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Surface Enhanced Raman Scattering of a Lipid Langmuir Monolayer at the Air–Water Interface

Abstract: Surface enhanced Raman spectra were recorded from a phospholipid monolayer directly at the air–water interface. We used an organized monolayer of negatively charged tetramyristoyl cardiolipins as a template for the electrochemical generation of silver deposits. This two-dimensional electrodeposition of silver under potentiostatic control was the substrate for enhancement of Raman spectra. We report the optimized conditions for the Raman enhancement, the microscopic observations of the deposits, and their characterization by atomic force microscopy. Laser excitation at 514.5 nm leads to intense and reproducible surface enhanced Raman scattering spectra recorded in situ from one monolayer of cardiolipin, using 0.5 mol % of 10N nonyl acridine orange or 5 mol % of acridine in the film, and demonstrates the possibility of estimating the pH at the metal/phospholipidic film interface. © 2004 Wiley Periodicals, Inc. *Biopolymers* 74: 136–140, 2004

Keywords: model membrane monolayers; surface enhanced Raman scattering; air–water interface; silver electrodeposits

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INTRODUCTION

Experiments with phospholipid monolayers spread at the air/water interface as a membrane model system have the advantage that the molecular arrangement can be very well controlled by changing not only the molecular area or the surface pressure of the monolayer but also the temperature and the composition of the subphase. Nevertheless, very few techniques can probe phospholipidic chain order or disorder and head-group interactions while the Langmuir monolayer remains *in situ* at the air–water interface. Until recently, grazing incidence X-ray diffraction, epifluorescence microscopy, and Brewster-angle microscopy were the only methods used to study monolayers. Considerable efforts have been made to enhance infrared spectroscopy sensitivity. Polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS) is now commonly used to obtain molecular information on lipidic monolayers at the air–water interface.¹ On the other hand, spontaneous Raman scattering is a low-sensitivity method and despite considerable improvements in instrumentation it has not been used much for monomolecular film characterization.

Surface enhanced Raman scattering (SERS) could be a powerful tool for studying these systems. However, this very sensitive method requires the use of roughened silver, copper, or gold surface or colloidal sols of these metals to obtain a large enhancement of Raman spectra up to 10 orders of magnitude.² Therefore, until now it has been used only to study monolayers transferred to SERS active substrates by the Langmuir–Blodgett technique.³

In the present work, we used two-dimensional (2D) electrochemical deposition of silver on a preformed Langmuir monolayer of tetramyristoyl cardiolipin (TMCL), a negatively charged phospholipid, as substrate for Raman enhancement. A systematic study was undertaken to optimize the Raman enhancement with the electrochemical deposition conditions: the silver ion concentration, the electric voltage, and the target surface pressure of the monolayer. A great variety of patterns and deposit morphologies were observed but we only report the characterization (morphology and growth rate) of the optimized ones.

We chose 10*N* nonyl acridine orange (NAO) to reveal SERS enhancement on these 2D silver substrates since it is a cardiolipin-specific fluorescent dye usually used to visualize negatively charged phospholipids in cells. We also demonstrate the possibility of estimating the pH at the metal/phospholipidic film interface by recording SER spectra of acridine *in situ*

from one monolayer of cardiolipin using less than 5 mol % of the probe.

MATERIALS AND METHODS

AgNO₃ (Aldrich, 99.9+%), acridine (Aldrich), synthetic TMCL (Avanti Polar lipids), and 10*N* NAO (Molecular Probes) were used as received. The phospholipids were dissolved in a chloroform/methanol 4/1 (v/v) mixture to a concentration of 1 mg/ml. The subphase water (pH 5.7) was distilled and filtered to a resistivity of 18 MΩcm using an Elgastat UHQ2 system.

Experiments were carried out using a Nima model 611*M* trough (Nima Technologies, England) equipped with a three-electrode potentiostatic electrochemical setup as shown in Figure 1. The reference (Ag/AgCl) and the counter (Pt wire) electrodes were immersed into the subphase (AgNO₃ 20 mM aqueous solution) prior to the monolayer being spread. After monolayer compression to the desired target pressure, the working electrode (Ag wire) was immersed into the subphase such that its tip just touched the surface. The potential was controlled during Ag colloid formation using a homemade potentiostat. A potential of -0.8 V versus Ag/AgCl was then applied to the working electrode. The morphology of the electrodeposits and the velocity of their growth were recorded *in situ* using a video camera. Once the colloid island reached a size that easily allowed laser beam access to the surface, the electrochemical cell was set to open circuit potential and Raman spectra were acquired.

The silver deposits were transferred onto glass slides by horizontal lifting through the air–liquid interface for further characterizations by optical microscopy (Olympus). Atomic force microscopy (AFM, Digital Instrument, Nanoscope III) was used in tapping mode to check the morphology, the homogeneity, and the roughness of the silver electrodeposits transferred by a horizontal Langmuir–Schäfer technique onto freshly cleaved mica substrates.

Raman spectra were recorded on a Dilor XY microspectrometer using an excitation line at 514.5 nm from a Spectra Physics Model 165 argon ion laser (ca. 10 mW at the sample). The size of the illuminated area is approximately 5 μm² with the objective used (50X, numerical aperture 0.55).

RESULTS AND DISCUSSION

The compression isotherm at 22°C of the TMCL monolayer over pure water subphase shows a characteristic plateau of a first-order phase transition between liquid expanded and liquid condensed states that appears at a surface pressure of about 1 mN · m⁻¹. The presence of 20 mM AgNO₃ in the subphase induces a shift of the isotherms of about 8 Å² toward higher areas per molecule and an increase of about 2 mN/m of the plateau surface pressure. Indeed, in-

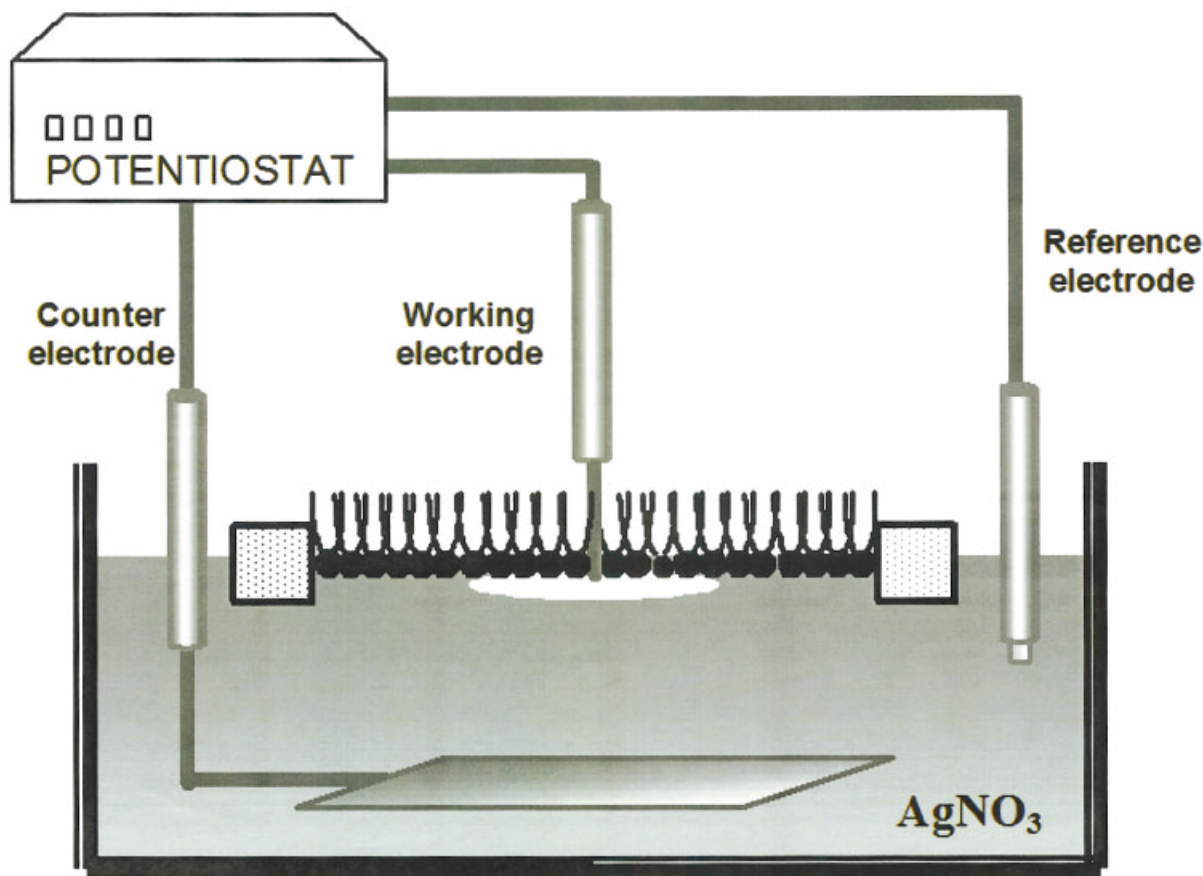


FIGURE 1 Schematic representation of the experimental setup.

creasing the ionic strength of the subphase changes the dissociation constant of the cardiolipin and leads to an increase of the ionized form of the lipid as previously reported for phosphatidylglycerol.⁴ This could explain why the monolayer is more expanded in the presence of AgNO₃ in the subphase.

Application of a negative potential to the working electrode resulted in the appearance of a 2D silver deposit under the monolayers of the negatively charged phospholipid TMCL. As previously reported in the literature,⁵ we found that 2D silver films could be formed at medium surface pressure of the monolayer and that the electrodeposits developed a 3D structure when the surface pressure was higher than 20 mN/m. The metallic aggregate grows concentrically, leading to larger and larger concentric disks. After roughly 400 s, it stops growing in a 2D manner along the interface and becomes darker and darker, suggesting that it thickens progressively with time. Following the point of view of Zeiri et al.,⁶ one can propose that the silver ions adsorbed under the TMCL monolayer may first be

reduced to form a seed deposit along the interface which would then induce a thin, surface-parallel growth of the metal deposit.

Optical microscopy observations (Figure 2A) of these latter structures, after they were transferred onto glass slides by horizontal lifting, reveal that the deposits exhibit a dendretic pattern with a rough surface in the center of the Ag layers (view A1) and fractal structure on the edges (view A2). Furthermore, one observes delicate veins running along the branches. These veins result from the 3D thickening of initially grown 2D deposits. An AFM image of the Ag deposits transferred by a horizontal Langmuir-Schäfer technique onto mica is shown in Figure 2B. The deposits appear as a continuous layer constituted of interacting silver particles ranging in diameter from 30 to 50 nm.

Figure 3 displays the SER spectra recorded directly at the air-water interface from TMCL Langmuir monolayers (containing 5 mol % acridine or 0.5 mol % NAO) compressed at 10 mN/m after growth of the 2D silver deposits. In the 1000–1700 cm⁻¹ range,

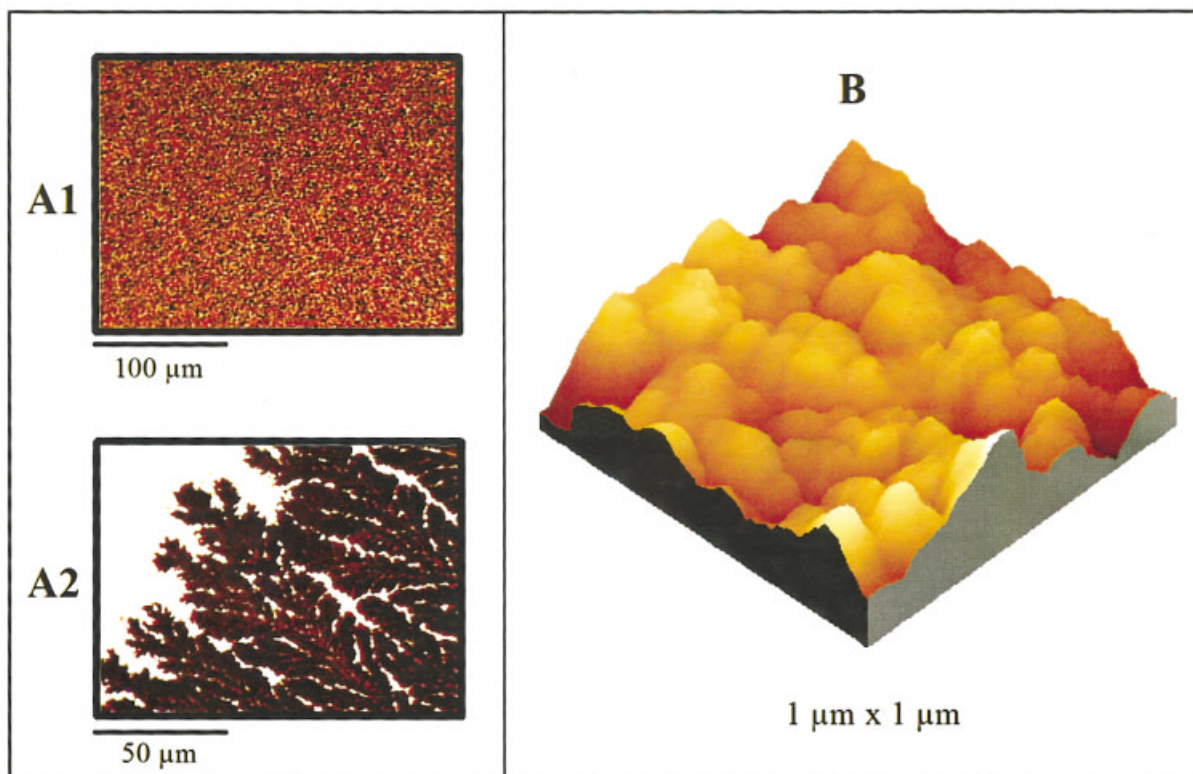


FIGURE 2 Microscopic views obtained in the center (A1) and on the edges (A2) of a silver electrodeposit grown from a 20 mM AgNO_3 solution. $\Delta V = -0.8$ V; $\pi = 10$ mN/m. AFM image (B) of the same deposit transferred by a horizontal Langmuir–Schäfer technique onto mica.

numerous Raman lines of acridine, at ca. 1167/1172, 1267/1277, and 1564/1585 cm^{-1} , are sensitive to pH variations. In particular, the SERS intensity variation of the ring stretching modes at ca. 1564 cm^{-1} and 1585 cm^{-1} can be used to reveal precisely the amount of

either neutral or protonated species and then to determine the apparent $\text{p}K_a$ of acridine in the presence of the TMCL monolayer from the measurement of the integrated intensity ratio of these bands by varying the pH of the subphase.⁷

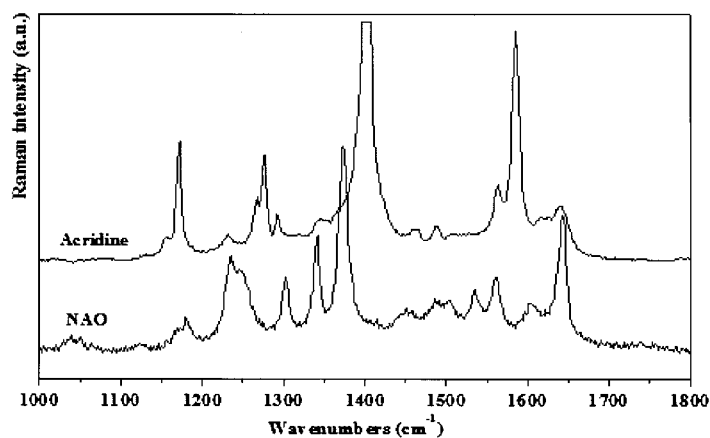


FIGURE 3 SER spectra of acridine (5 mol %) and NAO (0.5 mol %) in a TMCL monolayer recorded directly at the air–water interface.

CONCLUSION

This work demonstrates the usefulness of surface enhanced Raman scattering for the acquisition of high-quality Raman spectra from one Langmuir layer at the air–water interface after the electrochemical generation of silver deposits. This approach gives information that complements FTIR and vibrational sum-frequency generation studies of similar systems. Indeed, insight into the chemical environment of Langmuir layer head groups at the air–water interface can be provided. Grazing incidence X-ray diffraction experiments (line D41-B at the LURE, France) are currently underway to obtain information on the molecular organization of phospholipids in the presence of 2D electrodeposited silver.

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