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pH Regulates Pore Formation of a Protease Activated Vip3Aa from *Bacillus thuringiensis*

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Abstract

Vip3Aa insecticidal proteins are produced from *Bacillus thuringiensis* and exert a broad spectrum of toxicity against lepidopteran insect species. Although Vip3Aa has been effectively used as part of integrated pest management strategies, the mechanism of the toxin remains unclear. Here, we investigated the effect of pH in a range from 5.0 to 10.0 on the pore-forming activity of the trypsin activated Vip3Aa (actVip3Aa) by *in vitro* pore-forming assays. Based on calcein release assay, actVip3Aa could permeabilize the artificial neutral liposomes under all the pH tested, except pH 10.0. The maximum membrane permeability of actVip3Aa was detected at pH 8.0 and the permeability decreased and abolished when exposing to acidic and alkaline conditions, respectively. The planar lipid bilayer experiment revealed that actVip3Aa formed ion channels at pH 5.0-8.0 but no current signals were detected at pH 10.0, consistent with the observation from calcein release assay. The toxin formed ion channels with a diameter of 1.4 nm at pH 8.0 and pore size was gradually decreased when reducing the pH. This study provided a view of the molecular mechanism of Vip3Aa by which the pore-forming activity is regulated by pH.

Keywords: *Bacillus thuringiensis*; Vip3Aa; Membrane insertion; Planar lipid bilayers; Pore formation; Ion channels