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How much hydration is necessary for the stabilisation of DNA-duplex?

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ABSTRACT

A combination of NOESY and ROESY experiments show that the higher stabilities (T_m) of phenazine tethered matched (2) and G-A mismatched (4) DNA duplexes are due to the decrease of the exchange-rate (*i.e.* increase of the life-time) of the imino-protons and the reduced water activity in their minor grooves compared to their non-tethered counterparts (1) and (3).

INTRODUCTION

The ability of oligo-DNA analogues to act as antisense repressors at the transcriptional and translational level of gene expression is based on the strength and efficiency of the specific base pairing which stabilises the duplex formed between the oligonucleotide reagent and the target sequence[1]. One of the ways to improve the efficiency and specificity of the antisense oligonucleotides is to increase the stability of the DNA duplex by introducing polyaromatic systems to the oligonucleotide chain through a covalent linker. DNA duplexes tethered with 2-methoxy-6-chloro-9-aminoacridine (Acr)[2,3] and N-(2-hydroxyethyl)phenazinium (Pzn)[4] have been shown to strongly stabilise the DNA duplex by 10° to 20°C compared to their natural counterparts. No mechanism of such DNA duplex stabilisation due to tethered polyaromatic system is however known. It has been earlier shown[5] that the melting point (T_m) of Pzn-tethered [5′-PO_2-NH-$(CH_2)_2$-NH-Pzn] matched DNA duplex (**2**) (T_m = 50°C) and the corresponding G-A mismatched duplex (**4**) (T_m = 34°C) increase by several degrees compared to their parent DNA duplex (**1**) (T_m = 30°C) and (**3**) (T_m = 25°C). The solution conformation of these duplexes (**1**)–(**4**) was assessed[5] by 500 MHz ^{1}H-NMR, iterative hybrid relaxation matrix method (MORASS) combined with NOESY distances and torsion angle restrained molecular dynamics, which have shown that the Pzn residue stacks with both residues of the neighbouring G-C base-pair in the duplex (**2**) and contributes to its strong stabilisation, while the Pzn residue in (**4**) do not stack with the neighbouring G-A mismatch base-pair and adopts at least three different conformations in the NMR time scale. These studies[5] prompted us to address how do the stabilitities of duplexes (**1**)–(**4**) depend upon (a) the water activity in and around DNA, (b) the strength of hydrogen bond by Watson–Crick base-pairing, and (c) the stacking behaviour? The studies on the nature of hydration of oligo-DNA in aqueous solution are particularly important mainly because of the three following reasons: (1) mainly solid state data from X-ray crystallography have provided evidence for the presence of static water molecules (spine of hydration, ribbons of water etc) on a time average which have been thought to be the critical factor for the stabilisation of secondary and tertiary structure of polyelectrolite DNA[6]. It has been suggested that phosphate electrostatic repulsion is diminished by the solvation with water and hydrated counterions, which are also believed to be responsible for the stabilisation of the B-form of DNA[6a,7]. Clearly, these solid state data and theories need to be examined in the aqueous environment. (2) Indeed, very little information is known about the hydration of DNA in aqueous solution except for two recent studies[8,9] which for the first time gave some NMR evidence for DNA bound water in solution. (3) It is not clear how the nature of hydration and its salt dependent change act as the driving force for the transitions between A-, B- and Z-DNA and for their recognition and interaction with drugs and proteins[6a,7].

(1) [5′p[d(^{1}T^{2}G^{3}T^{4}T^{5}T^{6}G^{7}G^{8}C]3′]*[5′p[d(^{9}C^{10}C^{11}A^{12}A^{13}A^{14}C^{15}A]3′]
(2) [5′p[d(^{1}T^{2}G^{3}T^{4}T^{5}T^{6}G^{7}G^{8}C]3′]*[5′(Pzn)-p[d(^{9}C^{10}C^{11}A^{12}A^{13}A^{14}C^{15}A]3′]
(3) [5′p[d(^{1}T^{2}G^{3}T^{4}T^{5}T^{6}G^{7}G^{8}C]3′]*[5′p[d(^{9}A^{10}C^{11}A^{12}A^{13}A^{14}C^{15}A]3′]
(4) [5′p[d(^{1}T^{2}G^{3}T^{4}T^{5}T^{6}G^{7}G^{8}C]3′]*[5′(Pzn)-p[d(^{9}A^{10}C^{11}A^{12}A^{13}A^{14}C^{15}A]3′]

In this work, a combination of of NOESY[10] and ROESY[11] experiments have been used to allow a distinction[12] of the cross-relaxation process (chemical exchange, spin diffusion or direct magnetization transfer) between imino proton and water from the direct magnetization transfer process between imino protons and non-exchangeable protons. In these experiments[12], it has been possible to suppress the intense water resonance without obscuring the cross-relaxation process between water and DNA protons. It is a powerful tool[12] to identify a potential DNA–water interaction if no DNA protons resonate at the water chemical shift[8,9]. We herein report the rates of *fast exchanging* imino protons of DNA duplexes (**1**)–(**4**) without addition of any extra catalyst or altering the temperature, which can not be performed by the conventional saturation recovery experiment[13c,d,g]. We also provide the first unequivocal evidence that

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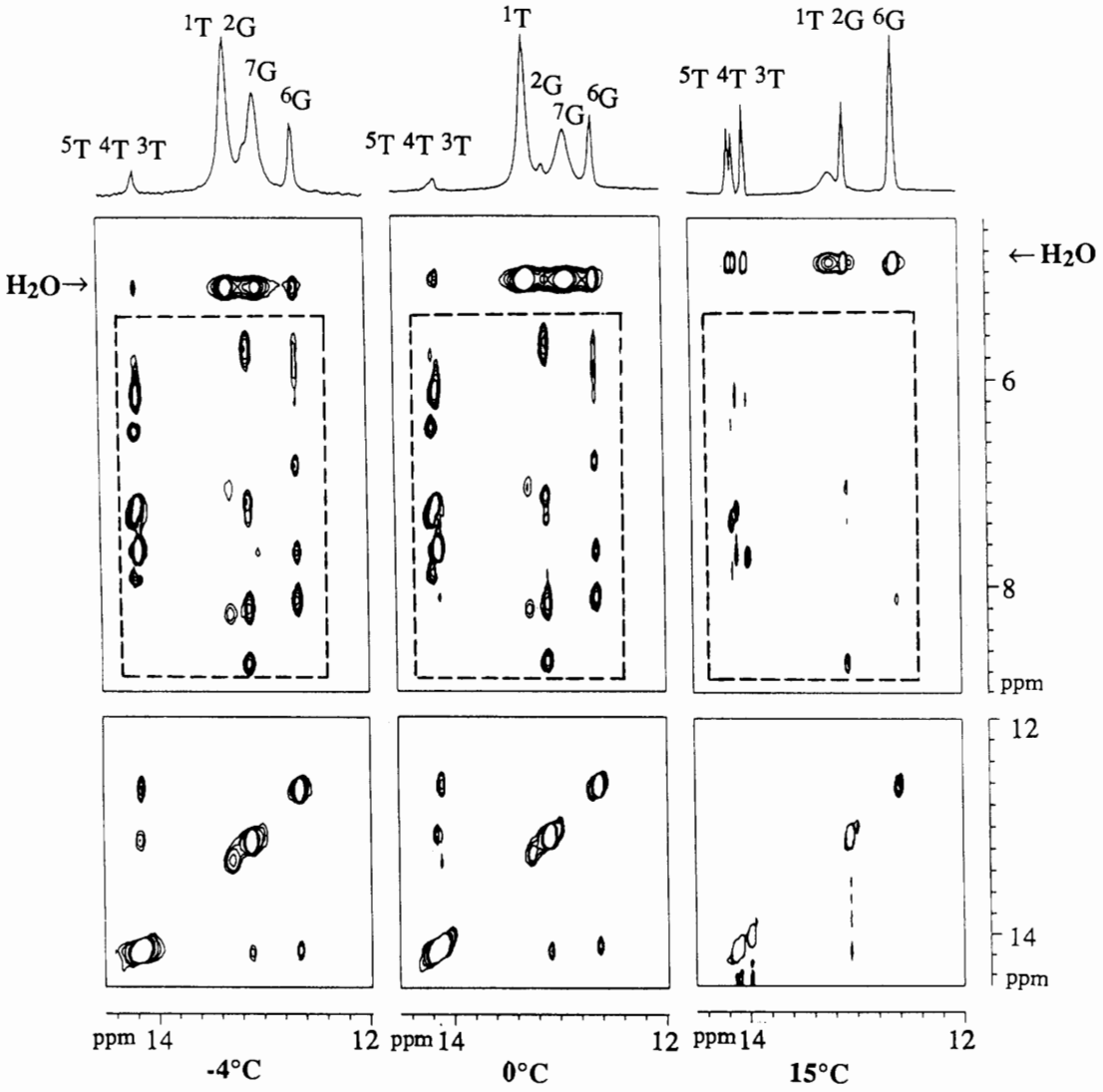

Figure 1. Plot of spectral region (f_1: δ14.5−12.0 ppm for imino protons; δ9.0−4.5 ppm for aromatic, amino and H1′ protons; f_2: δ14.5−12.0 ppm for imino protons) from contour plots of the NOESY[10] (τ_m = 60 ms) spectra of the matched duplex (**1**) at −4°C, 0°C and 15°C, recorded at 500 MHz (^{1}H) dissolved in 0.4 ml of the buffer [0.1 M NaCl, 10 mM NaD_2PO_4, 10 μM EDTA, pH 7.4 in 10%D_2O:90%H_2O, sample concentration: 10 mM]. The temperature-dependent water chemical shift is indicated by the horizontal arrow. Horizontal cross section along f_2 at the f_1 frequency of the water line in the NOESY spectrum showing cross-peaks of imino protons[5] with water. Note, as the temperature increases from −4°C to 15°C, the volumes of NOE cross-peaks from imino and aromatic protons decrease. Comparison of the spectra at −4°C and 0°C at the chemical shift of water clearly show that the intensity of the cross-peak between imino proton and water has increased because of the acceleration of the exchange rate even in this small range of temperature difference.

the stability of DNA duplex does not necessarily increase with a higher abundance of water inside the minor or the major groove in contrary to the common belief[6a,7]

In the equilibrium of non-catalytic exchange reaction of imino protons of DNA duplex with water at pH 7 [NH*···N⇄NH* + HOH⇄NH + HOH*⇄NH···N] , only the chemical shifts of the hydrogen-bonded imino protons (NH(H*)···N) are observed. It is generally accepted[13] that the limiting rate of the exchange process (k_{ex}) is dictated by the rate of opening of the duplex (k_{op}). The eqn. 1 have been shown[12] to be useful for the determination of k_{ex} by a combination of NOESY and ROESY spectra.

A set of NOESY experiments at −4°, 0° and 15°C at τ_m = 60 ms with duplexes (**1**)−(**4**) showed [see for *e.g.*, Fig. 1 for the matched-duplex (**1**)] that the dipole-dipole magnetization transfer by cross-relaxation process (direct magnetization transfer) between imino protons and the non-exchangeable aromatic protons were the dominant process at −4° or 0°C as evident by the cross-peaks (shown in the boxed region of the spectra in Fig. 1) but these direct magnetization cross-peaks with the non-exchangeable protons disappeared almost completely at 15°C while cross-peaks due to the magnetization transfer by exchange process (exchange) found at the chemical shift of water (marked by arrows in Fig. 1) increased considerably. Note that in the ROESY spectra at the same mixing time at 15°C, these direct magnetization cross-peaks are completely absent (data not shown). Since NOE cross-peaks in the NOESY spectra has opposite sign with respect to the ROESY[14] spectra because cross-relaxation rates (σ) between two spins in the NOESY and ROESY spectra are related by $2\sigma^{noe} = -\sigma^{roe}$ when

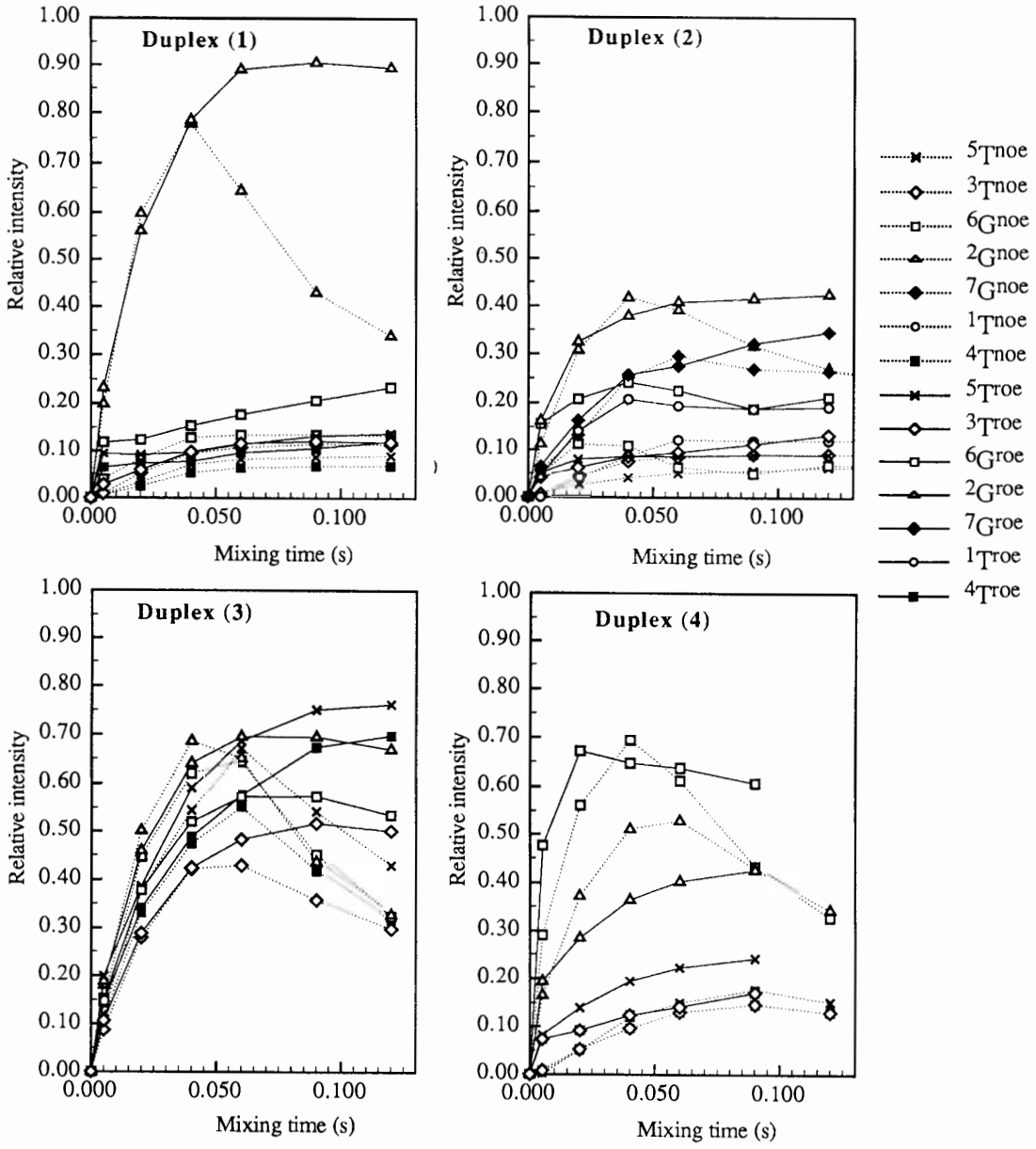

Figure 2. The relative intensity of the NOE buildup curves for the cross-peaks between imino protons and water in the phase-sensitive NOESY[10] (dotted line) and ROESY[11] [6.25-kHz rf field for all pulses (36.5 μs 90° pulse)] (solid line) spectra for (**1**)–(**4**) at 15°C are shown at τ_m = 5, 20, 40, 60, 90 and 120 ms, which were normalized to the diagonal intensity at τ_m = 0 ms (obtained by the extrapolation of the diagonal peaks at various mixing times to the zero mixing time). The NOE cross peak volumes in both NOESY and ROESY spectra were measured using the program AURELIA[19]. The sharp evolution of the NOE buildup in the ROESY spectra at τ_m = 5 ms for some slow-exchanging imino proton is most probably due to the influence of the spinlock at such a short mixing time. This cross-peak at 5 ms has not been used in our calculations shown in Table 1.

$\omega\tau_c >> 1$[15,16], therefore the $-\sigma^{roe}$ should have opposite sign with respect to the spin diffusion $\sim\sigma_{kj}\sigma_{jl}$ [multiple state magnetization transfer between sites *k* and *l* through the common neighbour *j* $(k \leftrightarrow j \leftrightarrow l)$][17]. This means that the contribution of direct magnetization process in the cross-peaks shown in the boxed region of Fig. 1 is much less than the diffusion contribution in the volume of the cross-peak between the imino and aromatic protons in the NOESY spectra in Fig. 1 at 15°C. This suggests that the contribution of direct magnetization process in the imino proton and water cross-peak is negligible and is excluded in eqn. 1[12].

$$4\left(\frac{a_{NW}^{noe}}{a_{N0}^{noe}}\right)-\left(\frac{a_{NW}^{roe}}{a_{N0}^{roe}}\right)\approx K_{ex}\tau_m\left[3-\left(\frac{4(R_{NN}^{noe}+R_{WW}^{noe})\tau_m}{2}-\frac{(R_{NN}^{roe}+R_{WW}^{roe})\tau_m}{2}\right)\right] \quad \ldots \quad (1)$$

The longitudinal relaxation rates have been calculated from diagonal peaks as follows:

$(R_{NN}^{noe}+R_{WW}^{noe})=\ln\left(\frac{a_{NN}^{noe}}{a_{N0}^{noe}}\right)/\tau_m$ and $(R_{NN}^{roe}+R_{WW}^{roe})=\ln\left(\frac{a_{NN}^{roe}}{a_{N0}^{roe}}\right)/\tau_m$, where $\frac{a_{NW}^{noe}}{a_{N0}^{noe}}$ and $\frac{a_{NN}^{noe}}{a_{N0}^{noe}}$ are

the cross-peak and diagonal peak intensities, respectively, normalised to zero mixing time intensity of diagonal peak in the NOESY spectra (with symbol *roe* in the ROESY spectra) between protons N (imino) and W (water).

Table 1. Rate constants for the exchange of the imino protons and the life-times of the duplexes (**1**)–(**4**) at 15°C using eqn. 1.

		Exchange rate			Life-time		
DNA duplex	Imino proton	k_{ex} (s^{-1}) (τ_m = 20 ms)	k_{ex} (s^{-1}) (τ_m = 40 ms)	k_{ex} (s^{-1}) (τ_m = 60 ms)	τ (ms) (τ_m = 20 ms)	τ (ms) (τ_m = 40 ms)	τ (ms) (τ_m = 60 ms)
Matched duplex (**1**)	^{1}T-^{15}A	#	#	#	#	#	#
	^{2}G-^{14}C	38.9	34.0	26.8*	26	29	37*
	^{3}T-^{13}A	2.6	2.5	1.9*	385	400	526*
	^{4}T-^{12}A	0.5*	1.2	0.9	2000*	833	1111
	^{5}T-^{11}A	0.7*	1.5	1.2	1429*	666	833
	^{6}G-^{10}C	3.6	3.2	2.1*	278	313	476*
	^{7}G-^{9}C	#	#	#	#	#	#
Pzn-Matched duplex (**2**)	^{1}T-^{15}A	3.7	2.6	1.9*	270	384	526*
	^{2}G-^{14}C	19.0	16.0	12.0*	53	63	83*
	^{3}T-^{13}A	1.8	1.8	1.5	556	556	667
	^{4}T-^{12}A	¥	¥	¥	¥	¥	¥
	^{5}T-^{11}A	0.7	0.7	0.6	1429	1429	1667
	^{6}G-^{10}C	4.0	1.6*	1.4*	250	625*	715*
	^{7}G-^{9}C	9.0	7.6	6.5*	111	132	154*
Mismatched duplex (**3**)	^{1}T-^{15}A	#	#	#	#	#	#
	^{2}G-^{14}C	34.0	35.0	42.0	29	29	24
	^{3}T-^{13}A	16.0	14.7	11.8	63	68	85
	^{4}T-^{12}A	19.0	16.3	15.6	53	61	64
	^{5}T-^{11}A	21.0	18.4	19.0	48	54	53
	^{6}G-^{10}C	31.0	32.7	44.6	32	31	22
	^{7}G-^{9}A	†	†	†	†	†	†
Pzn-mismatched duplex (**4**)	^{1}T-^{15}A	#	#	#	#	#	#
	^{2}G-^{14}C	22.6	18.0	14.0*	44	56	71*
	^{3}T-^{13}A	2.0	2.2	2.2	500	455	455
	^{4}T-^{12}A	2.0	2.2	2.2	500	455	455
	^{5}T-^{11}A	1.0*	2.6	2.4	1000*	384	417
	^{6}G-^{10}C	30.0	23.4	15.7*	33	43	64*
	^{7}G-^{9}A	†	†	†	†	†	†

the exchange process is very rapid which was evident by the absence of the diagonal peaks.
¥ no cross-peaks have been observed in the NOESY and ROESY spectra because of the slow exchange of imino protons.
† the imino proton of the ^{7}G-^{9}A mismatched base-pair are not involved in the imino-type hydrogen bonding (see ref. 5).
* It can be seen from fig. 2 that the approximations in eqn. 1 are not valid at this mixing time for this particular imino proton.

The correct range of mixing times τ^m, where the approximations[12,14] in eqn. 1 is valid, has been chosen by inspection of the NOESY and ROESY buildup curves of the cross-peaks between imino protons and water in duplexes (**1**)–(**4**) (Fig. 2). These curves show that it is not possible to choose a common mixing time for all imino protons in a short oligonucleotide duplex. For example, the choice of a short mixing time of $\leq$20 ms, would produce misleading results for the exchange rate of imino protons in the core part of the stable duplexes (**1**) and (**2**) where the process of exchange is very slow but in a rapidly exchanging imino protons of terminal residue, a mixing time of $\sim$15 ms is most probably correct. Table 1 shows the calculation of the rate of exchange (k_{ex}) and the life-time ($\tau = 1/k_{ex}$) for imino-protons in duplexes (**1**)–(**4**) from eqn. 1 at τ_m = 20, 40 and 60 ms. The results of these experiments in Table 1 have been assessed ($\sigma\tau_m < 1$) in conjunction with the NOESY and ROESY buildup curves in Fig. 2 to check the validity of eqn. 1.

Stabilties of the imino-protons in duplexes (I)–(IV)

Comparison of the exchange rates and life-times of hydrogen-bonded imino-protons (^{7}G-^{9}C base pair) in duplex (**1**) and Pzn-tethered duplex (**2**) show (k_{ex} and τ in Table 1) that for the former the exchange process has been found to be quite rapid (τ < 5 ms) while the introduction of the Pzn-tether in the latter enhances its life-time to 120 ± 10 ms. A similar comparison between ^{6}G-^{10}C, ^{5}T-^{11}A and ^{3}T-^{13}A show that the exchange rate of the imino protons become much slower in Pzn-tethered duplex (**2**). Note that the core imino proton of ^{4}T-^{12}A in duplex (**2**) becomes so stable that no exchange process is observable at all compared to (**1**). Our previous NMR and molecular dynamics studies[5] have shown that Pzn moiety is stacked on ^{7}G-^{9}C base-pair in the Pzn-tethered duplex (**2**) which along with the present study indicates that the stabilisation of ^{7}G-^{9}C and upstream base-pairs are partly due to intramolecular stacking interactions. One other surprising observation is that the imino proton of ^{1}T-^{15}A in duplex (**1**) which exchanges very quickly is considerably stabilised in duplex (**2**) by tethered Pzn group. This is presumably due to intermolecular head-to-tail stacking interaction. A comparison of mismatched duplex (**3**) and its Pzn-tethered analogue (**4**) show that the mismatched ^{7}G-^{9}A base-pair adjacent to Pzn residue is not at all stabilised which is consistent with our NMR and molecular dynamics studies[5] which shows that Pzn is not intramolecularly stacked with ^{7}G-^{9}A base-pair. A further comparison between (**3**) and (**4**) show most surprisingly that the core imino protons (^{5}T-^{11}A, ^{4}T-^{12}A and ^{3}T-^{13}A) becomes $\sim$8 times more stable in duplex (**4**) which has also shown much less water activity (Fig. 3) with the non-exchangeable aromatic protons in the minor groove *(vide infra)*. It is likely that this reduction of water activity inside the core of (**4**) is related to the

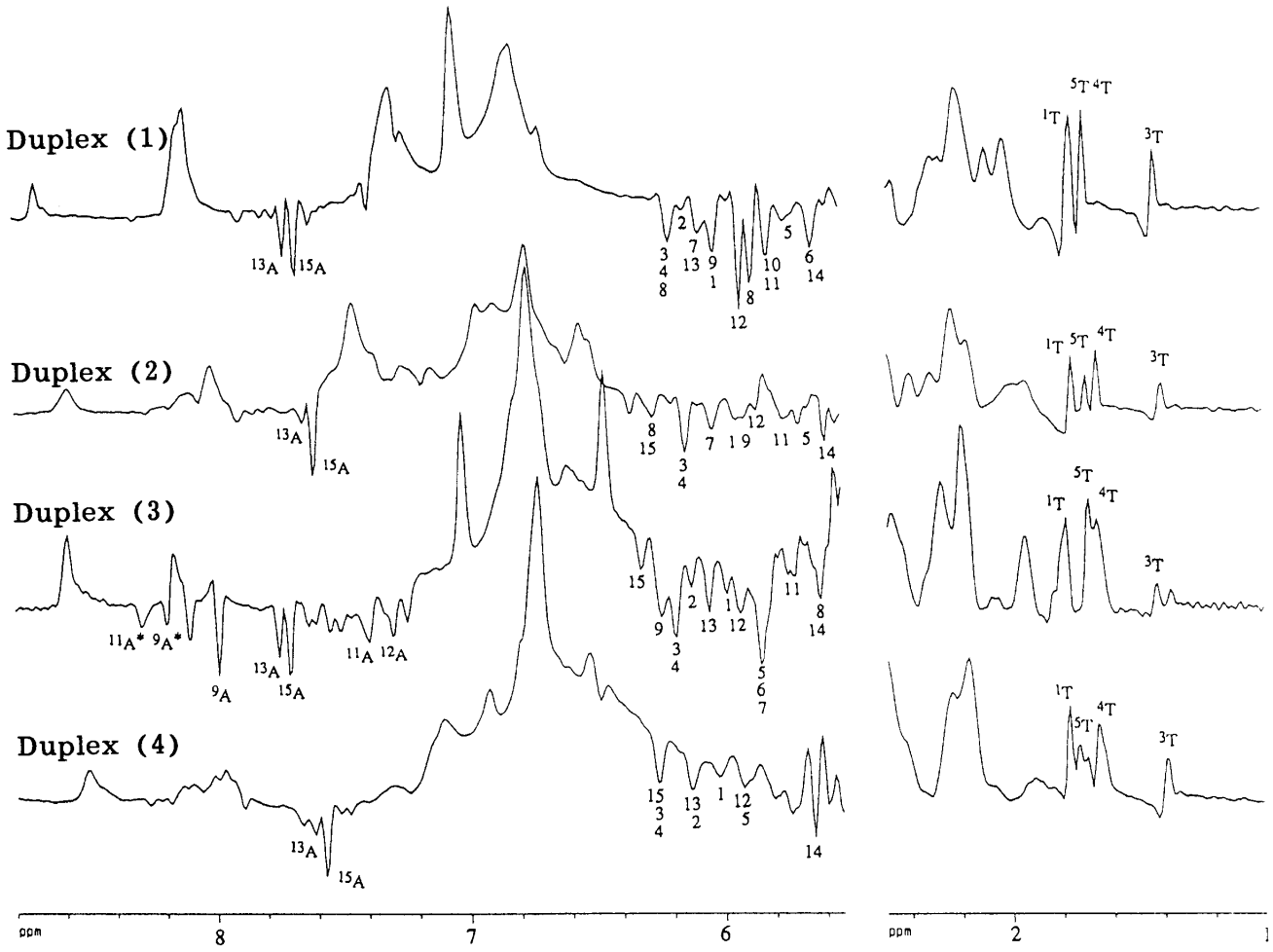

Figure 3. Proton NMR spectra showing NOEs through the cross section of the ROESY[11] spectrum along the f_2 (δ9.0−5.5 ppm and δ2.5−1.0 ppm) at the f_1 frequency of the water line with the spinlock duration of 0.06 s for duplexes (**1**)−(**4**). The spectra were baseline-corrected in both dimensions using polynomials and also corrected for the excitation profile, $\sin^3[2\pi\tau\omega(\delta-\delta_{off})]$[11], along the ω_2 frequency axis, where τ is the delay (187 μs), ω is the frequency of spectrometer (500.13 MHz), δ is the chemical shift in ppm, δ_{off} is the chemical shift of the carrier frequency on water. The upfield region from 2.5−1.0 ppm was inverted for improved readibility. Resonance assignments for the H1′ protons in δ 6.5−5.5 ppm region are indicated by the arabic numbers which are identical with the nucleotide number in the DNA sequence (**1**)−(**4**). Resonance assignments[5] for the aromatic H2 adenine protons (in δ 9.0−7.0 ppm region) and methyl thymine protons (in δ 2.0−1.0 ppm region) are indicated by the nucleotide number and base sequence whereas the aromatic H8 adenine protons are additionally labelled by symbol (*). See ref. 14 for the assignments of the signs of the cross-peaks and ref. 18 for the control experiments supporting the direct magnetization transfer between H2A and bound water.

hydrophobic character of Pzn residue which repels water from the polyelectrolyte DNA. This means that Pzn residue must also alter the nature of water in the first spine of hydration around the duplex.

Hydration pattern in the minor and major grooves of duplexes (I)−(IV)

It has been shown that the time frame of the exchange process of imino protons of DNA duplex and water takes place in a millisecond scale. There are however two types of water that have found to be interacting with DNA[8,9]: water that exchanges with the bulk solvent at a slower rate than the molecular tumbling correlation time scale (a few picoseconds) giving negative NOE, secondary fast exchanging water on the correlation time scale giving positive NOE[8,14]. Slow exchanging water on the NMR time scale has however the same chemical shift as the bulk water. The presence of relatively more immobilized water which exchanges only rather slowly with bulk water has been investigated by examining the cross section of the ROESY spectrum through the chemical shift of water resonance[8,9]. In duplex (**1**), the NOE contacts (*i.e.* first spine of hydration is within <5 Å) in the minor groove between water and ^{13}A(H2), ^{15}A(H2)[18] and with the methyl protons (^{3}T, ^{4}T and ^{5}T) in the major groove have been observed (Fig. 3). Additionally, cross-peaks between water and all anomeric protons have also been observed (Fig. 3). A qualitative comparison in Fig. 3 shows that the presence of the Pzn-tether in duplex (**2**) or (**4**), compared to their non-tethered counterparts (**1**) and (**3**), respectively, show a *much reduced water activity* in the proximity of H2 proton of adenine residues, methyl protons of ^{3}T, ^{4}T and ^{5}T and all anomeric protons[18]. A comparison of the hydration in matched duplex (**1**) (T_m = 30°C) with that of mismatched duplex (**3**) (T_m = 25°C) in Fig. 3 clearly shows that there is more water activity in the latter than in the former, which together with the T_m values, suggests that water, in general, contributes to the destabilization of the DNA duplex. Furthermore, it has been

found that the relative hydration in the major and minor grooves of matched duplex (**1**) (T_m = 30°C) is much larger than in matched Pzn-tethered duplex (**2**) (T_m = 50°C), which when compared with their corresponding T_m suggests that the increase of water activity has a negative effect on the stabilisation of the DNA duplex. In the mismatched duplex (**3**), all H2 protons of adenine moieties, both in the core and terminal parts, show strong NOE cross-peaks with water[18] suggesting that both the terminal and the core part are almost equally hydrated which is reminiscent of reported ribbons of water hydrating the ends of various B-form helices in crystal structures[6a-e]. On the other hand, in matched duplex (**1**), it is only the residues in the core-part [*i.e.* ^{13}A(H2) and ^{15}A(H2) which are located at the opposite end of the tether] that are hydrated. Note that the NOE of water with ^{15}A(H2) is stronger than with ^{13}A(H2) because of the location of the latter in the core part of the duplex. The fact that H2 protons of adenine show strong cross relaxations[18] with water strongly suggest that water is bound at the basic N3 nitrogen (pKa ~4.1) of adenine moiety which is consistent with the recent X-ray study[6b]. It is noteworthy that the Pzn tether in both matched (**2**) and mismatched (**4**) duplex reduces the level of hydration quite comparably in the minor groove compared to their non-tethered counterparts. The core ^{13}A(H2) in the Pzn-tethered matched duplex (**2**) shows a reduced level of hydration compared to the non-tethered counterpart (**1**) which means that the minor groove in (**2**) has a relatively poorer water activity because of the Pzn tether (Fig. 3). In both Pzn-tethered matched and mismatched duplex, the only proton which shows persistent hydration is ^{15}A(H2) which is far away from the Pzn residue, its intensity is not reduced in comparison with the non-tethered counterparts (although the life-time of imino proton of ^{1}T-^{15}A was found to increase in duplex (**2**)). The signs of cross-peaks[14] for nonexchangeable proton and water in the ROESY spectra (Fig. 3) compared with NOESY spectra (data not presented) show the relative rate of exchange of water molecules in first spine of hydration with the bulk solvent on the molecular tumbling correlation time scale[8,9]. The negative cross-peaks[14,18] in the ROESY spectra (and positive cross-peaks in the NOESY spectra) of aromatic protons in the minor groove (^{9}A, ^{11}A, ^{12}A, ^{13}A, ^{15}A) and anomeric protons with water show that the exchange rate is slow (a few nanoseconds) on the molecular tumbling correlation time scale[8,9]. The negative cross-peaks[14,18] in the ROESY and the NOESY spectra of methyl protons of ^{3}T, ^{4}T and ^{5}T with water in the major groove show that the exchange rates are fast with respect to the correlation time scale of DNA[8,9]. The presence of NOE with methyl protons of thymine residues and water suggests that water binds more strongly to the O4 than to N3/O2 of thymine residue which is also consistent with the recent X-ray study[6b]. The Pzn-tethered matched and mismatched duplexes and their non-tethered counterparts can be divided *on the basis of water activity in the major groove* in the following manner: mismatched-duplex (T_m = 25°C) > matched-duplex (T_m = 30°C) ≅ Pzn-mismatched-duplex (T_m = 34°C) > Pzn-matched-duplex (T_m = 50°C). *A comparison of the relative NOE intensities of both the aromatic and methyl protons (vide supra) with water in the set of four duplexes (1)–(4) suggest that it is only the least stable mismatched duplex (3) (T_m = 25°C) which has a continuous spine of hydration and ribbon structures through the core part and the terminals, which have been earlier implicated in stabilising the B-form of DNA[6a,7]. It can also be concluded that as the stability (T_m) of DNA duplex increases, the exchange-rate of the imino-protons decrease considerably, the life-time increases (Table 1) and the water activity in the minor groove decreases (Fig. 3).* These data for the first time show that the intrinsic stabilisation of DNA duplex depends both upon the economy of water activity, which dictate the strength of base-pairs, and the intramolecular base stacking interactions.

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REFERENCES

1. *Oligonucleotides and their analogues,* Ed. Eckstein, F, IRL Press, (1991).
2. Asseline, U., Toulme, F., Thuong. N.T., Delarue, M., Montenay-Garestier, T. and Hélene, C. (1984) *The EMBO Journal* **3,** 795–800.
3. Sun, J.-S., Francois, J.-C., Montenay-Garestier, T., Saison-Behmoaras, T., Roig, V., Thuong, N. T. and Hélène, C. (1989) *Proc. Natl. Acad. Sci. USA.* **86,** 9198–9202.
4. Zarytova, V.F., Kutyavin, I.V., Sil'nikov, V.N. and Shishkin, G.V. (1986) *Soviet journal of Bioorganic chemistry* **12,** 469–477.
5. Maltseva, T.V., Sandström, A., Ivanova, E.M., Sergeyev, D.S., Zarytova, V.F. and Chattopadhyaya, J. (1993) *J. Biochem. Biophis. Methods* **26,** 173–236.
6. (a) Saenger, W. *Principles of Nucleic Acid Structure*, Chapter 17, Springer-Verlag, Berlin, 1988, and references therein. (b) Narayana, N., Ginell, S.L., Russu, I.M. and Berman, H.M. (1991) *Biochemistry* **30,** 4449–4455. (c) Xuan, J.-C. and Weber, I.T. (1992) *Nucl. Acid. Res.* **20,** 5457–5464. (d) Edwards, K.J., Brown, D.G., Spink, N., Skelly, J.V. and Neidle, S. (1992) *J. Mol. Biol.* **226,** 1161–1173. (e) Yuan, H., Quintana, J and Dickersion, R.E. (1992) *Biochemistry* **31,** 8009–8021.
7. Berman, H.M. (1991) *Curr. Opin. strut. Bio.* **1,** 423–427.
8. Kubinec, M.G. and Wemmer, D.E. (1992) *J. Am. Chem. Soc.* **114,** 8739–8740.
9. Liepinsh, E., Otting, G. and Wüthrich, K. (1992) *Nucl. Acid. Res.* **20,** 6549–6553.
10. (a) Otting, G., Liepinsh, E. and Wuthrich, K. (1991) *Science* **254,** 974–980. (b) Otting, G. and Wüthrich, K. (1989) *J. Am. Chem. Soc.* **111,** 1871–1875. (c) Otting, G., Liepinsh, E., Farmer II*, B.T. and Wüthrich, K. (1991) *Journal of Biomolecular NMR* **1,** 209–215.
11. (a) Bax, A., Sklenár, V., Clore G.M. and Gronenborn A.M. (1987) *J. Am. Chem. Soc.* **1109** , 6511–6513. (b) Sklenár V. and Bax, A. (1987) *J. Magn. Reson.* **74,** 469–479.
12. Maltseva, T.V, Yamakage, S.-I, Agback, P. and Chattopadhyaya, J. *Nucleic Acid Res.* (submitted).
13. (a) Patel, D.J., Pardi, A. and Itakura, K. (1982) *Science* **216,** 581–590. (b) Leroy, J. L., Kochoyan. M., Huynh-Dinh. T. and Gueron. M. (1988) *J. Mol. Biol.* **200,** 223–238. (c) Johnston, P.D. and Redfield, A.G. (**1981**) *Biochemistry* **20,** 3996–4006. (d) Leijon, M. and Gräslund. A. (1992) *Nucl. Acid. Res.* **20,** 5339–5343. (e) Moe. J.G. and Russu. I.M. (1992) *Biochemistry* **31,** 8421–8428. (g) Lycksell, P.-O., Gräslund, A., Claesens, F., McLaughlin, L.W., Larsson, U. and Rigler, R. (1987) *Nucl. Acid. Res.* **15,** 9011–9025. (h) van de Ven, F.J.M., Janssen, H.G.J.M., Gräslund, A. and Hilbers, C.W. (1988) *J. Magn. Reson.* **79,** 221–235.
14. All spectra were corrected for the excitation profile of the particular NOESY or ROESY experiments, which means that the diagonal peaks are always positive. The distinction between different possible mechanisms of magnetization transfer has been based upon the comparison of NOESY and ROESY spectra in the same manner as prescribed in the literature protocol[8,10a,b,13h,15a,17] of ROESY and NOESY experiments. In the ROESY spectra chemical exchange and spin diffusion pathways shows positive cross peak (same as the diagonal), direct[10b] magnetization transfer (*i.e.* dipole-dipole relaxation) however shows negative cross peaks (*i.e* the σ^{roe} rate is always positive). In the NOESY spectra all of these cross peaks have positive signs for the macromolecule with $\omega\tau_c >> 1.12$ (*i.e.* the σ^{noe} rate is negative). For our DNA duplexes, τ_c is ~2 ns[5]. The only exception to this general observation in the NOESY experiment is when magnetization transfer through dipole-dipole interaction between non-exchangeable proton and water

is modulated by translation diffusion[10b,13h] when the cross-peaks have negative sign. Unfortunately, the simple rule for the determination of the type of magnetization transfer does not work if the transfer of magnetization is relayed[17]. The cross peaks arising from a ROE transfer followed by chemical exchange will have the same negative sign as a cross peak arising from a direct ROE transfer[17].

15. (a) Fejzo, J., Zolnai, Z., Macura, S. and Markley, J. L. (1989) *J. Magn. Reson.* **82,** 518–528. (b) Macura, S. and Ernst, R.R. (1980) *Molecular Physics.* **41,** 95–117.
16. Farmer II, B.T., Macura, S. and Brown, L.R. (1988) *J. Magn. Reson.* **80,** 1–22.
17. Farmer II, B.T., Macura, S. and Brown, L.R. (1987) *J. Magn. Reson.* **72,** 347–352.
18. Following control studies have been performed which supports our conclusion that the cross-peaks between H2A and water in Fig. 3 is indeed due to H2A and bound water which is also consistent with Wemmer's original observations[8]. To assign the direct ROEs between H2A and bound-water with effective $\tau_c > 1$ns[9,10a,b] (slow water molecules regime[9]) from the relay transfer, one should have a condition in which the imino proton should not be rapidly exchanging with the bulk water[9]. *This has been assessed in the following manner:* (1) Upon the reduction of the temperature the exchange rate of the imino protons with bulk water decreases. We have thus compared the ROESY spectra of all four duplexes at 0° (when the exchange of imino protons with the bulk water is almost negligible or very slow) and 15°C, and found no qualitative difference in the cross-peak volumes between H2A and water protons in any of the four duplexes. (2) The relay process can also be identifed performing build up curves from ROESY experiments[17]. We have therefore performed ROESY experiments at 200 ms and compared with those of 60 ms and 112 ms, and found that the signs of the cross-peaks have remained unchanged. (3) We have also compared the intensity of H2A-water cross-peaks with H1′-water cross peaks (see Fig. 3) since there is no pathway for exchange transfered NOEs from H1′ protons to water[8]. This comparison showed that they are either equal or only slightly different. All the above three lines of observations suggest that we indeed observe the direct dipole-dipole interaction between H2A and bound water.
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