyme. In this study we have cloned and sequenced the gene of FMK of *T. cruzi*. The amplicon with 1,431 bp, was inserted in pET-SUMO vector and cloned in *E. coli* Match1 and BL21 strains. After induction with IPTG 1mM, the expression of a protein with approximately 67.5 kDa was obtained in soluble and insoluble form. The protein was purified by Ni²⁺-HisTrap HP column on HPLC and after that utilized for susceptible BALB/c mice immunization to obtain polyclonal antibodies. This polypeptide has been used for mouse polyclonal antibody development and immunocytochemical studies. This work is of considerable relevance for the study of the parasite metabolism and for the development of new strategies for drug design against this pathogen.

Key-words: cloning, phosphomevalonate kinase, *T. cruzi*

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