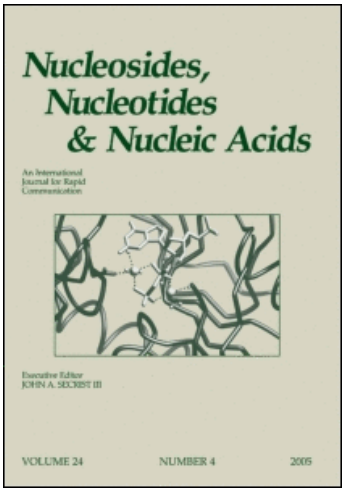

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TRIPLE HELIX BINDING OF OLIGODEOXYRIBONUCLEOTIDES CONTAINING 8-OXO-2′-DEOXYADENOSINE

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Abstract: Formation of a triple helix between an oligodeoxyribonucleotide containing 8-oxo-2′-deoxyadenosine and a DNA duplex target was studied as a function of pH. Above pH 7.4, the triple helix with the 8-oxo-2′-deoxyadenosine was more stable than the complex with an analogous oligodeoxyribonucleotide containing deoxycytidine instead of 8-oxo-2′-deoxyadenosine.

Introduction

Sequence-specific recognition of double stranded DNA by oligodeoxyribonucleotides is possible in special cases via the formation of triple helices.[1,2] These are readily formed when the DNA duplex contains a polypurine stretch. The triple helix involves Hoogsteen base pairing between a polypyrimidine oligodeoxyribonucleotide and the polypurine strand in double-stranded DNA via the triplets TAT and C^+GC. The third strand (i.e. the pyrimidine strand) binds in the major groove of the DNA parallel to the purine strand.

One problem with this approach is the requirement for a protonated deoxycytidine in the third strand to bind to G in the duplex and a following decreased stability of the triplex under physiological conditions.[3] Consequently, there have been a number of attempts to develop analogs of protonated deoxycytidine that can bind in a Hoogsteen sense to the GC base pair,[4,5] thereby allowing the formation of triple helices with duplex DNA containing both AT and GC base pairs at physiological pH. Recent publications on the use of N^6-

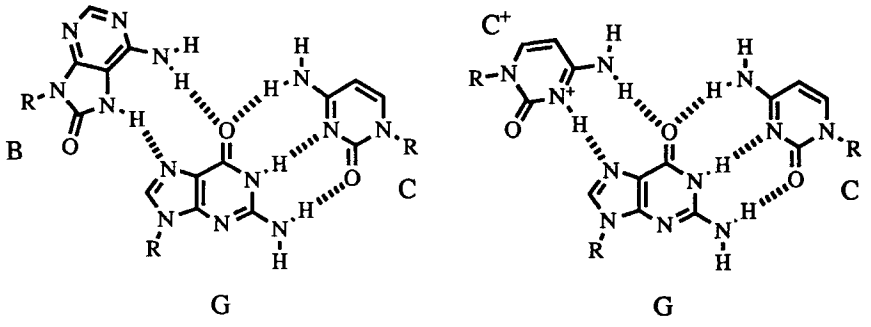

FIG. 1: Binding schemes for BGC and C+GC triplets.

methyl-8-oxo-2′-deoxyadenosine[6,7] and 8-oxo-2′-deoxyadenosine[8] as analogs of protonated cytosine prompts us to communicate our work on 8-oxo-2′-deoxyadenosine (B). We show here that B forms triple helices with duplex DNA containing GC base pairs. The putative binding scheme of 8-oxo-2′-deoxyadenosine, known to adopt predominantly the syn conformation,[9] in the BGC triplet is shown in Fig. 1.

Materials and Methods

Synthesis of the oligodeoxyribonucleotides

For the incorporation of 8-oxo-2′-deoxyadenosine into oligodeoxyribonucleotides via the cyanoethyl phosphoramidite method, 5′-O-(4,4′-dimethoxytrityl)-N^6-phenoxyacetyl-8-oxo-2′-deoxyadenosine 3′-[(2-cyanoethyl)-N,N-diisopropyl-phosphoramidite] was synthesized from 2′-deoxyadenosine following a modified procedure of Guy *et al.*[10] in an overall yield of 16% .

^{1}H-NMR (400 MHz, $CDCl_3$, 2 diastereomers): δ 9.32 (bs, 1 H, NH), 9.00 (bs, 1 H, NH), 8.23 (s, 0.5 H, H-(C2), isomer 1), 8.22 (s, 0.5 H, H-(C2), isomer 2), 7.43-6.72 (m, 18 H, arom. H), 6.42-6.38 (m, 1 H, H-C(1′), isomers 1 and 2), 4.94 -4.78 (2m, 1 H, H-C(3′), isomers 1 and 2), 4.67 (s, 2 H, $PhOCH_2$-), 4.24-4.18 (m, 1 H, H-C(4′)), 3.89-3.72 (m, 1H), 3.77-3.75 (m, 6 H, $(CH_3O)_2Tr$), 3.71-3.55 (m, 3 H), 3.44-3.38 (m, 1 H), 3.37-3.26 (m, 2H), 2.62-2.59 and 2.48-2.44 (2m, 2 H, CH_2CH_2CN, isomers 1 and 2), 2.39-2.27 (m, 1 H, H-C(2′)), 1.19-1.11 (m, 12 H, CH_3 of i-Pr)

^{31}P-NMR (161.9 MHz, $CDCl_3$, 2 diastereomers): δ 149.1, 148.9

Oligodeoxyribonucleotides were synthesized on an Applied Biosystems DNA Synthesizer using the solid-phase cyanoethyl N,N-diisopropyl phosphoramidite chemistry on a 1 μmol scale. The deprotected oligodeoxyribonucleotides were purified by HPLC on a reverse phase column (Aquapore Rp 300, 220 x 4.6 mm, 7 μm, Brownlee Labs) using gradients prepared from 0.1 M triethylammonium acetate, pH 7 (solvent A) and 0.1 M triethylammonium acetate, pH 7, containing 80 % acetonitrile (solvent B). The purified oligodeoxyribonucleotides were subsequently desalted using Sep-Pak C18 cartridges (Waters Millipore). The nucleoside composition of the oligodeoxyribonucleotides were confirmed by digestion of the oligodeoxyribonucleotides (~0.5 OD-units) with snake venom phosphodiesterase (10 μg, Boehringer Mannheim) and alkaline phosphatase (5 μg, Boehringer Mannheim) in 0.1 M Tris, 20 mM $MgCl_2$, pH 8.2 at 37 °C for 2 h and direct analysis by HPLC on a reverse phase column (Aquapore Rp 300, 220 x 4.6 mm, 7 μm, Brownlee Labs) using gradients prepared from 0.1 M triethylammonium acetate, pH 7 (solvent A) and acetonitrile (solvent B). Extinction coefficients of the oligodeoxyribonucleotides were determined by calculating the theoretical extinction coefficients as the sum of the nucleosides and multiplying with the experimental determined enzymatic hypochromicity.[11] The enzymatic hypochromicity was determined by digestion of the oligodeoxyribonucleotides (~0.3 OD-units) at 37 °C in 0.1 M Tris, 20 mM $MgCl_2$, pH 8.2 with snake venom phosphodiesterase (10 μg) and alkaline phosphatase (5 μg) until the absorbance at 260 nm was constant (about 1 h).

UV Measurements

UV absorbance versus temperature profiles were obtained on a Varian DMS 300 spectrophotometer at 260 nm. The cell holder was warmed at a rate of 0.5 °C/min. using a Colora thermostat equipped with a Colora PG6 programming unit. The temperature in the cell holder was monitored using a Kane-May 1201 thermocouple. The measurements were performed in 1 M NaCl, 10 mM sodium phosphate buffer at pH values of 6.35, 7 and 7.75. The concentrations of the oligodeoxyribonucleotides were 1.46 μM per strand. The melting points (T_m) were determined graphically as the midpoints of the melting curves. Mixing curves were recorded on a Shimadzu 240 UV spectrophotometer at 260 nm at 18 °C. The measurements were performed in 1 M NaCl, 10 mM sodium phosphate, pH 7. The mixing curve for the triplex was measured by adding aliquots of a solution of the third strand to a solution of the duplex (2

```
1    d-3'-C G T  T T C  T T T  C T T  T T C  T T G C-5'
2    d-5'-G C A  A A G  A A A  G A A  A A G  A A C G-3'
3       d-5'-T  T T B  T T T  B T T  T T B  T T-3'
4       d-5'-T  T T C  T T T  C T T  T T C  T T-3'
5       d-5'-T  T T A  T T T  A T T  T T A  T T-3'
                    *         *          *
```

FIG. 2: Sequences of the oligodeoxyribonucleotides. Oligodeoxyribonucleotides **1** and **2** form the target duplex. Oligodeoxyribonucleotides **3-5** are the third strand for the triplex. Positions containing B, C or A in the triplex strand are indicated.

μM) in a 1 cm path length cuvette. After each addition and mixing was completed, the resulting solution was equilibrated at 18 °C for 30 min. before the absorbance was measured. The mixing curve for the duplex was measured in a similar manner.

Results and Discussion

To examine the capability of oligodeoxyribonucleotides containing 8-oxo-2′-deoxyadenosine (B) to form BGC triplets in a triple helix five oligodeoxyribonucleotides were synthesized (Fig. 2).

The ability of oligodeoxyribonucleotides to form triple helices was examined by melting temperature studies. Fig. 3 shows the UV absorbance versus temperature profile for a mixture of the oligodeoxyribonucleotides **1,2** and **3** and **1,2** and **5**. In the case of the complex **1·2·3**, two resolved transitions were observed. The transition at higher temperature had a melting point identical to that of a mixture of **1** and **2** alone and was therefore assigned to the melting of the duplex. The stoichiometry of the complex formed by combining **1** and **2** was determined by UV mixing curves,[12] and had the expected 1:1 ratio. The stoichiometry of the complex formed by addition of **3** to the duplex formed by **1** and **2**, determined by UV mixing curves, showed also the expected 1:1 ratio. The UV melting profiles for a mixture of the oligodeoxyribonucleotides **1,2** and **4** showed also two well-resolved transitions. The second transition again was assigned to the melting of the duplex. The first transition exhibited the expected pH-dependence. UV melting profiles for a mixture of the

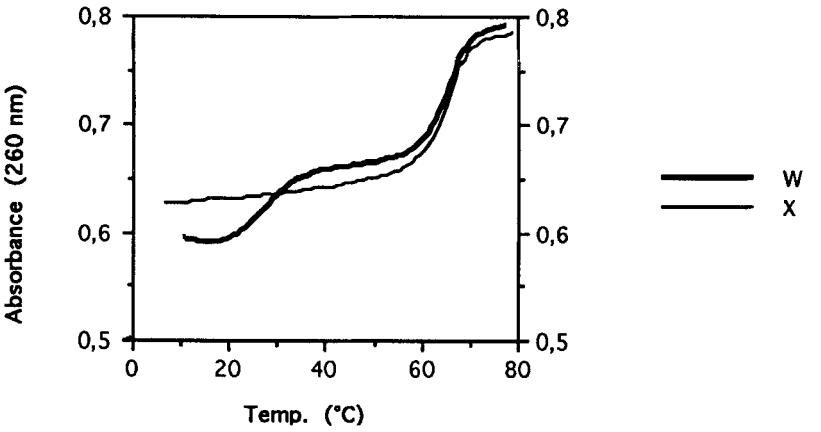

FIG. 3: UV absorbance versus temperature profile for the complexes **1·2·3** (W) and **1·2·5** (X) at pH 7, 1 M NaCl, 10 mM sodium phosphate.

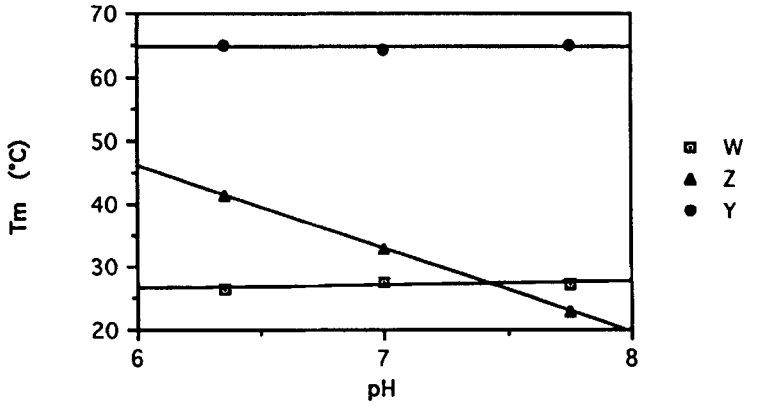

FIG. 4: pH-profile of T_m for the complexes of **1·2·3** (W), **1·2·4** (Z) and **1·2** (Y).

oligodeoxyribonucleotides **1**,**2** and **5** (see Fig. 3) showed only one transition, again the melting of the duplex.

Based on these observations we assigned the first transition, observed when combining the duplex with either **3** or **4**, to the melting of the corresponding triplex. Fig. 4 shows the pH-profile of the melting temperature of the complexes of **1·2·3**, **1·2·4** and **1·2**. In contrast to the oligodeoxyribonucleotide containing deoxycytidine the oligodeoxyribonucleotide containing 8-oxo-2'-

deoxyadenosine binds in a pH-independent manner to the target duplex. Above a pH of 7.4 there is a tighter binding of **3** compared to **4** to the duplex. The lower affinity of **3** compared to **4** at a pH below 7.4 might be due to the fact that the geometry of the hydrogen bonding in the BGC triplet is not as good as the one of the C^+GC triplet.

Krawczyk *et al.*[7] studied the ability of triplex formation of oligodeoxyribonucleotides containing either deoxycytidine, 5-methyl-deoxycytidine,[5] deoxyguanosine[13] or N^6-methyl-8-oxo-2´-deoxyadenosine under intracellular salt and pH conditions to a target duplex with a higher GC to AT base pair ratio than in the present study. They showed a much higher affinity of the oligodeoxyribonucleotide containing N^6-methyl-8-oxo-2´-deoxyadenosine to the target duplex compared to the oligodeoxyribonucleotides containing the other nucleotides. In a related study Koh and Dervan demonstrated[4b] that the use of their designed analog is superior to protonated deoxycytidine especially for the recognition of contiguous GC base pairs in the target duplex. In accordance with our results Miller *et al.* [8] showed that a more stable triple helix forms at pH 7 between the target duplex with a lower GC to AT base pair ratio and a third strand containing deoxycytidine and 5-methyldeoxycytidine instead of B.

Which of the bases is best suited for the recognition of GC base pairs by triple helix formation, either protonated deoxycytidine or one of the analogs presented so far ,[4-7] will probably depend on the conditions and properties of the target duplex.

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