Initial characterization of the proteolysis proteasome dependent in *Leishmania*

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Proteasomes are multi-subunit proteases involved in several mechanisms and thought contribute to the regulation of cellular homeostasis. Here, we report the partial biochemical characterization of proteasomes from *Leishmania chagasi*, *L. amazonensis* and *L. braziliensis*. To investigate the presence of an ATP-ubiquitin dependent proteolytic system in these parasites, we examined the rate of cellular protein degradation in crude extracts from promastigotes. Our results showed that the endogenous proteolytic activity in all species was not increased by stimulation with ATP plus ubiquitin. In addition, the presence of MG-132, a peptide aldehyde inhibitor of proteasome, caused a reduction of approximately 75% in basal endogenous protein breakdown. The levels of ubiquitin-protein conjugates and proteasome were analyzed by immunoblot and showed a specific profile of conjugates between the species analyzed and similar levels in the antibody reactivity when comparing 20S proteasomes between species. To further characterize the peptidase activities of *Leishmania* proteasomes, the protein crude extracts were subjected to assay using Suc-LLVY-AMC and N-Gly-Gly-Arg-AMC as substrate. These results showed that predominant tripsin-like activity. The effect of two proteasome inhibitors, MG132 and PSI on the growth of *L. amazonesis*, *L. braziliensis* and *L. chagasi* was examined. All these drugs blocked *Leishmania* growth in a concentration-dependent manner. PSI was most potent for the inhibition and were observed differences in the inhibitory effect when compared *L. braziliensis* and *L. chagasi*. The differences observed in the rate of hydrolysis of fuorogenic peptides, sensitivity to inhibitors and the lower ubiquitin-dependent proteolytic activity between *Leishmania* species suggest the existence of distinct mechanism for regulation of proteasome pathway and will be subjected to further exploration using proteomic approaches.

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