Research Article

Studies on Adsorption of DNA on Functional Ultrathin Films of Cationic Surfactant

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We have investigated the interaction of DNA with a highly ordered functional ultrathin layer of cationic surfactant, dioctadecyl ammonium bromide (DOAB). The ultrathin film of DOAB is fabricated by Langmuir-Blodgett technique onto the pretreated quartz crystal wafers. The solution of DNA in phosphate buffer saline (PBS) is injected through a flow cell in a quartz crystal microbalance (QCM) loaded with the functional ultrathin film. The QCM data indicate a slower kinetics (time constant = 162.2 seconds) for the adsorption of DNA on DOAB layer as compared to PBS on DOAB. The surface morphology of the aggregation of DNA over the DOAB layer is investigated using atomic force microscope (AFM). The AFM image indicates the trapping of DNA over the DOAB layer. Such trapping of DNA can be potentially employed in the field of genomics.

1. Introduction

The molecular interactions in the biological world govern numerous vital activities essential for physiological processes [1]. The field of biophysics relies on the investigation of molecular interactions at the relevant physiological conditions. The field of interaction of DNA with the biological relevant materials is matured. Understanding the molecular interactions of the biological relevant molecules is essential not only for the design of novel drugs but also devices for probing the systems rapidly and accurately. There are molecular specific interactions like antibody-antigen which work on the principle of lock-key mechanism. Such molecular specific interactions are potentially employed for the development of sensors [2, 3]. There are numerous studies on the interaction of DNA with proteins, lipids membranes, and cationic surfactants [4–7]. The phosphate backbone of the DNA strand acquires the negative charge in the aqueous environment. Such charged state of DNA can form complexes with the cationic surfactants due to the static charge-charge interaction. Due to complex formation, the DNA strands condense to form very small particles. These complexes may be employed for nonviral gene delivery vehicles in vaccines and gene therapy [8–11]. The efficiency and the sensitivity of the functional material increases manifold when the material

is spread into thin films. This is due to gain in surface-to-volume ratio when the bulk material is spread to form thin films [12, 13]. In this paper, we report our study on the interaction of DNA with the ultrathin film of a cationic surfactant using a quartz crystal microbalance (QCM) and atomic force microscope (AFM). A highly organized single layer of Langmuir-Blodgett (LB) film of cationic surfactant dioctadecyl ammonium bromide (DOAB) is formed such that the functional group ammonium bromide is exposed to air so as to facilitate its interaction with the DNA in aqueous environment. The QCM data indicate a slower kinetics of the adsorption of DNA on the DOAB. The AFM image shows the aggregates of DNA adsorbed on the DOAB layer.

2. Experimental

The cationic surfactant, dioctadecyl ammonium bromide (DOAB), and octadecanethiol (ODT) are purchased commercially from Sigma-Aldrich. The 5 MHz AT-cut quartz crystal wafers are deposited with gold film to facilitate the study on molecular specific interaction. The quartz wafers were cleaned in cold piranha solution ($\text{H}_2\text{SO}_4 : \text{H}_2\text{O}_2$ as 3 : 1) and rinsed successively with plenty of ion-free water, alcohol, and acetone (Piranha is very dangerous and it has to be

FIGURE 1: (a) Surface pressure area per molecule isotherm of Langmuir monolayer of DOAB on ultrapure ion-free water. (b) The in-plane elastic modulus (E) calculated from (a) using the relation $E = -A_m \times d\pi/dA_m$.

handled carefully). The gold surface on quartz wafer is made hydrophobic by depositing self-assembled monolayer (SAM) of ODT. The wafers were dipped into 1 mM solution of ODT in absolute alcohol for about 24 hours. The treated wafers were rinsed thoroughly by the absolute ethanol and stored in water for LB film deposition. The Langmuir monolayer and LB films were fabricated using a teflon trough equipped with double barriers and a dipper (Apex Instr., LB2007DC). The Langmuir monolayer of DOAB is formed onto ultrapure water subphase by spreading 1 mg/mL chloroform solution of DOAB using a microsyringe. The chloroform was allowed to evaporate completely from the surface of water leaving behind the dispersed DOAB molecules. The monolayer of DOAB was compressed symmetrically and the surface pressure (π), area per molecule (A_m), was recorded in the computer. The LB films were deposited onto the SAM-deposited quartz wafers. A single layer LB film was deposited at 20 mN/m such that the functional group, ammonium bromide, is exposed to the air. This is achieved by dipping the hydrophobic quartz wafer with the DOAB monolayer held at the target surface pressure at the air-water interface. On completing a downstroke of the dipper, the monolayer is removed from the water surface and the substrate is removed from the water carefully. This technique of deposition yields only one layer of the DOAB with the functional ammonium bromide exposed to air. Such arrangement of film is essential for establishing the cationic surfactant DNA interaction.

The functionalized quartz wafer is mounted in the quartz crystal microbalance (QCM) and the change in frequency (Δf) are recorded as a function of time. The QCM (QCM200, SRS, USA) is attached with a flow cell and a peristaltic pump for injecting fluids in a highly controlled manner. The DNA is dispersed in phosphate buffer saline (PBS) solution with a concentration of 100 ng/mL. The DNA solution is injected in the QCM at a rate of 75 μ L/min.

The morphology of the films is studied using an atomic force microscope (AFM, NTMDT, SolverPro) in semicontact

phase imaging mode. The images were obtained by scanning the films using silicon tips having resonant frequencies in the ranges of 180–270 KHz and spring constants between 10 and 20 N/m. The images were analyzed using the proprietary software of NTMDT.

3. Results and Discussion

The π - A_m isotherm of the Langmuir monolayer of DOAB is shown in Figure 1(a). The isotherm shows zero surface pressure at a very large A_m . This is the gas phase of the monolayer. On compression, the isotherm starts showing nonzero surface pressure at 0.65 nm². This is the onset of a liquid phase. The surface pressure rises slowly till 0.5 nm². The surface pressure increases monotonically thereafter on reducing A_m till the monolayer collapses at around 55 mN/m. The phase corresponding to the steep rise in surface pressure may correspond to a liquid-condensed phase. The average area occupied by the molecules in a phase is determined by extrapolating the corresponding region of the isotherm to the zero surface pressure on the A_m axis. The extrapolation of the steep region of the isotherm to zero surface pressure is called limiting area per molecule (A_o). This is the minimum area to which the molecules can be compressed on the water surface without collapsing the monolayer [14, 15]. The orientational state (tilt or untilt) of the molecules in a phase can be estimated qualitatively by comparing the extrapolated area per molecule with that of molecular cross-sectional area in the bulk single crystal [16]. The cross-sectional area of the vertically aligned DOAB molecule can be estimated to be around 0.5 nm². The estimated A_o value from the isotherm is found to be 0.45 nm². Therefore, the DOAB molecules are expected to be aligned vertical in the liquid-condensed phase. The in-plane isothermal elastic modulus (E) is a convenient quantity for identifying surface phases [17]. The

E as a function of A_m can be evaluated from a given isotherm using the following relation:

$$E = -A_m \frac{d\pi}{dA_m}. \quad (1)$$

The plot of E versus A_m for the Langmuir monolayer of DOAB is shown in Figure 1(b). The maximum values of E corresponding to liquid-like and liquid-condensed phases are found to be ~ 70 and ~ 185 mN/m, respectively. Liquid-condensed phase is more elastic and may possess high ordering as compared to liquid-like phase. Since the molecules are in a more ordered state in the liquid-condensed phase, we have chosen the LB film deposition of DOAB on the quartz wafer of QCM in this phase.

The modified quartz crystal wafers are mounted on the QCM and the change in frequency are recorded as a function of time. This is shown in Figure 2. The resonant frequency for the adsorbed layers like SAM of ODT (black curve) and one layer of LB film of DOAB on SAM (red curve) are found to be independent of time. We have injected the PBS solvent into the QCM using the flow cell with the LB film of DOAB as the functional sensing layer. As the PBS solvent enters the functional surface of DOAB, the resonant frequency changes rapidly (blue curve) yielding a very small value of time constant (τ) (~ 0.25 milliseconds). The value of τ is estimated by fitting the experimental curve using an exponential curve: $f(t) = A\text{Exp}(-t/\tau)$, where A and τ are the fitting parameters. In order to study the interaction of DNA with DOAB layer, the PBS solution of DNA is injected into the flow cell and the resonant frequency is recorded as a function of time (orange curve). The DNA adsorbed to the DOAB functional layer resulted in change in the resonant frequency. The kinetics are found to be slower as compared to those of pure PBS solvent. The fitted value of τ for the DNA interaction on the DOAB film is found to be 162.2 seconds. The resonance frequency of the quartz crystal is dependent on its mass. Any change in the mass due to adsorption of molecules on the surface of quartz is sensed as the change in the resonance frequency. The change in frequency (Δf) is directly proportional to the change in the mass per unit area (Δm). The mass per unit area on the surface of quartz crystal is estimated using the Sauerbrey equation [18] as follows:

$$\Delta f = -C\Delta m, \quad (2)$$

where C is the parameter dependent on the quartz crystal and the experimental condition. The change in mass per unit area of the adsorbed layer is tabulated in Table 1.

The surface morphology of the functional layers and the adsorbed DNA layer are studied using the AFM. The images are shown in Figure 3. The AFM image (Figure 3(a)) of the SAM of ODT reveals layer with few defects. The presence of defects in the height image is consistent with the phase imaging. The defects appear very small and distributed uniformly over the entire scan range of $10 \times 10 \mu\text{m}^2$. Due to the deposition of one layer of LB film of DOAB over the SAM of ODT, the uniformity in the texture is increased. The LB film yields uniform and homogeneous film (Figure 3(b)). On injection of DNA solution over the DOAB layer, due to

FIGURE 2: Quartz crystal microbalance measurement.

TABLE 1: Estimation of mass per unit area of the adsorbed layer at the different levels of the QCM measurement. The change in frequency is measured with respect to the previous adsorbed layer. The value of C for 5 MHz A-T cut quartz wafer is $56.6 \text{ Hz } \mu\text{g}^{-1} \text{ cm}^2$.

Adsorbed layer	$-\Delta f$, Hz	Δm , ng/cm^2
SAM of ODT	27	477
DOAB	22	389
PBS	250	4417
DNA	600	10601

the static charge-charge interaction of DNA and DOAB, the DNA strands are physioadsorbed onto the surface of LB film of DOAB. Figure 3(c) clearly shows the adsorbed DNA over the DOAB layer. The strands of DNA are clearly visible in both the height and phase images. Our study indicates the trapping of DNA over the highly ordered functional ultrathin layer of DOAB. Such trapped DNA can be processed for any genomics.

4. Conclusion

The functional film for the adsorption of DNA is formed very carefully. Here, in order to maintain the functional group, ammonium bromide exposed to the air such that DNA can get adsorbed from the solution, the gold deposited quartz crystal has been modified appropriately. Firstly the gold layer is made hydrophobic by forming SAM of ODT. Then a single layer of LB films of DOAB is deposited as discussed in the experimental section. The adsorption and the kinetics of adsorption is studied using the QCM. The adsorption of PBS solvent is very rapid. However, the DNA adsorbs with the slower time constant of about 162.2 seconds. The adsorption of DNA onto the DOAB is further confirmed by AFM imaging. Future work may include a study on controlling the selective adsorption of DNA over functional thin films.

FIGURE 3: AFM image of (a) SAM of ODT on gold deposited quartz crystal, (b) one layer of LB film of DOAB deposited over the SAM of ODT, and (c) DNA adsorbed over DOAB after the QCM measurement. The images were scanned in semicontact mode of the AFM. Left column represents the height images and right column is the corresponding phase image.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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