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RECOGNITION OF A G-C BASE PAIR BY α-N^7-DEOXYINOSINE WITHIN THE PYRIMIDINE-PURINE-PYRIMIDINE DNA TRIPLE HELICAL MOTIF

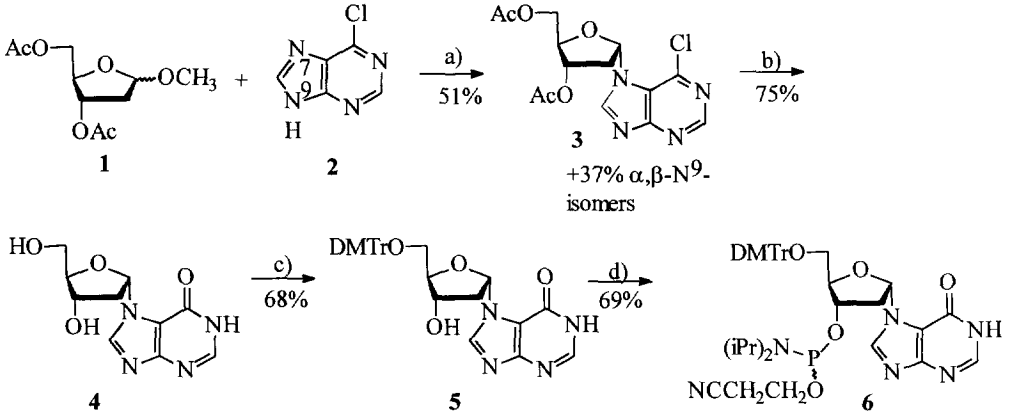
Judith Marfurt, Jürg Hunziker and Christian Leumann* [1])

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern

Abstract: The α-nucleoside 7-(2'-deoxy-α-D-ribofuranosyl)hypoxanthine, incorporated into an otherwise β-configured oligodeoxynucleotide that is designed to bind to a DNA duplex in the parallel motif, recognizes selectively and efficiently a G-C base pair, presumably via monodentate α-H^7•G-C base-triple formation.

Bidentate thymine-adenine (T-A) and protonated cytosine-guanine (C+-G) base recognition in the parallel Hoogsteen DNA triple helical motif (py•pu-py motif),[1,2] as well as A-A, T-A and G-G base recognition in the antiparallel reversed Hoogsteen motif (pu•pu-py motif)[3,4] are distinctly favored over other possible base-base combinations[5,6] and form the structural basis of the attractive interaction between a third DNA strand and a DNA duplex. Because of the restriction of both binding modes to homopurine and homopyrimidine sequences, much effort has recently been devoted to the search for a more general mode of DNA duplex complexation by oligonucleotides.[7] In this context we have embarked on a study of oligonucleotides containing the non-natural nucleoside 7-(2'-deoxy-α-D-ribofuranosyl)hypoxanthine (α-^{7}H) (**4**, *Figure 1*).

Figure 1



a) $(Me_3Si)_2NH$, TMSCl, $SnCl_4$, MeCN, r.t., 3h. b) NaOH in THF:MeOH:H_2O 5:4:1, 0-65°C, 5h. c) DMTrCl, C_5H_5N, r.t., 3h. d) $(iPr_2N)(NCCH_2CH_2O)PCl$, THF, r.t., 90 min.

[1]) *E-mail: leumann@ioc.unibe.ch, Fax: +41 (31) 631 3422*

By systematically exploring its properties in triple helix formation we also investigated its pairing in the context of the py•pu-py motif and found that a α-^{7}H residue within an otherwise β-configured pyrimidine oligonucleotide effectively recognizes a G-C base-pair with high selectivity.

α-^{7}H (**4**), as well as its phosphoramidite building block **6**, were conveniently prepared from the methylglycoside **1** and 6-chloropurine (**2**) using standard procedures in nucleoside chemistry (*Figure 1*). The nature of the glycosidic bond in **4** as well as its conformational preferences were safely established by x-ray analysis.[8] Building block **6** was incorporated into oligomer **8** by solid phase DNA-synthesis and its composition analyzed by MALDI-TOF mass spectrometry after isolation (M-1 calc: 4507.1, found: 4508.7). Oligomer **7** was prepared using standard DNA chemistry and used as a reference.

Triple helix binding affinity and specificity was determined by DNase I footprint analysis in analogy to known procedures.[9,10] A plasmid was constructed containing four triplex target cassettes each spaced by 13 random nucleotides, and each containing the consensus sequence shown in *Figure 2* displaying one of the four possible canonical base-pairs in the center. A ^{32}P-radiolabelled 229 bp fragment of this plasmid was used for the footprint assay with oligo **7** and **8** (100mM NaCl, 10 mM Bis-Tris.HCl, 0.25 mM spermine.4HCl, pH 7.0, 18°C).

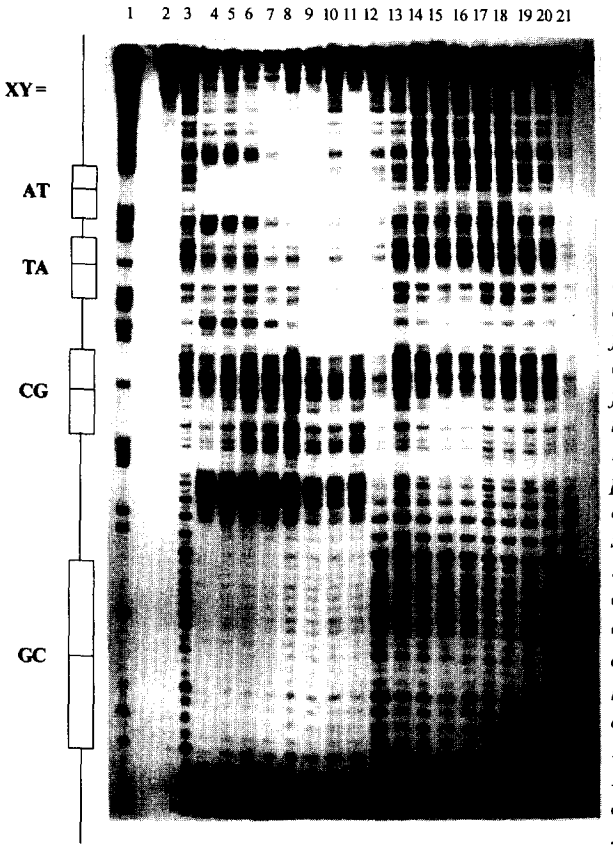
The corresponding autoradiogram (*Figure 2*) clearly shows protection from DNase I activity in the cassette containing a central G-C base pair at concentrations of **8** as low as 0.2μM, indicating strong binding of **8** to this cassette. Triplex formation of **8** is selective. No binding of **8** to the cassettes containing the T-A or the C-G central base-pair was observed but weak binding to the cassette containing the A-T base-pair occurred. Quantitation of binding of **8** to the G-C containing cassette, determined as described,[11] revealed an association constant ($K_{ass.}$) of 1.7 (± 0.9) x 10^6 M^{-1}. This compares to a $K_{ass.}$ of 7.4 (± 3.0) x 10^6 M^{-1} for the reference oligomer **7** binding to the same target cassette (data not shown). Therefore the exchange of a α-^{7}H residue for a methylcytidine in the context of the 15-mers studied here resulted only in a fivefold decrease in binding efficiency.

The decrease in affinity due to the replacement of ^{m}C for α-^{7}H in the third strand is less pronounced than for mismatches in purely β-configured oligomers containing only natural bases. It was shown previously that within the same sequence context, non canonical base triples (Z•G-C triple, Z=G, T, A) decrease binding efficiency by about 2 orders of magnitude with respect to the matched base triple ^{m}C•G-C.[5] Energetically the α-^{7}H•G-C base triple contributes more to the stability of the triplex than any of the 14 possible non canonical natural ones, the best of which (G•T-A) showing reduced binding by a factor of ca. 15.

We assume that base-base recognition occurs via one H-bond between N^1 of hypoxanthine and either N^7 or O^6 of guanine (*Figure 3*), favoring the $N^1H\cdots N^7$ model (*Figure 3*, left) because of the observable weak binding of α^{7}H to adenine (Figure 2). However, at this point we can not exclude other binding modes as e.g site selective intercalation. Computer model building within the given py•pu-py motif suggests that the sugar of the α-^{7}H residue adopts a 1'-endo conformation with the base in the (formal) syn orientation (O^6 of the base

oriented towards the α-face of the sugar). Additional factors that may contribute to the stability of the α-^{7}H•G base-pair, derived from this model, might comprise favourable stacking interactions between the imidazole moiety of α-^{7}H with the next, 5'-located cytosine base in the third strand. Furthermore, no repulsive interactions between H-2 of α-^{7}H and O^6 of guanine is expected in either arrangement (*Figure 3*).

Figure 2:

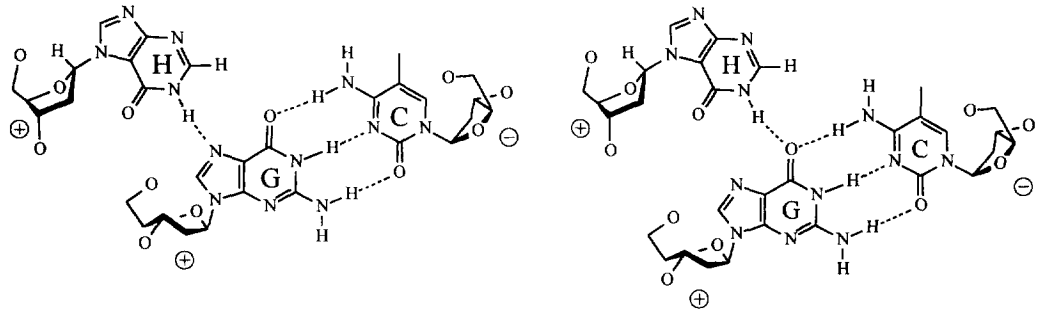


5'-TTTTT^{m}CTZT^{m}CT^{m}CT^{m}CT-3'

7: Z = ^{m}C
8: Z = α-^{7}H

*Top: oligonucleotide sequences and duplex target sites investigated, left: autoradiogram of an 8% denaturing polyacrylamide gel resulting from a DNase I footprinting experiment of oligonucleotide **8** with the 229 bp DNA fragment, that was ^{32}P-labelled at the 3'-end of the pyrimidine rich strand. The boxes to the left indicate the nature of the central base-pair and position of the 4 cassettes. Lane 1, products of an adenine and guanine specific Maxam-Gilbert sequencing reaction; lane 2, intact duplex; lane 3-21, DNase I digested duplex fragments obtained after incubation with different concentrations of oligonucleotide **8**. No oligonucleotide, lane 3; 40μM, lane 4; 20μM, lane 5; 8μM, lane 6; 4μM, lane 7; 2μM, lane 8; 800nM, lane 9; 400nM, lane 10; 200nM, lane 11; 80nM, lane 12; 40nM, lane 13; 20nM, lane 14; 8nM, lane 15; 4nM, lane 16; 2nM, lane 17; 800pM, lane 18; 400nM, lane 19; 200pM, lane 20; 80pM, lane 21.*

Figure 3:



In the series of N^7-β-configured purine nucleosides, guanine and a structural relative of this base were investigated in triple helix formation and were shown to act as base neutral cytosine replacements in the py•pu-py motif [10-12] or to a mismatch in the pu•pu-py motif.[13] It is well known that completely α-configured pyrimidine rich and purine rich oligonucleotides form triplexes with natural DNA.[14] On the level of DNA-duplex formation it was reported recently that a single α-configured deoxyadenosine residue can replace its β-anomer without loss of pairing energy.[15] Here, we show that an α-nucleoside within an otherwise β-configured third strand can selectively and efficiently recognize a DNA base-pair. Related work on oligonucleotides containing the corresponding β-configured N^7H-deoxynucleoside as well as both anomeric forms of it is currently in progress.

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