Calcium Sensitivity and Subcellular Localization of *Trypanosoma cruzi* Pyruvate Dehydrogenase Phosphatase (TcPDP)

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INTRODUCTION: Calcium ion (Ca^{2+}) is an important second messenger in trypanosomatids, essential for survival through their complex life cycle. In vertebrate cells, mitochondrial Ca^{2+} uptake is required to provide reducing equivalents to support oxidative phosphorylation through activation of dehydrogenases and the ATP synthase. Intramitochondrial Ca^{2+} stimulates pyruvate dehydrogenase phosphatase (PDP) that activates the pyruvate dehydrogenase (PDH), resulting in increased ATP production. This enzyme has not been studied in *Trypanosoma cruzi*, the causative agent of Chagas disease. However, TcPDH E1α subunit exhibits putative phosphorylation sites similar to those of the mammalian PDH, suggesting that, as the mammalian enzyme, it could be activated by calcium-stimulated dephosphorylation by TcPDP. **OBJECTIVES:** Investigate Ca^{2+} sensitivity of recombinant TcPDP and determine the subcellular localization of the enzyme by CRISPR/Cas9-mediated endogenous C-terminal tagging in *T. cruzi*. **MATERIALS AND METHODS:** *TcPDP* (gene ID: TcCLB.506315.100) was PCR-amplified and cloned into pET32 vector for heterologous expression in *E. coli*, which was further induced by IPTG addition to cells cultured on LB broth. Recombinant TcPDP was affinity purified under native conditions and enzymatic activity was assayed at different Ca^{2+} concentrations by quantitating phosphate release from a synthetic phosphopeptide corresponding to a segment of the TcPDH E1α subunit. For localization assays, we generated a *T. cruzi* cell line where endogenous *TcPDP* gene was modified by CRISPR/Cas9 genome editing, to encode the C-terminal tagged protein TcPDP-3xHA, to be used for immunofluorescence analysis using anti-HA antibodies and Mitotracker. **RESULTS:** Our results indicate that TcPDP is an intramitochondrial phosphatase that exhibits a peak of activity at 100 nM Ca^{2+}. **CONCLUSIONS:** TcPDP is sensitive to physiological Ca^{2+} concentrations *in vitro*, and it could be also calcium-stimulated *in vivo* to specifically activate mitochondrial pyruvate dehydrogenase, involved in energy metabolism. Further generation of a TcPDP knockout cell line will be useful to elucidate its role *in vivo*.

Keywords: Pyruvate dehydrogenase phosphatase, *Trypanosoma cruzi,* calcium sensitivity, CRISPR/Cas9
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