Ribose 5-phosphate isomerase from *Leishmania major*: molecular characterization and identification of native enzyme

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Leishmaniasis is a widespread endemic disease in several parts of the world and is caused by some species of *Leishmania*. There are some drugs used to treat this disease, but the parasite is becoming resistant to many of them. The development of new effective drugs against this parasite is therefore essential. Analogous enzymes are result of convergent evolution and, for this reason they carry out the same enzymatic activity. These enzymes show differences in their 3D structures that could be explored as possible drug targets. AnEnπ tool was used for the identification of putative cases of analogous enzymes between *Homo sapiens* and *L. major*. One of these enzymes is ribose 5-phosphate isomerase (R5PI) that is involved in the important step of the pentose phosphate pathway catalyzing the inter-conversion of D-ribose 5-phosphate and D-ribulose 5-phosphate. The parasite enzyme is a type B R5PI and human host has a structurally unrelated type A R5PI, and therefore has been proposed as an attractive drug target. In this study, we cloned the R5PI gene from *L. major* in the pBADThio/TOPO® vector, expressed the protein in insoluble form, purified it and produced polyclonal antibodies in order to identify the protein in promastigote forms. Using 2D-PAGE technique with soluble proteins from *L. major* promastigotes followed by western-blot allowed us identify a spot with MW=18.27KDa and pI=6.52, compatible with the predicted values (18.61 and 6.40, respectively). Sequencing of the RT-PCR product showed that R5PI´s mRNA is present in promastigote forms. Immunofluorescence data shows that the native enzyme is located in the promastigote cytoplasm, probably in small vesicular organelles called glycosome, as described for species from the Kinetoplastida order. Our data is in accordance with the genomic annotation, showing that this enzyme is expressed at least in promastigotes of *L. major*. Further molecular docking analysis will be performed to search for putative molecules that could bind in the catalytic pocket of this enzyme, and so could be tried initially in *in vitro* experiments for its inhibitory characteristics.

Keywords: *Leishmania major*, ribose 5-phosphate isomerase, AnEnπ, protein purification, protein identification

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