Novel G-Quadruplex Higher Order Assemblies Revealed by Electrospray Ionization Mass Spectrometry.

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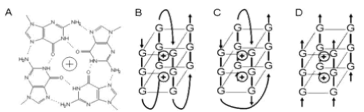
Overview

- Loops have an important role in G-quadruplex structures.
- To study the influence of loop length without the influence of individual bases, we used sequences containing random bases in the loops.
- Although all studied sequences can form intramolecular G-quadruplexes, multimers were also observed.

Introduction

Guanines form tetrads stabilized by Hoogsteen H-bond (A). Stacking of tetrads gives G-quadruplexes. Sequences containing 4 G-tracts can form intramolecular G-quadruplexes (B) while several short G-rich strands can associate to form intermolecular structures (C-D). G-quadruplexes are also stabilized by cations located between tetrads. Depending on the G-tracts orientations, structures can be parallel (D), antiparallel (B-C) or hybrid.



In intramolecular structures, the sequence and the length of the loops that connect G-tracts play an essential role in the structure and the stability of G-quadruplexes.

By using a sequence independant method, we show that the loop length also influence the formation of multimeric structures.

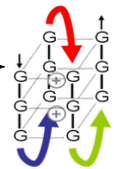
Materials and methods

Random base DNA sequences approach : every « sequence » is a mixture of several particular sequences :

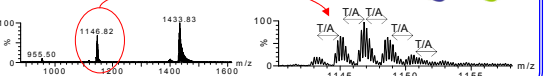
5'-d(GGG-W_i-GGG-W_j-GGG-W_k-GGG)-3'

With i-j-k = 1, 2, or 3 and W = T or A with equal probability.

28 groups of sequences (from ijk = 1-1-1 to 3-3-3) for a total of ≈ 2800 individual sequences, studied by C.D. (topology) and by M.S. and Gel electrophoresis (stoechiometry).



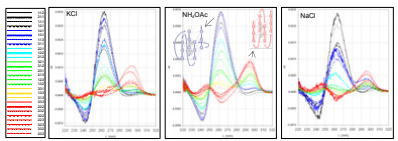
Sequences were heated at 80 °C for 5 min at high conc. and cooled at 4 °C at 50 µM OVN. They were diluted to 10 µM in 150 mM NH_4OAc – 20 % MeOH just prior injection.



Results

Circular dichroism

(discriminates parallel (min at 240 nm and max at 265 nm) from antiparallel G-quadruplexes (min at 265 nm and max at 295 nm).

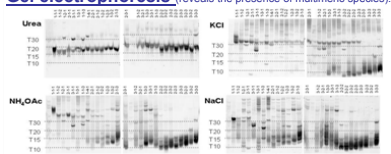


C.D. measurements of the 28 oligonucleotides. Oligonucleotides are named by the number of bases in each of their loops. [Oligos] = 5 µM in cation 150 mM and Li cacodylate 10 mM, pH 7.4.

- **K^+ favors the formation of parallel structures except for longest loops while Na^+ favors antiparallel structures except for shortest loops. NH_4^+ has an intermediate behavior.**
- **Sequences with short loops adopt parallel structures.**

Gel electrophoresis

(reveals the presence of multimeric species).

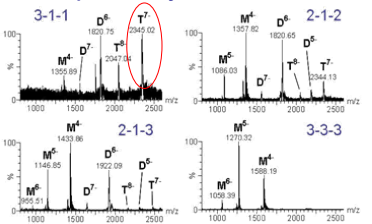


Gels of all oligos in urea, 150 mM K^+, NH_4^+, or Na^+. Mw are poly d(T).

- **K^+ favors the formation of intermolecular structures except for longest loops while Na^+ favors intramolecular structures. NH_4^+ has an intermediate behavior.**
- **Sequences with short loops form multimers.**

Results

Mass Spectrometry



Mass spectra of four oligonucleotides. (M = monomer, D = dimer, T = trimer). Measurements achieved on a Q-TOF Ultima Global (Micromass – Waters) using electrospray ionization in negative mode. Sample were diluted to 10 µM in 150 mM NH_4OAc – 20% MeOH.

- **MS shows that multimers are dimers, trimers and tertramers.**
- **First observation of trimers for sequences able to form intramolecular G-quadruplexes.**
- **Multimers are more abundant when loops are short, i.e. for sequences that exhibit parallel C.D. spectra.**

Ion Mobility

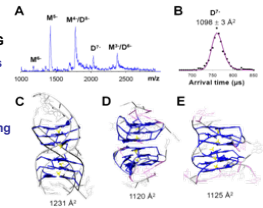
In vivo, G-quadruplexes would be surrounded by other sequences, thus **What happens if oligonucleotides begin and end by other bases than guanines?**

- **The c-Myc promoter sequence**

dGAGGGTGGGGAGGGTGGGGAAG

can also form multimeric structures but only dimers (A).

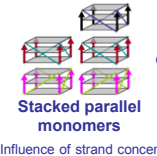
- **Ion mobility experiments (B) confirmed that these dimers are parallel but did not allow determining which type of parallel multimeric structure (D or E) is present.**



Nano-electrospray was used to perform ion mobility experiments. To make molecular dynamics, the AMBER 7 set of programs was used.

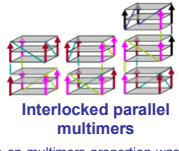
Discussion: Structure of multimers

Two models can be envisaged for parallel multimeric structures



Stacked parallel monomers

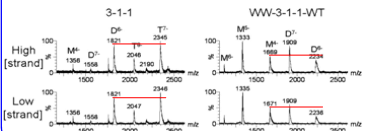
or



Interlocked parallel multimers

In the case of stacked monomers (rapid folding of intramolecualr G-quad.), the proportion of multimers would not be influenced by the initial concentration of unfolded strands while this is the case for the interlocked multimers.

Influence of strand concentration on multimers proportion was tested. The samples were heated at 80 °C for 5 min and diluted either to 20 µM or to 250 µM.



- **No influence of strand concentration for sequences beginning and ending by guanines. Multimers are probably composed by stacked monomers.**
- **Multimers could be interlocked for sequences with additional bases since the proportion of multimers is influenced by the strand concentration.**

Conclusions

1. **Formation of high order parallel G-quadruplex structures are favored by short loops and by K^+ > $NH4^+$ > Na^+. Longer loops favor intramolecular and more antiparallel structures.**
2. **For the first time, trimers have been observed for sequences containing four groups of three guanines. These structures are probably formed by the stacking of monomeric G-quadruplexes.**
3. **Sequences beginning and ending by others bases than G can form dimers but not trimers. These dimers could be formed by interlocked strands.**

References

Smargiasso et al., J. Am. Chem. Soc. (2008), accepted.

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