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Temperature dependence of diffusion in model and live cell membranes characterized by imaging fluorescence correlation spectroscopy

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ABSTRACT

The organization of the plasma membrane is regulated by the dynamic equilibrium between the liquid ordered (L_o) and liquid disordered (L_d) phases. The abundance of the L_o phase is assumed to be a consequence of the interaction between cholesterol and the other lipids, which are otherwise in either the L_d or gel (S_o) phase. The characteristic lipid packing in these phases results in significant differences in their respective lateral dynamics. In this study, imaging total internal reflection fluorescence correlation spectroscopy (ITIR-FCS) is applied to monitor the diffusion within supported lipid bilayers (SLBs) as functions of temperature and composition. We show that the temperature dependence of membrane lateral diffusion, which is parameterized by the Arrhenius activation energy (E_{Arr}), can resolve the sub-resolution phase behavior of lipid mixtures. The FCS diffusion law, a novel membrane heterogeneity ruler implemented in ITIR-FCS, is applied to show that the domains in the S_o–L_d phase are static and large while they are small and dynamic in the L_o–L_d phase. Diffusion measurements and the subsequent FCS diffusion law analyses at different temperatures show that the modulation in membrane dynamics at high temperature (313 K) is a cumulative effect of domain melting and rigidity relaxation. Finally, we extend these studies to the plasma membranes of commonly used neuroblastoma, HeLa and fibroblast cells. The temperature dependence of membrane dynamics for neuroblastoma cells is significantly different from that of HeLa or fibroblast cells as the different cell types exhibit a high level of compositional heterogeneity.

1. Introduction

The plasma membrane is a semipermeable boundary which separates the interior and exterior of the cell. The basic building block of the plasma membrane is a lipid bilayer where a number of other biomolecules including different proteins, and carbohydrates are embedded in an organized manner. A widely accepted model for membrane organization is the 'lipid raft' hypothesis which states that the plasma membrane is an inhomogeneous fluid where small nano-sized (20–100 nm) domains, the so-called rafts that are enriched in cholesterol and sphingolipids, are phase segregated from the surrounding phospholipid dominated fluid matrix [1–5]. These two phases are the liquid ordered phase (L_o) and the liquid disordered phase (L_d), respectively [6,7]. Certain kinds of membrane proteins, for example glycosylphosphatidylinositol (GPI) anchored proteins, are known to partition preferentially into rafts [5,8]. Lipid rafts are crucial to regulating important cellular processes including signal transduction, membrane trafficking, and pathogen entry [9–17].

The vast diversity of the lipids in the plasma membrane can be broadly categorized into three classes: low melting unsaturated acyl chain lipids, high melting saturated acyl chain lipids and cholesterol. The melting temperature (T_m) is defined as the temperature at which the hexagonal closed packed solid ordered or gel phase (S_o) transforms into a random array of liquid disordered or fluid phase (L_d) through *trans-gauche* isomerization of the acyl chain [18]. A ripple phase (P_{β}) can also exist for some lipids at much lower temperature than T_m [19]. The S_o phase is very compact, ordered and almost immobile while the L_d phase is less rigid, disordered and mobile. The L_o phase does not exist in the thermodynamic phase diagram of single component lipids. However, it is induced when cholesterol is mixed to either gel or fluid lipids. When cholesterol is mixed with gel lipids, it 'fluidizes' the system by disrupting the long-range (global) order. On the other hand, it 'condenses' the fluid lipids by inducing short-range (local) order [20]. This new phase which has intermediate order and fluidity is called the L_o phase. The formation of the L_o phase depends on temperature and on the molar ratio of the lipid/cholesterol mixture.

Abbreviations: FCS, fluorescence correlation spectroscopy; ITIR-FCS, imaging total internal reflection-fluorescence correlation spectroscopy; ACF, autocorrelation function; PSF, point spread function; SLB, supported lipid bilayer; F, fluid lipid bilayer; FC, fluid lipid:cholesterol bilayer; FG, fluid lipid:gel lipid bilayer; FGC, fluid lipid:gel lipid:cholesterol bilayer

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The above-mentioned three phases have distinct diffusion behavior, which stems from their differences in physical properties. The lateral mobility is essential to control membrane structure and function. The coexistence of the phases gives rise to intriguing diffusion features that depend on a range of physical properties including area per lipid, lipid expansivity, domain size and dynamics, and line tension. Thus diffusion measurements are good means to probe plasma membrane heterogeneity. The universal Stokes–Einstein model for 3D diffusion cannot be extrapolated to 2D systems to describe membrane diffusion (Stokes' Paradox) [21]. The best approximation was proposed by Saffman and Delbrück who considered the viscosity surrounding the lipid sheet as one of the mediators of the diffusion and treated the problem with 3D continuum hydrodynamic theory [22]. Recent studies have shown that the Saffman–Delbrück model is valid for proteins with a hydrodynamic radius of $\leq$8 nm [23–25]. For proteins with a hydrodynamic radius of more than 10 nm a Stokes–Einstein-like model, as proposed by Gambin et al. [26], describes the data better [27]. Although these models can describe the diffusion of proteins in the membrane, they fail to explain lipid diffusion since the latter is significantly affected by the inherent viscosity of the membrane, diffusants' size, and the tilt angle with respect to the bilayer normal. A more convenient model for lipid diffusion is the semi-quantitative 'free area model' [28]. This model is a direct extrapolation of the 3D free volume model for diffusion in a gas. According to this model, diffusion occurs in the following three steps. First, a transient void is created in the lipid matrix by thermal density fluctuations. Second, one of the surrounding lipids hops into the void which has to be larger than a certain critical size. The third step is the repletion of the void created by the second lipid by other surrounding lipids. Note that this model represents diffusion as a function of free area (void) instead of viscosity. Since the model is derived from the kinetic theory of gases, it has a weak dependence on temperature and no activation energy is implied in lipid hopping. However, lipid hopping must be an activated process due to the van der Waals' interactions with the surrounding lipids. Thus a more generalized approach, the so-called Macedo–Litovitz hybrid model, was proposed by including an activation energy (E_A) term [29,30]. E_A accounts for the energy barrier the lipids have to overcome for hopping to their new locations assuming both the states before and after hopping are in equilibrium. It also incorporates the viscous drag due to the opposite monolayers, the effect of the surrounding fluid or surface, and the energy required to create a void. Since both the hopping frequency and the density fluctuations are temperature dependent, diffusion is a thermally activated process. Thermally activated processes in reaction kinetics with the initial and final states at equilibrium are described by the Arrhenius equation. The effective activation energy (E_{Arr}) term in the Arrhenius equation is interpreted in a very similar fashion to that of E_A in the free area model. Computation of E_A from E_{Arr} has been done with the knowledge of free area and the cross-sectional area of the lipid under question, which shows that the temperature dependence of lateral diffusion in membranes can be successfully explained by the free area theory [31,32]. The degree of lipid packing (van der Waals' interactions), which is one of the major determinants of the membrane phase, is directly related to the availability of free area for diffusion. Thus the temperature dependence of diffusion can provide direct evidence of the membrane phase behavior.

Fluorescence correlation spectroscopy (FCS) is a widely used technique to study membrane dynamics at the single molecule level [33–35]. Here we used camera-based imaging total internal reflection fluorescence correlation spectroscopy (ITIR-FCS) to measure membrane diffusion [36]. ITIR-FCS possesses a number of important advantages over conventional single spot FCS. This calibration-free technique allows parallel measurements of diffusion coefficients at every diffraction limited spot over a large membrane area (in this study, $5 \times 5\ \mu m^2$) [37,38]. Moreover, TIR illumination significantly reduces background arising from the bulk since only molecules close to the surface are excited. In this article, we show the temperature dependence of diffusion in one, two and three component glass-supported lipid bilayer (SLB) model systems and the corresponding E_{Arr} values are calculated from the Arrhenius equation. We show that each phase has a characteristic E_{Arr} value. A phase change is directly indicated by a change in the magnitude of E_{Arr}. We also show that the spontaneous phase reorganization upon external perturbations, e.g. cholesterol extraction, can be detected from the change of E_{Arr}. FCS diffusion law analysis [38,39] is performed to observe the sub-resolution detail of membrane diffusion which is effectively mediated by lateral membrane organization. It shows that the size and dynamics of the domains are quite different for S_o–L_d and L_o–L_d phases. Finally, we extend our study to the plasma membrane of three commonly used live cells; namely HeLa, neuroblastoma (SH-SY5Y), and fibroblast (WI-38) cells.

2. Materials and methods

2.1. Lipids and dyes

The lipids used are 1,2-dioleoyl-sn-glycero-3-phophocholine (DOPC), 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol (Chol). Head group labeled rhodamine dye 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (RhoPE) was used as the fluorophore. All lipids and dyes were purchased from Avanti Polar Lipids (Alabaster, AL). Lipid and dye stock solutions were prepared in chloroform. Methyl-β-cyclodextrin (mβCD) was purchased from Sigma-Aldrich (Singapore). The stock solution of mβCD was prepared in buffer containing 10 mM HEPES and 150 mM NaCl (pH 7.4). DiI-C_{18} (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, C_{18}) was bought from Invitrogen (Singapore). The stock DiI solution was prepared in dimethyl sulfoxide (DMSO) and the concentration of the stock was calculated from the absorbance measurement in UV–Visible spectrometer (NanoDrop, Thermo Scientific, Singapore) assuming the molar extinction coefficient (ε) equals to 144,000.

2.2. Preparation of supported lipid bilayer (SLB)

Supported lipid bilayers (SLBs) were prepared by the vesicle fusion method [38,40]. In brief, calculated amounts of lipid(s) and RhoPE dye solutions were first mixed in a cleaned round bottomed flask and left in the rotary evaporator (Rotavap R-210, Buchi, Switzerland) to evaporate the solvent for at least 3 h. The thin lipid film left behind was then resuspended in 2 mL buffer containing 10 mM HEPES and 150 mM NaCl (pH 7.4). The milky lipid suspension was sonicated in a bath sonicator (FB15051 Model, Fisher Scientific, Singapore) until a clear solution was obtained, thereby forming large unilamellar vesicles. The vesicle solution was then stored at 4 °C if not used immediately. Before measurements are to be taken, the vesicle solution was first sonicated for 10 min and then 200 μL of it was placed on a cleaned cover glass ($24 \times 50-1$, Fisher Brand Microscope cover glass, Fisher Scientific, Singapore) containing 200 μL of the same buffer. The deposited vesicles were incubated at 65 °C for 20 min followed by cooling at room temperature for another 20 min. Unfused vesicles were then removed by washing with 200 μL of the buffer for at least 50 times. In the case of cholesterol depletion experiments, an mβCD solution was incubated with the sample for 30 min on the microscope stage to a final concentration of 2 mM and washed 5 times with buffer.

The cover glasses were cleaned as follows. They were first sonicated in a bath sonicator (FB15051 Model, Fisher Scientific, Singapore) with 10× diluted detergent (Hellmanex III, Hellma Analytics, Singapore) for 30 min. This was followed by rigorous washing with deioninzed (DI) water (resistivity 18.2 MΩ·cm). The cover glasses were then subjected to another sonication step for 30 min with 2 M sulfuric acid followed by