THE PRE-PRO REGION FROM A CYSTEINE PROTEINASE (TvCP) OF *TRICHOMONAS VAGINALIS* INHIBITS THE PROTEOLYTIC ACTIVITY OF CLAN CA CPs

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Introduction. Papain-like cysteine-proteinases (CPs) are enzymes that hydrolyze peptide bonds of target proteins by a catalytic mechanism involving a Cys residue of the catalytic triad that includes a His and an Asn residues (1). CPs are key virulence factors and the main proteolytic enzymes in many protist parasites. *Trichomonas vaginalis* is a protist parasite causative of the trichomonosis, one of the most common, non-viral sexually transmitted infections worldwide that has strong impact in the human health. *T. vaginalis* has many proteinases, mainly of the cysteine type (2). CPs are synthesize as inactive precursors (zymogen or proenzyme) that have a pre-pro region (that may include a signal peptide and a prodomain region) and a catalytic domain (mature enzyme). Diverse functions have been attributed to the pre-pro region of CP precursors: a) inhibition of enzyme activity through its interaction with the active site; b) folding assistance (3), and c) targeting of the precursors into the endosomal–lysosomal system (4).

The aim of this work was to determine the function of a recombinant pre-pro region (from one of the *T. vaginalis* CPs, dubbed TvCP), as a specific inhibitor for the CP proteolytic activity of *T. vaginalis*.

Methods. The DNA fragment encoding the pre-pro region of TvCP from *T. vaginalis* was cloned into the pColdI prokaryotic expression vector. The recombinant protein of the TvCP pre-pro region was expressed in *E. coli* as inclusion bodies, solubilized with Triton X-100 and urea, and purified under denaturing conditions by metal-affinity chromatography. Additionally, a protease-resistant extract with proteolytic activity was obtained from a clarified detergent extract from *T. vaginalis* at mid-logarithmic phase. To analyze the proteolytic inhibition, the recombinant pre-pro region and the protease-resistant extract were incubated for 20 min at 4 ºC and analyzed by 1- and 2-D substrate SDS–PAGE on 12% polyacrylamide gels copolymerized with 0.2% gelatin, renatured with Triton X-100 and activated at pH 4.5 in the presence of beta-mecaptho-ethanol as a reducing agent.

Results. The recombinant pre-pro region reduced the proteolytic activity in the >60, 39, and 30 kDa regions of a trichomonad protease-resistant extract of 1-D zymogram (Fig. 1). This inhibitory effect in the 2-D zymogram was against protein spots corresponding to *T. vaginalis* CPs of the papain-like family of clan CA (5). It did not inhibit spots corresponding to legumain-like CPs of clan CD on the 30 kDa region in the 2-D zymogram (5). These results showed that the recombinant pre-pro region of TvCP function as a specific inhibitor on CPs mainly of clan CA from *T. vaginalis*.

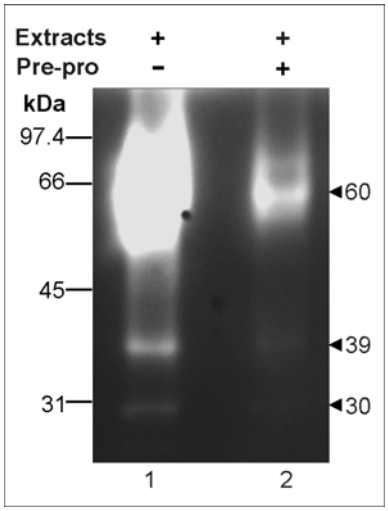

Fig.1 1-D zymogram shows the inhibitory effect of the recombinant pre-pro region of TvCP on the proteolytic activity of the >60, 39, and 30 kDa regions (lane 2) of *T. vaginalis* that appear as white bands against a dark background. The protease-resistant extract with proteolytic activity was used as control (lane 1)

Conclusions. The recombinant pre-pro region acts as a specific CP inhibitor of the proteolytic activity of some of the papain-like CPs from Clan CA of *T. vaginalis*.

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