Membrane Fusion Description Approaches

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From the very beginning at the stage of fertilization, through the tissue growth, hormone secretion and synaptic transmission, and sometimes morbid events of carcinogenesis and viral infections, membrane fusion regulates the whole life of high organisms. Despite that, a lot of fusion processes still lack the well-established model of the process and even a list of main actors. Merger of membranes requires their topological rearrangements controlled by elastic properties of the lipid bilayer. That is why continuum models of fusion based on theories of membrane elasticity are actively applied for the construction of physical models of membrane fusion. Started from the view on the membrane as a structureless film with postulated geometry of fusion intermediates, they developed along with experimental and calculation techniques to the powerful tool for prediction of the whole process with molecular accuracy. Modern approaches in this field allow continuum models of membrane fusion to stand shoulder to shoulder with molecular dynamics simulations to provide the deepest understanding of this process in multiple biological systems.

Keywords: membrane fusion ; lipid membranes ; theory of elasticity ; leaky intermediates ; fusion proteins ; pore formation

1. Introduction

A physical model of any biological process should not only explain the currently available experimental data, but should predict the results of future experiments as well, and allow one to plan experiments, which would determine necessary model parameters with the required accuracy. In the process of fusion, lipid bilayers of biological membranes inevitably severely deform, often with violation of their local integrity[1]. Their close approach, rupture, and further fusion do not proceed spontaneously, as the energy of thermal fluctuations is too small. For these processes to occur, some energy source, which is external to the membrane, is required. In biological systems, specialized proteins always participate in fusion processes[2]. The functioning of the influenza A virus fusion protein, hemagglutinin, during the viral infection seems to be the best studied. Another example is the protein complex SNARE, which facilitates the fusion of synaptic vesicles with the presynaptic membrane during synaptic signal transduction[3]. Certainly, the vast variety of fusion proteins is not limited to these two examples; more fusion proteins of interest are described in the reviews[4][5][6]. At the same time, despite the large diversity of protein machines involved in fusion, it is common among all of them that their work covers energy costs necessary to remodel fusing membranes. Most biological membranes are alike: they possess similar elastic parameters, degree of hydration, and are surrounded by solutions of the same ionic strength, and exist in a relatively narrow pH range. Thus, one may expect that the process of membrane fusion and, consequently, the consumption of energy in its course should be similar in different cases of biological fusion. This allows developing rather general theoretical models focusing on membrane metamorphoses only and avoiding dipping deep into biochemical details of the conformational changes of fusion proteins. Briefly, in a general theoretical model, one constructs an energy functional where fusion proteins are not taken into account explicitly. The state of the membrane is determined by the physicochemical properties of lipid bilayers only. Further, by minimizing the energy one obtains a system of Euler-Lagrange differential equations. The influence of fusion proteins at various stages of the fusion process is described by the corresponding boundary conditions for these equations.

2. Models of Membrane Fusion

Physical models of membrane fusion can be separated into two kinds. In the first one, a separate intermediate structure (or several discrete structures) is hypothesized. By the structure, we mean a membrane state, at which its free energy has a local minimum. The energy depends on system parameters: membrane elastic properties, surface electric charge, solution ionic strength and pH, hydrophobic and hydrophilic interaction, etc. It is calculated relative to some global fixed reference state (usually it is planar membranes before the fusion). A common reference state allows comparing energies of different intermediate structures; however, the transitions between them cannot be described. Generally speaking, the

structures can be separated by energy barriers of unknown arbitrary heights. Thus, we may make conclusions about the energetically preferable pathway for fusion but cannot say anything about its kinetic, about the rate of fusion. That kind of fusion description has been developed in numerous works[7][8][9][10]. Among them is the classical stalk fusion model[11][12].

In the second kind of models, the fusion is treated as a continuous process of system remodeling from two initially separate intact membranes to a final single bilayer. In the framework of those models, a generalized single reaction coordinate is considered. Then at fixed reaction coordinate the energy of the membrane is optimized with respect to its degrees of freedom and the system's energy along the reaction coordinate is thus calculated. As a result, the energy barriers separating intermediate structures and the total energy barrier of fusion can be estimated. The probability of the system transfer from the initial to the final state is proportional to the Boltzmann factor of the barrier height. A high degree of detailing makes the development of such models rather cumbersome[13]. In some works, short continuous trajectories between adjacent intermediate structures are modeled, rather than the trajectory of the entire fusion process. Models of this kind are designed in refs. [13][14][15][16]. Those partial trajectories are built taking into account all known energy inputs. The total energy along the fusion pathway may be considered as an upper limit estimation. Indeed, despite a thoroughly developed model, one can still miss some degrees of freedom. An additional degree of freedom may only lower (may not increase, speaking strictly) a system's energy. Herewith, there is always an unpleasant possibility that such a detailed description of the process is still insufficient, due to the incompleteness of available information on the physicochemical and elastic properties of the membranes. In that case, within a framework of a specific theoretical model, it is possible to take into account insignificant energy contribution and miss the major ones that are not yet available. As a result, the model that skips large unknown energy contributions may erroneously be accepted as energetically feasible, i.e., correct from a thermodynamic point of view.

A significant part of the fusion models uses a "macroscopic" continuum approach when the lipid membrane is considered as a continuous liquid crystal medium. In the simplest (and earliest) Helfrich approximation[17] any internal structure of the membrane is excluded from consideration, and a membrane effectively becomes an infinitely thin structureless elastic film. Since the work by W. Helfrich in 1973, the theory of elasticity of lipid membranes has been continuously developed and improved. The development of experimental techniques allowed observing new deformation modes, which were then introduced into the theories of elasticity. The improvement and development of the classical model of the fusion process by detailing contributions to the energy of the system and the evolution of the theory of elasticity of lipid membranes made it possible to construct continuous trajectories of the process from the initial state of flat parallel membranes to the final state of the fusion pore. Calculated heights of energy barriers of trajectories indicate the energy feasibility of developed models. Predicted dependencies of heights of energy barriers on elastic parameters of fusing membranes are in a good agreement with available experimental data[18][19][20]. Thusly, the conclusions drawn in the framework of continuum models have predictive power, allowing not only interpreting the experimental results, but also predicting the behavior of the entire system under the variation of the parameters.

In the most continuum models, to simplify calculations, highly symmetric intermediate states (usually axisymmetric) are considered[7][13][16]. Shape restrictions of membrane structures should generally lead to an overestimation of their calculated energies. Moreover, the characteristic spatial scale of the structures forming in the process of fusion is about a few nanometers, i.e., comparable to the lateral size of a single lipid molecule. Hence, a significant drawback of continuum approaches to the fusion description is their inevitable extrapolation to molecular spatial scales that is disputable for continuous models[21]. To make analytical computations in continuum models possible, linear elastic theories are usually used, which are applicable for small enough deformations only. However, calculated membrane deformations in the course of fusion can be so large that using the linear approximation to calculate membrane energy cannot be strictly justified. Surprisingly, it was recently shown that the linear generalized Helfrich model well describes strongly deformed membrane[22]. The modeling of the fusion process in the framework of the continuum approach in its current state involves "guessing" of qualitative properties of intermediate structures, followed by optimization of their quantitative characteristics. In other words, this approach does not provide tools to find optimal intermediate structures; one can only suggest a set of possible system configurations and then estimate physical (energetic) feasibility of the developed model.

Microscopic approaches based on the modern computational methods of numerical modeling, such as molecular dynamic of coarse-grain or all-atom lipid models or the Monte-Carlo method are widely applied to research membrane fusion. Such approaches can hardly be called theoretical ones; rather, they should be attributed to "numerical experiments". Numerical methods allow obtaining more detailed insight into structure and states of fusing membranes since they do not require "manual" building of intermediate states and enable visualization of these states directly during the simulation. Moreover, unlike continuum approaches, molecular modeling methods are not limited to symmetrical membrane structures. Also, molecular dynamics methods allow systematic study of alternative trajectories of the fusion process associated with the formation of leaky intermediates[23][24][25]. These data show that strongly asymmetric membrane structures can form

during the fusion, and the process may be accompanied by loss of the membrane barrier function, i.e. may occur with a leakage. The leakage could be transient, with the fusion process eventually ending with the formation of a fusion pore. Alternatively, a long-lived through pore can form in a membrane, which can be stabilized by fusion peptides, and the fusion process will stop. However, a numerical experiment does not allow for analyzing the influence of general non-specific parameters of the system, such as the elastic properties of membranes or an external force applied by fusion proteins. These approaches deal with systems of a specific composition and under specific conditions. Additionally, due to computational power limitations, molecular dynamic methods make it possible to consider only simplified systems with certain artificial restrictions. Most molecular dynamic simulations are performed on a system of limited size; as a result, this approach makes it possible to study the fusion of very small vesicles with a diameter of 15–30 nm only[26], which approximately corresponds to the lower size limit of liposomes produced experimentally. Moreover, to speed up the fusion process in simulations sometimes artificial membrane modifications, like dehydration of lipid headgroups to force attraction between fusing membranes are applied.

Another theoretical approach to calculate the fusion trajectory uses self-consistent field theory (SCFT)[27][28]. It is a microscopic approach that utilizes the statistical mechanics' tools to obtain the membrane state and calculate its energy. Therefore, a molecule behavior is not simulated, but analytical calculations are performed as far as possible. In that model, both solvent and "lipids" are modeled by simple Gaussian chains consisting of two mutually repulsing kinds of "atoms"—hydrophilic and hydrophobic ones. "Lipids" are amphiphilic, i.e., they are formed from two kinds of monomers (diblock copolymers), and the solvent consists of hydrophilic segments only. No hydrophilic repulsion is implemented. SCFT approximation is applied to calculate the partition function, and then the free energy of the ensemble of Gaussian chains. Thus, it is quite an artificial model that probably can reveal qualitative rather than quantitative features of fusion[28]. Even under that approximation, the solution of final equations and, hence, the energy and the intermediate states can be found only numerically, and within some restrictions imposed on the system (e.g., axial symmetry).

Theoretical models of the fusion of biological membranes were developed in 1980s. Starting with the simplest theories that consider lipid monolayers as structureless surfaces characterized by bending stiffness only, fusion models became continuously more complicated as new data on the structure and physicochemical properties of membranes accumulated, the theory of elasticity of lipid bilayers improved, and a theoretical description of hydrophobic and hydration interactions was developed. The development and improvement of continuum models have led to the possibility of describing strong deformations arising at molecular scales. These developments made significant progress in theoretical models of membrane fusion possible. Generally, the modern repertoire of continuum approaches allows a detailed description of the membrane fusion, predicting and analyzing various intermediate structures and process trajectories. The conclusions drawn in the framework of continuum models have predictive power, allowing not only interpreting the experimental results but also predicting the behavior of the entire system under the variation of the parameters.

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