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Self-assembly of phosphorylated dihydroceramide at Au(111) electrode surface

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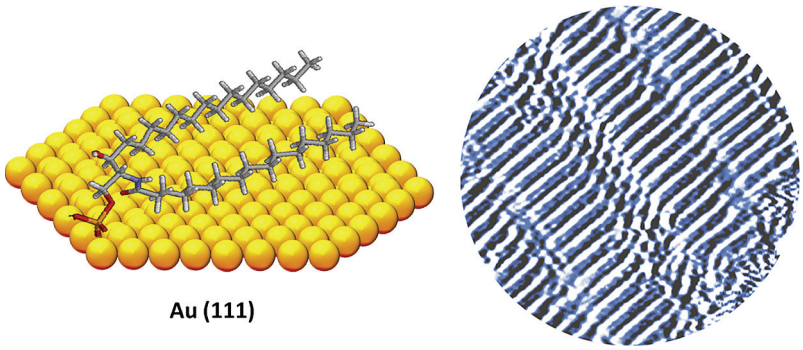
HIGHLIGHTS

- STM and AFM methods were used to examine adsorption of model lipid on Au(111).
- Self-assembly of model lipid leads to formation of highly organized molecular film.
- The model is proposed which reproduces the STM contrast.

GRAPHICAL ABSTRACT



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ABSTRACT

Although the adsorption of lipids on reconstructed Au(111) surface and formation of highly ordered stripe-like domains are well-known phenomena, the exact orientation of the molecules with respect to the substrate remains unclear. Therefore, in this study we have focused on the structure and arrangement of lipid molecules forming highly ordered stripe-like domains at gold electrode-electrolyte interface. *N*-palmitoyl-D-*erythro*-dihydroceramide-1-phosphate was selected as model compound since its ability to transform into hemimicellar structure is limited. This way it was possible to get very stable lipid film with characteristic stripe-like pattern. Application of complementary techniques such as atomic force microscopy and scanning tunneling microscopy enabled detailed characteristics of lipid adlayer adsorbed on Au(111) electrode. Based on careful analysis of the experimental results, we have proposed a model which describes the arrangement of the molecules within the film. In general, it assumes flat-lying orientation of the lipids but only one hydrocarbon chain of phosphorylated dihydroceramide is involved in direct interaction with gold.

1. Introduction

Interfacial self-assembly is one of the most commonly used methods to obtain functionalized surfaces with purposely tailored properties [1]. The latter depend on chemical nature and the arrangement of the adsorbate molecules on the substrate. Molecular films immobilized at solid surfaces are particularly attractive and they play a crucial role in many domains of fundamental research as well as technological applications. These include surface modification for sensors [2,3], biofuel cells [4,5], corrosion

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inhibition [6], heterogeneous catalysis [7], and investigation of mediated electron transfer [8,9]. Moreover, molecular layers adsorbed on a surface with well-defined crystallographic structure can act as a template for the construction of molecular electronics devices [10] and nanoscale level engineering [11]. Among many possible surface modifications, lipids self-assembled on metal substrates have emerged as an attractive platform to investigate membrane related processes [12–14] and design biosensing devices [15,16] or biomimetic interfaces [17,18]. Lipid monolayers and bilayers immobilized on gold surface already proved to be suitable systems for the studies of protein binding [19,20] interactions with antimicrobial peptides [21–25], as well as characterization of redox-active enzymes [26] and modelling of transmembrane ion transport [27,28]. Since the suitably tailored lipid films supported on metal substrates are increasingly used in a variety of fields, full control over their structure and properties becomes an important issue. Therefore, it is crucial to understand the fundamental mechanisms of lipid adsorption and film formation at solid-liquid interfaces.

Recently, we have demonstrated that formation of lipid bilayer on Au(111) electrode by spreading of small unilamellar vesicles involves several steps [29]. These include deposition of the vesicles on gold surface followed by release of lipid molecules which adsorb with flat-lying orientation and form stripe-like domains. The latter serves as a template for development of hemimicellar film which facilitates further adsorption and rupture of SUVs and the bilayer is spread over hemimicellar film. Finally, a single planar bilayer is formed due to the fusion between coupled layers. In this paper, we have focused on the initial step of this mechanism that is adsorption of lipid molecules with long molecular axis parallel with respect to the Au(111) surface. So far, this phenomenon was observed for 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) molecules either alone or in the presence of cholesterol and it was ascribed to preferential interactions between hydrophobic acyl chains and gold surface [30,31]. However, the exact orientation of the molecules with respect to the substrate plane and the extent of the molecule-substrate contact are still unclear. Moreover, the detailed examination of the stripe-like domains formed by DMPC is difficult due to their limited stability. As it was already mentioned, prolonged exposure of modified Au(111) electrode to lipid vesicles results in transition from stripe-like structure to hemimicellar film and then to planar bilayer. In order to suppress this transformation, we have selected phosphorylated dihydroceramide, which is *N*-palmitoyl-D-*erythro*-dihydroceramide-1-phosphate, as a model lipid. The size of the phosphate polar headgroup in this compound is smaller comparing with the diameter of the hydrophobic domain. Molecular shape affects supramolecular structural organization and therefore, the ability of the lipid to form hemimicellar structures will be limited. This assumption is reasonable since it was reported in the literature that closely related compounds such as phosphorylated ceramides, when added to lipid mixtures cause a reduction of the lamellar-to-inverted hexagonal phase transition temperature and have ability to induce negative membrane curvature [32]. Thus, by using *N*-palmitoyl-D-*erythro*-dihydroceramide-1-phosphate as a model lipid, we obtained stable stripe-like domains on reconstructed Au(111) surface. This allowed careful examination of their structure with scanning probe microscopy and based on it we have proposed detailed description of molecule-substrate contact.

2. Experimental

The chemicals used in this work were purchased from Sigma-Aldrich and Avantor Performance Materials SA. The only exception was *N*-palmitoyl-D-*erythro*-dihydroceramide-1-phosphate, which was purchased from Avanti Polar Lipids Inc. In all experiments we have used distilled water passed through a Milli-Q water purification system and its final resistivity was 18.2 MΩ × cm.

Surface pressure - molecular area isotherms for *N*-palmitoyl-D-*erythro*-dihydroceramide-1-phosphate monolayer at the air-water interface were recorded using a KSV LB trough 5000 (KSV Ltd., Finland) equipped with two movable Teflon barriers and a paper plate used as a surface pressure sensor. Trough and barriers were washed thoroughly before each experiment using the mixture of chloroform and methanol and finally rinsed with Milli-Q water. As a subphase 0.01 M phosphate buffer saline (PBS) aqueous solution was used. *N*-palmitoyl-D-*erythro*-dihydroceramide-1-phosphate was dissolved in a mixture of $CHCl_3$:CH_3OH:0.5N HCl (20:9:1). The resulting clear solution was spread on buffer surface and then it was left to evaporate the solvent. The compression of the monolayers was performed at the barriers speed of 10 mm/min at a constant temperature of 21 ± 1 °C.

In order to prepare the suspension of *N*-palmitoyl-D-*erythro*-dihydroceramide-1-phosphate in 0.05 M $KClO_4$ aqueous solution we have followed the same procedure as for preparation of small unilamellar vesicles [33]. However, the resulting product was a fine suspension of lipid aggregates. The protocol involved preparation of stock solution by dissolving 1.0 mg of *N*-palmitoyl-D-*erythro*-dihydroceramide-1-phosphate in 5 ml of hexane/ethanol 2:1 mixture. An aliquot of the solution (0.2 ml) was placed in a test tube and the solvent was evaporated by vortexing under argon stream. Subsequently, the test tube with dried lipid cake was placed for approximately 3 h in vacuum desiccator to remove the solvent. Finally, 1.5 ml of 0.05 M $KClO_4$ solution was added to the lipid cake and the sample was sonicated for at least 1 h. Portion of the resulting suspension was added to electrochemical cell of the microscope to give approximate lipid concentration of 4.0×10^{-6} M.

Scanning tunneling microscopy (STM) and atomic force microscopy (AFM) images were obtained with 5500AFM (Keysight Technology) and Dimension Icon (Bruker) instruments respectively. The experiments were performed at 21 °C. All images were recorded in 0.05 M $KClO_4$ under electrochemical control with single crystal Au(111) (MaTeck) as a working electrode, miniaturized Ag/AgCl (sat. KCl) as a reference and platinum wire as a counter electrode. The components of the electrochemical cell as well as metallic electrodes were cleaned in piranha solution (concentrated H_2SO_4/30% H_2O_2 3:1, v/v) for at least 2 h and then rinsed with copious amounts of Milli-Q ultrapure water. (*CAUTION*: *piranha solution reacts violently with organic materials and should be handled with extreme care*.) Single crystal Au(111) electrode was carefully flame annealed and then placed in electrochemical cell of the microscope. In order to facilitate the surface reconstruction, the electrode was held for approximately 30 min at the potential of −0.3 V directly before each experiment. For STM imaging, we have used electrochemically etched tungsten tips coated with polyethylene to minimize leakage currents. The AFM images were taken in PeakForce Tapping mode using qp-BioAC cantilevers (Nanosensors, CB2: nominal spring constant 0.06–0.18 N/m). Both STM and AFM imaging was performed on four independently prepared samples.

N-palmitoyl-D-*erythro*-dihydroceramide-1-phosphate molecule was modelled using Spartan '14 Version 1.1.4 (Wavefunction, Inc.). Molecular modeling was performed with the *ab initio* Hartree-Fock method using the 3-21G basis set available in the software package.

3. Results and discussion

The structure of *N*-palmitoyl-D-*erythro*-dihydroceramide-1-phosphate molecule is illustrated in Scheme 1. It consists of

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