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Comparison of the conformation of an oligonucleotide containing a central G–T base pair with the non-mismatch sequence by proton NMR

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ABSTRACT

We have recorded NOESY spectra of two non-selfcomplementary undecanucleotide duplexes. From the observed NOEs we do not detect any significant distortion of the helix when a G-C pair is replaced by a G-T pair and the normal inter-residue connectivities can be followed through the mismatch site. We conclude that the 2D spectra of the non-exchangeable protons do not allow differentiation between a wobble or rare tautomer form for the mismatch. NOE measurements in H_2O, however, clearly show that the mismatch adopts a wobble structure and give information on the hydration in the minor groove for the G-T base pair which is embedded between two A-T base pairs in the sequence.

INTRODUCTION

DNA base pair mismatches can occur *in vivo* as a consequence of (a) replication errors, (b) heteroduplex formation in the course of genetic recombination between homologous, but not identical sequences, (c) deamination of 5-methylcytosine to thymine giving rise to a G-T mismatch (1), (d) incorporation of base analogues, like br^{5}U or fl^{5}U. Strand discrimination for the mismatch repair system in *E. Coli* is determined by adenine methylation in GATC sequences (2,3). While transition mismatches (G-T and A-C) in phage λ are generally well repaired by the *E. Coli* mismatch repair system (4-6) repair of the G-T mismatch is more efficient in a G-C rich region than in a A-T region (7). Repair occurs preferentially on the unmethylated strand of hemimethylated duplexes (2-5, 8).

The present paper compares the conformation of two non-selfcomplementary undecamers: the one contains a central G-C base pair and the other a central G-T base pair, with all other base pairs being identical. We report NOESY spectra on the two duplexes to investigate any possible conformational differences induced when C is replaced by T and 1D spectra in H_2O to identify the nature of the hydrogen bonding between G and T. The repair efficiency by the *E. Coli* mismatch repair system of the G-T mismatch in

this sequence, obtained from a mutation in the cI gene of phage λ, has been reported (7).

MATERIALS AND METHODS

The three undecamers used in this study were synthesized by the phosphotriester method (9,10). The central base pair corresponds to the sequence position 208 from the amino terminus of the wild-type or mutant cI gene product.

5′-A A A T T C T C A A A^{11}
^{22}T T T A A G A G T T T-5′ Duplex I

5′-A A A T T T T C A A A^{11}
^{22}T T T A A G A G T T T-5′ Duplex II

The duplexes were annealed by heating to 80°C followed by slow cooling. Both were in 10mM phosphate buffer (pH 7.4), 150 mM NaCl and 0.2 mM EDTA. For both duplexes the strand concentration was 4 mM. NMR spectra were recorded in either 99.996% D_2O or 90% H_2O/ 10% D_2O. Chemical shifts were measured relative to internal tetramethylammonium chloride (3.18 ppm).

NMR spectra at 500 MHz were recorded on a Bruker WM-500 spectrometer. 2D NOESY spectra were recorded in the phase sensitive mode (11) with 2K data points in the t_2 dimension and 128 acquisitions per spectrum. 256 free induction decays were collected in the t_1 dimension with a mixing time of 250 ms. The residual HDO resonance was weakly presatured during the relaxation delay. After zero filling to give a 2K x 2K matrix, a slightly shifted sine bell function was applied to the data in both dimensions prior to Fourier transformation for the Figures shown below. For the measurement of cross peak volumes a $\pi/2$ shifted sine bell function was used in both dimensions in order not to distort relative volumes. Spectra in 90% H_2O were recorded using a 1-τ-1 hard pulse sequence (12).

RESULTS

Non-exchangeable protons.

The strategy for the sequential assignment of 2D spectra has been described in detail (13-16) and will not be repeated here. Fig. 1(a) shows the region of the NOESY spectra of duplex I for connectivities between base protons and thymidine methyl groups. We expect to observe connectivities between a T methyl group and its own H6 proton resonance and, depending upon the sequence, also with an H8 or H6 proton of the residue in the 5′ direction. The three methyl groups between 1.68 and 1.80 ppm can be connected, as

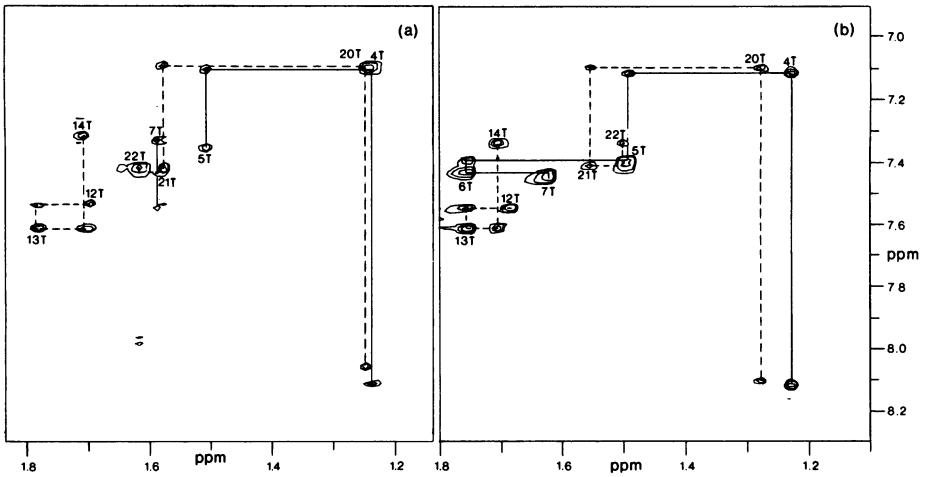

Fig. 1: Expanded NOESY contour plots of the region H8/H6-CH_3 (a) for duplex I and (b) for duplex II, at 23°C.

shown, and correspond to a TpTpT sequence. As no connectivity is observed to a purine H8 these three thymidines must correspond to residues 12 to 14. The two methyl resonances at ca. 1.25 ppm show each a connectivity with a purine H8 which, from the sequence must be an adenosine H8. In one case the connectivity continues to the T methyl at 1.51 ppm and stops. This must correspond to ApTpT, residues 3 to 5. The other shows a connectivity with a methyl group at 1.57 ppm and then, via the H6, to another at 1.62 ppm. The sequence ApTpTpT corresponds to residues 19 to 22. The resolution does not permit the relative assignment of the methyl resonances of 4T and 20T, and correspondingly of 3A(H8) and 19A(H8), but this is unambiguously established from connectivities in the base proton to anomeric proton region (see below). Finally the methyl resonance at 1.59 ppm shows a connectivity with a singlet in the resolution enhanced 1D spectrum, i.e. its own H6 proton at 7.34 ppm and a doublet, at 7.55 ppm, of a cytidine residue. This corresponds to 6C-7T.

The region corresponding to the connectivities between the base H8/H6 protons and the H1'/H5 protons for this duplex is shown in Fig. 2(a). The ambiguity described above is readily removed upon examination of this region. The highest field resonance in the aromatic region, assigned above to 20T(H6), shows a cross peak with an anomeric proton at 6.14 ppm which is also seen by the H8 resonance at 8.06 which must be assigned to 19A. Similarly

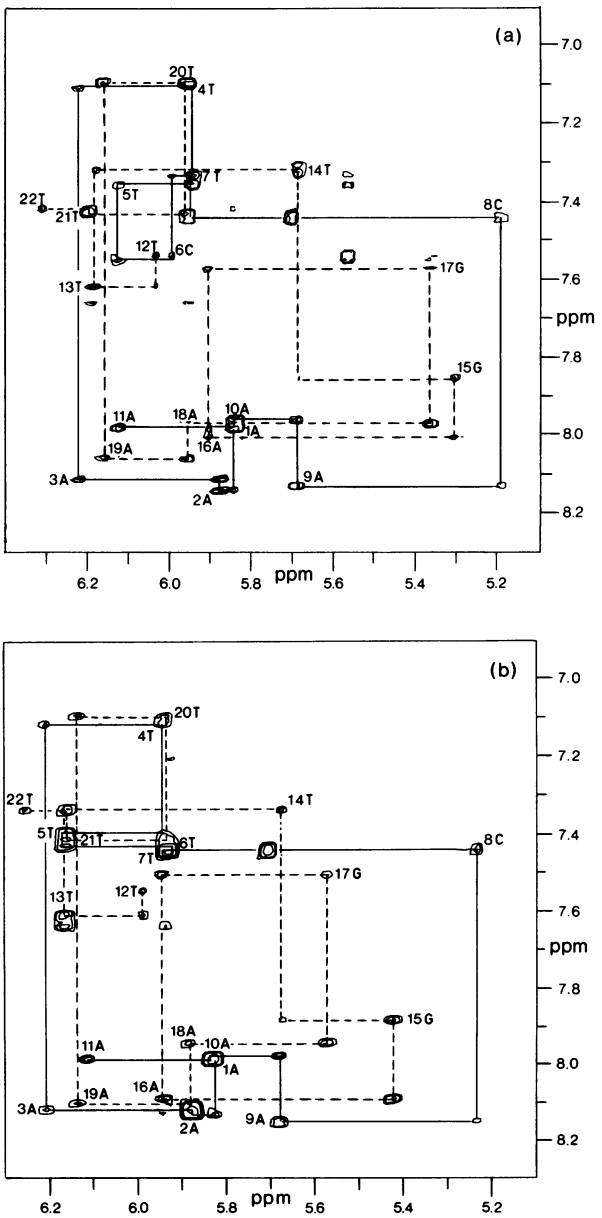

Fig. 2: Expanded NOESY contour plots of the region H8/H6-H1'/H5 (a) for duplex I and (b) for duplex II, at 23°C.

the second highest field aromatic resonance, assigned to 4T(H6) shows a cross peak with the anomeric proton at 6.20 pm as does the H8 resonance at 8.12 ppm wich must be assigned to 3A.

The assignment of the T(H6) resonances greatly assists in following the connectivities in this region. The chain from 12T to 17G can be followed without any ambiguity. Similarly, starting from the 3′ terminal end we can follow from 22T to 18A thus completing the sequential assignment of this strand. On the other strand we have already identified the base H8 or H6 resonances of 3A to 7T. The assignment of 8C follows by elimination as the sequence contains only two cytidine residues. There is no ambiguiy in the five remaining A residues.

The NOESY spectrum of mismatch duplex II was measured at the same temperature, 23°C, as for duplex I. We noted that the chemical shift and line width of a number of the resonances are highly sensitive to small variations in temperature. This is not surprising as the duplex contains 9 A-T base pairs and a destabilizing mismatch such that at this temperature the single strand population may not be negligible and/or end fraying may be important.

The aromatic proton/thymidine methyl region is shown in Fig. 1(b). The connectivities 14T-12T are highly conserved relative to duplex I. The connectivities starting from the cross peak between 19A(H8), 20T(CH_3) to 22T(H6), 22T(CH_3) show only a difference in the chemical shift of 22T(CH_3). Any possible ambiguity as to the assignment of 3A(H8) relative to 19A(H8) is resolved on analysis of the aromatic/H1′ region, Fig. 2(b), in exactly the same way as for duplex I.

The connectivities through the mismatch site from 3A(H8), 4T(CH_3) to 7T(H6), 7T(CH_3) are easily followed. We observe that the interresidue NOEs are very similar in intensity for 4T-5T, 5T-6T and 6T-7T. For the other strand we can compare the cross peaks between the aromatic H8 and anomeric protons. Fig. 2(b) shows this part of the NOESY spectrum. While it is certain that with a relatively long mixing time the cross peaks observed are significantly influenced by spin diffusion effects the similarity between Fig. 2(a) and (b) for the connectivities 15G to 19A is striking. For the other strand which has four consecutive thymidine residues the resolution does not permit measurement of the interresidue cross peak intensities around the mismatch site.

We note that the chemical shifts of both the aromatic and anomeric proton resonances are significantly different for the chain 15G-18A between duplex I and II but that the observed differences are much smaller on the op-

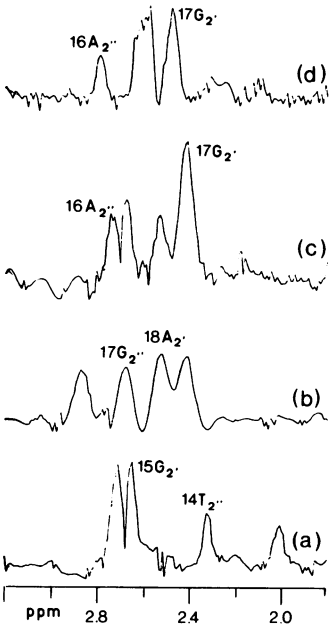

Fig. 3: H2'/H2" region of the rows through (a) 15G(H8) and (b) 18A(H8), for duplex II. Row through 17G(H8), (c) for duplex II and (d) for duplex I.

posite strand. The chemical shifts for both duplexes are summarized in table I.

We have examined all regions of the NOESY spectra looking for differences in cross peak volumes (see Materials and Methods) which might indicate a different conformation in the helix for the G-T base pair relative to the corresponding G-C pair. We have, naturally, concentrated on interactions which are direct - that is where spin diffusion will either very little or not at all influence the cross peak volumes. The spectral resolution does not always permit direct comparison between the two duplexes but some representative sections through the NOESY spectra are shown in Fig. 3. In general we observe that the intraresidue cross peak H8 to H2' is about double the intensity of the interresidue NOE, H8 to H2" of the residue in the 5' direction as shown for the row through 15G(H8) of duplex II, Fig. 3(a). The row through 18A(H8), Fig. 3(b) shows approximately equal intensities (and volumes when they are calculated) for the corresponding intra and interresidue effects. For the next step along the helix, the row through 17G(H8), Fig. 3(c), the same intra to interresidue NOE ratio is observed as for the refe-

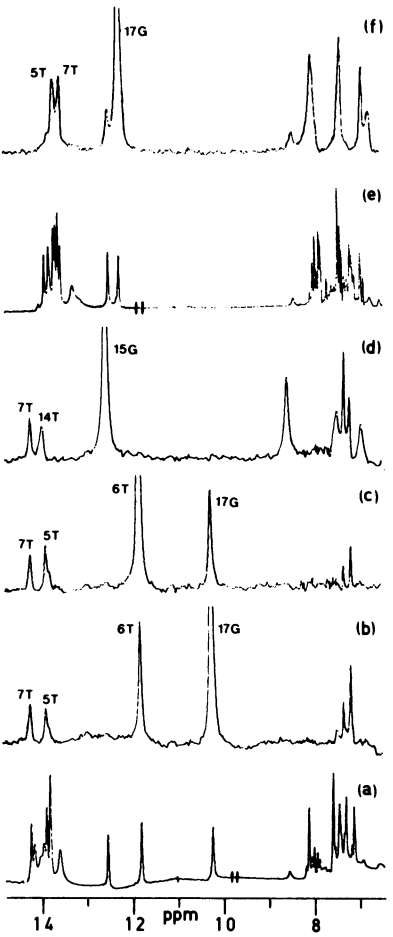

Fig. 4: (a) NMR spectrum of duplex II recorded at 0°C in 90% H_2O . The vertical scale is not the same for the imino and aromatic regions. Difference spectrum after presaturation for 0.5 s of the resonance at (b) 10.31 ppm, (c) 11.82 ppm and (d) 12.53 ppm. Spectrum of duplex I at 10°C in 90% H_2O (e). As above the vertical scale is not the same for the imino and aromatic regions. (f) Difference spectrum after presaturation at 12.46 ppm.

rence, Fig. 3(a), and the same is also observed for the same step in duplex I, Fig. 3(d). We do not observe, for any of the short distance connectivities, large differences between the spectra of duplexes I and II. As there is strong overlap of cross peaks in the cross section through 18A(H8) of duplex I in the H2'/H2" region we cannot directly compare the interresidue NOE to 17G(H2").

Adenosine H2 and exchangeable protons

The 1D spectrum of the G-T sequence is shown in Fig. 4(a). Assignment of the exchangeable resonances at 1°C was carried out by standard presaturation techniques. Presaturation for 0.5 s of the resonance at 10.81 ppm results in a large NOE to the resonance at 11.82 ppm, smaller ones to two thymidine imino protons and also to two H2 resonances at 7.34 ppm and 7.18 ppm, Fig. 4(b). Presaturation at 11.82ppm, Fig. 4(c), gives the reverse large NOE at 10.31 ppm and four smaller NOEs on the same resonances as observed in Fig. 4(b). All the difference spectra are normalized on the integral of the presatured resonance so that the magnitude of the NOEs can be directly compared. The integral, rather than the height of the resonance, was used as the line widths of the exchangeable resonances varies somewhat and as very low power was used, the extent of presaturation was not constant. We observe almost identical NOEs to the T imino protons for the two highfield resonances but significantly stronger NOEs to the AH2 protons in Fig. 4(b) than in Fig. 4(c). The strong NOEs observed between the resonances at 11.82 ppm and 10.31 ppm indicates a short interproton distance for these two protons in the G-T base pair.

Presaturation of the single resonance observed in the normal G imino region, at 12.53 ppm, Fig. 4(d), gives NOEs to one of the T imino protons and an H2 proton seen already in Fig. 4(b) and 4(c). They can thus be assigned to the A-T base pair between the G-C and the G-T pairs. The two other small NOEs, at 13.95 ppm and 7.22 ppm, must belong to the imino and AH2 of the 9A-14T base pair. Note also the NOEs to two exchangeable resonances in the aromatic region at 8.60 and 6.94 ppm which we assign to the WC and nWC protons of 8C. Although not every imino resonance can be selectively presatured we were able to assign all the imino and H2 resonances.

We have searched for a resonance or resonances which could be attributed to the G amino protons of the G-T base pair. A single resonance at 6.45 ppm was observed (17) for these protons in poly d(G-T). NOE experiments upon presaturation of the resonances at 10.31 and 11.82 ppm at different temperatures between 1-25°C failed to locate the amino protons. This could mean that the NOE is too small to be detected. We therefore made difference spectra between the normal 1D acquisition, Fig. (4a), and a spectrum following continuous solvent saturation. In the difference spectrum in the region 5.5-9.5 ppm only the two resonances assigned to the C amino group were observed.

The spectrum of duplex I at 10°C is shown in Fig. 4(e). The imino and H2 proton assignment was carried out in exactly the same way as described

Table I: Chemical shifts of non-exchangeable and exchangeable protons at 23° and 0°(*), respectively, of duplex I (first line) and II (second line)

	H8/H6	H2(*)/H5/CH_3	H1′	H2′	H2″	H3′	amino HC/nHC(*)	imino(*)
1A	7.98		5.83	2.45	2.69	4.96		
	8.00		5.83	2.44	2.73	4.97		
2A	8.14		5.87	2.72	2.87	5.05		
	8.14		5.88	2.71	2.87	5.05		
3A	8.12	7.69	6.20	2.58	2.92	5.01		
	8.13	7.67	6.20	2.57	2.94	5.01		
4T	7.12	1.24	5.93	1.99	2.57	4.85		13.86
	7.12	1.23	5.94	1.98	2.54	4.86		13.79
5T	7.36	1.51	6.10	2.23	2.58	4.85		13.90
	7.40	1.49	6.15	2.16	2.58	4.86		13.85
6C	7.55	5.55	5.98	2.09	2.53	4.82	8.24/6.99	
6T	7.44	1.74	5.92	2.02	2.42	4.86		11.82
7T	7.34	1.59	5.94	2.09	2.43	4.85		13.74
	7.46	1.62	5.93	2.23	2.41	4.86		14.22
8C	7.45	5.69	5.18	1.91	2.18	4.86	8.67/6.78	
	7.45	5.70	5.24	1.85	2.16	4.86	8.60/6.94	
9A	8.13	7.32	5.67	2.63	2.72	4.98		
	8.15	7.22	5.69	2.64	2.73	4.98		
10A	7.96		5.82	2.50	2.70	4.97		
	7.98		5.83	2.50	2.71	4.97		
11A	7.98		6.10	2.48	2.31	4.62		
	7.99		6.11	2.50	2.34	4.63		
12T	7.54	1.69	6.01	2.20	2.54	4.73		13.49
	7.56	1.68	5.98	2.19	2.56	4.72		13.46
13T	7.62	1.78	6.16	2.24	2.59	4.90		14.01
	7.62	1.76	6.15	2.25	2.58	4.89		14.00
14T	7.32	1.70	5.67	2.00	2.32	nd		14.00
	7.34	1.70	5.68	2.02	2.33	4.86		13.95
15G	7.86		5.30	2.64	2.66	4.96		12.69
	7.89		5.43	2.65	2.72	4.98		12.53
16A	8.01	7.60	5.89	2.57	2.78	5.01		
	7.99	7.34	5.94	2.55	2.75	5.01		
17G	7.58		5.36	2.47	2.61	4.95		12.46
	7.51		5.58	2.43	2.69	4.94		10.31
18A	7.97	7.12	5.94	2.60	2.90	5.01		
	7.95	7.18	5.89	2.54	2.87	4.99		
19A	8.06	7.65	6.14	2.56	2.91	5.00		
	8.11	7.67	6.13	2.56	2.87	5.00		
20T	7.11	1.25	5.95	1.99	2.57	4.86		13.80
	7.10	1.28	5.93	1.98	2.54	4.85		13.79
21T	7.44	1.58	6.17	2.22	2.59	4.90		14.10
	7.42	1.55	6.14	2.17	2.56	4.90		14.17
22T	7.43	1.61	6.29	2.27	2.27	4.80		13.49
	7.35	1.50	6.25	2.28	2.28	nd		13.46

above. For comparison, when the imino resonance assigned to the central G-C pair was presatured for 0.5 s, Fig. 4(f), we observed the T imino and AH2 resonances of the adjacent base pairs and the C amino resonances of 6C. All the chemicals shifts are summarized in table I.

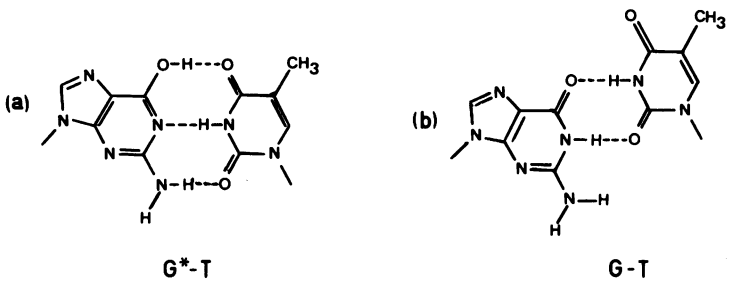

Fig. 5: Rare tautomer (a) and wobble (b) base pairing of G-T.

DISCUSSION

Comparison of the NOESY spectra of the two duplexes reveals only small differences. While many of the cross peaks observed at a mixing time of 250 ms are influenced by spin diffusion it is unlikely that major differences will appear at much shorter mixing times. The relative volumes of cross peaks due to direct NOEs are only slighty different. The differences are no greater than observed for oligonucleotides containing only normal base pairs which show internal variations depending upon the sequence. In so far as we are able to observe the G-T base pair occupies a normal position in the helix. These experiments do not give any information with respect to the phosphate backbone conformation which may be different for the two duplexes.

Is it possible to identify the nature of the hydrogen bonding between G and T in solution? Two types of hydrogen bonding have been proposed: the first involves retaining Watson-Crick geometry by the formation of a rare tautomer species, Fig. 5(a), (18,19). The rare tautomer can either be of guanine (as shown) or of thymidine. The second involves switching normal donnor and acceptor sites in a wobble structure, Fig. 5(b), such as proposed for RNA mismatches (20). Our NOESY spectra certainly do not permit us to eliminate the rare tautomer model, but on the other hand no convincing evidence for formation of this type of hydrogen bonding between normal bases has ever been presented.

It was concluded that the pairing in poly d(G-T) is of a wobble type based upon the chemical shifts of the exchangeable resonances (17). More recent NMR work has followed this line of thought (21-23) but it is not clear, if the rare tautomer model could be excluded in these studies.

We might expect to observe resonances corresponding to four exchangeable protons for the G-T base pair, as was observed for poly d(G-T) (17). But

only two have been observed here, as elsewhere (21-23). A number of possibilities have been considered to account for this: for a rare tautomer we could observe either (a) the hydroxyl and imino proton with the G amino protons being very broad via some exchange mechanism or (b) we could see the G amino protons with the others exchange broadened, while for the wobble structure we could observe either (c) the two iminos protons or (d) only the G amino protons.

Solution (a) can be immediately discounted. From model building we observe that the distance from the hydroxyl proton in the major groove to the AH2 protons (in both directions) in the minor groove is much greater than 5 A. No NOEs would be expected at such a long distance whereas we observe NOEs from both the resonances of the G-T pair. Solution (b) can be excluded for a number of reasons, firstly it would be difficult to explain the absence of the imino proton resonance, secondly the nWC G amino proton is exactly equidistant for the 3′ and 5′ neighbouring AH2 protons for Watson-Crick geometry and we do not observe equal NOEs in either Fig. 4(b) or 4(c) and lastly a shift from ca. 6.4 ppm in the monomer to 10-12 ppm observed here on hydrogen bonding is most unlikely. Solution (d) can be disgarded for the same reasons.

From Figs.4(b) and 4(c) we observe NOEs to the adjacent imino protons of the same intensity which suggests that these two imino protons are approximately equidistant from the helix axis. We do not attempt to determine absolute distances from NOEs to exchangeable protons as these may be influenced by exchange processes, especially next to a mismatch site. However, with this model we can say that the G imino proton will be ca. 0.5 A closer to the AH2 protons than will be the T imino proton. Further the G imino proton will be 0.3-0.5 A closer to the AH2 in the 3′ direction than that in the 5′ direction which is in agreement with the observed relative intensities and the assignment given in Table 1. We can thus unambiguously assign the imino proton resonance at 10.31 ppm to 17G. This is in agreement with an earlier assignment (21) based only on the observation that G imino resonance are found to higher field than T imino resonances in normal base pairs.

We conclude that we are observing a wobble structure and that the orientation of this base pair in the helix is similar, in so far as we can define it from NOEs in H_2O solution, to that found in some recent crystal structures (24,25).

Nevertheless one important aspect remains unexplained: why do we not observe the G amino protons of the wobble pair? These protons are observed

for the guanosine monomer and for poly d(G-T). In the absence of hydrogen bonding the amino group is free to rotate and a single resonance is seen. We have previously shown (26,27) that in G-C pairs hydrogen bonding slows down the rotation such that exchange is in the intermediate exchange region in the temperature range 0-20°C. But in the wobble structure this group is not involved in hydrogen bonding to T. Further we have observed that for haloU-G base pairs embedded between G-C pairs (G.V.F, M. Goodman & L. Sowers, unpublished results) a large NOE is observed from the G imino proton to the G amino proton resonance. In the crystal structure of a G-T pair between two G-C pairs (25) the G amino group is apparently not hydrogen bonded to a water molecule. On the other hand in A-T sequences the minor groove is strongly hydrated (28) with water molecules linking the base pairs which in turn are connected by a water spine. In this study we have examined a wobble pair between two A-T pairs and the absence of the G amino group from the spectrum is probably due to restricted rotation via solvent hydrogen bonding. On the other hand the amino group is observed for G-C rich sequences where the minor groove is very weakly hydrated.

The conclusions reached do not provide any definitive answer as to why the G-T base pair is better repaired in a G-C rich environment (7) but do provide basis for speculation. Whereas we concluded that the sequence dependence of the repair for G-A mismatches was due to an equilibrium between helical and looped out structure (7) with the latter strongly favoured in an A-T rich sequence, we have no evidence here for any structure other than a fully helical one.

Our data rather suggest a difference in the hydration, in the minor groove, of the G amino group. If the mismatch proof reading system involves examination of both grooves it may be that the non hydrogen bonded amino group is simply more visible in a G-C rich environment where it is not hydrated. It seems highly unlikely that any profound conformational change takes place as a function of the sequence. Further, although the data base is still small, we note that repair efficiency is higher the closer the conformation of the bases in the mismatch ressembles that of Watson-Crick geometry.

During the preparation of this manuscript our attention was drawn to a recent article describing a G-T mismatch in a self complementary oligonucleotide studied by 2D NMR techniques (29). We have not addressed the same questions as in this study but we are certainly in agreement with the conclusion that the G-T base pair is of a wobble structure.

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