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Review

Surface view of the lateral organization of lipids and proteins in lung surfactant model systems—A ToF-SIMS approach

Mohammed Saleem [1], Hans-Joachim Galla *

Institute of Biochemistry, University of Münster, Wilhelm-Klemm-Str. 2, 48149 Münster, Germany

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ABSTRACT

The lateral organization of domain structures is an extremely significant aspect of biomembrane research. Chemical imaging by mass spectrometry with its recent advancement in sensitivity and lateral resolution has become a highly promising tool in biological research. In this review, we focus briefly on the instrumentation, working principle and important concepts related to time-of-flight secondary ion mass spectrometry followed by an overview of lipid/protein fragmentation patterns and chemical mapping. The key issues addressed are the applications of time-of-flight secondary ion mass spectrometry in biological membrane research. Additionally, we briefly review our recent investigations based on time-of-flight secondary ion mass spectrometry to unravel the lateral distribution of lipids and surfactant proteins in lung surfactant model systems as an example that highlights the importance of fluidity and ionic conditions on lipid phase behavior and lipid–protein interactions.

Contents

1. Introduction

The molecular mechanisms underlying lipid–protein interactions are highly significant for studies of structure–function relationship in membranes. In particular, biological processes in which specific lipids are required for proper functioning has attracted much research. One such phenomenon, where specific lipid–protein interactions become indispensable, is to facilitate a continuous and effortless breathing process.

The alveolar interface is lined by a complex mixture of phospholipids and surfactant proteins, whose interplay works to reduce the surface tension at the alveolar surface, thereby, preventing alveolar collapse [1,2]. A significant amount of knowledge has been acquired on the physiological importance of lung surfactant in premature neonates and adults[3,4], and the role of phospholipids, surfactant proteins and their model peptides [5–12] on phase behavior [13–16] and their structure–function relationship [17–19]. The major phospholipid constituent of pulmonary surfactant is the phosphatidylcholine (PC),

* Corresponding author. Tel.: +49 251 8333200; fax: +49 251 8333206.
E-mail address: gallah@uni-muenster.de (H.-J. Galla).
[1] Present address: Physico-Chemistry Curie, Institute Curie, Paris, France.

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especially dipalmitoylphosphatidylcholine (DPPC) [20–22]. DPPC plays an important role in reducing surface tension to very low values and thus protecting the alveolus against collapse [2,23,24]. Other lipids that are essential for lung surfactant function are the anionic phosphatidylglycerol (PG), and phospholipids containing unsaturated acyl chains [22,25]. Besides phospholipids, surfactant proteins namely SP-A, SP-B, SP-C, and SP-D, have been found to be of immense importance in assembly and functioning of the lung surfactant [26–28]. SP-A and SP-D are hydrophilic proteins that play an important role in the storage and transport of lung surfactant and participate in host defense [29], whereas the hydrophobic proteins SP-B and SP-C have been shown to play an important role in promoting/enhancing the adsorption and spreading of monolayers containing large amounts of DPPC.

Attempts to understand the mechanism of surfactant function are numerous. However, the complexity of the system and the technical limitations has impeded its complete understanding. Nevertheless, efficient model systems have been established and studied that seem to closely mimic the properties of native composition. However, observations of specific lipid–protein interactions in various lung surfactant model systems have not been conclusive. To our knowledge, specific SP-B/DPPG interactions were first proposed based on fluorescence anisotropy studies in lipid bilayers [15], and it was proposed that SP-B interacts specifically with PG head groups [17]. Furthermore, electron paramagnetic resonance spectroscopy studies in vesicular systems also suggested preferential interaction between anionic DPPG and SP-B [30]. On the contrary, other bilayer studies based on ^{2}H nuclear magnetic resonance did not observe any preferential interaction of SP-B with either DPPC or DPPG [31]. Surprisingly, our recent monolayer studies based on time-of-flight secondary ion mass spectrometry (ToF-SIMS) imaging demonstrated that both SP-B and its mimetic peptide KL_4 preferentially tend to colocalize with the zwitterionic DPPC. This, however, did not rule out the existence of specific DPPG/protein interactions. Further studies to elucidate the possible influence of the ionic conditions, showed a reversal of surfactant protein colocalization with the anionic DPPG in the absolute absence of calcium ions [32].

In this review, we will mainly focus on the ToF-SIMS imaging as applied in biological systems and particularly its capacity for molecular identification and chemical imaging needed to unravel the lateral organization of membrane structure in model membranes. ToF-SIMS imaging provides a link between the contemporary techniques with atomic scale resolution and the optical microscopy has the unique advantage of providing direct insights into the chemical composition with a resolution down to less than 100 nm. In the following sections, we will briefly describe the working principle of ToF-SIMS, the chemical identification of biomolecules and the lateral organization of lipid–protein membrane systems. Finally, the exploitation of SIMS imaging to characterize the lipid–protein interaction in the lung surfactant model membranes during our studies over the last years will be described.

2. Time-of-flight secondary ion mass spectrometry (ToF-SIMS)—Overview

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a powerful technique for high resolution surface, interface and thin film analysis that enables label-free detection of individual components of a monolayer, transferred to a solid support. As shown in Fig. 1, ToF-SIMS involves rastering of a highly energetic electrically focused primary ion beam across the sample inducing a collision cascade that may lead to the release of charged molecules, and compound-characteristic secondary ions fragments [33]. The emitted fragment ions are then accelerated by an electric field in the time-of-flight analyzer leading to separation and detection of the ions according to their mass-to-charge ratios (m/z), thus, offering a high mass range, high mass resolution and a precise mass detection [34,35]. Eventual performance depends on critical factors such as the kind of primary ions source and modulation of its pulses. It is important to note that ToF-SIMS basically is a destructive technique as the highly energetic primary ion beam induces desorption of surface ions. However, use of very low intensity primary ion dose permits quasi-non-destructive analysis. We now briefly describe the primary ions sources: modulation, collision cascade, sputtering process and lateral resolution.

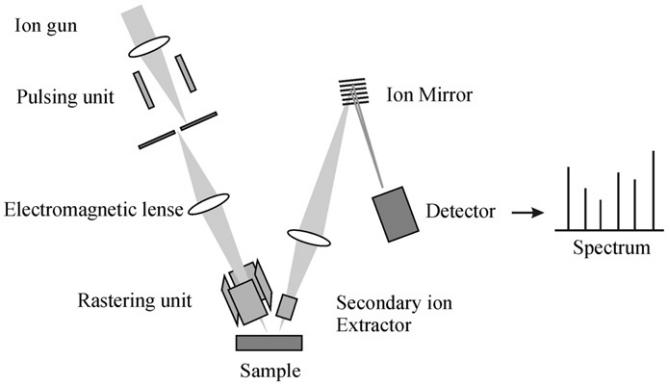

Fig. 1. ToF-SIMS instrumentation. A general scheme of ToF-SIMS showing the important components of the instrumentation.

2.1. Primary ion sources and modulation

The two commonly deployed primary ion sources in biomolecular research are electron impact (EI) sources and liquid metal ion guns (LMIG). Primary ion sources such as polyatomic SF_5^+ and fullerene ion beams (C_{60}^+, C_{60}^{2+}, C_{60}^{3+}) are generated by collision of the gaseous or vaporized source with the accelerated electrons (*electron impact*). The resulting ions have energies in the keV range and offer a significant secondary ion yield. In LMIG's, metals such as gallium, bismuth and gold flow from a reservoir to a small needle tip, thereby establishing a Taylor cone which is then ionized under high voltage to generate stream of metal cations. Bismuth and Gold have recently become the most popular ion source for LMIG's due to their ability to provide not only monoatomic singly charged ions (*like Gallium*) but also cluster of ions such as Bi_3^+, Bi_3^{2+} or Au_3^+ (*unlike Gallium*) [34].

Another essential aspect of ToF-SIMS involves the modulation of the primary ion beam in order to enable high lateral and mass resolution. Spatial focusing or pulsing of the primary ion is done to allow proper separation of secondary ion fragments desorbed off the surface according to there m/z ratio. This is also important to eliminate the varying accelerations of the primary ions resulting from mass and charge discrepancies. There are two kinds of operational modes of the ToF-SIMS which differ in pulse duration and sorting of the primary ions. In "burst alignment mode," the pulsing (10–100 ns), specific mass selection and focusing of the primary ion are optimized to achieve a high lateral resolution of ~ <100 nm [36]. On the contrary, the "bunched mode" involves a significant reduction of the pulse duration to shorter than 1 ns with a bunching device that enables conjunct flow of primary ions and forces primary ions with different speeds to hit the surface at the same time [34]. This allows a mass resolution of >10000 (i.e., $m/\Delta m$) [37]; however, chromatic abberation of the lense system in the bunching device tends to lower the lateral resolution to ~ 2–5 μm [36]. Thus, the above described operational modes could be effectively used to obtain high lateral resolution for chemical imaging of surfaces (*Burst alignment mode*) as well as high mass resolution for acquisition of spectra (*Bunched mode*).