Title: "Biochemical Characterization of the ChaC Family of γ-Glutamyl Cyclotransferases from *Leishmania major*"

Abstract

Leishmaniasis is a vector borne disease that currently affect millions of people worldwide every year especially population living in tropical countries. Unfortunately, India geographically lies in the affected region and most of its population is at risk. There are reports of these diseases becoming resistant to the currently used frontline drugs. Keeping this in mind the present research work has been designed with main objectives; parasite derived factor that modulates the pathogenicity of *Leishmania*. γ-glutamyl cyclotransferase (ChaC) like protein are considered as one of the parasite specific factors that modulates the pathogenicity of *Leishmania*.

Glutathione is a very important redox molecule throughout all kingdoms, its synthesis as well as degradation plays a key role for maintaining redox homeostasis. Although glutathione synthesis is well studied in trypanosomatids, glutathione degradation pathways are still missing in trypanosomal parasites. The ChaC family of γ-glutamyl cyclotransferase is conserved throughout all kingdoms which catalyzes the degradation of glutathione. Here we report two ChaC proteins ($LmChaC_{2a}$ and $LmChaC_{2b}$) in unicellular protozoan parasite *Leishmania*, which specifically degrade reduced glutathione (GSH), but no other γ-glutamyl peptides, trypanothione or oxidized glutathione (GSSG). Interestingly, HPLC activity measurements reveals that recombinant $LmChaC_{2b}$ (38 kDa) shows ~17 times more catalytic efficiency than $LmChaC_{2a}$ (28 kDa) towards GSH. Quantitative-PCR and western blot analysis suggests that $LmChaC_{2b}$ expression is depends on the sulfur stress, whereas $LmChaC_{2a}$ is constitutively expressed in the parasites. To understand its exact physiological role in *Leishmania major*, we have created overexpressed (OE_{2a} and OE_{2b}), knockout (KO) and complement cell lines (CM_{2a}, CM_{2b} and CM). Flow cytometric analysis suggests that null mutants (KO) have more reductive environment due to the presence of higher amount of GSH and lower amount of H_2O_2. Null mutant contains lower percentage of metacyclic promastigote and dead cells in late log phase but higher percentage of dead cells in stationary phase compared to control (CT) and complement cell lines. Growth curve analysis also reveals that KO cells grow very rapidly but can't survive for long time in aged culture. In addition, LmChaC-dependent controlled GSH degradation is crucial for intracellular survival of Leishmania following infection in macrophage or mice. This work clarifies how GSH homeostasis likely regulates chronic infection of the parasite.

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