

Effect of the Surface Hydrophilicity on the Formation of a Membrane-type Interface: Study Using an Acoustic Wave Device

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Received October 16, 2000. In Final Form: December 21, 2000

An acoustic wave device was used to study the deposition of lipid vesicles on solid surfaces of different hydrophilicity. The device operated at 103 MHz and consisted of a quartz substrate with a polymer overlayer acting as an acoustic waveguide and a thin layer of gold. The hydrophilicity of the device surface was controlled by modifying the gold surface with hexadecanethiol (HDT) for 16 h and mercaptoundecanol (MUO) for 10 min and 16 h in order to obtain a hydrophobic, relatively hydrophilic, and hydrophilic surface, respectively. The interaction of a vesicle suspension of 2-oleoylpalmitoyl-*sn*-glycero-3-phosphocholine (POPC) in PBS with each surface was monitored by recording the phase and amplitude of the acoustic wave in real time. The acoustic signal detected on the hydrophobic (HDT) and hydrophilic (16 h MUO) surfaces indicated the formation of a supported monolayer and bilayer, respectively, while a vesicle layer was detected on the less hydrophilic (10 min MUO) surface. The above findings were confirmed by using ^{14}C -labeled lipids and by monitoring the nonspecific binding of BSA on each surface. These experiments clearly showed that the hydrophilic properties of the solid surface are very important for designing a two dimensional (bilayer) or three-dimensional (vesicle) membrane-type interface layer. Furthermore, the simultaneous monitoring of the phase and amplitude of the acoustic wave was shown to provide complementary information related to mass and viscoelastic interfacial changes.

Introduction

The significance of cellular membranes in signal transduction processes imposes the need for studying membranes and membrane-associated interactions at a molecular level. The simplest way to carry out such studies involves the formation of a lipid bilayer on a solid support followed by the attachment of biomolecules, either after embedding them in or anchoring them on the lipid bilayer. Supported artificial membranes can be, thus, used to develop a cell-type biorecognition surface, which in combination with surface sensitive techniques can provide a model system for studying cellular events.^{1,2}

Given the potential of supported lipids in biomolecular studies, it is essential to study and elucidate the mechanism of their formation. A number of electrochemical^{1,3} and optical techniques such as surface plasmon resonance (SPR),^{2,4–6} total internal reflection fluorescence microscopy,^{7–9} and attenuated total reflection infrared spectroscopy¹⁰ have been used in these studies. Recently, acoustic wave devices have been also used in combination with supported lipid layers and shown to offer additional advantages to optical methods.^{11,12} This is based on the fact that acoustic

waves propagating at a solid/liquid interface are sensitive to viscoelastic and electrical changes¹³ in addition to being sensitive to mass deposition and can, therefore, provide more information that can be obtained with, for example, SPR.

Depending on the type of technique applied, two types of surface are mainly used as a solid support for the formation of a supported lipid layer: silica and gold. On those two surfaces supported bilayers can be formed through (1) successive addition of two monolayers using Langmuir–Blodgett (LB) films, (2) spontaneous vesicle fusion, (3) chemical attachment to the surface by using tethered lipids, or (4) a combination of the above.^{14,15} Of the available methods, vesicle fusion possesses a number of attractive properties. It is simple and rapid, the surface can be regenerated on addition of detergent, and it gives a 2 nm layer of water trapped between the solid support and the lipid bilayer,¹⁶ which is essential for the subsequent incorporation of membrane receptors. The spontaneous fusion of vesicles on a silica oxide surface to form a lipid bilayer has been extensively described by many investigators.^{16–19} However, the formation of a supported lipid bilayer on a hydrophilic thiol self-assembled gold

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layer has been little investigated, and there is doubt on whether this technique gives consistent results.

In this work, we use a high frequency acoustic wave device to study the interaction of a vesicle suspension with a solid surface. It is known that vesicles spontaneously form a lipid monolayer on a hydrophobic surface.¹⁴ Some studies suggest the spontaneous formation of a lipid bilayer on a hydrophilic self-assembled gold surface,^{4,6} while the effect of a surface with hydrophilic properties between the above two has not yet been reported. Our aim was to monitor the interaction of a vesicle suspension with a solid surface of well-defined hydrophilic properties. The surface properties were controlled by depositing a thin gold layer on top of the waveguide surface which was further modified with mercaptoundecanol ($\text{SH}(\text{CH}_2)_{11}\text{OH}$) and hexadecanethiol ($\text{SH}(\text{CH}_2)_{16}\text{CH}_3$). Vesicles consisting of POPC were used in this study since POPC has a low transition temperature ($T_c = -2^\circ\text{C}$), which allows the formation of fluid crystalline vesicles at the working temperature of 22.3°C . In all cases, a suspension containing extruded POPC vesicles of a diameter of 50 nm in PBS was applied to the device surface and the interaction was studied in real time by following simultaneously the amplitude and phase of an acoustic wave transmitted across the device.

Theoretical Background

Acoustic waves can be generated and detected on the surface of a piezoelectric solid by using a set of interdigitated transducers (IDTs). In the case where the acoustic wave consists predominantly of a shear horizontal component, i.e., the wave is polarized in the plane of the surface, it is possible to apply a liquid sample on the surface of the device without significantly damping the wave. Instead, the liquid sample in contact with the piezoelectric surface will oscillate with the surface, giving rise to an evanescent acoustic field at the solid/liquid interface. Any mechanical changes in the liquid which will affect the mass and viscosity at the interface will change the propagation characteristics of the wave.¹³ In addition, due to the piezoelectric nature of the solid, an evanescent electric field also exists at the solid/liquid interface, which is affected by the ions and dipoles in the solution. The acoustoelectric interaction can be eliminated by applying a thin gold layer to the device surface between the transducers.

For liquid-based applications, the interface layer is defined as the effective liquid thickness δ which is coupled to the surface acoustic wave and is given by $\delta = (2\eta/\rho\omega)^{1/2}$, where η and ρ are the liquid's viscosity and density and ω is the angular frequency of the shear wave. For a pure water sample and a device operating frequency of 103 MHz, δ is calculated to be 54 nm. In practice, changes in the mechanical properties of the interface will be detected as phase (or frequency) and amplitude changes of the acoustic wave. The former is mainly related to changes in the mass of the interface although bulk changes of the viscoelastic properties of the solution will also affect it. Amplitude is sensitive to viscoelastic changes and in combination with phase can provide a sensitive technique to distinguish between elastic (zero amplitude change) and viscoelastic mass loading.¹³

In this work we are using the Love wave device which is the most sensitive shear acoustic wave device reported so far.^{11,20} It is based on a waveguide structure consisting of a solid substrate overlaid by a material of a lower shear acoustic velocity than that of the substrate. Waveguide

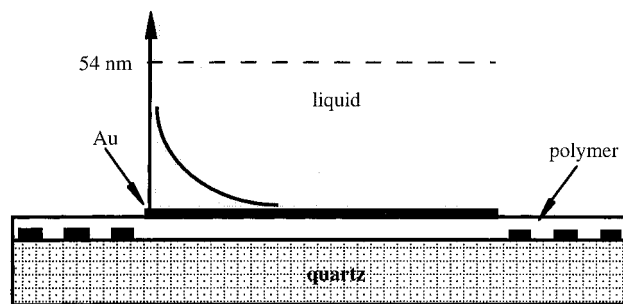


Figure 1. Schematic representation of the acoustic waveguide liquid sensor. A polymer layer ($\sim 1\ \mu\text{m}$) is deposited on the surface of an acoustic device which supports shear horizontal waves. A gold layer is deposited on top of the polymer between the interdigitated transducers (IDTs). Loading the gold layer with a liquid sample results in an acoustic evanescent field which extends $\delta = 54\ \text{nm}$ into the aqueous solution.

geometries are particularly sensitive to surface effects since they concentrate the acoustic energy preferentially in the overlayer, thus, circumventing the problem of energy scattering inside the bulk of the piezoelectric crystal.²⁰ In practice, a polymer-coated piezoelectric device was used to generate the waveguide geometry and the wave was excited at 103 MHz. A thin gold layer was deposited on the surface of the waveguide device which was further modified by self-assembling the appropriate thiol. In addition to providing a versatile substrate for chemical modification, the gold layer is also used to eliminate the acoustoelectric interaction. Figure 1 gives a schematic representation of the waveguide geometry and the acoustic evanescent field in liquid.

Materials

2-Oleoylpalmitoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Fluka, mercaptoundecanol (MUO) and hexadecanethiol (HDT) were purchased from Aldrich, medium molecular weight poly(methyl methacrylate) (PMMA), 2-ethoxy-(ethyl)acetate (2-EEA), cyclohexane, chloroform, phosphate buffer saline (PBS) tablets (to obtain 0.01 M phosphate, 0.0027 M potassium chloride, and 0.137 M sodium chloride, pH 7.4), bovine serum albumin (BSA), *tert*-octylphenoxypolyethoxyethanol (triton X-100), and sodium hydroxide were purchased from Sigma, ^{14}C -POPC was purchased from Amersham Life Sciences, Cryoscint scintillation fluid was purchased from ICN, and high quality water was purified using the USF ELGA Maxima system.

Experimental Procedures

(i) Device. The acoustic wave device consists of a single-crystal Y-cut (42.5°), z-propagating, 0.5 mm thick quartz. The input and output interdigital transducers have a 10 nm chromium adhesion layer and a 200 nm gold overlayer. The interdigitated transducers (IDTs) consist of 80 pairs of split fingers with a periodicity of $45\ \mu\text{m}$. Devices were patterned by conventional photolithographic techniques in the Southampton Microelectronics Centre. For the waveguide geometry, a solution of 22% (w/w) PMMA in 2-EEA was applied over the entire surface of the device by spin-coating at 4000 rpm for 40 s. The adhesion of the film was improved by heating the device in a conventional oven for 2 h at 180°C . A 20 nm gold layer was deposited on top of the polymer in the area between the IDTs by thermal evaporation in a vacuum evaporator chamber (Edwards Auto306) at a pressure of 5×10^{-6} mbar. Later work not discussed here showed that commercially available photoresist was also a suitable waveguide layer and that it had a higher solvent resistance than PMMA, making it suitable for experiments requiring long exposure to solvent.

(ii) Instrumentation—Experimental Setup. A Hewlett-Packard 4195A network analyzer was used to measure the amplitude and phase of the wave. Data were collected on a PC using LabView software. The device was placed on top of a Peltier plate inside a metal box. A temperature controller was used to

keep the temperature constant at 22.3 ± 0.1 °C. A Perspex flow-cell, a peristaltic pump and PVC tubing were used to pump through liquid samples. A rubber sleeve exposing an area of approximately 36 mm² was used to seal the cell to the acoustic device.

(iii) Self-assembly of Thiols on Gold. Two protocols were followed for the surface modification with thiol. In the first one, acoustic devices with a freshly evaporated gold layer were incubated in a 10^{-4} M solution of MUO in cyclohexane and HDT in ethanol for at least 16 h at room temperature. In the second one, ~ 100 μ L of a solution of 10^{-4} M MUO in ethanol was placed on the freshly evaporated gold surface for 10 min at room temperature. In both cases after the thiol incubation, the device surface was rinsed in the corresponding solvent and dried under nitrogen. The surface hydrophilicity was determined by measuring the advancing contact angle of 10 μ L of water on the device surface using a goniometer.

(iv) Preparation of Lipid Vesicles. The vesicle suspension was prepared by extrusion: stock solutions of lipids at 20 mg/mL in chloroform were dried onto the walls of a round-bottomed flask under flowing nitrogen for 1 h. The dried lipids were re-suspended in 100 μ L of PBS buffer during vortexing and sonication in a bath sonicator for few minutes and finally diluted to 1 mL. The milky suspension obtained was then extruded 21 times through a 50 nm pore polycarbonate filter in an Avestin Liposfast Basic extrusion apparatus to give a clear suspension at a lipid concentration of 2 mg/mL.

(v) Vesicle Adsorption: BSA Binding. The vesicle dispersion at a concentration of 0.2 mg/mL in PBS was applied to the device surface using a flow-through cell at a rate of 0.083 mL/min followed by a PBS rinse. In the case of the hydrophobic device, the surface was rinsed with 0.1% Triton in PBS prior to the application of the vesicle suspension. Supported lipid layers were removed from the sensor surface by rinsing with 0.1% Triton-containing PBS. After the formation of the supported lipid layer, 1 mg/mL of BSA in PBS buffer was applied to the device surface, followed by a buffer rinse.

(vi) Adsorption of C¹⁴-Labeled Vesicles. Radiolabeled liposomes were prepared as for normal liposomes but with the addition of 30 μ L of ¹⁴C-POPC (approximately 0.75 μ Ci). The lipid was accurately weighed after drying, and this figure was used to make up a 2 mg/mL suspension of lipid. This aqueous suspension was homogenized by vortexing and by sonication. An aliquot of this solution was taken and the radioactivity was counted by liquid scintillation with a Beckmann Coulter LS 1801 counter in order to calibrate the radioactivity to lipid mass. The aqueous lipid suspension was passed through the extruder to get the final POPC suspension. Static cells with silicone gaskets were used to apply the liposome solution at a final concentration of 0.2 mg/mL to a defined area on the surface of the 10 min MUO-gold modified device. The vesicle suspension was left in contact with the surface for 1 h at room temperature. Following deposition and copious washing with PBS, the static cell was removed and the device was immersed in a 1% Triton solution to solubilize the deposited lipid. The radioactivity of the detergent solutions was then counted by liquid scintillation as before and the mass of lipid present calculated.

Results

1. Adsorption of Vesicles on the Thiol-Modified Gold Surfaces. The phase and amplitude response of the acoustic wave on addition of 0.2 mg/mL of POPC are shown in Figures 2 and 3, respectively. The above concentration was experimentally determined after monitoring the POPC deposition rate as a function of the vesicle concentration. It was found that the rate of deposition was linear for up to 0.2 mg/mL of POPC, and there was no further rate increase at higher concentrations (data not shown). Figures 2 and 3 show that both signals dropped as soon as the solution reached the surface. In the case of the HDT-modified device, the surface was rinsed by injecting 0.1% Triton in PBS for 1 min before applying the suspension of POPC vesicles. This surface pretreatment only resulted in a very small signal change (data not shown), indicating that Triton was not deposited on the

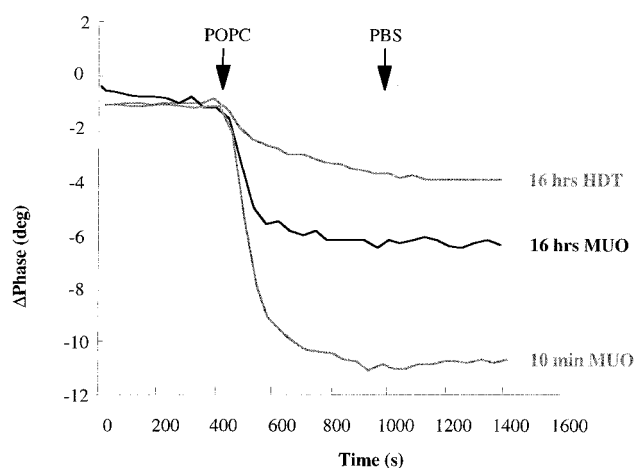


Figure 2. Phase change as a function of time during the application of 0.2 mg/mL of POPC in PBS on a gold coated device modified with (1) hexadecanethiol (HDT) for 16 h, (2) mercaptoundecanol (MUO) for 16 h, and (3) MUO for 10 min.

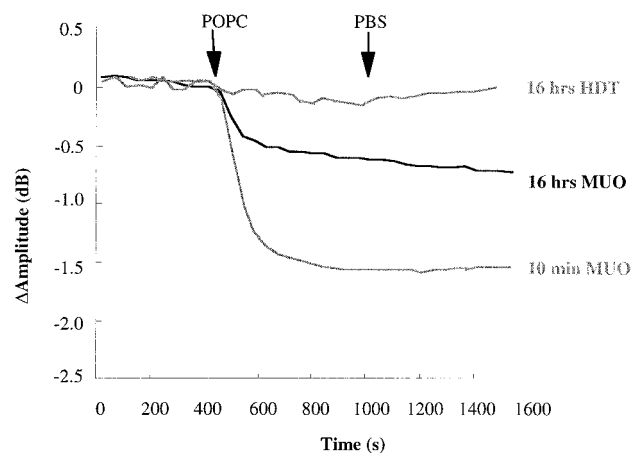


Figure 3. Amplitude change as a function of time during the application of 0.2 mg/mL of POPC in PBS on a gold coated device modified with (1) hexadecanethiol (HDT) for 16 h, (2) mercaptoundecanol (MUO) for 16 h, and (3) MUO for 10 min.

device surface to give an acoustically thick mass layer but only acted as a surface lubricant.

The different acoustic change associated with deposition on the three surfaces indicated the formation of different forms of supported lipid layers. Furthermore, the kinetics of the interaction of the vesicle suspension also varied considerably with the hydrophilic properties of the surface. The slowest kinetics were observed on the hydrophobic HDT surface, where equilibrium was reached after 12 min. Faster adsorption was observed on the 16 h and 10 min modified MUO surfaces where almost 80% of the deposition occurred in the first 2 min. In all cases, once the lipid adsorption had finished, the surface was rinsed with PBS for another 10 min.

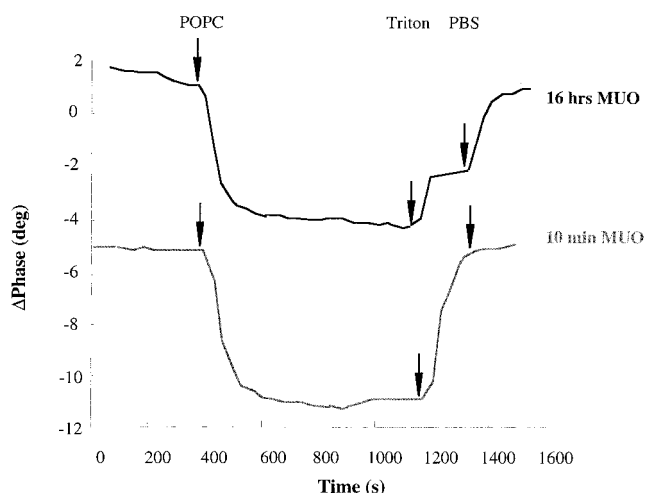
2. BSA Binding. The extent of nonspecific binding on the lipid-modified surfaces was studied by using BSA. After the formation of a supported lipid layer, 1 mg/mL of BSA in PBS buffer was applied to the device surface followed by a buffer rinse. A significant phase and amplitude change was detected on the 10 min MUO-modified device. However, no change was observed when the same solution was applied to the other two surfaces. The phase change observed during the nonspecific binding of BSA on each surface is summarized in Table 1.

3. Measurement of Lipid Surface Density. The surface density of the bound lipid layer was measured

Table 1. Summary of Data Related to the Interaction of a Vesicle Suspension with a Thiol-Modified Surface^a

surface	contact angle (deg)	POPC			BSA	
		Δ ampl (dB)	Δ phase (deg)	surf. dens (ng/mm ²)	Δ ampl (dB)	Δ phase (deg)
HDT 16 h	130	0.0	-1.6	1.2	0.0	0.0
MUO 16 h	5	-0.5	-3.8	3.6	0.0	0.0
MUO 10 min	50	-1.6	-9.4	8.6	-0.6	-5.0

^a Results include the following: (1) contact angle measurements on each thiol surface, (2) acoustic measurements during the interaction of POPC vesicles with each surface, (3) measured surface density of deposited POPC by using ¹⁴C-labeled lipids and, (4) acoustic measurements of BSA binding to the lipid layer.

**Figure 4.** Phase change as a function of time during the application of POPC vesicles on the 10 min and 16 h MUO modified surfaces, followed by a 0.1% Triton-PBS rinse.

directly by applying a mixture of ¹⁴C-labeled and nonlabeled POPC vesicles to the 10 min modified MUO surface. These measurements showed a POPC surface density of 8.6 ± 0.8 ng/mm². Since phase is predominantly related to changes in the mass at the interface, ¹⁴C measurements were used to calculate the lipid mass density on the other two surfaces and calibrate phase response to mass deposition. Results are summarized in Table 1.

4. Desorption of the Supported Lipid Layer. The deposited lipid layer was desorbed by washing the surface with 0.1% Triton in PBS. Figure 4 shows the phase response during the adsorption and desorption of a POPC lipid layer on addition of vesicles and detergent, respectively, on the 10 min and 16 h modified MUO surfaces.

Discussion

In this work, our first aim was to modify the acoustic waveguide device in order to obtain surfaces of very different hydrophilic properties. For this reason, a gold layer was deposited on the device surface and was further modified by self-assembling a thiol layer. The quality of the self-assembled layer was found to be critical for the subsequent deposition of supported lipid layers and was controlled by varying the incubation time and thiol solvent. Hydrophilic (contact angle $\sim 5^\circ$) and hydrophobic (contact angle $\sim 130^\circ$) surfaces were produced by incubating the freshly prepared gold-coated devices in a solution of mercaptoundecanol (MUO) in cyclohexane and hexadecanethiol (HDT) in ethanol, respectively, for 16 h. Such a long incubation time is known to be necessary in order to get a densely packed thiol layer. In addition, a surface with a contact angle of 50° was produced by leaving the gold-coated device in contact with mercaptoundecanol in

ethanol for 10 min. Although thiol has a high affinity for clean gold, such a short incubation time is not sufficient for the formation of a good thiol layer.

To study the interaction of a vesicle suspension with the device surface, it is important to establish good wetting of the surface by the liquid sample. This will enable the free transfer of vesicles from the solution to the surface and also ensure the continuity of the acoustic field from the piezoelectric surface to the adjacent liquid. The wetting of the hydrophobic surface was improved by first washing the thiol-modified surface with buffer containing 0.1% Triton detergent for 1 min. Longer exposure of the hydrophobic surface to Triton resulted in the deposition of detergent on the hydrophobic surface, which could not be removed by subsequent buffer rinse. Both the 16 h and 10 min modified MUO surfaces were used without the need to apply Triton prior to the vesicle suspension.

The overall phase and amplitude change detected during the interaction of the lipid suspension with each surface was measured from Figures 2 and 3 after equilibrium was reached. To investigate the structure of the lipid layer, the POPC surface mass density was measured directly on the 10 min MUO modified surface and used to calibrate phase change to POPC mass deposition. Finally, the nonspecific binding properties of each supported POPC layer were determined by monitoring the acoustic signals during the adsorption of BSA. It has been shown that hydrophilic surfaces suppress nonspecific binding,^{4,11} and thus, this measurement would be a good indication of the supported lipid structure. The hydrophilic properties of each surface, acoustic signal change during POPC deposition and BSA binding, and POPC surface mass density are summarized in Table 1. The combination of the above results clearly indicate that different amounts of lipid were deposited on each surface, presumably as a result of the different surface properties.

The large phase response observed on deposition of liposomes on the 10 min MUO modified surface clearly shows that more mass was deposited on that surface compared to the hydrophilic and hydrophobic surfaces. The measured POPC surface density (8.6 ± 0.8 ng/mm²) was much larger than the expected POPC surface density in a bilayer structure (4.2 ng/mm²),¹⁷ indicating the formation of a supported lipid multilayer or vesicle layer. However, the significant amount of nonspecific binding observed during application of BSA on the device surface rules out the possibility of a multilayer structure. Such a structure would expose the hydrophilic part of the top bilayer, which is known to have low nonspecific binding properties. In addition, comparison of the theoretical surface density of a closely packed layer of hollow spheres (9.2 ng/mm²)²¹ to what we measured shows that the two numbers are in good agreement. The final evidence that supports the formation of a supported vesicle layer is the large amplitude change observed during POPC deposition. This measurement indicates a significant interfacial viscoelastic change, which would be compatible with the presence of a supported vesicle layer consisting of soft lipid spheres surrounded by water.

The phase change observed after exposing the hydrophilic surface (16 h in MUO) to POPC vesicles corresponds to a surface mass density of 3.4 ng/mm². This is less than the reported surface density of a POPC bilayer.¹⁹ However,

(21) The mass ratio for a vesicle layer with respect to a bilayer was calculated to be 2.2, based on the ratio (τ) of the surface area of a sphere and the area projected by a sphere onto an underlying plane ($\tau = 4$) and on the fact that for a randomly packed layer of spheres such projections would be expected to occupy 55% of the total area of the plane. (Feder, J.; Giaver, J. *J. Colloid Interface Sci.* **1980**, *78*, 144.)

this discrepancy can be again explained on the basis of surface imperfections and/or miscalculation of the exact surface area. The zero acoustic response observed during the application of BSA on the deposited lipid layer strongly suggests that a supported lipid bilayer was formed on the hydrophilic device surface. Finally, the smaller amplitude change observed in this case, compared to that detected on the 10 min modified MUO surface proves that this lipid layer has different viscoelastic properties and, in fact, the interfacial viscoelastic perturbation is smaller. Supported lipid bilayers consist of lipids which are in the fluid state and are separated from the surface by a water layer of a thickness of 2 nm. Such a structure would exhibit some viscoelastic behavior, which, however, would not be as profound as that of a layer of vesicles.

The lipid layer deposited on the surface of the hydrophobic thiol gave a phase change that was half that observed during POPC deposition on the hydrophilic surface indicating the formation of a supported lipid monolayer. The following results strongly support the above hypothesis: (1) the suppression of nonspecific binding of BSA, which shows a surface with good biocompatible properties, and (2) the insignificant amplitude change observed during the deposition of POPC. The latter measurement shows that the deposited layer acts as an elastic mass layer, which is compatible with a lipid monolayer deposited directly on the hydrophobic surface with no water molecules trapped between the two.

The specific mechanism under which the lipid vesicles were adsorbed to and desorbed from the surface also depends on the surface properties. Figure 4 shows the adsorption and desorption of a lipid bilayer (16 h MUO) and vesicle layer (10 min MUO) on addition of POPC and 0.1% Triton, respectively. According to Figure 4, the adsorption kinetics are quite similar on both surfaces. It is interesting to note that the POPC bilayer seems to be formed spontaneously as soon as vesicles reach the surface and does not follow a two-step process where vesicles first adsorb and then fuse once a critical concentration is reached. The latter mechanism was observed on a hydrophilic silica¹² and monolayer modified quartz surface.⁸ However, according to Figure 4, the desorption kinetics of the supported lipid layers followed distinctly different patterns. In the case of the vesicle layer, the signal returns back to the original baseline as soon as the detergent reaches the surface and does not change any further on addition of buffer, indicating complete removal of the supported vesicles. In the case of the lipid bilayer, desorption kinetics followed two steps where some of the POPC was removed on addition of Triton and the rest after PBS rinse. A possible explanation is that the detergent disrupted the bilayer structure by removing some of the lipid mass but helped to stabilize the remaining lipid. The latter was only removed on addition of buffer.

Conclusions

The interaction of POPC vesicles in PBS buffer with surfaces of different hydrophilic properties was investigated by using the acoustic waveguide device as the main detection mode. It was found that a lipid monolayer and bilayer were formed on the hydrophobic and hydrophilic surfaces, respectively, while a vesicle layer was formed on the surface with hydrophilic properties between the above. Figure 5 gives a summary of the supported lipid structures obtained on each surface.

Our results suggest that the surface density of hydroxyl groups is important for vesicle fusion and that if it is not high enough adsorbed vesicles remain intact on the surface. One could assume that the mechanism of vesicle

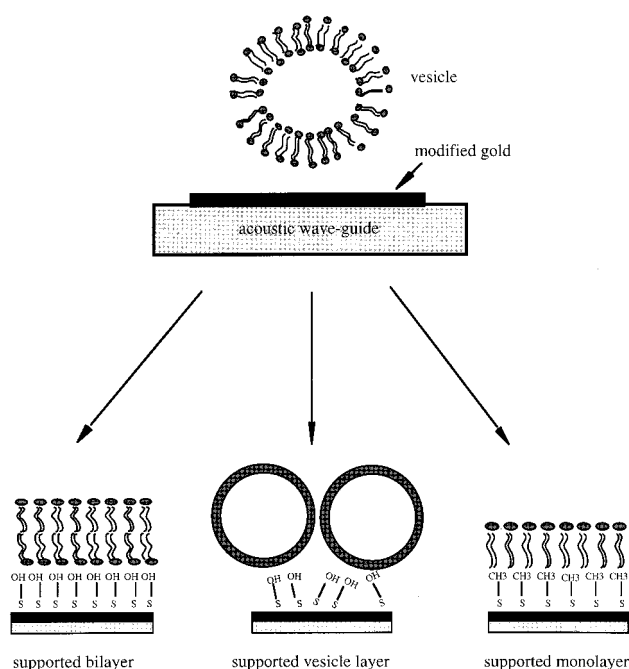


Figure 5. Schematic representation of the supported lipid layers obtained on gold as a function of the surface hydrophilicity. A lipid bilayer is formed on the 16 h modified hydrophilic surface (a), a vesicle layer on the 10 min MUO modified surface (b), and a lipid monolayer on the 16 h modified HDT surface (c).

adsorption and fusion on hydrophilic gold is similar to that proposed for silica; i.e., weak electrostatic interactions hold the zwitterionic bilayer on the hydrophilic surface due to the partly deprotonated POPC.⁹ However, since the surface charge of the self-assembled layer of aliphatic alcohols is very small ($pK_a \approx 19-20$), an interfacial potential in this case could be only established if gold, which develops a charge, affects the electric properties of the surface.

The formation of a supported vesicle layer on a fairly hydrophilic surface (oxidized gold) has been also reported and in that case was attributed to the small size of the vesicles used (diameter of 17 nm).¹² Small vesicles are under more stress and are more likely to break open to form a bilayer; however, our results suggest that apart from the size of the vesicles the surface density of hydroxyl groups is also critical for spontaneous vesicle fusion.

In relation to the acoustic measurements, it was shown that the acoustic waveguide can be used to follow interfacial changes in real time. This system possesses a number of advantages comparing to SPR. First, it can detect changes in both the mass and viscoelastic properties of the interface. Second, since phase responds mainly to mass and amplitude to viscoelastic perturbations it is possible to derive simultaneously information on each interaction by just monitoring the two signals. Finally, both amplitude and phase can clearly follow the kinetics of interfacial changes without exhibiting the out-of-scale response observed with SPR during the successive injection of samples of different bulk properties (such as refractive index). Given the above, it appears that the acoustic waveguide device would be a good candidate for studying surface-bound biomolecular interactions occurring on a cell membrane.

Acknowledgment. The authors would like to acknowledge the financial support of the Biotechnology and Biological Sciences Research Council (BBSRC).

LA001443J