Computational studies of influenza hemagglutinin

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Summary

Every year, influenza, also known as the common flu, infects about a tenth of the world population. Infection results in mild to severe disease symptoms and, for certain more vulnerable people, can lead to death. Genetic mutations within the virus have allowed it to evade the antiviral drugs that have been developed so far. By changing the biochemical appearance of the virus particles, these mutations generate new virus strains without impairing their infectivity. In fact, a highly infectious influenza strain could emerge this way at any time, potentially causing a pandemic. Only the development of a universal anti-influenza drug, effective against all virus strains, could prevent all further infections and eradicate the virus. Increased knowledge of the molecular details of influenza virus replication could aid in the rational design of such a drug. This thesis focuses on the operational mechanism of influenza hemagglutinin in mediating membrane fusion, a crucial step in the influenza virus replication cycle.

Hemagglutinin (HA) is the most abundant protein on the outside of the virus and is responsible for both target cell binding and membrane fusion during cell entry. It is therefore a natural target for the immune system, as well as for antiviral drugs. HA is anchored in the membrane that envelops the virus and consists of a globular binding subunit, HA1, which surrounds the fusion-active core, HA2. During infection, receptor binding domains in HA1 attach the virus to host-specific receptors on the outside of the target cell. Subsequently, the virus is internalized into the cell by endocytosis and transported within an endosome towards the cell nucleus. Acidification of this intracellular compartment triggers a series of conformational changes in the protein that ultimately lead to fusion of the viral and endosomal membrane. This allows the viral genome to enter the target cell nucleus to induce the production of new virus particles. These particles will be released to infect other cells, completing the replication cycle.

Biological membranes, consisting of two lipid monolayers, have a hydrophilic surface and a hydrophobic core. Fusing two of these membranes requires input of energy, because it involves membrane stretching and bending, overcoming repulsive hydration forces between the lipid head groups and unfavorable aqueous exposure of hydrophobic lipid tails. The HA fusion protein acts as a membrane fusion catalyst by providing this energy. Comparison of the molecular structures of HA at both neutral and low pH have led to a hypothesized pathway of the conformational changes in HA2 that mediate membrane fusion. After acidification, HA1 dissociates and an amphipathic fusion peptide at the N-terminus of HA2 is released. Helix formation within a previously unstructured loop in HA2 projects this fusion peptide over a distance of about 10 nm towards the target membrane, in which it can insert. This extended intermediate connects the two membranes. The protein then folds back on itself through a helix-to-loop transition and zippering of the globular bottom of HA2, thereby pulling the membranes together for fusion. (Reviewed in Chapter 2)

A number of open questions regarding the molecular details of HA-mediated mem-

brane fusion are treated in this thesis. In order to study the conformational changes of HA, we used molecular dynamics (MD) simulations. In MD, the positions and velocities of individual atoms are calculated from the instantaneous interatomic forces over femtosecond timesteps, based on a pre-defined force field. Because of differences in parametrization conditions, some force fields are more suitable for specific systems than others. In the case of the conformational changes of HA, we argue that the force field should produce the right balance between loop-to-helix and helix-to-loop transitions because they play such a central role. We show that the CHARMM36 force field can reproduce experimental helix-coil transitions of small peptides at room temperature, given that the correct water model is used. (Chapter 3)

Recent experimental findings indicate that multiple neighboring HAs are needed to jointly overcome the energy barrier to membrane fusion. It also appears that more than half of the available HAs is non-productive, presumably because the fusion peptides fail to insert into the target membrane during the fusion process. However, the exact molecular mechanism behind these non-productive events remain elusive. In this thesis, we explore the stability of the globular bottom of HA2 against mechanical unfolding. We argue that an untimely unfolding of the globular bottom can lead to non-productive refolding, because the extended intermediate would collapse before fusion peptide insertion. Using steered molecular dynamics simulations, we find that the stability of the globular bottom is governed by a network of salt bridges and we suggest a number of mutations that could potentially decrease HA productivity. (Chapter 4)

Another persistent open question in the field is how many productive HAs are needed for membrane fusion. This question is directly related to the amount of energy that a single HA can supply to the fusion process. We calculated this amount as the conformational free energy that is released during the transition from the extended intermediate to the postfusion structure, using the confinement free energy method. This method avoids the need to sample the enormous conformational space of this relatively large protein along the whole transition. Instead, the start and end states are confined to a system of harmonic oscillators, for which the free energy is known analytically. Although still computationally demanding, we can show convergence of the results. The resulting $34.2 \pm 3.4\, k_{\mathrm{B}}T$ of free energy that is available per HA is consistent with a model in which three neighboring HAs are needed for membrane fusion. (Chapter 5)