KINETIC CHARACTERIZATION OF ENZYMES FROM LATEX OF *CARICA CANDAMARCENCIS*

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Latex from *Carica candamarcencis* is a rich source of cysteine-proteinases. In earlier studies, by using chromatographic steps it was possible to separate three fractions each containing several proteinases (P1CMS, P2CMS, P3CMS). Only P2CMS displays mitogenic activity on mammals cells. The objective of this work was to establish criteria to characterize and differentiate these enzymes through kinetics and inhibition assays. Mice immunized with one of the mitogenic proteases (CMS2MS2) derived from P2CMS produced antibodies that cross-reacted with other cysteine proteases from the same fraction. Antibody mediated inhibition assays reduced by 50% the amidase activity of P2CMS and P3CMS and had no effect over P1CMS. The inhibition of P1-2CMS proteinases was also evaluated with egg white cistatin, a papain inhibitor. Only P1CMS and a papain positive control were inhibited but P2CMS was unaffected. We also compared the kinetic parameters of some of the purified enzymes derived from P1-P2CMS with L-D-BAPNA and Pyr-Phe-Leu-pNa substrates. The *Kcat/Km* of P1CMS enzymes with both substrates was significantly higher than P2CMS proteins. From these results we conclude that both, antibodies and synthetic substrates can discriminate between the enzymes from fractions P1CMS and P2CMS, suggesting that some structural differences occur between these fractions. In addition, selective inhibition by cystatin of papain and P1CMS suggest that the latter fraction share some papain determinants.

Supported by Cyted, Fapemig, CNPq, and Dr. Abraham Schnaiderman

Carica candamarcensis, cysteine proteinase, inhibition, kinetics