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Phospholipid lateral diffusion in phosphatidylcholine-sphingomyelin-cholesterol monolayers; Effects of oxidatively truncated phosphatidylcholines

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ABSTRACT

Oxidative stress is involved in a number of pathological conditions and the generated oxidatively modified lipids influence membrane properties and functions, including lipid–protein interactions and cellular signaling. Brewster angle microscopy demonstrated oxidatively truncated phosphatidylcholines to promote phase separation in monolayers of 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine (POPC), sphingomyelin (SM) and cholesterol (Chol). More specifically, 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (PazePC), was found to increase the miscibility transition pressure of the SM/Chol-phase. Lateral diffusion of lipids is influenced by a variety of membrane properties, thus making it a sensitive parameter to observe the coexistence of different lipid phases, for instance. The dependence on lipid lateral packing of the lateral diffusion of fluorophore-containing phospholipid analogs was investigated in Langmuir monolayers composed of POPC, SM, and Chol and additionally containing oxidatively truncated phosphatidylcholines, using fluorescence correlation spectroscopy (FCS). To our knowledge, these are the first FCS results on miscibility transition in ternary lipid monolayers, confirming previous results obtained using Brewster angle microscopy on such lipid monolayers. Wide-field fluorescence microscopy was additionally employed to verify the transition, i.e. the loss and reformation of SM/Chol domains.
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1. Introduction

Lipid oxidation has profound effects on the biophysical properties of lipid membranes, and alterations such as phase changes and phase separation in model membranes have been demonstrated. Interest in the effects of oxidized lipids on membrane biophysics was revived upon the availability of well-defined, stable oxidatively modified phospholipid derivatives together with recent advances in cell biology pointing to the involvement of oxidized lipids in processes such as inflammation and apoptosis and the molecular pathology of several neurodegenerative diseases [1,2]. Along these lines it was shown that the addition of oxidatively truncated phosphatidylcholines into fluid phase 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine (POPC) bilayers caused a loss of the permeability barrier function of the lipid bilayers, together with rapid loss of lipid asymmetry [3]. The latter finding is of significance as it undermines the notion of a specific transmembrane protein (flippase/scramblase) being responsible for the transfer of phosphatidylserine from the inner to the external leaflet of the plasma membrane lipid bilayer in apoptosis [4,5].This was substantiated in computer simulations, highlighting the importance of detailed understanding of lipid biophysics deciphering potential new physiologically meaningful functional consequences of changes in lipid composition, thus introducing a new paradigm to the analysis of cell behavior, which at present is almost exclusively being explained in terms of activities of specific proteins, enzymes, receptors, channels, and so on.

Along somewhat similar lines it was shown that the introduction of an oxidatively truncated phosphatidylcholine derivative PazePC promotes in lipid monolayers the phase separation of a lipid phase consisting of sphingomyelin and cholesterol. The aim of the present study was quantitative analysis of the diffusional dynamics of membranes containing the oxidatively truncated phosphatidylcholines (PazePC and PoxnoPC, Fig. 1) included into monomolecular films of sphingomyelin, cholesterol, and POPC. We could confirm that the

Abbreviations: AOM, acousto-optical modulator; AOTF, acousto-optical tunable filter; BSA, bovine serum albumin; Chol, cholesterol; FCS, fluorescence correlation spectroscopy; FRAP, fluorescence recovery after photobleaching; L_d, liquid disordered phase; L_o, liquid ordered phase; NMR, nuclear magnetic resonance; oxPC, oxidatively modified phosphatidylcholines; oxPL, oxidized phospholipids; PazePC, 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn* glycerol-3-phosphocholine; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; PoxnoPC, 1-palmitoyl-2-(9′-oxononanoyl)-*sn*-glycero-3-phosphocholine; SM, sphingomyelin; SPT, single particle tracking; WFM, wide-field fluorescence microscopy

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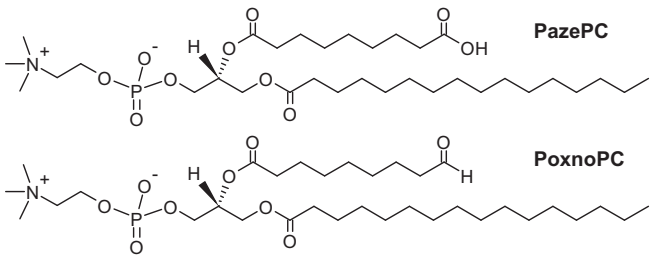

Fig. 1. Chemical structures of two oxidatively truncated phosphatidylcholines, PazePC and PoxnoPC.

miscibility transition pressure, the melting pressure of liquid-ordered (l_o) SM/Chol-domains, tends to increase with increasing the content of oxidized phospholipids (oxPLs) [6]. The results were interpreted in terms of line tension outweighing dipolar interactions in maintaining domain structure and shape due to the lack of differences in the average dipole density upon the mixing of the phases [7,8]. Most importantly, PCs with oxidatively truncated acyl chains increase the thickness mismatch between the coexisting phases, contributing to the line tension at the domain boundaries. This is further associated with the complete chain-reversal of the truncated acyl chains [9]. We aimed at investigating if the above also involves changes in lateral lipid mobility. Lateral self-diffusion is sensitive to a number of diverse changes in lipid bilayer properties and may give more insight into lipid film behavior during the compression and domain disintegration. To answer the question in which way is the vicinity of domains and oxPL affecting the lateral self-diffusion, we compared SM/Chol-enriched films with monolayers containing phospholipids only, while varying the content of oxPL in the phosphatidylcholine fraction.

For this utility, we assembled a fluorescence correlation spectroscopy (FCS) z-scan setup for continuous scanning of the fluorescence correlation time traces during compression of lipid monolayers. A similar system has previously been described by Gudmand et al. [10]. Several methods are available to measure membrane diffusional dynamics, the most popular being fluorescence recovery after photobleaching (FRAP), single particle tracking (SPT), and pulsed field gradient NMR [11–13]. Apart from the latter, these are all light microscopy methods. While FRAP measurements are affected by monolayer surface flow, and SPT can be experimentally demanding, FCS provides with the properties well applicable for monolayer experiments. Light microscopy diffusion methods are discussed in detail by Chen et al. [14].

2. Theory

In general, lateral self-diffusion due to the thermal collisions can be characterized by:

$$(r(t)-r(0))^2 = 4Dt^{\alpha}, \tag{1}$$

which describes the time dependence of the mean square displacement of a particle over long time scales. The diffusion coefficient D and anomalous factor α are sufficient to describe the diffusion of the particle. The parameter α can be assumed to be one for homogenous distribution of the self-diffusing molecules and <1 for hindered diffusion.

2.1. FCS

FCS is very well suited for use with Langmuir films, making it possible to observe anomalous dynamic components from the auto-correlation curves and still maintain statistical single-molecule sensitivity. The downside of this approach is the depth of the observation volume (~1–2 μm) in comparison with the monolayer thickness (nm), which makes it difficult to have the monolayer coincide with the scanner position. Likewise, while contact of the film with water corresponds to real molecular interactions of biomembranes, surface evaporation could be a challenge. As stated in previous studies, fluorescence correlation spectroscopy (FCS) is a powerful statistical method in analyzing lateral diffusion in membranes [15]. A confocal microscopy setup is utilized to focus laser light through an objective with high numerical aperture into a small detection volume (V ~ 1 fl), while the fluorescence light emitted from molecules diffusing through the detection volume is collected and analyzed. The number of fluorescent species has to be low enough in order to have substantial signal contribution from each diffusing molecule (usually in nanomolar range). For analysis, auto-correlation function is calculated for the detected signal.

For a two-dimensional sample, assumption of Gaussian detection profile yields:

$$G(\tau) = 1 + \frac{1}{N}\left(\frac{1}{1+\frac{\tau}{\tau_D}}\right), \tag{2}$$

where the fluctuating signal is analyzed with respect to its self-similarity after the lag time τ. N is the mean number of fluorophores in the focus, and τ_D is the average time for a single fluorescent molecule to diffuse through the detection area. The minimum of both these parameters is found in the exact beam waist of the confocal volume.

When other photophysical events (e.g. intersystem crossing) are contributing to the overall fluorescence signal, an average fraction of fluorophores in triplet state T and intersystem crossing relaxation time τ_T need to be implemented into Eq. (2) [16] and the autocorrelation function thus becomes:

$$G(\tau) = 1 + \left[1-T+Texp\left(\frac{-\tau}{\tau_T}\right)\right]\frac{1}{N(1-T)}\frac{1}{1+\left(\frac{\tau}{\tau_D}\right)}. \tag{3}$$

Moreover, in the case of two fluorescent species laterally diffusing through the detection volume, Eq. (3) has to be expanded:

$$G(\tau) = 1 + \left[1-T+Texp\left(\frac{-\tau}{\tau_T}\right)\right]\frac{1}{N(1-T)}\left(\frac{Amp}{1+\left(\frac{\tau}{\tau_{Da}}\right)}+\frac{1-Amp}{1+\left(\frac{\tau}{\tau_{Db}}\right)}\right), \tag{4}$$

where τ_{Da} and τ_{Db} correspond to the diffusion times of two fluorescent species with different diffusing properties and Amp is the amplitude of auto-correlation corresponding to faster diffusing species τ_{Da}. One can further relate the diffusion coefficient with the diffusion time by:

$$D = \frac{\omega^2}{4\tau_D}, \tag{5}$$

where ω is the radius of the cross-sectional area of the detection volume. Due to the variation in the axial positioning of the sample relative to the scanner, we could not determine the exact values of ω. Therefore we are limited to interpret individual diffusion times as a relative measure of the diffusional lipid dynamics. Large amount of auto-correlation functions in relation to the surface pressure, and area, were obtained and could be satisfactorily fitted by Eqs. (3) and (4), respectively.

2.2. Free-area model

Free-area model for self-diffusing particles has been known for decades and has proven to be useful in single-lipid studies [17–19]. Due to the discrepancies with the experiments involving multiple lipid species, we do not intend to use it for our ternary "raft mixture" studies [20,21]. Free-area model relates diffusion to the available free area in a lateral lipid lattice, $a_f = a - a_0$, where a is the molecular area obtained from Langmuir experiments, and a_0 is the core area of a single lipid

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