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L_o/L_d phase coexistence modulation induced by GM1

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ABSTRACT

Lipid rafts are assumed to undergo biologically important size-modulations from nanorafts to microrafts. Due to the complexity of cellular membranes, model systems become important tools, especially for the investigation of the factors affecting "raft-like" L_o domain size and the search for L_o nanodomains as precursors in L_o microdomain formation. Because lipid compositional change is the primary mechanism by which a cell can alter membrane phase behavior, we studied the effect of the ganglioside GM1 concentration on the L_o/L_d lateral phase separation in PC/SM/Chol/GM1 bilayers. GM1 above 1 mol.% abolishes the formation of the micrometer-scale L_o domains observed in GUVs. However, the apparently homogeneous phase observed in optical microscopy corresponds in fact, within a certain temperature range, to a L_o/L_d lateral phase separation taking place below the optical resolution. This nanoscale phase separation is revealed by fluorescence spectroscopy, including C_{12}NBD-PC self-quenching and Laurdan GP measurements, and is supported by Gaussian spectral decomposition analysis. The temperature of formation of nanoscale L_o phase domains over an L_d phase is determined, and is shifted to higher values when the GM1 content increases. A "morphological" phase diagram could be made, and it displays three regions corresponding respectively to L_o/L_d micrometric phase separation, L_o/L_d nanometric phase separation, and a homogeneous L_d phase. We therefore show that a lipid only-based mechanism is able to control the existence and the sizes of phase-separated membrane domains. GM1 could act on the line tension, "arresting" domain growth and thereby stabilizing L_o nanodomains.

1. Introduction

Cell plasma membrane contains hundreds of lipids and proteins designed to perform the functions that cells require. It is now established that these membranes are mosaics of different types of domains with different sizes, compositions, dynamics and functions [1,2]. Among the various types of membrane domains, several are based on lipid interactions, with the most documented being the so-called lipid rafts [3,4]. These appear to be involved in many biological functions involving cellular activation, membrane trafficking, and signal transduction.

Rafts are thought to be rich in sphingolipids, cholesterol, and specific proteins [5,6] with lipids that are in a phase state that is distinct from the surrounding membrane [7]. Indeed, it is often speculated that this raft organization arises from the tendency for lipids in membranes containing cholesterol to separate into coexisting liquid-ordered (L_o) and liquid-disordered (L_d) phases [8,9]. The raft hypothesis elevated lipids to a regulatory role in which they mediate protein clustering and restrict protein diffusion in the membrane [4]. While the key role of cholesterol and sphingolipids is clear, this definition implies long-lived structures with stable protein recruitment. However, because the characterization of these membrane heterogeneities in live cells has been challenged, the idea of what constitutes a lipid raft has evolved. Indeed, large-scale membrane domains are not optically observable without significant perturbation [10,11] and the biochemical methodology used to study rafts has generated ambiguous results [12,13]. Currently, lipids rafts are viewed as highly dynamic nanoscale assemblies [14,15] that could undergo coalescence into micrometer-sized domains during specific cellular activation process [16,17]. This feature may be pivotal to the raft function and raft size is of crucial importance in the study of cellular functioning.

Abbreviations: L_o, liquid-ordered phase; L_d, liquid-disordered phase; GUV, giant unilamellar vesicle; Chol, cholesterol; GPMV, giant plasma membrane vesicle; DSPC, distearoyl-phosphatidylcholine; DOPC, dioleolyl- phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; GM1, ovine brain monosialotetrahexosylganglioside; PC, egg yolk L-α-phosphatidylcholine; SM, egg yolk sphingomyelin; Laurdan, 6-dodecanoyl-2- dimethylaminonaphthalene; C_{12}NBD-PC, 1-acyl-2-[12-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoyl]-sn-glycero-3-phosphocholine; TR-PE, Texas red DPPE; GP, generalized polarization; LUV, large unilamellar vesicle; T_{IP}, temperature at the inflection point

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In contrast to in vivo membranes, L_o/L_d phase separation in model membranes can easily be induced by adjusting the composition and temperatures of lipid bilayers. In particular, giant unilamellar vesicles (GUVs) allow optical fluorescence discrimination of coexisting lipid phases. Using simple biologically relevant lipid model systems composed of a high transition temperature (T_m) lipid, a low-T_m lipid, and cholesterol (Chol), micron-scale domains (raft-like or L_o microdomains) have been observed on GUVs [9,18], and are believed to represent the in vitro equivalent of the rafts in natural membranes. The usual approach for studying the formation of L_o microdomains in GUVs is to use a range of lipid compositions for their preparation and/or to vary the temperature. These studies have yielded important results, culminating in the determination of several L_o/L_d phase diagrams for specific lipid mixtures [19,20]. GUVs containing L_o microdomains coexisting with an L_d phase, have become important tools for the modeling of properties and biological functions of lipid rafts [19,20]. These have been also instrumental in evaluating current ideas as well as making new proposals for raft-associated mechanisms [21–23].

However, the biological relevance of L_o microdomains on GUVs systems has been questioned because, in comparison to in vivo membranes, such domains are stable equilibrium structures with a larger micrometric size. First, it has to be noticed that the use of techniques other than optical microscopy to probe shorter length scales that are biologically relevant provides evidence for much smaller L_o domains [24–26], even with lipid mixtures and temperatures for which fluorescence microscopy indicates only the presence of a single homogeneous phase [27]. On the other hand, it has been shown that giant plasma membrane vesicles (GPMVs, same membrane composition as intact cells but lacking a cytoskeleton) formed from the plasma membranes of cultured mammalian cells can segregate in micrometer-scale fluid domains [28,29]. Coexisting fluid membrane phases in GPMVs show fluorescence probe partitioning behavior similar to model membranes with L_o/L_d phase coexistence. As emphasized by Hancock [30], "there is no a priori reason to assume that the basic lipid biochemistry and thermodynamics that operate in model systems are not the same as those that operate in the plasma membrane", and a systematic study of the factors affecting domain size is clearly needed.

Recent work from Feigenson et al. [31,32] has investigated a domain size transition in the four-component mixture DSPC/DOPC/POPC/Chol, which may yield insight into how cells are able to exploit lipid composition changes to alter the size and connectivity of domains. These studies establish that both the size and morphology of membrane L_o domains can be controlled by the concentration and the type of low-melting lipid in mixtures of cholesterol and high-melting lipid. Another fourth appropriate candidate to add to the classical raft-like-domain-making three component lipid mixtures could be the ganglioside GM1. Indeed, gangliosides are known as major players in the creation of lateral order within biological membranes [33]. They are essential components of rafts and are directly involved in several raft-associated cellular processes [34]. GM1 has a strong amphiphilic character due to the big saccharidic headgroup that bears a protonatable sialic acid moiety, and the saturated double-tailed hydrophobic moiety. GM1 can separate from unsaturated lipids as a result of immiscibility [35] and seems to be preferentially distributed in the L_o domains in model systems [36,37]. Moreover, because of the excluded volume effect of its bulky headgroup, GM1 can also segregate from other saturated lipids and be heterogeneously distributed in submicron-sized domains within the ordered phase [38]. Concerning the domain size modulation, AFM experiments show that asymmetrically-inserted GM1 on pre-formed supported lipid bilayers promotes not only a change in the dominant lipid component of the L_o phase but also, a decrease of the L_o domain area with increasing GM1 concentration [39]. These complex intermolecular interactions between GM1 and saturated lipids, unsaturated lipids, and cholesterol, allow the membrane structure to be modulated with an incredible diversity via distinct mechanisms. Because each method introduces a different set of issues and the same set of lipid mixtures are rarely used, further experiments on four-component mixtures containing GM1 are still needed to improve the role of GM1 on L_o domain size modulation.

In the present work, the effect of GM1 concentration on the lateral phase separation in the PC/SM/Chol (50:30:20) bilayers was studied by fluorescence microscopy and spectroscopy. As expected for this lipid mixture below 23 °C without GM1 [40], fluorescence microscopy shows the occurrence of micrometer-sized L_o domains in GUVs. Oppositely, GUVs displaying a homogeneous phase are observed at higher temperature. Interestingly, GM1 above 1 mol.% abolishes the formation of micrometer-scale L_o domains. We show however that the homogeneous phase observed in that case by optical microscopy corresponds in fact, for a certain temperature range, to a L_o/L_d lateral phase separation below the optical resolution. This nanoscale phase separation is revealed by fluorescence spectroscopy on LUVs, including of C_{12}NBD-PC self-quenching and Laurdan GP measurements, and is supported by Gaussian spectral decomposition analysis. This allowed us to determine the temperature of formation of L_o phase domains over an L_d phase. The determined demixing temperatures from L_d to L_o/L_d phase separation are shifted to higher values when the GM1 content increases, which is linked with the change of the dominant composition of L_o domains. A lipid only-based mechanism is consequently able to control the existence and the sizes of phase-separated membrane domains. This last feature is tentatively attributed to GM1 effect on the line tension, "arresting" domain growth and thereby stabilizing L_o nanodomains.

2. Materials and methods

2.1. Reagents

Lipids were obtained as follows and used without further purification: egg yolk L-α-phosphatidylcholine (PC), egg yolk sphingomyelin (SM), cholesterol (Chol) and ovine brain GM1 were from Avanti Polar Lipids. The fluorescent lipid analogue Texas red DPPE (TR-PE) was from Invitrogen and the lipophilic membrane probe C_{12}NBD-PC (1-acyl-2-[12-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]dodecanoyl]-sn-glycero-3-phosphocholine) was obtained from Avanti Polar Lipids. Fluorescent probe 6-dodecanoyl-2- dimethylaminonaphthalene (Laurdan) was from Molecular Probes, Inc. All others chemicals were of highest purity grade.

2.2. Giant unilamellar vesicle preparation and imaging

The electroformation method developed by Angelova and Dimitrov [41] was used to form the vesicles. We followed the particular protocol for giant unilamellar heterogeneous vesicle formation described elsewhere [42]. GUVs were prepared with the starting molar composition PC/SM/Chol 50:30:20. GM1 was substituted for SM in proportions from 1 to 10 mol.%. The vesicles were formed in a temperature-controlled chamber in 0.5 mM HEPES buffer, pH 7.4. The temperature was controlled with a water circulating bath. The vesicles were always formed at 45 °C at which a high yield of vesicles was consistently obtained. Texas red phosphatidylethanolamine (TR-PE) was used as a probe at a concentration of 0.25% (mol/mol). TR-PE partitions in favor of the L_d phase [9] which appears brighter while the L_o phase appears darker by fluorescence microscopy.

A Zeiss Axiovert 200M microscope (fluorescent unit fluo arc N HBO 103, Zeiss), equipped with a Lambda 10-2 unit (Sutter Instrument Co.), plus a CCD B/W chilled camera (CoolSNAP HQ, Photometrics) was used for GUV imaging. The setup was computer-controlled by the Metamorph 6.2 software (Molecular Devices). A 40× Ph LD Zeiss objective was used. The phase morphology transformations and dynamics in the heterogeneous GUV membranes were followed by fluorescence using Zeiss filter set 15 (E_x/E_m = 550/620 nm).

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