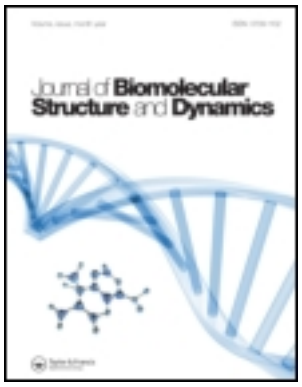

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The Residence Time of the Bound Water in the Hydrophobic Minor Groove of the Carbocyclic-Nucleoside Analogs of Dickerson-Drew Dodecamers

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Summary

The residence time of the bound water molecules in the antisense oligodeoxyribonucleotides containing 7′-α-methyl (T_{Me}) . carbocyclic thymidines in duplex (I), $d^5'(^1C^2G^3C^4G^5A^6A^7T_{Me}{}^8T_{Me}{}^9C^{10}G^{11}C^{12}G)_2{}^3'$, and 6′-α-hydroxy ($T_{OH}$) carbocyclic thymidines in duplex (II), $d^5'(^1C^2G^3C^4G^5A_{OH}{}^6A_{OH}{}^7T_{OH}{}^8\ T_{OH}{}^9C^{10}G^{11}C^{12}G)_2{}^3$, have been investigated using a combination of NOESY and ROESY experiments. Because of the presence of 7′-α-methyl groups of T_{Me} in the centre of the minor groove in duplex (I), the residence time of the bound water molecule is shorter than 0.3 ns. The dramatic reduction of the residence time of the water molecule in the minor groove in duplex (I) compared with the natural counterpart has been attributed to the replacement of second shell of hydration and disruption of hydrogen-bonding with O4′ in the minor groove by hydrophobic α-methyl groups, as originally observed in the X-ray study. This effect could not be attributed to the change of the width of the minor groove because a comparative NMR study of the duplex (I) and its natural counterpart showed that the widths of their minor grooves are more or less unchanged (r.m.s.d change in the core part is <0.63Å). For duplex (II) with polar 6′-α-hydroxyl groups pointed to the minor groove, the correlation time is much longer than 0.36ns as a result of the stabilising hydrogen-bonding interaction with N3 or O2 of the neighbouring nucleotides.

Introduction

DNA hydration study has attracted much interest both from X-ray (1-4) and NMR spectroscopists (5-10). Most of these investigations have been performed using self-complementary Dickerson-Drew dodecamer (3,11), $d(CGCGAATTCGCG)_2$. The X-ray (2,3,12) and NMR (13-15) studies indicate that Dickerson-Drew dodecamer adopts a B-type form with narrowing of the minor groove (4-5Å) (3,12) at its centre, and shows specific DNA-water interactions in the major and minor grooves (2,3,10,12,16,17). The width of the minor groove in B-DNA has been correlated with the order, number and the residence time of water molecules (7-9). Very little direct experimental evidence is however available by NMR regarding the nature of interaction that are responsible for the hydration of the DNA duplex compared to oligo-RNA (18,19) where hydrogen-bonding of water molecule with 2′-OH seems to be the dominating factor stabilising the spine of hydration.

To the best of our knowledge, no information is hithertofore available on the nature of hydration of the modified oligo-DNAs, which are potentially useful as antisense or antigene compounds. In this regard, it is of considerable interest to correlate the nature of hydration in the carbocyclic-modified oligo-DNAs, as antisense or antigene compounds (18,20,21), with their ability (i) to exhibit greater resistance to the enzymatic cleavage of the glycosidic linkage, (ii) their improved resistance to cellular nucleases, and most importantly, (iii) why the introduction of 6′-α-methyl-2′-deoxy-carbocyclic or 6′-α-hydroxy-2′-hydroxy-carbocyclic thymidine residues reduces the melting point of the duplex from 0.1° to 1.9° per modification in a

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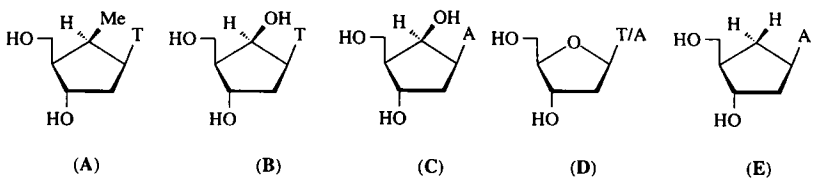

Figure 1: Structures of modified carbocyclic nucleosides (A, B, C and E) and natural counterpart (D). T = thymin-1-yl; A = adenine-9-yl.

sequence-dependent manner. Since both the X-ray crystal structure of two antisense DNA duplexes containing 6′-α-methyl- and 6′-α-hydroxy carbocyclic thymidines, duplex (I) $d^{5'}(^{1}C^{2}G^{3}C^{4}G^{5}A^{6}A^{7}T_{Me}{}^{8}T_{Me}{}^{9}C^{10}G^{11}C^{12}G)_2{}^{3'}$ and duplex (III) $^{5'}(^{1}C^{2}G^{3}C^{4}G^{5}A^{6}A^{7}T_{OH}{}^{8}T_{OH}{}^{9}C^{10}G^{11}C^{12}G)_2{}^{3'}$, as well as the NMR structure of the duplex (I) are now known, we argued that we have a good case in our hand to study if there is any correlation of anyone of the above properties of the carbocyclic-modified oligo-DNAs with their structure and the hydration pattern. We also argued that this study is additionally interesting because of the absence of O4′-oxygen in the carbocyclic moieties which makes them lipophilic as well as devoid of stereoelectronic anomeric and gauche effects (22) making them more flexible than the furanose counterpart, and thereby allowing the modified oligo-DNA to adjust smoothly to the geometrical constraints exerted by the phosphate backbones to mimic the structure of the B-form DNA of the natural counterpart.

The X-ray structures of antisense duplex (I) and (III) (20) show the following characteristic features: (i) They are isomorphous to the native Drew-Dickerson dodecamer (3), and they only have relatively small geometrical deviations. (ii) Nevertheless, the hydration patterns of the minor grooves of the two carbocyclic oligonucleotides are significantly different from their natural counterpart as a result of changing polarity in the minor grooves of duplexes (I) and (III). (iii) A comparison of the duplex (I) with the native counterpart shows that two second-shell water molecules (w33 and w35) are replaced by the four methyl groups in the former (Figure 2). This leads to the isolation of the first shell water molecule (w67) from the spine of hydration (20). It has been shown that this water molecule still has the hydrogen bonds with oxygens O2 of ^{7}T and ^{19}T residues. (iv) In the duplex (III), there are hydrogen bonds between the 6′-α-hydroxyl group of the carbocyclic thymidines and N3 or O2 atoms of 3′-adjacent A or T, respectively, in the central portion of the minor groove. Interestingly, the polar 6′-α-hydroxyl substituent in the duplex (III) distorts the minor groove hydration more severely than the nonpolar 7′-α-methyl group in the duplex (I). However, this is compensated by the 6′-hydroxyl group acting as the hydrogen-bonding acceptor for additional water molecules at the groove periphery. This additional hydrogen bond has been used to explain the higher thermodynamic stability of the duplex (III) than duplex (I).

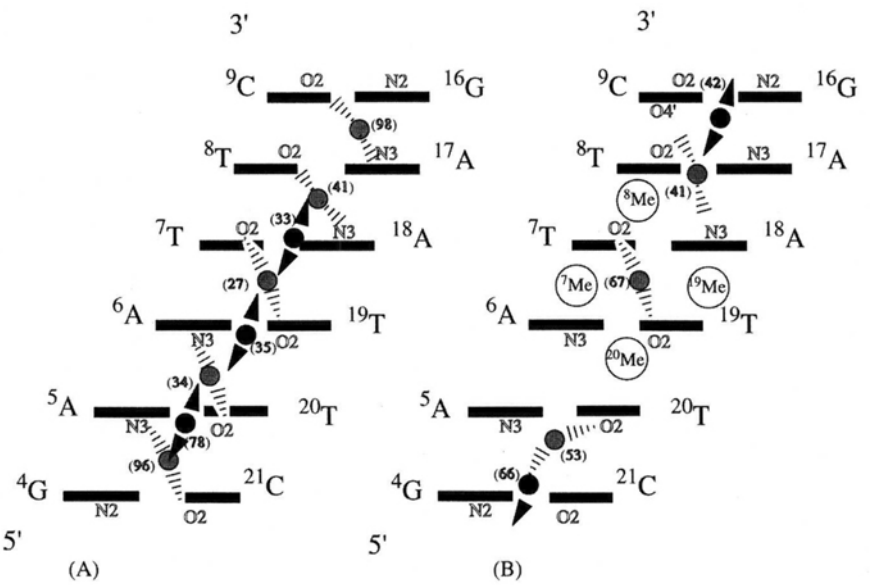

Figure 2: Schematic representation of the minor groove and its hydration in the natural Dickerson-Drew dodecamer in (A) and duplex (I), $d^{5'}(^{1}C^{2}G^{3}C^{4}G^{5}A^{6}A^{7}T_{Me}{}^{8}T_{Me}{}^{9}C^{10}G^{11}C^{12}G)_2{}^{3'}$ in (B). The numbering of water molecules has been used in a way similar to the one reported by (20) in their X-ray study.

In this work, we provide evidence through the use of the carbocyclic analogue of Dickerson-Drew dodecamer (I) that the favourable hydrogen-bonding interactions found in the first shell of hydration in the natural counterpart, d(CGCGAATTCGCG)$_2$ (3,20) are one of the main factors that are responsible for longer residence time of the bound water (~0.6 ns at 10°C) (23). This work also shows that the residence time of the bound water molecule near the 7′-α-methyl (T_{Me}) carbocyclic thymidines (Figure 1A) in the minor groove of duplex (I) is less than 0.36 ns because of the hydrophobicity of T_{Me} moieties (the oil effect), whereas in relatively more polar duplex (II), $d^{5'}(^1C^2G^3C^4G^5A_{OH}{}^6A_{OH}{}^7T_{OH}{}^8T_{OH}{}^9C^{10}G^{11}C^{12}G)_2{}^{3'}$, containing polar 6′-α-hydroxyl (T_{OH} and A_{OH}) blocks (Figures 1B and 1C), the correlation time of this hydroxyl group is much longer than 0.36 ns, which is very much similar to the native 12-mer DNA duplex (7,10) as well as to those found near H1′ in an oligo-RNA: r(CDCAAAUUUGCG)$_2$ (19).

Both NMR dispersion studies with ^2H and ^{17}O (23-25) as well as combination of NOESY and ROESY (26) experiments have been developed to assess the residence times of water in protein (27-31) and DNA or RNA (7,9,10,23,24). One essential assumption for the NMR dispersion (NMRD) study is the averaging of the correlation time (τ_c) of long-lived water molecules (23). To extract a single correlation time, a water molecule is displaced by chemical modification or mutation, and the correlation time is a function of the difference in the dispersion profiles of the parent and the modified compound (23-25). The information on residence time of bound water molecule from both the NMRD and the combination of NOESY and ROESY studies have been found to complement each other. In this communication, we have used a combination of NOESY and ROESY experiments as our initial attempt to study the residence time of water molecules in duplexes (I) and (II) because of the following reasons: (i) The use of 7′-α-methyl groups for the measurement (see Material and Method) of the relative residence time of the bound water in duplex duplex (I) allows us to disperse the water-DNA cross-peak at the chemical shift of water to estimate the individual correlation times based on both methyl groups as well as on H2A's. (ii) The 7′-α-methyl groups are pointed to the minor groove in duplex (I) just as the H2 proton of an adenine (A) moiety, and hence provide an independent marker. (iii) The 7′-α-methyl groups in duplex (I) are far away (>5.2Å) from the neighbouring labile exchangeable protons, hence they can be more confidently used for assessment of straight dipole-dipole interaction between bound-water and DNA-proton compared to NOEs from exchange relayed NOEs with labile DNA protons. We have also measured the residence times of the bound-water molecules in the minor grooves of both duplexes (I) and (II) by using H2A proton for the sake of comparison, although they are shown to be affected (32) by the two-step relay process via the neighbouring exchangeable protons under certain conditions, and hence relatively less reliable.

In analogy with the hydration pattern found in the X-ray structure of duplex (III), we herein conclude that the longer residence time of the water molecule in the minor groove in the duplex (II) suggests that they are potentially capable of forming the hydrogen bonds with the neighbouring N3 or O2 atoms, and can contribute in the thermodynamic stabilisation of the duplex II more than the duplex I.

The above conclusions are based on several unexpected interesting features that have been observed for the cross-peaks at the water chemical shift in the NOESY and ROESY spectra of duplexes (I) and (II) (Figure 3,4).

Results and Discussion

The NMR conformation of the carbocyclic analog of the Dickerson-Drew dodecamer [d(CGCGAAT*T*CGCG)]$_2$ containing 6′-α-Me carbocyclic thymidines (T*) has been determined by us (enclosed manuscript) and compared with that of

its X-ray structure. The solution structure of the 6′-α-Me carbocyclic thymidine modified duplex has also been compared with the solution structure of the corresponding unmodified Dickerson-Drew duplex solved by us under the same experimental conditions.

The assignment of all protons for duplex (II) has been also performed in a conventional manner, using NOESY and COSY-DQF experiments, and the data will be published elsewhere together with its full structural analysis.

(1) Water- 7′-α-methyl Contact of T_{Me} in Duplex (I)

In both NOESY and ROESY spectra of duplex (I) at 10°C [Figures 3(Bii) and 3(Biv)] the cross-peaks between bound water molecule and 7′-α-methyl of T_{Me} are negative with respect to the diagonal peaks, indicating that $\sigma_{nOe}/\sigma_{rOe} > 0$ (see Figure 5 for the phase distortion problem, and Material and Methods). This unambiguously indicates that the residence time of the bound water molecule located near 7′-α-methyl of T_{Me} groups is less then 0.36 ns (27,28,31). It is noteworthy that this is a well known behaviour (7b,10,33) for the water located in the major groove of native DNA in proximity with methyl groups of thymidine moieties (Me-T). Indeed, the cross-peaks corresponding to water-(Me-T) are negative for both duplexes (I) and (II) [Figure 3(Aii), 3(Aiv) and 3(Bii), 3(Biv)].

(2) Water-H2A Contact

(i) Duplex (I)

For duplex (I) the cross-peaks between water and H2A protons have vanished to zero in the NOESY spectrum, and they are negative in the ROESY spectrum for all temperatures between 5 and 20°C (Figures 3 and 4). These cross-peaks are interpreted as direct intermolecular nOe/rOe (see below) with a short residence time of ~ 0.36 ns for the bound water. However, a more detailed survey of the NOESY/ROESY spectra at 20°C of the water-2H^{5}A and water-2H^{6}A cross-peaks [Figure 4(Bi) and 4(Biii)] shows some minor differences: the intensity of the former cross-peak has slightly increased with increasing temperature and has a negative sign, showing that the hydration pattern is indeed different for ^{5}A and ^{6}A.

(ii) Duplex (II)

A completely different picture has been observed for duplex (II) with 6-α-hydrox-

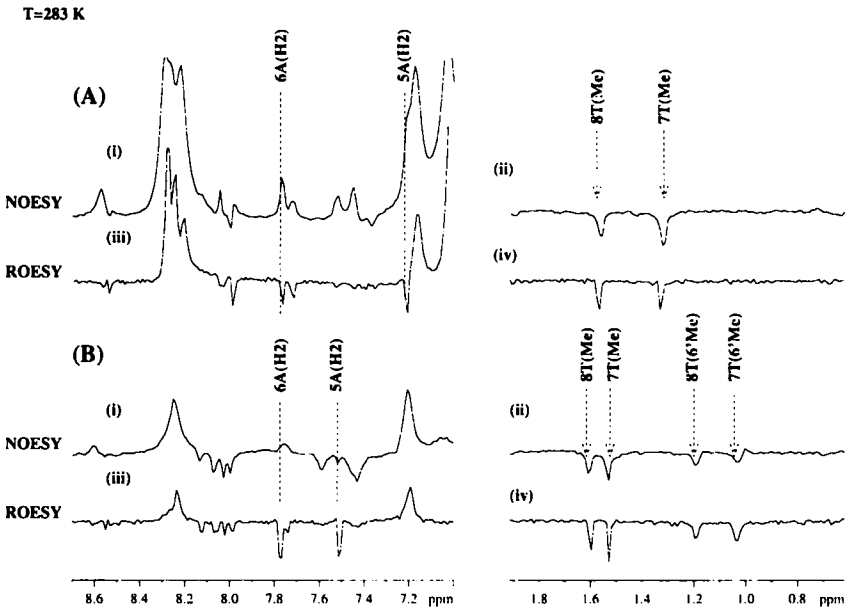

Figure 3: Comparison (at 10°C) of the cross-section through the NOESY [panels: A(i), A(ii), B(i) and B(ii)] and ROESY [panels: A(iii), A(iv), B(iii) and B(iv)] spectra of (A) duplex II, $d^{5'}(^1C^2G^3C^4G^5A_{OH}{}^6A_{OH}{}^7T_{OH}{}^8T_{OH}{}^9C^{10}G^{11}C^{12}G)_2{}^{3'}$, containing polar 6′-α-hydroxyl carbocyclic thymidine (T_{OH}) blocks, and (B) duplex I, $d^{5'}(^1C^2G^3C^4G^5A^6A^7T_{Me}{}^8T_{Me}{}^9C^{10}G^{11}C^{12}G)_2{}^{3'}$, containing 7′-α-methyl (T_{Me}) carbocyclic thymidines, showing nOe/rOe projection of cross-peaks between protons of the DNA and water. The NOESY and ROESY spectra were recorded at 200 ms and 100 ms mixing times, respectively. The low field region [panels: (i) and (iii)] between 8.7-7.0 ppm and higher field region [panels: (ii) and (iv)] between 1.9-0.6 ppm shows the water-DNA nOe/rOe cross-peaks with H2A or methyl protons, respectively. The assignments of the DNA protons are given at the top of the panel.

yl groups compared to duplex (I) with 7′-α-methyl substituents: The cross-peaks on the water line from $2H^5A$ and $2H^6A$ are very intense and have positive signs in the NOESY (τ_m = 200 ms) [Figure 3(Ai), 4(Ai)] and negative signs in the ROESY (τ_m = 100 ms) spectrum [Figures 3(Aiii), and 4(Aiii)]. Their intensities are almost identical, which is a sign of long correlation times (27,28,31) as found for covalently bound non-exchangeable protons in DNA. The recent X-ray structure of duplex (III) has shown that the 6′-α-hydroxyl group of $^8T_{OH}$ is hydrogen-bonded to O2 of $^7T_{OH}$ and 6′-α-hydroxyl group of $^7T_{OH}$ is hydrogen-bonded to N3 of $^6A_{OH}$ in the "first hydration sphere", and that the $^7T_{OH}$ and $^8T_{OH}$ also act as hydrogen-bonding acceptors for an additional water molecule, bridging the 6′-α-hydroxyl group of $^{20}T_{OH}$ and $^{19}T_{OH}$ in the opposite strand. In our duplex (II), we have different environments for $2H^5A$ and $2H^6A$ with respect to the opposite strand. Indeed for $2H^6A$, the 6′-α-hydroxyl group of $^7T_{OH}$ has the potential to bridge with the 6′-α-hydroxyl group $^{20}T_{OH}$ through a water molecule in the same manner as for duplex (III). On the other hand, a 6′-α-hydroxyl group of $^6A_{OH}$ is present around $2H^5A$, but there is no 6′-α-hydroxyl group-containing nucleotide on the opposite strand or in close proximity. Despite this different water environment in the second shell of hydration, we find that relative intensities of NOESY and ROESY cross-peaks on the water line of $2H^5A$ and $2H^6A$ to be almost identical (see Figures 3 and 4). This makes it tempting to suggest that the cross-peaks from $2H^5A$ and $2H^6A$ on the water line are perhaps owing to the dipole-dipole interaction between covalently bound 6-α-hydroxyl group and H2As, which is considered by Portmann et al as the model of the covalently bound water in the minor groove. We can not however rule out the contribution of the exchange mechanism in the above process (30). The fact that we see the cross-peaks from $2H^5A$ and $2H^6A$ on the water line allows us to put an upper limit for the residence time for any non-covalently bound water in the minor groove of any DNA duplex, which should be less than the correlation time of 6-α-hydroxyl groups or that of a non-exchangeable DNA protons (~6 ns). Hence, the ratio of intensities in NOESY and ROESY cross-peaks on the water line of $2H^5A$ and $2H^6A$ in duplex (II) have been used as a qualitative reference point for the estimation of the correlation time of the non-covalently bound water molecule in duplex (I) and in the natural counterpart.

The experimental data obtained in this work has shown that for duplex (I), the intensities of the water and 7′-α-methyl cross-peaks (Figures 3 and 4) are very similar. This indeed supports the previous X-ray study that the bound water molecule is located between four α-methyl groups involving $^7T_{Me}$, $^8T_{Me}$, $^{19}T_{Me}$ and $^{20}T_{Me}$ residues of both strands. The estimated distances [both from NMR (enclosed MS) and X-ray structure (20)], between water-oxygen (w67 in Figure 2) and carbon of

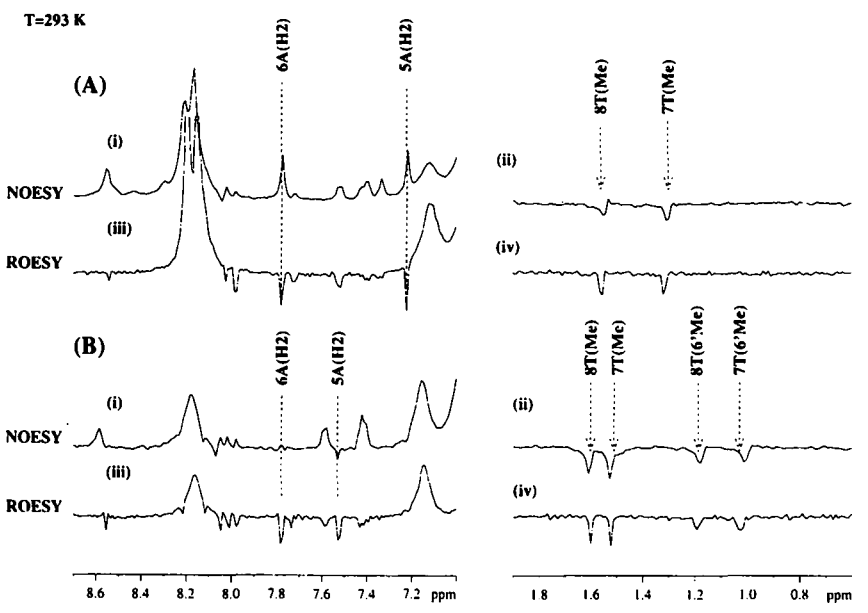

Figure 4: Comparison (at 20°C) of the cross-sections through the NOESY [panels: A(i), A(ii), B(i) and B(ii)] and ROESY [panels: A(iii), A(iv), B(iii) and B(iv)] spectra of duplex (II) in (A), and duplex (I) in (B). See Figure 2 for comparison and abbreviations at 10°C.

the 7′-α-methyl group of $^{7}T_{Me}$ are ~3.6Å. According to the X-ray data, the next water (w41) and the neighbouring 7′-α-methyl of $^{8}T_{Me}$ are more than 9Å away. This means that there is most probably one bound water molecule with a residence time of < 0.36 ns in the proximity of the 7′-α-methyl of $^{7}T_{Me}$, which is much shorter than the residence times of water (> 0.6 ns) (10°C) around ^{6}A /or ^{18}A, and ^{5}A /or ^{17}A in the natural dodecamer (10,23,25). For duplex (I), the residence time between water and the 7′-α-methyl of T_{Me} (0.36 ns) correlates well with those based on water-H2(^{6}A) results (0.3-0.5 ns). It is noteworthy that this residence time of water near H2(^{6}A) in duplex (I) is shorter than 0.6 ns for the same residue in the natural counterpart.

Our data has shown that the relative intensities in NOESY and ROESY for the water-H2(^{5}A) and water-H2(^{6}A) cross-peaks in duplexes (I) and (II) are indeed quite different. For duplex (II), they are similar to those observed in natural Dickerson-Drew duplex (10), where water molecules "live" longer than 0.36 ns. But for the duplex (I), they are more similar to the hydration pattern of the dodecamer with a central $(TTAA)_2$ position (7a) with a residence time of the water molecule of ~0.36 ns.

The occurrence of long-lived water molecules is believed (7a) to depend on the presence of a narrow minor groove. Our comparative NMR structural elucidation of duplex (I) and the natural Dickerson-Drew dodecamer duplex shows that their minor groove widths are similar, with an r.m.s.d of < 0.63Å. Additionally, it has been recently proposed (18) that the hydration kinetics in the r(CGCAAAUU-UGCG)$_2$ duplex may be dictated by hydrogen-bonding of water with 2′-OH groups than by the groove width. Moreover, the hydrogen bridging of bound water molecule with O2 atoms of $^{7}T_{Me}$ and $^{19}T_{Me}$ residues are found to be similar (20) (Figure 2) for duplex (I) and the natural Dickerson-Drew dodecamer duplex based on X-ray data. Additionally, in the X-ray structure of duplex (I), there are at least two water molecules present adjacent (~3.5Å) to H2(^{6}A) /or H2(^{18}A) (in Figure 2B, see

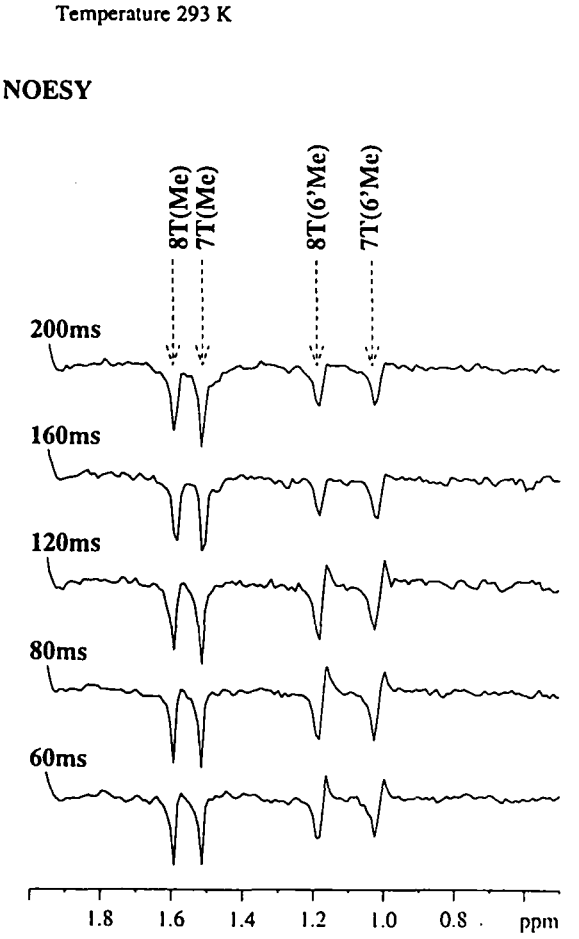

Figure 5: Comparison of the cross-sections through the NOESY spectra of duplex (I), d$^{5'}$(^{1}C^{2}G^{3}C^{4}G^{5}A^{6}A^{7}T$_{Me}$^{8}T$_{Me}$^{9}C^{10}G^{11}C^{12}G)$_2$$^{3'}$, containing 7′-α-methyl (T_{Me}) carbocyclic thymidines, taken along F2 at the F1 frequency of the water signal, showing nOe projection of cross-peaks between methyl and 7′-α-methyl protons of the DNA and water at different mixing times (marked at the left side of the panel) at 20°C. The assignments are shown at the top part of the panel.

w67 and w53 for 2H^{6}A and w67 and w41 for 2H^{18}A). In the neighbourhood of H2(^{5}A) /or H2(^{17}A), there are however four water molecules w53, w55 and w41, w84 (20). This hydration pattern is also quite similar to the natural Dickerson-Drew (3,20) dodecamer structure (Figure 2A). *This similarity of hydration patterns in the first shell of hydration found in the X-ray structure is actually in conflict with the much reduced residence time of water molecules found in the minor grooves of duplex (I) compared to the natural counterpart based on NMR.*

There are two possible explanations: (i) Since the NMR structure suggests that both the carbocyclic analogue and the natural 12-mer have almost the same minor groove width (r.m.s.d is < 0.63 Å), the replacement of the second shell of hydration is caused by the hydrophobic methyl groups of the carbocyclic nucleotide moieties, which, in turn, influence the life-time of the bound water molecule in the first shell of hydration, or, (ii) the water molecule in the proximity of the α-methyl of $^{7}T_{Me}$ has lost the extra hydrogen-bonded stabilisation with O4′ (34) in the sugar moiety because of its replacement by the hydrophobic α-methylcyclopentane moiety. It is also possible that both (i) and (ii) might contribute synergestically to the reduced residence time of water molecule in the minor groove in duplex (I). These suggestions are also supported by X-ray data (20) that the second shell of hydration in the centre of the modified duplex (I) is disorganised by the hydrophobic methyl groups compared with the natural counterpart (3). Moreover there was no hydrogen bond found between water molecule W67 and O4′ in duplex (I) compared with the natural counterpart (2,34). It appears unlikely that the bound water molecule can distinguish between structures with conformational variation of the minor groove smaller than 0.63Å as found between duplex (I) and its native counterpart.

The residence times of the water molecules located in the first shell of hydration around H2(^{6}A) /or H2(^{18}A) and H2(^{5}A) /or H2(^{17}A) protons are slightly longer (~0.36 ns) than for the water molecule located in the proximity of the 7′-α-methyl group of $^{7}T_{Me}$ (< 0.36 ns), which is consistent with X-ray data that the hydration pattern is restored (20) after ^{6}A/or ^{18}A residues in duplex (I). It is however not possible to rule out the absence of any relay effect contributing partially to the enhancement of crosspeaks of H2A's at the chemical shift of water, which could be one of the reasons also for the overestimation of the residence times of water molecule around H2A protons.

In contrary to duplex (I) with hydrophobic 7′-α-methyl group of T_{Me}, the correlation time of the 6′-α-hydroxyl groups in the minor groove in the duplex (II), with polar 6′-α-hydroxyl groups of A_{OH} or T_{OH} pointed to the minor groove, has been found to be much longer than 0.36 ns. Both the duplex (II) [T_m = 48.3°C at 1μM)] and the natural counterpart [T_m = 48.4°C at 1μM] have longer residence times of water molecules located near H2A, owing to their stabilisation by the hydrogen bonding with the 6′-α-hydroxyl group of A_{OH} or T_{OH} in duplex or with N3 and O2, which, in turn, culminate into their stronger thermodynamic stabilisation than duplex I [T_m = 44.1°C at 1μM] with T_{Me} groups.

The work is now in progress to elucidate the hydration pattern in an analogue of duplex (I) containing 2′-deoxyaristeromycin (Figure 1E), which will enable us to dissect and quantify the stereoelectronic (22) versus steric effects, and will also allow us to understand the importance of the hydrophobic effect in the design of antisense oligonucleotides.

Conclusion

The ability to chemically modify oligonucleotides provides a powerful tool in the design of the antisense and antigene therapy by controlling important properties such as their stability to cellular nucleases, stronger bonding to the target DNA or RNA as well as cellular penetration. Despite the fact that many modified oligonucleotides

have so far been prepared and their binding data to the opposite DNA or RNA strand have been reported, very little information is available regarding how the structure of these covalently modified duplexes change in aqueous solution vis-a-vis their natural counterpart, or what are the quantitative structural differences between the solution and the solid state structure of these modified duplexes. Our findings on the hydration of the carbocyclic- modified oligonucleotides are as follows:

(i) The residence time of the bound water molecule near the 7′-α-methyl (T_{Me}) carbocyclic thymidines in the minor groove of the self-complementary hydrophobic antisense oligodeoxyribonucleotides, *i.e.* duplex (I), is less than 0.36 ns because of the hydrophobic repulsion by T_{Me} groups (the oil effect) which induces the replacement of the second shell of hydration and the lose of the extra hydrogen-bonded of water molecule with O4′ in the sugar moiety caused by the hydrophobic methyl groups of the carbocyclic nucleotide.

(ii) In relatively more polar duplex (II), containing polar 6′-α-hydroxyl (T_{OH} and A_{OH}) blocks, the correlation time of this hydroxyl group is much longer than 0.36 ns because these hydroxyl groups are potentially capable of forming the hydrogen bonds with the neighbouring N3 or O2 atoms, and can contribute in the thermodynamic stabilisation of the duplex (II) more than the duplex (I).

Materials and Methods

(A) NMR Sample Preparation

The oligomers $d^{5'}(^{1}C^{2}G^{3}C^{4}G^{5}A^{6}A^{7}T_{Me}{}^{8}T_{Me}{}^{9}C^{10}G^{11}C^{12}G)_2{}^{3'}$ (duplex I) and $d^{5'}(^{1}C^{2}G^{3}C^{4}G^{5}A_{OH}{}^{6}A_{OH}{}^{7}T_{OH}{}^{8}T_{OH}{}^{9}C^{10}G^{11}C^{12}G)_2{}^{3'}$ (duplex II) were prepared as described before (20). Purified samples (~160 o.d units) were dissolved in 0.6 ml of the following buffer for NMR measurements: 100 mM NaCl, 10 mM NaH_2PO_4, 10μM EDTA, pH 7.0 in 90%H_2O:10%D_2O.

(B) NMR Experiment

^{1}H NMR spectra were recorded on a Bruker DRX 500 NMR spectrometer (^{1}H at 500 MHz). Phase-sensitive NOESY experiments with water suppression were achieved by the use of two short spinlock pulses, $SL_{\varphi 4}$ and $SL_{\varphi 5}$, as described by (26) using the following parameters: mixing times (τ_m) were varied between 0.03 and 0.200s to observe the spatial contact of the non-exchangeable protons with water; 4K complex data points in t_2, 512 complex data points in t_1, the relaxation delay between pulse sequence was 2.0s, $SL_{\varphi 4}$ and $SL_{\varphi 5}$ are equal to 0.5 ms and 3 ms, respectively, the delay between spinlock pulses τ is equal to 167μs, the carrier was set at the water frequency, 32 scans/FID were used for quadrature detection in F_1 - dimension with the time proportional phase incrementation (TPPI). 2D data sets for ROESY spectra with the water suppression are achieved with one short spinlock pulse, $SL_{\varphi 3}$ (26). During the mixing time sequence of n(π/6) pulses with length 3.4 μs separated by delay, Δ, (34.5μs) provides a similar effect as spin-lock $SL_{\varphi 4}$ of the NOESY experiment, so that the spectra were recorded with spinlock duration between 0.03 and 0.10s using 6.25 kHz rf field for all pulses and a recycle delay of 2s. Typically 4K data points were collected for each t_1 512 values during experiments. A 3 ms saturation pulse is applied after data acquisition. The spectral excitation profile in these experiment is proportional to sin(Ωt) where Ω is the angular frequency relative to the carrier and τ = 167 μs. The nonuniform spectral excitation in F2-dimension were corrected by multiplying with 1/sin(Ωt) function. The assignment of all protons of duplexes (I) and (II) were done in a conventional manner, using NOESY and COSY DQF experiments. They will be published elsewhere together with structural analysis.

The troubling difference between water-[7′-α-methyl of (T_{Me})] and water-(Me-T) cross-peaks is the phase behaviour in the NOESY spectra (Figure 5) at shorter mix-

ing time: water-[7′-a-methyl of (T_{Me})] cross-peaks were severely antiphase at short mixing times and became in-phase by increasing the mixing time to 200 ms. Simultaneously, water-(Me-T) cross-peaks remained inphase through all mixing times range. It is noteworthy that in ROESY experiment with correspondent mixing time, that phenomena was not observed (data not shown): all types of cross-peaks between the DNA and bound water were inphase. It should be also mentioned that in NOESY type experiment with a different approach of suppression of water through spin-locks (see experimental section and Figure 5) or WATERGATE sequence (data not shown) the effect persisted. Similar phenomena were observed earlier in the NOESY tritium spectra of the Dickerson-Drew dodecamer (17). The author pointed out the striking difference between tritium and homonuclear studies on bound water was because of the phase behaviour of the DNA-water cross-peak (17), A(^{3}H2)-water and, A(^{3}H8)-water at short mixing times (25-100ms). Possible sources of the phenomena have been discussed (17,35-37).

The ratio of NOESY and ROESY (ρ) cross-peak intensities was used to elucidate the correlation time, τ_c, (18,23,38). If the bound water molecule undergoes rapid / or large amplitude motion, the spectral density function becomes complex, and depends on the order parameter in the spectral density function (38). The theoretical evaluation of the transformation of correlation time from ratio/or sign of σ_{nOe} to σ_{rOe} is model-dependent (8,23). However, it has been shown (23) for the Dickerson-Drew dodecamer, using NMR dispersion (NMRD) and NOESY/ROESY methods, that there is a high degree of orientation order with little local motion during the residence time of water molecule in the minor groove. However, in major groove the local motion of water can be unrestricted. With this in mind, we have interpreted the ratio/or sign of σ_{nOe} to σ_{rOe} of water-DNA cross-peaks and correlation time in this work assuming the approximation of rigid binding for water in the minor groove for all three dodecamers (I), (II) and Dickerson-Drew, because of the width of the minor groove in the NMR spectra and the first-shell water position in the X-ray structure (duplex I) have remained unchanged. Hence, all through our evaluation of signs of crosspeaks of water-H2A or 7′-α-methyl (T_{Me}) of DNA in the NOESY and ROESY experiments, the following criteria were applied: The change of positive to negative nOe sign corresponds to the correlation time of ~0.36 ns (at 500 MHz) and the positive/or negative nOe sign are less/or more than 0.36 ns, respectively. Note, no comparison has however been used amongst the positive signs or the negative signs.

(C) Melting measurements. UV melting profiles were obtained by scanning A_{260} absorbency versus time at heating rate of 1°C/min and temperature gradient 20-80°C (60 min). The T_ms were calculated from the maximum points of the first derivatives of the melting curves. For thermodynamic calculations, an average of five T_m values were used at each concentration of 8, 12, 16, 20 and 24 μM. All measurements were carried out in 200 mM Na_2HPO_4/NaH_2PO_4, 1M NaCl buffer at pH 7.3. Before each melting experiment denaturation and renaturation of the samples were carried out by heating solutions to 80°C for 15 min followed by slow cooling to the RT and keeping it standing at RT overnight.

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