Adsorption behavior of DNA onto a cationic surfactant monolayer at the air–water interface

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This communication reports the adsorption of DNA to the preformed Langmuir monolayer of cationic surfactant Octadecylamine (ODA) at the air–water interface and thereby formation of ODA/DNA complex monolayer at the interface. Effect of concentration of DNA in the subphase as well as subphase pH on the adsorption of DNA onto ODA monolayer assemblies have been studied by monitoring the change in surface pressure of ODA/DNA complex monolayer as a function of time. The complex monolayer was also transferred onto solid substrate to prepare ODA/DNA Langmuir–Blodgett films which were analyzed by UV–vis absorption, ATR–FTIR spectroscopic techniques. The most significant observations is that the extent of interactions between ODA and DNA at the air–water interface increases with increasing concentration of DNA in the subphase and also subphase pH. At higher pH, hydrophobic interaction dominates over electrostatic interaction between DNA and ODA in the aqueous subphase. DNA immobilized in the backbone of ODA lies almost flat or extended onto solid substrate at neutral pH whereas, they lie aggregated and compacted coil rather than flat when adsorbed from high pH namely, 11.5 of the subphase. This was confirmed by atomic force microscopy of these complex LB films onto solid substrate.

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1. Introduction

Deoxyribonucleic acid (DNA) is well-known as a source of biological information depending on its base sequences. DNA is also interesting as a material that exists as double helical rodlike molecules consisting of base-pair (bp) stacking. Rod-like polymers such as polyglutamate [1–3] polysiloxane [4], alkylated cellulose, [5] and discotic crystals [6] have been reported to form Langmuir–Blodgett (LB) films in which rod-like molecules aligned in one direction during the compression process on the subphase [7,8] or the deposition process of monolayers [9]. DNA is a good candidate to form an oriented Langmuir monolayer when adsorbed from an aqueous subphase to the opposite charged surfactant monolayer at the air–water interface.

Monolayers of charged surfactant molecules at the air–water interface (Langmuir monolayers) have long been acknowledged to be the excellent media for the organization of large inorganic ions [10,11], colloidal nanoparticles [12], phospholipids [13] and biomacromolecules such as proteins/enzymes [14,15] and in the growth of oriented crystals [16]. The interaction of DNA with Langmuir monolayers has received considerable attention with a view to understand templated supramolecular organization as well as the transfer of DNA across biological bilayer membranes in gene therapy in cancer, VIH, Ebola or heart infarction [17–19]. Moreover, anchoring of DNA in a Langmuir–Blodgett film has made possible in its immobilization [20–22] and construction of DNA chip [23]. Studies on DNA immobilization at the air–water interface have hitherto concentrated on electrostatic complexation between DNA molecules with cationic surfactant Langmuir monolayers and hydrogen bonding between alkylated monolayer-forming nucleobases and complementary water-soluble bases and oligonucleotides. This electrostatic complexation between DNA and cationic surfactant sometimes very much sensitive to the different microenvironment from which it is adsorbed. There are some previous reports on the immobilization of DNA onto Langmuir monolayers onto hydrophilic solid substrate [24,25]. However the effect of subphase pH on the complexation of DNA and cationic surfactant is still not extensively studied. It is already believed that DNA-surfactant complexation is a non-viral method of gene delivery into the cell [26]. DNA immobilized onto solid substrates may have profound implication with respects to its biological functionalities and protein or enzymatic recognition [27,28].

In this present paper, the interaction mechanism of DNA on to the preformed Langmuir monolayer of cationic Octadecylamine (ODA) at the air–water interface as a function of various parameters like concentrations, pH of the subphase etc. and the successful transfer of the resultant complex Langmuir monolayer onto solid support have been demonstrated. pH of the medium can play important role for different headgroup interactions of surfactant in an aqueous subphase and can cause compaction or condensation of nucleotides while complexation with surfactant [29]. Adsorption of DNA onto ODA monolayer has been studied by monitoring the surface pressure (in mN/m) of the

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complex monolayer as a function of time elapsed. Fourier Transform Infrared Spectroscopy in Attenuated Total Reflection mode reveals the complexion of DNA with ODA in mixed monolayer deposited onto single crystal ZnSe substrate. Presence of DNA molecules in the ODA/DNA LB films on quartz substrates was further confirmed by UV–vis absorption spectroscopic results. The morphology of ODA/DNA complex LB films have been investigated by atomic force microscopy.

2. Materials and methods

Herring sperm sheared DNA (size nearly 2000 bp with approximate GC content 41.2%) was purchased from SRL India and was used as received. The purity of DNA was checked by UV–vis absorption and fluorescence spectroscopy before use. ODA (purity > 99%) was obtained from Aldrich Chemical Company, USA and was used without further purification. The molecular structure of DNA and ODA are shown in Fig. 1(a) and (b) respectively. Triple distilled deionized water (18.2 MΩ cm) was prepared using Millipore water purification system and used as the subphase for the complex Langmuir monolayer. Spectroscopic grade chloroform (SRL, India) was used as solvent for preparing ODA solution and its purity was checked by fluorescence spectroscopy before use. Langmuir–Blodgett (LB) film deposition instrument (LB2000C, Apex Instruments, India) has been used to study the adsorption isotherm of DNA onto preformed ODA monolayer in the Langmuir trough which is made of high quality Teflon to ensure complete removal of any organic contaminants. The upper surface of the trough is designed in such a way a single barrier can slide horizontally on the subphase surface. Effective working area of the trough is 30 cm × 15 cm = 450 cm². Depth of the dipping well of the trough is 10 cm. Maximum subphase volume in the trough is 1153.5 cc. Surface pressure of ODA/DNA complex monolayer at the air–water interface was monitored using a Wilhelmy plate attached to a microbalance, whose output was interfaced to a microcomputer which controls the movement of the barrier. pH of the subphase having DNA molecules was adjusted by using suitable amount of 0.5 M NaOH solution and the pH was measured by a high precision digital pH meter (Model 335, Make: Systronics, India).

Properly cleaned Langmuir trough was filled with triple distilled deionized water. The surface of water in the trough was cleaned by a suctioned pump so that the surface pressure attains some negative value. Appropriate amount of chloroform solution of ODA (concentration of 0.5 mg/ml) was spread by a microsyringe onto the air–water interface keeping the barrier fixed. After a delay of 15 min, to evaporate the solvent, ODA film at air–water interface was compressed slowly at 2 × 10⁻⁹ nm² mol⁻¹ s⁻¹ to record the surface pressure–area per molecule isotherm. When a desired surface pressure (0 mN/m) was achieved, DNA aqueous solution (concentration of 0.5 mg/ml) was spread on the water of the LB trough from the backside of the barrier as schematically shown in Fig. 1(c) and the corresponding increase in surface pressure with time was recorded. ODA/DNA mixed LB films were deposited onto quartz, ZnSe and hydrophilic Si substrates. These substrates were thoroughly cleaned by ultra-sonication for 15 min in a mixture (1:1) of isopropyl alcohol and acetone following rinsing by deionized water. The substrates were then dried under blowing N₂ gas. UV–vis absorption spectroscopic studies were performed by Perkin Elmer Lambda 25 UV–vis spectrophotometer. For ATR–FTIR spectroscopy of the LB films, the ODA/DNA complex Langmuir monolayer was deposited onto ZnSe single crystal. FTIR spectrophotometer (Spectrum-100, Perkin Elmer) was used for the ATR–FTIR measurement. Atomic force microscopy of LB films was performed in air with a commercial AFM (Inova AFM System, Bruker) using silicon cantilevers with sharp, high apex ratio tip (Ultralevers, Vecco Instruments) of phosphorous (resistivity 1–10 Ω-cm, frequency 240–308 kHz, spring constant 20–80 N/m, with backside Al coating of thickness 50 ± 10 nm). The AFM images presented in this article were obtained in intermittent-contact (tapping) mode. Hydrophilic Si substrates were used for monolayer deposition for AFM measurement.

3. Results and discussions

3.1. Adsorption isotherm of ODA–DNA complex monolayer at the air–water interface

To observe the adsorption of DNA molecules on to the oppositely charged ODA monolayer at the air–water interface, Langmuir–Blodgett film deposition instrument was used. The subphase surface was initially cleaned by sucking the impurities in such a high precession so that a negative surface pressure exists. Under this condition about 150 µl of ODA (concentration of 0.5 mg/ml) was spread onto the water surface of
LB trough and then 15 min time was allowed to evaporate the solvent before it was compressed to a desired surface pressure (0 mN/m) at the air–water interface. It has been observed that the surface pressure remains fixed for more than 500 min after attaining this desired surface pressure in absence of DNA (figure not shown). After attaining 0 mN/m surface pressure, the barrier was kept fixed and various amount (500, 1000, 2000 and 4000 μl) of DNA aqueous solution (concentration 0.5 mg/ml) were injected into the subphase slowly from the backside of the barrier as schematically shown in Fig. 1(c). Considering the total subphase volume, the concentrations of DNA in the subphase are calculated as 0.21633 microgram/ml, 0.43308 microgram/ml, 0.86542 microgram/ml and 1.7278 microgram/ml for each case respectively. Therefore, the ratios of ODA and DNA in the subphase became 3:10, 3:20, 3:40 and 3:80 respectively for each case. Since DNA is water soluble, initially it’s diffusion in the stationary subphase was slow. That is some DNA molecules contacted the ODA monolayer first as one side, which was near the barrier and then diffused to other side in the trough slowly. Fig. 2(a) shows the change in surface pressure for the formation of ODA/DNA complex LB monolayer at air–water interface at fixed subphase pH of 11. pH 11 was considered here as the interaction between ODA and DNA becomes larger as discussed later. From this figure it is observed that the surface pressure of the ODA monolayer increases with the time elapsed after spreading DNA onto the subphase.

Although DNA is water soluble, ODA/DNA complex is water insoluble. It is also evident that the area per molecule of this complex is greater than ODA area per molecule at a particular time [30]. However as the barrier was kept fixed, area per molecule of the monolayer could not be increased, and as a consequence, surface pressure began to rise and with increasing time the surface pressure showed a gradual increase which was an indication of adsorption of DNA to ODA monolayer and thereby formation of stable ODA/DNA complex Langmuir monolayer at the air–water interface. The rate of adsorption also increases with the concentration of DNA in the subphase. This indicates the availability of more number of DNA molecules in the subphase to interact with ODA monolayer by hydrophobic and electrostatic interaction. However, at pH 11, hydrophobic interaction between DNA sugar bases and ODA hydrophobic species dominates over electrostatic interaction as pKa of ODA is ~10.65 [31]. DNA is a kind of polyelectrolyte in the aqueous solution. In general, the entrapment of DNA molecules from subphase into ODA Langmuir monolayer is driven by electrostatic interaction between the negatively charged DNA and cationic ODA molecules at neutral pH (i.e. 7) i.e. the interaction between the negatively charged phosphate (PO4-) ion and positively charged amine (NH3+) group of ODA at the air–water interface.

Fig. 2(b) shows the surface pressure versus time elapsed plot of ODA/DNA complex LB monolayer at the air–water interface for different pH values (namely, 7, 8, 9.5 and 11) of the subphase. From the figure it is observed that the rate of adsorption of DNA molecules to the preformed ODA monolayer increases with increasing pH of the aqueous subphase. Near the neutral pH (i.e. 7), the interaction between DNA with ODA headgroups are mostly electrostatic and there is always an exchange of DNA molecules between the monolayer headgroups and the bulk water subphase. Therefore, it is also possible to suggest that electrostatic interactions provide a dynamic attachment of DNA to the monolayer [32] and so the rate of DNA adsorption was less at around pH 7 in the present study. However, at higher pH, the H–bond between the sugar bases of DNA becomes weaker and tries to separate the two strands of DNA [33]. At such higher values of subphase pH, there are more hydroxyl ions which also try to disrupt the H–bonds between the DNA base pairs. Additionally, ODA molecules lose their positive charges in this pH as pKa value of ODA is about 10.65 [31] and only few ODA molecules exist as protonated cationic species. This means weaker repulsive interaction in ODA monolayer at pH around 11. In this situation hydrophobic interaction between sugar bases of DNA and hydrophobic part of ODA dominates over the electrostatic interaction thereby causing aggregation of DNA in ODA/DNA complex LB monolayer [34]. Therefore the increase in surface pressure at higher pH of 11 is mainly due to the hydrophobic interaction between DNA and ODA molecules. It is important to mention that the isoelectric point of DNA is about 5 and DNA molecules are negatively charged due to phosphate groups when pH is above the isoelectric point [35,36]. Below the isoelectric point DNA loses the negative charges in their phosphate groups and so pH 7 was considered as the initial subphase pH in this present experiment.

3.2. UV–vis absorption spectroscopy

The UV–vis absorption spectroscopy is an important tool to determine whether there is any π–π stacking occurs within DNA. Fig. 3(a) shows the UV–vis absorption spectra of DNA aqueous solution along with pure bulk DNA sample deposited onto quartz substrates. From the figure it is observed that DNA double helix in aqueous solution has a specific absorption band from 220–300 nm and λmax at about 262 nm which is the characteristics of DNA absorption and is attributed to the π–π* transition of nucleic acid bases of DNA [37]. The corresponding
transition dipole moments lie along the short axis of base pair [38]. On the other hand, bulk sample of pure DNA onto quartz substrate gives somewhat broadened absorption profile with similar band pattern, only the peak position has been shifted to 270 nm.

The nucleic acid bases A and T are the strong UV chromophores and functional groups involved in H-bonding are part of chromophoric π electron system. For absorption spectrum of DNA in their bulk solid phase, the observed bathochromic shift is definitely due to the different π–π stacking structure as the closer association of several DNA strands and this also corresponds to the change in their π–π* transition [39,40]. Additionally, the spectral broadening is due to the change in H-bonding interaction and interchromophoric coupling [41] which corresponds to the hypochromic effect of DNA in their bulk solid phase when compared to the ssDNA where hyperchromic effect (DNA absorbs more UV energy) is observed. A. Bhowmik et al. [42] reported that several DNA strands overlap together to form bundles on the surface of the Langmuir–Blodgett films.

Fig. 3(b) shows the UV–vis absorption spectra of 10 bilayered ODA/DNA LB films lifted from the subphase having two different pH namely 6.8 and 11.5 and at a surface pressure of 20 mN/m. ODA/DNA LB films lifted from pH 6.8 show similar absorption band profile having almost identical peak position (262 nm) but only the change in their intensity distribution when compared to DNA solution or bulk sample absorption spectra. This certainly indicates that DNA molecules from the subphase interacted with preformed ODA Langmuir monolayer at the air–subphase interface and ODA/DNA complex monolayer was successfully transferred onto quartz substrates at that particular subphase pH. But for the film prepared with subphase pH 11.5, the absorbance intensity decreases abruptly. As mentioned earlier that at such high pH, the subphase having DNA molecules is rich in hydroxide ions, and these negatively-charged ions can pull off the hydrogen ions of molecules from the base pairs in DNA. This process disrupts the H-bonding that holds the two DNA strands together, causing them to separate from each other and DNA molecules were denatured and have random coil conformation [43]. Additionally most of the ODA molecules at such higher pH lose their protonated form (as pKa ~ 10.65) [31] thereby causing enhanced hydrophobic interaction between ODA and hydrophobic sugar bases of DNA. These interactions are stronger and more specific than electrostatic ones [44]. As a result, ssDNA had compacted or aggregated random coil like or globular shaped conformation [45] in ODA/DNA complex LB monolayer which were then immobilized onto quartz or Si substrates at 20 mN/m. Although ssDNA absorbs more UV energy than dsDNA (hyperchromic effect) [46], in our present study, UV absorption of ssDNA/ODA LB film decreases due to randomness and compactness of ODA/DNA complex assemblies. Because the magnitude of the resultant transition dipole moment of the assembly of ssDNA molecules in the backbone of ODA was sufficiently decreased in ODA/DNA LB films lifted at pH 11.5 and is manifested as abrupt fall of intensity of absorption peak around 262 nm as shown in Fig. 3(b). In one of our earlier works we have observed similar decrease in absorbance when ssDNA after thermal denaturation adsorbed on ultrathin films of some poly electrolyte [47]. The absorbance remains unaltered even after several days of fabricating ODA/DNA LB films. This indicates that stable ssDNA molecules have been successfully immobilized onto LB films.

3.3. FTIR spectroscopy

Fig. 4 shows the FTIR spectra of pure ODA and DNA in KBr pellets and also the ATR–FTIR spectrum (bottom image) of 10 layer of ODA–DNA complex LB film deposited onto ZnSe single crystal substrate for subphase pH of 7 and at a fixed surface pressure of 20 mN/m. The transfer ratio of the monolayer to the substrate was fixed around 0.98 by keeping the fixed surface pressure of the complex monolayer. DNA, being a complex molecule, bears a wealth of vibrational modes, and their disentanglement is not easy in some spectral region [47]. From figure we observe that pure DNA shows several distinct vibrational modes whose peaks are centered at 1064, 1216, 1380 and 1686 cm\(^{-1}\). The IR bands in the 1500–1800 cm\(^{-1}\) region originating to nucleobase vibrations appear which are extremely sensitive to base stacking and base pairing interactions. These bands mainly originate from in-plane double bond base vibrations, which include C=C, C=N and C=O stretching [48]. On the other hand, bands in the 1500–1250 cm\(^{-1}\) region, vibrational coupling between the base–sugar entities give rise to nucleoside specific information, reflecting glycosidic bond rotation, backbone conformation and sugar pucker. In the 1250–1000 cm\(^{-1}\) region, vibrations along the sugar–phosphate chain give rise to strong markers of backbone conformation. In the 1000–800 cm\(^{-1}\) region, sugar–sugar–phosphate vibrations result in reliable markers for the various sugar puckering modes. The FTIR spectra of ODA in KBr show strong prominent bands at 2847 and 2920 cm\(^{-1}\). These two bands are the diagnostic bands of ODA and identified as the methylene (CH\(_2\)) antisymmetric and symmetric stretching vibrations from the hydrocarbon chains of ODA [49,50].

Fig. 4 (bottom image) also shows the ATR–FTIR spectrum of ODA/DNA complex LB monolayer deposited onto ZnSe substrate. The complex LB monolayer film shows several band systems in the 800–3000 cm\(^{-1}\) region. Interestingly the intensity of IR bands of DNA

![Fig. 3](image-url)
was sufficiently reduced in the mixed monolayer. Additionally the observed slight shifts of the two IR bands position (2843 and 2896 cm\(^{-1}\)) of ODA in the ODA/DNA complex LB monolayer film deposited onto ZnSe substrate are due to the electrostatic complexation between the hydrophilic parts of positively charged ODA and negatively charged phosphate group of DNA molecules. Although electrostatic interaction plays major role in binding DNA with cationic ODA at the air–water interface, some kind of hydrophobic interactions are also prone to appear between hydrophobic species of ODA and hydrophobic domain of DNA sugar bases [32].

3.4. Atomic force microscopy

Atomic force microscopy is now a reliable and viable method for understanding surface morphology including roughness and thickness nano-dimensional film of various biological and other materials onto solid substrate [51–53]. Fig. 5(a) and (b) shows the atomic force micrographs of cleaned hydrophilic silicon substrate and pure ODA monolayer onto the substrate respectively whereas 5(c), 5(d) and 5(e) show the AFM topographic images of ODA/DNA complex LB film (one layer) onto hydrophilic Si substrates deposited at different surface pH namely 6.8, 9.2, and 11.5 respectively at a fixed surface pressure of 20 mN/m. Height profile analysis of these AFM topographic images is described in Table 1. From the analysis it is observed that the RMS roughness (Rq), average roughness (Ra) and maximum peak to valley (Rp-v) height were increased for ODA/DNA LB films deposited at various pH when compared to those of cleaned Si substrate and ODA monolayer onto the substrate. However the average height for ODA/DNA LB films deposited at pH 6.8 and 9.2 are almost similar showing similar surface morphology (as average height becomes 1.5 nm in both the cases). Interestingly at higher surface pH (11.5) ODA/DNA complex LB film shows aggregated and compacted morphology with a maximum peak to valley height of 20 nm. From this observation it can be concluded that at pH 6.8 DNA molecules can lie flat or extended conformation in the complex ODA/DNA LB films onto the substrate. On the other hand for pH 11.5 there is enhanced hydrophobic interaction between the hydrophobic sugar bases of DNA and ODA hydrophobic species in the complex monolayer. As a result aggregation and compactness of DNA occur in the monolayer, which is reflected as the increasing roughness and average height of ODA/DNA LB films at such higher pH. Additionally at this higher pH, due to alkaline denaturation, ssDNA had compacted random coil like or globular shaped conformation in the backbone of DNA molecules thereby decrease in UV absorption of complex ODA/DNA LB films deposited at pH 11.5. These observations suggest that DNA molecules in ODA/DNA LB films cannot lie flat or extended conformation onto the solid substrate at extremely higher pH.

4. Conclusions

In conclusion our results show that anionic polynucleotide DNA and cationic surfactant ODA can form stable complex Langmuir monolayer at the air–water interface. The adsorption of DNA on to the preformed ODA monolayer was confirmed by the rise in surface pressure as a function of time after spreading DNA aqueous solution into the subphase of Langmuir trough while keeping the barrier fixed. Near the neutral pH, the entrapment of DNA molecules from subphase into ODA Langmuir...
monolayer is mostly driven by electrostatic interaction between the DNA and ODA molecules. The extent of interaction was found to be dependent on concentration and pH of the subphase containing DNA. Hydrophobic interaction dominates over electrostatic interaction between DNA and ODA in the monolayer with increasing subphase pH. At higher pH aggregates of DNA was formed in the ODA/DNA complex monolayer. The complex monolayer was successfully and repeatedly transferred onto quartz substrates to get multilayered ODA/DNA LB films. UV–visible absorption spectroscopic studies also confirm the presence of DNA molecules in complex LB films deposited at both neutral and higher pH (namely 11.5) of the subphase. Interestingly, at pH 11.5 the absence of 262 nm band of DNA was possibly due to the random coil or globular shaped conformation of DNA in LB films. FTIR and ATR–FTIR spectroscopic measurements reveal the presence of DNA in ODA/DNA complex monolayer and LB films. The IR bands of both DNA and ODA were greatly influenced by the electrostatic complexation between ODA and DNA. AFM images of ODA/DNA complex LB films deposited at various pH give the clear surface morphology and formation of DNA aggregates especially at pH 11.5. At neutral pH DNA lies flat or extended in the backbone of ODA whereas at high pH such as 11.5 they do not lie flat as confirmed by height profile analysis of AFM images.

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<th>Table 1</th>
<th>Table for showing AFM topographic analysis of clean Si substrate, ODA monolayer, ODA/DNA LB films deposited onto Si substrate for various subphase pH.</th>
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<tr>
<td>Clean Si substrate</td>
<td>ODA monolayer onto Si substrate</td>
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<tr>
<td>RMS roughness(Rq)</td>
<td>0.0419 nm</td>
</tr>
<tr>
<td>Average roughness(Ra)</td>
<td>0.030 nm</td>
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<tr>
<td>Maximum peak to valley height(Rp-v)</td>
<td>0.5 nm</td>
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References


