

Determination Of NaCl Concentration On The Kinetic Parameters Of Miliin, A
Plant Thiol-Dependent Serine Protease From *Euphorbia milii*

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Proteases are ubiquitous proteins produced by microorganisms, plants and animals, representing around 60% of the commercialized enzymes. Due to their ability to hydrolyze proteins to peptides and free amino acids, they are incorporated in food, and pharmaceutical industries, besides being extensively used in biotechnology. Among several plant proteases, two are mostly used: papain from papaya (*Carica papaya*) latex and pineapple (*Ananas comosus*) bromelains. This work focuses on miliin (Moro et al., Protein and Peptide Letters, 2008), a protease extracted from the latex of Crown of Thorns, an ornamental plant widely used in fences and gardens. Previous data suggested a strong effect of ions on miliin activity, and accordingly we were motivated to investigate it. We used three fluorogenic substrates (Abz-KLISSKQ-EDDnp, Abz-KVRSSKQ-EDDnp and Abz-KHRSSKQ-EDDnp), carrying fluorescence measurements using a Shimadzu F-5301 spectrofluorophotometer in 50 mM glycine buffer, pH 9.5, at 37°C. We tested the effect of different concentrations of NaCl (10, 30 and 50 mM), carrying out determinations of kcat, Km and catalytic efficiency. The enzyme concentration was determined using 2,2-dithiopyridine. The results show, for all the substrates, an increase of Km, although there is no difference of the effect at 30 and 50 mM NaCl for KVRSSKQ and KHRSSKQ. On the other side, kcat decreased as salt concentration increased. The final effect was an inhibition of the catalytic efficiency up to 30mM NaCl, keeping the same values at 50mM. The changes of this parameter, between 5.9 and 2.4 fold, do not allow to conclude about specific ion effects.

keywords: proteases, kinetic, miliin.

Financial support: FAPESP/CNPq.