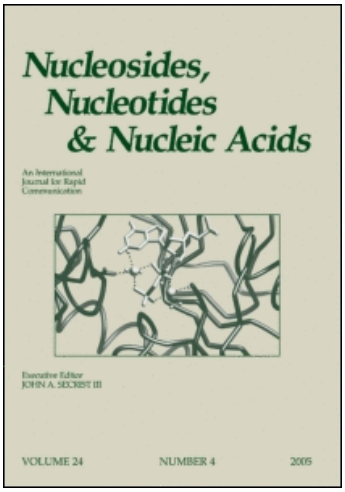
This article was downloaded by:
On: *26 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

Destabilization of DNA Guanine Quadruplex Structure by Foldback Triplex-Forming Oligodeoxynucleotides

Ekambar R. Kandimalla[a]; Sudhir Agrawal[a]
[a] Hybridon, Inc., One Innovation Drive, Worcester, MA

To cite this Article Kandimalla, Ekambar R. and Agrawal, Sudhir(1995) 'Destabilization of DNA Guanine Quadruplex Structure by Foldback Triplex-Forming Oligodeoxynucleotides', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 991 — 995
To link to this Article: DOI: 10.1080/15257779508012518
URL: http://dx.doi.org/10.1080/15257779508012518

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 991-995 (1995)

Destabilization of DNA Guanine Quadruplex Structure by Foldback Triplex-Forming Oligodeoxynucleotides.

Ekambar R. Kandimalla and Sudhir Agrawal
Hybridon, Inc., One Innovation Drive, Worcester, MA 01605.

Abstract: An oligodeoxynucleotide designed to bind to a single stranded guanine rich DNA sequence through Watson-Crick followed by Hoogsteen hydrogen bonds was found to destabilize quadruplex structure formed by the target sequence. However, the conventional antisense and antigene oligonucleotides were unable to destabilize the same quadruplex structure.

Downloaded At: 17:27 26 January 2011

Telomeres are known for their function to stabilize and duplicate chromosomes and for consisting of tracts of guanine residues, often interspersed with short stretches of thymines or adenines[1]. These G-rich tracts have been characterized as forming unusual self association complexes involving Hoogsteen-type hydrogen bonded guanine tetrads[2,3] not only by intermolecular association of four different strands[4] but also by intermolecular association of two hairpins[5] or even by intramolecular folding of a single strand[6]. In parallel with DNA G4 structures, RNA quadruplexes have been shown to occur[7]. The putative encapsidation-dimerization regions of 40 retroviruses possess guanine tracts[8]. Such sequences are known to occur two to five times in different HIV-1 and -2 isolates[8]. It is well established that dimerization of the retroviral genome is governed by RNA-RNA rather than RNA-protein interactions through quadruplex like structure formation involving G-rich sequences[9-11].

Such unusual nucleic acid forms might be good targets for rational drug design because of their transient and rare occurrence *in vivo*, which might allow for highly selective intervention. We have designed novel foldback triplex-forming antisense oligonucleotides (FTFO) that bind to single stranded nucleic acid targets by both Watson-Crick (duplex) and Hoogsteen (triplex) hydrogen bonding formation simultaneously[12, 13]. Now we have explored the possibility of destablization of some of the unusual structures, in this instance quadruplexes, using the FTFOs. The target oligonucleotide, **T** [5'-d(TAAGGCC**AGGGG GAAAGAAAAAA**TATAAAT) bold letters indicating the target site] contains a consensus sequence 5'-d(GGGGGAAAGA) known to occur in the HIV-1 genome several times that is necessary for dimerization through formation of quadruplex structure.

Copyright © 1995 by Marcel Dekker, Inc.

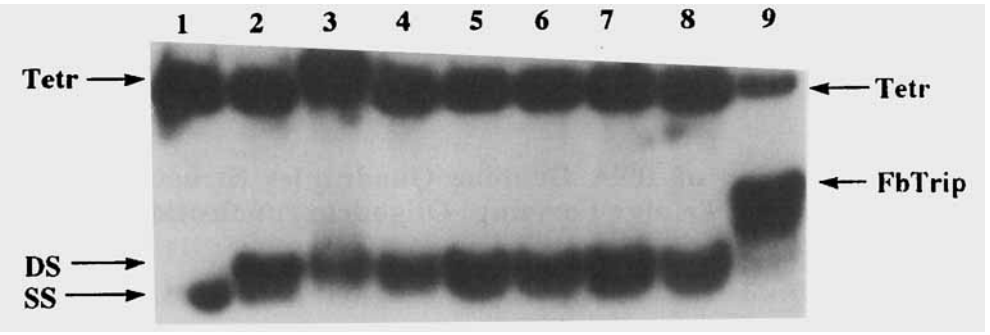


Fig. 1. Autoradiogram showing oligonucleotide **1** that can form only Watson-Crick duplex can not disrupt quadruplex structure. Concentration of the oligonucleotide **T** was 1 X 10^{-9} M and the ratios of target and oligomer **1** were - lane 1: **T** alone; lane 2: 1:0.1; lane 3: 1:0.5; lane 4: 1:1; lane 5: 1:2.5; lane 6: 1:5; lane 7: 1:10; and lane 8: 1:15. Lane 9 contained oligomer **2** instead of **1** at a ratio of 1:0.3. Typically both the oligonucleotides were mixed in 100 mM sodium acetate, pH 5.0 buffer, heated to 95°C for 15 min, allowed to cool down to room temperature and then stored at 4°C overnight before performing electrophoresis. DS, SS, FbTrip and Tetr stand for double stranded, single stranded, foldback triplex and quadruplex structures, respectively.

Downloaded At: 17:27 26 January 2011

Sequence **T** formed quadruplex structure that is characterized by retarded mobility on non-denaturing polyacrylamide gels compared to single and double stranded structures in buffers containing sodium and potassium ions. The formation of the slow moving band was dependent on the concentration of the oligonucleotide. The formation of this structure was kinetically slow, but once formed it was thermodynamically stable. Replacement of one or two of the Gs in the sequence 5'-d(GGGGGAAAGA) with other bases resulted in disappearance of the slow moving band indicating the requirement of the core sequence GGGGG for higher order structure formation. These properties of the slow moving band are consistent with formation of a quadruplex-like higher order structure under the experimental conditions[7, 14-17].

We wondered whether the Watson-Crick complementary sequence, **1** [5'-d(TTT TTTCTTTCCCCCT)] could destabilize quadruplex formation. Titration of the target oligonucleotide **T** with the conventional antisense oligomer **1** up to a ratio of 1:15 was unable to destabilize the superstructure T_4, except that it formed Watson-Crick duplex with the single strand fraction of the oligonucleotide, **T** in solution (Fig. 1).

The experiments with oligonucleotide **2** (FTFO) [5'-d(TCCCCCTTTCTTTTTT tctct TTTTTTCTTTCCCCCT) lower case letters indicating nucleotides in the loop that are not involved in hydrogen bonding] under the same conditions as above showed an

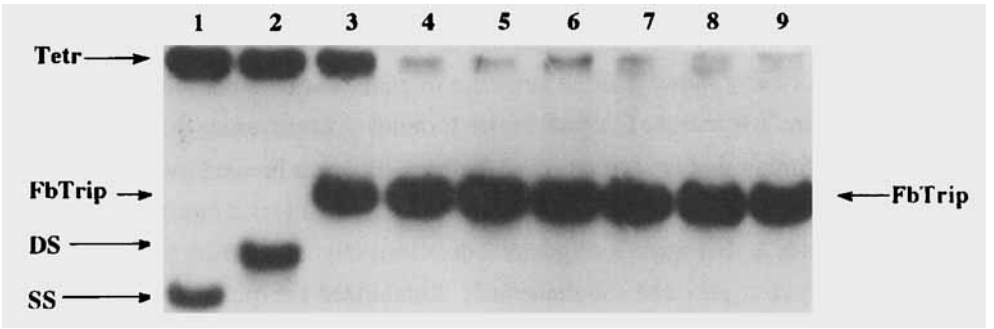


Fig. 2. Autoradiogram showing oligonucleotide **2** that can form foldback triplex destabilizes quadruplex structure. Experiment was carried out as described for Figure 1 but the ratios of target sequence **T** and **2** are - lane 1: **T** alone; lane 3: 1:0.25; lane 4: 1:0.5; lane 5: 1:1; lane 6: 1:2.5; lane 7: 1:5; lane 8: 1:10 and lane 9: 1:15. Lane 2 contained oligomer **1** instead of **2** at a ratio of 1:4.

Downloaded At: 17:27 26 January 2011

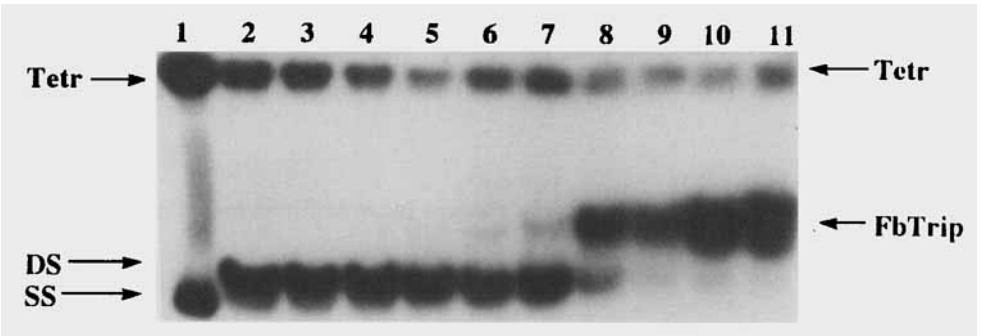


Fig. 3. Non-denaturing polyacrylamide gel showing oligonucleotide **2** destabilizes both duplex and tetraplex structures. To 1:1.2 mixture of oligomers **T** and **1** incubated overnight at 4°C in 100 mM sodium acetate, pH 5.0 buffer different concentrations of oligonucleotide **2** were added, mixed and incubated further at room temperature for 2 hrs before electrophoresing as described under Figure 1. Lane 1: T alone (3.1×10^{-10} M); lane 2: 1:1.2 of **T** and **1** and in other lanes oligomer **2** is added to 1:1.2 mixture of **T** and **1** in concentrations - lane 3: 3.5×10^{-12} M; lane 4: 8.7×10^{-12} M; lane 5: 1.7×10^{-11} M; lane 6: 2.6×10^{-11}; lane 7: 3.5×10^{-11} M; lane 8: 8.7×10^{-11} M; lane 9: 1.7×10^{-10} M; lane 10: 2.6×10^{-10} M and lane 11: 3.5×10^{-9} M.

intermediate band that moved slower than duplex and faster than quadruplex structures (Fig. 2). At the same time disappearance of a slow moving band that corresponded to quadruplex structure on gels is evident in Fig. 2 at a ratio of 1:1. In contrast in the presence of oligonucleotide **1** at 1:4 ratio (lane 2, Fig. 2) and up to 1:15 ratio (Fig. 1) the slow moving band intensity was unchanged but the band corresponding to single strand diminished. This clearly shows that the antisense oligonucleotide **1** does not destabilize quadruplex structure, whereas the foldback triplex forming oligonucleotide does.

To show further that the formation of foldback triplex is favored over duplex and quadruplex structures, the mixtures of oligonucleotides **T** and **1** (1:1.2 ratio) were titrated with oligonucleotide **2**. In Figure 3, oligomer **2** displaced oligomer **1** from its binding site and formed foldback triplex and simultaneously destabilized the quadruplex band also. However, in a reverse experiment where mixtures of **T** and oligonucleotide **2** (1:1 ratio) were titrated with oligonucleotide **1**, only the band corresponding to foldback triplex structure, but no bands corresponding to duplex and quadruplex structures were seen (data not shown), indicating that oligonucleotide **1** cannot disrupt foldback triplex structure.

In conclusion, we have demonstrated that a foldback triplex forming oligodeoxynucleotide can destabilize quadruplex structure. This opens up a new avenue to target unusual nucleic acid structures using oligonucleotides specifically to inhibit dimerization of retroviral RNA, thereby controlling encapsidation, affecting chromosomal crossover during meiosis in eukaryotes (cancers) and having possible application in gene therapy.

Downloaded At: 17:27 26 January 2011

Acknowledgments. The authors thank Ms. Patricia Iadarola for technical assistance and Dr. Wayne Keown for his comments.

References

1. Blackburn, E.H. Nature, 1991, **350**, 569-573.
2. Smith, F.W.& Feigon, J. Nature, 1992, **356**, 164-168.
3. Kang, C., Zahang, X., Ratliff, R., Moyzis, R. & Rich, A. Nature, 1992, **356**, 126-131; Laughlan, G., Murchie, A.I.H., Norman, D.G., Moore, M.H., Moody, P.C.E., Lilley, D.M.J. & Luisi, B. Science, 1994, **265**, 520-524.
4. Sen, D. & Gilbert, W. Nature, 1988, **334**, 364-366.
5. Sundquist, W.I. & Klug, A. Nature, 1989, **342**, 825-829.
6. Panyutin, I.G., Kovalsky, O.I., Budowsky, E.I., Dickerson, R.E., Rikhirev, M.E. & Lipanov, A.A. Proc. Natl. Acad. Sci. USA, 1990, **87**, 867-870.
7. Kim, J., Cheong, C. & Moore, P.B. Nature, 1991, **351**, 331-332.
8. Baudin, F., Marquet, R., Isel, C., Darlix, J.-L., Ehresmann, B. & Ehresmann, C. J. Mol. Biol., 1993, **229**, 382-397.

9. Prats, A-C., Roy, C., Wang, P., Erard, M., Housser, V., Gabus, C., Paoletti, C. & Darlix, J.-L. J. Virol., 1990, **64**, 774-783.
10. Sundquist, W.I. & Heaphy, S. Proc. Natl. Acad. Sci. USA, 1993, **90**, 3393-3397.
11. Awang, G. & Sen, D. Biochemistry, 1993, **32**, 11453-11457.
12. Kandimalla, E.R. & Agrawal, S. J. Biomolec. Struct. Dyn., 1993, **10**, a086.
13. Kandimalla, E.R. & Agrawal, S. Gene, 1994, **149**, 115-121.
14. Sen, D. & Gilbert, W. Nature, 1990, **344**, 410-414.
15. Lu, M., Guo, Q. & Kallenbach, N.R. Biochemistry, 1992, **31**, 2455-2459.
16. Sen, D. & Gilbert, W. Biochemistry, 1992, **31**, 65-70.
17. Wang, Y. & Patel, D.J. Biochemistry, 1992, **31**, 8112-8119.

Downloaded At: 17:27 26 January 2011