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FLUCTUATIONS, STABILITY AND FUSION IN BIOMEMBRANES

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ABSTRACT

In this article we try to introduce thermodynamical reasoning as an essential tool in the investigation of the phenomena of membrane fusion. We explore some necessary conditions for fusion to occur and mention possible ways for further advancement.

INTRODUCTION

Membrane fusion is involved in crucial cellular events such as cell separation during mitosis. Proteins, bacteria, viruses and a broad array of nutrients are imported into cells by processes in which regions of the cell membrane surround the particles and then detach and form intracellular vesicles. Vesicles fuse with the lysosomes triggering the degradation of the particles inside. Cells secrete various proteins by fusion of an intracellular vesicle with the plasma membrane of the cell. Intracellular vesicles fuse also with organelle such as the Golgi complex transporting to it macromolecules produced in another organelle, the endoplasmic reticulum [1]. The process of fusion is a simple one. It involves several steps in a sequence only one of which is the proper fusion.Other processes considered in this context are movement of the fusing membranes into close proximity, adherence of membranes, Ca^{+2} mobilization, triggering by ions and messengers, recognition of fusion site, steric constraints and their removal, membrane deformability and fluidity and forces between the approaching membranes [2]. The subject of our interest, the fusion process proper, is still obscure because of the many components and modes of organization existing in the fusing membranes (e.g the various phospholipids and proteins organized as cytoskeletal elements, integral proteins, etc.).

We introduce thermodynamics as a general theoretical frame for investigating the phenomena of membrane fusion. This includes discussion of stability

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conditions, role of fluctuations, characteristics of thermodynamic states and role of surface tensions. Using the theoretical frame we refer to investigations which were carried out on phospholipid bilayers as model systems [3] and explore how these can be correlated with the behaviour of biological membranes.

THEORETICAL FRAME

A system is fully characterized thermodynamically by an equation of the form $G = G(p,T,A,N_1,N_2,...,N_n)$ [4]. (G is the free energy, p and T are the pressure and the temperature, A is the surface area and the N_i's denote the mole numbers of the various components). When fusion occurs a characteristic equation of the fused membrane is obtained by transfomation from the characteristic equations of the original membranes. Also each intermediate stage is defined by such an equation. We are interested in transformations where the mole numbers are conserved but the energy may change. In general a transformation may involve changes in the free energy and changes in the way by which the free variables affect the free energy. We know however that fusion occurs even between identical membranes of simple composition, such as phosphatidylcholine bilayers [4]. This means that the changes which may accompany the transformation are not essential for the process to take place.

When two membranes approach each other, prior to fusion, there is a line at which three surfaces intersect (Fig. 1.). It can be shown [5] that the force on a point of the line is given by $F = \gamma_{12} - (\gamma_1' + \gamma_2')$ where γ_{12} is the surface tension of the interacting membranes, γ_1' and γ_2' are the components of the surface tensions of the original membranes in the direction of the force γ_{12}. It can be shown that since surface tensions are energies, F is a hypersurface in the n-dimensional thermodynamic field space. F=0 describes a system in mechanical equilibrium when $F<0$ there is a force acting on the interface 12 in order to stretch it. It follows that a necessary condition for fusion or for rupturing of the membranes is that $F<0$. Consider now the work of bringing together the two membranes 1 and 2. This involves the creation of a new surface phase 12 with energy γ_{12} and the abolishment of the surfaces 1 and 2. The work per unit area is $\gamma_1 + \gamma_2 - \gamma_{12}$ and this is a positive quantity meaning that work has to be invested at the initial stages of the fusion process and work is gained at the final stages of the process. From what is said here it follows that energy barriers are more important than free energy differences in the process of membrane fusion.

Membrane fusion is considered to be a catastrophic event[2]. Such a statement calls for the introduction of thermodynamic stability conditions in the investigation of the fusion phenomena. The stability conditions for any system at thermodynamic equilibrium is that the free energy at constant pressure and temperature is maximal from which it follows that for a stable system the heat capacity, the compressibility and the sum of all $\partial\mu_i/\partial\mu_j$ terms are definitely positive. A deeper insight into the meaning of stability from a statistical mechanical point of view is obtained by using fluctuation theory. The relation between thermodynamic fluctuation and the functions given above is

$$\overline{(x_j - \bar{x}_j)(x_k - x_k)} = -k(\partial x_k/\partial f_j)_{f \neq f_j} = -k(\partial x_j/\partial f_k)_{f \neq f_k}$$

where x_j and x_k are independent extensive variables and f_j, f_k are the corresponding intensive variables (the heat capacity C_v gives the fluctuation in internal energy and the compressibility is related to the fluctuation in density). In a stable system there is a large restoring force and the fluctuations are small. Near a critical point the fluctuations become enormous leading to catastrophic events. Should the reason for fusion be an instability then fluctuations will occur in some physical quantity which should be detected as noise in the quantitiy. Such instabilities have not yet been detected nor are they required when an outer force, such as the one described in the previous paragraph, is exerted on the area 12.

DISCUSSION

Using the frame given above it is possible to explain qualitatively features of membrane fusion. First we consider the barrier to fusion in phospholipid bilayers. In bilayers there is a repulsion between the head groups and attraction between the hydrophobic tails [6]. When two identical membranes are brought together we bring to the vicinity of the hydrophilic groups of one membrane identical hydrophilic groups from the other membrane. The groups will tend to mix with each other but this is counteracted by the repulsive interactions of these hydrophilic groups in the lateral direction. When the groups coming from either membrane interact work has to be invested. The repulsive interactions act to weaken the first CH_2 groups of the hydrophobic region in conjunction with the outside forces γ_1 and γ_2. Interaction between hydrophobic groups from the two membranes will lower the work needed for breaking any of the membranes but in this process the two original membranes fuse to give one membrane. Fusion therefore occurs as the result of lowering the activation energy of membrane rupture. Inspection of thermodynamic properties reveals [7] positive and large values of the compressibility in both the hydrophilic and the hydrophobic regions of the membranes. Hence it is unlikely that instability is the cause for fusion.

Support to the idea that membrane fusion involves the creation of an activated state can be found in the results of electron microscopy observations. It was found that fusing membranes form trilaminar picture in cross-section views [8]. That the event of fusion is a local event that is not driven by such thermodynamic gradients of mixing and phase transitions inside the membrane is provided by the results of the classical fusion experiment by Edidin [9]. It was shown then that no mixing occurs at the initial stages of the fusion process and hence that lateral mixing plays a minor role if any in the fusion process.

One possible mechanism to overcome the barrier for fusion is by coupling it to another more favourable reaction such as the binding of proteins to each other to form bridges between the fusing membranes. The binding and the activation occur in different surface elements. Some support to this mechanism is provided by the finding that the major determinants of the aggregation of natural membranes are proteins which protrude from the membrane surface and serve as points of contact [2]. Further support is furnished by the finding that lateral movement of glycolipids and glycoproteins occurs at the contact area between two opposing membranes [2]. It is interesting to note in this context that the free energy of contact is a function of active sites on each surface and the number of bound contacts and the chemical potential of each of the entities and that these attractive forces are counteracted by repulsion forces between the approaching membranes [10]. It is thus likely that the same attractive forces can do work against repulsive forces of the barrier facilitating fusion in this way.

CONCLUSION

It seems likely that the factor dominating fusion is a barrier of repulsive forces in the surface. The driving force for fusion is a net force in a lateral direction and the rate limiting step is the creation of the trilaminar state against the thermodynamical driving force of surface repulsion. It is not likely that fusion is driven by membrane instabilities.

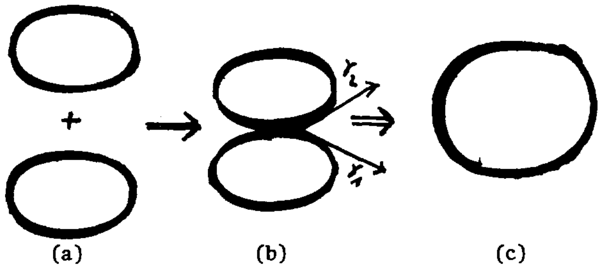

Fig. 1. A schematic description of the process of membrane fusion.
a) before fusion b) during fusion c) after fusion.

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