Analysis of Subsites Role and Specificity of a Recombinant Cathepsin L-like Proteinase of *Tenebrio molitor*

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**INTRODUCTION:** Cathepsin L (cysteine proteinase), is the major digestive proteinase in *Tenebrio molitor*. Previous studies of our group showed that there are three cathepsins L in *T. molitor* midgut: CAL1, which is lysosomal, and CAL2 and CAL3, which are digestive. The 3D structures of these two last enzymes were elucidated and we aim to study in details their specificities. **MATERIAL AND METHODS:** CAL3 was expressed as a zymogen, purified and activated. Activity assays were performed at 30 °C in citrate buffer, containing EDTA and cysteine, with 57 fluorescence resonance energy transfer (FRET) peptides as substrates. All peptides derived from the lead sequence Abz-KLRSSKQ-EDDnp, with an average of 19 amino acid replacements in each of the positions P2, P1 and P1'. The parameters $k_{cat}$ and $K_M$ were used in the determination of the thermodynamic parameters free energy of binding ($\Delta G_s$), activation energy ($\Delta G^\ddagger_T$) and subsite hydrophobicity (H).

**RESULTS, DISCUSSION AND CONCLUSIONS:** The data obtained suggest that the S2 subsite is hydrophobic (H = 1.3) and this is in agreement with the available data for cathepsin L, which is selective for substrates with hydrophobic amino acids at the P2 position. Both S1' and S1 are hydrophilic (H = -0.4 and -2.1, respectively). The slope (n) obtained in the plot of $\Delta G^\ddagger_T$ versus $\Delta G_s$ corresponding to each subsite indicates if it preferentially binds the transition state or substrate. The results suggest that the S2 and S1' subsites favor catalysis (n > 1), while S1 favors substrate binding (n < 1). The data agree with the known cathepsin L mechanism of catalysis. The S1' subsite apparently has no remarkable selectivity for any amino acid at this position, so this study will be extended to S2' and maybe to S3' in order to identify the primary specificity in the substrate unprimed side.

Keywords: Cathepsin L-like proteinase, *Tenebrio molitor*, FRET peptides

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