Functional Characterization of Fumarate Hydratase Isoforms in *Leishmania major*

Feliciano, P. R.[1], Baruffi, M. D.[2], Costa-Filho, A. J.[3] and Nonato, M. C.[1]

[1] Laboratório de Cristalografia de Proteínas - FCFRP – USP, Ribeirão Preto, SP, Brazil; [2] Departamento de Análises Clínicas, Toxicológicas e Bromatológicas - FCFRP – USP, Ribeirão Preto, SP, Brazil; [3] Grupo de Biofísica Molecular Sérgio Mascarenhas – IFSC – USP, São Carlos, SP, Brazil.

Fumarate hydratases (FH) are ubiquitous enzymes, which catalyses the stereospecific reversible hydration of fumarate to malate. Eukaryotes express two isoforms of FH, the mitochondrial isoform which performs this reaction as part of the tricarboxylic acid cycle and as such is central to aerobic respiration and the cytosolic isoform which is thought to be involved in the metabolism of fumarate. Recent studies in trypanosomatids, utilizing *Trypanosoma brucei* as model, suggest that fumarases are essential for survival of these parasites. In the present work, we have successfully cloned, expressed in *E.coli* and purified both FH isoforms of *Leishmania major* (*Lm*FH). In order to functionally characterize, the recombinant enzymes have been used to perform kinetic, biophysical and structural characterization of LmFHs. Circular dichroism studies have identified differences in secondary structure content when comparing both isoforms and electron paramagnetic resonance approach has identified the presence of an iron-sulfur cluster in only one isoform. In addition, significant differences in specific activities were found for both isoforms. Polyclonal antibodies have also been raised and subcellular localization studies for both isoforms are in progress. Our results suggest that LmFH isoforms, which share around 60% of sequence identity, besides being localized in different cell compartments, also display differences in protein folding and mechanism of action. The studies, here proposed, correspond to the first reported work for functional characterization of *Lm*FH isoforms.

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