DNA−Surfactant Complexes at Solid Surfaces

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In this work, we report on the adsorption of complexes between DNA of different molecular weight and a cationic surfactant, cetyltrimethylammonium bromide (CTAB), on hydrophobized and hydrophilic negatively charged silica surfaces as measured by ellipsometry. We will demonstrate how the adsorption is affected by the state of the DNA−surfactant complexes formed in bulk solution. High molecular weight DNA molecules, which condense (transform from coil to globule state) on addition of small amounts of cationic surfactants, do not adsorb on hydrophilic silica prior to phase separation. However, DNA−surfactant complexes formed from low molecular weight DNA were found to adsorb. For these complexes surfactants interact with DNA, without condensation of the DNA. Adsorbed DNA−surfactant complexes can easily be removed from the hydrophilic silica surface when replacing the bulk DNA−surfactant solution with pure salt solution. At the hydrophobic surface the DNA adsorbs without addition of cationic surfactant. However, with addition of a very low amount of surfactant, a rapid increase in adsorbed amount and a simultaneous decrease in adsorbed layer thickness are observed. This compaction of the adsorbed layer is to some extent reversible when replacing the bulk DNA−surfactant solution with pure salt solution.

Introduction

Complex formation between cationic surfactants and DNA has been studied extensively in recent years.[1−5] This is due to the large and increasing interest for using cationic liposomes as a possible way for in vivo gene transfer.[6,7]

In bulk, the binding of cationic surfactants to DNA appears to be analogous to binding of surfactants to oppositely charged synthetic polymers in general.[1] The DNA molecule, however, has a special structure compared to typical synthetic polymers. In water solutions of low ionic strength, DNA is a rodlike and highly negatively charged polymer with a persistence length of more then 50 nm. The binding of cationic surfactants proceeds in two stages.[1,8−10] In the first stage, surfactants exchange with counterions condensed at the DNA chain. At a critical surfactant concentration a highly cooperative binding of surfactant occurs which is typically followed by phase separation. For high molecular weight DNA the cooperative binding of surfactants causes a condensation (a discrete transition from coil to globule state) of the DNA chain. This means that the coil and globular forms coexist in a certain surfactant concentration interval, which has been demonstrated by fluorescence microscopy.[8] The cooperative binding of surfactants is believed to be a result of micellar-like aggregation on the DNA chain.[1,8−10] The rigidity of the DNA locally prevents the DNA chain to wrap itself around the surfactant aggregates. For short DNA chains (i.e., low molecular weight DNA) the DNA chain is too short and rigid to loop back to interact with surfactant aggregates on other parts of the polymer chain. Thus condensation of short chain DNA does not occur before inter molecular interactions with other complexes lead to phase separation (i.e. precipitation of DNA−surfactant complexes). This is indeed confirmed in studies of Dawson et al.,[9−12] where the interaction between low molecular weight DNA and cationic surfactants was investigated in great detail. They show that for a short chain DNA (220 base pairs) no surfactant induced condensation of the DNA chain occurs before phase separation. Instead, the surfactant molecules self-assembly on the surface of the DNA, according to the beads on necklace model.

The objective with the present study is to relate the interfacial behavior to the differences in the states of the DNA−surfactant complexes in bulk solution. The interaction between DNA and a cationic surfactant (cetyltrimethylammonium bromide (CTAB)) and the subsequent adsorption onto silica and hydrophobized silica surfaces have been followed. The studied DNA samples include short chain DNA, where surfactant-induced condensation is not expected to occur, as well as high molecular weight samples.

Experimental Section

Materials. Salmon sperm DNA (Sigma 10 000 base pairs), herring sperm DNA (Sigma 700 base pairs), and degraded herring

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sperm DNA (Sigma 100 base pairs (and shorter)) were used as received. The given average molecular weights of the samples were estimated by electrophoresis (agarose gels of different density using 0.7, 1, and 1.5 wt. % agarose). The concentration of DNA was determined by a spectroscopic method, using the molar extinction coefficient $\epsilon_{260} = 6600$ L mol^{-1} cm^{-1} at 260 nm. CTAB (Merck pa quality) and sodium bromide (Riedel-deHaen extra pure quality) were used as received. The water used was from a Milli-Q filtration system (Millipore).

Ellipsometry. The instrument used in this study was an automated Rudolph Research thin-film null ellipsometer, type 43603-200E. This is set up in a horizontal polarizer, compensator, sample, and analyzer arrangement as described in ref 13. The optical characteristics of the substrate (Si/SiO_2 plates) were determined at the beginning of each experiment by ellipsometric measurements in different ambient media (air followed by water). This substrate characterization procedure was described earlier.[13]

After characterization of the substrate, a known amount of DNA was injected into the cuvette, which originally contained 5 mL solution, and the ellipsometric angles Ψ and Δ were recorded continuously until plateau values were reached. The solution composition was then changed by addition of a known amount of surfactant or by rinsing with a flow of solution through the cuvette. All the measurements were performed at $\lambda = 4015$ Å, in a temperature-controlled cuvette (25 ± 0.1 °C) and under agitation with a magnetic stirrer at about 300 rpm.

The recorded Ψ and Δ were evaluated using a four layer optical model, assuming isotropic media and planar interfaces. The mean refractive index, n_f, and the ellipsometric thickness, d_f, of the adsorbed layer were calculated using a numerical procedure described earlier.[13] The adsorbed amount (Γ) was calculated from n_f and d_f by the formula first derived by de Feijter et al.,[14]

$$\Gamma = \frac{(n_f - n_0)d_f}{dn/dc} \quad (1)$$

where n_0 is the refractive index of the bulk solution and $dn/dc = 0.134$ g/cm^3. The refractive index increment were determined by measurements of the refractive index of different concentration of DNA in 10 mM NaBr solution at $\lambda = 5893$ Å. The values were then recalculated to $\lambda = 4015$ Å, as described by Mahanty and Ninham.[15]

The adsorbed amount Γ is the quantity most commonly extracted from ellipsometric measurements. This is due to the fact that, unlike n_f and d_f, the adsorbed amount is less sensitive to the optical model chosen for evaluation of the data. The values of n_f and d_f are as mentioned above calculated under the assumption of layer uniformity. Since the adsorbed material is in general distributed in some way normal to the surface, the calculations based on this model yield mean n_f and d_f values which must be interpreted with caution. For polymer systems, d_f tends to represent the inner dense part of adsorbed polymer layers and is expected to be lower than that determined by, for instance, light scattering or surface force measurements.

Surface Preparation.. Silica surfaces were prepared by the following procedure. Polished silicon wafers (p-type, boron-doped, resistivity 1−20 Ω·cm) were purchased from Okmetic Ltd. The wafers were oxidized thermally in oxygen atmosphere at 920 °C for ≈1 h, followed by annealing and cooling in an argon flow. This procedure results in a SiO_2 layer thickness of 300 Å. The oxidized wafers were then cut into slides with a width of 12.5 mm and cleaned according to the procedure described earlier.[13] Before use, the surfaces were dried under vacuum, 0.001 mbar, and then treated in a plasma cleaner (Harrick Scientific Corporation, model PDC-3XG) for 5 min prior to the start of the adsorption measurements.

Hydrophobized silica surfaces were obtained by placing oxidized cleaned and plasma-treated silicon slides in a reactor, which prior to the injection through a septum of 2 mL of dimethyloctylchlorosilane was evacuated from air by a water suction pump. The silicon slides were exposed to dimethyloctylchlorosilane for about 24 h at room temperature; the surfaces were then sonicated in ethanol and tetrahydrofuran repeatedly and, finally, stored in ethanol. Before use, the surfaces were dried under vacuum, 0.001 mbar. To avoid an air film sticking to the hydrophobic surface, ethanol was pumped through the cuvette before water was added. Note that reproducible measurements could not be obtained without this intermediate step. The ethanol was then rinsed of by a continuous flow of water, and prior to the start of the adsorption measurements both types of surfaces were allowed to stabilize in the aqueous solvent for at least 1 h.

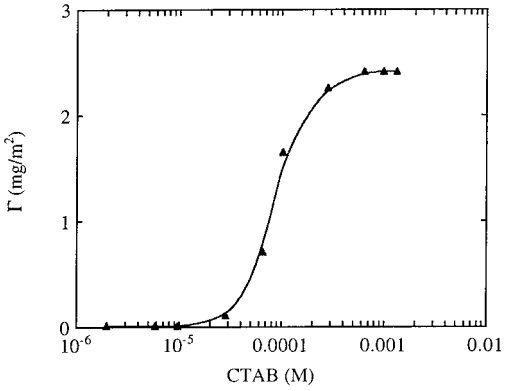

Figure 1. Adsorption isotherm for CTAB at the silica−water interface in 10 mM NaBr.

Results and Discussion

Adsorption at the Silica−Aqueous Interface. None of the studied DNA samples, in the concentration range between 0.02 and 10 mg/mL, adsorbed at the silica surface in absence of surfactant. This is expected as the negatively charged DNA is electrostatically repelled from the negatively charged silica surface. In cases where DNA has been shown to adsorb on a silica surface, it has been from solutions of higher ionic strength.[16] Under these conditions, the electrostatic repulsion is suppressed and the solubility of the DNA decreases.

A typical adsorption isotherm for CTAB[17] is shown in Figure 1. At low concentrations adsorption occurs through ion exchange and the adsorbed amounts are small due to the low surface charge density of silica under the used conditions (about pH 6). At higher concentrations, when approaching the CMC, the adsorption increases well above the ion exchange capacity and surface aggregates are formed. At concentrations above cmc the adsorbed amount finally reaches a plateau value independent of surfactant concentration.

We observed a clear difference in adsorption of DNA−surfactant complexes depending of molecular weight of the DNA. No adsorption of DNA−surfactant complexes was observed prior to phase separation, when the complexes were formed from high molecular weight DNA. For the low molecular weight DNA (Figure 2), on the other hand, the adsorption starts at a certain surfactant concentration, 7.0 10^{-6} M, which corresponds to a surfactant/ DNA charge ratio of 0.04, and then increases sharply with the surfactant concentration until a plateau in the amount adsorbed is reached. However, just prior to phase separation, the adsorbed amount again increases with the concentration. Changing the DNA concentration in the range 0.02 to 0.1 mg/mL did not significantly affect the value of the obtained adsorption plateau. The ellip-

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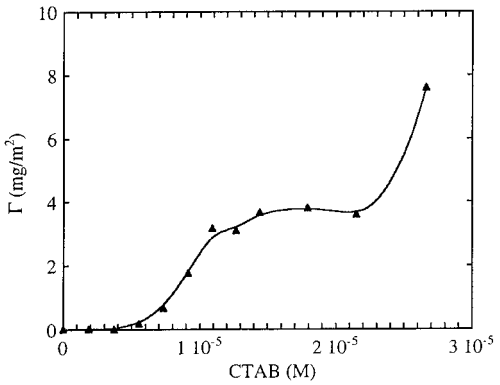

Figure 2. Adsorption of low molecular weight DNA−surfactant complexes at the silica−water interface in 10 mM NaBr. The DNA concentration was fixed at 0.06 mg/mL (corresponding to 0.18 mM charges), and the CTAB concentration was increased stepwise until a visible phase separation occurred.

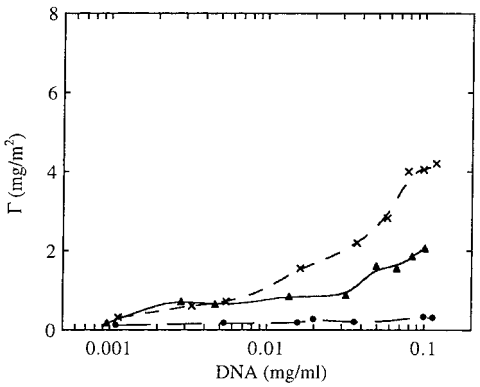

Figure 3. Adsorption isotherms of different size DNA, at a hydrophobic surface in 10 mM NaBr. The different DNAs are (crosses) 100 base pairs, (circles) 700 base pairs, and (triangles) 10 000 base pairs.

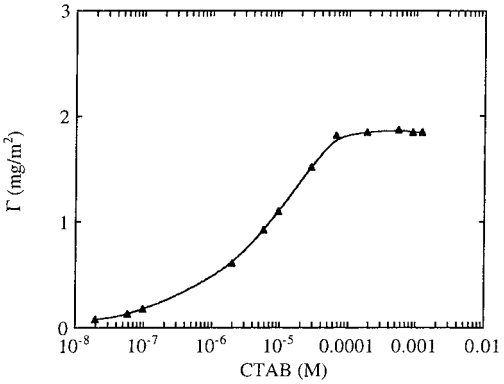

Figure 4. Adsorption isotherm for CTAB at the hydrophobized silica−water interface in 10 mM NaBr.

sometric thickness, at the adsorption plateau, was between 50 and 80 Å (some variations between different measurements) which indicates that the DNA−surfactant complexes are adsorbed in a rather compact form at the surface.

When phase separation occurs we registered a dramatic increase in adsorption for all the DNA samples; the data are very scattered and were found to be difficult to reproduce. However, we observed macroscopic precipitation on both the surface and the glass walls of the cuvette. When the cuvette was rinsed with 10 mM NaBr solution, the DNA−surfactant complexes were totally desorbed. Complete desorption was also observed for the low molecular weight DNA−surfactant complexes if rinsing was initiated prior to phase separation.

It is important to note that the total concentration of CTAB in this adsorption study always is very low (i.e. less than 3×10^{-5} M), 1 order of magnitude below the cmc for CTAB ($\approx 5 \times 10^{-4}$ M) at this ionic strength. The minimum DNA concentration used is 0.02 mg/mL, which corresponds to a negative charge concentration of 6×10^{-5} M. This implies that the free concentration of CTAB is much lower than the total concentration since most of the added CTAB molecules participate in the DNA−surfactant complexes. The adsorption isotherm for CTAB (Figure 1) reveals that the adsorption is negligible at these low concentrations. As described above, the DNA−surfactant complexes of the two high molecular weight DNA samples, which do not adsorb, are expected to condense in solution prior to phase separation. The low molecular weight DNA sample, on the other hand, will not condense. Instead the formed surfactant aggregates attached to the DNA−surfactant complex can interact with the silica surface, which leads to the observed adsorption. These findings also indicate that surfactant aggregates in the condensed complexes are not accessible for interaction with the negative silica surface.

Adsorption at the Hydrophobized Silica−Aqueous Interface. The adsorption isotherms for the different DNA samples on the hydrophobic surfaces are shown in Figure 3. For all samples the adsorption increases progressively with concentration in the concentration range of 0.001−0.1 mg/mL. If we compare the adsorption isotherms, we observe that the low molecular weight sample deviates by its high adsorption. The two high molecular weight samples follow the behavior normally observed for homopolymers, where the amount adsorbed increases with the molecular weight of the polymer.[18] The reason behind the large adsorbed amount of the low molecular weight DNA is not clear. One reason may be that the ends of the DNA molecule are more hydrophobic. This effect would only show up when the molecular weight is sufficiently low. Another possibility is that the degradation process has created defects in the DNA helixes where the more hydrophobic bases are available to interact with the hydrophobic surface.

Figure 4 displays the adsorption of CTAB to the hydrophobic surface. The adsorption starts already at very low CTAB concentrations and increase progressively with concentration until a plateau value is reached close to the cmc.

The interpretation of the ellipsometric data becomes more difficult than for the hydrophilic surface as DNA and CTAB as well as the DNA−surfactant complexes adsorb to the hydrophobic surface in the studied concentration range. Figure 5 shows the adsorption as a function of CTAB concentration for a given DNA concentration of 0.02 mg/mL. As was discussed above, all the DNA samples adsorb to the hydrophobic surface without addition of surfactants. However, a large increase in the adsorbed amount is observed at a very low CTAB concentration (i.e. $< 2 \times 10^{-6}$ M). Further increase in the CTAB concentration does only have minor affects on the adsorbed amount for the two high molecular weight DNA samples. Initially the adsorption of the low molecular weight DNA sample is similar. However, the amount adsorbed increases sharply at concentrations just before phase separation.

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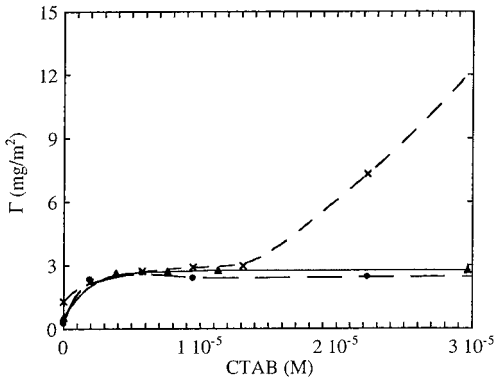

Figure 5. Adsorption of DNA−surfactant complexes at the hydrophobized silica−water interface in 10 mM NaBr. The DNA concentration was fixed at 0.02 mg/mL (corresponding to 0.06 mM charges), and the CTAB concentration was increased stepwise until a visible phase separation occurred. The different DNAs are (crosses) 100 base pairs, (circles) 700 base pairs, and (triangles) 10 000 base pairs.

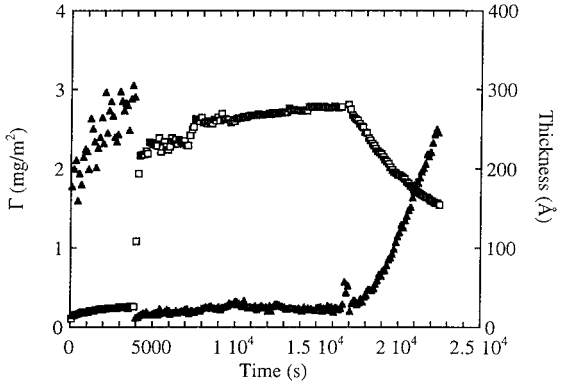

Figure 6. Adsorbed amount (open squares) and adsorbed layer thickness (triangles) as a function of time. The DNA (700 base pairs) is added at time 0 (0.06 mg/mL, corresponding to 0.18 mM charges), and at $t = 5000$ s CTAB is added to a concentration of 1.5×10^{-6} M. Then the CTAB concentration is increased in portions until at $t = 17\,000$ s where rinsing is started.

The large increase in adsorbed amount induced by very low CTAB concentrations is due to compaction of the adsorbed layer on the surface. This is visualized in Figure 6, where both the adsorbed amount and the adsorbed layer thickness are plotted against time. When the first portion of CTAB is added (2×10^{-6} M) after 5000 s of DNA adsorption, we observe a dramatic increase in adsorbed amount together with a decrease in adsorbed layer thickness. Further increase in surfactant concentration has only minor effects on the adsorption. The cell is flushed with pure salt solution after 17 000 s. We then observe that the compaction process of the adsorbed layer to some extent is reversible. The adsorbed layer thickness is more or less back at its initial values (before addition of CTAB) after 5000 s of continuous rinsing. The adsorbed amount is however still much higher than before the addition of surfactant. The initial decrease in adsorbed amount and increase in adsorbed layer thickness upon rinsing is mainly due to desorption of surfactant. This leaves the interfacial region in a nonequilibrium situation with an excess of DNA due to the low desorption rate of polymer.

The CTAB molecules are most likely attracted to the surface by the accumulation of negative charges due to the preceding DNA adsorption. The low adsorbed layer thickness (about 25−30 Å) after compaction of the adsorbed layer shows that the surfactant molecules interact with the hydrophobic surface and function as anchor points for the DNA molecules. This is analogous to adsorption of DNA molecules at surfaces of compressed cationic lipid films. Here a number of studies have shown that the DNA forms compact adsorbed layers with thicknesses around 25 Å.[19−22] The low thickness of the adsorbed layer shows that the surfactant molecule do not self-assemble into large surfactant aggregates in the adsorbed layer.

Adsorption Close to Phase Separation. At surfactant concentrations close to the phase separation the adsorption behavior of the low molecular weight DNA sample is different on the hydrophilic and hydrophobic surfaces. In both cases the adsorbed amount increases when the system approaches macroscopic phase separation. The increase in adsorbed amount is however observed at lower surfactant concentrations on the hydrophobic surface compared to the hydrophilic surface. The phase separation is a consequence of charge reduction of the DNA−surfactant complexes, which is due to incorporation of additional surfactant molecules in the complexes. The differences observed between the hydrophobic and hydrophilic surfaces are most likely due to the high adsorption of surfactant on the hydrophobic surface. An increase of surfactant adsorption on the hydrophobic surface renders the surface a slightly positive net charge that attracts the negatively charged DNA−surfactant aggregates. At the hydrophilic surface the adsorption of CTAB is, as we have pointed out before, negligible.

Conclusions

The adsorption of DNA−surfactant complexes is affected by the state of the complexes formed in bulk solution. Low molecular weight DNA, for which the surfactant interact with DNA without condensation of the formed complexes, was found to adsorb to a considerable extent. On the other hand, condensed complexes between DNA and surfactant do not adsorb on hydrophilic silica prior to phase separation. The DNA−surfactant complexes adsorb by interactions between the formed surfactant aggregates and the negative silica surface. The results also indicate that the surfactant aggregates in the condensed complexes are not accessible for interaction with the negative silica surface.

At the hydrophobic surface, a preadsorbed layer of DNA is compacted by addition of low amount of cationic surfactant. We observe a dramatic increase in adsorbed amount together with a decrease in adsorbed layer thickness. The surfactant adsorbs at the hydrophobic surface and acts as anchor points for the DNA molecules. The low thickness of the adsorbed layer indicates that no aggregation of surfactant occurs at the adsorbed DNA layer. The compaction of the adsorbed layer is to some extent reversible when the cell is rinsed by 10 mM NaBr solution. The surface excess after rinsing is however much higher than the initial adsorbed amount of the DNA before addition of surfactants.

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