Title : Membrane Permeabilization and Antimicrobial Activity of Phospholipase A_2 from Ant Venom

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ABSTRACT

Phospholipase A_2 (PLA_2), an enzyme commonly found in the venom of insects like ants, bees, wasps, and hornets, can break down phospholipids in cell membranes via hydrolysis, leading to membrane damage. In this study, the PLA_2 extracted from *Tetraponera rufonigra* venom was tested for its membrane permeabilizing ability and antimicrobial activity. To mimic cell membranes, we created liposomes made of phosphatidylcholine and phosphatidylethanolamine (POPC:POPE) at 1:1 ratio, encapsulated with a fluorescent dye called 5,6-carboxyfluorescein. These liposomes were tested with three substances, including antibiotic drug chloramphenicol, crude venom, and purified PLA_2. The results showed that crude venom and purified PLA_2 at 5 µg/mL caused a significant leakage in the liposomes, while chloramphenicol had little effect. These substances were also tested for their antimicrobial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* using broth dilution and agar dilution methods to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). As a result, crude venom had an MIC of 462.5 µg/mL against *E. coli*, while chloramphenicol was much more potent with an MIC of 3.12 µg/mL. Additionally, the disk diffusion method was used to measure the inhibition zones, indicating that the bacteria growth was inhibited. Crude venom of 440 µg exhibited a clear zone of 6.5 mm against *E. coli*, demonstrating its antibacterial activity. These findings suggest that crude venom could disrupt membrane and killed bacteria effectively, whereas the purified PLA_2 showed less effect suggesting that crude venom from *Tetraponera rufonigra*, which contain some proteins other than PLA_2 may have antibacterial activity. Therefore, PLA_2 from ant venom might be promising for further research, especially in drug development.

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