MOLECULAR CLONING OF THREE INSECT DIGESTIVE BETA-1,3-GLUCANASE

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Laminarinases are enzymes hydrolyzing β-1,3-glucans from fungal or plant cell walls, and are widespread among insects. Nevertheless, there is no knowledge about their primary structure. We designed a primer that anneals to a consensus sequence of animal β-1,3-glucanases from family 16 of glycoside hydrolases. Using it we cloned and sequenced cDNAs coding for putative midgut β-1,3-glucanases of *Spodoptera frugiperda* (Lepidoptera), *Diatraea saccharalis* (Lepidoptera) and *Periplaneta americana* (Dictyoptera). These enzymes are related to gram-negative bacteria binding proteins (GNBPs) from termites (60% identity), with respective molecular masses of 41, 40, 37 kDa, isoeletric points of 5.9, 6.2, 4.5, signal peptides with 20, 19, 23 amino acids, 1, 1, 0 or 1, 6, 0 sites for N- or O-glycosylation, respectively, and conserved residues from family 16 proteins, including the catalytic proton donor (E190, E189, E163) and the nucleophile (E195, E194, E168). Internal peptide sequencing from purified *P. americana* β-1,3-glucanases LAM and LIC1 resulted in sequences VTDSFSFVYGK, YGGEG (LAM) and NWVYGD (LIC1). The last sequence is coded by the cDNA clone obtained from *P. americana*, confirming that it corresponds to LIC1. Phylogenetic comparison of these sequences with insect proteins from family 16 suggests that some proteins classified as GNBPs are true glucanases, and that immune binding proteins and digestive glucanases diverged before the appearance of Hexapoda.

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