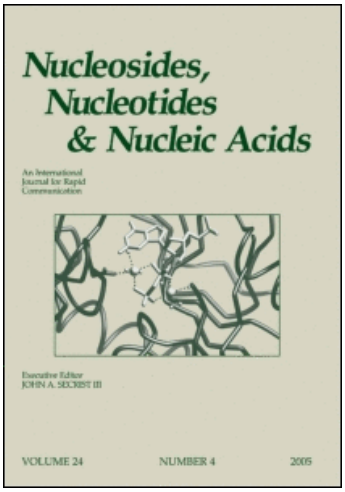

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

DNA Duplexes Containing Alpha Anomeric Nucleotides and Polarity Reversals: Coexistence of Parallel and Antiparallel DNA

Markus W. Gerrnann[a]; James M. Aramini[a]; Bernd W. Kalisch[b]; Richard T. Pon[b]; Johan H. van de Sande[b]
[a] Department of Pharmacology, Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia, PA [b] Department of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada

To cite this Article Gerrnann, Markus W. , Aramini, James M. , Kalisch, Bernd W. , Pon, Richard T. and van de Sande, Johan H.(1997) 'DNA Duplexes Containing Alpha Anomeric Nucleotides and Polarity Reversals: Coexistence of Parallel and Antiparallel DNA', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1481 — 1485
To link to this Article: DOI: 10.1080/07328319708006211
URL: http://dx.doi.org/10.1080/07328319708006211

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1481-1485 (1997)

DNA DUPLEXES CONTAINING ALPHA ANOMERIC NUCLEOTIDES AND POLARITY REVERSALS: COEXISTENCE OF PARALLEL AND ANTIPARALLEL DNA

Markus W. Germann,[†,*] James M. Aramini,[†] Bernd W. Kalisch,[§] Richard T. Pon,[§] and Johan H. van de Sande[§].

[†] Department of Pharmacology, Kimmel Cancer Institute, Thomas Jefferson University, Philadelphia, PA 19107,

[§] Department of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada T2N 4N1

ABSTRACT: Oligodeoxynucleotides that possess alpha anomeric nucleotides and polarity reversals show promise for application in the area of antisense therapy. Here we provide a survey of the spectroscopic, thermodynamic, and enzymatic techniques used in our laboratories to investigate model systems containing such unnatural features with the ultimate goal of designing a new class of more potent and effective antisense therapeutics.

INTRODUCTION

The attractive notion of employing synthetic oligodeoxynucleotides (ODNs) to control the expression of a gene in a specific and effective manner, introduced nearly two decades ago,[1] has given impetus for the development of "antisense" drugs. To be effective in an antisense capacity, an ODN must, among other things, be 1) highly nuclease resistant, and 2) capable of forming a stable complex with its messenger RNA target, that is 3) sensitive to cleavage in the RNA strand by RNase H.

To this end, our strategy,[2] and that of others,[3,4] is to employ a combination of alpha anomeric nucleotides and 3′-3′ and 5′-5′ phosphodiester linkages to generate a novel class of antisense therapeutics, which contain tracts of α- and β-DNA. The unusual phosphodiester bonds function to reverse the polarity of the α-tracts, thereby allowing them to effectively form Watson-Crick base pairs within an overall antiparallel duplex. Recent studies demonstrate that such an approach can lead to the design of ODNs that permit the action of RNase H, a property not displayed by hybrids containing exclusively α-DNA.[2,5] However, the precise structural parameters of DNA/DNA and DNA/RNA duplexes containing such modifications are as yet undetermined.

Here we present spectroscopic and thermodynamic studies of a model DNA decamer duplex system, in which a single α-anomeric nucleotide is inserted into each strand in a parallel configuration via 3′-3′ and 5′-5′ phosphodiester linkages (Figure 1A). Using this design, we systematically determine the consequences of an αT, αC, and αA nucleotide on the stability and structure of this construct.[6] In addition, we have investigated the RNase H sensitivity of a set of DNA/RNA hybrids, in which the DNA is composed of a short tract of β-nucleotides, flanked by α-nucleotides and polarity reversals (Figure 1B).

RESULTS AND DISCUSSION

1) SPECTROSCOPIC STUDIES. A number of spectroscopic techniques, including circular dichroism, hyperchromicity, imino ^{1}H NMR (Figure 2A), ^{31}P NMR (Figure 2B), and several 2D NMR techniques (NOESY, DQF-COSY, P. COSY, ^{1}H-^{31}P correlation) conclusively demonstrate that despite the insertion of one alpha anomeric nucleotide in a parallel orientation into both strands of the decamer sequences shown in Figure 1A above, the alpha-containing duplexes are capable of forming an overall antiparallel B-DNA structure, with perturbations to this motif localized to the regions encompassing the α-nucleotide and unusual phosphodiester linkages.[6] Such perturbations are exemplified by a break in the H2′/H2′′ (sugar) and H6/8 (base) "NOESY walk", between the α-nucleotide and the neighboring residue in all three alpha-containing duplexes (data not shown). Also, H1′-H2′ and H1′-H2′′ spin-spin coupling constants obtained from J-based experiments reveal that in all three alpha duplexes, the sugar ring puckering of the nucleotide following the 5′-5′ linkage is shifted somewhat from the "S-type" puckering toward the "N-type" conformation. This has also been observed in the DNA part of DNA/RNA hybrids.[7]

2) THERMODYNAMIC STUDIES. UV melting studies show that the alphaT duplex is only slightly less stable than the control, on the basis of the T_m and enthalpy, despite the unusual linkages and the presence of only a single α-nucleotide per strand (Table 1).[6] Preliminary results on the alphaC and alphaA sequences also indicate that the presence of the α-nucleotide is tolerated well by the alphaA construct; however, the thermostability of the alphaC duplex is somewhat lower (data not shown).

3) RNase H STUDIES OF α,β-DNA/β-RNA HYBRIDS. RNase H (*E.coli*) studies were carried out on the substrates shown in Figure 1B containing RNase H sensitive windows flanked by α-anomeric components. Windows containing 4 β-anomeric nucleotides were selected on the basis of our previous studies on similar systems. The major cleavage sites for both dT4 and dA4 are adjacent to the recognition modules and, remarkably, are across from the 3'-3' on the ODN strand, yielding 12- and 8-mers, respectively (Figure 3).

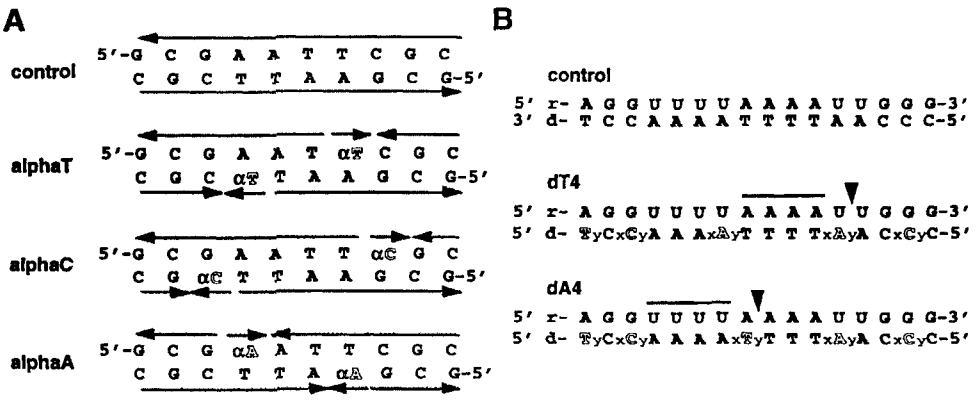

FIGURE 1. A: DNA duplexes for spectroscopic and thermodynamic studies. Arrows denote the polarity of the sequence; the 3′-3′ and 5′-5′ phosphodiester junctions are represented by arrows that are tail-to-tail and head-to-head, respectively. B: RNase H substrates. RNase H sensitive modules in dT4 and dA4 are denoted by bars. Major cleavage sites are marked by arrows. Alpha nucleotides are outlined; x = 5′-5′; y = 3′-3′.

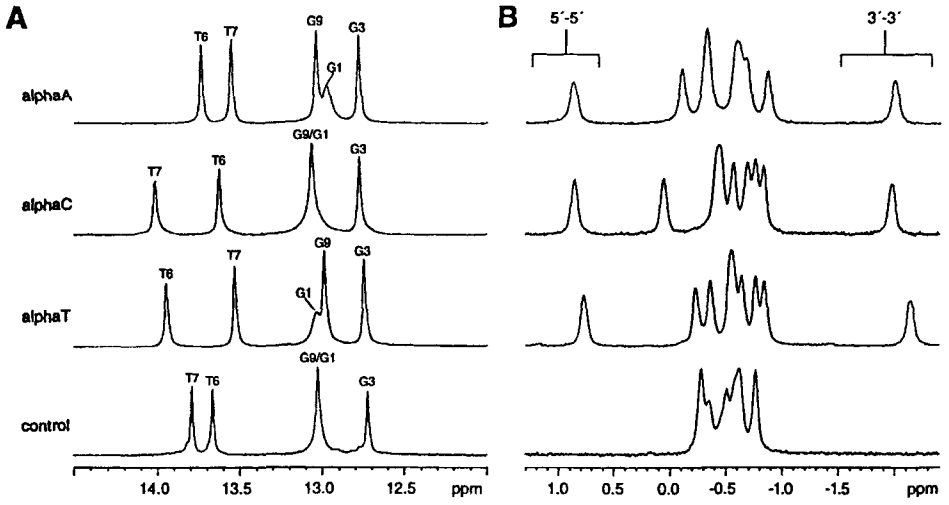

FIGURE. 2. A: imino ^{1}H (600.1 MHz) NMR spectra of the control, alphaT, alphaC, and alphaA duplexes in 50 mM NaCl, 10 mM phosphate, 0.1 mM EDTA, pH 6.5 at 293 K. B: ^{31}P (242.9 MHz) NMR spectra at 303 K. Assignments for the imino protons as well as the 3′-3′ and 5′-5′ phosphodiester linkages are shown.

TABLE 1. Thermodynamic data for the control and alphaT duplexes.[a]

	control	alphaT
T_m (°C)	59.8±0.1	54.7±0.3
$\Delta H°$ (kJmol^{-1})	343±12	330±15
$\Delta S°$ (kJmol^{-1}K^{-1})	0.940 ±0.033	0.946 ±0.021

[a] Melting temperatures reported are for 30 μM total strand concentration in 400 mM NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 6.5.

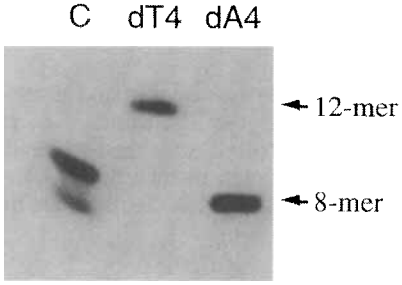

FIGURE 3: Denaturing gel electrophoretic analysis of the RNase H digestion (3h at 21°C) of the control dT4 and dA4 ODN/RNA hybrids.

Interestingly, the UUUU target in dA4 is cleaved more efficiently than the AAAA stretch in dT4.

Subsequent studies on the dA4 system have revealed that the ODN can catalytically destroy a 10 fold excess of the RNA target in the presence of the enzyme.

REFERENCES

1. Zamecnik, P. C., & Stephenson, M. L. (1978) *Proc. Natl. Acad. Sci. USA 75*, 280-284.
2. van de Sande, J. H., Kalisch, B. W., Quong B. Q., & Germann M. W. (1994) Antisense Oligonucleotides with Polarity and Anomeric Center Reversal. First International Antisense Conference of Japan, Kyoto, December 4-7.
3. Debart, F., Tosquellas, G., Rayner, B., & Imbach, J.-L. (1994) *Bioorg. Med. Chem. Lett. 4*, 1041-1046.

4. Koga, M., Wilk, A., Moore, M. F., Scremin, C. L., Zhou, L., & Beaucage, S. L. (1995) *J. Org. Chem. 60*, 1520-1530.
5. Boiziau, C., Debart, F., Rayner, B., Imbach, J-L. & Toulmé, J.-J. (1995) *FEBS Lett. 361*, 41-45.
6. Aramini, J. M., Kalisch, B. W., Pon, R. T., van de Sande, J. H., & Germann, M. W. (1996) *Biochemistry 35*, 9355-9365.
7. Gonzaléz, C., Stec, W., Kobylanska, A., Hogrefe, R. I., Reynolds, M., & James, T. L. (1994) *Biochemistry 33*, 11062-11072.