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Regular Article

The phase and charge of milk polar lipid membrane bilayers govern their selective interactions with proteins as demonstrated with casein micelles

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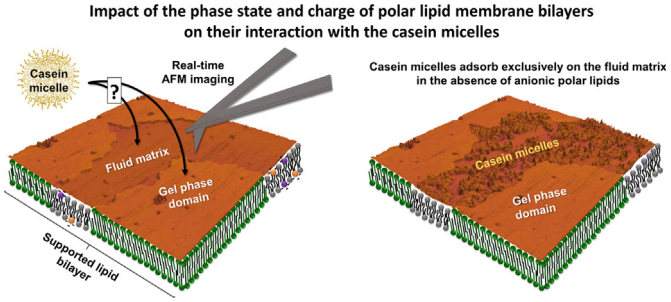
GRAPHICAL ABSTRACT



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ABSTRACT

The biological membrane surrounding fat globules in milk (milk fat globule membrane; MFGM) is an interface involved in many biological functions and interactions with the surrounding proteins or lipolytic enzymes in the gastro-intestinal tract during digestion. The MFGM exhibits lateral heterogeneities resulting from the different phase states and/or head-group charge of the polar lipids, which were both hypothesized to drive interaction with the casein micelles that is the major milk protein assembly. Atomic force microscopy (AFM) imaging was used to track the interactions of casein micelles with hydrated supported lipid bilayers of different composition, phase state and charge. Zeta-potential and Langmuir isotherms of the different polar lipids offered additional information necessary to interpret AFM observations. We showed that the negatively-charged casein micelles did not interact with milk sphingomyelin in the gel or liquid-ordered phases but did interact with polar lipids in the liquid-disordered phase (unsaturated polar lipids and milk sphingomyelin above its melting point). A wide intermolecular distance between polar lipids allowed protein adsorption on the membranes. However, the presence of the anionic polar lipids phosphatidylserine and phosphatidylinositol prevented any interaction with the casein micelles, probably due to electrostatic repulsion. These results open perspectives for the preparation of tailored emulsions covered by polar lipids able to modulate the interfacial interactions with proteins.

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1. Introduction

Many functions of biological membranes are governed by the interactions between polar lipids and proteins. However, they are far from being fully elucidated in particular as regards to the role played by the charge and physical phase state of lipids.

Biological membranes are highly dynamic interfaces composed of polar lipids and cholesterol organized into bilayers in which proteins and glycoproteins are embedded. The mammalian plasma membrane exhibits a complex lipid architecture, first visible in the asymmetry of the two leaflets composing the bilayer [1]. While unaltered plasma membrane exposes the zwitterionic polar lipids such as sphingomyelin (SM), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) to the outer leaflet, apoptosis or degradation leads to exposure of the anionic polar lipid phosphatidylserine (PS), which modifies the surface charge of the cell or vesicle and its interaction with signal proteins [2]. Furthermore, the composition of the membrane polar lipids is complex and associated to various physical properties. Individual gel to fluid phase transition temperatures (Tm) span from typically −25 °C for unsaturated polar lipid species to 80 °C for saturated ceramides. Cholesterol is an important structural component of biological membranes, able to interact with saturated polar lipids such as SM. This has been hold responsible for the lateral phase separation and formation of fluctuating domains or "rafts" of lipids in liquid-ordered phase, dispersed in a continuous matrix of lipids in the fluid phase [3,4]. This chemical and structural heterogeneity in polar lipid composition may be involved in the modulation of many membrane functions in particular the interactions with proteins, which are crucial in various biological functions such as cell signaling, trafficking or in the control of membranes physical stability or functional properties [5].

Among the biological membranes, the one enveloping milk fat globules (namely the milk fat globule membrane; MFGM) is of special interest since it is involved in various interfacial mechanisms and functions occurring in foods and in the gastrointestinal tract, and provides both nutritional and health benefits [6–8]. The MFGM is the interface between the core of triacylglycerols and the aqueous environment surrounding milk fat globules where interactions with bacteria, viruses and proteins (e.g. lipolytic enzymes involved in milk lipid digestion, milk proteins such as caseins and whey proteins) can occur.

The MFGM is structured as a trilayer of polar lipids and proteins, resulting from the milk fat globule secretion in the mammary epithelial cells [9]. A monolayer originating from the endoplasmic reticulum is in contact with the triacylglycerol core of fat globules, and an outer bilayer results from delivery of the fat globules through the apical plasma membrane of the mammalian epithelial cells. The major polar lipids found in the MFGM are the zwitterionic milk sphingomyelin (MSM: 20–35 wt%), phosphatidylcholine (PC: 20–35 wt%) and phosphatidylethanolamine (PE: 20–35 wt%), while the anionic phosphatidylserine (PS) and phosphatidylinositol (PI) are relatively less abundant (both around 11 wt% of total MFGM polar lipids) [8,10,11]. The transversal sorting of the polar lipids that compose the MFGM is currently poorly known [12]. Since the external bilayer of the MFGM originates from exocytosis, one can expect that the milk fat globule essentially exposes zwitterionic head-groups of PC, SM and PE [1]. However, the MFGM is known to bear high concentration of lactadherin, a PS-binding protein located in the external leaflet of the membrane [6,13,14]. Also, the MFGM polar lipids exhibit a significant negative zeta potential value at neutral pH [15,16]. These two elements indicate that the external bilayer may undergo resorting of the two leaflets after secretion, thereby exposing anionic PI and PS head-groups. The MFGM polar lipids are furthermore characterized by a wide diversity of saturated and unsaturated acyl residues [11] with distinct phase transition temperatures (Tm) leading to different physical states in the membrane. High-Tm saturated polar lipids, such as MSM with Tm ∼ 34 °C [17–19], are present in gel phase below Tm, in liquid-disordered (*ld*) phase above Tm, or in liquid-ordered (*lo*) phase in the presence of cholesterol (Chol) [17,18,20,21]. Unsaturated polar lipids (ie. PC, PE, PS and PI) have low Tm and are present in *ld* phase at positive temperatures. As a consequence of differences in the physical properties of milk polar lipids, the outer bilayer of the MFGM exhibits lateral phase separation with formation of MSM-rich gel phase and/or *lo* phase microdomains surrounded by a *ld* phase matrix of unsaturated polar lipids, as revealed *in situ* at the surface of milk fat globules using confocal microscopy [22–24]. This heterogeneity in the organization of polar lipids leads to a heterogeneity in their mechanical properties as revealed using force spectroscopy. MSM-rich gel phase and MSM/Chol *lo* phase domains are more rigid than the surrounding *ld* phase matrix composed of unsaturated polar lipids [20,21]. Hence, the MFGM is susceptible to show various physical phase states of the polar lipids (i.e. *ld* phase matrix; MSM-rich domains in the gel or *lo* phases), as well as heterogeneities in the local surface charge (i.e. negative charge bring by anionic polar lipids PS and PI, neutral charge of the zwitterionic MSM, PE, PC), depending on the composition and lateral packing of the polar lipids present in the outer bilayer. These physical and chemical heterogeneities of polar lipids occurring in the MFGM could govern the lipid – protein interactions at the surface of milk fat globules and have functional consequences.

The physico-chemical characteristics of the proteins are also of primary importance in the lipid-protein interactions, mainly their composition in amino acids that is responsible for both the charge as a function of pH and for the presence of hydrophobic regions, and their structure. The main proteins found in the aqueous phase surrounding fat globules in milk are the caseins (4 different caseins: κ-, β-, α_{s1}- and α_{s2}- caseins) that are organized as spherical hydrated colloids with an average diameter of 100–200 nm called the casein micelles [25–27] and appear as raspberry-like particles at pH 6.7 [28]. The casein micelles comprise about 80 wt% of the protein in bovine milk. They have a negative zeta potential in the range −15 to −20 mV at neutral pH [29–31].

Among other techniques that measure biomolecular interactions, such as quartz crystal microbalance and surface plasmon resonance techniques, atomic force microscopy (AFM) provides the unique ability to correlate interaction of proteins with local phase heterogeneity of the lipids, with nanometer resolution [5]. Model membrane systems such as lipid monolayers or bilayers have been used to mimic biological surfaces and investigate lipid-protein interactions [32]. Lipid monolayer films formed at the air/water interface allow controlling the lateral packing density of lipid molecules and to study the interaction and the penetration of proteins injected within the sub-phase by monitoring the change in surface pressure as function of time via Langmuir Blodgett approach [33]. Using AFM in air, authors investigated the partitioning and the insertion of β-casein into lipid monolayers composed of DPPC [34] or of natural complex mixtures of phospholipids extracted from bovine raw milk, raw cream, processed milk and butter milk powder [35]. Due to its small net negative charge at pH 6.7 and high hydrophobic part, β-casein inserted in the lipid monolayer at low surface pressure and after compression up to 20–25 mN m^{-1} partitioned in the ordered domains where it developed hydrophobic interactions with the hydrocarbon chains of the milk polar lipids [35]. Furthermore, supported lipid bilayers (SLB) have proven valuable models for biological membranes. SLB are stable in hydrated medium representative of physiological conditions, can exhibit lateral phase separation with a lamellar