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Molecular recognition of plant DNA: Does it differ from conventional animal DNA?

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ABSTRACT

The recognition mechanism of DNA with small drugs/ligands is an important field of research from pharmacological point of view. Such studies are ample with DNAs extracted from animal cells, but are rare for those extracted from plant cells. However, such a study is strongly demanding for the formulation of pesticides and other agrochemicals. In this contribution, for the first time, we report the interaction of two well-known DNA binder ethidium bromide (EB) and Hoechst 33258 (H33258) with two genomic DNAs extracted from the leaves of *Ricinus communis* L. (castor bean) and *Mangifera indica* (mango) using steady-state and picosecond-resolved fluorescence spectroscopy. The purity of the extracted DNAs is confirmed from gel electrophoresis and optical absorption studies. As evidenced from the circular dichroism (CD) measurements the DNAs retain physiologically relevant B forms. The well-known DNA intercalator EB has been found to show an additional electrostatic mode of binding with the DNAs, which is not present in the conventional animal DNAs. The binding affinity of EB is found to be even weaker for the DNA extracted from *M. indica* compared to that in *R. communis* L. On the other hand, the binding affinity of H33258 with the plant DNAs is found to be comparable to that of animal DNAs. The difference in interaction could be rationalized from the possible differences in the base sequences.

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1. Introduction

DNA is a polymer that preserves all the genetic information in living organisms. The double helical structure of DNA comprising of four nitrogenous bases has been discovered more than 50 years back [1]. The exact structure of DNA is very accurately known today, and irrespective of the source, the structure remains the same with only variable base sequences. This multiplicity in sequence is responsible for the genetic heterogeneity [2]. Knowledge about the exact sequence in DNA is important, specially a rational approach to drug design requires detailed knowledge of the molecular mechanism and specificity of drug induced DNA structural perturbation [3–5]. The acquisition of such information represents a challenging goal for molecular pharmacology, particularly if the DNA target is of chromosomal size and complexity [6]. DNA-binding drugs have numerous applications in the engineered gene regulation. However, the drug–DNA recognition is not always very well understood. Drugs can recognize specific DNA sequences not only through direct contacts, but also indirectly through sequence dependent conformation, in a manner similar to the indirect mechanism in protein–DNA interaction. The initial mode of interaction between these two is primarily electrostatic in nature, and depending upon the nature of the drug and the base sequence of the DNA, other modes of interaction, like intercalation (stacking of drug within the base pairs of DNA) or minor groove binding takes place. These interactions play the key role determining the function of the drugs. Such interactions have been studied in details for DNA extracted from animal cells [7]. But to the best of our knowledge such studies have never been attempted for DNA extracted from plant cells.

It could be argued that the double-stranded DNA retains its structure irrespective of whether it is extracted from plants or animals, but what could make the difference is the sequence of bases in the helix, which in turn affects the molecular recognition of the DNA. It might be taken into consideration that physiological properties of animal and plant cells are different, and there are several proteins and enzymes present in the plant cells (especially those used during photosynthesis) that are not found in animal cells. Thus there might remain a significant characteristic difference between the base sequence of plant and animal DNA, and how these differences affect the molecular recognition of small drugs/ligands is an interesting problem to be solved. It has been reported [8] that drugs like DNA intercalators, minor groove binders, etc. can trigger the expression of plant defense genes in the way similar to that of the fungal elicitors. But the detailed mechanism of such interaction has not been studied in details. Understanding of the mechanism of molecular recognition of plant DNA by these drugs

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can give us a detailed idea about the plant pathogen interaction leading to disease development as well as lead to newer approaches toward conferring disease resistance in plants of economic importance. In view of the above, to examine the nature of interaction of DNA extracted from plant cells with model drug/ligand, we have initiated a series of studies. As a primary step toward this, the present study involves the extraction and purification of two plant DNAs extracted from the leaves of *Ricinus communis* L. (castor bean, a common weed in Indian subcontinent), hereafter called DNA1 and *Mangifera indica* (mango, a very popular fruit in Indian subcontinent and southeast Asia), hereafter called DNA2, characterization of these and spectrophotometric investigation of their interaction with a well-known DNA intercalator, ethidium bromide (EB) and a minor groove binder, Hoechst 33258. Over recent years, the dynamics of solvation has evolved as an efficient technique to explore the binding interaction of ligands with biological macromolecules like DNA [7,9–11]. In the present paper we have made a detailed solvation dynamics study of both the drugs bound to the extracted plant DNAs in order to understand the mechanism of binding of them with plant DNA.

2. Materials and methods

All the chemicals used in the extraction procedure are of analytical quality purchased from reputed brands and used without any further purification. The DNA binders, ethidium bromide (EB) and Hoechst 33258 (H33258) (Scheme 1) are obtained from Molecular Probes. Water used throughout the study is triply distilled and autoclaved. The extraction and purification procedure of the DNAs has been given in the Supplementary Material section.

To investigate the structural conformation of the isolated plant genomic DNA, we have used CD spectroscopy (JASCO 815 spectrometer with a cell of path length of 1 cm). To check the purity of the extracted DNA we perform gel elctrophoresis and the details of electrophoresis experiment could be found in the Supplementary Material section. The nucleotide concentration of the DNA was determined by absorption spectroscopy (Shimadzu Model UV-2450 spectrophotometer) using the average extinction coefficient per nucleotide of the DNA (6600 M^{-1} cm^{-1} at 260 nm) [12]. The DNA concentration used in this work corresponds to the nucleotide concentration. Steady-state absorption and emission were measured with Shimadzu Model UV-2450 spectrophotometer and Jobin Yvon Model Fluoromax-3 fluorimeter, respectively. All fluorescence decays were taken by using picosecond-resolved time correlated single photon counting technique. The commercially available setup is a picosecond diode laser pumped time resolved fluorescence spectrophotometer from Edinburgh Instrument, UK. It has an instrument response function (IRF) ~80 ps. The details of time resolved measurements and construction of time resolved emission spectra (TRES) is given in the Supplementary Material section. The solvation correlation function, $C(t)$ is constructed according to the following equation:

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)}$$

where, $\nu(0)$, $\nu(t)$ and $\nu(\infty)$ are the emission maximum (in cm^{-1}) at time zero, t and infinity, respectively. The $\nu(\infty)$ values have been taken to be the emission frequency beyond which an insignificant or no spectral shift is observed. The $C(t)$ function represents the temporal response of the solvent relaxation process, as occurs around the probe following its photo excitation and the associated change in the dipole moment [13]. For anisotropy ($r(t)$) measurements, emission polarization is adjusted to be parallel or perpendicular to that of the excitation and anisotropy is defined as

$$r(t) = \frac{[I_{para} - G \times I_{perp}]}{[I_{para} + 2 \times G \times I_{perp}]}$$

G, the grating factor is determined following longtime tail matching technique [14]. All the anisotropies were measured at the emission maxima.

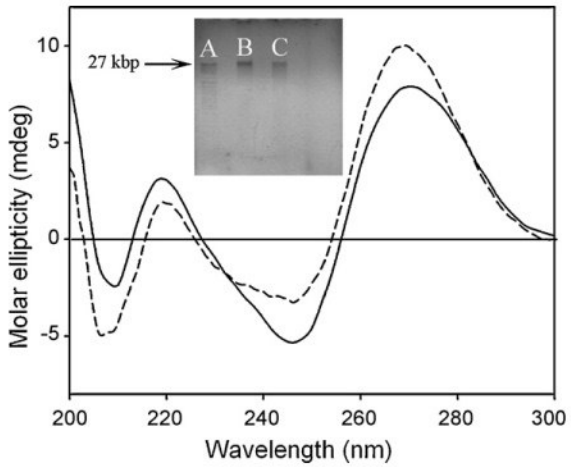


Fig. 1. Circular dichroism spectrum of the plant DNA extracted from *Ricinus communis* L. (solid line) and *Mangifera indica* (broken line). The gel electrophoresis picture is presented in the inset. Lane A: molecular weight marker; lane B: *Ricinus communis* L. DNA; lane C: *Mangifera indica* DNA.

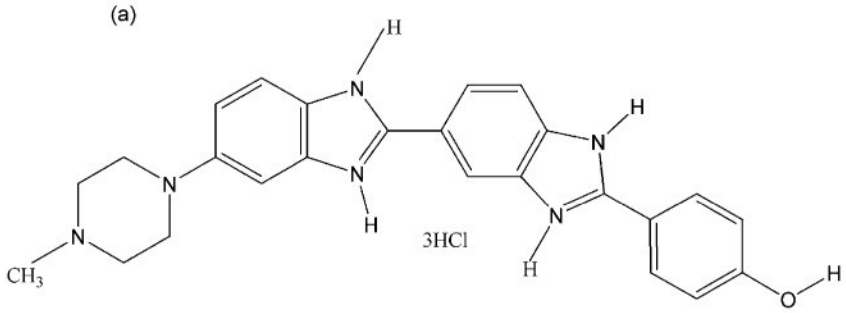
3. Results and discussion

3.1. Characterization of the extracted DNA

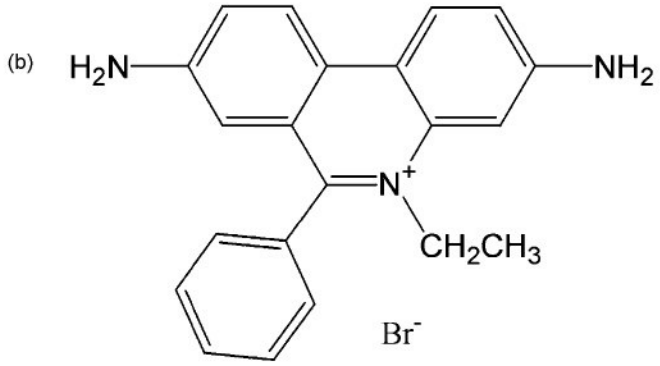
We have followed a standard procedure [15] to extract the DNA from plant leaves (see the Supplementary Material section for details). To check the purity of the extracted DNA, we perform absorption, circular dichroism and gel electrophoresis studies. The absorption spectrum of the DNA produces a single peak at 260 nm with absorption ratios of 260 nm/280 nm producing values higher than 1.8, indicating highly purified preparations of the DNA [12,16]. The gel electrophoresis of both the DNAs (inset of Fig. 1) shows single prominent bands indicating mainly single species present in the sample. Both the DNAs were found to be around 27 kbp of size. The circular dichroism (CD) spectra of the DNAs (Fig. 1) in the 200–300 nm range show negative band at 245 nm and positive band at 270 nm, which indicates a native B-form of the DNA at the experimental condition [17–20]. This positive negative pair of CD bands is sometimes attributed to degenerate exciton interactions of 260 nm absorption band among the stacked bases. Natural DNA contains four different bases and a total different $\pi \rightarrow \pi^*$ absorption bands contribute to the 258 nm envelop observed in the natural DNA. The total CD spectra are a convoluted picture of all these transition [20]. The above studies confirm the preparation to be highly purified and physiologically relevant DNA suitable for further spectroscopic studies.

3.2. Interaction between EB and plant DNA

EB, a phenanthridinium derivative, is a powerful probe of DNA dynamics and DNA-mediated electron transfer (ET) [21–23]. Two main modes of binding of EB to native DNA have been suggested based on the results of spectral and hydrodynamic studies. The primary and generally stronger mode of binding has been interpreted as "intercalation" where a part of the ethidium ion sandwiches between adjacent base pairs [24]. The second and generally weaker mode of binding is mostly evident at low salt and high EB con-



Hoechst 33258 (H33258)



Ethidium Bromide (EB)

Scheme 1. Molecular structure of (a) Hoechst 33258 and (b) ethidium bromide.

centration. This mode is an "electrostatic" interaction between the phosphate groups in the double-stranded nucleic acid backbone and the dye molecules [24]. The intercalated probe has different fluorescence properties from those of EB in bulk water [21,25]. Fig. 2a depicts the absorption and emission spectra of EB in absence and presence of DNA1. The [EB]:[DNA] ratio is fixed at 1:20. It could be noted that a blue shift is produced in the emission spectrum confirming interaction between EB and the DNA as the drug experiences less polar environment. This observation is similar to that obtained in case of animal DNA extracted from calf thymus and salmon sperm [26]. The absorption and emission spectra of EB in presence of DNA2 are presented in the Supplementary section (Figure S1). It is found that the absorption peak has only marginal (~1 nm) red shift than that in the bulk water, whereas the emission spectra show considerable blue shift. However, the blue shift obtained in DNA2 is smaller than that obtained in case of DNA1 indicating a weaker intercalative recognition of EB with the former. The temporal fluorescence decays of EB in DNA1 at three different wavelengths are shown in Fig. 2b. The decays can be fitted with time constants of ~25 ps, ~250 ps, ~2.5 ns and ~19 ns. For example, the decay transient at 610 nm is fitted tetra-exponentially as, 25 ps (42%), 280 ps (10%), 2.5 ns (13%) and 19.2 ns (35%). The corresponding decay transient of EB in presence of DNA2 at the emission peak (610 nm) can be fitted with time constants of 30 ps (28%), 400 ps (4%), 1.7 ns (65%) and 8.9 ns (3%) (Figure S1, Supplementary Material section). The observed temporal decays for both the DNAs are faster than those reported in the presence of animal DNA [26]. It could be noted that EB in water decays with a time constants of 1.6 ns and upon intercalation with animal DNA, the decay becomes extremely slow with a time constant of ~22 ns [26]. The 19 ns component for DNA1 in the present study signifies the intercalation of EB in the plant DNA interior. The 8.9 ns component in DNA2 might also represent a weak intercalation of the drug into the DNA. However, the presence of additional faster components in both the systems clearly indicates that intercalation is certainly not the sole mode of binding of EB with the plant DNA, even at a high DNA:EB ratio. Unlike animal DNA, the plant genomic DNA might have some sequence that makes the intercalation less acceptable. It is known that EB intercalates in the GC base pair rich region of genomic DNA [27] and the present system might not possess such a specific sequence to facilitate intercalation. The ultrafast components of the decay correspond to an ultrafast electron transfer process between the DNA base pairs and EB that quenches the fluorescence lifetime. The 2.5 ns component in DNA1 corresponds to the "electrostatic" mode of interaction between EB and DNA. Such interaction is observed in animal DNA only at low DNA concentrations. It could be noted that in case of DNA2, the decay transient is associated with 65% of a 1.7 ns component, similar to that of EB in bulk water indicating that a large fraction of the drug remains in the aqueous phase and is poorly recognized by the DNA. Herein, the difference in sequence of bases might have made non-intercalative mode of binding a favorable one even at a very high concentration of DNA. Our studies also indicate that the well-known animal DNA stain may not be very effective for the plant DNAs.

The drugs/ligands that deeply intercalate into DNA are not expected to show any solvation. As observed from the temporal decay, there exists other modes of interaction between EB and plant DNAs in which the drug might partially be exposed to the solvent, and we attempt to study solvation dynamics of this system. The temporal emission spectral shift of EB bound to DNA1 has been shown in the inset of Fig. 2c. It is observed that the emission spectra show a red shift of the peak with time clearly indicating solvation of the drug. The solvation correlation function (Fig. 2c) is well fitted bi-exponentially with time constants of 50 ps (85%)

and 1 ns (15%) with a considerable stokes shift of 680 cm^{-1}. We do not observe any solvation of EB in bulk water, possibly the solvation is very fast to be detected in our picosecond resolved experimental time window. The ultrafast component of the solvation denotes a possible exposure of the dye to the bulk water. The slow component of 1 ns is identical to the solvation of dyes at the micellar interface [28] indicating to the binding of the drug at the DNA surface. It could be noted that we obtain a very small stokes shift of ~50 cm^{-1} (figure not shown) in case of DNA2, showing a very weak interaction of the drug with the DNA. The temporal anisotropy of DNA1 bound drug can be fitted bi-exponentially with time constants of 45 ps (37%) and 450 ps (8%) along with an offset (Figure S3, Supplementary Material section). The presence of the offset indicates the intercalative mode of binding of the drug with the DNA, whereas the fast components are due to the wobbling motion of the drug in aqueous environment. The anisotropy study

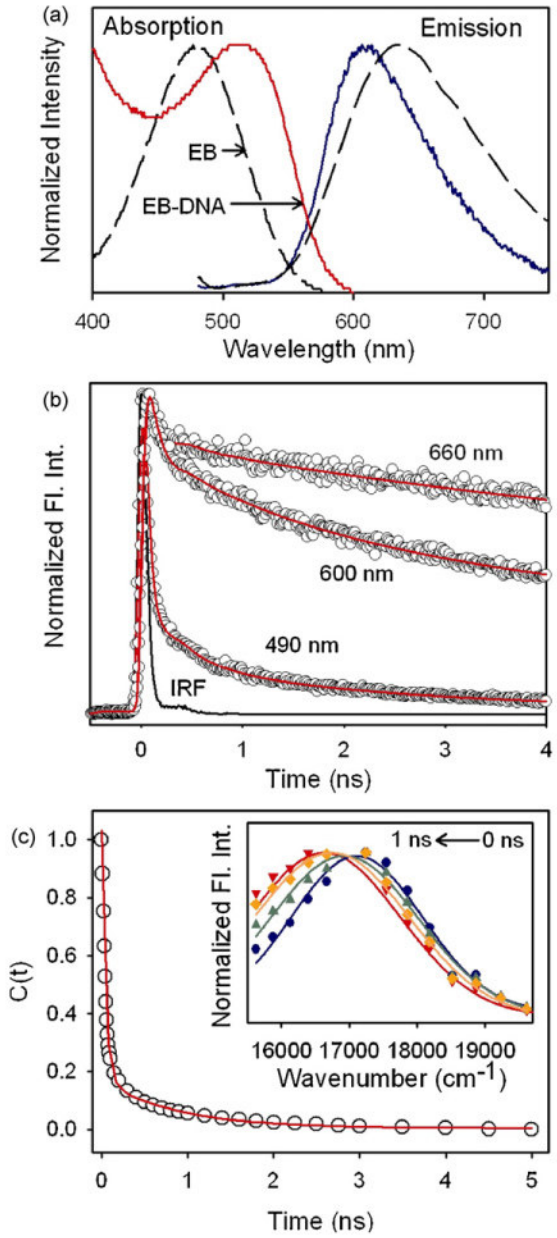


Fig. 2. (a) Absorption and emission spectra of EB in presence of the plant DNA extracted from *Ricinus communis* L. with [EB]:[DNA] = 1:20. The dotted lines represent the spectra of EB in water. (b) Fluorescence decay transients of EB bound to the DNA at three representative wavelengths. (c) Solvent correlation function, $C(t)$ of EB bound to the plant DNA. The TRES has been shown in the inset.

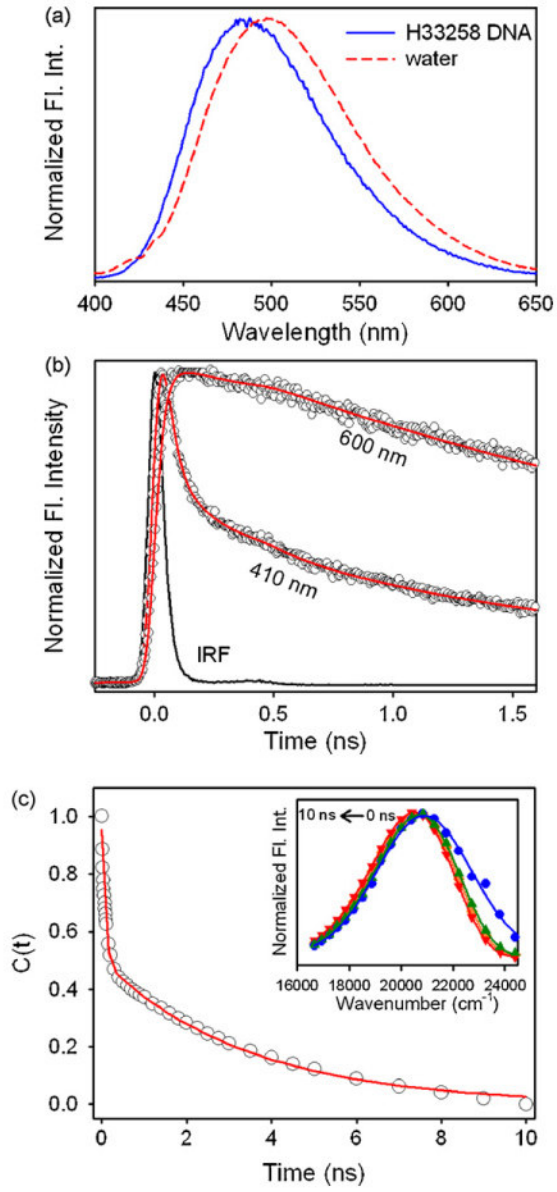


Fig. 3. (a) Emission spectra of H33258 in water and in presence of plant DNA extracted from *Ricinus communis* L. (b) Fluorescence decay transients of H33258 bound to the DNA at two representative wavelengths. (c) Solvent correlation function, $C(t)$ of H33258 bound to the DNA. The TRES has been shown in the inset.

thus also suggests the partial exposure of the drug towards bulk water revealing the additional "electrostatic" nature of the binding. For DNA2, the anisotropy decay time constants are 50 ps (51%) and 450 ps (10%) along with an offset (Figure S3, Supplementary Material section). The increased contribution of the shorter component in the latter case clearly indicates a weaker recognition of DNA2 compared to DNA1 as has been observed in steady-state and time resolved solvation studies.

3.3. Interaction between H33258 and plant DNA

As evident from the above discussion, EB shows some unusual binding characteristic with plant DNA as compared to conventional animal DNAs. Now we investigate the recognition of a well-known minor groove-binding drug, H33258, which displays sequence specificity, preferentially binds to the AATT stretch of DNA double helix [29–32]. Fig. 3a shows the emission spectra of the probe in presence of DNA2 at a high concentration

([H33258]:[DNA] = 1:100). As evidenced from the figure, H33258 in water produces an emission peak at ~500 nm, whereas in presence of DNA the peak gets blue-shifted to 485 nm, indicating strong recognition of the drug by the minor groove of the DNA. A similar shift is also obtained for DNA2 (Figure S2, Supplementary Material section). It could be noted that such blue shift is also observed in case of animal DNA [26,31] attributing to the exposure of the probe to a less polar environment. However, the blue shift observed in the plant DNAs are less than that reported for the animal DNA [26] indicating a less stronger recognition of the former. The temporal fluorescence decays of the DNA1 bound drug at two wavelengths are shown in Fig. 3b. It can be observed that the decay can be fitted tri-exponentially with time constants of ~100 ps, ~1.5 ns and ~4 ns. These time constants are in good agreement with those reported for animal DNAs [26]. H33258 has been reported to be a good solvation probe for the water present in the minor groove of DNA [31]. We herein attempt to measure the solvation dynamics of the drug in the presence of the plant DNA using time-resolved stokes shift technique. The time resolved emission spectrum of the drug in presence of DNA1 is shown in the inset of Fig. 3c. As can be observed from the figure, the emission peak suffers a red shift with time indicating to the solvation of the probe. The solvation correlation function can be fitted bi-exponentially with time constants of 80 ps (50%) and 3.4 ns (50%) (Fig. 3c). For DNA2, the obtained time constants are 70 ps (51%) and 3.2 ns (49%) (Figure S2, Supplementary Material section) indicating to a similar mode of recognition of both the DNAs with H33258. These time constants are of the same order of magnitude as that reported for animal DNAs [26,31] confirming an identical recognition of the drug in the minor groove of the plant DNA. The ~80 ps component is due to the solvation of the probe with the bound water in the minor grove [18] and the slower component (~3.4 ns) arises from the motion of the DNA backbone and ionic atmosphere [9,33]. However, the overall solvation is faster than that reported for animal DNA [26] indicating a less rigid binding of the drug at the DNA minor groove. A recent study from our group reports relatively faster solvation dynamics of H33258 (with time constants of 100 ps and 2.6 ns) bound to the AT rich region of an oligomer [34]. The temporal anisotropy decay is well fitted bi-exponentially with time constants of 450 ps (11.1%) and 3.9 ns (32.6%) with a considerable offset (Figure S3, Supplementary Material section). Identical time constants are also obtained for DNA2 (Figure S3, Supplementary Material section). The time scales are in the same order of magnitude as that obtained in animal DNA [26,31]. It could be noted that the rotational correlation time constant of H33258 in water is ~450 ps [31]. We herein assign the 450 ps component arising out of the free probe present in bulk water, and calculate the binding constant of H33258 in DNA1 using the formula,

$$K = \frac{[LD]}{[D - LD][L - LD]}$$

where $[L]$ and $[D]$ are the initial concentrations of the ligand (H33258) and DNA1 respectively, and $[LD]$ is the concentration of the H33258–DNA1 complex. We calculate a value of K to be $3.68 \times 10^4\ M^{-1}$, which is an order of magnitude smaller than that reported for animal DNAs [26]. This also corroborates well with the fact that the H33258–DNA recognition is weaker in plant DNAs as also evidenced from emission and solvation dynamics studies. It could also be noted that the spectral shift obtained with plant DNA ($\sim 600\ cm^{-1}$) is less than that obtained in case of calf thymus DNA ($\sim 1700\ cm^{-1}$) [26]. H33258 in water shows ultrafast solvation dynamics with time constants of 195 fs and 1.2 ps with a considerably high stokes shift of $3200\ cm^{-1}$ [30]. It can be argued that a considerable portion of the probe might be exposed to the bulk water, which shows ultrafast solvation dynamics (not recovered in our instrumental set up) and thus the stokes shift is also small.

4. Conclusions

In this study it is shown that the biologically relevant drugs ethidium bromide (EB) and Hoechst 33258 (H33258) show interaction with plant DNA. EB shows weak intercalative and electrostatic modes of binding with the plant DNA even at a high DNA concentration. This behavior of the plant genomic DNAs are different from those of conventional animal DNAs. On the other hand H33258 shows an identical but weaker recognition behavior as that of animal DNAs. Even when the plant and the animal DNAs are made of the same two base pairs, the differences in their base sequences must play some role to confer the differences in the modes of molecular recognition of the DNAs by the two drugs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijbiomac.2008.11.004.

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