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Protein Adsorption on Supported Phospholipid Bilayers

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Quartz crystal microbalance with dissipation (QCM-D) measurements were used to investigate the adsorption of human fibrinogen, human serum albumin, bovine hemoglobin, horse heart cytochrome *c*, human immunoglobulin (hIgG), and 10% fetal bovine serum on supported bilayers of egg-phosphatidylcholine (eggPC) lipids. For comparison the adsorption of fibrinogen and hIgG to eggPC bilayers was also studied with surface plasmon resonance (SPR). The supported bilayers were formed *in situ* by vesicle adhesion and spontaneous fusion onto a SiO_2 surface. The supported lipid bilayer is highly protein resistant: The *irreversible* adsorption measured with the QCM-D technique was below the detection level, while *reversible* protein adsorption was detected for all the proteins in the range 0.3–4% of the saturation coverage on a hydrophobic thiol monolayer on gold. The adsorbed amounts were slightly higher for the SPR measurements. Possible mechanisms for the protein resistance of eggPC bilayers are briefly discussed. © 2002 Elsevier Science

Key Words: **protein resistance; protein adsorption; lipid bilayer; supported biomembrane; hIgG; HSA; Cyt c; bov Hb; fibrinogen; fetal bovine serum.**

INTRODUCTION

Proteins are, after water and low-molecular-weight ions, the first molecules to come in contact with an artificial surface inserted into a biological system (1). Situations where this occurs are, e.g., medical implant surfaces in tissue, a ship's body in seawater, tubes and tanks in the food and biochemical processing industry, and biosensors/biochips used for medical diagnostics or process control. In these, and other similar situations, there is a need to control the nature of the protein-surface interactions; either to prevent adsorption (biofouling) or to specifically and selectively affect the amount, type, and function of proteins adsorbed out of a solution. In some applications, such as, e.g., biosensors and cell attachment, control of the spatial distribution of surface/interface-bound proteins is also desirable.

Protein-resistant surfaces are of considerable interest in this broad field. They are interesting because of their practical value, in preventing biofouling, e.g., *in vivo* on the walls of artificial blood vessels or on materials in the marine environment. They are also of basic science interest; for example, if one understands the surface-related properties/mechanisms constituting protein resistance it will also help to clarify the poorly understood general phenomenon of protein adsorption.

One attractive route to achieve surfaces that resist protein adsorption or promotes specific immobilization of proteins, structurally and functionally unaffected by the solid support, is to use (or mimic) supported phospholipid bilayer membranes (2, 3). Such membranes, e.g., the ones surrounding living cells, are chemically relatively passive and become biologically active primarily through membrane-bound molecules such as proteins, inserted into the membrane. Consequently they are likely to be passive toward protein adsorption ("protein adsorption" implicitly almost always means adsorption of water-soluble proteins). Lipid films at interfaces have actually previously been shown to be quite efficient in preventing protein adsorption (4–7), and have also been shown to limit cell attachment (8) (a fact that can probably be directly related to the low affinity to protein adsorption on the membrane; if proteins do not bind, cells will not find attachment sites). The actual mechanisms behind the protein resistance are, however, still poorly understood, although recent important experimental data have addressed, e.g., the role of the polarity of functional groups (9) interacting with proteins, surface wettability (10), and the role of the hydration layer of the protein-resistant surfaces (11). Recently the influence of surface polarizability and surface hydration layer thickness on the protein adsorption energetics was treated theoretically (12).

In the present work we investigate *in situ* formed supported phospholipid bilayers (SPBs) with respect to their reversible and irreversible (nonspecific) protein adsorption. The main method was the QCM-D technique (13–15) but complementary SPR measurements (16–18) were also performed for two proteins. We measured the adsorption tendency of a number of proteins on a supported phospholipid bilayer, composed of electrically neutral eggPC lipids in the liquid crystalline state, supported on a SiO_2 surface. We also measured the total amount of macromolecules adsorbed on the same surface from a solution of 10% fetal bovine serum (FBS) in buffer. The latter was motivated both because it is an interesting complementary study to the single protein adsorption measurements and because that solution is often used in cell culture studies.

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Our specific motivations, apart from the general ones above, are the following. (i) There is an interest in how low the protein adsorption can be on *in situ* prepared SPBs, e.g., for sensor development. (ii) We and others need this knowledge in studies of functionalized SPBs, with inserted active molecules in the SPB. For instance, specific binding to lipid films at interfaces has been proven efficient for protein 2-D crystal growth at both the air-liquid and solid-liquid interfaces (3, 19–22) (iii). Knowing the protein resistance quantitatively is of interest for correlation with cell culture studies on pure and "doped" SPBs ("doped" is used in the same sense as in semiconductor technology, i.e., the deliberate inclusion of a small amount of functional additive).

MATERIALS AND METHODS

Materials

Water in this work is equivalent to Milli-Q water (Millipore, Molsheim, France). It was used for the preparation of buffers, and for the final cleaning of QCM crystals and the measurement cell. QCM crystals (5 MHz) were purchased from Maxtek Inc. (Torrance, CA) and from Q-Sense AB (Göteborg, Sweden). SPR chips type Pioneer J1 were bought from Biacore AB (Uppsala, Sweden). All proteins and the egg yolk phosphatidylcholine (eggPC) were purchased from Sigma-Aldrich (Sweden) as well as tris(hydroxymethyl)aminomethane (Tris) and sodium chloride for buffers. Phosphatidylserine (PS) and bovine phosphatidylethanolamine (PE) were bought from Lipid Products, Surrey. Buffers will be referred to as "buffer pH 8.0" or "buffer pH 7.4." Both are 100 mM NaCl and 10 mM Tris and the different pH's were set by the addition of HCl. Fetal Bovine Serum was obtained from Gibco BRL. Other materials include silicon dioxide for electron beam evaporation (Balzers Process Systems, Sweden), octadecylmercaptan (98%, GC, Aldrich), *p.a.* grade solvents from Merck (chloroform, *n*-hexane, ammonia (25%), and hydrogen peroxide (30%)), ethanol (99.5% Kemetyl), and nitrogen gas (N48, Air Liquide, Sweden).

Preparation of SiO_2 Surfaces

The Maxtek QCM crystals were cleaned before surface preparation using the following procedure: Immersion in a 6 : 1 : 1 (vol/vol) solution of $H_2O : NH_3(25\%) : H_2O_2(30\%)$ at 70°C for 10 min followed by thorough rinsing with water and drying in a stream of nitrogen gas. The SiO_2 surfaces were either used as purchased from Q-Sense or prepared on Maxtek QCM crystals (provided with gold electrodes) by cleaning as described above followed by electron-beam evaporation of a thin adhesion layer of titanium (3 nm) and a thicker layer of SiO_2 (100 nm) on top. Similary, the SPR chips were cleaned as above (but at 60°C) before evaporation of 1 nm Ti and 30 nm SiO_2. Immediately before any measurement, the SiO_2-covered crystals or chips were UV/ozone treated for 2×10 min primarily to clean the surfaces from hydrocarbon contaminations. After 10 and 20 min they were rinsed with water and dried (N_2). Between runs, the crystals and chips were soaked in mild detergent and rinsed with water before UV/ozone treatment.

Preparation of Phospholipid Bilayers

The phosphlipid bilayers were formed *in situ* on SiO_2 surfaces as previously described (23) with the only exception that no dye-marked lipid was used. In short, small unilamellar vesicles (SUVs) of eggPC or a mixture of 50 : 45 : 5 mol% PE : PC : PS was formed in buffer pH 8.0 by sonication and centrifugation as described by Barenholz *et al.* (24). After mounting the QCM crystal or docking the SPR chip, the measurement chamber was filled with buffer pH 8.0 before exposure to the vesicle solution. When eggPC vesicles come in contact with the SiO_2-coated QCM sensor surface or SPR chip, they first adsorb as intact vesicles and then—when a sufficiently high surface coverage is reached—they transform spontaneously to a bilayer (23) (see Fig. 1). The temperature was held constant at 22°C during the

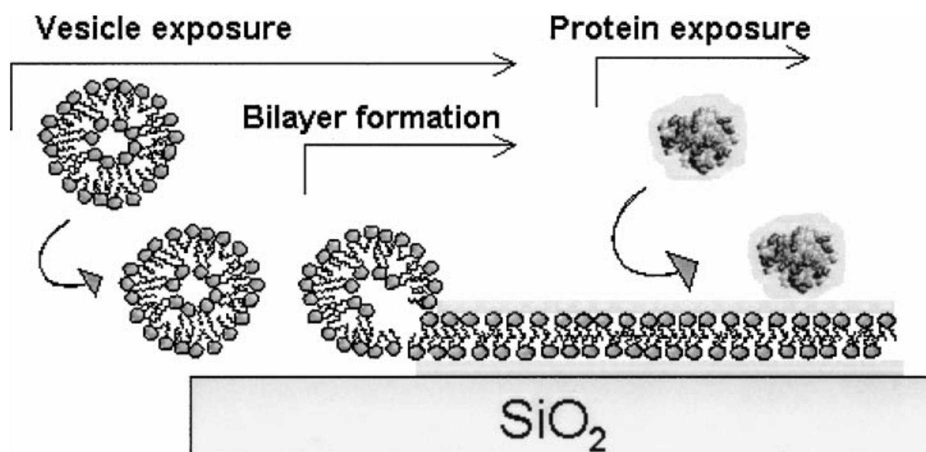

FIG. 1. Cartoon picture illustrating the measured sequence of events: vesicle adsorption followed by bilayer formation, and then protein exposure to the bilayer. The shaded areas indicate water shells.

experiments. After the formation of a bilayer on the surface, the system was rinsed with buffer pH 8.0 to check for bilayer stability before the protein adsorption experiment.

The QCM-D Technique

The sensing part of a quartz crystal microbalance (QCM) is a thin piece of AT-cut quartz crystal. This crystal can, thanks to its piezoelectric properties, be made to oscillate in thickness-shear mode at its very sharp resonant frequency. The resonant frequency, f, depends on the total mass of the system. A mass adsorbed on the crystal surface, from, e.g., a solution, is normally detected as a decrease in the resonant frequency, Δf. The QCM-D crystal can be driven at the fundamental frequency (in our case 5 MHz) or at an overtone. All data presented here were recorded at 15 MHz (i.e., the third overtone), for which a frequency shift of -1 Hz corresponds to a mass change of $\sim$6 ng/cm^2 according to the Sauerbrey equation (proportionality between mass and frequency change) (13). The detection limit of the present set-up, as determined from the noise level of the set-up, when operating at 15 MHz, is around 0.6 Hz. (For comparison, formation of a complete SPB measured at the same frequency causes a frequency shift of -78 Hz.) The QCM-D instrument used in the present work (Q-Sense AB, Göteborg, Sweden) simultaneously monitors a second important parameter, namely the damping, or the dissipation factor, *D*, of the oscillator. The dissipation measurements add qualitative and usually also quantitative information about the viscoelastic properties of the adsorbed layer (see, e.g., 14, 15) and allow for theoretical modeling of the QCM-D response (25, 26). In brief, the dissipation is generally high if the adsorbed film has a high viscous component. Such a film deforms more easily and dissipates the stored energy in the oscillator due to internal friction during the cyclic deformation at the atomic scale. It is also important to point out (see, e.g., 22), that the QCM-D senses both the mass of the adsorbed film *and* the mass of any water coupled to the system, such as buffer inside intact vesicles on the surface and trapped water in the adsorbed film or water shells around proteins adsorbed on the surface. In addition, the QCM-D is also sensitive to viscous changes in the bulk liquid in contact with the crystal.

For the conversion of frequency shifts to mass change, these factors must be considered. We have recently shown that for thin ($\sim<$25 nm), but very dissipative films ($|\Delta D/\Delta f|)\Delta 0.2 \times 10^{-6}\,\mathrm{Hz}^{-1}$), the mass (or thickness) can in fact be underestimated by a factor of up to 2 (26). Comparing the ratios of $|\Delta D/\Delta f|$ for the protein films studied in the present work, such underestimations can, for all proteins, be estimated to be less than $\sim$10%. Under the assumption that the adsorbed proteins do not slip on the surface (see below), the Sauerbrey equation is thus a fair approximation in the present case.

The measurements were conducted in a temperature-stabilized chamber with a volume of $\sim$0.2 ml, previously described in (27) or in a measurement chamber from Q-Sense AB. It includes a small container at the top of the measurement chamber, for temperature stabilization of the solution to be injected in the chamber. All injections are made by opening a valve between the container and the chamber, which allows a gravitationally driven rapid ($<$3 s) exchange of different solutions.

The SPR Technique

All SPR measurements were performed using a BIAcore 2000 (Biacore AB, Uppsala, Sweden). The BIAcore system includes automated handling of the buffer, vesicle, and protein solutions, flow rate, and temperature during measurement. The BIAcore system uses four very small ($50 \times 500\,\mu$m $\times$ 2.4 mm) silicone measurement cells that seal directly against the surface of the chip. All of the data presented here were taken at a flow rate of 30 μl/min and in multichannel mode with an injection volume of 325 μl.

A detailed account of the BIAcore system can be found in Refs. (16, 17). In brief, the SPR technique measures changes in refractive index within $\sim$230 nm of the surface of the chip. These changes are reported in response units, RU, a dimensionless quantity that is proportional to the change in refractive index, Δn, at the interfacial region. A change of 10 RU corresponds to the adsorption of 0.92 ng/cm^2 of eggPC (28) or 0.66 ng/cm^2 of proteins on a flat surface (16–18).

Protein Adsorption

All proteins were dissolved in buffer pH 7.4. After the formation of a phospholipid bilayer, a couple of rinses was performed with buffer pH 7.4 to get the proper environment for the proteins. In the case of SPR measurements, the buffer (pH 7.4) flows constantly after the injection of protein solution has ended. The final concentrations of proteins used were 0.5 mg/ml for human serum albumin (HSA), horse heart cytochrome *c* (Cyt c), and bovine hemoglobin (bov Hb) while it was 0.25 mg/ml for human immunoglobin (hIgG) and fibrinogen. The adsorption of macromolecules from a more complex solution was also studied with the QCM-D: buffer pH 7.4 containing 10% FBS.

RESULTS AND DISCUSSION

QCM-D Measurements

A typical complete QCM-D experiment, including (i) bilayer formation on SiO_2, followed by (ii) change of buffer and (iii) exposure to a protein solution, is shown in Fig. 2. At $t = 0$ s, the pure SiO_2 surface is exposed to the vesicle solution, resulting in a rapid and large decrease in frequency, f (mass uptake due to vesicle adsorption), and increase in energy dissipation, D. A minimum in f and a maximum in D are reached at $\sim$40 s, which is (approximately) the coverage at which adsorbed intact vesicles start to fuse and transform into a bilayer (23, 29, 30). Before the maximum/minimum, f increases due to the mass load of the adsorbed, intact vesicles, and D increases due to the high internal energy dissipation in the soft vesicles, subject to the oscillatory shear motion of the sensor surface. As the vesicles fuse to a bilayer (at $\sim 40 < t < 250$ s), they effectively loose mass since

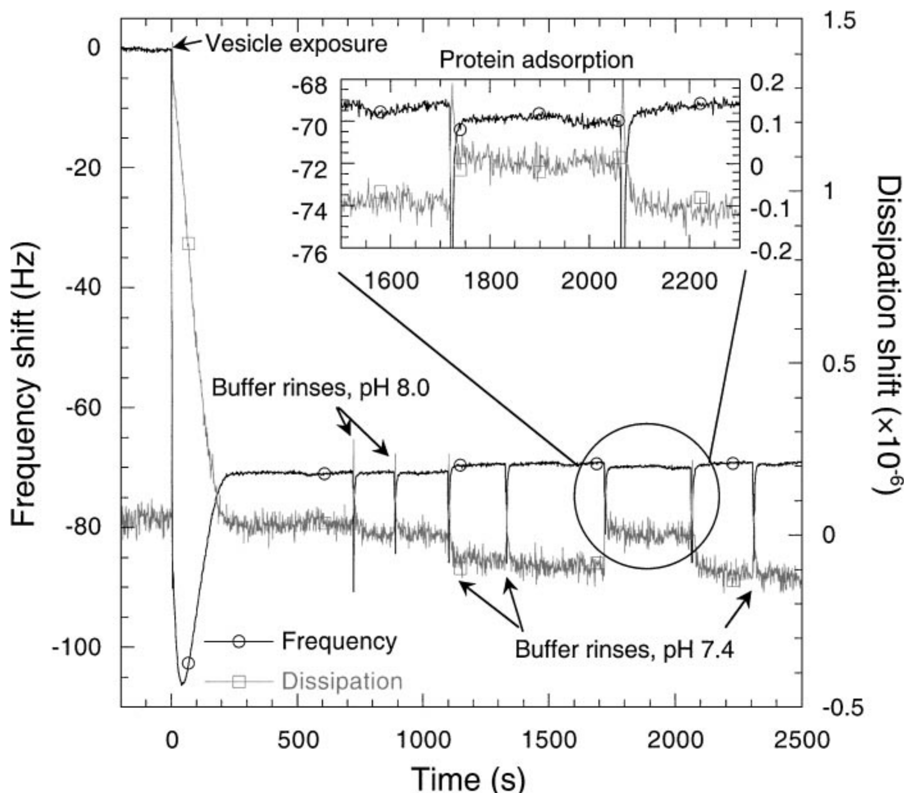

FIG. 2. Changes in frequency, Δf (right axis), and dissipation, ΔD (left axis), of the QCM-D as a function of time, due to (i) *in situ* bilayer formation, followed by (ii) buffer exchange and (iii) protein (fibrinogen) exposure: First the SiO_2 surface is exposed to eggPC SUVs which at low coverage adsorb intact on the surface (large frequency decrease and large dissipation increase). Around the minimum in frequency/maximum in dissipation, the vesicles transform to a bilayer, which eventually is completed around 250 s. All other events in the figure are buffer rinses. (See text for details.) The actual protein exposure begins at 1720 s and is shown at higher magnification in the inset. Only a small frequency change is detected, reflecting the high protein resistance of the bilayer.

the trapped water inside the vesicles is lost. Simultaneously the adlayer becomes more rigid, and consequently the dissipation goes down. This part of the experiment has already been reported and subjected to a detailed analysis (23, 29, 30). In this work we investigate the protein adsorption properties of the SPB formed in this way.

At ∼1100 s in Fig. 2, the buffer solution is changed to buffer pH 7.4 which is the one used in the subsequent protein adsorption experiment.

At ∼1720 s, the supported bilayer is exposed to a 0.25 mg/ml fibrinogen solution (magnified in the inset of Fig. 2). The addition of the protein solution results in a quick, small change in the frequency which stabilizes at about −1 Hz, and a simultaneous increase in D of about 1.1×10^{-7} (mean value of three runs: 1.3×10^{-7}); see inset of Fig. 2. At about 2060 s the protein exposure is interrupted by exchange to pure buffer pH 7.4. All other spikes are transients arising from buffer rinses.

The inset of Fig. 2 shows a magnification of the fibrinogen exposure and subsequent rinsing period. The difference in frequency before protein exposure (at 1720 s) and after rinsing (at 2060 s) is not experimentally significant since the short-term (<10 s) and longer-term (>100 s) fluctuations/drift for the present experiment were about 0.2 and 0.6 Hz, respectively. We thus conclude that there is no measurable *irreversible* adsorption of fibrinogen. The measured frequency shift of −1 Hz upon protein exposure corresponds to a small, *reversible* amount of weakly adsorbed proteins, since the frequency returns to the original value upon rinsing. The reversibly adsorbed amount is 0.5, 4, <0.8, <2, and <0.3% of the saturated coverage of hIgG, Cyt c, bov Hb, HSA, and fibrinogen, respectively, on a methyl-terminated monolayer on gold. Since fibrinogen is a protein known to have very high affinity to most surfaces these results signal a very protein-resistant SPB surface.

The same experiment as performed with fibrinogen, shown in Fig. 2, was performed with several other proteins (Bov Hb, HSA, hIgG, and Cyt c) and with 10% FBS (see below). For comparison two additional experiments were performed, one with Cyt c on a modified SPB and one with Cyt c on a methyl-terminated thiolated gold surface. The upper three panels in Fig. 3 show, from top to bottom, typical uptake runs for fibrinogen, hIgG, and Cyt c on the SPB. The corresponding reversible and irreversible frequency shifts are, respectively, for hIgG ≈ 1 and <0.6 Hz and for Cyt c ≈ 1 and <0.6 Hz. (The irreversible adsorption is not experimentally significant; see below.) The fourth panel

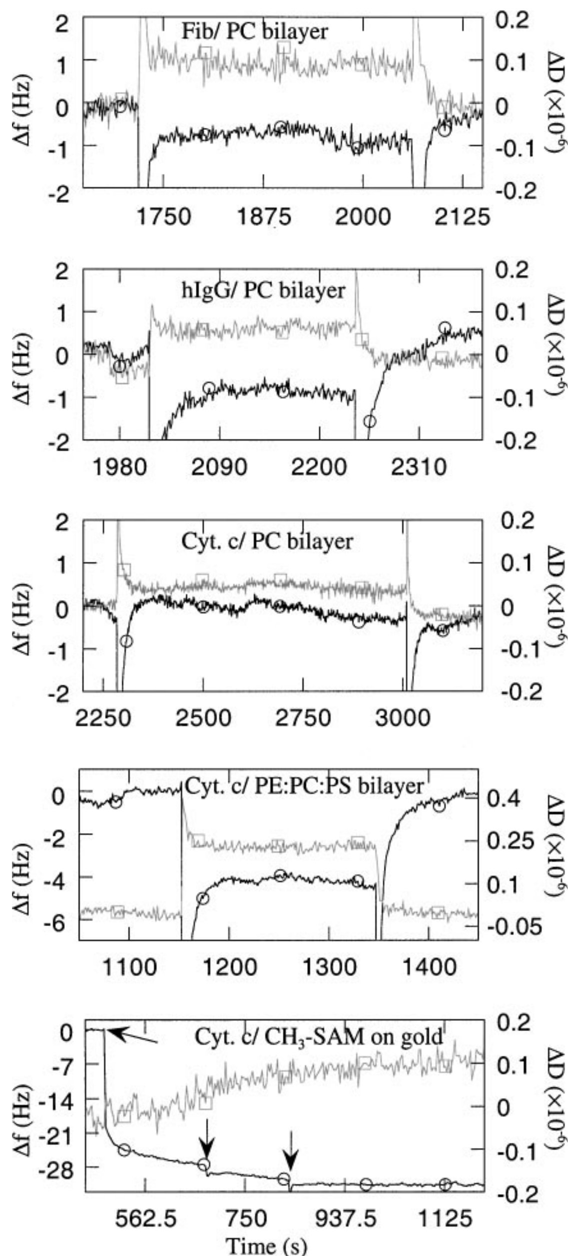

FIG. 3. The upper three panels in Fig. 3 show, from top to bottom, typical uptake runs for fibrinogen, hIgG, and Cyt c on the pure eggPC SPB. The fourth panel from the top shows Cyt c adsorption on a bilayer containing about 5% of negative lipids (50 : 45 : 5 mol% PE : PC : PS). The bottom panel shows the adsorption of Cyt c on a methyl-terminated thiol monolayer on gold. The arrows indicate injections of a protein solution of 50 μg/ml.

from the top shows Cyt c adsorption on a bilayer that was modified to increase the adsorption capacity of Cyt c (31) by adding about 5% of negative lipids (50 : 45 : 5 mol% PE : PC : PS). This lipid composition was chosen to make the bilayer more active for protein adsorption; it is a mimic of the mitochondria membrane, with which Cyt c is naturally interacting. In this case the reversible adsorption is considerably higher (≈6 Hz). The dissipation shift is larger than in the other cases (mean value of three runs: 2.4×10^{-7}). The irreversible adsorption is still insignificant.

Obviously this lipid composition causes a somewhat stronger, but still only reversible protein binding. The bottom panel in Fig. 3 shows, for comparison, the f and D shifts measured for Cyt c on a methyl-terminated thiol monolayer on gold (prepared as described in (23)), in a 50 μg/ml solution. In this case the total uptake at saturation, interpreted as a full monolayer of irreversibly bound Cyt c, causes a frequency shift of ∼31.5 Hz, i.e., much larger than on the SPB. The dissipation shift is 1.1×10^{-7}, about twice that on the pure eggPC SPB.

All QCM-D reversible and irreversible frequency shifts measured on the SPBs are summarised in Table 1 (mean values from 3–4 runs with each protein on freshly prepared SPBs). In no case is there an experimentally significant irreversible uptake. The reversible uptakes are above the detection level, only for hIgG and Cyt c and for the Cyt c on the bilayer composed of 50 : 45 : 5 mol% PE : PC : PS.

These measurements with single proteins were complemented with an experiment using the 10% FBS protein mixture solution. Also in this case the irreversible protein adsorption was below the detection limit, while the reversible frequency shift was about twice as large as for Cyt c adsorption on the mixed bilayer.

In summary for the QCM-D results we find that the SPB is remarkably protein resistant. None of the proteins explored cause an irreversible deposition of proteins on the SPB. The reversible adsorption is typically in the percentage of a monolayer range for the used (quite high) protein concentrations and the proteins desorb very quickly upon rinsing, demonstrating the weak binding to the SPB. The modified bilayer binds about 5 times more Cyt c than the pure eggPC SPB, which is attributed to a somewhat stronger (but still reversible) binding to the presence of 5% negative phospholipids. The relatively large reversible frequency shift for the FBS solution does not mean that the number density (proteins per unit area) is larger than for, e.g., Cyt c on the mixed bilayer, since it is likely that heavy proteins adsorb preferentially due to larger van der Waals interaction (cf. the Vroman effect) out of the FBS protein mixture, causing a larger frequency shift per protein.

Comparison with SPR Measurements and Literature Data

We also performed SPR measurements for two of the studied proteins, hIgG and fibrinogen. The SPR measurements differ from the QCM-D measurements primarily through a different cell geometry and flow conditions. In addition, the gold films under the SiO_2 layer were evaporated in different systems.

The measured reversible/irreversible uptakes are shown on the second row of Table 2. In order to compare the QCM-D and SPR data we need to convert the observed frequency shifts to mass per unit area. This was done using the Sauerbrey relation, well aware of its limitations (see our discussions in, e.g., 27). Two comments are particularly important in the present context: (i) based on our earlier published work and recent

TABLE 1
Frequency Shifts in Hz (QCM-D at 15 MHz) for the Adsorption of Different Proteins on eggPC Bilayers

QCM-D	hIgG	Fibrinogen	HSA	bov Hb	Cyt c PC	Cyt c mixed[a]	10% FBS
Reversible (Hz)	-1.1 ± 0.2	<0.6	<0.6	<0.6	-1.2 ± 0.8	-5.8 ± 0.4	-11.5 ± 0.4
Irreversible (Hz)	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6

Note. All numbers are given as the mean of 3–4 runs ± standard deviation. The protein concentrations were 0.5 mg/ml for HSA, Cyt c, and bov Hb and 0.25 mg/ml for hIgG and fibrinogen.

[a] Mixed denotes a SPB with the lipid composition 50 : 45 : 5 mol% of PE : PC : PS.

quantitative comparisons among QCM-D, SPR, and ellipsometry (26) and among QCM-D, ellipsometry and OWLS (32), we know that QCM-D generally yields *higher* mass uptake values than the optical technique by 1.5–3 times for proteins, because the QCM-D measures also the bound water in the protein adlayer (hydration shell and trapped water). (ii) Although not observed hitherto in any of our previous protein adsorption experiments, there is a possibility that proteins that slip on the surface during the shear motion of the sensor surface cause a reduction in mass sensitivity.

Comparing the frequency-to-mass converted QCM-D data with the SPR data (Table 2 rows 1–2) we find the same order of magnitude values but significantly smaller uptakes for the QCM-D. This difference is amplified if we take into account the just-noted overestimation of the uptake by QCM-D due to hydration water. There are two possible explanations for the difference. The first one is that we might, in principle, have a case of slip; i.e., the associated proteins do not couple fully to the oscillating sensor surface. This would be both remarkable and interesting, since slipping requires very special conditions (33). At present we cannot entirely exclude slip, although we regard it quite unlikely that the protein would slip on the surface, given the high amount of possible attachment points between the protein and the lipid bilayer.

A more likely cause is the differences with regard to SPB preparation in the SPR and the QCM-D experiments. We frequently note that the quality of the SPBs are quite sensitive to the preparation conditions. In the QCM-D system there is a more direct injection of the vesicle solution to the sample, compared to the polymer-based micro-fluidics system in the SPR equipment used in this work, and we believe this affects the quality of the SPB. Furthermore we can in the QCM-D system directly from the shape and quantitative values of the f and D shifts judge the quality of the SPB preparation. This is not the case with the SPR curve, which contains less information (29).

To put our results into the context of earlier measurements on protein-resistant surfaces we compare them in Table 2 with ellipsometry results reported by Malmsten (5), for spin-coated PC (third row in Table 2), and for PC triple layers on hydrophobic surfaces made by Langmuir-Blodgett deposition (5) (fourth row in Table 2). For fibrinogen the results are quite consistent with ours, and it is the SPR results that stick out as somewhat high. For hIgG the uptake detected by ellipsometry is largest followed by SPR and QCM-D. However, it should be emphasized that these differences, which between themselves are considerable, are for very small adsorbed amounts that generally are around or below a few percent of complete monolayer coverage, and only for data taken prior to rinse (i.e., they include reversible adsorption). We believe that the major factor influencing the data spread is a combination of the inherent spread in each technique, working relatively close to the sensitivity limit, and the perfectness of preparations, i.e., how one can avoid defects in the adlayer preparations. In our view the result with the mixed bilyer (PE : PC : PS) supports this hypothesis; the negative phospholipids act as local binding sites in the matrix of the pure eggPC SPB.

TABLE 2
Compilation of Data from the Literature Together with Our Results

Surface[a]	Technique	hIgG	Fibrinogen	HSA	bov Hb	Cyt c PC	Cyt c mixed[b]	10% FBS	Ref.
SPB	QCM-D	6/<4	< 4/<4	< 4/<4	< 4/<4	7/<4	34/<4	69/<4	This work
	SPR	11/4	18/11	-/-	-/-	-/-	-/-	-/-	This work
SC	Ellipsometry	20/-	5/-	30/-	-/-	-/-	-/-	-/-	(5)
LB	Ellipsometry	-/-	8/-	26/-	-/-	-/-	-/-	-/-	(5)

Note. The values are given in ng/cm^2 as "prior to"/"after" rinse as detected by the given techniques. The first two rows show the present work. These results were extracted from 3 to 4 measurements per protein on *in situ* made PC bilayers. The QCM-D values of Table 1 were converted to mass using the Sauerbrey equation. The standard deviation of the values obtained by SPR was $\leq 11\%$.

[a] Surface: all surfaces listed are PC-lipid surfaces. SPB, supported planar bilayer on SiO_2; SC, spincoated PC on hydrophobic silica; LB, Langmuir-Blodget deposition of three lipid layers on hydrophobic silica.

[b] Mixed denotes a SPB with the lipid composition 50 : 45 : 5 mol% of PE : PC : PS.

Discussion

The most important result of the present study is the lack of measurable irreversible and very small reversible protein adsorption for several proteins on *in situ* formed supported phospholipid bilayers. Even these small amounts may be governed by defects so our values should be regarded as upper limits for a perfect SPB. Such surfaces are useful for a variety of experiments and practical situations where resistance to protein adsorption is desired, as discussed in the introduction. It also provides a basis for controlled protein adsorption on top of the bilayer by incorporating modified, more active groups for protein adsorption in the bilayer (34).

One reason for the highly protein-resistant bilayers obtained in this work may be that all measurements were performed with freshly deposited bilayers with *in situ* control on the preparation, via the typical f and D traces signifying "good" preparations. It is well known that supported lipid bilayers are destroyed if they come in contact with air (35). We also know that their quality is critically influenced by the cleanliness of the SiO_2 surface.

It has been suggested that one reason for the protein resistance of PC lipids is the zwitterionic nature of the headgroup, which makes it electrically neutral in a large pH range ($3 < \mathrm{pH} < 10$) (4). It is known from the literature that a supported PC bilayer on glass or silica binds a certain amount of water to the lipid head groups facing the solution (36, 37). The latter is another candidate reason for the protein resistance of PC head groups (4). However, comparing lipid bilayers on SiO_2 with polyethylene oxide-terminated SAMs, which are also hydrophilic and protein resistant (10), an important difference may be that the molecules of a SAM are covalently bound to the surface, while the lipids in a bilayer are generally free to diffuse laterally on the surface as long as the temperature is above the gel-to-liquid crystalline transition temperature of the lipids. According to literature, the lateral mobility of lipids in supported membranes depends strongly on the way by which they have been associated to the surface (38, 39). In an earlier study from our group, FRAP (fluorescence recovery after photobleaching) was used to show that the lipids of the bilayers used in this work have high lateral mobility (23).

The current atomic level understanding of protein adsorption and resistance to protein adsorption is still at a rudimentary stage. It is, however, clear through recent systematic experimental work and earlier experimental documentation that certain hydrophilic surfaces are quite protein resistant (5, 9–11, 40–42). Consequently, the bonding and structure of water at the surfaces, with which proteins interact, are important. (Note, though, that hydrophilicity alone is definitely not enough to achieve protein resistance (see, e.g., 10.) We have recently theoretically explored some basic aspects of protein resistance by considering the van der Waals interaction for various cases of surface polarizibility, thickness of separating water layers, etc. (12). The groups of Grunze and Whitesides (9–11, 40) have addressed these issues in several recent papers both experimentally and theoretically, and on a more qualitative level this has been discussed long ago by several authors (4, 42).

The picture emerging is that a strongly bound water layer on a surface with otherwise low polarizability, and with a high mobility/flexibility of functional groups, is a strong candidate for protein resistance. The PC head groups of eggPC serve this water-binding function on the surface. The contribution from van der Waals interactions will be weak, if the rest of the bilayer close to the proteins has very low polarizability, which is the case for the hydrocarbon chains of the lipid molecules. Furthermore, as noted above, the lipids in the bilayer are quite mobile. It appears that the SPBs nearly ideally produce this combination of a strongly bound water spacer, a low polarizability of the inner part of the bilayer, and a lateral mobility of the monomers.

SUMMARY

Supported eggPC bilayers, formed with *in situ* kinetic control of the bilayer formation, are highly protein resistant ($\leq$ one or a few percent of a monolayer). This result is tentatively attributed to the water structure on top of such layers, the mobility of the lipids in the layer, and the low polarizibility of the bulk of the bilayer.

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