Identification, in silico characterization and expression in a heterologous system of a cysteine protease of Echinococcus canadensis

Naidich, Ariel[1]; Salas, Emir[2]; Bouvier, Leon[2]; Niemirowicz, Gabriela[2]; Alvarez, Vanina[2]; Gutierrez, Ariana[1]

1- Department of Parasitology. INEI-ANLIS "Dr. Carlos G. Malbran". Av. Velez Sarsfield 563. CP 1281. City of Buenos Aires. ARGENTINA.

2- Institute of Biotechnological Research-UNSAM. Martín de Irigoyen 3100 CP 1650. San Martín, Province of Buenos Aires, ARGENTINA.

Human hydatidosis is a zoonosis caused by the complex of species Echinococcus granulosus sensu lato. Humans are accidentally infected by ingestion of water and vegetables contaminated with parasite eggs. During the invasion stage these eggs must pass through tissues, degrade proteins of the extracellular matrix, adhere and produce cysts in the host's target organ. Several parasite proteases from different species have been identified in these roles during the different stages of development. Cysteine proteases are synthesized as precursors, with an amino-terminal region; The propeptide that collaborates in the correct folding of the protein and the mature region which includes the catalytic site (Cys-His-Asn). In addition, the propeptide is a potent specific inhibitor for enzymatic activity. The purpose of this work was to identify, in silico characterize and express in an heterologous system of a cysteine protease of Echinococcus canadensis. Protoescolices of E. granulosus were collected aseptically from hydatid cysts from livers of naturally infected pigs. They were washed 3 times with PBS, treated with pepsin and the mRNA was extracted. This mRNA was used to construct a cDNA library by RT-PCR employing oligo-dT. Two PCR reactions were performed a) Amplification of a fragment using primers for generic cysteine proteases (EgCLPg); B) Amplification of complete cysteine peptidase employing cathepsin-L primers for E. multilocularis (EgCLPc). The fragments were cloned and sequenced. We searched for sequence similarity in NCBI databases (BLASTn and BLASTp). Both fragment sequences translated into amino acids, were highly identic to cathepsins L of evolutionarily related organisms. Less similarity was found to sequences from hosts such as man, pig and dog, which could suggest that would be possible to selectively block the action of the parasite protease. We looked for conserved domains with the Conserved Domains Search tool (CDS), the translated sequence of EgCLPg showed the presence of aminoacidic residues essentials for cathalysis, located in usual positions for peptidase domain C1A (MEROPS nomenclature). In addition, the CDS analysis of the fragment corresponding to the mature protein also showed an inhibitory domain I29. The three-dimensional structure predicted by homology modeling, using human procatepsin L as template (selected for quality parameters) presents the usual characteristics for cathepsins L and its propeptide. The EgCLPc fragment was cloned into pBAD and transformed in E. coli BL21. The recombinant protein (38kDa) was expressed in inclusion bodies at 37°C. An activity assay was performed in the absence and presence of E-64 on the purified protease. The specificity for the substrate assayed was compatible with the prediction made by bioinformatics from its sequence. The study and characterization of EgCLP1, as a prophylactic target, may contribute to the treatment and prevention of human hydatidosis.