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Triple Helix Stabilization Properties of Oligonucleotides Containing 8-Amino-2'-Deoxyguanosine

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Abstract : Analysis of the Hoogsteen base pairs of guanine and 8-aminoguanine with cytosine in the gase phase shows a strong stabilization of the 8-aminoguanine•cytosine base pair due to the formation of an extra H-bond. Melting profiles of oligonucleotides carrying 8-amino-2'-deoxyguanosine confirm the triple helix stabilization properties of 8-aminoguanine.

Several years ago, it was shown that oligonucleotides could bind to homopurine-homopyrimidine sequences of double stranded DNA by forming triple helices. The formation of nucleic acid triple helices offers the possibility of designing sequence specific DNA binding molecules, which may have therapeutic uses.[1, 2] A large effort has been devoted on the design and preparation of modified oligonucleotides in order to enhance triple helix binding stability.[1, 2]

Recent results have shown that the introduction of an amino group at position 8 of adenine increases the stability of triple helix due to the combined effect of the gain in one Hoogsteen purine-pyrimidine H-bond,[3-5] and to the ability of the amino group to be integrated into the "spine of hydration" located in the mM groove of the triplex structure.[3, 6] A similar behaviour may result by adding an amino group at position 8 of guanine. 8-Amino-2'-deoxyguanosine can be found in cellular DNA as result of the carcinogenic action of nitroalkanes and it is considered an important intermediate in the mutagenesis caused by these compounds.[7,8] Triple-helix binding properties of 8-aminoguanine (8AG) have been investigated on homopolymers.[9, 10] Poly (8-aminoguanylic) acid did not form triple helix with poly(C) because of the high stability of a hemiprotonated G.G self-structure[9] but a very stable complex was observed between 8-aminoguanosine monophosphate and 2 strands of poly(C).[10] The preparation of oligonucleotides containing 8-amino-2'-deoxyguanosine has been described but the binding properties of 8AG oligonucleotides were not reported.[11] In the present communication we describe the base-pairing properties of 8AG using theoretical calculations and by analysis of the melting profiles obtained with 8AG oligonucleotides. Both methods show that 8AG indeed stabilizes triple helix.

In order to evaluate the stability of the different models of Hoogsteen-like recognition of guanine or 8AG and cytosine, we have computed the base pairing energies, enthalpies (T= 298°K) and free energies (T= 298°K) of the different Hoogsteen base pairs of G or 8AG and C in the gas phase (see Figure 1). All base pairs were fully optimized at the B3LYP/6-31G(d) level[12], the energies being computed at the same level.[13] Frequency analysis (performed also at the B3LYP/6-31G(d) level) were performed to verify the minimum

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nature of the different optimized conformations, as well as to determine thermal, and entropic corrections to the SCF energy. For thermodynamic calculations a standard reference state (ideal gas at P= 1 atm) was used. The basis set superposition errors (BSSE) were corrected using the Counter-Poisse method,[14] and introducing explicitly geometry distortion contributions to binding. Calculation of every ΔE^{SCF} value implies 3 full geometry optimization runs with frequency calculations, plus 4 single point calculations.

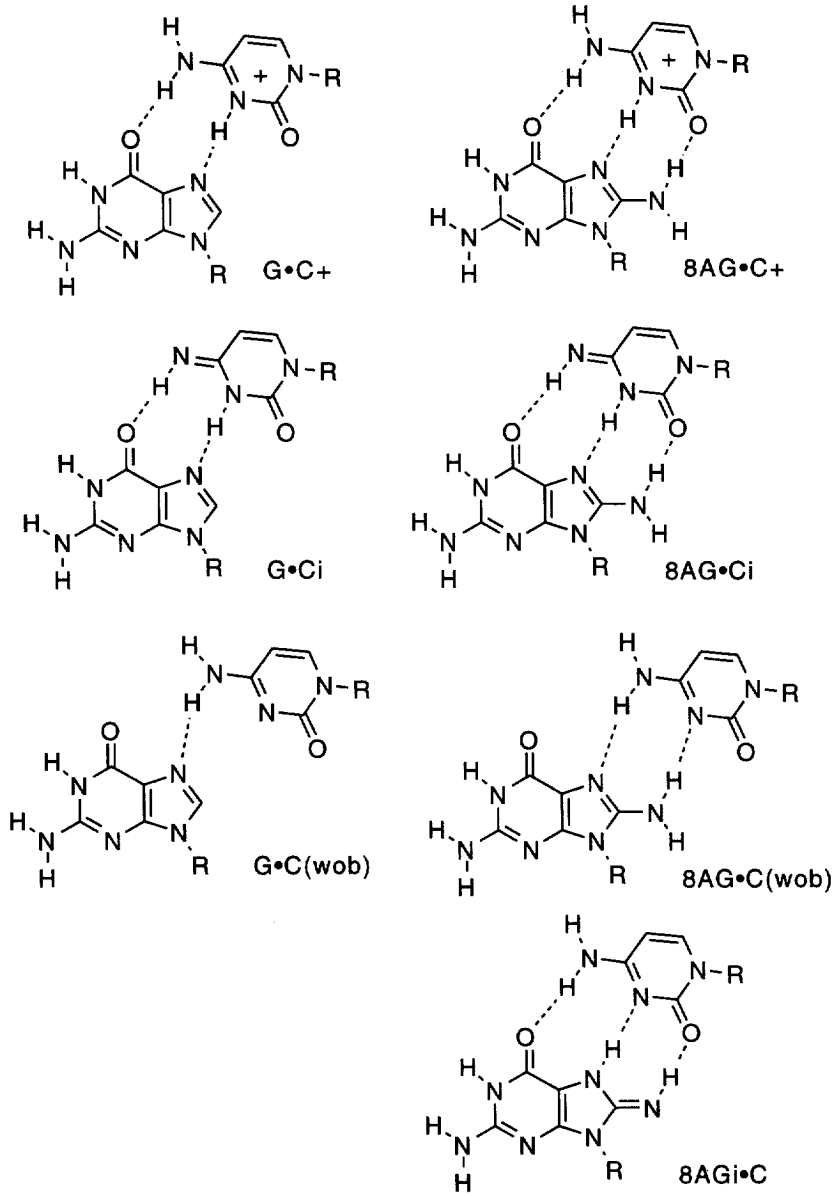

Figure 1: Hoogsteen base pairs between guanine or 8-aminoguanine and cytosine.

B3LYP/6-31G(d) calculations allows to obtain a high quality estimate of base pairing thermodynamics in the gas phase.[15] Such information cannot be translated directly to a DNA environment,[16] but is very useful to determine the intrinsic stability of different H-bonding recognition patterns.

Results in Table 1 and 2 suggest that the Hoogsteen binding of G and 8AG to protonated cytosines are the most stable Hoogsteen base pairs in the gas phase. However, the situation might change in physiological conditions, specially at neutral or basic pH, and for poly-guanine sequences (Soliva *et al.* to be published). In any case, comparison of thermodynamic data for G•C and 8AG•C base pairs with protonated cytosine reveals that the presence of the amino group at position 8 leads to a stabilization of around 4.6 kcal/mol in the free energy of dimerization. Such a stabilization is probably due to the formation of an extra H-bond (see Table 1) between O2(C) and N8(8AG). The formation of this extra H-bond (d(N8-O2)=2.97 Å) leads only to a small distortion in the O6-N4 and N7-N3 distances (around 0.1-0.2 Å, see Table 1).

Table 1. H-bond distances (in Å) in the gas phase for different Hoogsteen complexes of guanine or 8-aminoguanine with cytosine.

Dimer	d(O6-N4)	d(N7-N3)	d(N8-O2)	d(N7-N6)	d(N8-N3)
G•C+	2.50	2.70	-----	-----	-----
8AG•C+	2.69	2.80	2.97	-----	-----
G•Ci	3.62	2.92	-----	-----	-----
8AG•Ci	3.18	2.79	2.89	-----	-----
G•C(wob)	-----	-----	-----	2.97	-----
8AG•C(wob)	-----	-----	-----	2.92	2.98
8AGi•C	2.83	2.96	3.24	-----	-----

As it can be seen in Figure 1, Hoogsteen-binding of G or 8AG with C might be obtained at neutral or basic pH by stabilization of the imino tautomer of C. Thermodynamic data in Table 2 shows that the recognition of G or 8AG to the imino cytosine is exothermic (ΔH from -9 to -14 kcal/mol), but the large magnitude of the entropic term makes the base pairing process non-spontaneous (at the conditions used). Once again the interesting point is to analyze the effect of the mutation from G to 8AG. Results shown in Table 2 demonstrate that such a substitution leads to a stabilization of around 4 kcal/mol in free energy of dimerization. Analysis of Table 1 demonstrate again that this is probably due to the formation of an extra H-bond (d(N8-O2)=2.89 Å).

An interesting possibility for Hoogsteen-like H-bonding of G or 8AG and C in the absence of protonated cytosine is the formation of wobble-Hoogsteen H-bonds as shown in Figure 1. This type of structure can be made using the most stable ionic and tautomeric form of G and C. But, on the other hand they are stabilized by one H-bond less compared with G•C+ or G•C(i) base pairs (see Figure 1 and Table 1). Thermodynamic results in Table 2 demonstrated that this type of wobble recognition is surprisingly stable, even for G, which might be attributed to a certain acidity of H8 which favor a "pseudo H-bond" with O-2 of the Hoogsteen cytosine. Once again, the effect of the amino group at position 8 is the stabilization (around 4.4 kcal/mol from Table 2) of the base pair due to the formation of an extra H-bond.

Table 2. Energies, enthalpies (T= 298°K) and free energies (T= 298°K) of dimerization of different Hoogsteen-type of guanine (or 8-aminoguanine)•cytosine base pairs in the gas phase (reference state: ideal gas at P= 1 atm). All values are in kcal/mol.

Dimer	ΔE_{SCF}	ΔH^{298}	ΔG^{298}
G•C+	-40.98	-39.70	-27.91
8AG•C+	-46.13	-45.11	-32.51
G•Ci	-10.44	-8.80	+1.41
8AG•Ci	-15.64	-13.98	-2.53
G•C(wob)	-11.16	-9.57	+0.32
8AG•C(wob)	-16.37	-14.92	-4.11
8AGi•C	-16.90	-15.11	-2.87

Finally, the last possibility for the stabilization of 8AG•C Hoogsteen base pair involves the presence of the 8-amino tautomer of 8AG (see Figure 1). This type of recognition is not possible for G. Thermodynamic data in Table 2 demonstrate that this base pair is also possible, having a similar stability to the base pair 8AG•C (i).

The last interesting issue raised from results in Tables 1 and 2 is which species stabilize the G•C•C triad in triplex DNA. It seems that under conditions favoring the presence of protonated cytosines, the G•C+ or the 8AG•C+ base pairs are the most stable. However, what happens where the sequence or pH makes the existence of protonated cytosine very unlikely?.

Results in Tables 1 and 2 suggests that all the three binding models studied here are possible, and probably contribute to the stability of the base pair. However, the best base pairing are obtained for the wobble pair, rather than for the base pairs with imino tautomers. This result is surprising since *a priori* the binding with imino tautomers allows the formation of a greater number of H-bonds (see Figure 1). However, inspection of Table 1 demonstrate that, the H-bonds involving imino groups are weak, as seen on the O6-N4 distance for G•Ci and 8AG•Ci and the N8-O2 distance for 8-AGi•C.

It is interesting to realize that the base pairing of imino species implies also an extra penalty due to their intrinsic lower stability. Thus, according to our B3LYP/6-31G(d) calculations the imino tautomers are less stable than the amino tautomers ($\Delta\Delta G_{taut}$ = 3.5 kcal/mol for C and 4.5 kcal/mol for 8AG, similar results were obtained using high level ab initio methods[17]). It seems that, in the gas phase, the wobble binding would be favored by 5-6 kcal/mol (G•Ci) compared with the imino binding models. It needs to be determined, if this also happens under physiological conditions, and whether the distortion in the structure needed for accommodating a wobble pairing can be tolerated by the triple helix.

Preliminary results presented here should be taken with caution, since the study is focused only in intrinsic binding preferences, neglecting, solvent and steric effects which are expected to have a key role in the Hoogsteen G•C recognition in the triplex. However, our results are clear enough as to predict that the presence of an amino group at position 8 of guanine leads to a strong stabilization of around 4-5 kcal/mol in the free

energy of Hoogsteen-base pairing of G and C in the gas phase. Based in our experience[3] this stabilizing effect is going to be slightly reduced when the DNA environment is considered.

The stabilization obtained by the presence of the 8-amino group is similar for neutral and protonated base pairs, even in percentage is much larger for the neutral forms of the base pairs. Finally, our theoretical results have raised the possibility that G•C•C triads could be stabilized at neutral or basic pH due to the existence of a G•C wobble Hoogsteen pairs. Calculations currently performed will determined whether or not this suggestion is compatible with the DNA triplex structure.

In order to confirm the triple helix stabilizing properties of 8AG predicted by calculation, oligonucleotide carrying 8AG were synthesized. The phosphoramidite derivative of 8-amino-2'-deoxyguanosine (**1**) protected with dimethylaminomethyliden (dmf) groups was prepared as previously described.[11] In order to analyze the stability of the dmf groups to ammonia the dimer 5' **1**T 3' was prepared. The dimer was assembled on controlled-pore glass using standard phosphoramidite protocols. Aliquots of dinucleotide-supports were treated with concentrated ammonia at room temperature and at 55°C. HPLC analysis of the resulting products followed by mass spectrometry analysis showed a complex deprotection pattern. One dmf group is removed rapidly but the second dmf group needed a long treatment (at least 1 day) at 55°C. Other minor products were also observed. When ammonia treatment is performed at 55°C the desired compound is obtained[18] together with small amounts of degradation products that became more evident if the deprotection was prolonged for 2 or 3 days at 55°C. For these reasons the deprotection conditions used for oligonucleotides containing 8AG were at 55°C for 1 day.

Oligonucleotide sequences containing 8-amino-G A (5' AG**1**CT 3'), B (5' GCAATGGA**1**CCTCTA 3') C (5' GAAG**1**AGGAGATTTTTCTCCTCCTTC 3') and D (5' GAAG**1**A**1**GA**1**ATTTTTCTCCTCCTTC 3') were prepared using phosphoramidite chemistry on an automatic DNA synthesizer. After deprotection with concentrated ammonia the products were purified by reverse phase HPLC using the DMT on and DMT off protocols. In all cases a major peak was obtained and collected. The purified oligonucleotides were analyzed by mass spectrometry (electrospray and MALDI-TOF) and by enzyme digestion followed by HPLC analysis of the resulting nucleosides. Purified oligonucleotides presented the correct nucleoside composition and the expected mass.[18]

Duplexes having 8AG base pairs with the four natural bases were analyzed.[19] As expected the most stable base pair is formed between the guanine derivative and cytosine and the stability of the 8AG base pairs are very similar to the stability found in G base pairs.

Table 3 : Melting temperatures* (°C) for the triplex h_{26}:s_{11} containing 8-amino-2'-deoxyguanosine.

h_{26}: 5'GAAG-XAXGAX-ATTTTTCTCCTCCTTC3'

s_{11}: 3'CTTCCTCCTCT5'

Sequence	-xaxgax-	pH 5.5	pH 6.0	pH 6.5	pH 7.0
h_{26}:s_{11}	-gaggag-	40	≈20	--	--
C:s_{11}	-**1**aggag-	50	35	25	--
D:s_{11}	-**1**a**1**ga**1**-	59	47	40	32

*1 M NaCl, 100 mM sodium phosphate/ citric acid buffer. The duplex Tm values of h_{26} occurred between 82 °C and 75°C.

On the other hand, triple helix stabilization properties of 8AG have been investigated using a triple helix model formed by a self-complementary hairpin of 26 bases (h_{26}) and a all pyrimidine single stranded oligonucleotide (s_{11}) described previously.[20] Substitution of G by 8AG in triple helix results in a stabilization of 5-8°C per base in the range from pH 5.5 to pH 7.0 (see table 3). The degree of stabilization is of the same order of magnitude than the one found in oligonucleotides containing 8-aminoadenine.[3, 4] As a consequence of the stabilizing properties of 8AG, it is possible to observe triple helical structures at neutral pH which is not possible with natural bases.

In conclusion, oligonucleotides carrying 8AG residues form stable triple helices in agreement with the formation of a third hydrogen bond between C and the amino group introduced in position 8. These results confirm that substitution of position 8 of purines by amino compounds leads to new products with important triple helix stabilization properties. Since this modification is introduced in the target strand, the significance of this modification in "antigene strategy" may be limited.

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18. Mass spectra (electrospray) of dimer 1T: 586.2 (expected for $C_{20}H_{27}N_8O_{11}P$: 586.4), pentamer A: 1518.2 (expected for $C_{49}H_{63}N_{21}O_{28}P_4$: 1517.9), pentadecamer B: 4591.1 (expected for $C_{146}H_{185}N_{59}O_{87}P_{14}$: 4591.4).

19. Melting temperatures of 8AG duplexes (0.15 M NaCl, 50 mM Tris-HCl buffer pH 7.5) were measured in the following duplex TAG AGG XTC CAT TGC 3' • 3' ATC TCC YAG GTA ACG 5'. The Tm (°C) were: X=G Y=C 61, X=8AG Y=C 61, X=G Y=A X=8AG Y=A 54, X=G Y=G 57, X=8AG Y=G 55, X=G Y=T 52, X=8AG Y=T 52.

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