Expression in *E. coli*, Purification and Fluorescence Studies of Tryptophan Mutants of the *Leishmania major* Macrophage Migration Inhibitory Factor

Melo M. T.¹, Sousa L. F¹, Cunha, E. M. F.², de Oliveira, A. H. C.¹

¹ Departamento de Química, FFCLRP-USP, Universidade de São Paulo, Ribeirão Preto, SP, Brazil. ² Instituto Federal de Educação, Ciência e Tecnologia de Rondônia, IFRO, Ji-Paraná, RO, Brazil.

Migration Inhibitory Factor (MIF) was the first cytokine to be identified from the human T cells and participates in innate and adaptive immune response. This protein has been considered an important factor in the control of parasites infections, presenting a beneficial or a detrimental role, depending on the pathogen. Interestingly, MIF homologues were identified in several parasites, including *Leishmania* (LmMIF), and have been suggested a possible modulating function of the host immune response to benefit the development of the pathogen. The characterization in solution of the LmMIF and of mutants can help understand the function of this protein in the host/parasite biochemical interactions. We have used site-direct mutagenesis of Trp residues coupled with spectroscopic studies to investigate the conformations changes in the LmMIF. The recombinant LmMIF and Trp mutants (W66L and W108F) were expressed in *E. coli*, purified from soluble extract by affinity chromatography and analyzed by circular dichroism. Intrinsic tryptophan fluorescence spectroscopy showed that 70% of the protein total fluorescence is resulting from Trp66 emission which was shown to be quenched possibly by W108 presence in the wild LmMIF. The pH effect was analyzed by fluorescence quenching wherein was observed an increased quenching at low pH, except for the W108F with acrylamide. Further W66L mutant presented the higher differences in the quencher access, suggesting the W108 residue as intrinsic probe for the LmMIF conformational changes studies. The results may be useful for understanding of the structural arrangements involved in the LmMIF oligomerization and its interaction with the membrane receptor of the host cell during the modulation of the immune response.

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