Fusion Flashes Illuminate Kinetics

Joshua Zimmerberg
Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland USA

Membrane fusion, the merging of two biological membranes, is the underlying event in synaptic transmitter release, fertilization, and enveloped viral entry. Despite progress in identifying, crystallizing, and determining the structure for one fusion protein (influenza HA), and intense electrophysiological study of fusion pores, the physical and molecular mechanisms of membrane fusion remain obscure (1). One traditional route of investigating mechanisms of biological phenomena is kinetics. The most elegant studies of membrane fusion kinetics have come from the neuromuscular junction, where individual fusion events could be detected electrically and the underlying statistical distributions determined. Even there, however, it is not possible to control intracellular concentrations of ligands or to vary the lipid composition of either membrane in a defined manner.

HA-mediated fusion to lipid vesicles, where the target membrane can be varied, has been investigated. However, the fusion kinetics are not isolated from binding rates in these systems. HA-mediated fusion has been followed in a cuvette in a fluorimeter by measuring the fluorescence of a population of viruses or cells whose membranes are loaded with a membrane-soluble dye at high and self-quenched concentrations. Fusion of a labeled and an unlabeled membrane leads to dye dilution, dequenching, and an increase in fluorescence. Kinetic schemes of fusion derived from such studies are complex and multicomponent (2, 3). Subsequently, it was shown that the rate of dequenching of each individual fusion event within that population is often comparable to the rate of initiation of fusion, hence confusing the analysis of cuvette experiments (4, 5). Fusion kinetics are better measured by collecting data from a large number of individual cells and viruses (4, 6, 7).

The system of planar phospholipid bilayer membranes, originally developed to study transport properties of lipid membranes, is ideally suited for studying membrane fusion because the aqueous compartments bathing the membrane, and the lipid composition of the membrane, can be experimentally controlled. In addition, the planar surface is an optimal optical specimen. Phospholipid vesicles, planar membranes, and viruses have been studied fusing to planar membranes (1, 7–9). Niles and Cohen (8), using image processing algorithms for pattern recognition, have developed an automated dequenching "flash" detector which determines the initial moment of dye dequenching for each individual fusion event. In their analysis of the fusion of influenza virus to planar phospholipid bilayer membranes, they find fusion to be a simple, single-exponential, two-state process (or at least rate-limited by a single process). Moreover, this kinetic structure is dependent upon both low pH and the presence of sialate receptor. Without these, a much more complicated scheme is found.

The dependence of the kinetic scheme on sialate in the target membrane calls into question the wisdom of the current nomenclature. The sialate-containing glycoproteins in the cell surface membrane are referred to as the receptors for HA. However, HA binds to any sialic acid residues, so there is little specificity in the cell surface molecule. From a biophysical point of view, a receptor is defined in terms of ligands. For example, the nicotinic acetylcholine receptor, a bona fide cell surface receptor for the ligand acetylcholine, is an allosteric molecule. Binding of the allosteric effector acetylcholine, the ligand, changes the molecule's energy such that the channel opens.

What is the case for HA? From the point of view of the virus or cell expressing HA, HA is the receptor, while sialate and protons are the ligands that alter conformational energy. HA is the allosteric protein whose conformation determines function. Protonation of HA kicks out the fusion peptide, thereby starting a process that is rate-limited by a single state when HA is bound to surface sialate. In the absence of binding sialate, the kinetics are not so simple, and are not explained well by any number of single exponentials. Fusion occurs, albeit at a 20-fold-reduced efficiency. However, it is the lipids (and ultimately water) that are missing from this view. HA binds to membranes—cellular or pure phospholipid, with or without sialate-bearing membrane components. The insertion of the fusion peptide into the target membrane leads to fusion pore formation. Thus a complex of lipid and protein is responsible for the initiation of fusion (1).

Initial studies with the extraviral domain of HA (BHA) showed conformational changes for the molecule when pH is lowered. But all of these studies were done without the target membrane, and may represent changes that occur after fusion, or do not occur when the target membrane is present. It is likely that HA is conformationally altered by binding to the membrane. Having a stronger membrane binding, with sialate present in the target membrane, does not merely lower the energy barrier for fusion. In the absence of strong binding, a completely different underlying kinetic structure is seen: new states are visited by the system (8).

Nor is the effect of sialate merely that of an allosteric regulator of HA, since preincubation with small, soluble sialate-containing groups prevents fusion altogether (7). Binding may also be interrupted. The intriguing possibility remains that tension may develop within the target membrane at a location between the sialate binding sites and the fusion peptide binding sites, to pull apart the target membrane at the point of contact. This would be analogous to the tension needed to fuse phospholipid vesicles to planar phospholipid membranes (9). Without the pulling by both the sialate binding sites of HA and the fusion peptide of HA, fusion occurs much less frequently and by an alternate mechanism. In any case, binding energy may be a key driving force of fusion.

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