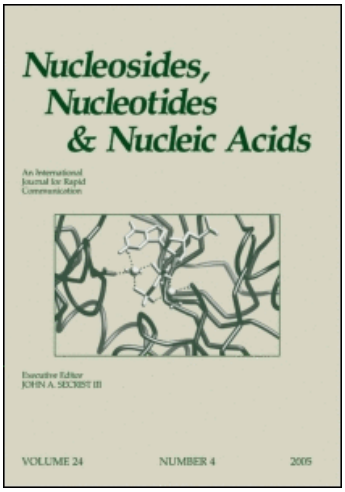

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Structural Studies on LNA Quadruplexes

Antonio Randazzo[a]; Veronica Esposito[a]; Oliver Ohlenschläger[b]; Ramadurai Ramachandran[b]; Antonella Virgilio[a]; Luciano Mayol[a]
[a] Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Napoli, Italy [b] Institut für Molekulare Biotechnologie, Abt. Molekulare Biophysik/NMR-Spektroskopie, Jena, Germany

To cite this Article Randazzo, Antonio , Esposito, Veronica , Ohlenschläger, Oliver , Ramachandran, Ramadurai , Virgilio, Antonella and Mayol, Luciano(2005) 'Structural Studies on LNA Quadruplexes', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 795 — 800
To link to this Article: DOI: 10.1081/NCN-200060279
URL: http://dx.doi.org/10.1081/NCN-200060279

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):795–800, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200060279

Taylor & Francis
Taylor & Francis Group

STRUCTURAL STUDIES ON LNA QUADRUPLEXES

Antonio Randazzo and Veronica Esposito ▫ *Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Napoli, Italy*

Oliver Ohlenschläger and Ramadurai Ramachandran ▫ *Institut für Molekulare Biotechnologie, Abt. Molekulare Biophysik/NMR-Spektroskopie, Jena, Germany*

Antonella Virgilio and Luciano Mayol ▫ *Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Napoli, Italy*

▫ *LNAs (locked nucleic acids) are new DNA analogues with higher binding affinities toward nucleic acids than the canonical counterparts mainly due to the characteristic conformational restriction arising from the 2′-O, 4′-C methylene bridge. In light of the promising therapeutic applications and considering the advantageous characteristics of LNAs, such as their high water solubility, easy handling, and synthetic accessibility through the conventional phosphoramidite chemistry, we undertook a study concerning the capability of these nucleic acid analogues to form quadruplex structures. Particularly, we have been investigating the LNA/DNA chimeras corresponding to the well-known DNA sequences 5′-GGTTGGTGTGGTTGG-3′, capable of forming an unimolecular quadruplex. This article deals with the study of the sequence 5′-ggTTggTGTggTTgg-3′ (upper and lower case letters represent DNA and LNA residues, respectively), which, according to CD spectroscopy, is able to fold into a quadruplex structure.*

Keywords LNA, Locked Nucleic Acids, Quadruplex, Thrombin Binding Aptamer

INTRODUCTION

Noncoding repeat sequences of guanine-rich DNA are particularly important at the ends of chromosomes, where they form protein-DNA assemblies, well known as telomeres. Telomeric DNA contains runs of guanine bases that can adopt an important structural motif based on the association of four guanines. In this arrangement each guanine interacts with two neighbors via Hoogsteen-like

This work is supported by Italian M.U.R.S.T. (P.R.I.N. 2003 and 2004) and Regione Campania (L.41, L.5). The authors are grateful to "Centro Ricerche Interdipartimentale di Analisi Strumentale", C.R.I.A.S., for supplying NMR facilities.

Address correspondence to Luciano Mayol, Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Napoli, Italy; Fax: +39-081-678552; E-mail: mayol1@unina.it

hydrogen bonding, resulting in a square planar configuration called G-quartet. Quadruplex structures are composed of stacks of G-quartets, which can be realized in different ways, as far as strand stoichiometry and orientation are concerned. Strand stoichiometry variation allows G-quadruplexes to be formed by association of one, via intramolecular folding,[1–3] two, by dimerization of a folded-back hairpin,[4,5] or four separate strands.[6,7] It has been demonstrated by footprinting, strand mixing and cross-linking studies,[8–10] along with NMR spectroscopy and X-ray crystallography,[6,11,12] that single dGn segments can form a tetramolecular complex with all G bases in the *anti* conformation and all strands parallel to each other in monovalent cation containing solution. In contrast, the folded-back dimeric quadruplex has alternating *syn* and *anti* nucleotide conformations along each strand and two pairs of adjacent parallel strands.[4,5,13,14] Furthermore, oligonucleotides, forming an unimolecular quadruplex, as the thrombin-binding DNA aptamer (TBA) 5′-GGTTGGTGTGGTTGG-3′, for example, show the residues of the tetrads in an *anti-syn-anti-syn* conformation with alternating antiparallel strands.[13,15,16]

In addition to their presence in the telomeric ends of eukaryotic chromosomes,[17,18] runs of dGs may also be involved in recombination and mutation hot spots processes,[19–23] gene regulation,[24] and various human diseases.[25,26]

Considering that a wider biological relevance for quadruplex DNA is now becoming apparent and that the G-tetrad repeating motif tolerates a surprisingly broad range of structural features, it may be interesting to investigate changes in structure and activity that occur upon chemical modification. In order to explore this case, we have undertaken a study concerning the capability of locked nucleic acids (LNAs) to form quadruplex structures. LNAs are new DNA analogues characterized by a 2′-O, 4′-C methylene bridge (Figure 1) that is mainly responsible for their enhanced hybridization performance[27] and for their exceptional biostability.[28] The presence of the methylene bridge confers a RNA-like C3′-endo (N-type) conformation to the sugar moiety of the modified nucleotide and reduces its conformational flexibility, thus expanding the degree of local organization of the

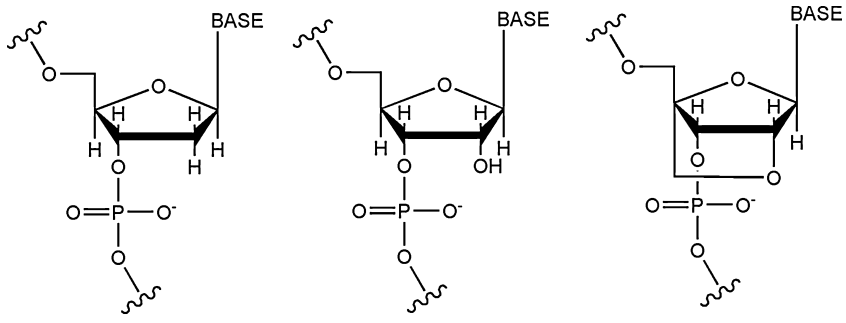

FIGURE 1 Chemical structures of DNA, RNA, and LNA residues.

phosphate backbone. These unprecedented hybridization properties suggest that LNAs could be powerful agents for fine tuning drugs with very specific target potential, thus providing a new class of therapeutics.[29–31] The solution structure of a locked nucleic acid quadruplex, formed by the oligomer 5′-tgggt-3′, where lower case letters represent LNA residues, has been already reported.[32] NMR and CD spectroscopy, as well as molecular dynamics and mechanic calculations, were used to characterize the complex. The molecule adopts a parallel stranded conformation with a fourfold rotational symmetry, showing a right-handed helicity and the guanine residues in an almost planar conformation with three well-defined G-tetrads (Figure 2). A T_m increment of 20°C with respect to the corresponding unmodified quadruplex $[d(TGGGT)]_4$ was observed. Preliminary studies on antiparallel LNA quadruplexes are reported here. We have first focused our attention on the unimolecular quadruplex. Particularly we analysed the LNA counterpart of the well-known DNA sequences 5′-GGTTGGTGTGGTTGG-3′ (TBA), since this sequence was found to be a potent inhibitor of thrombin with an EC_{50} of 20 nM in a purified fibrinogen clotting assay.[33] Unfortunately, the LNA sequence 5′-ggttggtgtggttgg-3′ (the lower case letters represent LNA residues) is not able to fold into quadruplex structures (data not shown), most probably due to the lower flexibility of the loops. For this reason, we designed and synthetized the chimera 5′-ggTTggTGTggTTgg-3′, where the upper and lower case letters represent DNA and LNA residues, respectively.

CD spectra of 5′-ggTTggTGTggTTgg-3′ were acquired at 20 and 75°C (Figure 3). Particularly, while the spectrum at 75°C is characteristic of an unstructured molecule, the spectrum at 20°C shows a maximum and minimum Cotton effect at 262 and 241 nm, respectively, indicating the presence of a quadruplex structure in solution.[34,35]

In order to estimate the effects of structural modification on the thermal stability of the quadruplex formed by 5′-ggTTggTGTggTTgg-3′, CD melting and annealing

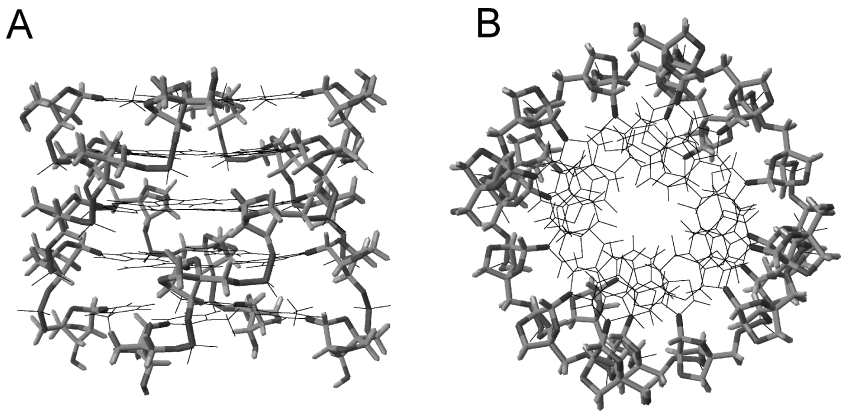

FIGURE 2 Side (A) and top (B) view representations of the best NMR structure of the tetramolecular LNA quadruplex formed by the oligomer 5′-tgggt-3′ (lower case letters represent LNA residues). Backbones are depicted in colored "stick" (carbons, green; nitrogens, blue; oxygens, red; hydrogens, white), whereas bases in black "lines."

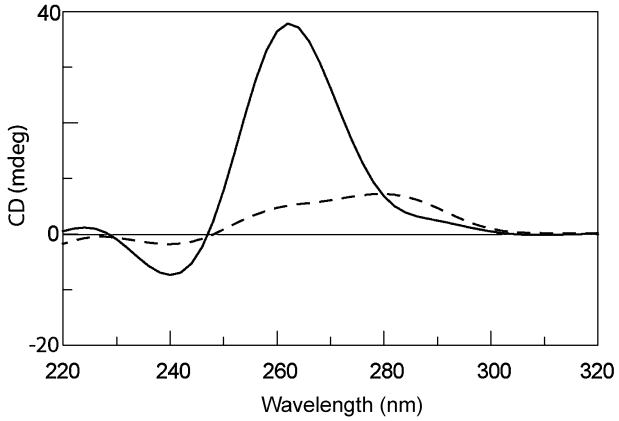

FIGURE 3 CD spectra of 5′-ggTTggTGTggTTgg-3′at 20°C (continous lines) and 75°C (dashed lines). Lower case letters represent LNA residues.

experiments were performed. All the measurements were performed at a concentration of 5×10^{-5} M. Taking into account that the rates of quadruplex formation/dissociation are extremely slow, to avoid a kinetic influence on the collected data, we acquired all the experiments at a very low scan rate (10°C/h). The curves for both 5′-ggTTggTGTggTTgg-3′ (Figure 4) and its natural counterpart 5′-GGTTGGTGTGGTTGG-3′ (Figure 5) were obtained collecting data in the range 5–90°C. The CD signals at 262 and 293 nm for 5′-ggTTggTGTggTTgg-3′ and 5′-GGTTGGTGTGGTTGG-3′, respectively, were reported as a function of temperature.

The very small observed hysteresis in the case of 5′-ggTTggTGTggTTgg-3′ indicates that, in spite of the very low scan rate used in the experiments, the cooling

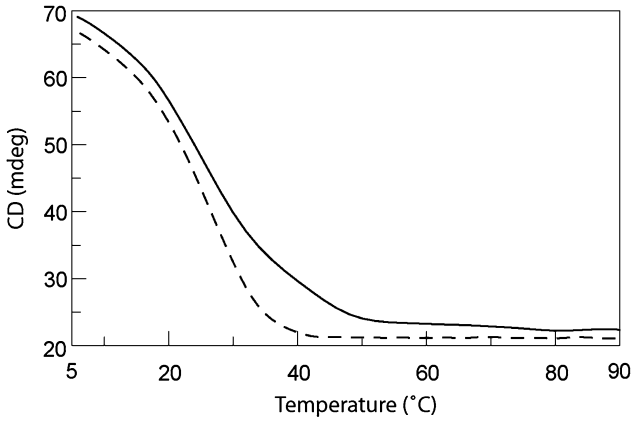

FIGURE 4 Melting (continous lines) and annealing (dashed lines) CD experiments of 5′-ggTTggTGTggTTgg-3′. Lower case letters represent LNA residues.

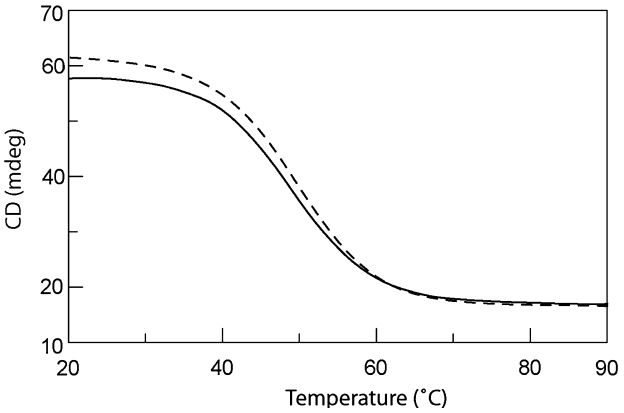

FIGURE 5 Melting (continous lines) and annealing (dashed lines) CD experiments of 5′-GGTTGGTGTGGTTGG-3′.

and heating curves are not completely in a thermodynamic equilibrium; i.e., the temperature change is faster than the rate at which the folding/unfolding processes reach a new equilibrium. On the other hand, the hysteresis reflects a difference in kinetics of association versus dissociation, as is often found for multistranded nucleic acid structures. Even if a more detailed physicochemical study has not been yet accomplished, it is not unreasonable to hypothesize a considerably slower kinetics of association versus dissociation. For this reason we believe that the melting profiles provide a better descriptive picture of the situation at the equilibrium than the annealing curves.

It is noteworthy that, according to CD melting experiments, 5′-GGTTGGTGTGGTTGG-3′ is characterized by higher thermal stability than 5′-ggTTggTGTggTTgg-3′. Particularly, a ΔT_m of 20°C has been observed. The structural features of the LNA-quadruplex examined may open new perspectives for the biological application of LNAs as novel versatile tools to design aptamers.

REFERENCES

1. Henderson, E.; Hardin, C.C.; Walk, S.K.; Tinoco, I., Jr.; Blackburn, E.H. Telomeric DNA oligonucleotides form novel intramolecular structures containing guanine-guanine base pairs. Cell **1987**, *51*, 899–908.
2. Macaya, R.F.; Schultze, P.; Smith, F.W.; Roe, J.A.; Feigon, J. Thrombin-binding DNA aptamer forms a unimolecular quadruplex structure in solution. Proc. Natl. Acad. Sci. U. S. A. **1993**, *334*, 3745–3749.
3. Wang, Y.; Patel, D.J. Solution structure of the Oxytricha telomeric repeat d[$G_4(T_4G_4)_3$] G-tetraplex. J. Mol. Biol. **1995**, *251*, 76–94.
4. Schultze, P.; Flint, W.S.; Feigon, J. Refined solution structure of the dimeric quadruplex formed from the Oxytricha telomeric oligonucleotide d(GGGGTTTTGGGG). Structure **1994**, *2*, 221–233.
5. Smith, F.W.; Feigon, J. Quadruplex structure of Oxytricha telomeric DNA oligonucleotides. Nature **1992**, *356*, 164–168.
6. Aboul-ela, F.; Murchie, A.I.H.; Norman, D.G.; Lilley, D.M.J. NMR study of a parallel-stranded tetraplex formation by the hexadeoxynucleotide d(TG_4T). J. Mol. Biol. **1994**, *243*, 458–471.
7. Patel, P.K.; Koti, A.S.R.; Hosur, R.V. NMR studies on truncated sequences of human telomeric DNA: observation of a novel A-tetrad. Nucleic Acids Res. **1999**, *27*, 3836.

8. Sen, D.; Gilbert, W. Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis. Nature **1988**, *334*, 364–366.
9. Sen, D.; Gilbert, W. A sodium-potassium switch in the formation of four-stranded G4-DNA. Nature **1990**, *334*, 410–414.
10. Sen, D.; Gilbert, W. Novel DNA superstructures formed by telomere-like oligomers. Biochemistry **1992**, *31*, 65–70.
11. Laughlan, G.; Murchie, A.I.; Norman, D.G.; Moore, M.H.; Moody, P.C.E.; Lilley, D.M.J.; Luisi, B. The high-resolution crystal structure of a parallel-stranded guanine tetraplex. Science **1994**, *265*, 520–524.
12. Phillips, K.; Dauter, Z.; Murchie, A.I.; Lilley, D.M.J.; Luisi, B. The crystal structure of a parallel-stranded guanine tetraplex at 0.95 Å resolution. J. Mol. Biol. **1997**, *273*, 171–182.
13. Wang, Y.; Patel, D.J. Solution structure of the human telomeric repeat d[$AG_3(T_2AG_3)_3$] G-tetraplex. Structure **1993**, *1*, 263–282.
14. Catasti, P.; Chen, X.; Moyzis, R.K.; Bradbury, E.M.; Gupta, G. Structure-function correlations of the insulin-linked polymorphic region. J. Mol. Biol. **1996**, *264*, 534–545.
15. Kang, C.; Zhang, X.; Ratliff, R.; Moyzis, R.; Rich, A. Crystal structure of four-stranded Oxytricha telomeric DNA. Nature **1992**, *356*, 126–131.
16. Schultze, P.; Macaya, R.F.; Feigon, J. Three-dimensional solution structure of the thrombin-binding DNA aptamer d(GGTTGGTGTGGTTGG). J. Mol. Biol. **1994**, *235*, 1532–1547.
17. Henderson, E.R.; Blackburn, E.H. An overhanging 3′ terminus is a conserved feature of telomeres. Mol. Cell. Biol. **1989**, *9*, 345–348.
18. Blackburn, E.H. Structure and function of telomeres. Nature **1991**, *350*, 569–573.
19. Nikaido, T.; Yamawaki-Kataoka, Y.; Honjo, T. Nucleotide sequences of switch regions of immunoglobulin C epsilon and C gamma genes and their comparison. J. Biol. Chem. **1982**, *257*, 7322–7329.
20. Jeffreys, A.J.; Wilson, V.; Thein, S.L. Hypervariable "minisatellite" regions in human DNA. Nature **1985**, *314*, 67–73.
21. Collier, D.A.; Griffin, J.A.; Wells, R.D. Non-B right-handed DNA conformations of homopurine.homopyrimidine sequences in the murine immunoglobulin C alpha switch region. J. Biol. Chem. **1988**, *263*, 7397–7405.
22. Hastie, N.D.; Allshire, R.C. Human telomeres: fusion and interstitial sites. Trends Genet. **1989**, *5*, 326–331.
23. Akman, S.A.; Lingeman, R.G.; Doroshow, J.H.; Smith, S.S. Quadruplex DNA formation in a region of the tRNA gene supF associated with hydrogen peroxide mediated mutations. Biochemistry **1991**, *30*, 8648–8653.
24. Nickol, J.M.; Felsenfeld, G. DNA conformation at the 5′ end of the chicken adult beta-globin gene. Cell **1983**, *35*, 467–477.
25. Simonsson, T.; Pecinka, P.; Kubista, M. DNA tetraplex formation in the control region of c-myc. Nucleic Acids Res. **1998**, *26*, 1167–1172.
26. Hammond-Kosack, M.C.; Kilpatrick, M.W.; Docherty, K. Analysis of DNA structure in the human insulin gene-linked polymorphic region in vivo. J. Mol. Endocrinol. **1992**, *9*, 221–225.
27. Wengel, J. Synthesis of 3′-C- and 4′-C-branched oligonucleotides and the development of locked nucleic acid (LNA). Acc. Chem. Res. **1998**, *32*, 301–310.
28. Singh, S.K.; Nielsen, P.; Koshkin, A.A.; Wengel, J. LNA (locked nucleic acids): synthesis and high-affinity nucleic acid recognition. Chem. Commun. **1998**, *4*, 455–456.
29. Koller, E.; Gaarde, W.A.; Monia, B.P. Elucidating cell signaling mechanisms using antisense technology. Trends Pharmacol. Sci. **2000**, *21*, 142–148.
30. Braasch, D.A.; Corey, D.R. Novel antisense and peptide nucleic acid strategies for controlling gene expression. Biochemistry **2002**, *41*, 4503–4510.
31. Opalinska, J.B.; Gewirtz, A.M. Nucleic-acid therapeutics: basic principles and recent applications. Nat. Rev., Drug Discov. **2002**, *1*, 503–514.
32. Randazzo, A.; Esposito, V.; Ohlenschlaeger, O.; Ramachandran, R.; Mayol, L. NMR solution structure of a parallel LNA quadruplex. Nucleic Acids Res. **2004**, *32*(10), 3083–3092.
33. Bock, L.C.; Griffin, L.C.; Latham, J.A.; Vermaas, E.H.; Toole, J.J. Selection of single-stranded DANN molecules that bind and inhibit human thrombin. Nature **1992**, *355*, 564–566.
34. Dapic, V.; Abdomerovic, V.; Marrington, R.; Peberdy, J.; Rodger, A.; Trent, J.O.; Bates, P.J. Biophysical and biological properties of quadruplex oligodeoxyribonucleotides. Nucleic Acids Res. **2003**, *31*, 2097–2107.
35. Jing, N.; Xiong, W.; Guan, Y.; Pallansch, L.; Wang, S. Potassium-dependent folding: a key to intracellular delivery of G-quartet oligonucleotides as HIV inhibitors. Biochemistry **2002**, *41*(17), 5397–5403.