NANO HIGHLIGHT
Functional Reconstitution of Ion Channel Sensing Elements in Polymerizable Lipid Membranes

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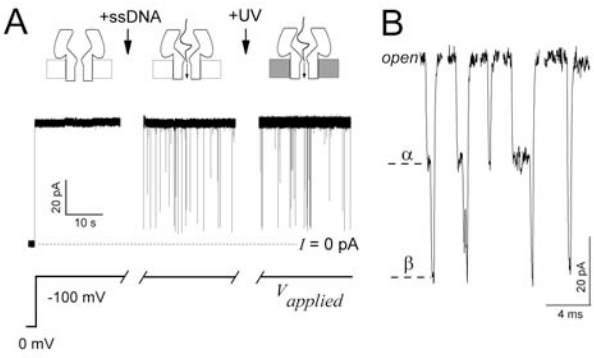
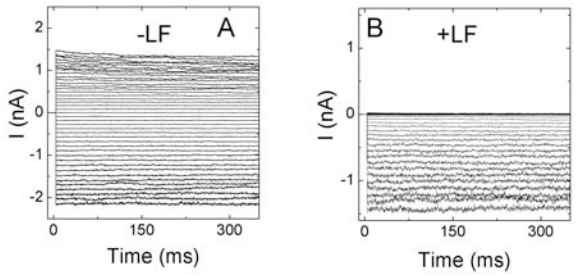
For the past four decades, planar lipid bilayer membranes have enabled the study of ion and macromolecular transport through protein ion channels. It is in such matrices that nanopore channels have recently been used to detect and characterize different ions, polynucleotides and specific proteins. Because only weak intermolecular interactions stabilize liquid-crystalline phospholipid membranes (*i.e.*, the membranes are extremely fragile), nanopore-based sensing applications would benefit if channels could be functionally reconstituted and immobilized in robust ultra-thin films. Our study demonstrates that two different protein ion channels formed by *Bacillus anthracis* protective antigen (PA) and *Staphylococcus aureus* α-hemolysin are fully functional in two different polymerizable lipid membranes in the liquid crystalline state. Moreover, one of these pore-forming toxins functions even after the membrane is polymerized.

Both PA and α-hemolysin spontaneously formed highly conducting channels in membranes prepared from either of two polymerizable lipids, DC8,9PC and PTPE, and a nonpolymerizable control, diphytanoyl phosphatidylcholine (DiPhPC). UV exposure of the polymerizable membranes causes diynes in the hydrocarbon chains to covalently link across neighboring lipids. The α-hemolysin ion channel remained completely functional in PTPE bilayers before and after polymerization. Current recordings (panel A, left) show that the ionic current through a single α-hemolysin channel is quiescent in pure buffer. Adding single-stranded 50-mer poly[dT] DNA causes transient current blockades that occur at random intervals (center in A). Following UV illumination of the PTPE membrane, the polynucleotide-induced current blockades persist (A, right). Five of the characteristic poly[dT]-induced blockades after UV illumination are shown in panel B. The three predominant states (fully open, α and β) were observed before and after UV irradiation.



To gain insight into the mechanism by which *B. anthracis* kills hosts, we studied the interaction between activated PA (PA_{63}) and either Lethal factor (LF) or Edema Factor (EF) – which in conjunction form the molecular basis of anthrax-induced cell death. LF converts the current-voltage relationship of PA_{63} from slightly nonlinear to highly rectifying. Similar experiments over a wide range of LF concentration suggests that LF forms a very tight complex with the PA_{63} channel (*i.e.*, $K_d < 50$ pM). In addition, a known antibody against PA_{63} prevents LF blocking of the channel. This suggests that PA_{63} reconstituted in robust membrane mimics might be utilized to detect LF and to rapidly screen for therapeutic agents against any of the three anthrax proteins.



References: See NIRT 0304062 Overview