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CHARACTERIZATION OF THE HIGH pH WOBBLE STRUCTURE OF THE 2-AMINOPURINE·CYTOSINE MISMATCH BY N-15 NMR SPECTROSCOPY

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Transition mutations induced by the base analogue 2-aminopurine arise via the formation of AP·C base pairs during DNA replication. We report here the results of N-15 NMR studies on a duplex oligonucleotide containing N-15 enriched AP and C residues. At high pH (8.6) the AP·C base pair is predominantly wobble. This is the first report on use of a site specifically N-15 enriched oligonucleotide as a probe of aberrant base pairing in DNA.

2-Aminopurine (AP) is a mutagenic base analogue which causes primarily transition mutations in vivo (1). It is believed that transition mutations caused by 2-aminopurine proceed via formation of 2-aminopurine·cytosine mispairs (2-4). It was originally proposed by Freese (2) that the mutagenicity of 2-aminopurine resulted from an enhanced tendency to form the unpreferred imino tautomer. More recent theoretical studies indicated that 1) 2-aminopurine is less likely to form the imino tautomer than the normal base, adenine (5), and 2) that a protonated base pair formed by AP and C was more likely under physiological conditions than structures involving rare tautomers (6). Results of a proton NMR study (7) indicated that the AP·C mispair was most likely protonated at neutral pH.

A more direct and sensitive probe to distinguish among possible base pairing schemes would be N-15 NMR spectroscopy of site specifically enriched oligonucleotides containing the AP·C mispair. We have prepared duplex oligonucleotides containing the AP·C mispair in which ring nitrogen and amino groups of both AP and C are N-15 enriched. Results of N-15 NMR studies at high pH are reported here.

MATERIALS AND METHODS

The preparation of enriched 2-aminopurine deoxynucleoside (APdR, ref. 8) and deoxycytidine are reported elsewhere. Oligonucleotides containing N-15 enriched derivatives were prepared by phosphotriester methods with modifications for synthesis of oligonucleotides containing APdR as described previously (9).

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The sequence of the duplex oligonucleotide studied here is:

5' C G G AP G G C
3' G C C C C C G

Three separate duplexes of the same sequence were prepared and studied. Duplex 1 contained N-15 AP, 40% enriched in the N1 position and 60% enriched at the amino group. In Duplex 2, the C residue opposite the AP was 98% enriched in the N3 position, and in Duplex 3, the C opposite the AP was 98% enriched on the amino group.

Oligonucleotides were 4mM in strand concentration and were dissolved in 90% H_2O/10% D_2O/ 150mM NaCl/10mM phosphate pH 8.6. N-15 NMR spectra were obtained with a Bruker 300MSL spectrometer operating at 30.416MHz. Chemical shifts are reported in ppm downfield from external N-15 aniline in deuterated acetonitrile.

RESULTS AND DISCUSSION

Complementary oligonucleotides containing AP and C residues were mixed to form duplexes 1-3. We first attempted to obtain N-15 spectra at neutral pH, however, under a variety of different NMR conditions, no resonances were observed. Upon increasing the solvent pH to 8.6, N-15 spectra for all enriched sites were observed as shown in Fig. 1.

At pH 8.6, AP and C residues assume neutral, amino conformations.

Both protonation and tautomer formation induce large changes in the chemical shifts of N-15 resonances. Protonation of AP and C results in upfield shifts of ring nitrogen resonances of 81 and 59 ppm respectively (Table 1). Conversion to the imino tautomeric conformation would induce upfield chemical shift changes for the ring nitrogens of 50 to 80 ppm (10,11). As can be seen by comparing the chemical shifts reported in Table 1, chemical shift differences between neutral monomers and AP and C residues in the duplex at pH 8.6 have shifted by 5 ppm at most. This data eliminates either protonated or imino tautomeric conformations for both AP and C residues at pH 8.6.

Proton coupled N-15 spectra shown in Fig. 1 confirm the amino conformation of each base. Both exocyclic nitrogens are split into triplets with coupling constants (J N-H, 89 Hz) consistent with those

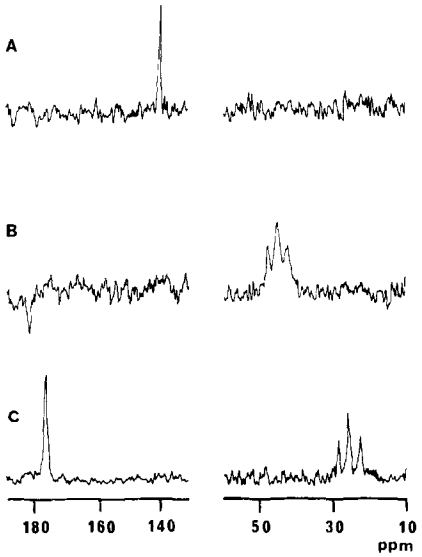

Fig. 1 Proton-coupled N-15 NMR spectra of AP·C duplexes containing N-15 enriched AP or C at pH 8.6, 4°C; A) 98% enriched at N3 of C, B) 98% enriched at the C amino group, and C) N-15 enriched AP (40% N1, 60% amino).

TABLE 1

N-15 Chemical shifts of AP and C (ppm)

	protonated deoxynucleoside (pH 1.5)	neutral deoxynucleoside (pH 7.5)	AP·C duplex pH 8.6
AP N1	93	174	175
AP amino	26	21	26
C N3	85	144	143
C amino	45	35	40

expected for amino nitrogens (12) demonstrating that each exocyclic nitrogen has two attached protons. The ring nitrogens of AP and C are both singlets. If either AP or C were in the imino conformation, both ring and exocyclic nitrogens would appear as doublets.

AP and C are hydrogen bonded in the duplex at pH 8.6.

Previously, James et al. (13) demonstrated that the chemical shifts of nitrogen resonances change upon base pair formation in duplex DNA. The largest effects are observed for those nitrogens which form base-base hydrogens bonds. Recently, Kupferschmidt et al. (14) demonstrated that the amino nitrogen resonance of C shifts downfield 6.6 ppm when paired with guanine. The amino resonance of adenine is reported to move downfield by 1.9 (15) to 2.4 ppm (14) when paired with thymine, depending upon the flanking sequences. We observe, for the AP·C mispair at pH 8.6, resonances of both amino groups shift downfield (AP, +4.5; C, +5 ppm). This data demonstrates hydrogen bonding between AP and C. The only conformation in which AP and C can be neutral, amino and paired is the wobble structure shown in Fig. 2.

Ring nitrogen resonances for adenine (N1) and cytosine (N3) shift upfield by 2 to 10 ppm upon formation of Watson-Crick base pairs (13,15). We observe, for the AP·C pair, that the ring nitrogen resonance of C shifts slightly upfield (-1.0 ppm) whereas the ring nitrogen of AP shifts slightly downfield (+1.0 ppm). In Watson-Crick bases pairs, the ring nitrogens are hydrogen bonded to imino protons. In the AP·C wobble pair hydrogen bonds are formed with the less acidic amino protons, which may explain the reduced magnitude of the observed chemical shift changes.

Conclusion

The N-15 data presented here clearly demonstrate the wobble base pairing of AP and C in a duplex oligonucleotide at pH 8.6. As imino tautomer formation does not involve net gain or loss of protons

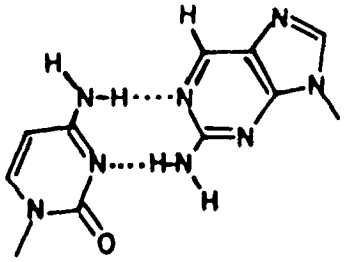

Fig. 2 The wobble base pair formed between AP and C in an oligonucleotide at pH 8.6.

with solvent water, tautomeric equilibrium should be pH independent. Thus, if AP and C are not observed to form a base pair in Watson-Crick geometry involving unpreferred imino tautomeric forms at pH 8.6, it is unlikely that imino tautomers would be observed at lower pH.

The observation that N-15 spectra are lost with decreasing solvent pH indicates that, at lower pH, there are multiple species in exchange with one another. One of the species may include the protonated base pair previously described (7), however, due to line broadening of N-15 resonances with decreasing pH, we are unable to confirm the protonated AP·C pair. In other oligonucleotide sequences containing modified bases, we have been able to follow pH dependent structural changes from one predominant structure to another using proton NMR spectroscopy (16,17).

In addition to a possible pH-dependent conformational change within the A·PC mispair, the oligonucleotide sequence studied here could potentially form additional complex structures. The strand containing five consecutive C residues could self associate to form two G·C and four hemiprotonated C·C base pairs (18,19). The apparent pK value for the hemiprotonated C·C pairs in poly dC is 7.4 (19). Additionally, either the G-rich or C-rich strands could associate with the duplex to form triple stranded structures. The triplex with an additional C-rich strand requires cytosine protonation. The apparent pK value for triplex structures involving protonated cytosines is between 7.0 and 7.4 (20, 21). Work is currently in progress to further delineate the system described here at neutral pH.

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REFERENCES

1. Ronen, A. (1979) Mutation Res. *75*, 1-47.
2. Freese, E. (1959) J. Mol. Biol. *1*, 87-105.
3. Rudner, R. (1960) Biochem. Biophys. Res. Commun. *3*, 275-280.
4. Watanabe, S.M., and Goodman, M.F. (1981) Proc. Natl. Acad. Sci. USA *78*, 2864-2868.
5. Danilov, V.I., Kruglyak, Y.A., Kupriyevich, A.A., and Shiramko, D.V. (1967) Biofizika *12*, 726-719.
6. Sowers, L.C., Shaw, B.R., Veigl, M., and Sedwick, W.D. (1987) Mutation Res. *177*, 210-218.
7. Sowers, L.C., Fazakerley, G.V., Eritja, R., Kaplan, B.E., and Goodman, M.F. (1986) Proc. Natl. Acad. Sci. USA 83, 5434-5438.
8. Sowers, L.C., Mhaskar, D., Khwaja, T., and Goodman, M.F. (1988) Nucleosides and Nucleotides (1989) *8*, 23-34.
9. Eritja, R., Kaplan, B., Mhaskar,D., Sowers, L.C., Petruska, J., and Goodman, M.F. (1986) Nucleic Acids Res. *14*, 5869-5884.
10. Levy, G.C. (1979) in "Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy". Wiley, New York, p.74.
11. Gonnella, N.C., Nakanishi, H., Holtwick, J.B., Horowitz, D.S., Kanamori, K., Leonard, N.J., and Roberts, J.D. (1983) J. Am. Chem. Soc., *105*, 2050-2055.
12. Markowski, V., Sullivan, G.R., and Roberts, J.D. (1977) J. Am. Chem. Soc. *99*, 714-718.
13. James, T.L., James, T.L., and Lapidot, A. (1981) J. Am. Chem. Soc., *103*, 6748-6750.
14. Kupferschmidt, G., Schmidt, J., Schmidt, Th., Fera, B., Buck, F., and Rüterjans, H. (1987) Nucleic Acids Research *15*, 6225-6241.
15. Gao, X., and Jones, R. (1987) Nucleosides and Nucleotides *6*, 433-435.
16. Sowers., L.C., Eritja, R., Kaplan, B.E., Goodman, M.F. and Fazakerley, G.V. (1988) J. Biol. Chem. *263*, 14794-14801.
17. Sowers., L.C., Goodman, M.F., Eritja, R., Kaplan, B.E., and Fazakerley, G.V. (1989) J. Mol. Biol. *205*, 437-447.
18. Gray, D.M., Cui, T., and Ratliff, R.L. (1984) Nucleic Acids Research *12*, 7565-7580.
19. Inman, R.B. (1964) J. Mol. Biol., *9*, 624-637.
20. Povsic, T.J., and Dervan, P.B. (1989) J. Am. Chem. Soc. *111*, 3059-3061.
21. Rajagopal, P. and Feigon, J. (1989) Nature *339, 637-640.*