KINETIC STUDIES AND EVALUATION OF POTENTIAL COMPOUNDS FOR THE CHEMOTHERAPY OF LEISHMANIASIS USING *Ld*NH-MBP

Rennó, M.N.[1], da Silva, N. B.[2], Borja-Cabrera, G.P.[3], Palatnik-de-Sousa, C.B.P. [3], Tinoco, L.W.[2], Figueroa-Villar, J.D.[1];
[1] Departamento de Química, Instituto Militar de Engenharia, Rio de Janeiro, Brazil. [2] Núcleo de Pesquisas de Produtos Naturais and [3] Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Protozoan parasites rely exclusively on purine salvage from the host for DNA and RNA synthesis and nucleoside hydrolases (NHs) are the enzymes that catalyze the N-ribosyl hydrolysis of all commonly occurring purine and pirimidine nucleosides, thus being excellent targets for the design of antiparasitic compounds. The general aim of our work with *Leishmania donovani* NH (*Ld*NH) is to find new inhibitors for this enzyme as potential agents for the chemotherapy of visceral leishmaniasis. In this part of the work we expressed *Ld*NH bound to maltose-binding protein (MBP) in *E. coli* using the pMAL-C2x vector. After purification by affinity chromatography the enzyme activity was monitored by UV (280 nm) and ^{1}H NMR spectroscopy using inosine as substrate. All the assays were carried out at 25 ^{o}C in phosphate buffer (pH 8.0) in water (UV) and D_2O (NMR). Our results show that *Ld*NH-MBP behaves kinetically in the same way as it have been reported for free *Ld*NH, thus confirming that *Ld*NH-MBP maintains the appropriate folding and activity of the enzyme active site, thus being a good model to develop and evaluate new inhibitors of *Ld*NH. As an example, the kinetics tests with AZT have shown that this compound is not an effective inhibitor of this enzyme.

Acknowledgements: CAPES, FAPERJ, CNPQ, FINEP, IMBEBB.

Keywords: nucleoside hydrolase, NMR, *Leishmania donovani*.