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NMR Studies on an Oligodeoxynucleotide Containing 2-Aminopurine opposite Adenine[†]

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ABSTRACT: A heteroduplex containing the mismatch 2-aminopurine (AP)–adenine has been synthesized and studied by proton NMR. The mismatch was incorporated into the seuquence d[CGG(AP)GGC]·d-(GCCACCG). One-dimensional nuclear Overhauser effect measurements in H_2O and two-dimensional nuclear Overhauser effect spectra in D_2O show AP·A base pairs in a wobble structure in which both bases are in the anti conformation. The adenine is stacked well in the helix, but the helix twist between the adenine and neighboring cytosine in the 3' direction is unusually small. As a result, the aminopurine on the opposite strand is somewhat pushed out of the helix. From the measurements of the imino proton line widths, the two adjacent G·C base pairs are not found to be significantly destabilized by the presence of the purine–purine wobble pair.

2-Aminopurine (AP),[1] a base analogue of adenine, is a strong mutagen causing predominantly A·T ↔ G·C transitions in vivo (Ronen, 1979). AP preferentially forms base pairs with T during DNA synthesis (Bessman et al., 1974; Clayton et al., 1979; Watanabe & Goodman, 1981). The mutagenicity of AP occurs because it can form base mispairs with C (Freese, 1959; Rudner, 1960) when present either as a template base (A·T → G·C pathway) (Watanabe & Goodman, 1981) or as a substrate for DNA polymerase (G·C → A·T pathway) (Mhaskar & Goodman, 1984). We have recently demonstrated by proton NMR that AP and T pair with normal Watson–Crick geometry (Sowers et al., 1986); the preferred base pairing of AP and C involves protonation of the mispair also having normal Watson–Crick geometry (Sowers et al., 1986). In contrast with previously accepted models that invoke the presence of disfavored tautomeric forms to account for AP·C mispairs (Freese, 1959; Topal & Fresco, 1976,), we find that a second hydrogen bond is formed by acquisition of a proton from solvent water (Sowers et al., 1986).

We decided to investigate the possibility of additional hydrogen bonding interactions of AP with A because of the presence of higher T_m values observed for the case of oligomers containing an AP·A mispair in place of an AP·C mispair at

the same location (Eritja et al., 1986). We were further interested in the AP·A interaction as we have previously shown that, to a small extent, DNA polymerase incorporates AP opposite template A residues (Mhaskar & Goodman, 1984). Also, weak transversion mutations have been reported to result following mutagenesis with AP in vivo (Persing et al., 1981).

If, as seems likely, base pairing occurs between aminopurine and adenine, two possibilities exist (Figure 1). In both cases, a wobble-type pairing is involved, but this may be Watson–Crick or Hoosteen. Unlike the G·T wobble (Brown et al., 1985) in which the two hydrogen bonds are formed with imino protons, for AP·A both would be amino protons. The G·A base pair has been shown to adopt a Watson–Crick wobble structure (Kan et al., 1983; Patel et al., 1984), to give Hoogsteen pairing with the A in a syn conformation (Kennard, 1985) or to provoke looped-out structures depending upon the DNA sequence (Fazakerley et al., 1986).

In order to investigate the possible base pairing of AP with A, we have synthesized the heteroduplex structure

	1	2	3	4	5	6	7
5'	C	G	G	AP	G	G	C
3'	G	C	C	A	C	C	G
	14	13	12	11	10	9	8

containing the AP·A pair which has been studied by proton NMR in H_2O and D_2O. The predominant structure of the AP·A pair is wobble in which both bases are in the anti conformation.

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[1] Abbreviations: NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; WC, Watson–Crick; nWC, non-Watson–Crick; 2-D, two dimensional; NOESY, two-dimensional nuclear Overhauser effect spectroscopy; AP, 2-aminopurine.
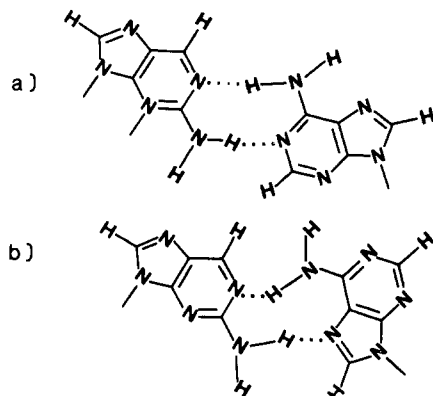
FIGURE 1: Possible hydrogen-bonding schemes between A and AP: (a) wobble, both bases oriented anti; (b) Hoogsteen pairing with A syn.

MATERIALS AND METHODS

The oligonucleotides containing AP were synthesized by a modified triester method (Eritja et al., 1986).

NMR spectra were recorded at 500 MHz on a Bruker WM-500 at the Southern California Regional NMR Facility at the California Institute of Technology, Pasadena, CA, and the Centre d'Etudes Nucleaires de Saclay, Gif-sur-Yvette, France. For spectra in H_2O the solvent peak was suppressed by a $1-\tau-1$ hard-pulse sequence (Moore & Kim, 1983).

Oligonucleotides were 4 mM in strand concentration and dissolved in D_2O or 90% H_2O/10% D_2O, 150 mM NaCl, and 10 mM phosphate, pH 7.2 (unless otherwise stated). Chemical shifts were reported relative to internal tetramethylammonium chloride at 3.18 ppm.

The NOESY spectra were recorded at 20 °C with 1024 complex points in t_2 and 256 points in t_1. A total of 96 scans were averaged with a mixing time of 300 ms and a recyle delay of 2.5 s. The data were multiplied by a 15° shifted sine bell function in t_2 and an unshifted sine bell function in t_1 before Fourier transform. Zero filling gave a final matrix of 1K × 1K real points.

RESULTS

Exchangeable Proton Resonances. The resolution-enhanced spectrum of the regions 5.5–10 and 12.5–13.5 ppm for the AP·A duplex measured at 4 °C is shown in Figure 2a. At no temperature have we observed any resonances between 10 and 12.5 ppm.

In the imino region four resonances corresponding to the imino protons of the dG·dC base pairs 2, 3, 5, and 6 are observed. Even at −8 °C the imino resonances of the terminal base pairs are very broad. We do not observe an imino proton resonance for the AP·A base pair for, if such a base pair forms, the hydrogen-bonded protons will be from amino groups.

Between 8 and 8.6 ppm a number of exchangeable resonances are observed and one nonexachangeable resonance at 8.065 ppm. This is the region where the hydrogen-bonded Watson–Crick (WC) cytidine amino proton resonances of dG·dC base pairs are normally found. To lower field, at ca. 9.1 ppm, we observe a very broad resonance. Its line width, ca. 250 Hz, is much greater than that of the exchangeable resonance at 8.52 ppm, ca. 35 Hz. This could be due to either enhanced exchange with solvent or the fact that this proton belongs to an amino group that is rotating.

Between 6.3 and 7 ppm a number of poorly resolved exchangeable proton resonances are observed. This is the region where the non-Watson–Crick (nWC) cytidine amino protons of dG·dC base pairs are normally found.
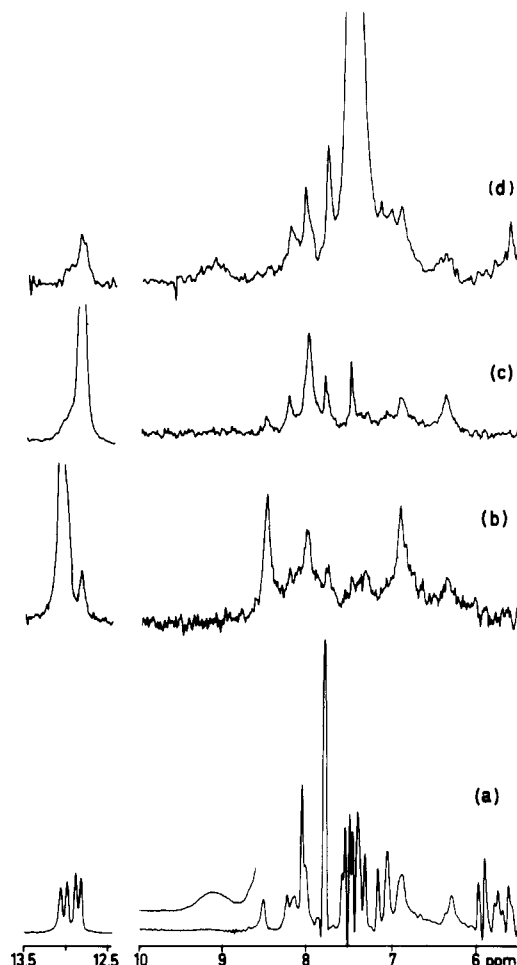

FIGURE 2: (a) Resolution-enhanced spectrum of the AP·A duplex, regions 5.5–10 and 12.5–13.5 ppm recorded at 4 °C in 90% H_2O; (b) NOE difference spectra after 0.4-s preirradiation of the imino resonance at 13.055 ppm; (c) as in (b) but with preirradiation at 12.814 ppm; (d) as in (b) but with preirradiation at 7.51 ppm.

We have first carried out the assignment of the exchangeable proton resonances of the dG·dC base pairs. Presaturation at low power, less than 50% saturation, for 0.4 s of the lowest field imino resonance at 13.055 ppm results in a small NOE to the imino resonance at 12.814 ppm, Figure 2b. The large NOE observed at 8.51 ppm must arise from the cytidine WC proton of the same base pair and the smaller one at 8.02 ppm from the same proton of an adjacent base pair.

The NOE observed at 6.93 ppm is from the cytidine nWC proton paired to the resonance at 8.51 ppm. A weak NOE at 6.37 ppm arises from the nWC proton of the adjacent base pair.

Presaturation at 12.814 ppm results in NOEs to the same four cytidine amino resonances, Figure 2c, with the relative intensities of the pairs of resonances (WC plus nWC) reversed as expected. In addition, two NOEs at 7.811 and 7.506 ppm of nonexchangeable protons are observed. Due to partial saturation of the adjacent imino resonance at 12.88 ppm, an NOE to its own cytidine amino resonance at 8.24 ppm is observed.

The only two nonexchangeable proton resonances in this duplex that can give NOEs from the imino resonances for Watson–Crick base pairing are the H-6 of AP and the H-2 of A. This is a case of a purine–purine wobble base pair in which both bases are anti. It is possible that the A is syn, giving a Hoogsteen type base pair, and this is discussed below. Thus, the imino resonance at 12.814 ppm is one of the base pairs adjacent to the AP·A. Repeating the presaturation of

Table I: Chemical Shifts of Observed Exchangeable Protons of the AP·A Duplex at 4 °C[a]

	3′	5′		imino	C_{WC}	C_{nWC}	A_{WC}	A_{nWC}
14	G	C	1		8.0	6.8		
13	C	G	2	13.055	8.51	6.93		
12	C	G	3	12.814	8.02	6.37		
11	A	AP	4				9.1	6.5
10	C	G	5	12.878	8.24	6.92		
9	C	G	6	12.977	8.04	6.32		
8	G	C	7		8.15	6.8		

[a] The solution was 4 mM in strand concentration, 150 mM NaCl, and 10 mM phosphate at pH 7.2 in 9:1 H_2O/D_2O.

the imino resonance at 13.055 ppm but for a longer time, 1 s, gives additional NOEs (not shown) to the amino protons of the terminal base pair.

Repeating the experiments on the other two dG·dC imino resonances gives the assignment of all the cytidine amino protons and the four observed imino protons, and these are given in Table I.

We have carried out NOE experiments on presaturation of the two nonexchangeable resonances observed in Figure 2c. Selective presaturation of the resonance at 7.811 ppm is impossible as at least four H-8 proton resonances are found between 7.79 and 7.82 ppm. The resonance at 7.506 ppm is better resolved but is close to several C H-6 resonances. Presaturation at 7.506 ppm, Figure 2d, gives rise to NOEs to the dG·dC imino resonances of the adjacent base pairs at 12.8 ppm. NOEs are also observed on the cytidine WC and nWC proton resonances of the adjacent base pairs, and a relatively large NOE, when taking into account that this resonance has a line width of 250 Hz, to the resonance at 9.1 ppm is observed. Presaturation at 7.81 (not shown) gives similar NOEs to the adjacent imino proton resonances. The NOEs observed in the anomeric region are similar in magnitude, ca. 2% to those shown in Figure 2d. The magnitude of the NOEs observed to the anomeric proton resonances is consistent with anti conformations for the bases.

We have searched for other resonances of exchangeable protons which could be attributed to the AP·A base pair. When the temperature is lowered to −8 °C, the resonance at 9.1 ppm sharpens to 120 Hz although the other resonances in the spectrum show line widths increased by about 10–15 Hz. We have observed the buildup of NOEs as a function of the presaturation time which was varied from 0.04 to 0.5 s when the resonance at 9.1 ppm was irradiated.

For a 40-ms presaturation time the largest NOE, Figure 3a, is observed at 6.51 ppm. This resonance does not coincide with any of the cytidine amino protons and has the same line width as the resonance at 9.1 ppm. The apparent NOE is already 50% even at this short preirradiation time. To optimize signal to noise, this experiment was carried out with the carrier placed at 14 ppm and by using a double excitation envelope, giving a first null at ca. 11 ppm and a second one at the water frequency. Under these conditions the effective flip angle is similar at 9.1 and 6.5 ppm and ca. 80% of that at the center of the envelope at 8 ppm. Thus, the magnitude of the NOE between these two resonances is little affected by the nonlinear pulse excitation. The NOE observed on the nonexchangeable proton resonance at 7.46 ppm (corresponding to 7.51 ppm at 4 °C) is ca. 15%. The resonance observed at 8.55 ppm arises from a direct effect of the preirradiation as a relatively high power had to be employed to obtain satisfactory signal to noise. The corresponding nWC proton resonance is seen at 6.95 ppm. Relative to the integrated intensity at 9.1 ppm that at 8.55 ppm remains constant as a function of the presaturation time.
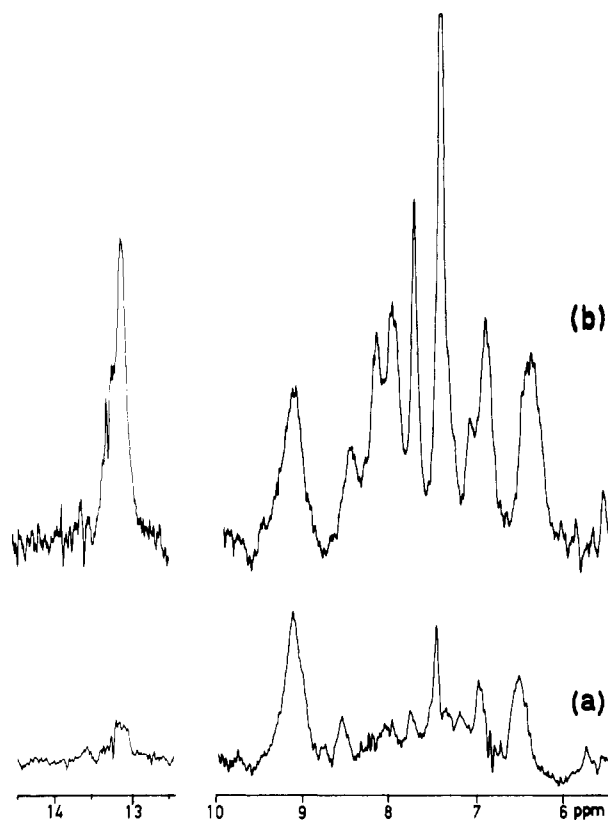

FIGURE 3: NOE difference spectra following (a) 40- and (b) 500-ms preirradiation at 9.1 ppm recorded at −8 °C.
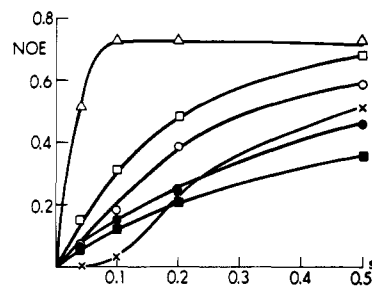

FIGURE 4: Buildup of NOEs as a function of time after presaturation at 9.1 ppm for the AP·A duplex: (△) A nWC amino proton, (□) AP H-6 proton, (○) C WC amino proton at 8.02 ppm, (✕) WC amino proton at 8.23 ppm, (●) A H-2 proton, and (■) G imino protons of base pairs 3 and 5.

For the longest presaturation time, 500 ms, we observe, Figure 3b, NOEs to the cytidine WC protons of the adjacent base pairs at 8.02 and 8.23 ppm, one of the corresponding nWC protons at 7.1 ppm with the other probably hidden under the NOE at 6.5 ppm. An NOE is observed on the second nonexchangeable proton at 7.78 ppm and also to the two adjacent imino protons. The NOE buildup is shown in Figure 4. As the two imino proton resonances cannot be resolved at this temperature, it has been assumed to be equal in both of them.

In order to investigate whether the AP·A base pair caused a destabilization of the adjacent base pairs (3 and 5) relative to base pairs 2 and 6, the line widths of the imino proton resonances as a function of temperature have been recorded, and these are shown in Figure 5.

2-D NOESY Spectra. In order to investigate the conformation of the helix around the AP·A base pair, we have measured the 2-D NOESY spectrum with a mixing time of 300 ms at 20 °C. The general principles of 2-D NOESY spectra (Jeener et al., 1979; Macura et al., 1981) and their
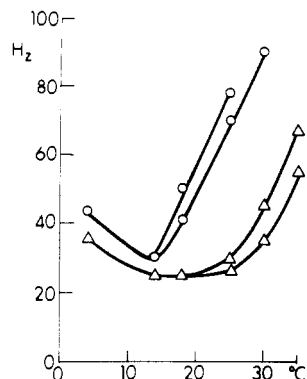
FIGURE 5: Line widths of the imino resonances in the AP·A duplex as a function of temperature: (O) base pairs 2 and 6; (△) base pairs 3 and 5.
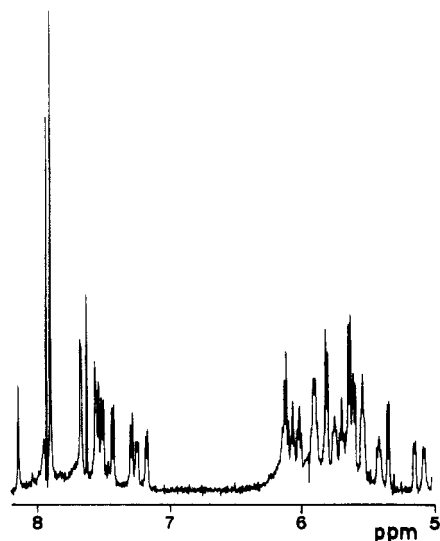

FIGURE 6: Resolution-enhanced 1-D spectrum, 5–8.2 ppm, of the AP·A duplex in D_2O at 20 °C.

FIGURE 7: Portion of the NOESY spectrum of the AP·A duplex showing cross-peaks between protons in the aromatic and anomeric regions.

application to the assignment of proton spectra of oligonucleotides (Hare et al., 1983; Feigon et al., 1983; Frechet et al., 1983; Scheek et al., 1984) have been described and will not be repeated here. For a B-DNA conformation an H-6 or H-8 base proton is close to its own H1′ and H2′ protons and also to the H1′ and H2″ protons of the residue in the 5′ direction. Thus the proton assignment can be made for each strand of a duplex structure by a chain of interresidue NOEs. However, in the presence of a mismatch, and certainly one that must significantly alter the internuclear distances when two purines are base paired, the normal scheme of connectivities will be modified in a manner that cannot necessarily be predicted.

The one-dimensional spectrum, 5–8.2 ppm, is shown in Figure 6. The region of the NOESY spectrum corresponding to interactions between the aromatic and anomeric region is shown in Figure 7. The residues are numbered as shown in Table I. The six cross-peaks arising from cytidine H-6 to H-5 interresidue NOEs are easily identified as they are the strongest.

As a starting point for the assignment of the spectrum, we looked for a purine H-8 (11-A) with a cytidine on each side and the additional requirement of continuing to a further cytidine in both directions. Only one sequence of cross-peaks satisfies these requirements where 11-A H-8 is at 7.89 ppm; the connectivities can be followed between 13-C and 9-C as shown in Figure 7. The assignment of 8-G and 14-G follows without ambiguity.
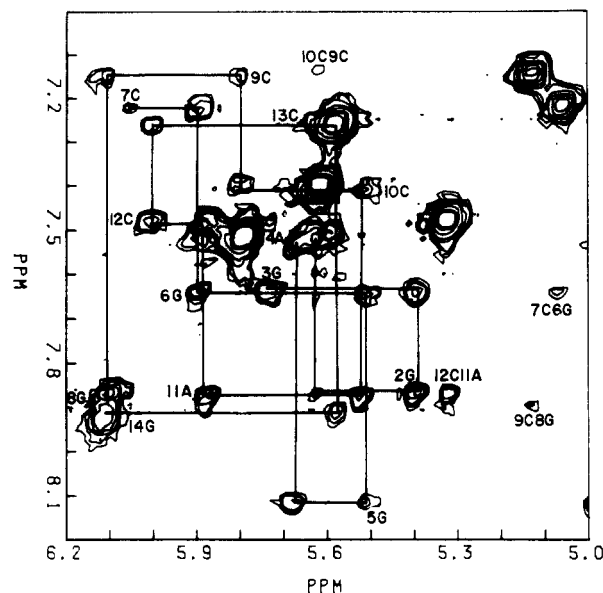
For the other strand there is a clear starting point. The cytidine H-6 at 7.24 ppm gives cross-peaks to its own anomeric proton and to a purine anomeric proton, thus identifying it as 7-C H-6.

The connectivities can be followed via 6-G to the 5-G H-8, which has an unusually low field chemical shift, and on to the 4-AP H-8. But here the chain is broken. As there is only one remaining cytidine residue, the H-6 at 7.54 ppm, the connectivities can be followed in the other direction from 1-C to 3-G. Clearly, there is no cross-peak connecting 3-G and 4-AP.

A number of other cross-peaks are observed in the spectrum which confirm the assignment given above: between 10-C H-5 and 9-C H-6; 7-C H-5 and 6-G H-8; and 9-C H-5 and 8-G H-8. In addition, a strong cross-peak is observed between 12-C H-5 and 11-A H-8 which indicates an unusually small helix turn between these two bases. Two of the other minor cross-peaks that correspond to the same type of interaction, C H-5 to purine H-8, are on the limit of detection. Lastly, it should be noted that the intensities of the intraresidues cross-peaks, base to anomeric proton, are without exception typical for anti conformations. No cross-peaks is observed that could be compatible with a syn conformation and Hoogsteen pairing between AP and A.

In the aromatic region of the NOESY spectrum (not shown) a strong cross-peak close to the diagonal is observed between the resonance assigned to 3-G H-8 and a resonance at 7.625 ppm. The latter corresponds to the resonance observed at 7.51 ppm at 4 °C, and this can be assigned to 4-AP H-6.

DISCUSSION

The observation of NOEs to two nonexchangeable proton resonances upon presaturation of the imino resonance of an adjacent base pair, Figure 2c, shows unambiguously that both bases are inside the helix and must be base paired. If either were extrahelical, one of these protons would be much too far away to experience an NOE.

While we have found all of the exchangeable proton resonances of the dG·dC base pairs, not all of the exchangeable resonances were observed with the AP·A base pair. At –8 °C, from the extremely rapid NOE buildup to the resonance at 6.50 ppm upon presaturation at 9.1 ppm, we can conclude that these two resonances correspond to two protons of an amino

group. The next most rapidly building up NOE is that on the nonexchangeable resonance at 7.46 ppm. However, for the wobble model, there is a pseudosymmetry with the base pair, A NH_2 and AP H-6 on the one side and AP NH_2 and A H-2 on the other.

If either of the two nonexchangeable proton resonances were attributed to the A H-8 of the Hoogsteen model, then presaturation of this proton resonance would result in a large NOE to its anomeric proton as it must be in a syn conformation. Presaturation of the resonance at 7.51 ppm, Figure 2c, gave rise to only small NOEs in the anomeric region. These must arise from partial saturation of nearby H-8 or H-6 resonances; their magnitude is entirely consistent with an anti conformation for these residues. Effects of very similar size were observed upon presaturation at 7.81 ppm. For a syn conformation we would expect an NOE to an anomeric proton of much greater intensity under these conditions. The absence of a syn conformation is confirmed by the analysis of the NOESY spectrum, Figure 7. We find no evidence to support a Hoogsteen model, and indeed, it is difficult to see how the energy required to turn the A syn would be recuperated relative to the wobble model.

Presaturation at 7.51 ppm also shows that this proton is close to the cytidine amino protons of the adjacent base pairs. These NOEs are much less pronounced upon presaturation at 7.81 ppm. We do not know the precise orientation of the base within the helix. Apart from inevitable distortion of the phosphate backbone, the twist necessary to form two hydrogen bonds will change the normal base stacking pattern as has been observed for the G·T wobble (Brown et al., 1985). This twisting results in an unique cross-peak in the aromatic region of the NOESY spectrum between 3-G H-8 and 4-AP H-6 which resolves the ambiguity as to the assignment of the two nonexchangeable resonances seen in the NOE spectra in H_2O.

With the resonance at 7.51 ppm (at 4 °C) assigned to the H-6 of AP, that at 7.81 must be the H-2 of A for a Watson–Crick wobble pair. Preirradiation at 9.1 ppm gave a large NOE to the resonance now assigned to the H-6 of AP. In the wobble structure, the adenosine amino WC proton is close to the H-6 of AP. Thus, the resonances at 9.1 and 6.5 ppm can most probably be assigned to the A amino WC and nWC protons, respectively. Both Figure 3b and Figure 4 show an NOE to a second nonexchangeable proton, the H-2 of A. However, this NOE is for all preirradiation times much smaller than that observed on the H-6 of AP. This most likely arises from spin diffusion, which is very efficient at −8 °C. An alternative explanation would be that in addition an amino resonance of AP is present, unresolved, between 8.8 and 9.5 ppm, and this cannot be excluded. These two resonances are 4–5 times broader than the A amino resonances of an A·T base pair in d(GGATCC) (Fazakerley et al., 1985).

We have previously observed in oligonucleotides (Fazakerley et al., 1984, 1985) that, while rotation of the C amino group is not observed, the A amino group rotates via transient breaking of the hydrogen bonds, giving rise to line broadening. The process is faster still for guanine amino groups, and even at 0 °C, the G resonances are not observed.

From the line widths of the resonance at 9.1 ppm the lifetime for rotation of the A amino group of the AP·A pair is ca. 1.5 ms (4 °C), and at −8 °C it is ca. 3 ms.

We have observed four (two exchangeable and two nonexchangeable) of the proton resonances out of the six that might be observed for the AP·A base pair. In a previous study (Sowers et al., 1986) on the "normal" base pair between AP and T, we observed separate resonances for the WC and nWC

amino protons of AP although these were broad. For the monomers both AP and A, unlike C, show a single amino proton resonance. Duplex formation slows down the rate of rotation, but not to the slow-exchange limit. Rotation is slowed down less in this wobble pair than for a normal base pair, and the AP amino resonance may be very broad.

From the line widths of the imino proton resonances as a function of temperature, Figure 5, the opening rate of base pairs 3 and 5 is considerably slower than for base pairs 2 and 6. Their relative rates of opening show normal behavior. We have no means of measuring the opening rate for the AP·A base pair, but we can say the helix melts, or frays from the ends rather than the center.

The NOESY spectrum supports the conclusion of wobble pair formation and gives some information concerning the orientation of the base pair relative to its neighbors. The unusually strong cross-peak between 11-A H-8 and 12-C H-5 shows that the helix turn between these two adjacent residues is much smaller than usual and that the 11-A must lie at a normal distance relative to the helix axis. In consequence, the 4-AP H-8 will be pushed out of the helix, and that plus the unusual orientation relative to 3-G results in a longer 3-G H-1′ to 4-AP H-8 internuclear distance. As a result, this cross-peak is absent from the NOESY spectrum. The observation of a cross-peak between 3-G H-8 and 4-AP H-6 supports the conclusion that the aminopurine base is pushed somewhat out of the helix. Despite the helix distortion introduced by the purine–purine wobble pair, the neighboring base pairs are not significantly destabilized judging from the imino proton line widths as a function of temperature, Figure 5.

Thermal melting profiles were previously obtained for DNA oligomers used in this study (Eritja et al., 1986). In the case of the AP·A mispair, cooperative melts were observed at 330 (absorption of AP only) and 275 nm, indicating base pair formation. Data from this study confirm that a base pair is formed and clearly indicates that the AP·A mismatch is a wobble structure in which both bases are oriented anti.

The mutagenicity of 2-aminopurine has been known for thirty years [see Ronen (1979) for a review]; however, it is only recently that we have been able to determine the thermodynamics and nature of hydrogen bond formation between 2-aminopurine and the normal DNA bases (Petruska & Goodman, 1985; Sowers et al., 1986). The structural and thermodynamic data are important in attempting to establish a relationship between the fidelity of DNA synthesis and relative stabilities of mispaired bases in DNA.

It is to be expected that DNA polymerase strongly favors the formation of base pairs that conform to Watson–Crick geometry, and within the class of Watson–Crick base pairs, the more thermodynamically stable appear to be favored. Thus, A·T, the most thermodynamically stable, is favored over AP·T (Bessman et al., 1974; Clayton et al., 1979). AP·T, a neutrally charged Watson–Crick base pair (Sowers et al., 1986), is, in turn, strongly preferred by the polymerase over the less stable AP·C (Watanabe & Goodman, 1981, 1982), a protonated Watson–Crick base pair (Sowers et al., 1986). These base pairs are discriminated against by the enzyme almost entirely by the ratio of K_m values for the binding of substrate dNTPs to the polymerase–primer–template complex (Clayton et al., 1979; Watanabe & Goodman, 1982); V_{max} values are roughly similar for the formation A·T, AP·T, and AP·C base pairs (Clayton et al., 1979; Watanabe & Goodman, 1982).

In contrast, the AP·A mismatch is thermodynamically more stable than AP·C; however, DNA polymerase incorporates AP

opposite A at a significantly lower rate than AP opposite C (Mhaskar & Goodman, 1984). We have shown in this paper that the geometry of the AP·A mismatch is wobble with both bases oriented anti. Perhaps the apparent preference of DNA polymerase to form the less stable AP·C mispair is an example of how active site geometric constraints allow the polymerase to discriminate against even relatively stable base mispairs that form other than Watson–Crick geometry.

Registry No. AP, 452-06-2; d[CGA(AP)GGC]·d(GCCACCG), 105356-93-2; adenine, 73-24-5; guanine, 73-40-5; cytosine, 71-30-7.

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NMR Studies of the Stable Mismatch Purine–Thymine in the Self-Complementary d(CGPuAATTTCG) Duplex in Solution[†]

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ABSTRACT: One- and two-dimensional nuclear Overhauser effect experiments demonstrate that a single hydrogen bond between a T imino proton and purine N3 is sufficient to hold the base pair dPu·dT in d(CGPuAATTTCG) by a Watson–Crick fashion rather than a Hoogsteen type. In addition, the dPu·dT base pair is well stacked with neighboring base pairs. The spin–lattice relaxation measurements at 30 and 35 °C of two decamers, d(CGPuAATTTCG) and d(CGAAATTTCG), reveal that the elimination of two single hydrogen bonds of dA·dT base pairs (due to the substitution of adenine for purine) in the sequence results in an increase in the overall imino proton exchange rate from 7 to 36 s⁻¹ at the site of mismatch.

Recently we have demonstrated by dissociation kinetic experiments that the incorporation of a single mismatched base pair destabilizes DNA duplexes to some extent, depending on the nature of the mismatched base pair incorporated (Ikuta

et al., 1987). In that system, stable mismatched G·A and G·T slightly destabilize a duplex while unstable mismatches of A·A, T·T, C·T, and C·A significantly destabilize it relative to a perfectly matched duplex. Similar high-stability duplexes involving guanosine residues have been reported for homopolymer and oligonucleotide duplexes containing G·A and G·G interactions (Dodgson & Wells, 1977; Gilliam et al., 1975). Aboul-ela et al. (1985) reported on studies of mismatched bases and concluded that G·G as well as G·T (Patel et al.,