Immobilization of single strand DNA on solid substrate

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Received 2 April 2007; in final form 16 October 2007
Available online 26 November 2007

Abstract

This Letter reports the immobilization of DNA onto a solid support by electrostatic interaction with a polycation poly(allylamine hydrochloride) (PAH). The films were analyzed by UV–vis spectroscopy. The significant observation is that single stranded DNA gets immobilized on the PAH backbone of Layer-by-Layer (LbL) films when the films are fabricated above the melting temperature of DNA. Singly stranded DNA immobilized in the LbL films is not restored into double stranded DNA at room temperature.

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1. Introduction

DNA (deoxyribonucleic acid) is an interesting anionic polyelectrolyte with unique double helix structure [1] whose base sequence controls the heredity of life. Immobilization of DNA onto a solid substrate has profound biological and technological advantages having a wide range of research areas including work on nanoparticles [2], DNA computing [3,4], DNA chip technologies and single molecule biology [5]. Now a day’s immobilization of DNA on an electrode is also used to produce biosensors [6,7]. Some of the common substrates for the immobilization of DNA are latex particles [8], glass substrates [9], silanized mica substrate [9], gold nanoparticles [10], polystyrene microspheres [8] etc. The requirement for reproducible stable surfaces has created increasing interest in designing modified surfaces to immobilize DNA. The techniques used for immobilization of DNA onto solid substrates are Layer-by-Layer (LbL) self assembly by electrostatic interaction [11] as well as also by Langmuir–Blodgett (LB) technique [12].

LbL films are predominantly assembled by the alternate deposition of positively and negatively charged polymers facilitated by electrostatic interaction [13,14]. In one of the previous studies on DNA–LbL films [15] the control of the morphology of the film was achieved by manipulating the extent of hydrogen bonding and repulsion between the negatively charged phosphate backbones. In another work, hydrogen bonding and hydrophobic interaction were used to facilitate LbL assembly of uncharged polymers [11]. The thermal separation of DNA strands, commonly known as the denaturation or melting [1], has been experimentally observed by taking the UV–vis absorption spectra of aqueous solution of DNA at different temperatures. The specificity of hydrogen bonding between the bases along with the phosphodiester bond maintain the basic double helical structure of DNA and at higher temperature due to the increase in thermal energy, breaking of only hydrogen bonds occur. As a result the two DNA strands are separated. The denaturation of DNA changes the intensity of its 260 nm absorption band. Due to vertical unstacking intensity of 260 nm band in single stranded DNA solution is much higher than that of the double stranded DNA solution [1]. Utilizing this principle the present investigation has been carried out to study the photophysical characteristics of DNA–LbL films fabricated by electrostatic interaction of polyamionic native and denatured DNA with a polycationic poly(allylamine hydrochloride) (PAH). PAH being a photophysically inert
substance, UV–vis absorption spectroscopic studies exhibit the characteristics of DNA organized in LBL films. The films fabricated by changing various parameters have been the subject of this investigation.

2. Experimental

Herring sperm sheared DNA purchased from SRL India, was used as received. The purity of DNA was checked by UV–vis absorption and fluorescence spectroscopy before use. Poly(allylamine hydrochloride) (PAH) was used as the polycation. PAH (molecular weight = 70000, purity >99%), purchased from Aldrich Chemical Co., USA, was used without any further purification. The deposition bath was prepared with 0.2 mg/ml DNA and 10⁻³ M (based on the repeat units for polyion) of PAH aqueous solutions using triple distilled deionised (18.2 MΩ) Millipore water.

Layer-by-Layer (LbL) self-assembled films were obtained by dipping thoroughly cleaned fluorescence grade quartz substrates alternately in solutions of the polyelectrolyte PAH and oppositely charged DNA. PAH was used as polycation for the fixation of the DNA molecules on to the substrate. Initially the quartz substrate was cleaned by standard procedure[13] and immersed in the PAH solution for 15 min followed by rinsing in water bath for 2 min. This rinsing washes off the surplus cation attached to the surface. The substrate was then immersed in DNA solution for 15 min followed by same rinsing procedure. After each deposition and rinsing procedure sufficient time was allowed to dry up the film and their UV–vis absorption spectra were recorded to monitor the film growth. Deposition of successive PAH (cation) and DNA (anion) layers resulted in one bilayer of self-assembled PAH–DNA–LbL film. The whole sequence of the film deposition procedure was repeated for the preparation of desired number of bilayer LbL films. Denaturation of DNA was studied by dipping one layer PAH film into DNA solution at different temperature keeping the immersion time in DNA solution constant for 15 minutes. The UV–vis absorption spectra of the LbL films as well as solutions were recorded using Lambda-25 UV–vis spectrophotometer, Perkin-Elmer. AFM picture was taken by Atomic force Microscope (Veeco Digital Instrument CP II Microscope) installed at Indian Association for the Cultivation of Science, Jadavpur, Kolkata.

3. Results and discussion

Fig. 1 shows the UV–vis absorption spectra of the aqueous solution of DNA (10⁻³ M), aqueous solution of DNA–PAH mixture (1:1 volume ratio) and DNA microcrystal.

Absorption spectrum of DNA–PAH mixed aqueous solution shows similar band pattern. It may happen due to the fact that there is no interaction between PAH and DNA in solution phase. However, there is clear evidence of interaction between PAH and DNA in LbL films as discussed later. It was also evident that DNA molecules were successfully adsorbed in PAH films. Incorporation of DNA–PAH multilayers only increases the intensity of absorption bands (Fig. 2), however no changes in band pattern and band position are observed. Therefore the most possibility is that DNA interacts with PAH even in solution phase. PAH–DNA interaction occurs with the cationic NH₃⁺ group of PAH molecule through the phosphate (PO₄³⁻) ions tagged at the sugar bases of DNA, whereas the purine and pyrimidine bases remain unaffected. As a result there are no changes in absorption band pattern and position in case of DNA–PAH solution.

DNA microcrystal absorption spectrum gives broadened absorption profile with almost similar band pattern. However the peak position is shifted to about 270 nm. This may be due to the change of microenvironment of individual DNA molecules in the microcrystal and in solution or in films. It has also been reported that several DNA strands overlap together to form bundles on the surface of the Langmuir–Blodgett films [5].

Fig. 2 shows the absorption spectra of different layered PAH–DNA–LbL films starting from 1 bi-layer to 33 bi-layers. In all the cases absorption bands give the similar spectral profile with peak at 262 nm. This is a clear indication that DNA molecules are successfully incorporated into...
the PAH–DNA–LbL films due to the interaction with PAH molecules.

The inset of Fig. 2 shows the plot of integrated intensity of absorption maximum (262 nm) as a function of layer number. It is evident that the intensity of this band increases linearly for lower number of layers (up to 10 layers) indicating uniform deposition of DNA molecules onto quartz substrate. However for higher number of layers (>10 layers) the intensity does not increase linearly indicating less amount of DNA deposition at higher number of layers. This may be due to the fact that as the number of layers increases, the surface roughness of the film increases. As a consequence the electrostatic interactions do not work as well and amount of DNA deposited decreases.

The absorption spectra of PAH–DNA one bi-layer LbL films with different dipping time in DNA solution is shown in Fig. 3a. Here in all the cases the deposition time of polymer was kept fixed at 15 min. However the DNA deposition times were taken from 1 min and by varying different time interval up to a maximum time limit of 50 min. From the figure it has been observed that the intensity of absorption spectra increases initially with DNA deposition time and gets saturation at 12 min. This is evident from the plot of the intensity of absorption maximum versus time (inset of Fig. 3a) of 262 nm peak. The amount of DNA adsorbed by PAH layer at saturation (i.e. after 12 min) of absorption maximum has been found to be 35.2 μg using the intensity of absorption maximum and following Beer–Lamberts law.

Moreover, from the saturation value of the intensity of the absorption maximum (Inset of Fig. 3a) it is evident that the interaction of DNA molecules with the PAH layer is completed by 12 min and no PAH molecule remains free within the film after 12 min for further interaction with the DNA molecules. As a result a complex PAH–DNA layer is formed.

To investigate the surface topography and roughness of PAH–DNA Layer-by-Layer self assembled films, we have taken AFM image of LbL films having outer DNA layer and shown in Figs. 3b and 3c. From the figure it is observed that DNA molecules have closed packed conformation in the LbL films. It also gives a clear visual evidence that DNA molecules cover the whole film area after the interaction with PAH is completed. The scanned surface area on the film was 0.1761 mm² whereas the projected area was 0.1705 mm² and the root mean square (RMS) (Rq) surface roughness was calculated as 1.864 nm. Maximum peak to valley height (Rpv) was found to be 21.31 nm. The line roughness parameters for the PAH–DNA–LbL multilayer films have been calculated by computing the spatial power spectrum of the image and the root mean square (RMS) (Rq) roughness in this case has been found to be 1.351 nm.

It is a well-known fact that at and above 60 °C, breaking of hydrogen bonds of the double stranded DNA starts and around 90 °C DNA denaturation or melting [1] occurs resulting in complete separation of two strands.
In order to check whether the two DNA strands can be immobilized, we performed a unique experiment of fabricating the LbL films of DNA at various temperatures on a preassembled PAH layer onto quartz substrate.

DNA monolayer was deposited on PAH layer at different temperature starting from 60 °C to 95 °C with an interval of 5 °C. The dipping time was kept fixed at 15 min.

UV–vis absorption spectra of these LbL films are shown in Fig. 4. The most interesting part of this observation is that although the band pattern are similar in all the cases, the absorbance intensity increases to a large extent starting from the film fabricated at 60 °C to the film fabricated at 90 °C. However the maximum absorbance intensity was observed around 90 °C in our experimental design. With further increase in temperature to 95 °C, the absorbance intensity is abruptly reduced. Films cannot be fabricated above 95 °C due to technical difficulties.

Again films fabricated by decreasing the solution temperature (from 95 °C to 60 °C with an interval of 5 °C) also give the similar absorbance intensity pattern as shown in Fig. 4 (dotted line). In this case the freshly prepared DNA aqueous solution was taken initially to 95 °C temperature and then the temperature was decreased systematically and films were fabricated at various desired temperatures.

Fig. 5 gives a schematic representation of DNA molecules in the LbL films fabricated at various temperatures. Several authors discussed about the schematic representation of DNA molecules below and above the denatured temperature [18–20]. Fig. 5a shows the schematic diagram of DNA molecules in the PAH–DNA–LbL films formed at room temperature where the initial double helix structure of DNA prevails. The transition dipole moment of 262 nm absorption band is directed along the short axis of horizontal base pair [17] and the resultant transition dipole moment of this band is due to the vector sum of all the individual transition dipole moment of nucleic acid base pairs in the DNA strands. Under twisted condition, these individual transition dipole moments are directed differently. As a result, the magnitude of the resultant transition dipole moment is reduced and the intensity of the 262 nm absorption maximum is substantially reduced at low temperature.

Above 60 °C temperature, due to thermal agitation breaking of hydrogen bond starts and the two DNA
strands get separated away from each other as shown in Fig. 5b. The DNA strands increase dramatically both in length and diameter accompanied by a corresponding increase in the length of the transition dipole moment vector [20]. This is because the effective dipole moment of DNA is known to increase with the increase in length of DNA strands [21]. The most plausible explanation is that during unwinding condition of the DNA strands, most of the individual transition dipole moments along the short axis tend to orient along the same direction and the magnitude of resultant dipole moment increases. The resultant increase in transition dipole moment is manifested by the observed increase in absorbance at the 262 nm band in the absorption spectra of DNA–PAH–LbL films deposited onto quartz substrates at different temperatures.

However around 95 °C the single stranded DNA molecules, resulting from denaturation, form random coil without a regular structure [20]. Fig. 5c shows the most possible representation of DNA molecules on the PAH backbone of the solid substrate in the films fabricated above denaturation temperature. In such a state the individual dipole moment vector of the bases is oriented in different direction and the length of the resultant dipole moment vector is reduced to a considerable extent. Consequently the absorbance intensity falls abruptly.

DNA molecules once immobilized at higher temperature in the PAH–DNA mixed LbL films, their organization remain unaffected even if the films are taken back to normal temperature, as is evidenced from the absorbance intensity shown in Fig. 4.

Stabilization studies also indicate that once the DNA are immobilized on the PAH films in different organization, they remain so even after 15 days (figure not shown).

The most interesting observation in our work is that once the denatured single strands DNA molecules are immobilized on the PAH backbone of the LbL films at higher temperature, they remain immobilized on the films even when the films are again taken back to the room temperature. This is manifested by the increase in the intensity of the absorption band in LbL films fabricated at higher temperature than that fabricated at lower temperature, although all the absorption spectra have been taken at room temperature. It has also been observed that once the denatured single strand DNA are immobilized by PAH on the LbL films, the organization of DNA remains unaffected even after 15 days as is evidenced by the unaltered absorbance intensity.
4. Conclusion

In conclusion our results show that DNA molecules can be immobilized on the PAH backbone of a solid substrate by electrostatic interaction. Absorption spectroscopic studies definitely confirm the successful incorporation of DNA molecules in PAH–DNA–LbL films. Spectroscopic characteristics also exhibit the nature of DNA organization in LbL films. Time dependent studies indicate that the whole process of formation of DNA layer on PAH backbone takes only 12 min after which no PAH molecule remains free to interact with the excess DNA molecules in the solution. The most significant observation in our work is that once the denatured single strand DNA molecules get immobilized on the PAH backbone of the LbL films at higher temperature, they remain immobilized on the films even when the films are again taken back to the room temperature. This is manifested by the increase in the intensity of the absorption spectra of the films fabricated at higher temperature than that fabricated at lower temperature, although all the absorption spectra are taken at room temperature. It has also been observed that once the denatured single strand DNA are immobilized by PAH on the LbL films, the organization of DNA remains unaffected even after 15 days as has been evidenced by the unaltered absorbance intensity.

Acknowledgements

The authors are grateful to DST and CSIR, Government of India, for providing financial assistance through FIST-DST Project No. SR/FST/PSI-038/2002 and CSIR Project Ref. No. 03(1080)/06/EMR-II.

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