

A secretory PLA₂ associated with tobacco hornworm hemocyte membrane preparations acts in cellular immune reactions

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Abstract

We report on a secretory phospholipase A₂ (sPLA₂) associated with membrane-enriched fractions prepared from hemocytes of the tobacco hornworms, *Manduca sexta*. Virtually no PLA₂ activity was detected in serum of immunologically naive or bacterially challenged hornworms. PLA₂ activity was detected in cytosolic and membrane-enriched fractions prepared from hemocytes. PLA₂ activity in the cytosolic fraction (1.2 pmol/mg/h) was approximately 4-fold greater than in the membrane-enriched fraction. The cytosol-associated PLA₂ activity was strongly inhibited in reactions conducted in the presence of the specific cytosolic PLA₂ inhibitor methylarachidonyl fluorophosphate (MAFP) but not in the presence of the sPLA₂ inhibitor *p*-bromophenacyl bromide (BPB). Conversely, the membrane-associated PLA₂ activity was inhibited in reactions conducted in the presence of BPB but not in the presence of MAFP. While the cytosol-associated PLA₂ was independent of calcium, the membrane-associated sPLA₂ required calcium for full catalytic activity. Hornworms treated with either BPB, MAFP or the glucocorticosteroid dexamethasone were severely impaired (by 50 to 80% relative to controls) in their ability to form nodules in reaction to bacterial challenge. However, the immune-impairing influence of the inhibitors was reversed by treating larvae with arachidonic acid, a precursor for eicosanoid biosynthesis. We infer that the biological significance of the sPLA₂ (as well as the previously characterized cytosolic PLA₂) relates to hydrolysis of polyunsaturated fatty acids from cellular phospholipids. Moreover, this enzyme may be the target of immunity-impairing factors from the bacterium *Xenorhabdus nematophila*. The fatty acids serve as precursors for the generation of eicosanoids responsible for mediating and coordinating cellular immune reactions to infection.

Keywords: insect immunity; eicosanoids; phospholipase A₂; hemocytes; *Manduca sexta*;
Xenorhabdus nematophila