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Ultrafast charge transfer and solvation of DNA minor groove binder: Hoechst 33258 in restricted environments

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Abstract

Picosecond resolved photophysical studies on a DNA minor groove binder, H33258 in bulk buffer and various restricted media including DNA reveal excited state charge transfer as an important mode of excited state relaxation. The charge transfer is found to be essentially associated with intramolecular twisting of the probe, being absent in SDS micellar environment and in DNA where twisting is hindered. Solvation and rotational dynamics of the probe in various restricted media including DNA are explored. A significantly longer component (8.5 ns) in DNA-solvation dynamics, which is well known to be associated with δ-relaxation of the DNA, is identified.

1. Introduction

Dynamics of a biological macromolecule is crucial for its structure and functionality [1]. Luminescent probes are widely used in biophysical studies in order to explore the dynamics of a biomolecule [2]. Commercially available probe Hoescht-33258 [H33258, 2′-(4-hydroxyphenyl)-5-[5-(4-methylpiperazine-1-yl)-benzimidazo-2-yl-benzimidazole] is widely used as fluorescent cytological stain of DNA. Since it has affinity for the double stranded DNA [3,4], H33258 can affect transcription/translation [5], and block topomerase/helicase [6,7] activities. The X-ray crystallographic studies on H33258-DNA complex [8] reveal that the probe selectively binds to the minor groove of the host DNA. The minor groove recognition of H33258 is further supported by a structural NMR study on the probe complexed with a dodecamer DNA ($d(GTGGAATTCCAC)_2$) in buffer solution [9]. The fluorescence Stokes shift of the probe heavily depends on the polarity of the host environments and has been exploited to determine the polarity of the minor groove of a DNA [10]. The study [10] showed that the minor groove of the DNA is non-polar with dielectric constant similar to propyl-alcohol ($\varepsilon = 20$). Recently, the probe was used [11] to study the dynamics of water molecules (hydration) in close vicinity of DNA (synthesized and genomic) and recognized their importance in drug binding.

Wide applications of the probe [3–7,11–13] warrant detailed photophysical studies on H33258. In recent years attempts have been made to explore the photophysics of the probe [10,14,15] in various solvents and DNA media. In a report the dependence of pH of the host buffer on the excited state relaxation of the probe was studied [14] and concluded that the nonradiative deactivation mechanism involved rotation along bisbenzimide axis at low pH values and when complexed to DNA. However, at higher pH a keto structure was possible, which was in agreement with a previously reported study [16]. The excited state deactivation through various twisting modes of the probe was in agreement with the X-ray crystallographic investigation [8], which essentially identified three rotational axes in the probe molecule. The formation of radicals upon photoexcitation and the possibility of crossover to the triplet state were discussed in another study [15]. Aforesaid studies outlined the basic photo-processes associated with deac-

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tivation of the excited probe. However, exact nature of the excited state charge transfer and its consequences in various restricted media including DNA needs further investigation.

2. Materials and methods

DNA (from salmon testes, sodium salt), bis(2-ethylhexyl) sulphosuccinate (AOT) and phosphate buffer were purchased from Sigma. H33258 was from Molecular Probes and isooctane from Spectrachem. Hydrochloric acid (HCl) and sodium chloride (NaCl) were from Merck. Sodium dodecyl sulfate (SDS) was from Fluka. All aqueous solutions were prepared using Millipore water. The sample solutions were prepared in 0.1 M phosphate buffer. Fluorescence transients were measured and fitted by using commercially available spectrophotometer from Edinburgh Instrument, UK (375 nm, 80 ps instrument response function (IRF)). The observed fluorescence transients were fitted by using a nonlinear least square fitting procedure to a function $(X(t) = \int_0^t E(t')R(t-t')\mathrm{d}t')$ comprising of convolution of the IRF, $(E(t))$ with a sum of exponentials $R(t) = A + \sum_{i=1}^{N} B_i \mathrm{e}^{-t/\tau_i}$ with preexponential factors (B_i), characteristic lifetimes (τ_i) and a background (A). Relative concentration in a multiexponential decay is finally expressed as, $c_n = \frac{B_n}{\sum_{i=1}^{N} B_i} \times 100$. The quality of the curve fitting was evaluated by reduced chi-square and residual data. Construction of decay associated spectra (DAS), time resolved emission spectra (TRES), solvent correlation function, $C(t)$ were detailed elsewhere [2,17–20]. For anisotropy $(r(t))$ measurements, emission polarization was adjusted to be parallel or perpendicular to that of the excitation and anisotropy is defined as, $r(t) = \frac{[I_{\mathrm{para}} - G \times I_{\mathrm{perp}}]}{[I_{\mathrm{para}} + 2 \times G \times I_{\mathrm{perp}}]}$. G, the grating factor is determined following longtime tail matching technique [21] to be 1.05. The integrity of the time constants of $r(t)$ decays is further checked by the methodology described in reference [22] and found to have good agreement with the former method.

3. Results and discussion

Fig. 1(i) shows the absorption and emission spectra of the dye in various environments. The emission spectrum of the dye in buffer shows a peak at 505 nm, which becomes blue-shifted in hydrophobic environments (475 nm in SDS micelles and 457 nm in DNA). The probe notoriously undergoes dimerization in the bulk water [23]. A decay component of time constant <80 ps, an indication of emission from dimer decreases (from 29% to 10%) with the dilution of the probe in the solutions (from 100 μM to 1 μM, Fig. 1(ii)). It was found that the concentration of the probe where no significant dimerization occurs is 1 μM. In order to avoid complications due to dimer emission we maintained 1 μM concentration of the probe in all sample solutions.

Fig. 1(iii) shows fluorescence transients of the probe in buffer solutions of various pH values. The transients of

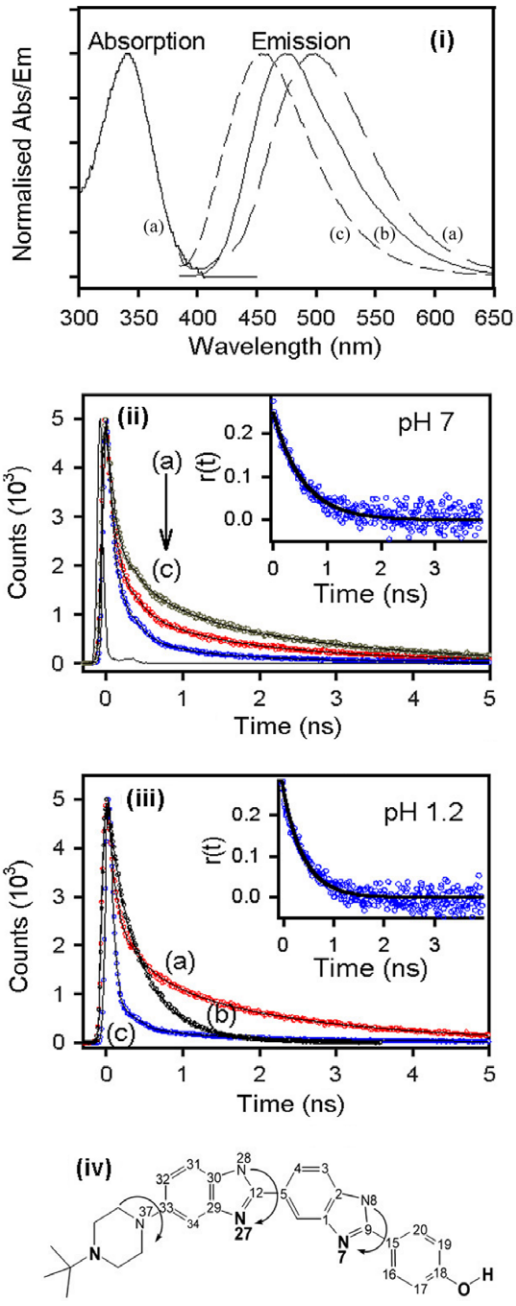

Fig. 1. (i) Absorption and emission spectra of the dye in buffer (a), 100 mM SDS (b) and DNA (c). (ii) Fluorescence transients of H33258 at various concentrations 1 μM (a), 10 μM (b) and 100 μM (c). Instrument response function (IRF) is also shown for comparison. (iii) Fluorescence transients of H33258 at different pHs, pH 1.2 (c), pH 7 (a) and pH 12 (b). Insets show fluorescence anisotropy of H33258 in the respective medium. (iv) Schematic diagram of H33258 showing the different protonation centres (bold) and twisting axes (arrows).

H33258 in buffer at pH 1.2 with a systematic series of wavelength detection (data not shown) show three distinct time scales of 50 ps (95% at 610 nm and 40% at 460 nm), 550–950 ps (550 ps (21%) at 460 nm and 950 ps (5%) at 610 nm) and ~3 ns (40% at 460 nm, vanishes at 610 nm). It is known that in the ground state the probe at pH 1.2 is protonated at both the nitrogens (N27 and N7,

Fig. 1(iv)) of benzimide rings, the latter being at a torsional angle of 43° [14,16,24]. Upon photoexcitation charge transfer occurs from phenyl to the distant benzimidazole moiety resulting in a stronger and planar bond between two benzimidazole units [14,16]. The possibility of charge transfer from phenyl to its nearest benzimidazole moiety is ruled out because there is a significant increase in the basicity of the N7 atom in the excited state of the probe compared to that of N27 [16]. Thus for the charge transfer reaction twisting of two benzimidazole planes is essential for the required planarity.

The experimental observations are in agreement with excited state intramolecular charge transfer (TICT) of the probe as detailed for similar dye molecules [25]. The nanosecond component (~3 ns) predominant in the blue end is consistent with the lifetime of the locally excited (LE) state. In the case of the probe in restricted media including DNA (see below) the excited state dynamics is essentially dominated by LE state nanosecond decay. The faster 50 ps component present in the transients (heavily at the red side) is assigned to the population decay of the charge transferred (CT) state coupled with an underlying triplet state of the probe [15] and/or radiationless transition to ground state [16]. This component is absent in higher pH solutions (pH 7 and pH 12) where the possibility of CT state formation is vanishingly small [14]. The observed 550–950 ps components represent the twisting motion of H33258 in a barrier crossing from initial to the final state of charge separation. The molecule twists toward the CT state and stabilized by the polar solvents [25]. The total available energy in the molecule above the twisting barrier decreases with time. The resulting twisting time becomes longer, consistent with the gradual increase in the decay time from the blue side (550 ps) to the red side (950 ps) of the emission. The $r(t)$ of the probe in the solution (pH 1.2) shows a decay constant of 500 ps (inset, Fig. 1(iii)), further confirming rotational motion of the probe due to twisting in the solution.

As evidenced in the fluorescence decays, at pH 7 (decay components 110 ps (14%), 480 ps (13%) and 2.24 ns (72%)) and at pH 12 (decay components 100 ps (9.7%), 350 ps (46.5%) and 710 ps (43.8%)) and in the $r(t)$s (0.53 ns in pH 7 and 0.45 ns in pH 12) of the probe in high pH solutions, the twisting relaxation still exist even in the absence of the CT state. Higher quantum yields and similarity of temporal decays in the blue and red ends of the emission spectra of the probe in higher pHs are also in agreement with the absence of CT state. In order to study the contribution of the LE and CT states in the overall steady state fluorescence spectrum, we constructed DAS (data not shown) of the slower and faster components of the TRES of the probe at pH 1.2. DAS studies for the faster component (50 ps) associated with CT state population dynamics gives a peak around 18 600 cm^{-1} (537 nm) close to the steady state peak value of 18 720 cm^{-1} (534 nm) obtained in buffer solution of pH 1.2. The DAS for the slower decay components responsible for LE state emission shows a peak at 19 900 cm^{-1} (502 nm) close to the steady state peak values of the probe in buffer solutions of pH 7 (502 nm) and pH 12 (500 nm).

In order to investigate the geometrical restriction on the CT reaction, we studied temporal fluorescence dynamics of the probe in anionic SDS micelles at various pH conditions. The affinity of the probe towards the micellar environment is established by a post-CMC (8 mM) blue shift in the steady state emission spectra in SDS solution at pH 7 (data not shown). Time resolved fluorescence studies were carried out in the 100mM SDS prepared in different pH values (Fig. 2(a)). At the surface of an ionic micelle the effective pH can be calculated using the formula, $C_s = C_b \exp(-e\psi/kT)$ [26,27], C_s, C_b, ψ, k and T being hydrogen ion concentrations in bulk and at the micellar surface, surface potential, Boltzmann constant and absolute temperature, respectively. Thus the surface of the SDS micelle at pH 1.2 should acquire a value lower than 1.2. At the micellar surface the probe does not show either 50 ps component reflective of CT species or the components around 550 ps indicative of intramolecular twisting relaxation. The observations indicate that the twisting, which is essentially hindered at the micellar surface is prerequisite for the formation of CT state in the restricted environment even at pH 1.2.

The temporal nature of $r(t)$s in 100 mM SDS solutions at different pH (Fig. 2(b)–(d)) are similar being around 2 ns which is in good agreement with the values reported for other dyes in SDS micellar environment [28]. The observations confirm the residence of the probe at the micellar surface. The residual anisotropy reflects geometrical restriction of the probe (rotational motion in a cone) in a microenvironment [25]. However, a steady state emission peak of the probe (18 700 cm^{-1}) in the micelles at pH 1.2 was observed to be similar to that in bulk buffer (18 720 cm^{-1}) indicating a significant solvatochromic shift [10] at the micellar surface with large proton concentration (ionic environment). The solvatochromic shift toward red side of the emission spectrum is further confirmed by fluorescence of the probe in the micellar solution (pH 7) in presence of 1 M–NaCl.

Fig. 3(a) and (b) shows the steady state emission spectra and the fluorescence decay profiles of the probe in 100 mM-AOT reverse micelles (RM) with $w_0 = 2.5$ and $w_0 = 40$, respectively. The $r(t)$ (Fig. 3(c) and (d)) of the probe in $w_0 = 2.5$ RM (6.2 ns (57%) with a significant residual offset) shows single exponential decay in contrast to biexponential decay in $w_0 = 40$ RM (1.7 ns (24%), 12.85 ns (76%)) reflecting larger contribution of tumbling motion of the probe in the bigger sized RM. The higher offset in the smaller sized RM is due to incomplete rotation of the host RM, which could be rationalized in terms of higher concentration of micelles in $w_0 = 2.5$ RMs (aggregation-number < 43) compared to that in $w_0 = 40$ RMs (aggregation-number > 800). In the case of $w_0 = 40$ RM, 12.85 ns decay is due to global motion of the RM. Note that the value of the rotational anisotropy do not approach

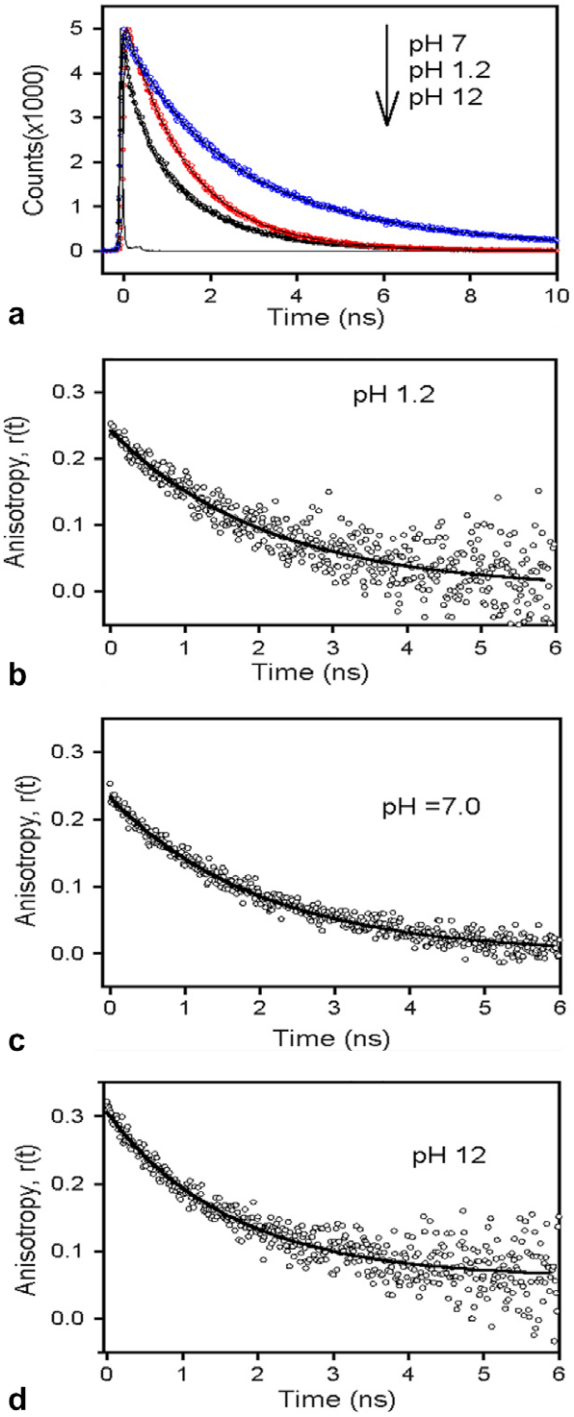

Fig. 2. (a) Fluorescence transients of H33258 in 100 mM SDS at various pHs. (b)–(d) show fluorescence anisotropies of H33258 in 100 mM SDS at various pHs.

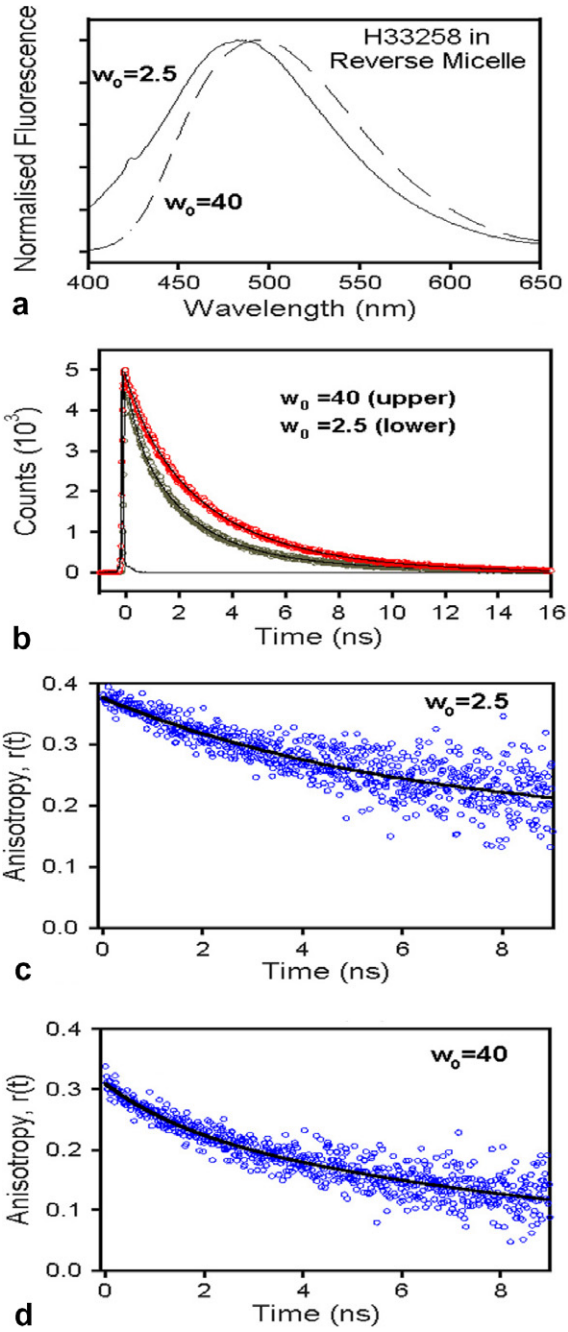

Fig. 3. (a) The steady state emission spectra of H33258 in RMs. (b) Fluorescence transients of H33258 RMs. (c), (d) show fluorescence anisotropies of H33258 in RMs.

to that of bulk water (0.53 ns) even at $w_0 = 40$ RM indicating interfacial affinity of the probe. The probe in $w_0 = 2.5$ RM shows significantly slower solvation dynamics. The constructed TRES of the probe (Fig. 4(a)) in the RM shows significant shift of 1000 cm^{-1} in 15 ns time window. The $C(t)$ (Fig. 4(b)) shows biexponential decay with time constants 0.59 ns (25%) and 4.9 ns (75%). Slower and faster components might be attributed to those water molecules near the ionic head group and in the central region of the water pool, respectively. The solvation dynamics in $w_0 = 40$ RM does not show any slower components.

Upon complexation (minor groove binding) with a DNA the probe shows only nanosecond components in the fluorescence decay reflecting insignificant role of either twisting or CT type of intramolecular dynamics in the microenvironment. Thus the probe with the DNA is an attractive choice to explore the internal (minor groove) dynamics of the DNA. An attempt to use the probe has already been made to explore the dynamics of hydration of genomic and syn-

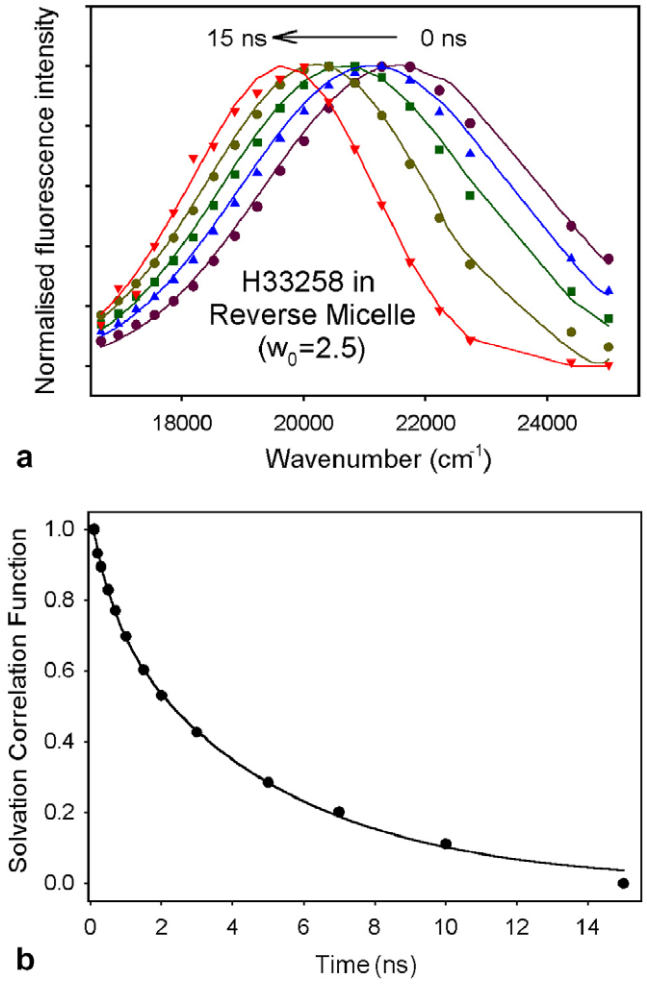

Fig. 4. TRES (a) and solvation correlation function, $C(t)$ (b) of H33258 in $w_0 = 2.5$ RM.

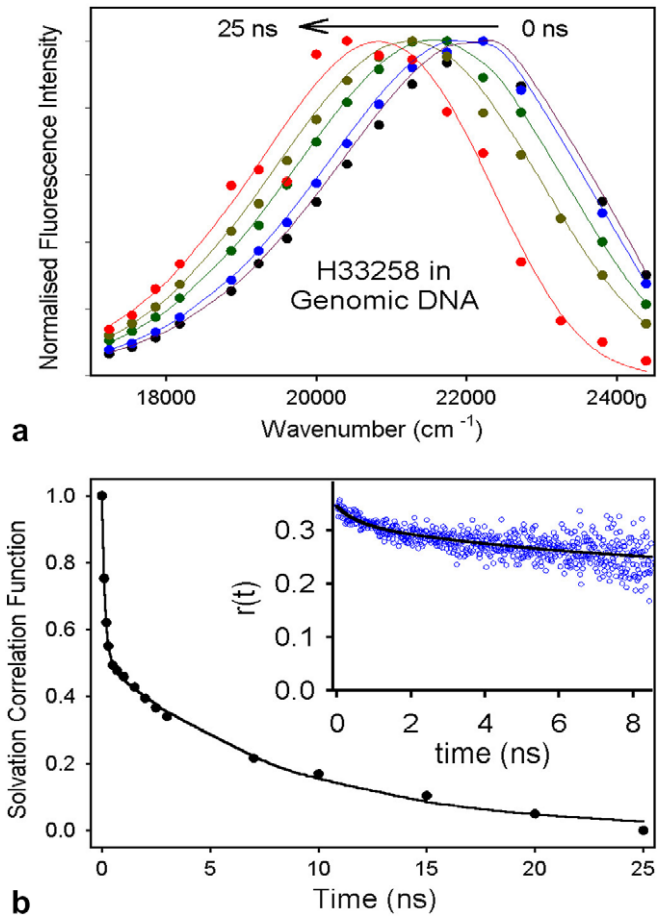

Fig. 5. TRES (a) and solvation correlation function, $C(t)$ (b) of H33258 in DNA. Restricted physical motion of the probe is evident from $r(t)$ (inset).

thesized DNA by using femtosecond resolved fluorescence spectroscopy [11]. However, limited experimental window (up to 200 ps) restricts the exploration of relatively slower dynamics due to local reorganizational motion of the DNA environment. Recently, studies of solvation dynamics have been reported for an extrinsic chromogenic probe, inserted into DNA either by covalent adduction of coumarin dye [29] or hydrophobic intercalation of acridine dye [30]. The fluorescence results [29] give two relaxation time constants of 300 ps (47%) and 13 ns (53%), both measured with 100-ps time resolution and attributed to the local reorganization in the modified DNA.

In order to explore the reorganizational motion of a genomic DNA by using H33258, solvation studies were carried out in 100 μM salmon sperm DNA. Fig. 5(a) shows the constructed TRES giving a shift of 800 cm^{-1} in a 25 ns time window, essentially indicating that the probe in the excited state is stabilized due to the solvation by the DNA molecule. The plot of $C(t)$ (Fig. 5(b)) was fitted with a biexponential curve yielding decay components of 150 ps (49%) and 8.5 ns (51%). The temporal decay of the $C(t)$ is similar to that of a coumarin molecule embedded in DNA [29]. The time constant of 8.5 ns obtained in our experiment is slow for even low frequency vibrational motion of the DNA or quasiharmonic oscillation of the fluorophore [29]. However, the longer time constant (8.5 ns) is consistent with the solvation due to the δ-relaxation of the DNA environment, which is attributed to the diffusion of counter ions along the DNA chain [29,31].

4. Conclusion

Our present study explores the nature of excited state charge transfer processes of a DNA minor groove binder, Hoechst 33258 in bulk and restricted environments including DNA. The experimental results are consistent with TICT as the dominant mode of relaxation of the dye in bulk buffer at low pH. It also explores the possibility of using the probe to study slower relaxation dynamics in bio-mimetics and DNA, and identifies the slower nanosecond solvation dynamics due to δ-relaxation of a genomic DNA.

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