Surface Acoustic Wave Biosensor as a Tool to Study the Interaction of Antimicrobial Peptides with Phospholipid and Lipopolysaccharide Model Membranes

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Surface acoustic wave biosensors are a powerful tool for the study of biomolecular interactions. The modulation of a surface-confined acoustic wave is utilized here for the analysis of surface binding. Phase and amplitude of the wave correspond roughly to mass loading and viscoelastic properties of the surface, respectively. We established a procedure to reconstitute phospholipid and lipopolysaccharide bilayers on the surface of a modified gold sensor chip to study the mode of action of membrane-active peptides. The procedure included the formation of a self-assembled monolayer of 11-mercaptoundecanol, covalent coupling of carboxymethyl−dextran, and subsequent coating with a poly-L-lysine layer. The lipid coverage of the surface is highly reproducible and homogeneous as demonstrated in atomic force micrographs. Ethanol/triton treatment removed the lipids completely, which provided the basis for continuous sequences of independent experiments. The setup was applied to investigate the binding of human cathelicidin-derived peptide LL32, as an example for antimicrobial peptides, to immobilized phosphatidylserine membranes. The peptide−membrane interaction results in a positive phase shift and an increase in amplitude, indicating a mass increase along with a loss in viscosity. This suggests that the bilayer becomes more rigid upon interaction with LL32.

Introduction

Supported lipid bilayers on a biosensor surface are a useful and versatile tool to study the intrinsic properties of biological membranes and their interaction with a variety of compounds. During past years, simpler biosensor technologies and newly designed surfaces became available allowing one to measure interactions of liposomes with proteins and enabling one to extract kinetic data.[1] Baird et al.[2] showed that liposome capturing measured with surface plasmon resonance (SPR) sensor technology is highly reproducible. Furthermore, drug binding responses were directly proportional to the amount of liposomes bound to the surface. BIAcore even created a carboxymethyl (CM)−dextran surface equipped with lipophilic groups, based on the principle of Hämmerling and Westphal[3] applied to the direct capture of liposomes. This method allows one to follow the binding and fusion process of liposomes at their surface.[4] Alternative biosensors applied mostly are based on quartz crystal microbalance with or without dissipation (QCM-D and QCM). Contrary to SPR methods, the QCM-D technique offers the possibility to study not only mass changes, but also viscoelastic properties of bound materials. The adsorption of vesicles can, thus, be differentiated from the viscoelastic properties of the respective bilayers.[5−7]

The resulting signals from the S-sens K5 biosensor system (Biosensor GmbH, Göttingen, Germany) used for these experiments are based on the modulation of the amplitude and phase of a surface acoustic wave (SAW, love-wave) induced by the adsorption or desorption of molecules on the surface of the sensor chip. This technique has a higher sensitivity and enables separation of signals resulting from mass changes from viscoelastic properties, as has been shown for thrombin−antithrombin aptamer interaction.[8] A two-frequency mode enables precise detection with very little effect from buffer contents (e.g., salts, viscose properties) on the phase signal.[9]

A large number of biologically important interactions take place between lipid membranes and peptides/proteins. The reproducible reconstitution of bilayers on the surface of the SAW sensor chip is a prerequisite for investigations on such interactions. Different strategies have been developed on gold or silica surfaces. These include lipid bilayers directly formed on the solid support,[10] or on a polymer (cushioned bilayer),[11] as well as hybrid bilayers with a lipid monolayer on a self-assembled monolayer (SAM)[12] or on a tethered monolayer.[13]

Thus, in a first step we used different approaches to adsorb lipid membranes on the sensor surface acting as a solid support, and in a second step we applied the SAW biosensor to study the interaction of antimicrobial peptides with these lipid membranes. This interaction is believed to be a key step in bacterial killing by these compounds.[14−18] Antimicrobial peptides are part of the innate immune system and are found in almost all organisms,

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including humans.[19] They provide a first line of defense against invading pathogens, e.g., on epithelial surfaces.[20,21] The peptide LL32 comprises a C-terminal natural fragment of human cathelicidin.[22] It is an α-helical peptide with pronounced antibacterial activities.[23] The cell envelope of Gram-negative bacteria, such as *Salmonella* and *Escherichia coli*, consists of a phospholipid membrane surrounded by a peptidoglycan layer, and an additional outer membrane whose outer leaflet is composed solely of the complex glycolipid lipopolysaccharide (LPS). LPS is the causative agent of bacterial sepsis and the septic shock syndrom and is thus also termed endotoxin.[24] To investigate the mode of action and the lipid specificity of antimicrobial peptides, we analyzed their interaction with model membranes composed of phospholipids and LPS to mimic the cytoplasmic and the outer bacterial membrane.[18,25,26]

In this study, we provide the proof of principle that homogeneous bilayers composed of various phospholipids and of bacterial LPS can be easily formed on a functionalized sensor chip, and that binding and change of the viscolelastic properties of the bilayer upon interaction with the peptide LL32 can be monitored. Data obtained by SAW biosensor are compared with those from SPR spectroscopy, and the surface of the sensor chip is analyzed at different steps of functionalization and lipid binding by atomic force microscopy (AFM) and force spectroscopy.

Materials and Methods

Lipids. Rough mutant LPS from *Escherichia coli* strain WBB01 and *Proteus mirabilis* strain R45 were used. LPS was extracted by the phenol/chloroform/petroleum ether method,[27] purified, lyophylized, and transformed into the triethylamine salt form. Phosphatidylcholine (PC) from egg yolk and phosphatidylserine (PS) from bovine brain were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification.

Peptide Synthesis and Purification. Peptide LL32 (sequence: LLGDF FRKSK EKIGK EFKRI VQRIK DFLRN LV-$CONH_2$) was synthesized with an amidated C-terminus by solid-phase synthesis utilizing an automatic peptide synthesizer (model 433 A; Applied Biosystems, Darmstadt, Germany). Synthesis according to the fastmoc synthesis protocol of the manufacturer was done on a standard 9-fluorenylmethoxy carbonyl (Fmoc)-amide resin. The peptide was cleaved from the resin by treatment with 90% trifluoroacetic acid (TFA), 5% anisole, 2% thioanisole, and 3% dithiothreitol. It was precipitated with diethylether at 0 °C, centrifuged, and washed several times with diethylether. Purification was done by reversed-phase high-performance liquid chromatography with an Aqua-C_{18} column (Phenomenex, Aschaffenburg, Germany) using a gradient of 0 to 70% acetonitrile in 0.1% TFA. LL32 was lyophilized, and purity was confirmed by mass spectrometry. The peptide was stored at −20 °C.

Preparation of Lipid Aggregates/Liposomes. Aggregates/liposomes were prepared as 1 mM aqueous dispersions of the phospholipids or LPS in buffer (5 mM Hepes, 100 mM KCl, pH 7.0) as follows. The lipids were dissolved in chloroform, and the LPS was dissolved in chloroform/methanol (10:1) to a concentration of 1 mg/mL. The solvent was evaporated under a stream of nitrogen, the lipids were resuspended in buffer, mixed thoroughly, and sonicated with a Branson sonicator for 1 min (1 mL solution). Subsequently, the preparation was cooled for 30 min at 4 °C, heated for 30 min at 56 °C, and recooled to 4 °C. Preparations were stored at 4 °C overnight prior to measurements.

Functionalization of the Biosensor Chip Surface. SAMs on the gold shielding were formed by incubation of gold-coated chips (S-sens K5 Biosensor Quartz Chips, Biosensor GmbH, Bonn, Germany) in 2 mM ethanolic solutions of either 11-mercapto-1-undecanol (Aldrich), 11-mercapto-1-undecanoic acid, or 1-octadecanthiol (Sigma)[8] for at least 12 h at room temperature (RT). A CM−dextran layer was prepared on top of the 11-mercapto-1-undecanol SAM.[28] The alcohol groups of the 11-mercapto-1-undecanol SAM were activated with 0.6 M epichlorhydrine (Sigma) dissolved in a 1: 1 mixture of 0.4 M NaOH and diglyme (Fluka) for 4 h at RT. The chip surface was then washed with water, ethanol, and water, and a dextran solution (0.3 mg/mL, from *Leuconostoc mesenteroides*, average MW 8500−11 000 Da, Sigma) in 0.1 M NaOH was allowed to react for 17 h at RT. After extensive washing with water at 50 °C, the dextran on the chip was carboxylated by incubation in 0.9 M bromoacetic acid (Sigma) in 2 M NaOH for 17 h at RT. The chip was washed extensively with water at 50 °C, dried under a gentle stream of nitrogen and stored until use at 4 °C.

Love-Wave Sensor Measurements. A functionalized chip was incubated overnight in measuring buffer (5 mM Hepes, 100 mM KCl, pH 7.0), placed into the reader unit of the S-sens K5 readout system (Biosensor GmbH, Bonn, Germany, Germany) integrating a high-frequency unit, a control unit, and all fluidic components required for a systematic buffer and analyte solution handling. This enabled fluidic and electrical contacting of the chip at a stable temperature of 22 or 37 °C ($\Delta T = 0.05$ °C by means of four peltier elements). Mass loading and viscoelastic alterations resulting from biomolecular interaction processes on the surface of the sensor chip result in changes of phase and amplitude of the surface acoustic waves based on the inverse piezoelectric effect. Signals were recorded in real-time using a double-frequency measurement mode.[9] Measurements were performed at a continuous buffer flow of 20 μL/min at 22 °C if not indicated otherwise. By injection of 200 μL of poly-L-lysine (PLL, 60 μg/mL, Fluka, Basel, Switzerland) a positively charged layer was formed on top of the negatively charged CM−dextran matrix. Phospholipid liposomes and LPS aggregates (100 μM) of varying composition and charge states were bound to the positively ionized surface. All injections, but those containing LL32, were performed with an initial burst, an increase in flow to 150 μL/min.

Surface-Plasmon Resonance Spectroscopy Experiments. The SPR technique was used to compare the results with those of the SAW biosensor data. The same buffer and the same preparations of lipids and LL32 were used. A Biacore 3000 (GE Healthcare/Biacore AB, Uppsala, Sweden) equipped with a CM3 sensor chip was used at a continuous buffer flow of 10 μL/min, if not indicated otherwise, at 25 °C.

Atomic Force Microscopy. The AFM was used to characterize the surface of the SAW biosensors at the steps bearing a CM−dextran surface, and modified with PLL, and finally a phospholipid membrane. A CM−dextran-coated chip was imaged using a MFP 3D (Asylum Research, Santa Barbara, CA). An RC 800 PSA (Olympus, Japan) cantilever with a spring constant of 0.7 N/m (determined via thermal noise analysis) was driven in AC mode at frequencies of about 15 kHz. PLL was added to the dextran-layered chip to a final

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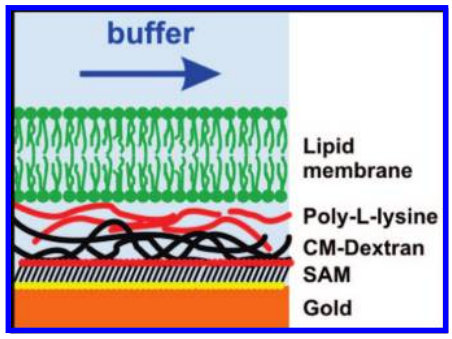

Figure 1. Schematics of the functionalization of the gold surface by a SAM, a layer of CM−dextran, PLL, and a phospholipid membrane.

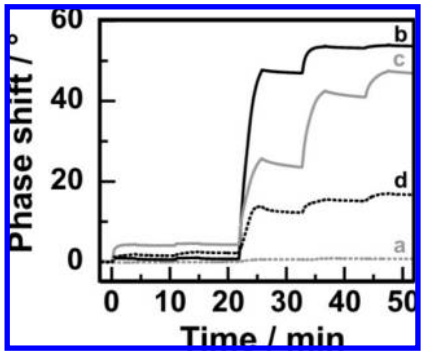

Figure 2. Immobilization of PS liposomes on differently functionalized gold surfaces of love-wave biosensors: (a) CM−dextran matrix, (b) CM−dextran + PLL, (c) 11-mercapto-1-undecanoic acid + PLL, and (d) 1-octadecanthiol + PLL. Injection of 100 μL of PLL (60 μg/mL) at time points 0 and 10 min, and of 100 μL of PS (100 μM) at time points 21, 32, and 42 min.

concentration of 60 μg/mL, and after 15 min the solution (2 mL) was exchanged successively with a total volume of 10 mL of buffer. After imaging, PS liposomes were added to a final concentration of 100 μM, and after 15 min the solution was exchanged against 10 mL of buffer. In addition to imaging, force spectroscopy was performed in DC mode with a velocity of 1 μm/s. All experiments were done in buffer (5 mM Hepes, 100 mM KCl, pH 7.0) at 22 °C.

Results and Discussion

To perform binding experiments between lipid membranes and peptides using biosensor chips, the first prerequisite is the adsorption of a stable lipid bilayer that covers the chip surface homogenously. The most successful of the approaches tested for adsorbing lipid bilayers on the gold surface of the SAW biosensor chip is shown schematically in Figure 1. A CM−dextran cushion was prepared on top of a 11-mercaptoundecanol SAM formed on the gold surface. A layer of PLL was adsorbed in the first two injections, positively ionizing the negatively charged CM−dextran matrix. The phospholipids and LPS used in this study were negatively charged or zwitterionic, and, thus, an electrostatic interaction between the positively charged PLL and the lipids enhances the adsorption. Other surface modifications were tested, including SAMs of 11-mercapto-1-undecanoic acid or of 1-octadecanthiol. Both were used with (Figure 2, traces c and d) and without additional PLL injections. Figure 2 shows that the binding of PS liposomes was negligible on an unmodified CM−dextran matrix (Figure 2, trace a). Only minor binding of PS to the two SAMs was observed without PLL addition under the conditions applied (data not shown). The lipids bound to both SAMs and the CM−dextran surface after positive ionization with PLL. Interestingly, the binding kinetics as well as the

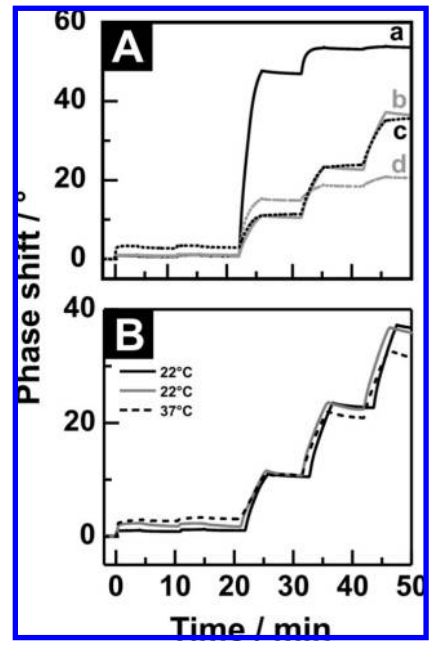

Figure 3. (A) Immobilization of various phospholipid liposomes and LPS aggregates on a biosensor surface functionalized with a CM−dextran matrix and a PLL layer. Injection of 100 μL of PLL (60 μg/mL) at time points 0 and 10 min, and of 100 μL of lipid liposomes (100 μM) at time points 21, 32, and 42 min. (a) PS, (b) LPS WBB01, (c) LPS R45, and (d) PC. (B) Reproducibility and temperature dependence of the immobilization of *E. coli* LPS WBB01 aggregates on the biosensor surface functionalized with a CM−dextran matrix and PLL. Injection of 100 μL of PLL (60 μg/ml) at time points 0 and 10 min, and of 100 μL of LPS aggregates (100 μM) at time points 21, 32, and 42 min.

maximal shift in the phase differed. In the literature, changes in the phase of the acoustic wave are interpreted as changes in the mass of the adsorbed material on the sensor surface.[9] However, as shown later, a clear distinction between changes in mass and in the viscosity of the material bound to the surface can not be made for the bilayers used in this study. The most significant adsorption of PS bilayers could be observed in the case of a PLL-coated CM−dextran surface (Figure 2, trace b). Surfaces prepared according to this method were used in the following experiments. After association of the lipids to the surface, the dissociation in pure buffer was relatively low. The adsorbed bilayers were stable over hours. Figure 3A shows the adsorption of various phospholipid liposomes, including PS, PC, and two different LPS aggregates. The biggest change in both phase shift and amplitude (not shown) was observed for PS and the two LPS. For the various lipids and LPS, the individual saturation kinetics differed. For the phospholipids, the sensor surface was nearly saturated after the first injection. However, after each of the three injections of LPS aggregates, an increase of the phase signal was measured. This effect depends on the flow rate and whether an initial burst, a short increase in flow rate to 150 μL/min, was applied. The five channels of the chip are connected in series. Application of such a burst reduces the time difference of the individual sensor elements to come in contact with and to react on the added reagents. The adsorption signals are highly reproducible (Figure 3B), while the influence of the temperature changes are relatively low. For LPS from *E. coli* WBB01, the phase transition temperature is $T_c = 36$ °C. Thus, the adsorption below and above T_c is comparable.

Various antimicrobial peptides were injected to analyze their binding to the adsorbed bilayers. Representatively, the interaction of the peptide LL32 with a PS-covered surface is shown here (Figure 4). The curve sections show (a) the adsorption of PLL,

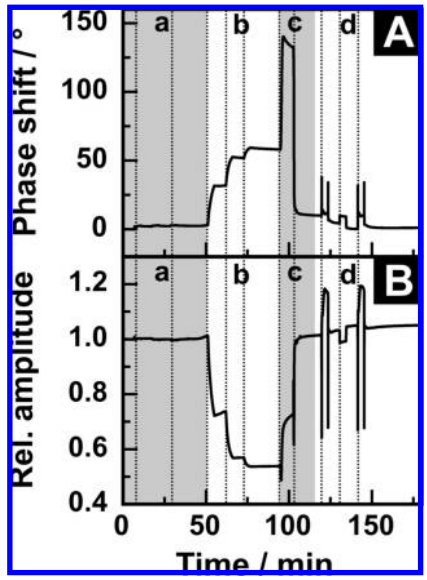

Figure 4. Binding of the antimicrobial peptide LL32 to immobilized PS on a CM−dextran/PLL matrix and regeneration of sensor surfaces in a SAW biosensor experiment. Injection of (a) 100 μL of PLL (60 μg/ml) at time points 10 and 30 min, of (b) 100 μL of PS liposomes (100 μM) at time points 50, 63, and 76 min, of (c) 20 μL of LL32 (100 μM) at time point 95 min (without initial burst), of 60 μL of LL32 (100 μM) at time point 102 min (with initial burst), and of (d) 100 μL of ethanol (100%), 100 μL of 0.1 M glycine, 0.3% triton X-100, pH 12, and 100 μL of ethanol at time points 120, 130, 140 min, respectively.

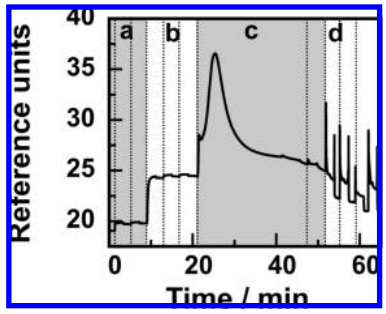

Figure 5. Binding of the antimicrobial peptide LL32 to immobilized PS on a CM−dextran/PLL matrix and regeneration of sensor surfaces in an SPR spectroscopy experiment. Injection of (a) 20 μL of PLL (60 μg/ml) at time points 1 and 5 min, of (b) 20 μL of PS liposomes (100 μM) at time points 9, 13, and 17 min, of (c) 20 μL of LL32 (100 μM) at time point 21 min with a reduced flow of 1 μL/min and at time point 46 min with a standard flow of 10 μL/min, and (d) of 20 μL of ethanol (100%), 20 μL of 0.1 M glycine, 0.3% triton X-100, pH 12, and 20 μL of ethanol at time points 52, 55, 59 min, respectively.

(b) the adsorption of PS liposomes, (c) the binding of LL32 to the bilayer, and (d) the dissociation of the peptide and the lipid using a cleaning procedure. These data are compared with results determined from well-established SPR experiments (Figure 5). The adsorption of PS led to an increase of the phase and a decrease in the amplitude. It has been described for the adsorption of proteins (see Introduction), the phase shift corresponds to a defined change in mass loading (Figure 4A), while the amplitude (Figure 4B) reflects a change in viscoelastic properties.[9] For thin layers adsorbed to a surface, mass and viscosity can be calculated after calibrating the sensor system using a 5% glycerol solution.[9] In our experiments, the assumption of a thin layer not damping the acoustic wave is not fulfilled. In the future, the model for the adsorption of lipid bilayers needs to be customized. The injection of LL32 using a constant flow rate of 20 μL/min led to a drastic

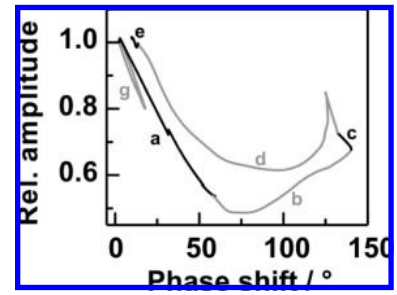

Figure 6. Amplitude/phase diagram of the adsorption and desorption of PS and LL32 on a CM−dextran/PLL matrix. The data were taken from the experiment shown in Figure 4. The five traces show (a) adsorption and desorption of PS during the three injections, (b) adsorption of LL32 (without initial burst), (c) desorption of LL32, (d) adsorption of LL32 (with initial burst), (e) desorption of LL32, and (g) injection of a solution of 5% glycerol in buffer.

increase of both phase and amplitude. This can be interpreted as a strong binding of the LL32 molecules to the membrane. The lipid-to-peptide mass ratio calculated from the respective phase shifts is approximately 1:1. Using different lipid and peptide concentrations, we could show that there is an almost linear correlation between the phase shift and the concentration (data not shown). Thus, we propose that the phase shift is almost linearly correlated to the mass adsorbed to the senor surface. Furthermore, we suggest that the cross-linking of a number of PS molecules by LL32 molecules led to an increase of the membrane rigidity as well as a decrease of the viscosity. The second injection of LL32 was performed using an initial burst with a flow rate of 150 μL/ml. The phase dropped down from about 130° to 10°, and the amplitude increased to the initial value. It has been previously described that LL32 can lead to the formation of membrane lesions and to a destabilization of lipid bilayers.[25] Therefore, we propose that LL32 destabilizes the bilayer. At fast flow, the shear force becomes strong enough to remove the bilayer completely or most of it from the sensor surface. In control experiments it could be shown that binding of LL32 to the CM−dextran matrix with adsorbed PLL can be neglected (data not shown). Thus, the binding of LL32 shown in the experiment in Figure 4 can be addressed to specific binding to the lipid matrix and not to an unspecific adsorption to potentially uncovered areas of the chip surface. The amplitude/phase diagram (Figure 6) shows the relation between amplitude and phase more clearly and helps to interpret the different interaction steps. The relation between amplitude and phase in the three binding steps of PS to the PLL matrix is almost linear, demonstrating a homogeneous adsorption of PS. Interestingly, the adsorption of LL32 in the first injection shows a two step behavior: In the first step, LL32 leads to an increase in phase and a decrease in amplitude, and in the second step to an increase in phase and amplitude. Thus, we propose that the interaction between PS membranes and LL32 takes place in a concentration-dependent two step mechanism: (i) at low concentration, LL32 binds to the PS bilayer leading to a more elastic membrane and (ii) at higher concentrations a conformational change of LL32 in the membrane or changes of the whole lipid/peptide complexes occur. The destabilization of the bilayer during the second injection of LL32 also shows different steps of interaction, which cannot be interpreted in detail. A solution of 5% glycerol was used as a liquid with almost Newtonian behavior (Figure 6, trace g).

To verify the results obtained using the SAW biosensor system, the experiments were repeated in SPR experiments using the same surface modifications (Figure 5). The flow rate was 1 μL/min (first injection of LL32) or 10 μL/min (second injection of

LL32). Because of the different geometries of the flow cells in the SAW and the SPR instruments, different flow rates were used in the two systems. Qualitatively, the data obtained from the SPR experiments result in the same conclusions as the phase shifts of the SAW biosensor experiments: adsorption of lipids to the surface and a strong binding of LL32 to the membrane followed by a destabilization of the matrix. After adsorption of the peptides to the lipid bilayers, the surfaces were recovered by a cleaning procedure using subsequent injections of (i) ethanol, (ii) a solution containing 0.1 M glycerol and 0.3% triton X-100 (pH 12), and (iii) ethanol. It has to be mentioned that pure ethanol might cause problems because of the formation of gas bubbles in the tube; in this case ethanol/water solutions with a lower amount of ethanol should be used. After the cleaning procedure, almost the initial values of the phase and the amplitude or of the reference units were reached. Probably not all PLL could be removed by this procedure, because, in a second loading of the same chip, slightly reduced increases in the phase signal could be observed.

For the bacterial killing the binding of LL32 to LPS membranes is the initial step of permeabilization of the outer membrane of Gram-negative bacteria. In SAW as well as in SPR experiments the interaction between LL32 and LPS WBB01 showed comparable results to those obtained in the case of the PS membranes (data not shown). In future investigations, a comparative study of the binding of LL32 to LPS obtained from sensitive and resistant strains might help one to understand the underlying mechanisms of bacterial resistances. Our results clearly demonstrate that LL32 strongly interacts with negatively charged lipid membranes, leading to destabilization of whole lipid matrices. In experiments using planar lipid bilayers, we could show earlier for LL32 and various fragments of the human and rabbit cathelicidine that these peptides are able to induce the formation of lesions and lead finally to a disintegration of the lipid membrane.[25,29,30] Probably, this strong interaction of LL32 with bacterial membranes is already sufficient to kill bacteria.

For an interpretation of the data, it is necessary to understand how the lipid liposomes are adsorbed to the chip surface. Therefore, we imaged a sensor chip covered with CM−dextran by AFM. The CM−dextran-covered gold chip showed a typical surface covered with spherical elevation (Figure 7A). In the force spectroscopic experiments, only short rupture events originating from the CM−dextran could be observed (Figure 7B). The subsequent addition of PLL led to slight changes in the roughness of the surface and to a significant increase in the number of rupture events as well as their rupture length (Figure 7C,D, respectively). This indicates that PLL covers the whole surface as a thin film. The final addition of PS liposomes led to a significant reduction of the surface roughness (Figure 7E). Because of the mechanical interaction between the surface and the hard tip of the cantilever, it is likely that the tip deforms the PLL as well as the lipid membrane. However, the images show homogeneous changes of the surface topography after addition of PLL and the lipid liposomes, demonstrating that the surface modifications do not lead to the formation of clusters on the surface. Moreover, the pulling curves show a behavior that is typical for a single lipid bilayer with a thickness of about 5 nm (Figure 7F).[31] In the retraction curve, a region with an attractive force of about 40 nm could be observed. This behavior cannot be observed if a bilayer

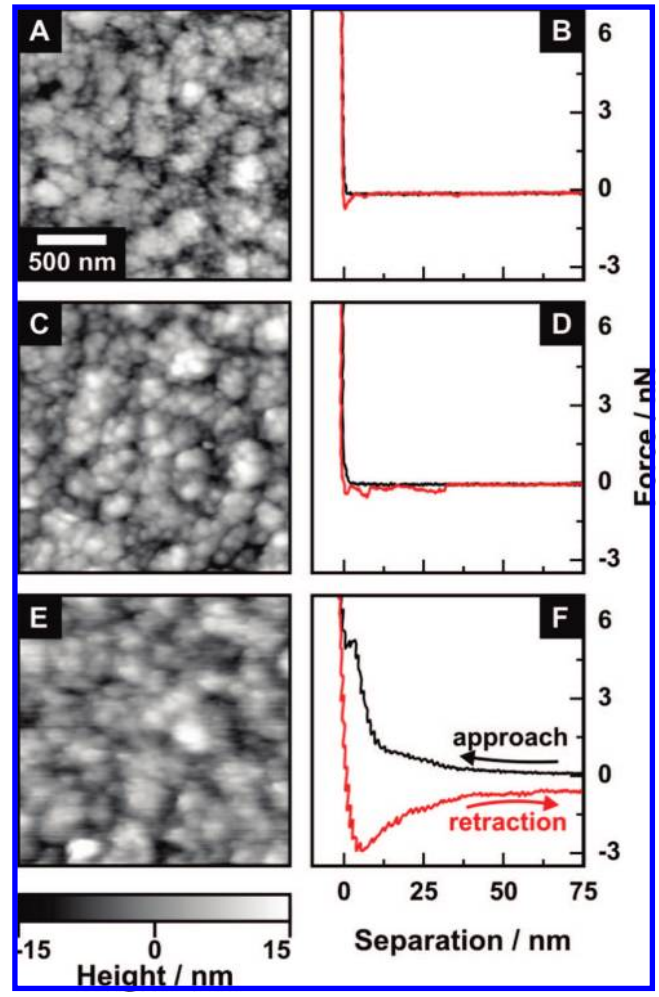

Figure 7. Characterization of the SAW biosensor surface by AFM. The left column shows 2 × 2 μm^2 AFM height images taken in AC mode, and the right column shows force spectroscopy curves performed on the respective samples (black line: approach to the surface; gray line: retraction from the surface). (A) and (B) show the results from the surface of the CM−dextran matrix immobilized on the gold layer of the sensor, (C) and (D) show that of the CM−dextran matrix after the addition of PLL (60 μg/mL), and (E) and (F) show that of the CM−dextran/PLL matrix after the subsequent addition of PS liposomes (100 μM).

is adsorbed on an unmodified solid surface such as gold or mica and is indicative for a fluid bilayer that can be deformed by the retracting cantilever tip. Thus, we propose that this reconstitution technique is suitable to generate homogeneous bilayers adsorbed on a solid support, which should allow the binding and intercalation of membrane-active peptides. The interaction of antimicrobial peptides with lipid bilayers directly formed on the silica surface of a QCM crystal has been reported before for bovine lactoferricin and nisin.[32,33] This is the first report where lipid bilayers were formed on a CM−dextran/PLL cushion. The membrane formed in our experiments is more flexible (see Figure 7), which highly resembles the biological system.

In conclusion, we developed a procedure for the reproducible and reversible adsorption of phospholipid liposomes and LPS aggregates resulting in bilayer formation on a functionalized SAW biosensor surface. Dependent on their lipid composition, these bilayers may serve as easy-to-handle model systems for bacterial or other biological membranes. Two measuring parameters, e.g., phase shift and amplitude of the acoustic waves, provide information about mass loading on the surface and their viscoelastic properties, respectively. To quantify these two parameters, the development of new models describing the

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influence of thick, viscous layers on the phase and amplitude will be a challenging task for the future. In particular, the modulation of the latter parameter appeared to be of importance for a better understanding of the interaction of antimicrobial peptides with biomembranes.

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