An NMR structural study of deaminated base pairs in DNA

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ABSTRACT

The structurally aberrant base pairs TG, UG and TI may occur in DNA as a consequence of deamination of 5-methylcytosine, cytosine and adenine respectively. Results of NMR spectroscopic studies are reported here for these deaminated base pairs in a model seven base pair long oligonucleotide duplex. We find that in all three cases, the DNA helix is a normal B form and both mispaired bases are intrahelical and hydrogen bonded with one another in a wobble geometry. Similarly, in all three cases, all sugars are found to be normal C2′ endo in conformation. Symmetric structural perturbations are observed in the helix twist on the 3′ side of the mispaired pyrimidine and on the 5′ side of the mispaired purine. In all three cases, the amino group of the G residue on the 3′ side of the mispaired pyrimidine shows hindered rotation. Although less thermodynamically stable than helices containing only Watson-Crick base pairs, these helices melt normally from the ends and not from the mispair outwards.

INTRODUCTION

Besides the canonical GC and AT Watson-Crick base pairs, occasionally, this set is expanded to include the 5-methylcytosine.guanine base pair. Structurally aberant base pairs may be generated from the normal Watson-Crick base pairs by simple hydrolysis of primary amino groups of the base moieties cytosine, 5-methylcytosine and adenine.

In aqueous solution, 5mC, C and A are deaminated to U, T and hypoxanthine respectively (1−4). While G may be deaminated by nitrous acid (5), G is not observed to deaminate at measurable rates in aqueous solution (1). Upon deamination, GC, 5mCG and AT base pairs would become GU, GT and IT base pairs, respectively.

The mutagenic consequences of deaminated bases in DNA have been addressed by several laboratories. Uracil is removed by uracil glycosylase (6) and T in TG pairs (arising through misincorporation of T opposite G or deamination of 5mC) is removed by a specific glycosylase (7). Inosine in DNA is similarly presumed to be removed by a specific glycosylase, however, such an activity has not yet been characterized.

Of the base pairs which may result in DNA via hydrolytic deamination, only the TG base pair has been characterized previously by NMR spectroscopy and X-ray crystallography (8−10). In this paper, we present NMR structural data on the TG mispair as well as the remaining deaminated base pairs which may exist in DNA, UG and TI.

MATERIALS AND METHODS

The heptanucleotides were synthesized by a classical phosphotriester method (11). Appropriate pairs of oligonucleotides were heated to 80°C followed by slow cooling to form the following five duplexes.

5′(C1 A2 G3 Y4 G5 G6 C7)
3′(G14 T13 C12 R11 C10 C9 G8)

where (1) Y4.R11 is T4.I11, duplex TI, (2) Y4.R11 is U4.G11, duplex UG, (3) Y4.R11 is T4.G11, duplex TG, (4) Y4.R11 is C4.G11, duplex CG and (5) Y4.R11 is T4.A11, duplex TA. The duplexes were 4mM in strand concentration dissolved in 10 mM phosphate buffer, pH 7.4 (unless otherwise specified), 150 mM NaCl and 0.2 mM EDTA. NMR spectra were recorded in either 99.99% D_2O or 90% H_2O/10%D_2O. Chemical shifts were measured relative to the internal reference tetramethyl ammonium chloride, 3.18 ppm.

NMR spectra were recorded on either WM500 or AM600 Bruker spectrometers. NOESY spectra were recorded with mixing times of either 60 or 400 ms for spectra in D_2O and 250 ms for spectra recorded in H_2O, in the phase sensitive mode (12). After zero filling the data were multiplied by either an unshifted or only slightly shifted sine bell function (with the exception of the 60 ms spectra for which a sine bell shifted by $\pi/2$ was used prior to Fourier transformation. For spectra recorded in H_2O the observation pulse was replaced by a jump and return sequence (13) and the pulse maximum placed at 15 ppm. This gave high quality spectra with virtually no base plane distortion.

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Table 1. Chemical shifts of non-exchangeable protons at 23°C and of exchangeable protons at 1°C. For the central three base pairs the chemical shifts for the three duplexes are given in the order indicated for the central base pair.

	H8/H6	H5/H2 CH3	H1′	H2′	H2″	H3′	H4′	NH	NH2
C1	7.61	5.87	5.53	1.82	2.28	4.67	4.03		8.21,6.90
A2	8.28	7.75	6.00	2.80	2.92	5.03	4.37		7.90,6.55
G3	7.70		5.94	2.46	2.73	4.98	4.20	12.75	
G3	7.68		5.89	2.45	2.65	4.95	4.37	12.92	
G3	7.67		5.92	2.44	2.71	4.94	4.40	13.00	
T4.I	7.13	1.62	5.48	1.91	2.18	4.80	4.07	11.43	
U4.G	7.36	5.44	5.44	1.90	2.16	4.95	4.26	11.68	
T4.G	7.11	1.64	5.43	1.92	2.13	4.80	4.02	11.74	
G5	7.86		5.71	2.70	2.79	5.00	4.37	13.26	8.08,5.40
G5	7.84		5.67	2.67	2.76	4.96	4.38	13.30	8.25,5.40
G5	7.83		5.70	2.69	2.77	4.96	4.34	13.40	8.30,5.40
G6	7.69		5.90	2.50	2.67	4.97	4.37	13.07	
C7	7.24	5.05	6.09	2.20	2.20	4.48	4.00		8.13,6.37
G8	7.90		5.93	2.62	2.74	4.83	4.26	12.90	6.00
C9	7.50	5.33	6.04	2.21	2.50	4.86	4.24		8.25,6.35
C10	7.58	5.68	5.68	2.25	2.48	4.88	4.13		8.51,6.85
C10	7.53	5.64	5.69	2.20	2.47	4.84	4.13		8.49,6.82
C10	7.55	5.68	5.74	2.23	2.50	4.86	4.16		8.54,6.83
I11.T	8.27	7.81	6.28	2.71	2.90	5.02	4.48	12.04	
G11.U	7.89		5.98	2.58	2.72	4.81	4.26	10.43	5.99
G11.T	7.89		6.00	2.60	2.75	4.96	4.38	10.61	6.01
C12	7.36	5.39	5.84	1.89	2.42	4.80	4.28		8.20,6.90
C12	7.37	5.39	5.99	1.92	2.41	4.73	4.28		8.10,6.80
C12	7.36	5.38	5.87	1.92	2.42	4.86	4.26		8.19,6.89
T13	7.30	1.68	5.82	1.99	2.33	4.83	4.05	14.12	
G14	7.90		6.13	2.64	2.39	4.70	4.18	12.93	

TOCSY spectra were recorded in the phase sensitive mode (14) with 25 or 80 ms mixing times.

RESULTS

Duplex TI

The region corresponding to interactions between the base H8/H6/H2 protons and the H1′/H5 protons of the 400 ms NOESY spectrum recorded at 23°C is shown in Figure 1. The general principles and strategy for the assignment of proton spectra of oligonucleotides has been described in detail (15–18). Starting from the 3′ terminal C residue at 7.24 ppm the connectivities can be followed without ambiguity through to C1. Similarly on the other strand the connectivities can be followed from G14 through I11 to G8. Four interbase cross peaks, A-D in Figure 1, are observed corresponding to interactions H8/H6-H5 for the pairs G6-C7, G8-C9, C9-C10 and I11-C12 respectively. The I11 H2 proton at 7.81 ppm gives a strong cross peak with the C12 H1′ proton and a weak one with its own H1′ proton. The A2 H2 proton at 7.75 ppm gives only a weak cross peak with its own H1′ proton.

Figure 2 shows the region of interactions between the base H8/H6 protons and the H2′/H2″/CH3 protons. Analysis of this region confirms the assignment of the base protons shown in Figure 1. Two interbase cross peaks are observed, G3 H8-T4 CH3, peak E and C12 H6-T13 CH3, peak F.

The assignment of the H3′ and H4′ resonances was obtained by analysis of the TOCSY spectra (not shown).

The observed interresidue cross peaks show that the T.I bases stack into the helix and that the helix is a right handed B form. The T.I pair does not induce any major deformation of the helix.

Analysis of the 60 ms NOESY spectrum gives the relative assignment of the H2′ and H2″ resonances as for all sugar

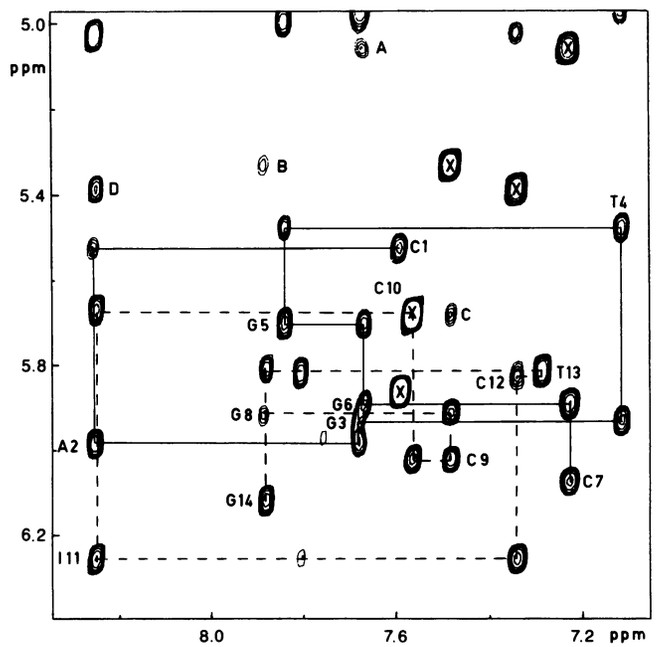

Figure 1. Part of the 400ms NOESY spectrum of the duplex TI recorded at 23°C. The cross peaks marked × correspond to CH6-CH5 interactions and the peaks labelled A-D are described in the text.

conformations the distance H1′-H2″ is always smaller than H1′-H2′. The observed chemical shifts are given in the Table.

The cross peak volumes corresponding to the interresidue interactions H8/H6-H2″/H2′ are very sensitive markers for the helix geometry. For a normal B DNA the ratio of the cross peak

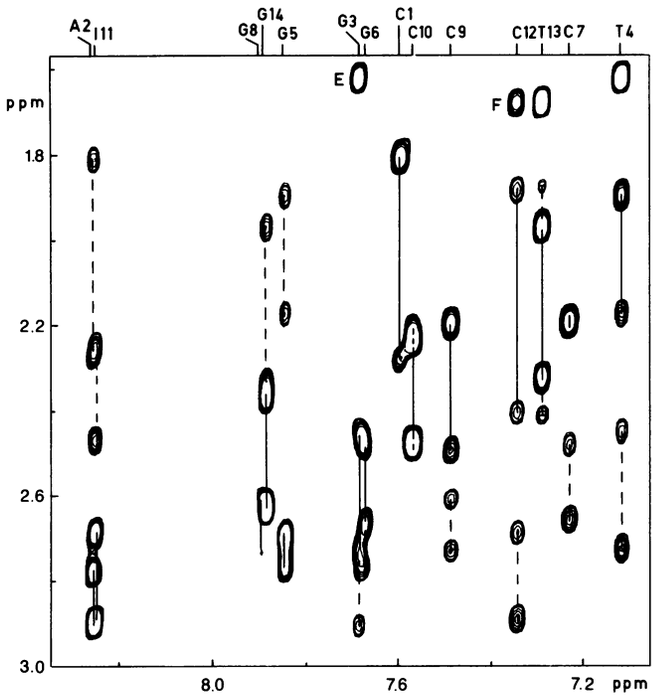

Figure 2. Part of the 400 ms NOESY spectrum of the duplex TI. Intraresidue interactions are linked by solid lines and interresidue interactions by broken lines. Peaks E and F are described in the text.

volumes H8/H6-H2'' to H8/H6-H2' should be of the order of 15 in the absence of spin diffusion effects. In the 60 ms spectrum we observe that this ratio is typically ca. 10, with one exception, confirming that spin diffusion influences little the spectrum and that the helix adopts a normal B form. The exception that we observe is for the interaction G5 H8-T4. For this step the above ratio is 0.8. The corresponding interaction on the other strand I11 H8-C10 we cannot measure due to resonance overlap, as shown in Figure 2. One explanation for the observed cross peak volume ratio would be that the sugar of T4 has a much larger than normal C3' *endo* conformation. We have previously shown (19) that significant changes in the C2' *endo*-C3' *endo* equilibrium can be detected by measuring the ratio of the cross peak volumes for the intraresidue interactions H8/H6-H2' and H8/H6-H3'. The ratio that we observe for T4 is, within experimental error, the same as we observe for all the other non-terminal residues and corresponds to a predominantly C2' *endo* conformation. Thus the unusual interresidue interactions that we observe must arise rather from the orientation of the base of G5 relative to the sugar of T4.

Figure 3 shows two regions of the 250 ms NOESY spectrum recorded in H_2O at 1°C and pH 6.3. The imino proton resonances of the terminal base pairs are strongly attenuated. From the unique A.T base pair for which the T13 imino proton is found at 14.12 ppm we can follow the chain of inter-imino connectivities through G3 to the two high field imino resonances corresponding to the T.I base pair. We note the very strong imino-imino NOE between these two protons. The chain can then be followed until G6. Only very weak NOEs are observed involving the imino protons of the terminal base pairs.

In the other region of Figure 3 we observe the interactions between the imino protons and those resonating between 9 and 5 ppm. The T13 imino proton shows NOEs to the A2 H2, to

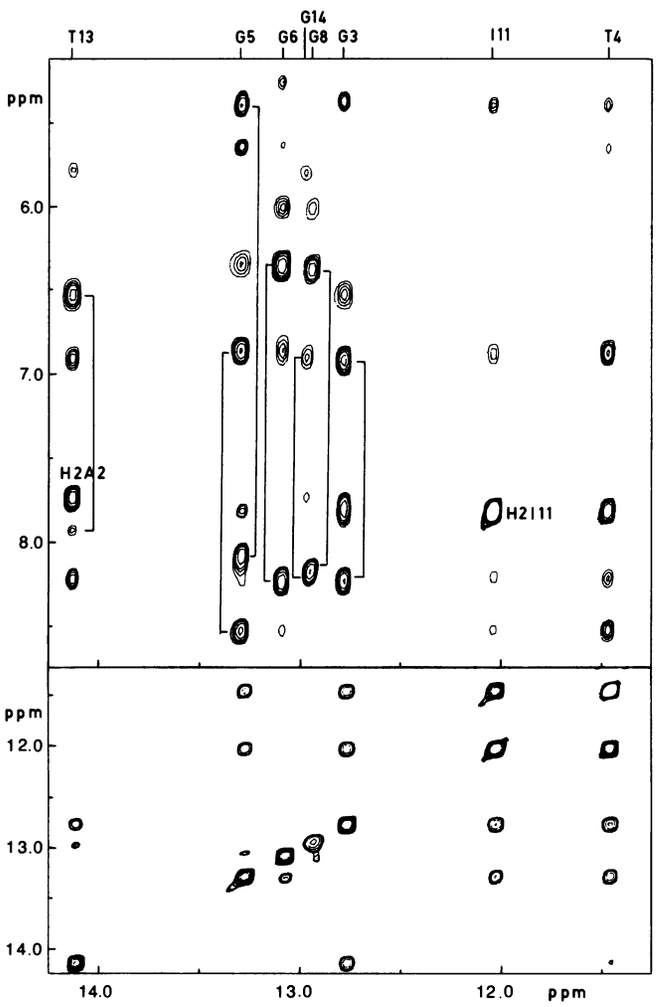

Figure 3. Lower part. Part of the 250 ms NOESY spectrum of duplex TI recorded in H_2O at pH 6.3 and 1°C. This region shows interactions between imino proton resonances. Upper part. The region of the same spectrum showing interactions between the imino protons and the base and amino protons. Intraresidue connectivities with amino proton resonances are connected by solid lines. The unlabelled cross peaks observed between 5.0 and 5.8 ppm arise from spin diffusion to CH5 protons.

the neighbouring C12 amino protons and to two other exchangeable protons which are not the amino protons of C1. They must therefore be attributed to the adenosine amino protons. In general, even at 1°C these proton resonances are broad due to flipping of the amino group (20). It is not obvious why the NOE to the close hydrogen bonded amino proton, at 7.90 ppm, should be weaker than to the non-hydrogen bonded proton at 6.55 ppm. The relative assignment is, however, certain as hydrogen bonding will shift the proton resonance downfield as observed previously (20).

The two imino protons of the T.I base pair both show NOEs with the I11 H2 resonance at 7.81 ppm but that from the resonance at 12.04 ppm is much stronger and this must be the I11 imino proton. This is confirmed by the observation that the most intense cross peaks with the adjacent C amino protons are seen from the imino proton at 11.43 ppm. In addition both of these imino protons give an NOE with a resonance at 5.40 ppm (see below).

All the remaining C amino protons have been assigned, as indicated, including those of the terminal base pairs. Although

imino-imino cross peaks involving these base pairs are very weak, cross peaks with the very slowly exchanging amino protons are seen. NOEs to CH5 and in some cases CH6 protons arising from spin diffusion are also observed. At this point four cross peaks remain unassigned. Two of these arise from an interaction with the G5 imino proton and are found at 8.08 and 5.40 ppm. In the region for interactions between amino protons we observe cross peaks corresponding to each C amino group, for the single A amino group and also a cross peak between these two unassigned exchangeable protons. These must arise from the G5 amino group. At this temperature these are generally in the intermediate exchange rate (8), because of flipping as for adenosine amino protons but even slower, and are not observable. It should be noted that only the amino protons of G5 (for the non-terminal base pairs) are observed for which a mechanism must exist which slows down considerably the flipping of this amino group. Thus the two cross peaks observed from each of the two T.I imino protons to the resonance at 5.40 ppm can be attributed to an NOE with the G5 non-hydrogen bonded amino proton.

Two other cross peaks are observed from the G6 and G8 imino protons to a resonance at 6.00 ppm. While at or close to 6.00 ppm several H1′ resonances are observed an NOE between an imino resonance and C9 H1′, the only vaguely possible candidate appears highly unlikely. The direct distance is far too long and no obvious spin diffusion pathway is available. Further we do not observe this type of interaction elsewhere. A much more likely explanation is that the interaction is with the amino group of G8. In the amino-amino region no cross peak is found between a resonance at 6.00 ppm and another in the region 7.5–9.0 ppm indicating that the 6.00 ppm resonance corresponds to both amino protons with rapid flipping of the amino group. This is reasonable for a terminal residue due to fraying but we do not observe corresponding NOEs for the other end of the duplex. For the sequence studied here we would expect that stacking of C1.G14 over A2.T13 would create a clash or steric hindrance in the minor groove while the stacking of G6.C9 over C7.G8 would have a similar effect but in the major groove. This would explain the greater freedom for flipping for the amino group of G8 relative to that of G14.

We have measured, at pH 7.4, the imino proton line widths as a function of temperature between 10 and 30°C. At low temperature all the imino proton resonances have the same line width. On raising the temperature we observe that the helix melts from the ends and that the T.I base pair is the last to show fast exchange with the solvent. For example, at 30°C the line widths of the T.I imino protons are ca. 24 Hz whereas those of the neighbouring base pairs are ca. 45 Hz.

Duplex UG

We have carried out the same series of experiments on the sequence containing a central U4.G11 pair. Figure 4 shows the

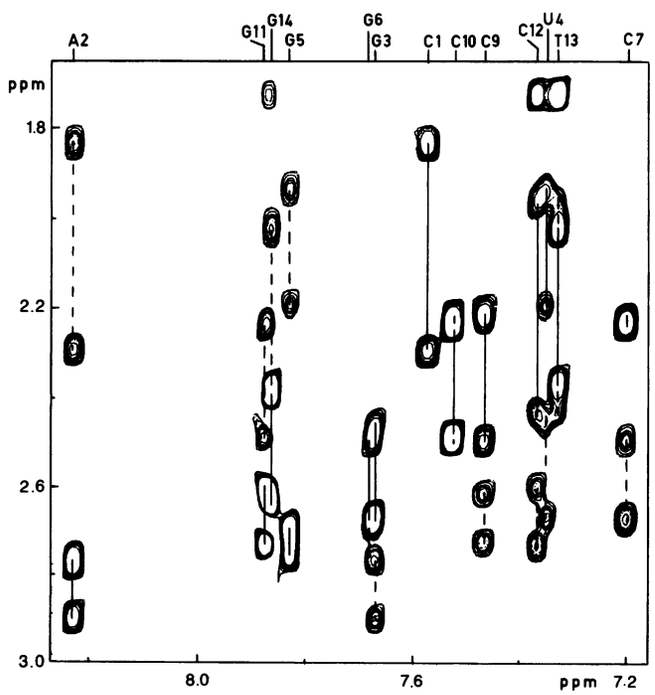

Figure 4. Part of the 400 ms NOESY spectrum, recorded at 23°C, of the duplex UG corresponding to interactions between the base H8/H6 protons and the H2′/H2″/CH3 protons. Intraresidue interactions are connected by solid lines and interresidue interactions by broken lines.

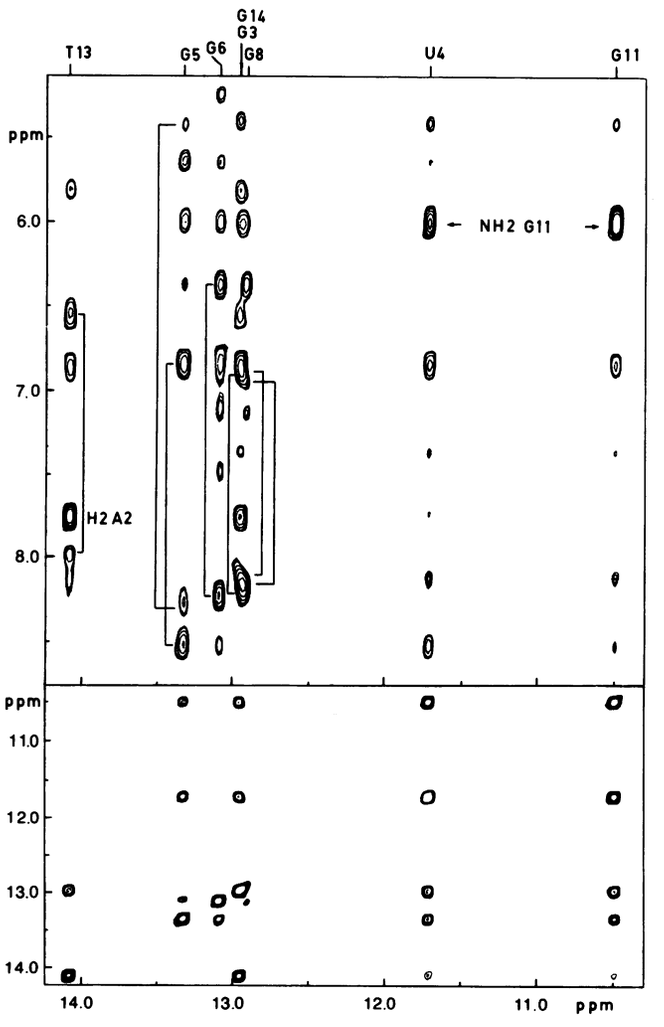

Figure 5. Lower part. Part of the 250 ms NOESY spectrum of the duplex UG recorded in H_2O at pH 6.3 and 1°C. This region shows interactions between the imino protons. Upper part. A region of the same spectrum showing interactions between the imino protons and the amino protons and certain base protons. Interactions with a pair of amino protons of the same base pair are linked by solid lines.

region of the 400 ms NOESY spectrum, recorded at 23°C, corresponding to interactions between H8/H6 protons and H2'/H2''/CH3 protons. Comparison with Figure 2 shows that for the proton resonances of the six Watson-Crick base pairs only minor changes in chemical shifts are observed. The interresidue NOEs for the central segment G5-G3 and C12-C10 show that no major change in the helix conformation is present. We have recorded the spectrum with a 60 ms mixing time. Integration of cross peak volumes does, however, reveal two steps showing abnormal contacts. The ratio of the cross peak volumes for G5 H8 to U4 H2'' relative to that to U4 H2' is 0.5. On the other strand the corresponding ratio for G11 H8 to C10 H2''/H2' is 1.0. This shows a modified stacking of the U.G base pair within the helix. All the other interresidue interactions are those expected for a normal B DNA.

Two regions of the 250 ms NOESY spectrum recorded in H_2O at 1°C and pH 6.3 are shown in Figure 5. As previously we can follow the chain of connectivities from the T13 imino resonance through G3 to the two high field imino protons of the U.G base pair which show a very large NOE between them. The chain can then be continued to G6. The other region shown of this spectrum is less well resolved relative to that of duplex IT. The imino proton resonance of G3 is coincident with those of G8 and G14. Nevertheless the central part of the duplex is well defined. The imino proton of G5 shows NOEs with the C10 amino protons and weak NOEs with the neighbouring C9 amino protons. We again observe a cross peak with a resonance at 5.40 ppm which from analysis of the amino-amino region gives an NOE with a peak at 8.25 ppm. The cross peak at 8.2−8.25 ppm consists of two overlapping peaks. As previously we assign the pair of peaks 8.25 and 5.40 ppm to the G5 amino group which rotates slowly. The G5 imino proton also shows a cross peak with a resonance at 6.00 ppm which is much more strongly seen by the two high field imino protons. These give weak NOEs with the neighbouring C amino protons and also the non-hydrogen bonded G5 amino proton. The remaining intense cross peak at 6.00 ppm can only arise from the G11 amino group. As this amino group cannot be involved in hydrogen bonding it will be more free to rotate. This also provides the relative assignment of the two imino protons. That, at 10.43 ppm gives a much stronger NOE to the amino group and must be the G imino proton. The G6 imino proton probably shows a cross peak with the G8 amino group but due to resonance overlap this can not be confirmed. The observed chemical shifts for the central three base pairs are given in the Table.

The evolution of the imino proton linewidths as a function of temperature at pH 7.4 is exactly the same as that observed for duplex IT.

Duplex TG

We have carried out the same series of experiments on the sequence containing a central T4.G11 base pair. The TG base pair is the prototype wobble structure which we have included for purposes of comparison. Not suprisingly the spectra ressemble very strongly those obtained for the duplex UG. Analysis of the 60 ms NOESY spectrum shows normal B DNA interactions except for the two steps as observed above. For the interaction G5 H8-T4 we observe that the ratio of the NOEs to T4 H2''/H2' is 0.4. On the other strand the ratio G11 H8 to C10 H2''/H2' is 1.4.

Figure 6 shows the 250 ms spectrum recorded in H_2O at pH 6.3 at 1°C. We observe the chain of imino-imino connectivities as observed above with the expected strong NOE between the two high field imino protons of the T.G base pair. The G amino resonance is found at 6.01 ppm and from the relative intensities of the imino to G amino NOEs the imino resonance at 10.61 ppm can be assigned to the G11 imino resonance. As for the two duplexes described above both of the T.G imino protons show an NOE with a resonance at 5.40 ppm which is also observed from the G5 imino proton. For the reasons described above we can assign the resonance at 5.40 ppm to the non-hydrogen bonded G5 amino proton.

Duplexes CG and TA

We have previously studied these two duplexes in D_2O (Fazakerley and Sowers, unpublished results) and we observed no abnormal interresidue interactions corresponding to those observed above. In the light of what we have observed above we have now recorded spectra in H_2O under the same conditions as above (not shown). We do not, for either of these duplexes, observe the G5 amino protons.

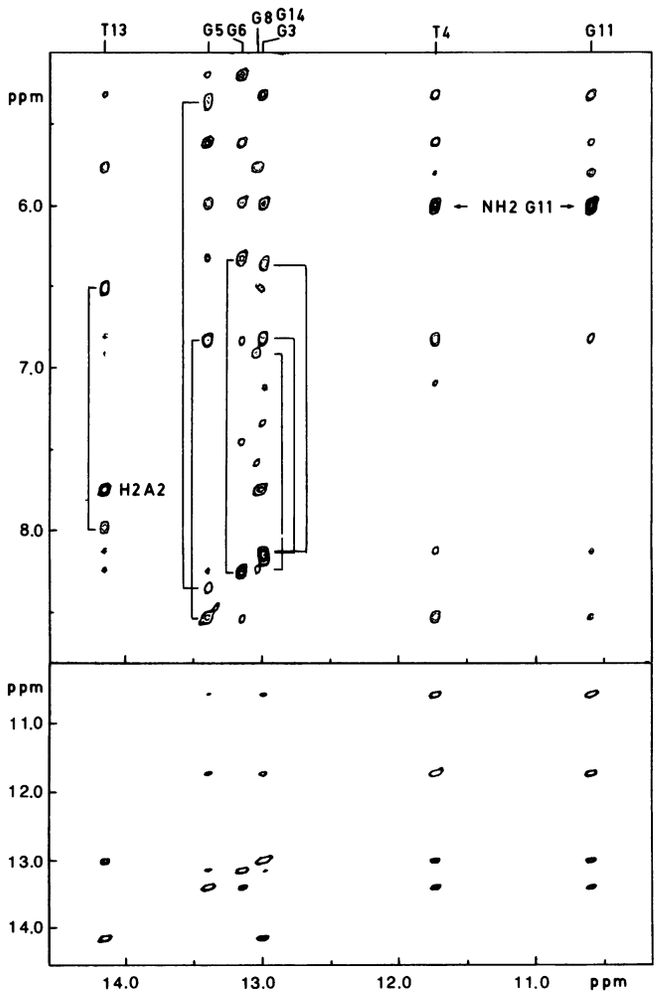

Figure 6. Lower part. Region of the 250 ms NOESY spectrum of the duplex TG recorded at 1°C and pH 6.3 corresponding to imino-imino interactions. Upper part. Region of the same spectrum corresponding to interactions between imino and amino protons. Pairs of amino protons are linked by solid lines.

DISCUSSION

Three chemically stable mismatches, TG, UG and TI, may be generated in DNA via simple hydrolytic deamination of normal Watson-Crick base pairs. Proton NMR spectra obtained in both H_2O and D_2O for model DNA duplexes containing these mispairs demonstrate, in all three cases, that the mispaired bases are stacked into the helix. There are two imino protons for each base mispair, and the magnitude of the interbase NOE between these two protons indicates that they are in close proximity. The line width of the imino resonances at the mismatch site indicate slow proton exchange, indicative of base-base hydrogen bonding. These data clearly indicate that all of the deaminated base pairs assume a geometry which is predominantly wobble pair at physiological pH as shown in Figure 7.

In a wobble conformation, one or both bases must be displaced relative to Watson-Crick geometry. In a attempt to more clearly define the structural perturbations which result from the presence of the mispair, we have searched the 60 ms NOESY spectra for interactions which show structural modification relative to a B DNA conformation. Although most of the interproton interactions throughout the 7 base pair long duplexes are consistent with a normal B form helix, we observe one interresidue interaction on each strand, adjacent to the mismatch site, which indicates significant perturbation.

In a normal B DNA helix, the magnitude of the interresidue NOE between a purine H8 or pyrimidine H6 proton and the H2′ proton of the residue in the 5′ direction is, in the absence of spin diffusion, greater than the corresponding interaction with the H2″ proton by a factor of 10 to 15. In all three systems studied here, the conformational modification observed is between the pyrimidine (position 4) and G5 and the purine (position 11) and C10. The ratio of the cross peak volumes, H8/H6-H2′ to H8/H6-H2″ for these interactions have been reduced significantly. Reduction of these NOE cross peak volume ratios indicates that the distances between the H8/H6 proton and the H2′ and H2″ protons of the residue in the 5′ direction are now nearly equal.

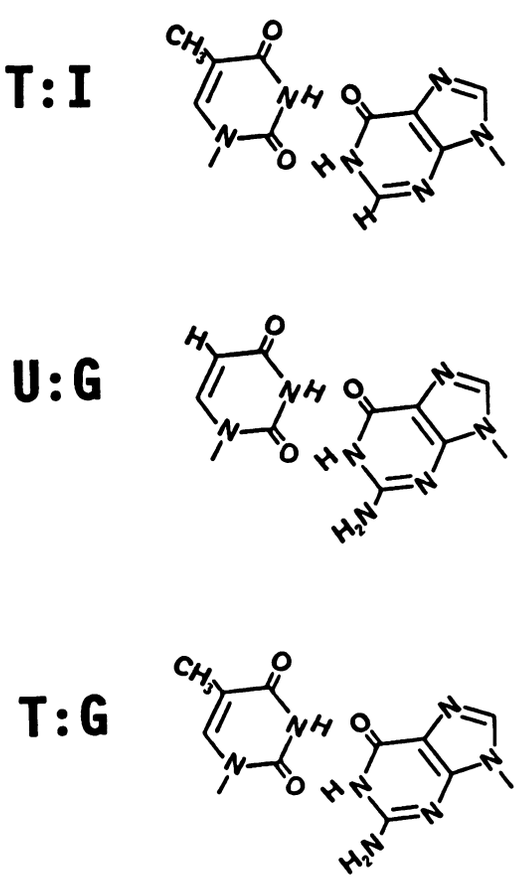

Figure 7. The wobble structures of the deaminated base pairs of DNA: T.I, U.G and T.G.

The observed anomalous H8/H6 to H2′/H2″ interresidue interaction could result from either altered sugar conformation at or adjacent to the mismatch site or from shifting of the bases within the helix. By examining the intraresidue H8/H6 to H2′/H3′ cross peak volumes, we conclude that all sugars, including those at or adjacent to the mismatch site remain predominantly normal, C2′ *endo* in conformation.

Alternatively these unusual interproton distances could result from shifting of the bases within the helix in order to accomodate the wobble geometry. We have performed preliminary model building studies based upon a crystal structure study of the TG pair in B DNA (9). We observe that the lateral displacement of the pyrimidine into the major groove and the purine into the minor groove, generating the wobble structure, will satisfy the observed proton interactions. Similar base displacements are consistent with the data obtained for the UG and TI systems as well.

In the TG and UG wobble systems, the amino group of the mismatched G is not involved in base-base hydrogen bonding. Consistent with this structure, the proton resonance corresponding to the two amino protons, in both cases, is observed as a single peak, indicating that the amino group of the mispaired G residue is freely rotating on an NMR time scale. Previously, rapid rotation of the G amino group in halouracil-G wobble structures, in which the mismatched pair was embedded between GC base pairs, has been reported (21,22). However, this was not the case for a TG mismatch embedded between AT base pairs (8) where hydration in the minor groove slows down amino group rotation.

Normally, rotation of the G amino group of a GC base pair is in the intermediate range on the NMR time scale at low temperature, rendering the amino proton resonances extremely broad or undetectable. In all three systems containing a wobble mispair studied here, the amino group of the G residue of the GC base pair 5′ to the mispair (G5) displays hindered rotation. Possible explanations for the observed slow rotation include 1) formation of a hydrogen bond by the non Watson-crick amino proton of G5, 2) anomalous hydration in the minor groove due to the presence of the mispair, or 3) a steric barrier to rotation.

Formation of an additional hydrogen bond by the non Watson-Crick G amino proton has been observed. In an X-ray study of an oligonucleotide containing GA mismatches (23), a hydrogen bond was found between the amino group of G of the GA mispair and a keto group of T on the opposite strand of the adjacent base pair. In our case, such a hydrogen bond would have to be between the G5 amino group and the G11 (or I11). It is, however, difficult to build a model of such a structure.

Although we cannot totally eliminate the possibility that hindered rotation might result from anomalous hydration in the minor groove, as observed for a TG mismatch between AT base pairs (8), it is unlikely in this case. First, we would not expect that the minor groove of this part of the molecule to be especially heavily hydrated. Secondly, the G amino group at the mismatch

site, directly above the G5 amino group, is rotating rapidly, arguing against a strong water lattice in the vicinity.

The third, and most likely explanation for the anomalous slow rotation of the G amino group adjacent to the mismatch is that generation of the mispair causes a steric barrier to amino group rotation. We have previously argued that both cytosine amino protons and the G imino proton may exchange with water through a mechanism which calls for a scissor opening of the bases, in plane, into the major groove (24). Similarly, propeller twist of an intact GC pair could stretch the G amino-C carbonyl hydrogen bond sufficiently to allow the amino group to rotate. Broadening of G amino proton resonances in normal GC pairs results from rotational exchange of amino protons, as opposed to chemical exchange with solvent at low temperature. In all sequences studied here containing a wobble mispair, the purine base at the mispair site shifts into the minor groove. Such displacement would place it more directly over the G5 amino group. In such a structure, G5 would be wedged between purine 11 and G6, and thus less likely to propeller twist. Such a constraint could then be the source of the slow rotation of the G5 amino group.

CONCLUSION

We have studied the three base mispairs which may arise in DNA as a consequence of hydrolytic deamination. When examined within the same surrounding base context, similar results are observed for all cases. Each base mispair is predominantly in a wobble geometry. The wobble geometry is accomodated without modification of the global helix conformation by lateral displacement of the purine towards the minor groove and the pyrimidine towards the major groove. While we do not wish to overinterpret our NOESY data, as we have not measured NOE build up slopes, the magnitude of the lateral displacement of each base appears to be similar in magnitude.

Our results are consistent with previously reported X-ray structural data on wobble systems in B DNA. In both a T.G mismatch (9) and an A.C mismatch (25) the purine was observed to move into the minor groove and the pyrimidine into the major groove. The angle between the glycosidic bond and the C1′-C1′ vector is increased for the pyrimidine and decreased for the purine relative to the Watson-Crick base pairs.

The data obtained in this study on deaminated base pairs in DNA in solution is consistent with the crystallographic data although we cannot measure by NMR the angles referred to above. The common structural perturbations revealed by these studies may shed light on the mechanisms by which repair enzymes recognize base mispairs in DNA.

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REFERENCES

1. Shapiro, R. and Klein, R.S. (1966) Biochemistry, **5**, 2358–2362.
2. Lindahl, T. (1967) J. Biol. Chem., **242**, 1970–1973.
3. Lindahl, T. and Nyberg, B. (1974) Biochemistry, **13**, 3405–3410.
4. Wang, R.Y.-H., Kuo, K.C., Gehrke, G.W., Huang, L.-H. and Ehrlich, M. (1982) Biochem. Biophys. Acta, **697**, 371–377.
5. Schuster, H. and Schramm, G. (1958) Z. Naturforschung, 13b, 697.
6. Caradonna, S.J. and Cheng, Y.-C. (1980) J. Biol Chem., **255**, 2293–2300.
7. Wiebauer, K. and Jiricny, J. (1989) Nature, **339**, 234–236.
8. Quignard, E., Fazakerley, G.V., van der Marel, G., van Boom, J.H. and Guschlbauer, W. (1987) Nucleic Acids Res.,**15**, 3397–3409.
9. Hunter, W.N., Brown, T., Kneale, G., Anand, N.N., Rabinovich, D. and Kennard, O. (1987) J. Biol. Chem., **262**, 9962–9970.
10. Hare, D., Shapiro, L. and Patel, D. (1986) Biochemistry, **25**, 7445–7456.
11. Gait, M.J. (1984) Oligonucleotide synthesis, a practical approach. IRL Press, Oxford, U.K.
12. Bodenhausen, G., Kogler, H. and Ernst, E.E. (1984) J. Magn. Reson., **58**, 370–388.
13. Plateau, P. and Guéron, M. (1982) J. Am. Chem. Soc., **104**, 7310–7311.
14. Davis, D.G. and Bax, A. (1985) J. Am. Chem. Soc., **107**, 2820–2821.
15. Hare, D.R., Wemmer, D.E., Chou, S.H., Drobny, D. and Reid, B.R. (1983) J. Mol. Biol., **181**, 319–336.
16. Feigon, J., Leupin, W., Denny, W.A. and Kearns, D.R. (1983) Biochemistry, **22**, 5943–5951.
17. Fréchet, D., Cheng, D.M., Kan, L.S. and Ts'o, P.O.P.(1983) Biochemistry, **22**, 5194–5200.
18. Scheek, R.R., Boelens, R., Russo, N., van Boom, J.H. and Kaptein, R. (1984) Biochemistry, **23**, 1371–1376.
19. Cuniasse, Ph., Sowers, L.C., Eritja, R., Goodman, M.F.,Cognet, J.A.H., Le Bret, M., Guschlbauer, W. and Fazakerley, G.V. (1989) Biochemistry, **28**, 2018–2026.
20. Fazakerley, G.V., Téoule, R., Fritzsche, H., Guy, A. and Guschlbauer, W. (1985) Biochemistry, **24**, 4540–4546.
21. Sowers, L.C., Goodman, M.F., Eritja, R., Kaplan, B. and Fazakerley, G.V. (1989) J. Mol. Biol.,**205**, 437–447.
22. Sowers, L.C., Eritja, R., Kaplan, B., Goodman, M.F. and Fazakerley, G.V. (1988) J. Mol. Biol., **263**, 14794–14801.
23. Privé, G.G., Heineman, U., Chandrasegaran, S., Kan, L-S., Kopka, M. and Dickerson, R.E. (1987) Science, **238**, 498–504.
24. Fazakerley, G.V., van der Marel, G.A., van Boom, J.H. and Guschlbauer, W. (1984) Nucleic Acids Res., **12**, 8269–8279.
25. Hunter, W.N., Brown, T., Anand, N.N. and Kennard, O. (1986) Nature, **320**, 552–555.