78e Global Intertrimer Cooperativity of Influenza Hemagglutinin Conformational Change

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Membrane fusion is an important step in many biological activities and numerous studies have been performed to investigate the mechanism of fusion proteins. Enveloped viruses express membrane-bound fusogenic proteins that share the common feature of a sequence of hydrophobic amino acids called the fusion peptide. The fusion peptide is masked until an environmental signal induces a dramatic conformational change in the fusion protein, leading to fusion peptide exposure and fusion between the virus and target cell membranes. Influenza virus, one of the most studied viruses, bears a fusogenic receptor protein called hemagglutinin (HA) that is activated by pH. HA exists as a homotrimer with the fusion peptide of HA is buried inside a trimeric structure at neutral pH; acidic conditions induce an irreversible conformational change, freeing the fusion peptide to interact with the target membrane. We have developed a mammalian cell expression system where the conformational change of Fowl Plague Virus HA can be easily detected by indirect immunofluorescence. A ten amino acid epitope tag sequence has been substituted at various places in the fusion peptide surface expression of these mutants on 3T3 cells has been analyzed by flow cytometry. One of these mutants shows pH-induced exposure of the epitope tag. This method is advantageous from a protein engineering perspective because it allows more accurate quantitative analyses of the HA conformational change compared to assays of membrane fusion. Kinetics of the conformational change have been analyzed, and our results show that the HA activation depends on the surface density of HA, suggesting intertrimer cooperativity and in agreement with prior studies of HA-mediated membrane fusion. Most significantly, our assay demonstrates that a single cell acts as a discrete switch with respect to the conformational change, indicating global cooperativity between trimers on a length scale that spans the entire surface of the HA-expressing cell. We hope to elucidate the structure-function replationship of this protein by indentifying mutations leading to altered functions via directed evolution.