J Fluoresc (2009) 19:1111–1118
DOI 10.1007/s10895-009-0512-9

ORIGINAL PAPER

Sequence Dependent Femtosecond-Resolved Hydration Dynamics in the Minor Groove of DNA and Histone—DNA Complexes

Debapriya Banerjee · Abhinandan Makhal · Samir Kumar Pal

Received: 24 February 2009 / Accepted: 22 June 2009 / Published online: 30 June 2009

Abstract Understanding the sequence dependent molecular recognition of DNA is crucial for the rational design of many drugs. Femtosecond resolved studies on the hydration dynamics of the dodecamer duplexes having sequences $(CGCGAATTCGCG)_2$ and $(CGCAAATTTGCG)_2$, and their complexes with the nucleic protein histone 1 (H1) reveal significant correlation of the molecular recognition of the DNA and DNA-protein complexes with the dynamics of hydration. The different molecular recognition of DNA and DNA-protein complexes is also borne out by circular dichroism (CD) and fluorescence detected CD measurements.

Keywords Hoechst · Sequence dependent molecular recognition · Femtosecond-resolved hydration dynamics

Introduction

Minor groove of a duplex DNA is the host of a number of drugs. An important category of such drugs are those which possess anti-tumor and anticancer properties, and improvisations in the development of these drugs duly embrace a lion's share in current biomedical research. Majority of these anticancer drugs bind noncovalently in the DNA minor groove and over the years the binding of ligands in the DNA minor groove has been a subject of intense and active research. X-ray crystallographic studies [1, 2] on oligonuleotide duplexes of various length and sequences suggest that majority of the minor groove binding drugs occupy AT rich tract. The studies reveal that majority of the minor groove binders are crescent shaped molecules that slide into the complementary structure of the DNA groove without considerable structural perturbation. NMR studies corroborate similar binding of the drugs in solution phase [3]. The study of the thermodynamic parameters, through calorimetric [4] and relatively recent acousto-densimetric techniques [5] show that the minor groove binding is an entropy driven process [6]. Studies on femtosecond resolved environmental dynamics [7, 8] suggest that the dynamical transitions between free and ordered waters present in the minor groove assisted biomolecular recognition.

Attempts to study the sequence dependent minor groove binding in DNAs have produced a wealth of x-ray crystallographic images of minor groove binding drugs bound to synthetic oligomers with different combinations of AT and CG base pairs [1, 9–11]. In a direct attempt to compare the x-ray crystallographic structures of minor groove binding in oligomer duplexes having the sequences $(CGCGAATTCGCG)_2$ and $(CGCAAATTTGCG)_2$, Neidle et al. have shown that the minor groove binding drugs berenil [12] and Hoechst 33258 (Hoechst) [13] show small but significant differences in their binding sites in the different duplexes. It is well established from both x-ray crystallographic and volumetric studies that the binding of these drugs in the minor groove in the dodecamer duplexes is accompanied by the expulsion of bound waters from the DNA minor groove [2, 5], further emphasizing that the process is entropy-driven [6]. Since the dynamical transition between bound and free waters are crucial for the molecular recognition of the dodecamer duplexes by drugs, the questions that naturally arise whether this difference in the binding sites of the drugs in the two dodecamer duplexes leaves its imprint in the associated hydration

D. Banerjee · A. Makhal · S. K. Pal (✉)
Unit for Nano Science & Technology, Department of Chemical, Biological & Macromolecular Sciences,
S. N. Bose National Centre for Basic Sciences,
Block JD, Sector III, Salt Lake,
Kolkata 700 098, India
e-mail: skpal@bose.res.in

dynamics? How is this sequence dependent binding manifested in DNA-histone complexes? In this present study, an attempt has been made to exploit the fluorescence solvochromism of the DNA minor groove binder Hoechst [14] to study the ultrafast environmental dynamics of the DNA minor groove in the above two oligomer sequences and their corresponding histone complexes, using femtosecond resolved fluorescence spectroscopy. The overall structure and the local structure around the probe of dodecamer and dodecamer-histone have been monitored through Circular Dichroism (CD) and Fluorescence Detected CD (FDCD) studies.

Materials and methods

Phosphate buffer, calf thymus histone 1 (H1) and the dodecamer DNAs, having sequences CGCGAATTCGCG and CGCAAATTTGCG, are obtained from Sigma Aldrich. The dodecamers have been purified by Reverse Phase Cartridge (RPC) technique and checked by gel electrophoresis. The gel electrophoresis result indicated a single spot consistent with pure DNA. In order to rehybridize the single strand DNA into self-complementary ds-DNA [$(CGCGAATTCGCG)_2$, O1 and $(CGCAAATTTGCG)_2$, O2], thermal annealing is performed. The fluorescent dye Hoechst 33258 (Hoechst) is obtained from Molecular Probes. All the solutions are prepared in 50 mM phosphate buffer using water from the Millipore system. The probe-DNA solutions are prepared by adding requisite amount of the probe in DNA solution and stirring for 1 h. DNA-H1 complexes (O1-H1 and O2-H1) are obtained by dropwise addition of H1 solution to the pre-equilibrated solutions of ligand-DNA with vigorous stirring.

Steady-state absorption and emission are measured with Shimadzu UV-2450 spectrophotometer and Jobin Yvon Fluoromax-3 fluorimeter respectively. Circular dichroism (CD) and Fluorescence detected circular dichroism (FDCD) are done in a JASCO 815 spectrophotometer. The femtosecond-resolved fluorescence spectroscopy is measured using a femtosecond upconversion setup (FOG 100, CDP) in which the sample is excited at 365 nm (0.5nJ per pulse), using the second harmonic of a mode-locked Ti-sapphire laser with an 80 MHz repetition rate (Tsunami, Spectra Physics), pumped by 10 W Millennia (Spectra Physics). The fundamental beam is frequency doubled in a nonlinear crystal (1 mm BBO, $\theta=25°$, $\varphi=90°$). The fluorescence emitted from the sample is upconverted in a nonlinear crystal (0.5 mm BBO, $\theta=10°$, $\varphi=90°$) using a gate pulse of the fundamental beam. The upconverted light is dispersed in a double monochromator and detected using photoncounting electronics. A cross-correlation function obtained using the Raman scattering from water displayed a full width at half maximum (FWHM) of 165 fs. The femtosecond fluorescence decays are fitted using a Gaussian shape for the exciting pulse. The details of the picosecond-resolved spectroscopic measurement and the construction of time resolved emission spectra (TRES) can be found elsewhere [15]. The time dependent fluorescence Stoke's shifts, as estimated from TRES are used to construct the normalized spectral shift correlation function or the solvation correlation function C(t) defined as,

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \tag{1}$$

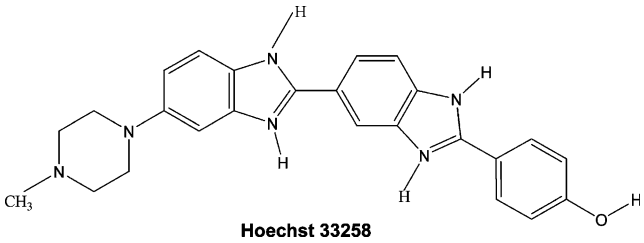
where, $\nu(0)$, $\nu(t)$ and $\nu(\infty)$ are the emission maximum (in cm^{-1}) at time zero, t and infinity. The $\nu(\infty)$ values had been taken to be the emission frequency beyond which an insignificant or no spectral shift was observed. The C(t) function represents the temporal response of the solvent relaxation process, as occurs around the probe following its photo excitation and the associated change in the dipole moment. Picosecond resolved fluorescence anisotropy (r(t)) has been measured with commercially available time correlated single photon counting (TCSPC) setup from Edinburgh Instrumenets (instrument response function (IRF)=60 ps). For anisotropy (r(t)) measurements, emission polarization is adjusted to be parallel or perpendicular to that of the excitation and anisotropy is defined as,

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

G, the grating factor is determined following longtime tail matching technique [16]. For the estimation of G through the tail matching technique, the decay of the fluorescence anisotropy of a probe in such a solvent is chosen in such a way that the probe fluoresces at the wavelength of interest (here 500 nm), and the anisotropy of the probe in the host solvent should decay down to zero within the experimental time window. In this condition, the tails of the parallel and perpendicular fluorescence decays should match. The value of G required to match the tails at the long time is considered as the estimated value of G factor. Here, we have taken coumarin 500 in methanol to estimate the G factor. The time-resolved anisotropy of a fluorophore/probe reveals the physical motion of the probe in a microenvironment. We fit the anisotropy decay by using multi-exponential decay model, where the time constants reflect rotational correlation time of the probe in the microenvironment.

Results and discussion

The fluorescence of the probe Hoechst (Scheme 1) is sensitive to its environment. Fig. 1 shows the fluorescence



Scheme 1 The schematic representation of the probe Hoechst 33258

characteristics of the probe in the minor groove of the dodecamer duplexes having sequences $(CGCGAATTCGCG)_2$ (O1) and $(CGCAAATTTGCG)_2$ (O2) and the corresponding duplex-histone complexes. The emission maximum of Hoechst in O2 (Fig. 1b) shows a blue shift of 5 nm compared to that in O1 (Fig. 1a). The observation is consistent with the x-ray crystallographic image which reveals that the minor groove of O2 is narrower than that in O1. The drug, which occupies the minor groove in both the complexes is less exposed to the water molecules and is closer to the hydrophobic residues [13] in O2, compared to that in O1. The emission spectra of Hoechst in both O1-H1 and O2-H1 (Fig. 1) complexes show blue shift, compared to its emission in the dodecamers. The excited state of the probe is more polar than the ground state, therefore, the decrease in stability of the excited state in the more hydrophobic dodecamer-histone complexes accounts for a blue shift in the emission spectrum. It is to be noted that the magnitude of the blue shift is marginally greater in O1-H1 complexes, pointing out the possibility of different local environment of the probe in the two dodecamer-histone complexes. Since the time constants associated with the decay of fluorescence anisotropy of the probe in buffer and when bound to dodecamer duplexes are widely separated in time scales [17], it provides an useful measure to confirm the binding of the probe in oligomer duplexes and the corresponding dodecamer histone complexes. Femtosecond resolved decays of fluorescence anisotropies (data not shown) do not decay appreciably in 250 ps, suggesting that there is no faster motion of the probe in the different environments. The insets of Fig. 1 show the decay of picosecond resolved fluorescence anisotropies in the different dodecamers and the dodecamer-histone complexes. In bulk buffer, the probe shows rotational motions having a time scale of 500 ps [18]. However, the presence of longer rotational time times of the probe in the dodecamer duplexes and in their corresponding histone complexes indicate that the probe is in the bound state. In the dodecamer duplexes O1 and O2, the rotational time scale of ~5 ns (Table 1) represents the global tumbling motion of the probe-DNA complex [15]. The longer component (~50 ns, Table 1) reflects the rotational motion of the probe-DNA complex which is not complete within the fluorescence lifetime of the probe. Since the rotational motion represents the global tumbling motion of the DNAs, no significant difference is expected for oligomers of the same size, as is borne out by the similarity of the rotational time constants, tabulated in Table 1. In O1-H1 and O2-H1 complexes, the percentage of the long component (~80 ns) increases (Table 1) suggesting that a greater part of the rotational motion of the protein-DNA complex is not complete within the fluorescence lifetime of the probe. The slower rotational motion is consistent with the larger size of the dodecamer-histone complex [19].

The studies on the fluorescence anisotropies suggest that the probe remains as an essential part in both duplexes and their corresponding histone complexes and hence can report their environmental dynamics. Figure 2 shows the femtosecond resolved fluorescence transients along the emission profile of Hoechst in the different systems. The transients in the dodecamers and their corresponding histone complexes show fast decays (2–30 ps) in the blue end and corresponding rises in the red end characteristic of environmental stabilization [20]. The temporal evolution of the emission spectrum (shown in the time resolved emission spectrum (TRES), insets of Fig. 3) in the different environments is expressed as the decay of the solvation correlation functions, C(t) (Fig. 3). The time constants of C(t) decay associated with the ultrafast environmental relaxation in the minor groove of the studied dodecamer duplexes and their

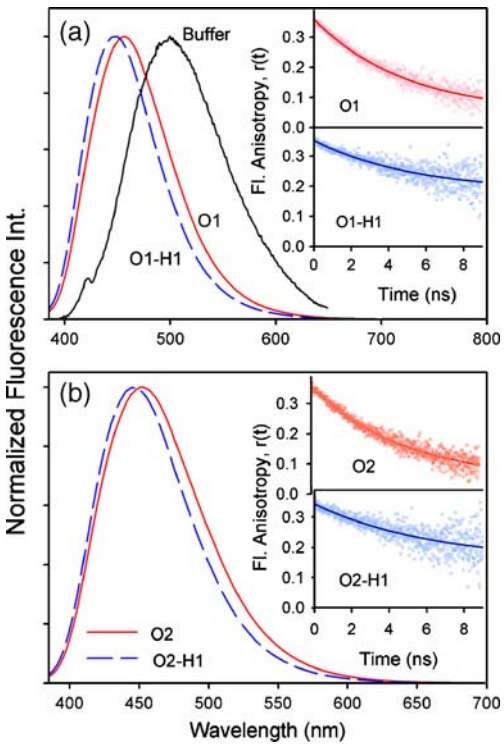


Fig. 1 **a** The emission spectra of Hoechst-O1 and Hoechst-O1-H1 complexes. The emission in buffer is also shown for comparison. **b** The emission spectra of Hoechst-O2 and Hoechst-O2-H1 complexes. (Inset) the decay of fluorescence anisotropy of Hoechst in different environments

Table 1 Fitting parameters for the ultrafast environmental dynamics and rotational dynamics of Hoechst in dodecamer duplexes and duplex-histone complexes

System	Solvation correlation function, C(t)			Fl. Anisotropy, r(t)	
	τ_1(ps)	τ_2 (ps)	τ_3(ps)	τ_{rot1} (ns)	τ_{rot2} (ns)
H-O1	1.16±0.11 (50%)	28.31±2.83 (50%)	–	4.9±0.49 (93%)	50±5.0 (07%)
H-O1-H1	2.01±0.20 (41%)	30.93±3.09 (25%)	0.24±0.02 (33%)	5.2±0.52 (50%)	80±8.0 (50%)
H-O2	1.99±0.20 (61%)	28.5±2.85 (39%)	–	5.0±0.5 (94%)	50±5.0 (06%)
H-O2-H1	1.98±0.20 (70%)	29.7±2.97 (30%)	–	5.9±0.59 (50%)	80±8.0 (50%)

respective histone complexes are tabulated in Table 1. A pioneering study [7] by Zewail et al. on the ultrafast relaxation dynamics in the minor groove of the dodecamer duplex $(CGCAAATTTGCG)_2$ (O2 in our study) reveals that the ultrafast dynamics is associated with the environmental relaxation of bulk-like water (1.4 ps, 64%) and ordered water (20 ps, 36%) present in the DNA minor groove, the dynamical interconversion between which is proposed to be crucial for the minor groove recognition by the ligand. The time constants associated with the environmental relaxation in O2 (2 ps (61%) and 28 ps (39%)) show striking similarity with the results obtained by Zewail et al. [7].

It seems logical that a comparison between the environmental dynamics in the minor groove of the dodecamer duplexes O1 and O2 should be preceded by the comparison of their x-ray crystallographic structures. Analyses of the crystal structures of Hoechst-O1 and Hoechst-O2 complexes by Neidle et al. [13] have revealed that the drug has distinct binding sites in the two duplexes. Hoechst shows a strong preference for the central AATT site in the duplex O1 (Scheme 2). The binding of Hoechst to the central AATT tract in O1 is corroborated from high field NMR study [21]. In the NMR study, the evidence of the chemical shifts of the C1 hydrogen atoms present in the minor groove coupled with the internal Nuclear Overhauser Effect (NOE) peaks provides strong evidence for drug binding in the central AATT tract. However, in O2, the drug occupies a unique non disordered position fully covering the lower four AT base pairs (the sequence 5′-ATTTG) with the

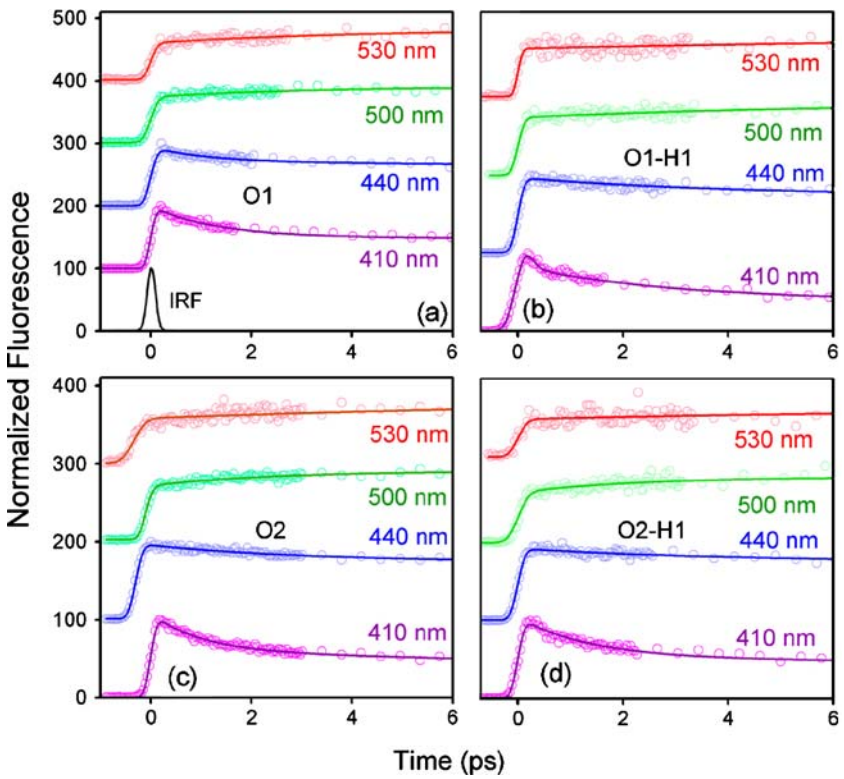


Fig. 2 The femtosecond resolved fluorescence transients at different wavelengths of Hoechst in different environments. Baseline of each transient is shifted for clarity. The instrument response function (IRF) is also shown in black (Fig. 2(a))

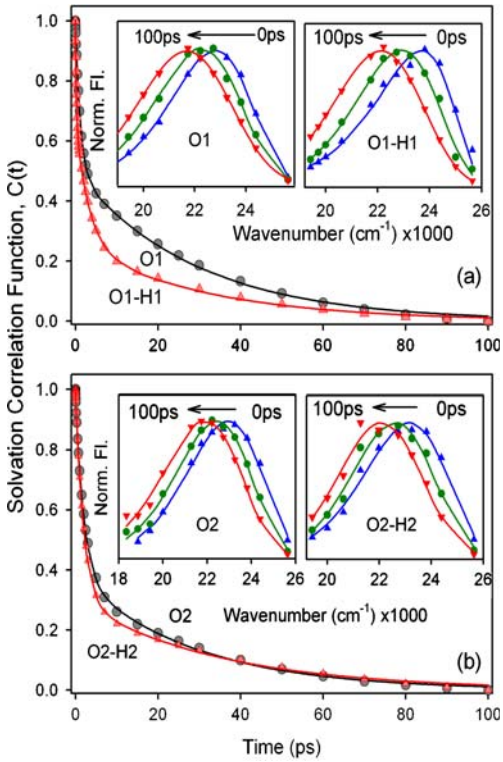
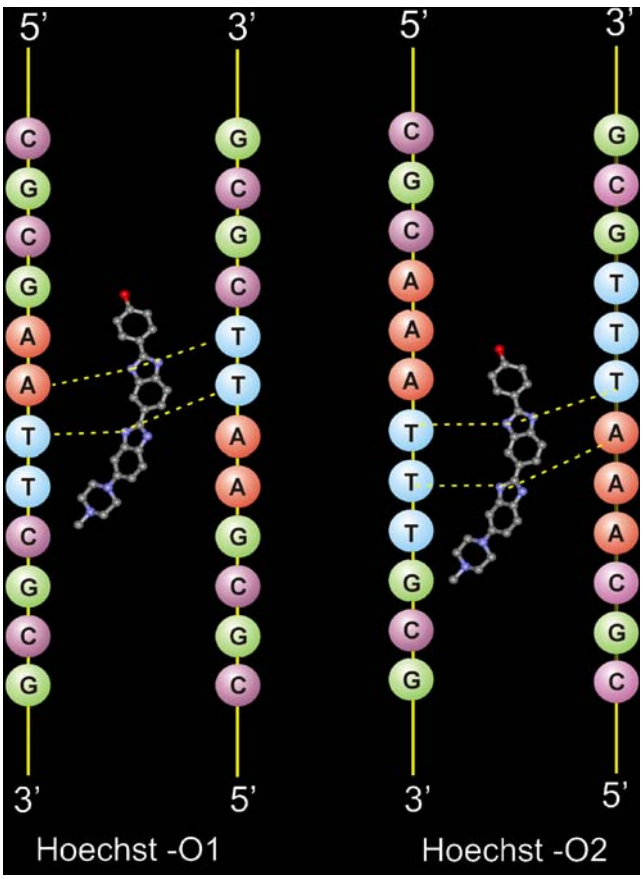


Fig. 3 The temporal decay of the solvation correlation function in **a** Hoechst-O1 and Hoechst-O1-H1 complexes **b** Hoechst-O2 and Hoechst-O2-H1 complexes. The insets show the constructed TRES in different environment

methyl piperazine ring reaching the edge of lower adjacent CG base pair (piperazine down orientation). It is known that extended AT tracts in DNA sequences tends to have high propeller twists and result in narrow minor grooves [22, 23]. The central sequence of 5′-AAATTT in O2 imposes small but significant differences compared to that in 5′-AATT, which forces Hoechst to have a different binding site, with narrowed minor groove in the 3′ direction of O2 favoring drug binding in that direction (Scheme 2). The structure of the hydration waters associated with these sequences also show small but significant differences. The "spine of hydration" [1] of O1 modifies itself into a more flattened "ribbon of hydration" [2] in O2, the details of which are discussed in the respective references (references 1 and 2). The waters revealed in the x-ray crystallographic images are the structurally observed waters [8] which may have reorientation times from hundreds of picoseconds to several nanoseconds. However, acousto-densimetric techniques sense a much broader population of water molecules than crystallographically ordered waters [5]. These techniques show that there are ~25 water molecules associated with each base pair of DNA duplexes. Thus, it is evident that in addition to these structurally ordered waters, there are dynamically ordered waters present at the surface of a biomolecule. These dynamically ordered molecules are constantly in exchange with the bulk. The presence of such dynamically ordered molecules at the surface of the proteins [24] and DNA [7, 25] have been revealed from femtosecond-resolved spectroscopic studies. Molecular dynamics simulation study [26] on the hydration of myoglobin have also revealed the existence of water molecules having considerable mobility, and breaking and reforming bonds with the protein. The dynamically ordered waters at a biomolecular surface are characterized by a solvation correlation time of 20–40 ps [7]. The contribution of these ordered waters to the overall solvation of the probe decreases from 50% in O1 to 39% in O2 (Table 1), and possibly reflects lesser number of dynamically ordered waters in the narrower minor groove of O2. The faster counterpart, reflecting the dynamics of free type water present in the groove decreases from 2 ps in O2 to 1 ps in O1 (Table 1), suggests faster motion of free type waters in the wider groove of the latter.

At this juncture, it would be interesting to compare the situation in histone-DNA complexes. Since the DNA condensation in vivo stems from the interaction of DNA



Scheme 2 The schematic representation of the probe Hoechst 33258 in the dodecamer duplexes O1 and O2. The yellow dotted lines represent the hydrogen bonds between the dye and the DNA bases. The DNA bases are represented as circles for clarity of presentation

fragments with histone [27, 28], DNA-histone complexes are the closest mimics of the condensed DNA to be realized in practice. Figure 3a and b represents the temporal decay of the solvation correlation function associated with the environmental relaxation in O1-H1 and O2-H1 complexes. A comparison of the associated time constants (Table 1) makes it evident that the difference in hydration dynamics between O1 and O1-H1 complexes are more pronounced than that between the hydration dynamics of O2 and O2-H1 complexes. The environmental dynamics in Hoechst-O2-H1 complexes are marginally faster than that of the Hoechst-O2 complexes, with increase in the bulk water dynamics. However, the environmental dynamics of Hoechst-O1-H1 complex shows an additional 240 fs component in contrast to the dynamics in Hoechst-O2 complex.

It is to be noted that the solvation of Hoechst in buffer is associated with time constants of 195 fs (33%) and 1.2 ps (67%) [7]. The appearance of water-like component in the environmental dynamics in the Hoechst-O1-H1 complex might indicate expulsion of the probe in bulk water. However, absence of picosecond components, (characteristic of the rotation of the probe in bulk buffer), in the rotational anisotropy of the probe in the O1-H1 and O2-H1 complexes as discussed above, negates the possibility of the expulsion of the probe in buffer. The position of the emission maximum of Hoechst in the O1-H1 complex (Fig. 1) is also blue shifted compared to that in buffer, indicating the location of the probe in a much hydrophobic and rigid environment than in buffer [18]. Solvation dynamics studies on 2-(p-tolidino)naphthalene-6-sulphonate (TNS) and 5-(dimethylamino)naphthalene-1-sulfonyl chloride (DC) bound the N and C terminal portions of the histone molecule shows that the solvation occours as fast as in bulk solvents [20], indicating the minor role of rigid water structure in the binding sites. Since the N and C termini with high densities of positive charges are involved in the recognition of DNA by electrostatic interactions, these random coils optimize the attraction by ulilizing labile bulk water [20]. Thus in O1-H1 complexes, the presence of water like component suggests that the probe in the wider minor groove senses the dodecamer-histone interaction more effectively, than in the O2-H1 complexes, where due to the location in the narrower groove, it shows only a marginal increase in the bulk-like water component. Also, it is to be noted that the histone H1, used in our study is rich in lysine and hence shows affinity towards GC tracts in DNA [29]. The possibility of better interaction of the protein with O1, having a higher GC content, can not be ruled out.

In order to study the different interaction of the dodecamer duplexes with the protein, we compared the circular dichroism (CD) spectra. Figures 4a and b show

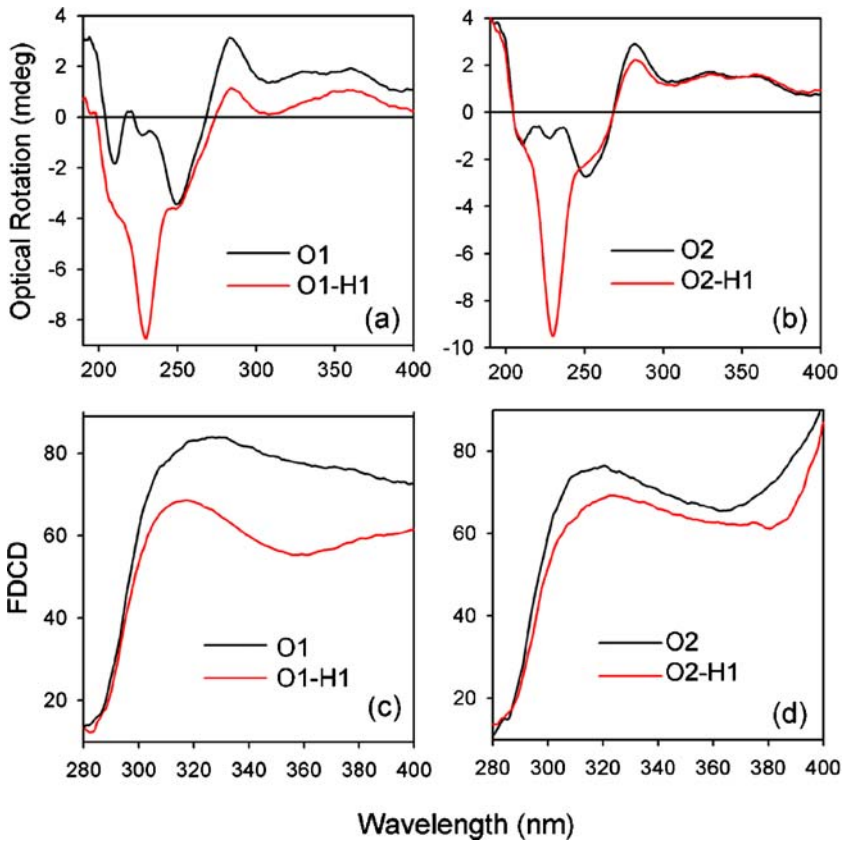


Fig. 4 The circular dichroism (CD; a and b) and fluorescence detected circular dichroism (FDCD; c and d) spectra in different systems

the CD spectra of the Hoechst-O1-H1 and Hoechst-O2-H1 complexes. The CD spectra of the dodecamers with the probe show general characteristics having positive peaks around 350 nm and 281 nm and a negative peak around 250 nm. The positive peak around 280 nm and the negative peak at 250 nm are associated with the structure of B form DNA [19]. The twin positive peaks around 320 nm and 360 nm reveal the induced chirality of Hoechst in DNA environments [30], which is absent in bulk buffer (data not shown). From the CD spectra of the dodecamer-histone complexes, it is evident that the structure of O1 is more perturbed in its histone complex than O2 in the complex. Most likely, O1 reveals a mixture of B and C form DNA [19] in the complex. Relatively larger negative peak at 230 nm bears the signature of H1. From the spectra it is evident that the immediate environment of the probe in the O1 complex is heavily affected by the complexation, which is reflected in the change in the induced chirality (peaks around 350 nm) of the probe in the complex. In order to further confirm the change in the induced chirality of Hoechst in O1 and O2 and upon complexation with H1, FDCD measurements on the probe in the dodecamers and in the dodecamer-histone complexes (Fig. 4c and d) are performed. The FDCD spectra of Hoechst in dodecamer and dodecamer-histone complexes reveal greater perturbation in the O1-H1 complex, consistent with those obtained from femtosecond studies.

Conclusion

In the present work, we have studied molecular recognition of two dodecamer duplexes $(CGCGAATTCGCG)_2$ and $(CGCAAATTTGCG)_2$, and in their respective histone complexes by the drug Hoechst through comparison of their femtosecond resolved environmental dynamics. The modest difference between the molecular recognition of the two dodecamer duplexes by Hoechst gets amplified in the dodecamer-histone duplexes, suggesting a possible difference between drug action in vivo and in vitro. The change in molecular recognition as evidenced from femtosecond experiments is corroborated from CD and FDCD studies.

Acknowledgement DB, AM thanks CSIR, India for fellowship. We thank DST for financial grant (SR/SO/BB-15/2007).

References

1. Pjura PE, Grzeskowiak K, Dickerson RE (1987) Binding of Hoechst 33258 to the minor groove of B-DNA. J Mol Biol 197:257–271. doi:10.1016/0022-2836(87)90123-9
2. Edwards KJ, Brown DG, Spink N, Skelly JV, Neidle S (1992) Molecular structure of the B-DNA dodecamer d(CGCAAATTTGCG)$_2$ An examination of propeller twist and minor-groove water structure at 2·2 Å resolution. J Mol Biol 226:1161–1173. doi:10.1016/0022-2836(92)91059-X
3. Lane AN, Jenkins TC, Frenkiel TA (1997) Hydration and solution structure of d(CGCAAATTTGCG)$_2$and its complex with propamidine from NMR and molecular modelling. Biochim Biophys Acta 1350:205–220. doi:10.1016/S0167-4781(96)00161-3
4. Haq I, Ladbury JE, Chowdhry BZ, Jenkins TC, Chaires JB (1997) Specific binding of Hoechst 33258 to the d(CGCAAATTTGCG)$_2$ duplex: calorimetric and spectroscopic studies. J Mol Biol 271:244–257. doi:10.1006/jmbi.1997.1170
5. Han F, Taulier N, Chalikian TV (2005) Association of the minor groove binding drug Hoechst 33258 with d(CGCGAATTCGCG)$_2$: volumetric, calorimetric, and spectroscopic characterizations. Biochemistry 44:9785–9794. doi:10.1021/bi047374f
6. Privalov PL, Dragan AI, Crane-Robinson C, Breslauer KJ, Remeta DP, Minetti CASA (2007) What drives proteins into the major or minor grooves of DNA? J Mol Biol 365:1–9. doi:10.1016/j.jmb.2006.09.059
7. Pal SK, Zhao L, Zewail AH (2003) Water at DNA surfaces: ultrafast dynamics in minor groove recognition. Proc Natl Acad Sci USA 100:8113–8118. doi:10.1073/pnas.1433066100
8. Pal SK, Zewail AH (2004) Dynamics of water in biological recognition. Chem Rev 104:2099–2123. doi:10.1021/cr020689l
9. Vlieghe D, Sponer J, Meervelt LV (1999) Crystal structure of d(GGCCAATTGG) complexed with DAPI reveal novel binding mode. Biochemisty 38:16443–16451. doi:10.1021/bi9907882
10. Quintana JR, Lipanov AA, Dickerson RE (1991) Low-temperature crystallographic analyses of the binding of Hoechst 33258 to the double-helical DNA dodecamer C-G-C-G-A-A-T-T-C-G. Biochemistry 30:10294–10306. doi:10.1021/bi00106a030
11. Wood AW, Nunn CM, Czarny A, Boykin DW, Neidle S (1995) Variability in DNA minor groove width recognised by ligand binding: the crystal structure of a bis-benzimidazole compound bound to the DNA duplex d(CGCGAATTCGCG)2. Nucleic Acids Res 23:3678–3684. doi:10.1093/nar/23.18.3678
12. Brown DG, Sanderson MR, Garman E, Neidle S (1992) Crystal structure of a berenil-d(CGCAAATTTGCG) complex: an example of drug-DNA recognition based on sequence-dependent structural features. J Mol Biol 226:481–490. doi:10.1016/0022-2836(92)90962-J
13. Spink N, Brown DG, Skelly JV, Neidle S (1994) Sequence-dependent effects in drug-DNA interaction: the crystal structure of Hoechst 33258 bound to the d(CGCAAATTTGCG)2 duplex. Nucleic Acids Res 22:1607–1612. doi:10.1093/nar/22.9.1607
14. Jin R, Breslauer KJ (1988) Characterization of the minor groove environment in a drug-DNA complex: bisbenzimide bound to the poly[d(AT)] poly[d(AT)]duplex. Proc Natl Acad Sci USA 85:8939–8942. doi:10.1073/pnas.85.23.8939
15. Banerjee D, Pal SK (2007) Direct observation of essential DNA dynamics: melting and reformation of the DNA minor groove. J Phys Chem B 111:10833–10838. doi:10.1021/jp074697n
16. O'Connor DV, Philips D (1984) Time correlated single photon counting. Academic, London
17. Banerjee D, Pal SK (2007) Simultaneous binding of minor groove binder and intercalator to dodecamer DNA: importance of relative orientation of donor and acceptor in FRET. J Phys Chem B 111:5047–5052. doi:10.1021/jp0715427
18. Banerjee D, Pal SK (2006) Ultrafast charge transfer and solvation of DNA minor groove binder: Hoechst 33258 in restricted environments. Chem Phys Lett 432:257–262. doi:10.1016/j.cplett.2006.10.018
19. Sarkar R, Pal SK (2007) Interaction of Hoechst 33258 and ethidium with histone1-DNA condensates. Biomacromolecules 8:3332–3339. doi:10.1021/bm700690p

20. Zhong D, Pal SK, Zewail AH (2001) Femtosecond studies of protein-DNA binding and dynamics: histone I. ChemPhysChem 2:219–227. doi:10.1002/1439-7641(20010417)2:4<219::AID-CPHC219>3.0.CO;2-K
21. Parkinson JA, Barber J, Douglas KT, Rosamond J, Sharples D (1990) Minor-groove recognition of self-complementary duplex d(CGCGAATTCGCG)$_2$ by Hoechst 33258: a high field NMR study. Biochemistry 29:10181–10190. doi:10.1021/bi00496a005
22. Privé GG, Yanagi K, Dickerson RE (1991) Structure of the B-DNA decamer C-C-A-A-C-G-T-T-G-G and comparison with isomorphous decamers C-C-A-A-G-A-T-T-G-G and C-C-A-G-G-C-C-T-G-G. J Mol Biol 217:177–199
23. Alexeev DG, Lipanov AA, Skuratovskii IY (1987) Poly(dA).poly(dT) is a B-type double helix with a distinctively narrow minor groove. Nature 325:821–823. doi:10.1038/325821a0
24. Kamal JKA, Xia T, Pal SK, Zhao L, Zewail AH (2004) Enzyme functionality and solvation of subtilisin *carlsberg*: From hours to femtoseconds. Chem Phys Lett 387:209–215. doi:10.1016/j.cplett.2004.02.011
25. Pal SK, Zhao L, Xia T, Zewail AH (2003) Site and sequence selective ultrafast hydration of DNA. Proc Natl Acad Sci USA 100:13746–13751. doi:10.1073/pnas.2336222100
26. Gu W, Schoenborn BP (1995) Molecular dynamics simulation of hydration in myoglobin. Proteins 22:20–26. doi:10.1002/prot.340220104
27. Ramakrishnan V (1997) Histone structure and the organization of the nucleosome. Annu Rev Biophys Biomol Struct 26:83–112. doi:10.1146/annurev.biophys.26.1.83
28. Richmond TJ, Davey CA (2003) The structure of DNA in the nucleosome core. Nature 423:145–150. doi:10.1038/nature01595
29. Laigle A, Chinsky L, Turpin PY, Liquier J, Taillandier E (1982) In vitro recognition of DNA base pairs by histones in histone-DNA complexes and reconstituted core particles: an ultraviolet resonance Raman study. Nucleic Acids Res 10:7395–7404. doi:10.1093/nar/10.22.7395
30. Moon JH, Kim SK, Sehlstedt U, Rodger A, Norden B (1996) DNA structural features responsible for sequence-dependent binding geometries of Hoechst 33258. Biopolymers 38:593–606. doi:10.1002/(SICI)1097-0282(199605)38:5<593::AID-BIP5>3.0.CO;2-N