Purification, characterization and biotechnological applications of enterolobin

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Enterolobin is a cytolytic protein found in seeds of the tree *Enterolobium contortisiliqumm* which presents sequence homology with pore-forming proteins from the aerolysin family. The primary sequence of enterolobin was previously determined by Edman degradation, being composed of 485 amino acid residues, and its molecular mass, 52,905.2 Da, was determined by MALDI-TOF mass spectrometry. Enterolobin, initially discovered for its hemolytic activity, is capable of lysing other cell types and has pro-inflammatory and insecticidal activities. Interestingly, its activity on leukocytes is selective, lysing most of them, but preserving T lymphocytes. This set of activities reveals a great biotechnological potential for enterolobin. Protein purification used extraction in 0.9% saline solution, precipitation in ammonium sulfate (fraction L1), ion exchange (DEAE-cellulose) in batch (fraction L2), and anion exchange chromatography (Mono-Q) in FPLC (fraction L3). The L3 fraction was shown to be pure by SDS-PAGE. The three fractions were tested in cell viability assays against lines of acute promyelocytic leukemia, glioblastoma, prostate and breast cancer, showing outstanding antiproliferative activity, which was proportional to the purity of enterolobin. Enterolobin was also tested against murine macrophages which did not have their viability affected. Assays were also carried out to verify the production of total reactive oxygen species (ROS) in human neutrophils and minimum inhibitory concentration (MIC) on Gram-positive and negative bacteria. The results indicated that enterolobin induced the activation of human neutrophils associated with an increase in ROS production and that it also has antimicrobial activity, specifically against *Staphylococcus aureus*. Presently, enterolobin is being subjected to proteolytic digestions for subsequent LC-MS/MS analysis of the resulting peptides, in order to ratify its primary structure and search for the presence of post-translational modifications. These tests are being carried out in parallel with analyzes of the cytolysin gumiferin, isolated from *E. gummiferum* seeds.

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