Development of a sensor to study the DNA conformation using molecular logic gates

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HIGHLIGHTS

- Effect of DNA conformation on Fluorescence Resonance Energy Transfer (FRET) between two laser dyes.
- Increase in pH cause the denaturation of DNA followed by coil formation of single stranded DNA.
- Change in FRET efficiency due to change in DNA conformation.
- Idea about DNA conformation from the change in FRET efficiency using molecular logic gate.

GRAPHICAL ABSTRACT

Change in DNA conformation with increasing pH of solution.

ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form 15 May 2014
Accepted 22 October 2014
Available online 30 October 2014

Keywords:
FRET
Deoxyribonucleic acid (DNA)
Denaturation
Coil formation
Molecular logic gate
Sensor

ABSTRACT

This communication reports our investigations on the Fluorescence Resonance Energy Transfer (FRET) between two laser dyes Acriflavine and Rhodamine B in absence and presence of DNA at different pH. It has been observed that energy transfer efficiency is largely affected by the presence of DNA as well as the pH of the system. It is well known that with increase in pH, DNA conformation changes from double stranded to single stranded (denaturation) and finally form random coil. Based on our experimental results two different types of molecular logic gates namely, XOR and OR logic have been demonstrated which can be used to have an idea about DNA conformation in solution.

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Introduction

Like electronic computers and human brains, molecules can also manipulate and process information using logic [1–3], known as molecular logic. Due to the influence of some external chemical or biological materials, molecules can undergo changes in ground or exited state [4]. Familiar Boolean logic can be utilized for the realization of these kinds of changes [5]. Of late molecular logic gates are enormously used for the sensing of different organic [6], inorganic [1] and biological [2,3,7] materials. One of the most fascinating applications of molecular logic gates is the investigation of inside components of a cell, where silicon – based analogues are hardly able to reach [8]. The development of techniques for sensing and monitoring of different biological materials like DNA,
RNA, proteins etc is in great demand to have some structural as well as functional ideas [6].

Fluorescence Resonance Energy Transfer (FRET) is a very powerful tool for the sensing of different chemical and biological analytes. If any of the FRET parameters changes due to the introduction of an external analyte, then it will result in a change of FRET efficiency. Now that change in FRET efficiency can be calibrated for the sensing of that particular external analyte. Recently the calibration of that change in FRET efficiency can easily be done by using molecular logic gates. FRET process is one of the most important strategies for the detection of DNA. Combining FRET with optical microscopy, it is possible to determine the approach between two molecules within nanometers. Based on DNA quadruplexes Margulies et al. [5] reported about a homogenous sensing ensemble, where it can generate and provide a direct analysis of the properties of the target proteins. Due to DNA hybridization or denaturation the donor–acceptor moieties are brought closer together or moved further apart and as a result there is a change in measuring the fluorescence intensity of the FRET pair. There are many reports where the detection and characterization of DNA involves FRET process. Fujimoto et al. reported the detection of target DNAs by excimer–monomer switching of Pyrene using the FRET process [9]. DNA based nanomachine was reported by Liu et al. using the FRET phenomenon [6]. Also for encrypting messages on DNA strands, various methods have been accomplished [3,6]. Packaging of DNA in the cell relates to gene expression. Of late a lot of efforts have been given to understand the physicochemical behavior of DNA in different conditions. In one of our earlier work we have demonstrated molecular logic based DNA sensor using FRET [10]. Still a lot of research efforts have to be done in order to understand its behavior from the point of view of its functions in the cell as well as its technological applications.

Present communication reports the effect of DNA and DNA denaturation on FRET between two laser dyes Acriflavine (Acf) and Rhodamine B (RhB). It has been observed that the incorporation of DNA in the FRET pair modulates the FRET efficiency. This has been used to design the molecular logic gate, which is capable of sensing the presence of DNA [10]. On the other hand the increase of pH of DNA solution changes the FRET efficiency between Acf and RhB. This is because cationic dyes Acf and RhB are attached onto phosphate backbone of DNA. With change in pH, the DNA conformation changes. Accordingly the FRET efficiency also changes. Based on the presence and absence of different chemicals and FRET efficiency level we have demonstrated two chemical logic viz XOR and OR. The output of these gates is mimicking their electronic counterpart. By observing the output of such logic it is possible to sense the DNA conformation. Although there are several reports on investigations of DNA using FRET [3,6,9,10], however, molecular logic gate based DNA conformation sensor have never been reported.

Material and methods

Material

Both the dyes Acriflavine (Acf) and Rhodamine B (RhB) were purchased from Sigma Chemical Co., USA and used as received. The dyes used in these studies are positively charged. Ultrapure Milli-Q water (resistivity 18.2 MΩ-cm) was used as solvent. The DNA used is Salmon sperm DNA, purchased from SRL India and was used as received.

Experimental details

The UV–Vis absorption and fluorescence spectroscopy was used to check the purity of DNA before use. In all cases the dye concentration was 10⁻⁶ M (for both Acf and RhB) and DNA concentration was 1 µg/ml in aqueous solution. To study the pH dependent behavior of the dyes, the pH of the mixture of Acf and RhB (1:1 volume ratio) solution was changed. On the other hand to study the pH dependent behavior of DNA, we mix DNA solution of different pH to the mixture of Acf and RhB (1:1 volume ratio). For increasing the pH of the solution, NaOH has been used and HCl has been used for the decreasing the pH. All the experiments were repeated 4–5 times and found almost reproducible.

UV–Vis absorption and fluorescence spectra measurement

UV–Vis absorption and fluorescence spectra of the solutions were recorded by a Perkin Elmer UV–Vis Spectrophotometer (Lambda-25) and Perkin Elmer Fluorescence Spectrophotometer (LS-55) respectively. For fluorescence measurement the excitation wavelength was chosen as 420 nm (close to the absorption maxima of Acf).

Results and discussion

FRET between Acf and RhB in presence and absence of DNA

The molecules Acf and RhB used in this study are in principle suitable for energy transfer. Both the dyes are highly fluorescent. There exists sufficient overlapping between the absorption spectrum of Acf and the fluorescent spectrum of RhB. In one of our earlier work we have studied the FRET between Acf and RhB in detail and quantified different FRET parameters [11]. The absorption and emission maxima of Acf are centered at 449 and 502 nm respectively which is assigned due to the Acf monomers [7]. On the other hand RhB absorption spectrum possess prominent intense 0–0 band at 553 nm along with a weak hump at 520 nm which is assigned due to the 0–1 vibronic transition of the monomer [12]. The RhB fluorescence spectrum shows prominent band at 571 nm which is assigned due to the RhB monomeric emission [12]. The corresponding absorption and emission spectra of the above results are shown in Fig. 1 of the Supporting information.

Fig. 1a shows the fluorescence spectra of pure Acf (Curve-1), pure RhB (Curve-2) and Acf + RhB mixture (1:1 volume ratio) in aqueous solution in absence (Curve-3) and in presence (Curve-4) of DNA. Spectra shown in Fig. 1 were recorded with excitation wavelength 420 nm (close to the monomer absorption of Acf). This excitation wavelength was selected in order to avoid the direct excitation of the RhB molecules. With this excitation wavelength Acf shows prominent fluorescence (Curve-1) where as RhB fluorescence intensity is almost negligible (Curve-2). However, from the fluorescence spectrum of Acf + RhB mixed aqueous solution, it was observed that the RhB fluorescence intensity increases (Curve-3 Fig. 1a) even with this excitation wavelength (420 nm) and Acf fluorescence decreases compared to their pure counterpart. Transfer of energy from Acf to RhB may be the reason of the above mentioned phenomenon. This transferred energy excites more RhB molecules followed by light emission from RhB, which is added to the original RhB fluorescence. As a result the RhB fluorescence intensity gets sensitized. In order to confirm this, excitation spectra was measured with excitation wavelength fixed at Acf (500 nm) and RhB (577 nm) fluorescence maximum, in case of Acf + RhB mixed aqueous solution (Fig. 2 of Supporting information). Interestingly both the excitation spectra are almost similar to the absorption spectra of Acf having characteristic bands of Acf monomers. This confirms that the RhB fluorescence is mainly due to the light absorption by Acf and corresponding transfer to RhB monomer. Thus FRET between Acf to RhB has been confirmed. It has already been reported that energy transfer occurred from Acf to RhB in solution and ultrathin films [7,11]. It is interesting to observe that in presence of DNA the RhB fluorescence increased.
FDA is the fluorescence intensity of the donor in the absence of the acceptor. Acf (1)

The FRET efficiencies were calculated from Fig. 1 using the equation given below [13]

\[ E = 1 - \frac{F_{DA}}{F_D} \]

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

It has been observed that the FRET efficiency increases from 11.4% to 79.6% in presence of DNA (Table 1). It is interesting to mention in this context that FRET strongly depends on the distance between donor–acceptor pair [10]. Both the dyes used in the present study are cationic. In presence of DNA, the dyes are adsorbed onto phosphate backbone of DNA strands through electrostatic interaction. This results an increase in close proximity of the dyes Acf and RhB in presence of DNA compared to that in absence of DNA. Consequently, the energy transfer efficiency increases [10] in presence of DNA. Among the molecules under current investigation Acf is pH sensitive because of its basic nature of the central nitrogen atom [14]. The fluorescence spectra of Acf are affected with change in pH [11,15]. This may in turn cause a change in spectral overlapping of the donor fluorescence and acceptor absorbance resulting a change in FRET efficiency. In order to check the effect of pH on FRET process, fluorescence spectra of Acf–RhB mixture in aqueous solution prepared at three different pH (pH = 6.5, 10 and 12) were measured (Fig. 1b). Energy transfer efficiency was found to be 11.4%, 47.2% and 57.2% respectively (Table 1) for these three pH. It was observed that the Acf fluorescence was red shifted with decrease in pH. It has also been found that acriflavine mainly remains in double protonated form due to the high local proton concentration [16]. Larger red shift in Acf fluorescence has been observed and explained due to the change in the dipole moments of the excited state of the double protonated Acf [17] and due to the broad distribution of pKa [17]. In the present case at lower pH red shift of Acf fluorescence is observed. At lower pH Acf molecules mainly remains in double protonated form due to the increase in local proton concentration with decreasing pH. Accordingly the dipole moments of the excited state of double protonated Acf have been changed. This change in dipole moments may be responsible for the observed large stoke shift/red shift of the Acf fluorescence. The electron donor type functional group of Acf become more basic with increase in pH in the excited state, consequently the fluorescence spectra shifts towards shorter wavelength providing a larger spectral overlap and increase in FRET efficiency at higher pH. In one of our earlier work we have demonstrated that the FRET between Acf and RhB is pH sensitive over a wide range of pH variation based on the hypochrometic shift of DNA absorbance [18,19].

In order to investigate the effect of DNA denaturation and coil formation on the FRET between Acf and RhB, we have prepared Acf + RhB (1:1 volume ratio) mixed solution in presence of DNA at three different pH. Curve 1, 2 and 3 of Fig. 1c shows the fluorescence spectra of Acf + RhB mixed solution at pH 6.5, 10 and 12 respectively in presence of DNA. At higher pH the denaturation of DNA occurred resulting in an increase in absorbance intensity [18,19]. There are several reports on the denaturation and renaturation of DNA with pH variation based on the hypochrometic shift of DNA absorbance [18,19].

It is interesting to mention in this context that due to increase in pH the H-bond in the double stranded DNA is disrupted [10]. As a result the DNA strands are no longer held together, resulting denaturation of the DNA [10]. After denaturation the DNA strands separate from each other. Finally they form random individual coils.

Further and Acf fluorescence decreased in case of Acf–RhB mixture fluorescence spectrum. This indicates the increase in extent of energy transfer in presence of DNA.

The FRET efficiencies were calculated from Fig. 1 using the equation given below [13]

\[ E = 1 - \frac{F_{DA}}{F_D} \]

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

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>FRET efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acf + RhB (normal pH)</td>
<td>11.4</td>
</tr>
<tr>
<td>Acf + RhB (with DNA of normal pH)</td>
<td>79.6</td>
</tr>
<tr>
<td>Acf + RhB (pH = 10)</td>
<td>47.2</td>
</tr>
<tr>
<td>Acf + RhB (with DNA of pH = 10)</td>
<td>27.45</td>
</tr>
<tr>
<td>Acf + RhB (pH = 12)</td>
<td>57.2</td>
</tr>
<tr>
<td>Acf + RhB (with DNA of pH = 12)</td>
<td>41.5</td>
</tr>
</tbody>
</table>

Fig. 1. Fluorescence spectra of (a) Acf (1), RhB (2) and Acf + RhB mixture (1:1 volume ratio) in aqueous solution (3), in DNA solution (4); (b) Acf + RhB mixture (1:1 volume ratio) in aqueous solution at natural pH (pH = 6.5) (1), in aqueous solution of pH = 10 (2) and in aqueous solution of pH = 12 (3); (c) Acf + RhB mixture (1:1 volume ratio) in DNA solution at natural pH (pH = 6.5) (2) DNA solution of pH = 10 (1) and in DNA solution of pH = 12 (3). Dye concentration was 10 \(^{-6}\) M and DNA concentration was 1 \(\mu\)g/ml.

Fig. 2. FRET efficiency of Acf and RhB mixture for normal pH (1), presence of DNA of normal pH (2), higher pH (3) and presence of DNA of higher pH (4). Here normal pH = 6.5 and higher pH = 10.

Fig. 2. FRET efficiency of Acf and RhB mixture for normal pH (1), presence of DNA of normal pH (2), higher pH (3) and presence of DNA of higher pH (4). Here normal pH = 6.5 and higher pH = 10.
Table 2
Truth table of XOR gate for detection of the denaturation of DNA at pH = 10.

<table>
<thead>
<tr>
<th>INPUT A (NaOH)</th>
<th>INPUT B (DNA)</th>
<th>OUTPUT (FRET efficiency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>(FRET efficiency less than reference level)</td>
</tr>
<tr>
<td>absence of NaOH</td>
<td>absence of DNA</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>(FRET efficiency greater than reference level)</td>
</tr>
<tr>
<td>presence of NaOH</td>
<td>presence of DNA</td>
<td>1</td>
</tr>
</tbody>
</table>

[18]. The course of this dissociation can be followed spectrophotometrically because the relative absorbance of the DNA solution at 260 nm increases as much as 40% as the bases unstack [20]. UV absorption of the bases in the DNA is due to the π-electron transition. In double stranded DNA the aromatic bases are staked together and they interact via their p-electron clouds. This results a decrease in π-electron transition probability. As a result the UV absorption intensity is also less. Whereas in case of single stranded DNA the unstacking of aromatic bases of DNA decreases the interaction between the p-electrons resulting in an increase in π-electron transition probability. As a result the UV absorption intensity increases. At pH values greater than 10, extensive deprotonation of the bases occurs, destroying their hydrogen bonding potential and denaturing the DNA duplex [21]. Beyond pH 12 the absorbance intensity decreases abruptly indicating the helix to coil transition due to the ionization of the guanine moieties of the DNA [21].

In presence of DNA the FRET efficiencies decreases quite remarkably at pH 10 in compared to normal pH (79.6–27.45%) indicating the separation between the strands of the DNA resulting in an increase in the separation between the dye molecules attached electrostatically with the DNA strands. But increasing the pH to 12 results some increase in FRET efficiency (41.5%) between the Acf–RhB pair. This is because at pH 12 the single stranded DNAs start converting into coil and as a result the separation between the dye molecules decreases providing a slight increase in FRET efficiency. This situation has been explained schematically in the later part of the manuscript. The corresponding FRET efficiencies have also been calculated from Fig. 1 and shown in Table 1.

Design of molecular logic gates

Based on the efficiency of FRET between Acf and RhB in presence and absence of DNA at different pH, two molecular logic gates have been proposed, namely XOR and OR logic gates. These molecular logic gates, unlike digital counter parts; sense the presence of NaOH and DNA which acts as an input signals. The output signal is the FRET efficiency. Using these logic gates it is possible to have idea about DNA conformation in solution. The level of 30% of the FRET efficiency is considered to be the reference level. In this case the presence of either NaOH or DNA (i.e. input = 0, 1 or 1, 0) provides a FRET efficiency greater than the reference level (output = 1). But the presence of both NaOH and DNA (input = 1, 1) or the absence of both NaOH and DNA (input = 0, 0) provides a FRET efficiency less than the reference level (output = 0).

Thus an effective XOR gate can be developed which can sense the presence of DNA in aqueous solution having concentration as low as 1 μg/ml at pH10 (denatured DNA). Thus by observing the FRET efficiency it is possible to detect the denaturation of DNA.

Design of OR gate for the sensing of DNA coil formation at higher pH

Fig. 3 shows the variation of FRET efficiencies between Acf and RhB in presence and absence of DNA at two different pH 6.5 and 12. Based on this figure we have designed a chemical OR logic gate. Table 3 shows the corresponding function table. Here also the inputs are NaOH and DNA. The level of 30% of the FRET efficiency is considered to be the reference level. In this case the presence of either NaOH (i.e. input = 1, 0) or DNA (i.e. input = 0, 1) or presence of both DNA and NaOH (input = 1, 1) provides a FRET efficiency greater than the reference level (output = 1). But the absence of both NaOH and DNA (input = 0, 0) provides a FRET efficiency less than the reference level (output = 0). Thus an effective OR gate can be developed which can sense the presence of DNA in aqueous solution having concentration as low as 1 μg/ml at pH 12 (coiled DNA). Thus by observing the FRET efficiency it is possible to detect the coil formation of DNA.

Schematic diagram

DNA is comprised of two long polymer strands and repeating units called nucleotides (or bases). There are four types of bases, and they are Adenine (A), Thymine (T), Guanine (G) and Cytosine (C). The bases lie horizontally between the two spiraling polymer strands. The distance between two consecutive base pairs is
0.34 nm [23–25]. The negatively charged phosphate deoxyribose backbones on either side of the base pair can be labeled with different functional groups or dye molecules [23–25]. Again double stranded DNA can be denatured at higher pH which results the separation of the two DNA strands. After denaturation DNA may exist as single stranded or by forming coil. Here we have attached two cationic dyes (FRET pair) with the negative phosphate backbone of DNA and FRET between them has been studied in order to have an idea about DNA conformation. A schematic representation showing the attachment of RhB (A) and Acf (D) on the negatively charged phosphate backbone of double stranded DNA is given in Fig. 4a. In presence of DNA both the cationic dyes Acf and RhB are attached with the negatively charged phosphate backbone of DNA through the electrostatic attraction (Fig. 4a). We have measured the FRET efficiency between Acf and RhB in presence of DNA at different pH (Figure not shown). Before denaturation of DNA the FRET efficiency between Acf and RhB found to remain almost constant. This is because the distance between Acf and RhB attached onto the anionic DNA backbone is almost constant. Again after denaturation (at high pH) the FRET efficiency decreases. After denaturation the DNA strands are separated and remain as single stranded resulting an increase in separation between the phosphate backbones and hence the dyes. This has been shown schematically in Fig. 4b. However beyond the pH 12 the FRET efficiency again increases slightly. At this stage the single stranded DNA forms coiled structure resulting slight decrease in separation.

<table>
<thead>
<tr>
<th>INPUT A (NaOH)</th>
<th>INPUT B (DNA)</th>
<th>OUTPUT (FRET efficiency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (absence of NaOH)</td>
<td>0 (absence of DNA)</td>
<td>(FRET efficiency less than reference level)</td>
</tr>
<tr>
<td>0 (absence of NaOH)</td>
<td>1 (presence of DNA)</td>
<td>(FRET efficiency greater than reference level)</td>
</tr>
<tr>
<td>1 (presence of NaOH)</td>
<td>0 (absence of DNA)</td>
<td>(FRET efficiency greater than reference level)</td>
</tr>
<tr>
<td>1 (presence of NaOH)</td>
<td>1 (presence of DNA)</td>
<td>(FRET efficiency greater than reference level)</td>
</tr>
</tbody>
</table>

Table 3: Truth table of OR gate for detection of the coil formation of DNA at pH = 12.
between the dyes compared to the single stranded form. This situation has been shown in Fig. 4c.

Conclusion

On the basis of experimental studies it is observed that presence of DNA increases the FRET between two laser dyes Acf and RhB. Change of pH affects the FRET efficiency. Based on the experimental observations, two types of molecular logic gates, namely, XOR and OR gate have been designed. XOR gate can be efficiently used for the detection of denaturation of DNA. On the other hand the OR logic can be used to sense the coil formation of the single stranded DNA.

Acknowledgements

The author SAH is grateful to DST – India and CSIR – India for financial support to carry out this research work through DST Fast-Track project Ref. 386 No. SE/FTP/PS-54/2007 and CSIR project Ref. 03(1146)/09/EMR-II.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2014.10.086.

References