Biochemical characterization of a potential Trypanosoma cruzi nitric oxide synthase

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Post-translational modifications (PTMs) decorate proteins influencing several physicochemical properties and playing a key role in numerous biological processes. More than 450 PTMs have been described from prokaryotes to eukaryotes. Among these PTMs is protein S-nitrosylation (SNO) which is generated by the addition of nitric oxide (NO) to specific cysteine thiols in target proteins. Nitric oxide (NO) is a highly diffusible and short-lived cellular messenger produced by the nitric oxide synthase (NOS) family of proteins. The sites and stoichiometry of SNO regulates a variety of important pathophysiological responses aiming to maintain cellular homeostasis. Recently, our group identified S-Nitrosylated proteins in trypomastigote forms of Trypanosoma cruzi, the etiological agent of Chagas disease, and host proteins following the incubation of the parasites with the host extracellular matrix (ECM), suggesting the presence of NOS and roles of NO in the host\'s extracellular environment. Based on previous results from our laboratory, we identified a single copy of the putative oxido-reductase protein (NOS putative gene) located on chromosome 9 in T. cruzi Y strain, encoded by 1878 bp. In this context, the study aims to characterize the nitric oxide synthase enzyme from T. cruzi. We have designed specific primers containing restriction enzyme sites (HindIII and XbaI) to amplify the putative T. cruzi NOS gene using T. cruzi genomic DNA as the template. The first step was to optimize the amplification conditions by performing a primer gradient of annealing temperatures, and we chose 65°C to proceed to the next steps. The NOS gene was amplified by PCR and purified using the Wizard® SV gel and PCR clean-up system (Promega). The NOS putative gene was inserted into the pTREXn vector containing HA tagging, and the construct was cloned in E. coli DH5? with subsequent miniprep plasmid purification using the Wizard® Plus SV Minipreps DNA Purification System (Promega). The pTREXn-NOS was confirmed by sequencing analysis. These results will direct the NOS localization and functional characterization in T. cruzi providing detailed information about the SNO pathway in this organism. In the future, this could contribute to the targeting of the putative TcNOS for the development of new drugs to control Chagas disease.

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