Structural Characterization and Immunolocalization of Recombinant Procathepsin L 3 from *Tenebrio molitor* Midgut

Beton, D.[1],Guzzo, C. R.[1], Ribeiro A. F.[2], Farah, C. S.[1] and Terra, W. R.[1]
[1]Departamento de Bioquímica, Instituto de Química, USP, Brazil
[2]Departamento de Biologia, Instituto de Biociências, USP, Brazil

Cathepsin L corresponds to the major digestive proteinase in the midgut of *Tenebrio molitor* larvae. In our laboratory, three procathepsin L-like proteinases (pCALs) were cloned and sequenced from a *T. molitor* midgut cDNA library: pCAL1 (lysosomal CAL), pCAL2 and pCAL3 (digestive enzymes). The cDNA coding pCAL3 were cloned and expressed at a high level in *E. coli.* The recombinant proenzyme was purified and the activation of the pCAL3 to the active CAL3 occurs under acidic conditions. For crystallographic studies we expressed pCAL3 as an inactive Cys26→Ser mutant to prevent self-processing. In this work pCAL3Cys26Ser was crystallized by vapor diffusion in sitting drops against 0.1-1.6 M mono-ammonium dihydrogen phosphate. The crystals are monoclinic, belonging to space group C2, with cell parameters: a = 59.425 Å, b = 91.894 Å, c = 72.084 Å, $\alpha = \gamma = 90°$, $\beta = 91.86°$ and contain one molecule in the asymmetric unit. The structure has been determined by molecular replacement using the structure of *Fasciola hepatica* procathepsin L (42.5% identity) as a model. The model was refined at 2.0 Å resolution with an R factor of 0.18 (R_{free} = 0.22). Immunoblot analysis of different *T. molitor* larval tissues demonstrated that pCAL3 and CAL3 occurs in the anterior two-thirds of midgut tissue of *T. molitor* larvae. Immunoblot experiments of midguts contents of *T. molitor* larvae showed that anti-pCAL3 serum recognized CAL3 in the anterior and posterior midgut contents. Immunolocalization studies indicate that cathepsin 3 occurs in vesicles in the anterior midgut and peritrophic membrane in posterior midgut.

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