**On the interaction of LaSirR2P1, a sirtuin of *Leishmania amazonensis*, with resverastrol**

Fessel R. M.; Peres, B. R.; Tavares, R.C.A.; Ramos, C.H.I.

Institute of Chemistry, University of Campinas, SP, Brazil

**Introduction** *Leishmania amazonensis*, an etiologic agent of American tegumentary leishmaniasis, is a parasite that has two developmental forms, promastigote and amastigote. LaSir2RP1 is a sirtuin of the *L. amazonensis*, which is cytoplasmic and secreted from both parasite forms. Sirtuin proteins are NAD+-dependent lysine deacetylases that display essential cellular and developmental roles in *Leishmania*.

**Methodology** A His-tagged recombinant LaSir2RP1 was expressed in *Escherichia coli* BL21(DE3) and purified. The interaction of resverastrol with LaSir2RP1 was studied by intrinsic and extrinsic fluorescence and UV-Vis spectroscopic. The effect of resverastrol on the deacetylase activity of LaSir2RP1 was studied by using a p53-derived peptide as an acetylated substrate.

**Results and Discussion** Previously we showed that resverastrol has a leishmanicide activity and here we show that resverastrol inhibit the deacetylase activity of recombinant LasSir2RP1 by about 20%, suggesting that this protein may have an important role in the parasite survival. Thus we investigated whether resverastrol interacted or not with the protein and its possible site of binding. Titration with resverastrol affected the intrinsic fluorescence profile of LaSir2RP1 and a plot of intensity versus inhibitor concentration was fitted with the Stern-Volmer equation indicating a 1:1 stoichiometry and a constant of interaction of 0.06±0.03x10^6 M^-1. The results supported the hypothesis of direct binding to the protein. Since both resverastrol and bis-ANS affected the intrinsic fluorescence of LaSir2RP1 we tested whether the compound affected or not the fluorescence of bis-ANS. We found that resverastrol affected the extrinsic fluorescence of bis-ANS, a result that indicated that resverastrol bound near the tryptophan residue, which was predicted to be localized at the active site of the protein. **Conclusion** Our results suggested that resverastrol had an inhibitory activity LaSir2RP1 likely by competing with the substrate for the active site. These finds may be relevant for the *in vivo* function of the protein.

Key Words: LaSir2RP1, sirtuin, *Leishmania amazonensis*, resveratrol

Supported by: Fapesp, CAPES and CNPq