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Effect of denaturation of DNA on the molecular organization of a fluorescent dye in ultra thin films

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ABSTRACT

Acridine Orange (AO) forms H-dimer in solid state and in ultra thin films. However, H dimer of AO reduces its efficiency as a usefull material for fluorescence probe. In the present work detailed investigations has been done on the interaction of AO with different forms of DNA in order to check the effectiveness in controlling the dimeric sites of AO in the Layer by Layer (LbL) self assembled film. It was found that single stranded DNA (ssDNA) is most effective than the dsDNA and coil-shaped DNA in controlling the dimeric sites of AO in LbL film.

KEYWORDS

Acridine Orange; dsDNA; ssDNA; coil-shaped DNA; layer-by-layer (LbL) self assembled film; UV-vis spectroscopy

1. Introduction

Deoxyribonucleic acid (DNA) is a well-known hereditary material present in all living organisms. It is an anionic polyelectrolyte having double helix structure [1]. Its base sequence controls the heridity of life. Interaction of DNA with dyes is a topic of hot research interests, which include a wide range of areas in biology, physics, chemistry, polymer engineering and also in the application of biosensors [2–6]. Due to ionic nature it can interact with oppositely charged molecules. Based on this interaction DNA can be anchord onto solid substrates also. Each DNA molecule consists of two biopolymer strands coiled around each other to form a double helix structure. Hydrogen bonding between the biopolymer strands is responsible for the double helix structure. At high temperature (>60°C) and also in high alkaline aquous solution (pH > 10) breaking of hydrogen bonds between the two strands started. This results in the unwinding of the two strands of DNA. This is known as denaturation of DNA. At the starting of denaturation, DNA becomes partially unwound and at the final stage (>90°C or > 13 pH) double-stranded DNA (dsDNA) is transformed into totally denatured single stranded DNA (ssDNA). When complete denaturation occurs ssDNA forms random coil without a regular structure [7–9]. When denaturation was made by increasing the pH of the medium, it is called alkaline denaturation and when by raising the temperature of the medium, it is called melting denaturation.

Being anionic DNA can easily interact with the cationic molecules by electrostatic interaction. There are two interaction processes by which DNA can interact with cationic molecules. In the first process, small cationic molecules are intercalated between the base pairs of DNA and in the second process small cationic molecules are bound onto the negatively charged

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backbone of DNA [10, 11]. In the first process, the main force reponsible for the intercalation is the short range Van der Waals force and in the second process the main acting force is the long-range electrostatic coulombic force [10,11].

Acridine orange (AO) used in our study is a cationic fluorescent dye. AO is widely used as a stainer for the characterization of biopolymers [12, 13]. The interaction of AO with anionic polyelectrolytes has vast applications in the technical field [13, 14]. Interactions between gold nanoparticles and AO has been studied in detail [15]. The cytotoxicity of Titanium Dioxide Nanoparticles in Mouse Fibroblast cells has been studied using AO as a stainer [16]. There are several reports about the intercalation of AO into the base pairs of DNA [17, 18]. It was reported by several authors that AO molecules can intercalate into the base pairs of DNA in double-stranded form (dsDNA), while in single-stranded form (ssDNA), cationic molecules are bound on the negatively charged backbone of ssDNA by electrostatic interaction [19–22].

At 10^{-4} M concentration of the aquous solution of AO, UV-vis absorption spectrum shows intense dimeric band along with monomeric band. It clearly shows that even in aqueous solution, dimeric sites predominant to a larger extent. In solid state and also in the thin film, AO gives intense dimeric band and monomeric band reduces to a weak hump.This is due to the predominance of H-dimeric sites in the solid state of AO. H-dimeric sites reduce the fluorescence intensity resulting in the decrease of efficiency of AO as a biological stainer. The H-dimer of AO is very sensitive to the environment and thus AO can be used as a probe to investigate the intermolecular interactions between the dye and DNA. Dye – DNA interactions result in the organisation of dye molecules either onto the phosphate backbone of DNA or into the base pairs of DNA as intercalation.

In the present communication, a detail investigations have been made to study the role of DNA in controlling the dimeric sites of AO in ultra thin films fabricated by Layer-by-Layer (LbL) self assembled technique. Investigations have been made to study the effect of denatured DNA in controlling the dimeric sites of AO. Denaturation of DNA was made by increasing the temperature as well as also by changing the pH of the aqueous solution. Depending on the interaction between AO and DNA, spectral characteistics of AO change. Normally in LbL film, AO formed a large number of H-dimeric sites. This is due to the self-aggregation of AO molecules in the LbL film. Observed results showed that by introducing DNA the dimer formation of AO in LbL film can be controlled. We have also studied the effect of dsDNA, ssDNA, and coil-shaped DNA in controlling the dimer sites of LbL film of AO. It was observed that ssDNA was more effective in controlling the H-dimeric sites of AO in LbL film.

2. Experimental

2.1. Materials

DNA used is sheared Salmon sperm DNA having a size of nearly about 2,000 bp with approximate GC content 41.2%, purchased from SRL India and was used as received. AO (M.W. = 301.8), purity > 99%, poly (allylamine hydrochloride) (PAH), purity > 99%, were purchased from Aldrich Chemical Co., and were used as received. The purity of DNA and AO was checked by UV-vis absorption spectroscopy before use.

2.2. Methods

Electrolytic solution baths were prepared using triple-distilled deionized (electrical resistivity 18.2 MΩ-cm) Millipore water. In order to make the mixture of DNA (con. = 0.1 mg/ml)

and AO (con. = 10^{-4}M) solution, they were mixed with different volume ratios. The UV-vis absorption of mixed solutions as well as mixed LbL films was taken. For mixed AO – DNA LbL film preparation quartz substrates were first dipped into the cationic PAH solution for 15 min. Then it was taken out and sufficient time was allowed for drying and then rinsing in water bath for 2 min so that the surplus cations attached to the surface were washed off. The slides were then dipped into mixed solution of AO – DNA for 30 min followed by similar rinsing in a separate set of water bath to result monolayer PAH – AO – DNA LbL film. All the adsorption procedures were carried out at room temperature (25°C).

In case of pH and temperature variation of DNA, the quartz slide was first dipped into electrolytic polycation (PAH) aqueous solution for 15 min followed by same rinsing in water bath and drying procedure and then dipped into the anionic DNA (con. = 0.1 mg/ml) solution of different pH (6.8, 11, 13) and temperature (30°C, 70°C, 95°C) which is again followed by rinsing action in water bath. The dried DNA films were then dipped into cationic AO (con. = 10^{-4}M) solution for 30 min followed by similar rinsing in a separate set of water bath to result in mixed AO – DNA LbL films. For increasing the pH of DNA solution, NaOH was used. For increasing the temperature of DNA solution, an automatic controlled hot water bath was used.

2.3. UV–vis absorption spectra measurement

UV–vis absorption spectra were recorded using a spectrophotometer (Lambda-25, Perkin Elmer). For absorption measurement, the LbL films were kept perpendicular to the incident light.

3. Results and discussions

3.1. Effect of dsDNA on the molecular organization of AO in solution phase

Figure 1 shows the normalized UV-vis absorption spectra of aqueous solution of AO (10^{-4}M) and mixed AO – DNA solutions at different volume ratios. Concentration of DNA was kept fixed at 0.1 mg/ml. UV-vis absorption spectrum of pure aqueous solution of AO shows two distinct and prominent bands with intense peaks at 470 and 490 nm. These are in well agreement with the reported results [23, 24]. The longer wavelength peak at 490 nm is due to monomeric absorption of AO. The higher energy peak at 470 nm is due to the formation of H-dimer of AO. In the AO – DNA mixed solution, with increasing the volume concentration of DNA, the dimeric peak was gradually reduced in intensity while monomeric peak became intense. It is also interesting to note that monomeric peak was gradually red shifted and at 1:6 volume ratios of AO and DNA in aqueous solution, it was red shifted to 500 nm. The 10-nm red shifting of monomeric peak in AO – DNA aqueous solution has been attributed as due to the intercalation and organization of AO molecules into dsDNA [17]. Inset of Fig. 1 shows the decrease of dimer/monomer ratio with the increasing percentage of volume fraction of DNA.

3.2. Effect of dsDNA on the molecular organization of AO in LbL film

LbL film of pure AO was fabricated by using a polyanionic poly (acrylic acid) (PAA). PAA being photophysically inactive, only the AO bands were observed. Hybrid AO – DNA LbL film was fabricated by using a polycationic poly (allylamine hydrochloride) (PAH).

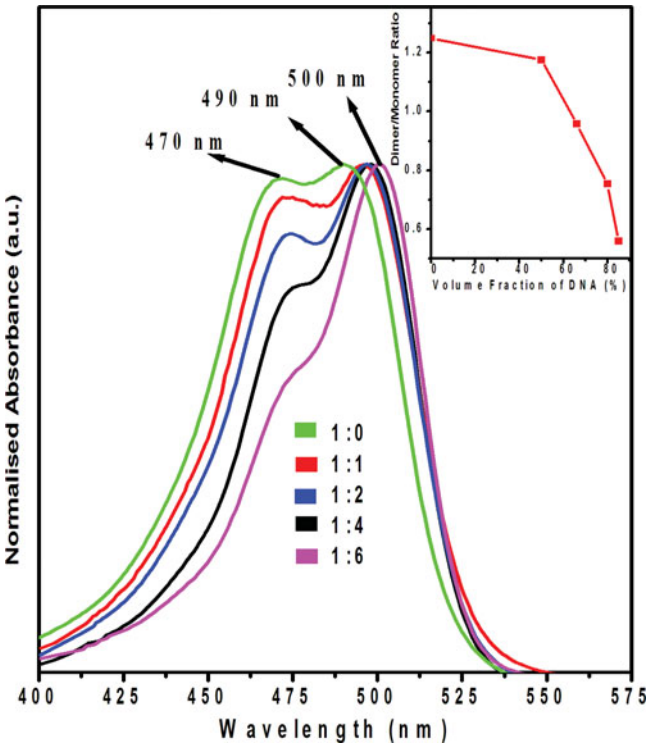

Figure 1. Normalized UV-vis absorption spectra of aqueous solution of AO (10^{-4}M) and mixed AO-DNA solution. AO mixed with DNA in different volume ratios. AO : DNA, 1:1, 1:2, 1:4, 1:6. Concentration of DNA was 0.1 mg/ml. Inset shows the decrease of dimer/monomer ratio with the increasing percentage of volume fraction of DNA in solution phase.

In pure AO LbL film, the cationic parts of AO molecules interacted with the anionic parts of the PAA molecules, and thus formed the hybrid molecules. AO molecules became closer side by side resulting in a favorable condition for aggregation and consequent H-dimer formation of AO molecules. Hence pure AO LbL film showed intense dimeric absorption band at 470 nm and the monomeric band at 500 nm reduced to a weak hump.

In AO – DNA mixed hybrid LbL film AO molecules were intercalated into the base pairs of dsDNA and also organized on the negatively charged phosphate backbone of dsDNA. It may also be mentioned in this context that at low AO concentration, AO molecules were mainly intercalated into the base pairs of dsDNA. With increasing AO concentration along with intercalation, AO molecules were also organized on the phosphate backbone of dsDNA. As shown in Fig. 2 at higher AO concentration dimeric band predominated, while at lower AO concentration (AO : DNA = 1 : 6) monomeric band predominated and the dimeric band reduced to a weak hump. As discussed above this was due to the intercalation of AO molecules into the base pairs of dsDNA. Inset of Fig. 2 also supports the decrease of the dimeric band with respect to the monomeric band.

3.3. A comparative study of the effect of dsDNA, ssDNA, and coiled form DNA on AO

A comparison has been made to study the effect of double-stranded DNA (dsDNA), single stranded DNA (ssDNA) and coiled form DNA in controlling the H-dimeric sites of AO molecules in the LbL film. At higher temperature >60°C and also at high pH > 10 breaking

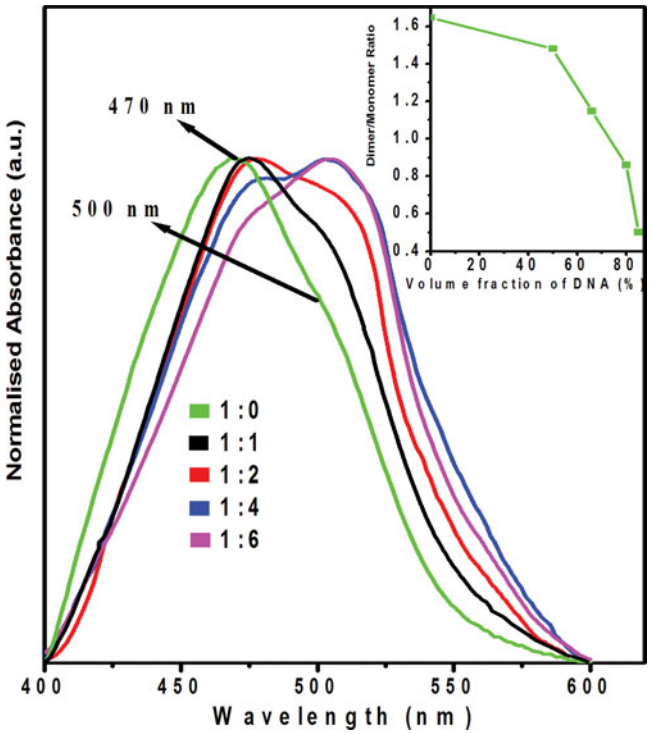

Figure 2. Normalized UV-vis absorption spectra of monolayer LbL film of pure AO (10^{-4}M) and mixed AO – DNA LbL film. AO : DNA = 1:1, 1:2, 1:4, 1:6. Concentration of DNA was 0.1 mg/ml. Inset shows the decrease of dimer/monomer ratio with the increasing percentage of volume fraction of DNA in LbL film.

of hydrogen bonding started resulting in the formation of single stranded DNA. This is called denaturation of DNA. Temperature dependent denaturation is known as melting denaturation and pH dependent denaturation is known as alkaline denaturation. Greater than 90°C and > 13 pH complete denaturation occurs. This results in the formation of fully denatured single-stranded DNA (ssDNA). The fully denatured ssDNA forms irregular-shaped coil-like structure. Interactions of AO with three different types of DNA structures have been investigated by employing UV-vis absorption spectroscopic technique.

Figure 3(a) and Fig. 4(a) show the UV-vis absorption spectra of monolayer LbL film of AO adsorbed onto three different types of DNA during alkaline and melting denaturation respectively. AO : DNA volume ratio was kept fixed at 1:1. At normal pH and also at 30°C temperature, DNA remains in the double-stranded form (dsDNA). UV-vis absorption spectra of AO – dsDNA in both the cases showed two distinct bands with intense dimeric band at 475 nm and the monomeric band at 500 nm was reduced to a weak hump. At pH 11 and at 70°C, denaturation occurred resulting in the formation of ssDNA. Due to electrostatic interaction AO molecules were adsorbed onto the phosphate backbone of ssDNA. The AO molecules thus arranged on the phosphate backbone of ssDNA remained separated from each other to a comparatively larger distance than in case of dsDNA. Thus, the closer association of AO molecules was restricted resulting in the decrease of dimeric sites. UV-vis absorption spectra of AO – DNA show a broad band which is the overlapping of both dimeric and monomeric bands as shown in both the Fig. 3(a) and 4(a). At pH > 13 and at 95°C temperature, complete denaturation of DNA occurred and single strands of DNA formed random coil-shaped structures. AO adsorbed on the phosphate backbone of these coil-shaped structures might come

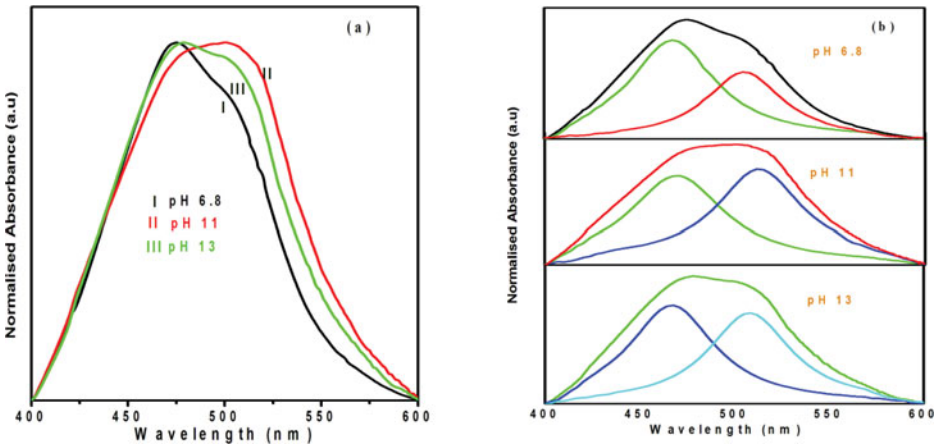

Figure 3. (a) Normalized UV-vis absorption spectra of monolayer LbL film of AO – DNA (1 : 1) at three different pH values of DNA solution (i) 6.8, (ii) 11, (iii) 13 and pH value of AO solution was 6.8. (b) Deconvoluted UV-vis absorption spectra of mixed AO – DNA monolayer LbL film at three different pH values.

into closer proximity with each other, increasing the number of dimeric sites. Thus the UV-vis absorption spectra show intense dimeric band with decreasing intensity of monomeric band in both the cases of alkaline and melting denaturation.

Sufficient confirmation about the overlapping components present in a spectrum can be obtained from the deconvolution of the spectrum [25]. Fig. 3(b) shows the deconvolution of the absorption spectra of monolayer LbL film of AO – DNA at three different pH values namely pH 6.8, 11, and 13. Also Fig. 4(b) shows the deconvoluted spectra of monolayer LbL film of AO – DNA at three different temperatures of 30°C, 70°C, and 95°C respectively. It is clearly observed that each deconvoluted spectrum was resolved into two Gaussian curves, one for the dimeric band and the other for the monomeric band. At normal pH and at 30°C

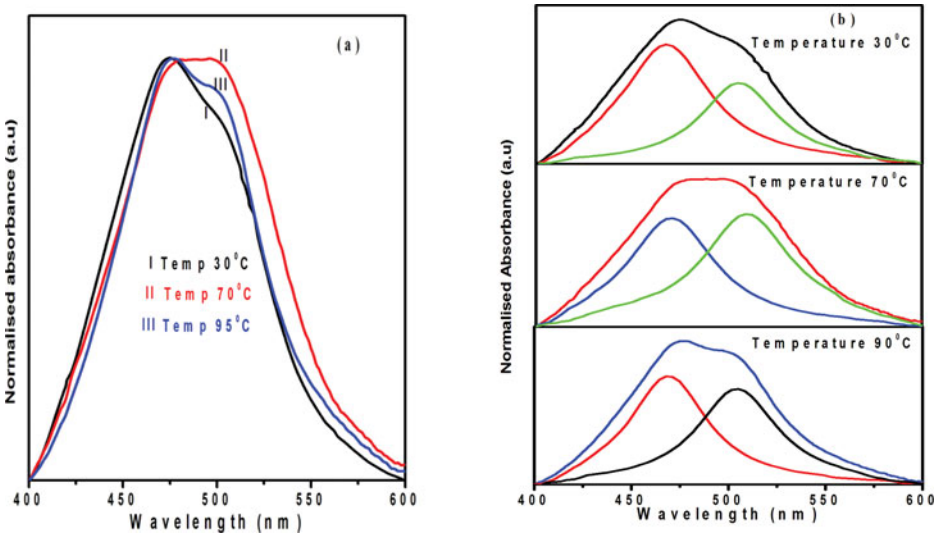

Figure 4. (a) Normalized UV-vis absorption spectra of monolayer LbL film of AO – DNA keeping the temperature of DNA solution at three different values (i)30°C, (ii)70°C, (iii)95°C and temperature of AO solution was 30°C. (b) Deconvoluted UV-vis absorption spectra of mixed AO – DNA monolayer LbL film at three different temperatures.

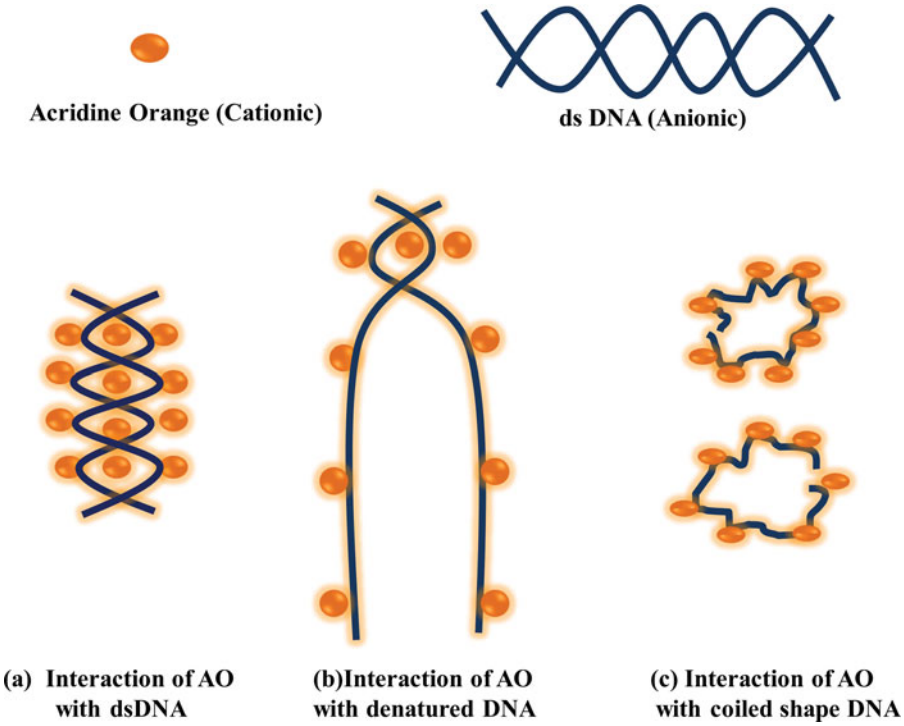

Figure 5. Schematic representation of interaction of AO with three different types of DNA (a) dsDNA, (b) denatured DNA, and (c) coil-shaped DNA in monolayer LbL film.

temperature, dimeric band became intense in comparison to the monomeric band. While at pH 11 and at temperature 70°C, monomeric band became intense with respect to dimeric band and again at pH 13 and at temperature 95°C, dimeric band became intense. It clearly shows different types of organization of AO molecules onto DNA. At normal pH, DNA remains in double-stranded form and AO molecules intercalated as well as also adsorbed onto the phosphate backbone. In ssDNA form, AO molecules were adsorbed on the phosphate backbone of the elongated single strand of DNA and in the coil form DNA, AO molecules were associated on the phosphate backbone of the compressed coil structure.

3.4. Schematic representation

Figure 5(a) shows the interaction of AO molecules with dsDNA. In this case AO molecules were intercalated in between the double strand of the DNA as well as also adsorbed onto the phosphate backbone of the DNA. In the double-stranded form DNA, strands made loop-like compressed structures. Thus the AO molecules adsorbed on the backbone of dsDNA came comparatively closer to each other. Adsorption and consequent organization of AO molecules onto the ssDNA has been shown in Fig. 5(b). Due to elongated structure of single-stranded DNA, the adsorbed AO molecules remain at comparatively larger distance from each other. This results in a favorable condition for increasing monomeric sites and decreasing of dimeric sites. In Fig. 5(c) adsorption of AO molecules onto the phosphate back bone of coil shaped DNA structures has been shown. Due to compressed length of coil-shaped DNA, the adsorbed AO molecules came closer to each other resulting the increase of dimeric sites. Thus it can be concluded that the elongated structure of ssDNA is more effective in controlling the dimeric sites of AO in the LbL film.

4. Conclusion

In conclusion, our results show that the dimeric sites of AO can be effectively controlled by adsorbing it on DNA. In aqueous solution and also in the LbL film by changing the volume ratio of AO : DNA, dimeric sites can be controlled. Also in the LbL film it has been shown that ssDNA has a greater influence in controlling the dimeric sites. This is because in ssDNA form the single-stranded DNA remained in elongated structure. UV-vis absorption spectroscopic studies confirm this.

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