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Ligand binding to tetra-end-linked $(TGGGGT)_4$ G-quadruplexes: an electrospray mass spectroscopy study

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ABSTRACT

The binding properties of a series of known G-quadruplex ligands have been studied by ESI-MS experiments. The tetramolecular $(TG_4T)_4$ quadruplex and its analogues I and II blocked, respectively, at the 3' or 5'-end by a tetra-end-linker (TEL) unit were chosen as the ligands targets. The stoichiometries of the obtained complexes as well as the ligand affinity and selectivity to the different quadruplexes were determined to deduce the ligand binding site. The TEL derivatives I and II allowed the probing of the grooves contribution to the binding of ligands to G-quadruplexes, demonstrating that the 3' and 5' quartets are not equivalent binding sites for ligand end-stacking.

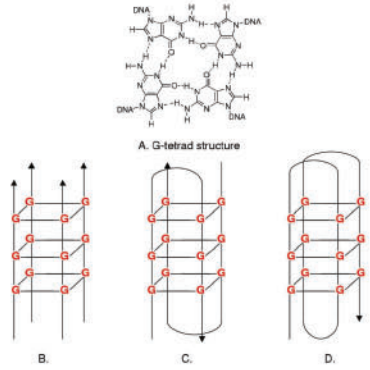

Fig. 1 a) G-tetrads; b) tetramolecular parallel quadruplex; c) bimolecular antiparallel quadruplex; d) monomolecular antiparallel quadruplex.

INTRODUCTION

Guanine-rich oligonucleotides (GROs) tracts of nucleic acids can form four-stranded structures, also known as G-quadruplexes, made up of G-quartet subunits with four coplanar guanines (G) linked together by Hoogsteen hydrogen bonds.[1,2] G-rich strands can adopt a variety of folds, that can be intra- or intermolecular[3] (Fig. 1). Evidence for the in vivo formation of G-quadruplex structures is increasing.[4] In particular, molecules able to induce G-quadruplex structures are intensively studied for their ability to inhibit telomerase, thus acting as potential antitumoral agents. Several classes of small molecules that induce and/or stabilize G-quadruplex structures and inhibit human telomerase have already been characterized.[5] The rapid screening of the interaction between ligands and simple G-quadruplex models is surely relevant to assess the binding mode and the structural specificity. Electrospray mass spectrometry (ESI-MS) of non covalent complexes has found important application as a screening tool in drug-nucleic acid interactions.[6,7]

RESULTS AND DISCUSSION

ESI-MS has been used to study the interactions between some known G-quadruplex ligands and two $(TG_4T)_4$ analogues in which the 3' or 5' ends, respectively for **I** and **II**, are attached to a tetra-end-linker (TEL) unit[8] (Fig. 2 and 3). These analogues represent useful models to investigate the ligand interaction sites of $(TG_4T)_4$ parallel quadruplex, because the presence of the TEL at the top of the 3' or 5' G-quadruplex face is expected to decrease or prevent the end-stacking of the ligands (Fig. 3). We focused on the stoichiometries and on the signal intensities of the complexes formed between the ligands and **I** or **II**, compared to that formed with $(TG_4T)_4$, to obtain information about ligand affinities and selectivities for the 3' or 5' face. The ligands chosen for this study are the Distamycin A, PIPER and TMPyP4.

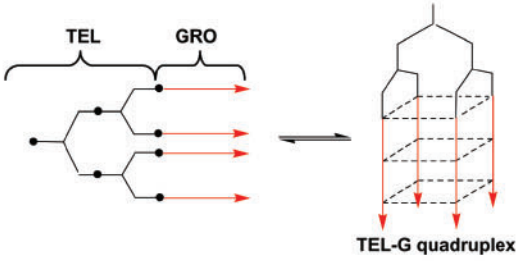

Fig.2 Schematic representation of TEL-oligonucleotides. Unfolded form (left side) and folded TEL-G-Quadruplex (right side).

Distamycin A can associates with G-quadruplex DNA either via stacking interactions with the terminal guanine residues at the ends of G-runs[9] or by binding to quadruplex grooves.[10,11] We observed that the binding sites of Distamycin A to **$(TG_4T)_4$** are still present in the linked quadruplexes (**I** and **II**, Fig.2), but the affinities are decrease as **$(TG_4T)_4$** > **II** > **I**, indicating that some TEL steric hindrance occurs.

In the case of PIPER, for which end-stacking is the main binding mode,[12] we observed that **$(TG_4T)_4$** can accommodate two ligands with high affinity and one with low affinity. **I** and **II** interact with only one PIPER, and with a lower affinity than the two preferred sites of **$(TG_4T)_4$**. This result suggests that PIPER could interact with **I** and **II** by the third site of **$(TG_4T)_4$**, probably a groove binding site.

Two interacting mode are credited for TMPyP4: external stacking to the end of G-quadruplex and externally binding in the groove.[14] In this case the TEL quadruplexes can still accommodate TMPyP4 in their two binding sites as **$(TG4T)_4$** and the scale of affinity follows the order **II** > **$(TG4T)_4$** > **I**. These results suggest either that TMPyP4 binding sites are in the grooves, or that the insufficient linker rigidity does not prevent end-stacking on the TEL side.

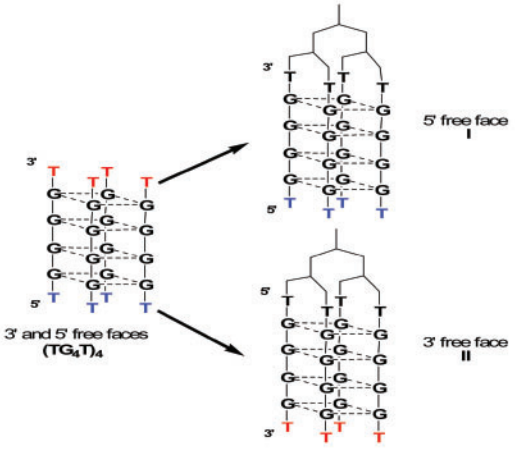

Fig. 3 Comparison between **$(TG_4T)_4$**, **I** and **II.**

Table 1 ESI-MS results (150 mM NH_4Cl, pH 7.0).

Ligand	$(TG4T)_4$	I	II
Dystamicin A	[Q+L]; [Q+2L]	[Q+L]; [Q+2L]	[Q+L]; [Q+2L]
PIPER	[Q+L]; [Q+2L]; [Q+3L]	[Q+L]	[Q+L]
TMPyP4	[Q+L]; [Q+2L]	[Q+L]; [Q+2L]	[Q+L]; [Q+2L]

MS experiments using **I** and **II** with shorter and less flexible linkers are under way to better distinguish between different ligand interaction possibilities.

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