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Studies on the influence of inversion of polarity sites on the dG residues glycosidic conformation in quadruplex structures

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ABSTRACT

Insights into the influence of inversion of polarity sites on the dG residues glycosidic conformation in quadruplexes is presented. The NMR studies concern modified oligodeoxynucleotides based on the quadruplex forming sequence TGGGT.

INTRODUCTION

G-quadruplexes are higher-order DNA and RNA structures formed by G-rich sequences based on tetrads of hydrogen bonded guanine bases.[1] The important role of these structures in biological systems relies mainly on three features, since they are probably (in several cases definitely) involved in: (i) the architecture of telomeres of many organisms;[2] (ii) G-rich sequences that are present within a wide range of genes;[3] (iii) the scaffolds of several oligonucleotide aptamers.[4] G-quadruplexes are structures characterized by a great variability.[5] Their structural differences involve several aspects, which are often mutually interconnected. For example, the relative orientation of the strands affects the glycosidic conformation (*syn* or *anti*) of the guanosine residues forming the scaffold of the quadruplex structure. The structural variability of the quadruplexes has been further increased by introducing modification on both bases[6] and sugar-phosphate backbone.[7] In the latter frame, some of us reported the description of quadruplex structures formed by the sequence TGGGGT containing a 3'–3' or 5'–5' inversion of polarity site in the G-stretches, namely $^{5'}$TGG$^{3'}$-$^{3'}$GGT$^{5'}$ (Q33),[8] $^{3'}$TGG$^{5'}$-$^{5'}$GGT$^{3'}$ (Q55),[8] $^{5'}$TG$^{3'}$-$^{3'}$GGGT$^{5'}$ (QS33)[9] and $^{3'}$TG$^{5'}$-$^{5'}$GGGT$^{3'}$ (QS55).[9] Among these, Q33 and QS33 show parallel-like quadruplexes each characterized by four all G-*anti* tetrads. On the other hand, although Q55 maintains a parallel-like structure, it possesses an all G-*syn* tetrad adjacent to the inversion of polarity site, while QS55 shows a two-fold symmetric structure in which a tetrameric antiparallel quadruplex is embedded between two parallel tracts. In order to get further insights into the influence of the inversion of polarity site on the glycosidic conformation of the adjacent G residues, we have synthesized six oligonucleotides containing inversion of polarity sites (ODNs) based on TGGGT sequence (Tab. 1) and investigated the nature of the quadruplex structure adopted by them.

Table 1 ODNs forming quadruplex structures. The underlined residues indicate a *syn* glycosidic conformation.

sequence	
$^{5'}$dS$^{3'}$-$^{3'}$GGGT$^{5'}$	**I**
$^{5'}$T$^{3'}$-$^{3'}$GGGT$^{5'}$	**II**
$^{5'}$TG$^{3'}$-$^{3'}$GGT$^{5'}$	**III**
$^{3'}$dS$^{5'}$-$^{5'}$GGGT$^{3'}$	**IV**
$^{3'}$T$^{5'}$-$^{5'}$GGGT$^{3'}$	**V**
$^{3'}$TG$^{5'}$-$^{5'}$GGT$^{3'}$	**VI**

RESULTS AND DISCUSSION

The ODNs **I-VI**, synthesized by a reported procedure,[9] can be grouped in two series: the 3'-3' series (**I-III**) and the 5'-5' series (**IV-VI**). In ODNs **I** and **IV**, dS indicates a sugar-mimic, namely dSpacer (Fig. 1).

All ODNs are able to form four-fold symmetric parallel-like quadruplex structures characterized by three G-tetrads, as clearly indicated by the ^{1}H-NMR spectra showing mainly three signals in the region 10.5-12 ppm, attributable to imino protons involved in Hoogsteen hydrogen bond (for an example see Fig. 2).

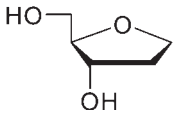

Fig. 1 Structure of dSpacer (dS) introduced in ODNs **I** and **IV**.

However, in order to obtain more information about the main structural features, most of the ^{1}H-NMR resonances of quadruplexes have been assigned by means of the analysis of 2D TOCSY and 2D NOESY experiments (for an example see Tab. 2). The NOE patterns of the quadruplexes formed by ODNs **I**, **II**, **III** (series 3'-3') and **IV** (series 5'-5') turned out to be similar to that of other parallel quadruplex structures. The lack of strong NOEs between any G H8 and H1' of the same residue, in comparison to those observed between each G H8 and its ribose H2'/H2'', indicates that all G residues are in the *anti* glycosidic conformation. As for ODNs **V**, and **VI** (series 5'-5'), useful information about the quadruplex structures

adopted arose from an in-depth analysis of the 2D NOESY spectrum. Firstly, an intense H8–H1' NOEs (diagnostic of a *syn* glycosidic conformation) was clearly observable for the two quadruplex structure (for an example see Fig. 3).

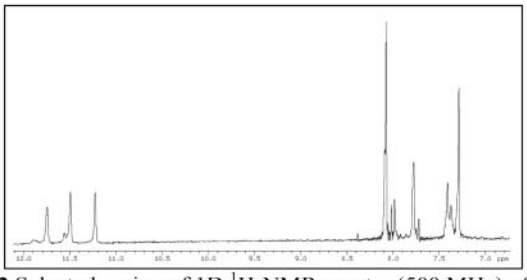

Fig. 2 Selected region of 1D ^{1}H-NMR spectra (500 MHz) of **III**.

The entire sets of NOE crosspeaks allowed us to assign these signals to the first G residue of the $^{5'}$GGGT$^{3'}$ (**V**) and $^{5'}$GGT$^{3'}$ (**VI**) tracts. In both cases the all-*syn* tetrads are adjacent to the 5'-5' inversion of polarity site. In agreement with literature data, the H8 resonance of these *syn* G residues is upfield shifted with respect to those of the *anti* ones.

Table 2 ^{1}H-NMR resonance values of **V** (3'-T_1-5'-5'$G_2G_3G_4T_5$-3') (500 MHz, T = 25°C)

	H8/H6	H1'	H2' H2"	H3'	H4'	H5' H5"	CH_3
T_1	7.51	6.06	2.12	4.37	4.15		1.38
G_2	7.37	5.96	3.00 3.60	4.95	4.13	3.59	
G_3	8.10	5.73	2.62 2.68	5.01	4.39	4.09 4.19	
G_4	7.60	6.22	2.45 2.60	4.81	4.43	4.21	
T_5	7.32	6.00	2.10	4.39	3.98		1.56

Data concerning quadruplex structures formed by ODNs **I**-**III** (3'-3' series) clearly indicate that all dG residues show an *anti* glycosidic conformation. Such a result is in agreement with those pertaining to quadruplex structures Q33[8] and QS33[9] (see above) in which only *anti* 2'-deoxyguanines occur. On the other hand, the behaviour of ODNs **V** and **VI**, belonging to the 5'-5' series, is quite different. In fact, quadruplexes formed by them show an all-*syn* tetrad adjacent to the inversion of polarity site.

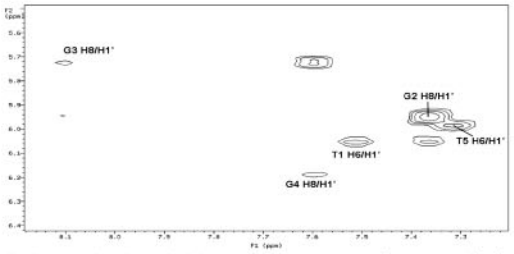

Fig. 3 2D NOESY (500 MHz) contour plot correlating base sugar H1' protons in **V** (3'-T_1-5'-5'$G_2G_3G_4T_5$-3').

Interestingly, in quadruplex from ODN **IV**, in which an abasic site (dSpacer) replaces the nucleoside, only G-*anti* tetrads are present. The NMR data indicate that in quadruplexes **V** and **VI**, the 5'-5' inversion of polarity site promotes the formation of an all-*syn* tetrad adjacent to the site, on condition that a nucleoside is present in the other position adjacent of the 5'-5' inversion of polarity site.

CONCLUSION

The NMR results reported here describe the structural features concerning the glycosidic conformation of dG residues in a series of quadruplex structures based on sequence TGGGT and containing inversion of polarity sites. The whole collected data suggests that a 5'-5' inversion of polarity site is able to promote the formation of an adjacent all-*syn* tetrad. However, the crucial role of the base next to the 5'-5' inversion of polarity site clearly comes out for **IV** since this ODN, having a sugar-mimic moiety next to the 5'-5' inversion of polarity site instead of a nucleoside, forms a quadruplex containing only *anti* dG residues, although it belongs to the 5'-5' series. To get further information about the role of bases adjacent to the inversion of polarity sites in these structural systems, a detailed thermodynamic analysis of the quadruplexes formed by ODNs **I**-**VI** is in order and it is in progress in our laboratories.

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