Structure-function Relationship Studies of *Leishmania major* Dihydroorotate Dehydrogenase

[1]Leite, F.P., [1]Feliciano, P.R., [1]Nonato, M.C.
[1]Laboratório de Cristalografia de Proteínas de Ribeirão Preto, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil.

Dihydroorotate dehydrogenase enzyme (DHODH) catalyzes the fourth step of the *de novo* biosynthetic pathway of pyrimidine nucleotides, through the conversion of dihydroorotate into orotate. DHODH, essential in this pathway, is considered a promising therapeutic target to cytostatic and antiparasitic drugs. Though, as an important tool against Leishmaniasis, our project combine site-directed mutagenesis, kinetic and crystallographic experiments in order to establish a correlation between structure and function of *Leishmania major* DHODH (LmDHODH). In the present work, we have initiated our studies in order to identify the residues involved in the dimerization state of LmDHODH and evaluate the relevance of this oligomerization form for its catalytic activity. The C174H*Lm*DHODH mutant, where the histidine 174, located at the dimer interface, has been replaced by a cystein residue, was constructed in pET28 expression vector, purified by affinity chromatography with excellent yield and kinetically characterized. The structure determination by X-ray diffraction approach is in progress. The kinetic studies reveal a significantly decrease in specific activity for C174H*Lm*DHODH mutant, suggesting an important role of hystidine 174 and the dimerization form for enzyme activity. Furthermore, our preliminary crystallographic studies reveal the existence of a pocket located at the dimer interface that could be explored for the search of ligands that could disrupt the dimer or simply alter molecule dynamics, interfering with the catalytic function of LmDHODH.

This work was supported by FAPESP.