Recombinant Expression and Immunocytochemical Localization of a Digestive ß-1,3-Glucanase from *Spodoptera frugiperda* Larvae

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β-1,3-glucanases are widespread enzymes that hydrolyze β-1,3-glucans from fungal or plant cell walls. Nevertheless, little is known about their structure or physiological role in insects. ß-1,3-glucanase activity is similar along the midgut lumen of *S.frugiperda* with an specific activity of 0.20 mU/µg in the anterior and 0,16 mU/ug in the posterior midgut. In the midgut epithelium, the specific activity of the enzyme is 0.35 mU/ug in the anterior and 0.85 mU/ug in the posterior region. The cDNA corresponding to a beta-1,3-glucanase from *S. frugiperda* was previously cloned from a midgut cDNA library and used to produce the recombinant protein (SLAM) in *Pichia pastoris* GS115 cells using a pPIC9K expression system. SLAM has a K_m of 0.13±0.08% (w/v) and optimum pH of 9.0 with laminarin as susbstrate, that are similar to the kinetic parameters previously obtained for the ß-1,3-glucanase purified from the larval midgut. The action of the enzyme against several substrates was tested, being laminarin by far the best substrate. The straight specificity of the enzyme may have resulted due to adaptation to fungal polysaccharides. Thus, the enzyme may help in preventing midgut infections by fungi, by disrupting the ß-1,3-glucan of the cell wall. With anti-serum generated in rabbits against recombinant SLAM, the enzyme was immunolocalized in columnar cells, associated to the cell glycocalyx and in the large secretory vesicles from posterior midgut. This result agrees with β-1,3-glucanase activity, that predominates in posterior midgut epithelium.

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