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DNA RECOGNITION BY NON-NATURAL OLIGONUCLEOTIDES

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ABSTRACT: Non-natural oligonucleotides were synthesized by modified phosphoramidite procedure and tested in triple helix-mediated DNA recognition.

Targeting of DNA *via* oligonucleotide-directed triple helix formation is still mainly restricted to uninterrupted oligopurine tracts¹. We attempt to circumvent the difficulty of pyrimidine recognition by binding the opposite purine of the Watson-Crick base pair in a non-natural triple helix context. Indeed, a third-strand oligonucleotide *centered* in the major groove would be able to « read » guanine and adenine of either strands by switching from one target DNA strand to the other through the β/α anomerism.

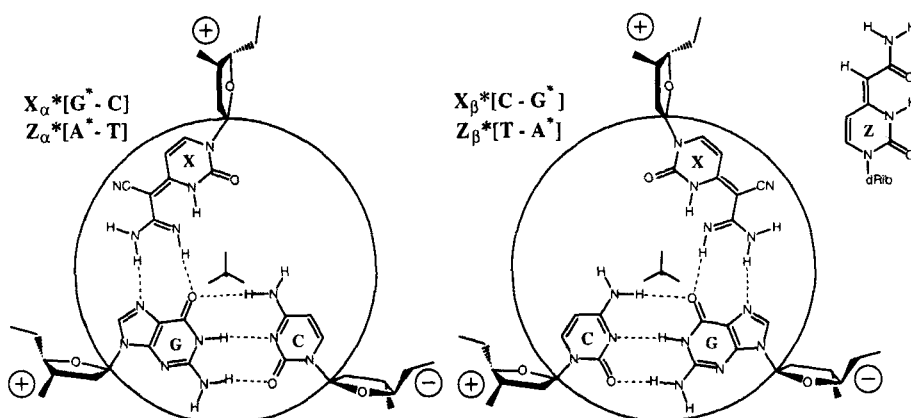


FIG.1 Recognition of a random DNA sequence within a non-natural triple helix may be achieved with only two targeting residues: **X** for guanine and **Z** for adenine (not shown) in their α/β anomeric forms.

A couple of non-natural nucleosides (amidine **X** and amide **Z**) were prepared² that fit within the geometrical constraints of the new triplex context and recognize guanine and adenine, respectively (FIG.1). The **X** and **Z** residues have very similar structures, and perfectly isomorphous base triplets should be obtained.

The nucleosides **X** and **Z** were shown not to require any nucleobase protection. After their tritylation and phosphitylation, oligonucleotides β -d(**XXXZ**)₃**XXX** (**1**) and β -d(**Z**)₁₅ (**2**) were synthesized by solid-phase phosphoramidite chemistry on a 1 μ mol scale. The oligomerization conditions were as follows: two 15 min coupling cycles per step, and 0.5 M (1S)-(+)(10-camphorsulfonyl)oxaziridine solution in acetonitrile for 4 min for oxidation³. Despite excellent 'trityl-on-line' data, HPLC profiles of reaction mixtures revealed considerable quantities of truncated products. The full-length oligonucleotides could nevertheless be isolated by anion-exchange HPLC (ca. 10 nmol of each, 1% yield). Both **1** and **2** are substrates of T4 polynucleotide kinase and have the same electrophoretic mobility as a natural 15-mer oligonucleotide (data not shown).

Various techniques were used to detect triple helix formation. S1 nuclease digestion experiments⁴ resulted (FIG.2) in a statistically significant and reproducible protection of oligonucleotide **1** from enzymatic degradation in the presence of the target duplex (compare lanes 5-7 with digestion in the presence of the mismatch duplex, lanes 2-4), thus indicating sequence-specific hybridization of **1**.

However DNase I or DMS footprintings as well as gel mobility shift assays failed to detect any complex formation, thus tempering the S1 nuclease-derived conclusions.

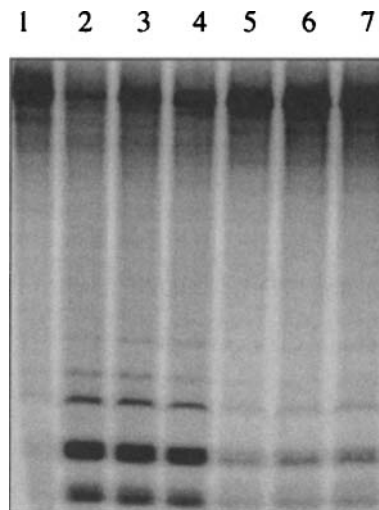


FIG.2 ³²P-Storage image of the 19% denaturing PAGE of the S1 nuclease digestion fragments of oligonucleotide **1**.

REFERENCES AND NOTES

1. Thuong, N. T.; Hélène C. *Angew. Chem. Int. Ed. Engl.*, **1993**, 32, 666-690. Doronina S. O.; Behr, J.-P. *Chem. Soc. Rev.*, **1997**, 63-71.
2. Blanalt-Feidt, S.; Doronina, S. O.; Behr, J.-P., *Nucleosides & Nucleotides*, this issue.
3. M. Manoharan, ISIS Pharmaceuticals, personal communication.
4. Digestion conditions: 10⁻⁹ M 5'-³²P-**1**, 10⁻⁴ M dT₁₅ and 10⁻⁴ M duplex in 50 mM Tris-HCl (pH 7.2), 100 mM NaOAc, 10 mM MgCl₂, 1 mM ZnCl₂, 10 mM Co(NH₃)₆, 2 mM 1,12-bisguanidino-4,9-diazadodecane in 10 μ L were treated with 10 U/ μ L of S1 nuclease for 2h at 0°C.
Target sequence for **1**: 5'-CTTCGTGCTGGCGGGAGGGAGGGAGGGCGTGCTCC
mismatch sequence: 5'-CTTCGTGCTGGCGGAGGGGAGGGAGCGTGCTCC