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Sensing of DNA conformation based on change in FRET efficiency between laser dyes

Dibyendu Dey[a], Jaba Saha[a], Arpan Datta Roy[a], D. Bhattacharjee[a], Sangram Sinha[b], P.K. Paul[c], Santanu Chakraborty[a], Syed Arshad Hussain[a,*]

[a] Thin Film and Nanoscience Laboratory, Department of Physics, Tripura University, Suryamaninagar 799022, Tripura, India
[b] Department of Botany, Tripura University, Suryamaninagar 799022, Tripura, India
[c] Department of Physics, Jadavpur University, Kolkata 700 032, India

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ABSTRACT

This communication reports the effect of DNA conformation on fluorescence resonance energy transfer (FRET) efficiency between two laser dyes in layer by layer (LbL) self assembled film. The dyes Acraflavine and Rhodamine B were attached onto the negative phosphate backbones of DNA in LbL film through electrostatic attraction. Then FRET between these dyes was investigated. Increase in pH or temperature causes the denaturation of DNA followed by coil formation of single stranded DNA. As a result the FRET efficiency also changed along with it. These observations demonstrated that by observing the change in FRET efficiency between two laser dyes in presence of DNA it is possible to detect the altered DNA conformation in the changed environment.

1. Introduction

By measuring the fluorescence signals it is possible to detect DNA hybridization where the dye molecules are intercalated into a DNA double helix [1,2]. But the inherent limitation of this method is the lack of specificity for many particular duplex [3,4]. Another most important strategy for the detection of DNA hybridization involves fluorescence resonance energy transfer (FRET). FRET between two molecules is an important physical phenomenon, where transfer of energy occurs from an excited donor fluorophore to a suitable acceptor fluorophore [5,6]. Combining FRET with optical microscopy, it is now possible to determine the distance between two molecules in nanometers. The basic requirements for the FRET to occur are (i) sufficient overlap between the absorption band of acceptor fluorophore and the fluorescence band of donor fluorophore and (ii) both the donor and the acceptor molecule must be in close proximity of the order of 1–10 nm [5,6]. The interference of solvent or other macromolecules has little effect on the FRET efficiency [7–9]. Literature survey suggests that FRET process can be used to investigate molecular mechanisms [10,11] as changes in the distance between the donor and the acceptor molecules effect the FRET efficiency.

Double stranded DNA is an interesting anionic polyelectrolyte with unique double helix structure whose base sequence controls the heredity of life [12]. DNA, the eternal molecule shows autocatalytic property i.e., self replication. The process involves the partial separation of two individual strands known as denaturation of DNA.

During annealing and thermal denaturation of duplex DNA the donor–acceptor moieties are brought closer together or moved further apart and as a result changes occur in the fluorescence intensity of the FRET pair. Using this principle various researchers reported the detection of target DNAs by excimer–monomer switching of Pyrene and by DNA based nano-device [7–9].

The denaturation of DNA can also be done by changing the hydrogen ion concentration of the medium. Though extensive researches were carried out in the field of thermal denaturation of DNA [12–14] comparatively less attention has been paid in alkaline denaturation of DNA [15–17]. The stability of a duplex DNA has a relation with environmental pH [17]. Increase in alkaline pH level of the cells may cause denaturation of DNA which eventually affects gene expression.

The present communication focuses on the changes in FRET efficiencies between two dyes Acriflavine (Acf) and Rhodamine B (RhB) associated in LbL films in presence of double stranded DNA as well

* Corresponding author. Tel.: +91 381 2375317; fax: +91 381 2374802.
E-mail addresses: sa_h153@hotmail.com, sah.phy@tripurauniv.in (S.A. Hussain).

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as denatured DNA. In the present study, the denaturation of the DNA has been studied in a wide range of changes in pH and temperature. This technique has potential applications in optoelectronic and thin film devices [18,19] and is also important for better understanding of some biological systems. The present investigation is, therefore, aimed at detection of altered DNA conformation in the changed environment which could be used in logic gates operations, sensing purpose, nano transporting and above all in nano medicine [20–23].

2. Materials and methods

2.1. Materials

The purity of Salmon sperm DNA (SRL India) was checked by UV–vis absorption and fluorescence spectroscopy before use. All other reagents viz., Acf, RhB, poly acrylic acid (PAA) and poly allylamine hydrochloride (PAH) were purchased from Sigma Chemical Co., USA for experimental purposes. Ultrapure Milli-Q water (resistivity 18.2 MΩ cm) was used as solvent.

2.2. Film preparation

Electrolytic deposition bath of cationic dye RhB and Acf were prepared with 10^{-4} M aqueous solution using triple distilled deionized Millipore water. The anionic electrolytic bath of PAA was also prepared with triple distilled deionized Millipore water (0.25 mg/mL). LbL self assembled films were obtained by dipping clean fluorescence grade quartz substrate first in the solutions of anionic PAA for 30 min. Then it was taken out and sufficient time was allowed for drying. It was followed by thorough rinsing in water bath for 2 min so that the surplus anions attached to the surface were washed off. The dried substrate was then immersed in to the cationic dye mixture of RhB and Acf (1:1) followed by same rinsing procedure. Deposition of PAA and RhB – Acf layers resulted in one bi-layer of self assembled film. The incorporation of DNA into the LbL film was done with the help of aqueous PAH solution (0.25 mg/mL). For each experimental set, the quartz slide was first dipped in to the electrolytic aqueous solution of polycation (PAH) for 30 min followed by same rinsing in water bath and drying procedure and then dipped into the anionic DNA (con. = 0.25 mg/mL) solutions of different pH (5–13.5) which was again followed by rinsing action in water bath. NaOH and HCl were used to increase and decrease the pH of DNA solution. The slide thus prepared was dipped into the cationic electrolytic solution of RhB and Acf (1:1). Due to electrostatic interaction, cationic Acf and RhB were adsorbed onto the negatively charged surface of the DNA in LbL films. LbL method utilizes the Van der Waals interactions between the quartz slide and PAA as well as electrostatic interaction between PAA and cationic dyes [24].

2.3. UV–vis absorption and fluorescence spectra measurement

UV–vis absorption and fluorescence spectra were recorded by a Perkin Elmer Lambda-25 Spectrophotometer and Perkin Elmer LS-55 Fluorescence Spectrophotometer respectively. For absorption measurement the LbL films were kept perpendicular to the incident light and fluorescence from the sample surface at an angle of 45° (front face geometry) was recorded. All the spectra were recorded with excitation wavelength at 420 nm.

2.4. AFM measurement

Atomic force microscopy (AFM) image, in intermittent contact (tapping) mode, of one bi-layer LbL film was taken in air with commercial AFM system (Bruker Innova). Typical scan area was 1 μm × 1 μm. The Si-wafer substrate was used for the AFM measurement.

3. Results and discussions

3.1. FRET between Acf and RhB in presence and absence of DNA

Acf and RhB dyes are highly fluorescent and, in principle suitable for FRET [25]. The fluorescence spectrum of Acf sufficiently overlaps with the absorption spectrum of RhB. There are few reports on the investigation of FRET between these two dyes [25]. In our laboratory we have also studied FRET phenomenon using these two dyes [26]. The absorption and fluorescence spectra of Acf and RhB in LbL films (Fig. S1) suggest that both the dyes remain as monomers in the LbL films [25,27]. The corresponding absorption and fluorescence maxima are shown in Table 1. Fig. 1 shows the fluorescence spectra of (i) pure Acf and RhB and, (ii) Acf–RhB mixed LbL films in presence and absence of DNA. The excitation wavelength was selected in order to excite the Acf molecules directly and to avoid any direct excitation of the RhB molecules. Acf shows strong fluorescence (curve 1 of Fig. 1) since it absorbs light in this excitation range. On the other hand, the RhB fluorescence (curve 2 of Fig. 1) is almost negligible. It is interesting to note that for Acf–RhB mixed LbL films (curve 3 of Fig. 1) the RhB fluorescence intensity increases and the Acf fluorescence intensity decreases with respect to their individual excitation behavior. It is likely that some energy is transferred from Acf to RhB which causes excitation of more RhB molecules followed by emission of light. Thus an increase in RhB fluorescence intensity and decrease in Acf fluorescence intensity was found. This has been confirmed by measuring the excitation spectra, where the monitoring emission maxima are 525 nm (Acf) and 580 nm (RhB) in case of Acf-RhB mixed LbL films. It has been observed that both the excitation spectra are almost similar (Fig. S2) and possess characteristic absorption bands of Acf monomers. This validates FRET between Acf and RhB. Further increase in RhB fluorescence and decrease in Acf fluorescence in Acf–RhB fluorescence spectra (curve 4 of Fig. 1) have been observed for the Acf–RhB mixed LbL films prepared in presence of DNA. This is an indication that the presence of DNA enhances the FRET. In one of our previous work we reported the influence of DNA in the FRET between Acf and RhB in solution phase and based on that we also developed a DNA sensor [28].

The FRET efficiencies were calculated from Fig. 1 using the equation given below [29]

$$E = 1 - \frac{F_{DA}}{F_D};$$

where F_{DA} is the relative fluorescence intensity of the donor in the presence of acceptor and F_D is the fluorescence intensity of the donor in the absence of the acceptor.

Our findings indicate that in presence of DNA, FRET efficiency of the dye pair increases from 28.42% to 44.62%. FRET is a distance dependent process and so, when inter molecular distance between donor and acceptor pair decreases the transfer of energy from donor to acceptor becomes more efficient [5,6]. Thus proximity of cationic Acf and RhB molecules in presence of DNA, by their electrostatic attachment to the phosphate moiety of negatively charged DNA, creates favorable condition for efficient energy transfer. This has been shown schematically in the later part of the manuscript.

3.2. Effect of pH on DNA and FRET

In order to investigate the effect of DNA denaturation on the FRET between Acf and RhB, we have prepared DNA LbL films at different pH and Acf–RhB mixed LbL films in presence of DNA at different pH. Fig. 2(a) shows the variation of absorbance intensity of the 260 nm band of DNA-PAH LbL films with increasing pH range