

Development of Rapid Diagnostic Method for Detecting Phospholipase A2 Inhibitors Using *in vitro* Hemolymph Melanization and Phenoloxidase Activity

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Phospholipase A2 (PLA2) is responsible for releasing arachidonic acid (AA) from cellular phospholipid, and is thought to be the first step in eicosanoid biosynthesis. Eicosanoids are important mediators related with insect cellular immune response in response to bacterial infection. *Xenorhabdus nematophilus*, which is a symbiotic bacterium of entomopathogenic nematode, *Steinernema carpocapsae*, directly suppressed PLA2 activity in the fifth instar larvae of *Spodoptera exigua*. Survival of larvae infected with symbiotic bacteria increased with exogenous injection of AA, while it decreased with dexamethasone as an inhibitor of PLA2. Here, we devised a rapid diagnostic method for detecting PLA2 inhibitor(s) secreted by *X. nematophilus* without any use of radioisotopes. Principle of this assay is the functional positive correlation between PLA2 activity and phenoloxidase activation, which leads to hemolymph melanization. A potent PLA2 inhibitor should block this pathway. To prove a specific inhibition of PLA2, an effective dose of AA should recover the inhibitory pathway caused by the PLA2 inhibitors. A reaction mixture (10l) consists of hemolymph (7l), laminarin (1l), PLA2 inhibitor (1l), and AA or 50% ethanol (1l). Hemolymph melanization was graded from 0 to 5 scales according to darkening intensity. When dexamethasone was used as a PLA2 inhibitor, it delayed hemolymph melanization by more than 100% (ex, from 12 min to 25 min) at a dose-dependent manner. This inhibitory effect was rescued by addition of AA, which also showed a dose-dependent response. This simple bioassay of PLA2 inhibition is currently applied to a colorimetric method using spectrophotometer by measuring phenoloxidase activity. This colorimetric method would give us a continuous measurement variable to discriminate the inhibitory activities of different PLA2 inhibitor fractions obtained from *X. nematophilus*-growing medium.