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"Effects of Abasic Sites on Triple Helix Formation Characterized by Affinity Cleaving"

by

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Effects of an abasic site on triple helix formation characterized by affinity cleaving

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ABSTRACT

The stability of triple helical complexes between oligodeoxyribonucleotides containing one or two abasic 1,2-dideoxy-D-ribose (Ø) residues bound to single 15-17 base pair sites within short duplex (30 mer) or plasmid DNA (4.9 kbp) was examined by affinity cleaving. The triplets Ø·AT, Ø·GC, Ø·TA and Ø·CG are significantly less stable than triplexes having the matched counterparts, T·AT, C + GC and G·TA. Generally, the decrease in binding produced by an abasic residue is at best equivalent to that observed with imperfectly matched natural base triplets with Ø·AT and Ø·GC being less stable than Ø·TA and Ø·CG triplets.

INTRODUCTION

Oligodeoxyribonucleotide-directed triple-helix formation offers a chemical approach for the sequence-specific binding of double-helical DNA that is 10^6 times more specific than restriction enzymes. (1-4) Because triple-helix formation by pyrimidine oligodeoxyribonucleotides is limited to mostly purine tracts (T·AT, C + GC triplets), it is desirable to find a general solution whereby oligodeoxyribonucleotides (or other analogs) could be used to bind all four base pairs of intact duplex DNA (37°C, pH 7.0). Approaches toward such a goal include the search for other natural triplet specificities (5,6), oligonucleotides capable of binding alternate strands of duplex DNA by triple-helix formation (7), the design of nonnatural bases for completion of the triplet code, and the incorporation of universal bases for nonreading of certain base pairs.

1,2-Dideoxy-D-ribose (Ø), possessing a furanose ring with the same stereochemistry at C-3' and C-4', as that of the natural nucleosides would substitute a hydrogen atom in place of a base in a triple helical complex. We address here the issue whether this abasic site in the third strand of a local triple helix could be used as a null position. Recent, calorimetric data suggest that the enthalpy for a T·AT and C + GC triplet is 2.2 kcal/mol. (8) The incorporation of a 1,2-dideoxy-D-ribose (Ø) residue in the third strand would result in the loss of two stacking interactions which flank the modified position as well as base pairing at that location in the major groove of DNA. The influence of abasic sites in Watson-Crick double helical DNA has been characterized by spectroscopic and calorimetric techniques. (9-12) Two of these studies conclude that the abasic site is a highly localized lesion that does not alter the overall duplex structure or significantly perturb flanking base pairs. (10-11) The third, however, reveals a sequence dependent looping out of pyrimidine bases and abasic residues when placed across each other. (12) In all cases, a global decrease in Tm values is observed. We report the effect on the stability of pyrimidine oligonucleotides containing abasic (Ø) sites on triple helix formation characterized by affinity cleaving. The relative stabilities of Ø·AT, Ø·GC, Ø·TA and Ø·CG with the other 16 possible triplets are compared.

MATERIALS AND METHODS

Synthesis of EDTA·Oligodeoxyribonucleotides

The fully protected oligodeoxynucleotides (1μMol) were synthesized on a Beckman System 1 Plus oligonucleotide synthesizer using standard β-cyanoethyl phosphoramidite chemistry. The 5'-O-DMT-protected thymidine EDTA (T*) triethylester (13) and 1,2-dideoxy-D-ribose (Ø) nucleoside (14) phosphoramidites were prepared according to published methods. Deprotection of the polymer-bound oligonucleotide and ethylester hydrolysis were accomplished by treatment of the support with 0.1M NaOH (1.5mL, 55°C, 24 h). The supernatant was neutralized (~6μL of acetic acid), desalted (Sephadex G10-120) and lyophylized. Purification of the crude DNA EDTA·oligonucleotides by denaturing 20% polyacrylamide gel electrophoresis produced one major band by UV shadowing. The band was excised and eluted with 0.2 M NaCl containing 1mM EDTA for 24h at 37°C. Filtration followed by extensive dialysis (5 days, 4°C) gave pure EDTA·oligodeoxyribonucleotides 1-7 in 20-25% yield.

Cleavage of Oligonucleotide 30-mer Duplexes

The cleavage reactions were carried out by combining a 2 min preincubated mixture of oligonucleotide·EDTA (1μM), spermine (1mM), and Fe(II) (25μM, $Fe(NH_3)_2(SO_4)_2 \cdot 6H_2O$) with the 5' ^{32}P-labeled 30-oligomer duplex (0.5 μM in base pairs) in a solution of tris-acetate, pH 7.0 (25 mM), NaCl (100 mM), calf thymus DNA (100μM in base pairs) and 40% ethanol. After equilibration at 27°C for 1h the cleavage reactions were initiated by addition of dithiothreitol (DTT) (4 mM) giving a total reaction volume of 20μL. The reactions were allowed to proceed for 8h (27°C), stopped by freezing and lyophylization and the cleavage products were analyzed by gel electrophoresis. Individual bands were quantitated by densitometry.

Cleavage of Linear Plasmid DNA

pHIV-CAT DNA (4.95 kpb) was Bam H1 linearized and 3'-end-labeled at both ends. The cleavage reactions were carried out by combining a mixture of oligonucleotide·EDTA (1μM), spermine·4HCl (1mM) and Fe(II) with the ^{32}P-labeled linearized plasmid (0.1 μM m base pairs) in a solution of tris-acetate, pH 7.0 (50 μM), NaCl (100 mM), calf thymus DNA (100 μM in base pairs) and 10% ethanol. The mixture was equilibrated for 1h at the reaction temperature indicated. Cleavage reactions were initiated by addition of ascorbate (1mM) and allowed to proceed 18h at 0° and 23°C. Ethanol precipitation followed by 1% agarose gel electrophoresis separated the double strand cleavage products.

RESULTS AND DISCUSSION

Cleavage of Oligonucleotide 30-mer Duplexes. Binding Affinities of Containing Abasic Triplets.

The relative affinity of the abasic (Ø) nucleoside for all four base pairs within a pyrimidine triple helix motif was examined by affinity cleaving. Oligonucleotides **1-5** equipped with the DNA cleaving moiety, EDTA·Fe(II) (T*) at a single thymidine position and differing at one base position $d(T_7NT_7)$ [N = T, C, A, G, or Ø were prepared in order to compare the relative stabilities of triple helix formation

with 30-bp DNA duplexes containing the 15 base pair target sites $d(A_7XA_7)\cdot d(T_7YT_7)$ (XY = AT, GC, CG, or TA) (Fig 1A). The 30-bp oligodeoxyribonucleotide duplexes were labeled with ^{32}P at the 5' end of the Watson-Crick target-site strand $d(T_7YT_7)$. Reaction conditions were chosen to distinguish between stabilities of the variable base triplets (pH 7.0, 27°C, 40% ethanol). (1,4) The most intense cleavage patterns were observed for oligonucleotides containing the base triplets TAT, CGC and GTA at the variable position (Fig. 1, lanes 3, 8 and 14). In contrast, oligonucleotide 5 containing 1,2-dideoxy-D-ribose residue (Ø) produced moderate to weak cleavage indicating overall weaker binding, presumably due to loss of base stacking and hydrogen bonding (Fig. 2). Similar results are observed for the thirteen additional triplet mismatches.

Within the pyrimidine triple helix motif there exists a preference for TA and CG over AT and GC base pairs across the basic site (Ø·TA, Ø·CG > ØAT, ØGC) suggesting that local conformational effects may play a role in overall triplex stability (Fig. 1B, lanes 19-22 and Fig. 2). The decrease in cleavage for N·TA (N = A,C and T) and N·CG (N = A,G and T) triplet imperfections relative to Ø·TA and Ø·CG sites suggests that further destabilizing (steric) interactions exist between certain mismatched bases (Fig. 2). In contrast, an abasic site across GC (ØGC) destabilizes triplex formation to a significantly greater extent than a T·GC triplet. Finally, examination of the cleavage ratio for triplexes containing G·TA versus Ø·TA demonstrates that G contributes a positive interaction across TA base pairs rather than effectively being the most tolerable of triplet imperfections (Fig. 1A, lanes 14 and 22 and Fig. 2). This confirms our hypothesis that both base stacking and hydrogen bonding are implicated in stabilizing the G·TA triplet within the pyrimidine motif (4). It should be noted that the stability of the G·TA triplet is dependent on flanking sequences. We would anticipate that nearest neighbor interactions will be important for abasic sites in the third strand.

Site-Specific Cleavage of pHIV-CAT

In order to study the effect of local triple helical interactions at a single binding site within large pieces of DNA, the binding of a 17 nt oligonucleotide to a 17 base pair sequence in 4.9 kilobase pair DNA was examined. The plasmid pHIV-CAT was digested with Bam HI to produce a 4.95-kbp fragment which contains the 3' LT of HIV with the 17 bp site d(AGATAAGATAGAAGAGG) located 1.54 and 3.41 kbp from the ends (Fig. 3). The ^{32}P end-labeled DNA was allowed to react

with Fe(II)·EDTA-oligonucleotides **6** and **7** in the presence of ascorbate at 0° or 23°C (pH 6.2 to 7.0). Separation of the cleavage products by agarose gel electrophoresis revealed one major cleavage site producing two DNA fragments, 1.54 and 3.41 kbp in size (Fig 3A). Both oligonucleotides **6**, containing two GTA triplets, and to a lesser extent **7**, containing two ØTA triplets, produced cleavage at pH 6.2, 0 °C (lanes 3 and 7). However, with an increase of 0.4 pH units only oligonucleotide **6** generated cleavage. Oligonucleotide **7**, which maintains a high degree of specificity, possesses two abasic sites that results in a significant decrease in binding affinity.

CONCLUSION

Analogous to duplex DNA, it is apparent that the stability of a triplex is dependent upon base stacking contributions as well as base pairing of its composite bases. (8) The substitution of a hydrogen atom for the proper base is not sufficient in maintaining overall stability of the triplex which implies that an abasic site would unlikely be used as a universal base for triple helix formation. More appropriately, an abasic residue will serve as a diagnostic tool in conjunction with other bases and synthetic heterocycles for characterizing key elements of novel base triplets. Characterization of the abasic site by direct physical methods (such as ^{1}H NMR) and calorimetric analyses remain to be elucidated.

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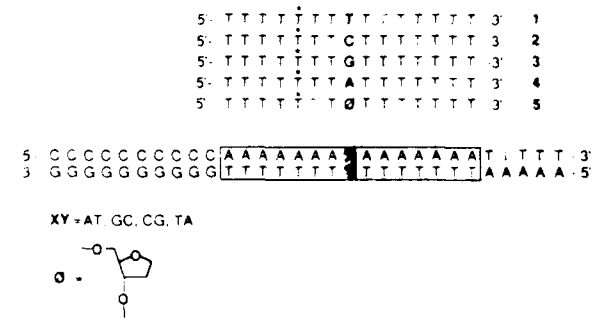
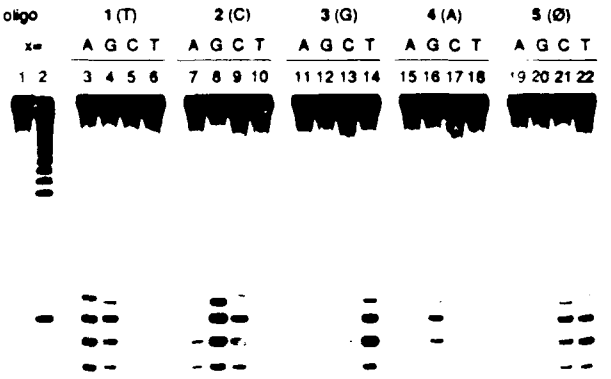
FIGURE LEGENDS

Figure 1 (Top) Sequence of oligonucleotide-EDTA 1- 5, where T* is the position of thymidine-EDTA. The oligonucleotides differ at one base position indicated in bold type. DNA cleavage pattern derived by densitometry of the autoradiogram. The heights of the arrows represent the relative cleavage intensities at the indicated bases. The box indicates the double stranded sequence bound by oligonucleotide-EDTA·Fe 1 - 5. The Watson-Crick base pair (AT, GC, CG, or TA) opposite the variable base in the oligonucleotide is shaded. (Bottom) Autoradiogram of the 20% denaturing polyacrylamide gel. (Lanes 1 to 22) Duplexes containing 5' end-labeled $d(A_5T_7YT_7G_{10})$. (Lane 1) Control showing intact 5' labeled 30-bp DNA standard obtained after treatment according to the cleavage reactions in the absence of oligonucleotide-EDTA; (lane 2) products of Maxam-Gilbert G + A sequencing reaction; (lanes 3 to 22) DNA cleavage products produced by oligonucleotide-EDTA·Fe(II) 1 to 5; 1 (lanes 3 to 6), 2 (lanes 7 to 10), 3 (lanes 11 to 14), 4 (lanes 15-18), 5 (lanes 19-22). XY = AT (lanes 3,7,11,15 and 19); XY = GC (lanes 4,8,12,16 and 20); XY = CG (lanes 5,9,13,17 and 21); XY = TA (lanes 6,10,14,18 and 22).

Figure 2 Histograms depicting relative cleavage intensities (normalized) for the twenty base triplets. The data are obtained from scintillation counting and densitometric analysis of the autoradiogram shown in Figure 1B.

Figure 3(A) Illustration of the triple helix complex between a single site in pHIV-CAT and oligonucleotide-EDTA·Fe **6** or **7** located 1.54 and 3.41 kbp from the ends. **(B)** Autoradiogram of double strand cleavage of pHIV-CAT (4.95 kbp) analyzed on a 1% agarose gel. (lane 1) Controls containing Bam H1 linearized pHIV-CAT and 3'end- labeled at both ends control without oligonucleotide-EDTA·Fe(II); (lane 2) DNA size markers obtained by digestion of Bam H1 linearized pHIV-CAT with Hind III Xho 1: 4950 (undigested DNA), 3725, 3003, 1947, 1225; (lanes 3 to 6 and 7 to 10) DNA cleavage products produced by oligonucleotide-EDTA·Fe(II) **6** and **7**, respectively; (lanes 3, 4, 7 and 8) at 0°C, (lanes 5, 6, 9 and 10) at 23°C, (lanes 3, 5, 7, and 9) at pH 6.2, (lanes 4, 6, 8, and 10) at pH 6.6.


5' T T T T T T T T T T T T T T T 3' 1
5' T T T T T T T C T T T T T T T 3' 2
5' T T T T T T T G T T T T T T T 3' 3
5' T T T T T T T A T T T T T T T 3' 4
5' T T T T T T T Ø T T T T T T T 3' 5
5' C C C C C C C C C C A A A A A A A A A A A A A T T T T 3'
3' G G G G G G G G G G T T T T T T T T T T T T T A A A A 5'
XY = AT, GC, CG, TA
Ø =


oligo
1 (T)
2 (C)
3 (G)
4 (A)
5 (Ø)
x=
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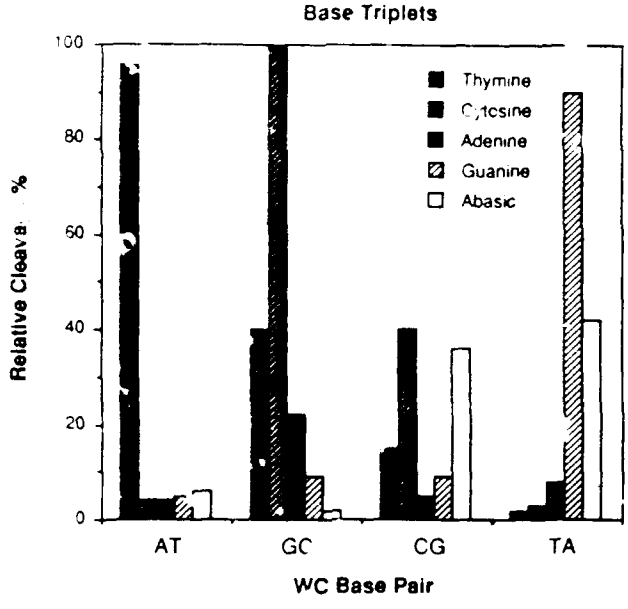
Base Triplets
100
80
60
40
20
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Relative Cleava %
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Cytosine
Adenine
Guanine
Abasic
AT
GC
CG
TA
WC Base Pair

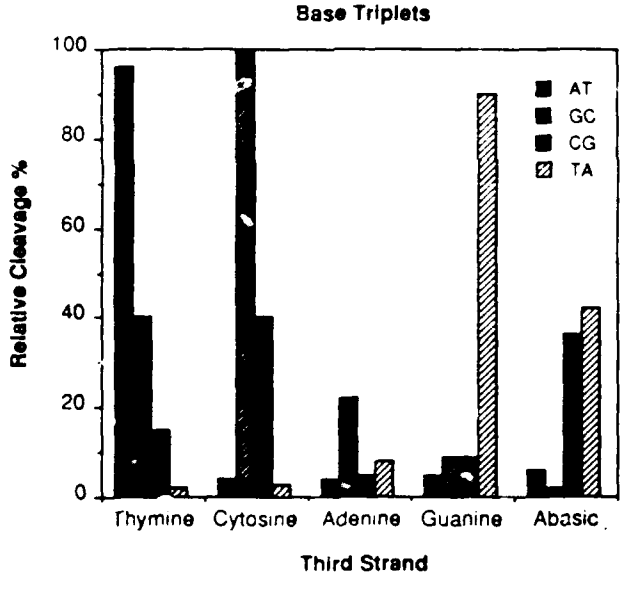
Base Triplets
100
80
60
40
20
0
Relative Cleavage %
AT
GC
CG
TA
Thymine
Cytosine
Adenine
Guanine
Abasic
Third Strand

FIG 2

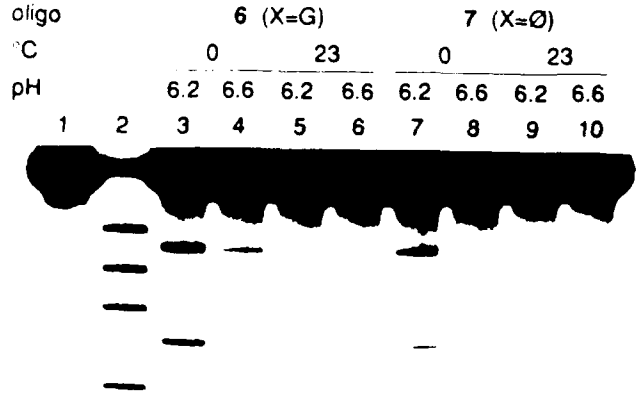

FIG 3A

5'-Ṫ C T G T T C T G T C T T C T C C - 3' 6

5'-Ṫ C T Ø T T C T Ø T C T T C T C C - 3' 7

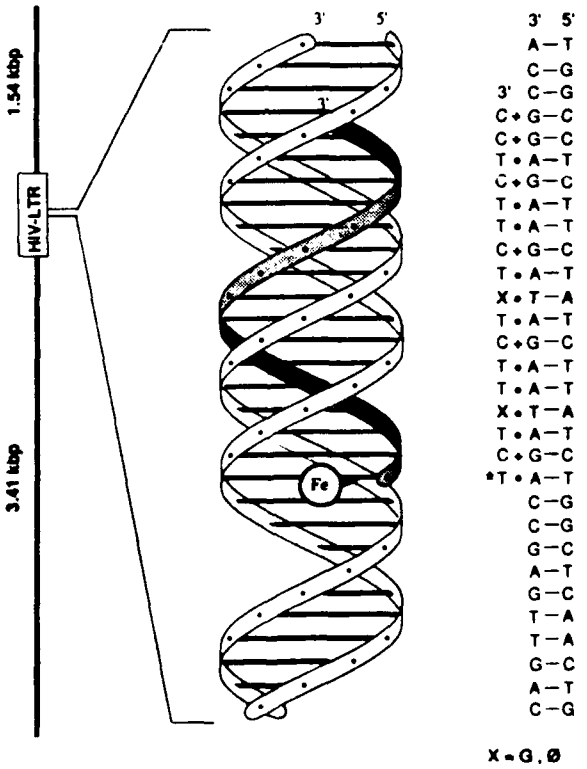

FIG 3B