Recombinant Expression and Initial Characterization of a Sugarcane Legumain

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Legumains (LEG) are cysteine peptidases which hydrolyze preferentially peptide bonds after an asparagine residue. In plants LEG act in the propolypeptide processing, storage protein mobilization during seedling growth, protein processing and degradation during senescence, programmed cell death and stress response. Given the importance of this enzyme to the plant development and defense this work characterizes the first sugarcane legumain (CaneLEG). The CaneLEG has a 1467 pb ORF encoding 488 amino acids (aa) residues. A fragment of cDNA of 1401 bp encoding 466 aa of CaneLEG (excluding the sequence for the signal peptide) was cloned in pET29a to recombinant expression in *Escherichia coli* Rosetta(DE3). The recombinant protein expressed in *E. coli*, with a molecular mass of 58,5-kDa, was predominantly insoluble. However, the recombinant CaneLEG expressed in *E.coli* was electroeluted from SDS-PAGE gels and used to antibody production in mice. In attempt to obtain the protein in a pure and active form its expression was performed in *Pichia pastoris*-(KM71H) utilizing the vector pPICZaA that contain the secretion signal a-factor. The clones expressing CaneLEG were confirmed by Western blotting and the his-tagged protein with a molecular mass around 50-kDa was purified and its activity analyzed by gelatin zymography. CaneLEG was functionally expressed in *P. pastoris* and its activity inhibited by the recombinant sugarcane cystatins CaneCPI3 and CaneCPI4. CaneLEG inhibition by CaneCPI3 was expected by the presence of a C-terminal extension similar to others cystatins that inhibited legumain. Surprisingly, the enzyme was inhibited by CaneCPI4, which does not possess a C-terminal extension. This is the first report of the presence of papain and legumain-like inhibitory motifs in the same amino acid phytocystatin domain.
Keywords: cysteine protease, legumain, sugarcane, cystatin.

Supported by CEPID (CBME-FAPESP Proc. 98/14138-2), Capes