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Investigating lipid–lipid and lipid–protein interactions in model membranes by ToF-SIMS

L. Zheng [a,*], C.M. McQuaw [a,1], M.J. Baker [b], N.P. Lockyer [b], J.C. Vickerman [b], A.G. Ewing [a,c], N. Winograd [a]

[a] Department of Chemistry, The Pennsylvania State University, 104 Chemistry Building, University Park, PA 16802, United States
[b] Surface Analysis Research Centre, Manchester Interdisciplinary Biocentre, University of Manchester, P.O. Box 88, M60 1QD, UK
[c] Department of Chemistry, Göteborg University, Kemivägen 10, S-41929 Göteborg, Sweden

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ABSTRACT

With the chemical imaging capability of ToF-SIMS, biological molecules are identified and localized in membranes without any chemical labels. We have developed a model membrane system made with supported Langmuir–Blodgett (LB) monolayers. This simplified model can be used with different combinations of molecules to form a membrane, and thus represents a bottom-up approach to study individual lipid–lipid or lipid–protein interactions. We have used ternary mixtures of sphingomyelin (SM), phosphatidylcholine (PC), and cholesterol (CH) in the model membrane to study the mechanism of domain formation and interactions between phospholipids and cholesterol. Domain structures are observed only when the acyl chain saturation is different for SM and PC in the mixture. The saturated lipid, whether it is SM or PC, is found to be localized with cholesterol, while the unsaturated one is excluded from the domain area. More complicated model membranes which involve a functional membrane protein glycophorin are also investigated and different membrane properties are observed compared to the systems without glycophorin.

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1. Introduction

There is an increased interest in how lipids interact with each other and with membrane proteins/peptides and how these interactions lead to various cell membrane functions. ToF-SIMS is a unique technique to investigate these interactions by chemically identifying the location of each molecule [1–3]. However, it is extremely difficult to characterize the native structure of cell membranes due to their innate complexity, i.e., the eukaryotic cell membranes consist of up to 500 different lipid species [4]. Model membrane systems, such as supported lipid bilayers and Langmuir–Blodgett (LB) monolayers, have been proven to be good mimics of cellular membranes [3,5–7]. These simplified models can be used with different combinations of lipid and protein molecules to form a membrane, which represents a bottom-up approach to study individual interactions.

The LB model membrane is especially suitable for ToF-SIMS characterization owing to its stability in the ultra-high vacuum environment [8]. Previous studies in our lab have already shown the capability of ToF-SIMS to identify the localization of molecular species in the supported LB films [3,6,9,10]. Here, we report on a novel method of studying lipid–lipid interactions by ToF-SIMS. Ternary mixtures of sphingomyelin (SM), phosphatidylcholine (PC), and cholesterol (CH) with varying tail group saturation for SM and PC are characterized by ToF-SIMS. Domain structures are observed when either SM or PC is saturated while uniform lateral distributions are found for all the lipids in the systems when SM and PC are both saturated or both unsaturated. The location of SM and PC with respect to CH elucidates the mechanism of interactions between CH and SM/PC molecules in the membrane. Ternary mixtures containing a membrane protein glycophorin are also investigated by ToF-SIMS. Dipalmitoyl-phosphatidylcholine (DPPC) and dipalmitoyl-phosphatidyl ethanolamine (DPPE) together with CH are assumed to be present primarily in the outer and inner leaflet of the plasma membrane, respectively. The results demonstrate the capability of ToF-SIMS to identify membrane proteins and the possibility to understand lipid–protein interactions in the model membrane system.

* Corresponding author.
E-mail address: luz109@psu.edu (L. Zheng).
[1] Current address: Volume Institute and Department of Microbiology Immunology, Oregon Health and Science University, Portland, OR 97220, United States.

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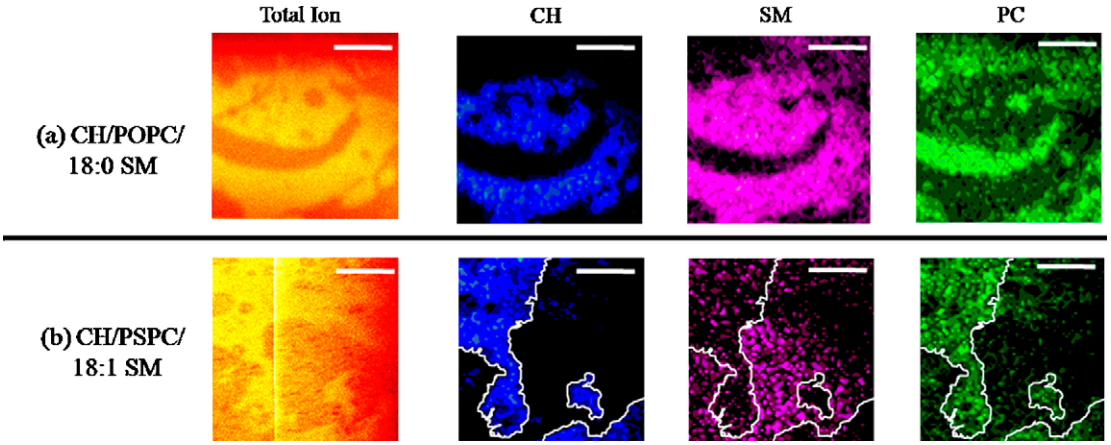

Fig. 1. ToF-SIMS positive ion images of the lipid LB films containing (a) 23% CH/30% POPC/47% 18:0 SM, (b) 23% CH/30% PSPC/47% 18:1 SM. The field of view is 300 μm × 300 μm with 256 × 256 pixels for the total ion images and 128 × 128 pixels for the molecular-specific images. The total ion images contain all ions within *m*/*z* 1–1000. CH, SM, and PC are represented by *m*/*z* 369 in blue, *m*/*z* 264 in pink, and *m*/*z* 224 in green, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

2. Experimental

2.1. Sample preparation

The following materials were used without further purification: DPPC, DPPE, cholesterol, POPC, 18:1 SM (all purchased in powder form from Avanti Polar Lipids, Inc., Alabaster, AL), 18:0 SM (Matreya LLC, Pleasant Gap, PA), 16-mercaptohexadecanoic acid and Glycophorin A (both purchased from Sigma–Aldrich Co., St. Louis, MO), methanol, chloroform. The water used was purified by a Nanopure Diamond Life Science Ultrapure Water System (Barnstead International, Kubuque, IA) and had a resistivity of 18.2 MΩ-cm.

The LB films of lipid or lipid–protein mixtures were prepared using a Kibron μTrough S-LB (Helsinki, Finland). The LB films were built on acid-terminated self-assembled monolayers on gold. The details of substrate and LB film preparation have been described elsewhere [6].

2.2. Sample analysis

All processes of self-assembly and LB film preparation were confirmed with a single wavelength (632.8 nm, 1 mm spot size, 70° incident angle) Stokes ellipsometer LSE (Gaertner Scientific Co., Skokie, IL). The films were analyzed by an imaging ToF-SIMS instrument equipped with a 15 keV Ga^+ liquid metal ion gun (described previously [11]). Mass spectra were acquired at each pixel by rastering the ion beam across the sample surface to generate an ion image of the area being analyzed. The imaging experiments were performed at room temperature with an ion dose $<10^{12}$ ions/cm^2.

3. Results and discussion

3.1. Lipid–lipid interactions

Ternary model membrane systems of PC, SM, and CH are considered to be the archetypical lipid raft mimics since SM and CH are the major constituents of detergent-insoluble fractions isolated from cell membranes [12,13]. The 4 ternary systems that were investigated in this study are 23% CH/30% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)/47% 18:0 SM, 23% CH/30% 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (PSPC)/47% 18:1 SM, 23% CH/30% PSPC/47% 18:0 SM, and 23% CH/30% POPC/47% 18:1 SM. Each system contains CH while the SM and PC are varied by the tail group saturation, i.e. one double bond at the same position of the tail group. ToF-SIMS is able to identify each molecule in the LB film of the ternary mixtures. The SIMS peaks used for CH, SM, and PC are *m*/*z* 369, *m*/*z* 264, and *m*/*z* 224, respectively.

The images for CH/PSPC/18:0 SM and CH/POPC/18:1 SM, in which the SM and PC are both saturated or both unsaturated, show uniform lateral distribution of all lipid molecules (data not shown). However, domain structures are observed for the LB films of CH/POPC/18:0 SM and CH/PSPC/18:1 SM (Fig. 1). The 18:0 SM is co-localized with CH and POPC is anti-localized with CH in CH/POPC/18:0 SM film while the 18:1 SM is excluded from the CH/PSPC domain in the CH/PSPC/18:1 SM film. The saturated lipid, whether it is SM or PC, co-localizes with CH and the unsaturated one is excluded. SM and PC used in the study have the same headgroup and acyl chain length. Thus the differences between them are found in the head–tail linkage region and the tail group saturation. Our results show that CH does not differentiate between the SM and PC when they have the same saturation level of tail group. However, when SM and PC in the system differ only by a double bond on the 18-carbon chain at the same position, domain structures are observed in the film images due to phase separation in the film, which means CH interacts with the saturated lipid significantly more strongly than the unsaturated one. This can be explained by preferential hydrophobic match between the saturated acyl chain and the steroid ring. Double bonds create kinks in the tail structures which sterically prevents part of the acyl chain from interacting with CH. The location and functional role of SM in the formation of lipid rafts are still under debate. Our results show that the tail group saturation dominates the interaction between SM and CH. Thus the co-localization of SM and CH is more probably due to the high saturation level of the SM tail group rather than the hydrogen bonding capability of the head–tail linkage region of SM.

3.2. Lipid–protein interactions

Membrane proteins are major components of cellular membranes and play important functions. Incorporation of membrane proteins into model membrane systems is an essential step towards synthesizing a more representative mimic and better

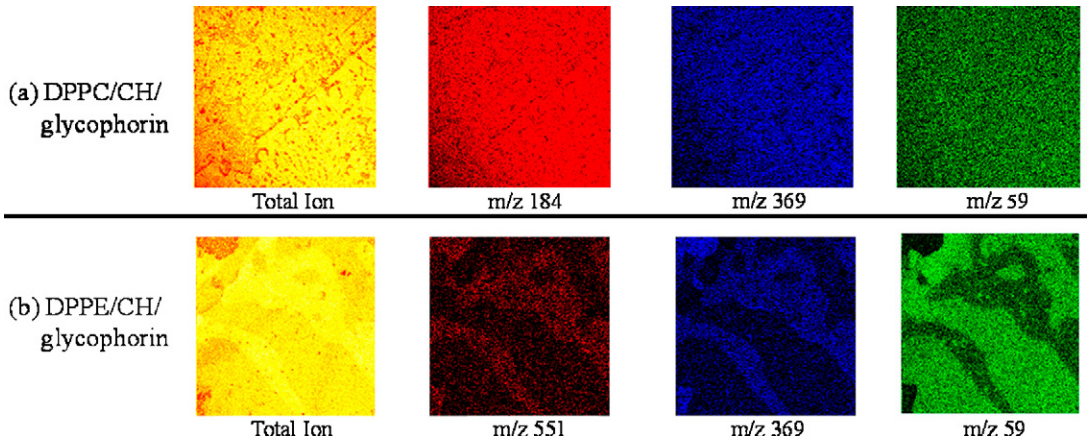

Fig. 2. ToF-SIMS positive ion images of the lipid LB films containing (a) 60% DPPC/38% CH/2% glycophorin and (b) 65% DPPE/33% CH/2% glycophorin. The field of view is 400 μm × 400 μm with 256 × 256 pixels and there are 20 shots/pixel. The total ion image contains all ions within the *m*/*z* 1–1000. *m*/*z* 184, *m*/*z* 551, *m*/*z* 369, and *m*/*z* 59 represent DPPC, DPPE, CH, and glycophorin, respectively.

understanding of lipid–protein interactions. We have investigated the ternary systems containing an integral membrane protein, glycophorin A. Glycophorin A contains 131 amino acids and presents its amino-terminal end at the extra-cellular surface of the human red blood cell [14]. DPPC and DPPE, together with CH, are also included in the mixtures to represent the outer and inner leaflet of the plasma membrane [6]. The molar ratio of each component is 2% for glycophorin A (which mimics the 49.2% of protein by weight in the red blood cell plasma membrane [15]), 38% for CH and 60% for DPPC/DPPE. The lipids CH, DPPC, and DPPE are identified at *m*/*z* 369, *m*/*z* 184, and *m*/*z* 551, respectively, by ToF-SIMS. High mass molecular ions of glycophorin are not observed in the mass spectra; however, several low mass peaks, i.e., at *m*/*z* 59 (a fragment peak for valine and arginine), from the amino acid fragments can be used for identification. Other fragment peaks of glycophorin, such as *m*/*z* 72 (val) and *m*/*z* 101 (arg), are also observed, but their ion images have much lower contrast than that of *m*/*z* 59 which is mainly due to DPPE and CH also contributing to these peaks. The ToF-SIMS images (Fig. 2))show that the lipids and glycophorin are evenly distributed in the DPPC/CH/glycophorin membrane. Domain structures are observed in the DPPE/CH/glycophorin system, in which the DPPE and CH are co-localized with each other and the glycophorin is excluded. A previous study of the DPPE/CH binary system at the molar ratio of 2:1 DPPE/CH, shows that two immiscible phases of DPPE and DPPE/CH coexist in the system. The addition of glycophorin changes the phase behaviour and leads to the disappearance of the DPPE phase. The results also indicate that glycophorin interacts with DPPC/CH better than with DPPE/CH since it is excluded from the DPPE/CH domain.

4. Conclusions

By examining the model membranes containing various combinations of lipid molecules and membrane proteins, we have shown that it is possible to study lipid–lipid and lipid–protein interactions in the LB supported monolayer membrane system by utilizing the chemical imaging capability of ToF-SIMS. The results of the CH/SM/PC system suggest that the tail group saturation is the dominating factor for lipid–cholesterol interactions. The study of glycophorin-containing systems indicates the importance of membrane proteins for the physical properties and functions of cellular membranes.

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