Published in final edited form as:

Colloids Surf B Biointerfaces. 2010 December 1; 81(2): 607–613. doi:10.1016/j.colsurfb.2010.08.008.

EXCESS FIBRINOGEN ADSORPTION TO MONOLAYERS OF MIXED LIPIDS

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Abstract

Adsorption of fibrinogen to the monolayers of mixed lipids, dipalmitoyl phosphatidyl choline (DPPC) and eicosylamine (EA) was measured at a surface pressure of 20 mN/m by an *in situ* surface plasmon resonance technique. Pressure-area isotherms of DPPC+EA mixtures on water and buffer subphases indicated good lipid miscibility and some contraction of the monolayers at intermediate and higher surface pressures. Surface electric potential of the DPPC+EA monolayers showed excess values for intermediate DPPC:EA ratios. Fibrinogen adsorption and its adsorption rates from a dilute solution (0.03 mg/ml) were proportional to the fraction of EA in the monolayer indicating that protein binding was primarily driven by electrostatic interactions between positive EA charges in the monolayer and a net negative protein charge. At a higher protein concentration (0.06 mg/ml) both the fibrinogen adsorbed amount and its maximum adsorption rate showed excess values relative to the pure EA for 1:1, 2:1 and 3:1 DPPC+EA monolayers. This excess adsorption could be explained, in part, by the contraction of the monolayers with intermediate DPPC:EA ratios which resulted in an excess surface electric potential.

Keywords

fibrinogen; adsorption; mixed lipids monolayer; SPR; surface potential

Introduction

Interactions between blood plasma proteins and lipid membranes are important because they are believed to play a pivotal role in blood coagulation [1]. One of the major protein in the coagulation is fibrinogen, a large (340 kDa) circulating protein present in blood at concentration of 2.0–4.0 mg/ml. The molecule has an elongated dimeric structure with two sets of three polypeptide chains [2]. The platelet integrin receptor $\alpha\text{IIb}\beta 3$ recognizes and binds fibrinogen from solution only after the receptor is activated by agonists such as ADP or thrombin [3–5].

Adsorption of fibrinogen to biomaterials is known to result in platelet adhesion and activation [6–8] indicating that, if fibrinogen is adsorbed to a surface, inactive platelet integrins will bind to it, leading to platelet adhesion and activation [9–11]. However, the non-specific interaction between fibrinogen and cell membranes are less well understood. It is known that the sequence:

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non-specific binding to cell membrane -> 2-D diffusion -> receptor encounter and binding, increases the probability of finding the receptor compared with pure diffusion to capture from solution [12]. Fibrinogen binding to and 2-D diffusion on the platelet membrane may increase the probability of fibrinogen - integrin receptor encounter and subsequent platelet activation.

Lipid monolayers at the air/water (A/W) interface often serve as *in vitro* mimics of biological membranes [13,14]. Laboratory studies using lipid monolayers at the A/W interface of a Langmuir trough can be performed under well-defined experimental conditions that allow for the control over the interfacial microstructure [15]. The advent of analytical surface sensitive techniques such as surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) has motivated us to combine these techniques with the Langmuir trough and thus measure molecular events occurring at the monolayer-solution interface *in* and *ex situ* [16,17]. For example, we have used *in situ* SPR to monitor the efficacy of packing in poly(ethyleneoxide) containing monolayers to suppress protein binding [16]. We have also used *ex situ* QCM to measure protein binding to lipid monolayer mixtures at the A/W interface [17] and assess the potential of protein-monolayer interactions for imprinting the interaction images of protein molecules into the mixed lipids monolayers [18].

The aim of this study was to elucidate the effects that a composition of two lipids in the monolayer have on fibrinogen adsorption. For any mixture of lipids in a monolayer, the expected values of lipid mixture properties, such as mean molecular area, MMA, or surface electrical potential difference, $\Delta\psi$, can be estimated in the first approximation by applying the addition rule to the properties of the pure lipid components. Often, a mixture of lipid components shows a deviation of the observed values from such addition estimates and this can then be used to determine the molecular state of the monolayer [19,20]. In a very similar manner, protein binding to the interfaces with mixed chemical characteristics sometimes differ from the addition estimates obtained from the binding of the same protein to uniform surfaces [21].

This paper describes the adsorption of fibrinogen (Fgn) to the monolayers of mixed lipids, dipalmitoyl phosphatidyl choline (DPPC) and eicosylamine (EA), at the A/W interface measured by *in situ* surface plasmon resonance (SPR). The initial hypothesis was that Fgn adsorption to positively charged EA + zwitterionic DPPC monolayers is driven by the electrostatic interactions between EA and Fgn, and therefore proportional to the fraction of EA in the monolayer, χ_{EA} .

Materials and methods

1,2 dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, Avanti Polar Lipids) was used without further purification to prepare 1 mM solutions in HPLC-grade, acid-free chloroform. Eicosylamine (EA, EGA-Chemie), was purified by re-crystallization and reconstituted in acid-free chloroform. All lipid solutions, stored in sealed glass vials at -20°C , were warmed to room temperature prior to their use. Binary mixtures of lipids DPPC+EA were prepared from DPPC and EA solutions in molar ratios (DPPC:EA) of 1:1, 2:1, 3:1, 4:1, 5:1 and 6:1. All the lipid mixtures were prepared prior to each experiment to minimize the effects of degradation in storage.

Human fibrinogen (Fgn, plasminogen-free, Sigma) was used without further purification. Fgn solution was prepared in 10 mM phosphate buffer (PB, no NaCl), pH 7.4, and was filtered through a $0.22\ \mu\text{m}$ filter under positive pressure to avoid foaming. The solution was then dispensed into smaller aliquots for single use, and was stored at 4°C . Prior to use, the solution was warmed to 25°C in a water-bath. Fibrinogen solutions were consumed within 1 week of preparation.

For lipid-miscibility studies, surface pressure – mean molecular area (π -MMA) isotherms were obtained for DPPC, EA, and their binary mixtures using a Langmuir trough (Minitrough-2, KSV Instruments Ltd., Finland). The trough had a centrally positioned dipping well and was used in conjunction with Pt Wilhelmyplate film balance with a sensitivity of 0.004 mN/m. The instrument was housed in an opaque enclosure to minimize air-currents and reduce interference from external light sources during SPR measurements. The trough temperature was maintained at 25°C. Surface potential, $\Delta\psi$, was measured in parallel with the surface pressure using a Kibron Micro-Trough X with a vibrating capacitor surface probe (Kibron Inc., Finland). Surface potential of 0.01 mM PBS was taken as zero reference point.

SPR studies were performed using a SPR system (SPREETA®, Nomadics) with a 12-bit, single-channel DSP controller. To make the SPR sensor gold surface reactive and hydrophilic, it was first cleaned in oxygen plasma for 1 minute and then immersed in a 1 mM octadecanethiol (ODT) in ethanol solution for 30 min to render it hydrophobic. The SPR sensor was attached to a mechanical assembly, which lowered the sensor with precision onto the surface of Langmuir trough. Fibrinogen adsorption studies involved preparing the lipid monolayer by holding the trough barriers in the feedback mode to maintain a desired surface pressure, followed by lowering the ODT-modified SPR sensor onto the lipid monolayer (Fig. 1a). Each lipid monolayer was compressed to a surface pressure of 20 mN/m at 25°C on 10 mM PB pH 7.4 subphase. To minimize the adsorption reaction volume, a 2-inch diameter polystyrene Petri dish connected to a movable mechanical arm was raised up slowly through the film held at a target surface pressure. The dish sequestered a smaller area of the monolayer around the SPR sensor. After the SPR sensor was lowered onto the monolayer, the recording of the changes in refractive index was initiated. Fibrinogen was injected into the subphase through the monolayer and its adsorption kinetics was measured under stirring conditions for 30 minutes from either 0.03 mg/ml or 0.06 mg/ml solution by continuously monitoring the changes in the refractive index. An actual SPR trace recorded for the SPR-Langmuir trough experiment, starting with SPR sensor calibration and ending with protein binding segment is shown Fig. 1b.

Results

Lipid miscibility

Lipid miscibility in the two-component-lipid monolayers was determined from pressure-area (π -MMA) isotherms measured at 25°C both on the deionized double-distilled (DDD) water as well as on the 10 mM PBS pH 7.4 subphases. Figure 2a shows the π -MMA isotherms of DPPC, EA and their mixtures on DDD water subphase. The isotherms of pure lipids, DPPC and EA, were in agreement with those published in the literature [22], while those of the DPPC+EA mixtures were intermediates of the two pure lipid isotherms both in shape and in position. The π -A isotherms of the DPPC+EA mixtures displayed a higher collapse pressure than either DPPC or EA, which was an indicator of the monolayer stability and high miscibility between DPPC and EA in the mixtures.

The ideal or additive mean molecular areas (MMA) for the lipid mixtures were calculated by linear extrapolation between the pure DPPC and EA π -MMA isotherms at four different surface pressure values (10, 20, 30 and 40 mN/m). Miscibility diagrams (i.e., isobars) were constructed as the difference (Δ MMA) between the additive MMA estimate and the experimental MMA value. The Δ MMA is plotted in Fig. 2b as a function of monolayer EA fraction, X_{EA} , to yield a miscibility diagram. Maximum negative Δ MMA values were observed at low surface pressure of 10 mN/m. The MMA differences were smaller at higher surface pressures. The fact that the mixtures had lower MMA than the individual lipid components was taken as evidence of non-additive but intimate mixing of the DPPC and EA lipids in the binary monolayers on the water subphase.

Isotherms of DPPC, EA and their mixtures obtained on 10 mM PB pH 7.4 subphase are shown in Fig. 3a. A comparison with the isotherms measured on the DDD water subphase revealed that each isotherm on PB was shifted to larger areas, indicating that corresponding MMAs were larger for the pure lipids as well as for the DPPC+EA mixtures on PB. The mixtures also exhibited higher collapse pressures. The miscibility diagram (Fig. 3b) showed that the Δ MMAs were highly dependent on surface pressure. At low surface pressures, Δ MMAs were larger and, unlike the deviation obtained on water subphase, positive. Upon the increase of surface pressure, the MMA differences transitioned from positive to negative and became progressively more negative at higher surface pressures.

The surface potential-area ($\Delta\psi$ -MMA) isotherms of DPPC, EA and DPPC+EA monolayers were recorded simultaneously with the surface pressure-area (π -MMA) isotherms on 10 mM PB subphases. The ψ -MMA and π -MMA data were first used to construct π - $\Delta\psi$ isotherms and the $\Delta\psi$ - X_{EA} isobars were then calculated at the four different surface pressures as was done for Δ MMA calculation. The isobars were used to find the difference of surface potential between addition estimates for each DPPC+EA mixture, $\Delta\Delta\psi$. This, so-called “excess” surface potential, $\Delta\Delta\psi$, is plotted as a function of monolayer EA fraction, X_{EA} , in Fig. 4. The lipid mixture monolayers, 1:1, 2:1 and 3:1 (DPPC:EA), showed the greatest excess surface potential that was even more pronounced at lower surface pressures, where the monolayers were less compact than at higher surface pressures.

Fibrinogen adsorption

The Fgn binding kinetics from 0.03 mg/ml solution on the monolayers of DPPC, EA, and their mixtures are shown in Fig. 5a. The Fgn adsorption, measured by the refractive index changes, is plotted against time, t , with $t = 0$ being defined as the moment of protein injection. The highest Fgn adsorption was observed on the pure EA monolayer, while the lowest adsorption was observed on the pure DPPC monolayer. Fgn adsorbed amount decreased monotonously for all lipid mixtures with increasing DPPC:EA ratio with the least amount of protein bound to the 6:1 DPPC:A mixture, close to that observed for the pure DPPC monolayer. To further elucidate the effect of the lipid composition on Fgn adsorption, the rate of refractive index change, $d(\Delta RIU)/dt$, (i.e. parameter proportional to the adsorption rate) was plotted as a function of the refractive index change, ΔRIU (Fig. 5b).

Fig. 6a shows Fgn adsorption kinetics from the solution concentration of 0.06 mg/ml. In contrast to the adsorption from the solution of 0.03 mg/ml (Fig. 5a) Fgn adsorption to mixed lipids monolayers of 1:1 and 2:1 DPPC:EA exceeded the adsorption to pure EA monolayer. Fgn adsorption was lowest on the 6:1 DPPC:EA mixture and on pure DPPC monolayer, similar to what was observed at the Fgn concentration of 0.03 mg/ml. The respective rates of refractive index change, $d(\Delta RIU)/dt$, plotted as a function of the observed refractive index change, ΔRIU , are shown in Fig. 6b.

Discussion

Pressure-area isotherms of monolayers of DPPC, EA and DPPC+EA mixtures on DDD water subphase indicated high lipid miscibility (Fig. 2). All Δ MMAs were negative with the highest values ($\sim 10 \text{ \AA}^2$) observed at the surface pressure of 10 mN/m. This contraction of the two lipids in the mixtures is evidence of ideal, albeit non-additive, mixing of DPPC and EA molecules on the water subphase. The greatest deviation from linear mixing was for $X_{EA} \sim 0.14$ to 0.16 at all four surface pressures, indicating that this is the ratio for the most efficient packing of the relatively large, zwitterionic DPPC and the more compact cationic EA. DPPC exhibits a rich phase behavior with a liquid expanded to liquid condensed plateau that begins as the surface pressure approaches 10 mN/m (Fig 2a). This explains why the largest Δ MMA value of approx $-10 \text{ \AA}^2$ was observed at the surface pressure of 10 mN/m since this surface

pressure corresponds to a first order phase transition that allows for the change in MMA without corresponding change in surface pressure.

In contrast to the DDD water subphase, Δ MMA were greater in magnitude on 0.01 mM PB subphase and also *positive* at the surface pressure of 10 mN/m indicating an expansion of the monolayers under these conditions (Fig. 3). This is highly intriguing because this low surface pressure behavior is exactly opposite of that observed on the water subphase. At higher surface pressures Δ MMA became negative and also large in magnitude; for example Δ MMA = $-20 \text{ \AA}^2$ at $X_{\text{EA}} = 0.25$, and $\pi = 40 \text{ mN/m}$. Thus on PB subphase the maximum deviation from linear mixing is twice that observed on DDD water, and also occurs at a greater EA fraction ($X_{\text{EA}} = 0.25$ on PB vs. $X_{\text{EA}} = 0.14 - 0.16$ on DDD water for max Δ MMA). This indicates that the presence of buffer ions allows more EA to be accommodated within a DPPC matrix. Indeed, this maximum film contraction of $20 \text{ \AA}^2$ is approximately equivalent to an area occupied by a single acyl chain. The most obvious question is whether single-chained EA molecules were being lost to the subphase upon compression. We argue that the probability of EA molecules being squeezed from the monolayer into a solution micelle is very low. Namely, such a loss of monolayer material would have implied a phase-separation between the mixture components and a partial collapse of the mixed-lipid monolayer, none of which were apparent in the observed π -A isotherms (Fig. 3a). As already suggested, a more likely possibility is that certain ratios of DPPC+EA mixtures led to favorable interactions between the head-groups of the two lipids due to electrostatic effects and that these electrostatic effects were different on the two subphases. For instance, interaction between the positively charged amine head-groups of EA with the negatively charged phosphate groups of DPPC could have led to optimal packing at higher PB ionic strength due to a reduction in electrostatic repulsion between the head-groups. This effect would cause the additional reduction of MMA on the PB subphase as observed.

One interesting Δ MMA feature was found for the DPPC+EA mixture at $X_{\text{EA}} = 0.5$, where Δ MMA decreased in magnitude with increasing surface pressures, whereas at lower EA fractions the opposite trend was observed (Fig. 3b). Given the charged nature of the lipid head-group the packing of the molecules in the mixed lipids monolayer was strongly dependent on lipid ratios. This is supported by an inflection point around $X_{\text{EA}} \sim 0.45$ where the deviation of experimental MMA from the additive calculated MMA appears invariant with surface pressure. In other words, the shape of the isotherm for the mixed monolayer with $X_{\text{EA}} \sim 0.45$ parallels the shape calculated based on additive behavior of the pure components. In contrast, for $X_{\text{EA}} < 0.45$ the deviation of MMA from linear mixing is enhanced due to dominance of DPPC: as surface pressure is increased the presence of EA is somewhat hidden by the rich phase behavior of DPPC. Likewise, for $X_{\text{EA}} > 0.45$ the EA lipid exceeds the ability of the DPPC matrix to best accommodate it and the Δ MMA values approach zero.

As the DPPC+EA monolayers displayed overall negative Δ MMA values due to the contraction of the monolayer at intermediate and higher surface pressures, the mixtures also had a correspondingly greater number of lipid molecules occupying the same area. In terms of electrostatics this resulted in a larger, positive surface excess electric potential protein $\Delta\Delta\psi$, as shown in Fig. 4.

All protein experiments were preformed at an intermediary surface pressure, $\pi = 20 \text{ mN/m}$, where the lipids on PB subphase appear to be ideally miscible with the Δ MMA values comparable to Δ MMA on water subphase (Fig. 2b), except at $X_{\text{EA}} = 0.25$. Comparison of fibrinogen binding kinetics from 0.03 mg/ml and 0.06 mg/ml solutions showed profound differences (Figs 5a vs. 6a). From Fgn solution of 0.03 g/ml the maximum protein adsorption decreased monotonously with decreasing fraction of EA in the monolayers. This effect disappeared when the adsorption took place from 0.06 mg/ml solution: while the adsorption remained unchanged on EA, DPPC, and on 6:1 and 5:1 DPPC+EA monolayers, the amount of

protein bound to monolayers of 1:1, 2:1 and 3:1 DPPC+EA mixtures was higher and the adsorption process was faster. The binding kinetics on 1:1 and 2:1 DPPC+EA mixtures also displayed kinks at $\Delta\text{RIU} \sim 0.003$. These features are further exemplified in the $d(\Delta\text{RIU})/dt$ vs ΔRIU plots (Figs 5b vs 6b). The rate of adsorption, $d(\Delta\text{RIU})/dt$, from less concentrated protein solution (0.03 mg/ml) quickly reached its maximum and then fell off close to zero as Fgn binding approached its steady state (Figs 5b). From 0.06 mg/ml fibrinogen solution $d(\Delta\text{RIU})/dt$ for monolayers of 1:1, 2:1 DPPC+EA mixtures displayed large variability due to the kinks in the adsorption kinetics (Figs 6b).

A comparison between adsorption, ΔRIU , at $t = 1200$ s and the highest adsorption rate, $(d(\Delta\text{RIU})/dt)_{\text{max}}$ at two fibrinogen solution concentrations, each plotted as a function X_{EA} , is shown in Figure 7. Both the adsorbed amount (Fig. 7a) and the fastest adsorption rates (Fig. 7b) from 0.03 mg/ml fibrinogen solution show moderate deviations from the expected values.* For lower Fgn concentration, the positive excess adsorbed amount (Fig. 7a) can be explained by the contraction of the monolayer (Fig. 3b), which resulted in a higher number of lipids per area and consequently in a higher positive surface excess potential (Fig. 4). Thus, the contraction leads to more charged lipids per area so that Fgn molecules have a greater number of binding sites available for adsorption, leading to a corresponding increase in ΔRIU .

The contraction of the monolayers can not explain the adsorption results from the more concentrated Fgn solution (0.06 mg/ml) where the adsorbed amount (Fig. 7a) and the maximum adsorption rates (Fig. 7b) were more than doubled for both 1:1 and 2:1 DPPC+EA mixtures. Since the adsorbed amount from the two protein solution was quite similar for all DPPC+EA monolayers except for 1:1, 2:1 and 3:1 DPPC+EA mixtures, one can speculate that for these three lipid mixtures, the adsorption from a more concentrated solution filled up additional binding sites that were not occupied by the adsorption from 0.03 mg/ml solution. It is possible that a spatial matching of electrostatic interactions between protein and lipids mixtures is responsible for the excess adsorption. Fibrinogen molecule carries net negative charge of $-20e^-$ at neutral pH based on recently resolved human fibrinogen structure, 3GHG.pdb [2]. Based on its molecular size a close-packed monolayer of adsorbed Fgn contains about $0.6\text{--}0.7$ ng/cm². When the magnitude of ΔRIU signal is converted into the adsorbed mass using a previously published procedure [23], the maximum adsorbed amount from a 0.06 mg/ml concentration on a 1:1 DPPC+EA monolayer was 0.68 ng/cm², thus implying a complete surface coverage of fibrinogen. However, the kinks in the adsorption kinetics on 1:1 and 2:1 DPPC+EA mixtures may also indicate that some fibrinogen molecules form aggregates in the adsorbed state on these two monolayers; a feature similar to the onset of fibrin formation on self-assembled monolayers [24].

In the SPR experiments, due to the local protein injection the mass transport of protein to the lipid monolayer could not have been rigorously controlled, even under identical stirring conditions. One expects the adsorption rates for adsorption from a more concentrated solution to be overall higher compared with a more dilute protein solution. This has indeed been observed for all lipid mixtures (Fig. 7b). Interestingly, the ratios between $(d(\Delta\text{RIU})/dt)_{\text{max}}$ measured for 0.06 mg/ml vs. 0.03 mg/ml Fgn solutions more than doubled for 1:1, 2:1 and 3:1 DPPC+EA mixtures: 3.5, 2.5 and 3, respectively. Such a large increase of protein binding rate may indicate that these mixed lipids monolayers attracted Fgn molecules due to higher surface electric potentials. However, at 10 mM PB the electrostatic interaction is effective over about 6 nm, i.e. very close to the surface. In addition to the mass transport variability, another explanation for the observed higher binding rates is that the smaller binding rates are the result of an energy barrier which is lowered in the case of 1:1, 2:1 and 3:1 DPPC+EA mixtures, thus

*The expected values, indicated by dashed lines in Fig. 7, were calculated using the addition rule from the respective values for pure DPPC and EA monolayers.

enabling faster protein binding. The desorption kinetics were difficult to measure due to the confined volume and SPR geometry, but preliminary experiments (not shown here) showed very limited desorption of Fgn (~5%) from each lipid monolayer.

Although the number of extra lipid molecules per area due to monolayer contraction accounts for some excess Fgn adsorption to the mixed lipids monolayers, the observed adsorption on 1:1, 2:1 and 3:1 DPPC+EA monolayers could not be explained solely by the excess number of available binding sites per area. We speculated that the spatial distribution of charges in 1:1, 2:1 and 3:1 DPPC+EA monolayers better matched the charge distribution on the protein surface. An alternative explanation is that the interactions between the positively charged primary amine head group of EA and the negatively charged phosphate headgroup of the zwitterionic DPPC molecule might have resulted in an different exposure of DPPC zwitterionic headgroup to the PB subphase, thus making more binding sites for Fgn available.

Conclusions

Pressure-area isotherms of DPPC+EA mixtures on water and buffer subphases indicated good lipid miscibility and some contraction of the monolayer at intermediate and higher surface pressures. The contraction of DPPC+EA monolayers resulted in an excess surface electric potential at intermediate DPPC:EA ratios. Adsorption of fibrinogen to the DPPC+EA monolayers showed excess binding dependent on the solution protein concentration. From a dilute solution (0.03 mg/ml) the adsorbed Fgn amount and the rate of adsorption increased with the increasing fraction of EA in the monolayer thus indicating that the binding was primarily driven by electrostatic interactions between positive EA charges in the monolayer and net negative protein charge. At this condition, the adsorbed amount was below the calculated Fgn monolayer surface coverage. In the case of a higher protein concentration (0.06 mg/ml) both the fibrinogen adsorbed amount and its maximum adsorption rate showed excess values (relative to the pure EA monolayer) for binding to 1:1, 2:1 and 3:1 DPPC+EA monolayers. This excess adsorption could be explained, in part, by the contraction of the monolayers which resulted in an excess surface electric potential of monolayers with intermediate DPPC:EA ratios.

Acknowledgments

This work was supported by the National Institute of Health (R01 HL84586).

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Figure 1.

a) Schematics of Langmuir trough – SPR experimental set-up for measuring Fgn adsorption on mixed lipids monolayers. b) An actual SPR trace recorded for the whole SPR-Langmuir trough Fgn adsorption experiment.

Figure 2.

a) Isotherms of DPPC, EA and their mixtures on deionized, double-distilled water subphase.
 b) Miscibility diagram of DPPC+EA monolayers on DDD water subphase showing the deviations from the expected MMA, as a function of X_{EA} .

Figure 3.

a) Isotherms of DPPC, EA, & their mixtures on 10 mM phosphate buffer (no NaCl). b) Miscibility diagram of DPPC+EA monolayers on 10 mM phosphate buffer (no NaCl), showing deviation from the expected MMA, as a function of X_{EA} .

Figure 4. “Excess” surface potential, $\Delta\Delta\psi$, of DPPC EA and DPPC+EA monolayers on 10 mM phosphate buffer (no NaCl). See the text about the $\Delta\Delta\psi$ calculation.

Figure 5.

a) Adsorption kinetics of fibrinogen (0.03 mg/ml) on DPPC, EA, and DPPC+EA monolayers.
 b) Rates of refractive index change, $d(\Delta RIU)/dt$, shown as a function of the observed refractive index change, ΔRIU .

Figure 6.

a) Adsorption of fibrinogen (0.06 mg/ml) on DPPC, EA, and DPPC+EA monolayers. b) Rates of refractive index change, $d(\Delta RIU)/dt$, shown as a function of the observed refractive index change, ΔRIU .

Figure 7.

a) Comparison of Fgn adsorption (shown as ΔRIU at $t = 1200$ s) measured on DPPC+EA monolayers from 0.03 and 0.06 mg/ml solutions shown as a function of X_{EA} . The expected ΔRIU values, shown by the dashed lines were calculated using the addition rule from the respective ΔRIU values for pure DPPC and EA monolayers. b) Comparison of maximum Fgn adsorption rates (shown as maximum $d(\Delta RIU)/dt$) measured on DPPC+EA monolayers shown as a function of X_{EA} . The expected values, shown by dashed lines, were calculated using the addition rule from the respective $d(\Delta RIU)/dt$ values for pure DPPC and EA monolayers.