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Surface enhanced Raman spectroscopy of self-assembled layers of lipid molecules on nanostructured Au and Ag substrates

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ABSTRACT

In this work surface enhanced Raman spectroscopy (SERS) has been used for the investigation of the self-assembled layers of lipid molecules (SALLMs) deposited on the nanostructured Au and Ag surfaces. The SALLMs were prepared from one part of 1,2-dioleoyl-sn-*glycero*-3-phospho-L-serine (DOPS) and four parts of 1,2-dioleoyl-sn-*glycero*-3-phosphocholine (DOPC) lipids. The synthesis of Au and Ag SERS substrates was based on the direct gold and silver ions reduction onto HF etched silicon wafers. Au SERS substrates were not suitable for the formation of SALLMs because of the inappropriate contact angle of surface. It was found that the formation of the SALLM does not take place on Au SERS substrate. However, it has been shown that the modification of Au SERS substrate with 1-dodecanothiol layer allows building the SALLM on its surface. In the case of Ag SERS substrate, the SALLM was deposited directly on its surface. The SERS spectra of the SALLMs were recorded in the C—H stretching (2800–3000 cm^{-1}) and the fingerprint (<1.800 cm^{-1}) regions. It has been demonstrated that the SERS spectra of the SALLM recorded on Au substrate differs from that one recorded on Ag SERS substrate. These spectral differences were found to be determined by the different interaction mechanisms of the lipid molecules with nanostructured surfaces.

1. Introduction

The lipid membranes (LM) of living cells are essential components responsible for specialized biochemical functions such as energy transfer, immunological recognition, biosynthesis, etc. It is important to study the changes of structural and dynamic properties of lipid membranes in order to determine and early diagnose neurodegenerative diseases such as Parkinson's or Alzheimer's (Farooqui and Farooqui, 2011; Evangelisti et al., 2013), also for studying nanotoxicity, pharmaceutical research, etc. It is very likely that the damages of lipid bilayer, caused by the interaction of various nanoparticles with membrane components, the aggregation and conformation of specific proteins on the lipid membranes, are directly involved in the pathogenesis of such diseases (Taylor et al., 2014; Axelsen et al., 2011). Therefore, bilayer lipid membranes are increasingly accepted as experimental models, commonly employed for both fundamental and applied studies (Sweetenham and Notingher, 2010; Vemula et al., 2015). The most common methods used to characterize lipid membranes are fluorescence-based techniques, such as confocal microscopy, fluorescence correlation spectroscopy (FCS), Förster resonance energy transfer (FRET), total internal reflection fluorescence (TIRF) or two-photon microscopy (Sezgin and Schwille, 2011). However some of these methods require labelling, they also provide limited data about the structure of LMs. Vibrational spectroscopies, such as infrared (IR) or Raman are eligible analytical tools for the investigations of LMs because of their ability to provide information about the structure of the LM as well as its chemical composition (Baeten, 2010; Lewis and McElhaney, 2013). However, IR analysis of the samples prepared in aqueous medium is complicated due to the strong and temperature dependent absorption of water, while Raman spectroscopy allows avoiding this problem. This aspect is important with regard to biological samples, including lipid membranes, requiring maintaining native conditions. However, the analysis of lipid membranes by Raman spectroscopy is complicated due to its low sensitivity and spatial resolution. This problem can be facilitated by the usage of Surface enhanced Raman scattering (SERS) spectroscopy enabling to enhance Raman signal of lipid membranes. In recent years a number of reports have been published showing the successful application of SERS for the investigation of lipids-proteins interactions (Petersen and Nielsen, 2009) and lipid membranes using silver and gold nanoparticles (Suga et al., 2015; Kühler et al.,

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2014; Kundu et al., 2009; Ren et al., 2011; Li et al., 2014). It has been shown that in cases when the gold nanoparticles (NP) are used, the formation of LM is complicated. The modification of SERS substrates should be done because Au NPs are hydrophilic and immiscible in organic solvents or lipid systems (McClements, 2014), and the contact angle of surface are often inappropriate to form the lipid membrane. In order, to create a lipids monolayer around Au NPs, they must be modified with ligands, mostly alkanethiols, to increase the hydrophobicity and the dispersibility of Au NP within lipid phases (Kundu et al., 2009; McClements, 2014). The other way is to use thiol-modified lipids for formation of bilayer LM on gold nanoparticles (Castellana et al., 2011) or to modify gold nanoparticles surface with halide ions (Kah et al., 2012; Orendorff et al., 2009).

The aim of this work was to investigate the formation of self-assembled layers of lipid molecules (DOPS and DOPC) onto the nanostructured gold and silver surfaces. It is known that the lipid molecules can self-assemble into the mono-layered or bi-layered structures thus forming the lipid membranes (Xu et al., 2009). Therefore, the investigation of the SALLMs deposited onto the SERS substrates can provide the essential knowledge required for the better understanding of the lipid membranes formation as well as their interaction with the nanostructured surfaces. The SERS spectrum of DOPC lipids was reported before (An et al., 2014). However, to the best of our knowledge, there are no publications reporting the investigation of the DOPS:DOPC lipids based self-assembled layers of lipid molecules deposited onto the gold and silver SERS substrates. DOPC lipid has been chosen because it is the most abundant lipid in animal cell membranes which provides the structural framework, while negatively charged DOPS was used as the anionic lipid (Richter et al., 2003a, 2003b; Yesylevskyy et al., 2013). The membranes synthesized from the charged phospholipids can be used as the model membranes to bind oppositely charged molecules, such as DNA or proteins (Olson et al., 2001; Groves et al., 1996) and for further investigation of neurodegenerative diseases such as Parkinson's or Alzheimer's.

2. Materials and methods

2.1. Materials

All the reagents and solvents used in this work were of analytical grade. 1-dodecanethiol, phosphate buffered saline (PBS), Tris buffer, EDTA, sodium chloride, calcium dichloride, sodium nitride, 2-propanol, water, hydrofluoric acid, silver nitrate, hydrogen tetrachloroaurate (III), ethanol, lipids 1,2-dioleoyl-sn-*glycero*-3-phospho-L-serine (DOPS) and 1,2-dioleoyl-sn-*glycero*-3-phosphocholine (DOPC) were obtained from Sigma Aldrich. 1,2-dioleoyl-sn-*glycero*-3-phosphoethanolamine (DOPE) was obtained from ATTO-TECH.

2.2. The preparation of Ag and Au SERS substrates

The preparation of silver and gold SERS substrates was based on the direct silver and gold ions reduction by elemental silicon. Silicon wafers were cut into small pieces (1.5 cm × 1.5 cm) cleaned by immersing them into pure ethanol and dried under the nitrogen flow. Hydrofluoric acid was diluted with water to the final concentration of 24%.

Silver precursor solution was prepared by diluting $AgNO_3$ with water to the final concentration of 2.0 mM. Silicon wafers were immersed into prepared HF solution for 10 s then immediately transferred to $AgNO_3$ solution for 5 s, washed with water and finally dried under the nitrogen flow.

Gold solution was prepared by diluting hydrogen tetrachloroaurate (III) with water to the final concentration of 2.5 mM. Silicon wafers were immersed into prepared HF solution for 20 s, then immediately transferred to hydrogen tetrachloroaurate (III) solution for 20 s, washed with water and finally dried under the nitrogen flow.

2.3. The preparation of the self-assembled layers of the lipid molecules

The self-assembled layers of the lipid molecules were prepared based on the modified protocol reported by Richter et al. (2003a), Richter et al. (2003b). DOPS and DOPC lipids in chloroform were used as received and mixed in the ratio of 1:4 respectively. The lipids were dried in order to remove the solvent and then re-suspended in Tris buffer (149 mM NaCl, 5 mM $CaCl_2$, 10 mM Tris buffer) to a final concentration of 0.1 g/L. The prepared solution was extruded 27 times through a membrane (pore size 0.1 μm) to produce unilamellar vesicles. The vesicle solution was placed in the measurement cell containing the glass or silver/gold SERS substrate. The solution was left for 1 h and then replaced with the pure Tris buffer. Subsequently, the solution was exchanged again by a Ca^{2+} free Tris buffer (10 mM Tris, 133 mM NaCl) containing 5 mM EDTA in order to remove the Ca^{2+} ions, and finally by PBS. All buffers contained 0.05% NaN_3 and were adjusted to pH 7.4.

For supercritical angle fluorescence microscopy (SAF) and Fluorescence Correlation Spectroscopy (FCS) measurements the labelled lipids (DOPE) were added. DOPS, DOPC and DOPE in chloroform were used as received and mixed in the ratio of 35:65:0.000016.

2.4. The formation of 1-dodecanethiol binding layer on Au SERS substrate

The modification of the Au SERS substrates was performed as follows. 3 ml of 1-dodecanethiol was diluted to 5 ml with ethanol. The prepared Au SERS substrates were immersed into the solution of 1-dodecanethiol for 2 h. The formation of the binding layer took place through the covalent attachment of sulphur to the gold nanoparticles. The proposed formation of the binding layer is shown in Fig. 1:

2.5. Atomic force microscopy, UV–vis spectroscopy and scanning electron microscopy imaging of the SERS substrates

The morphological characterization of Au and Ag SERS substrates were carried out by atomic force microscopy (AFM) in a semi-contact tapping mode using commercial silicon cantilevers (resonant frequency 150 kHz, tip radius <10 nm, force constant 5 N/m). Scanning electron microscopy (SEM) (Helios

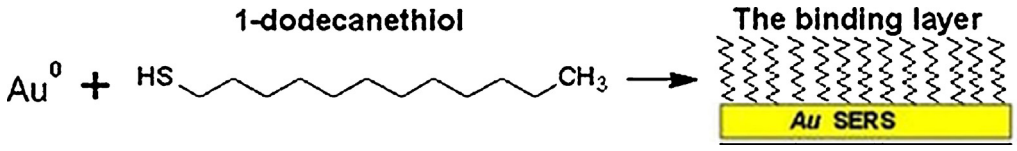

Fig. 1. The formation of 1-dodecanethiol binding layer on Au SERS substrate.