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Quadruplexes of human telomere $dG_3(TTAG_3)_3$ sequences containing guanine abasic sites

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ABSTRACT

This study was performed to evaluate how the loss of a guanine base affects the structure and stability of the three-tetrad G-quadruplex of 5′-$dG_3(TTAG_3)_3$, the basic quadruplex-forming unit of the human telomere DNA. None of the 12 possible abasic sites hindered the formation of quadruplexes, but all reduced the thermodynamic stability of the parent quadruplex in both NaCl and KCl. The base loss did not change the Na^+-stabilized intramolecular antiparallel architecture, based on CD spectra, but held up the conformational change induced in $dG_3(TTAG_3)_3$ in physiological concentration of KCl. The reduced stability and the inhibited conformational transitions observed here *in vitro* for the first time may predict that unrepaired abasic sites in G-quadruplexes could lead to changes in the chromosome's terminal protection *in vivo*.

1. Introduction

Abasic sites are the most common and consistently repaired lesions in genomic DNA. The sites are formed by spontaneous base loss, mainly by depurination, and as intermediates in the enzymatic base excision repair process of various base lesions [1,2]. Thousands of purine bases are released from DNA in every human cell daily, and if not repaired the AP sites are highly mutagenic [1]. Natural AP sites exist in equilibrium states between hemiacetals and aldehyde forms, and due to the aldehydes the AP sites are unstable that lead to DNA chain-breaks through beta-elimination [1]. Therefore, stabilized forms of AP sites, mainly the tetrahydrofuranyl analog are preferentially studied in DNA models. Thermal and thermodynamic stability [3–6], conformation [6–8], as well as repair and replication [5,9,10] of natural and synthetic AP sites have been widely studied in the canonical, double-helical oligodeoxynucleotide models. In the non-double helical, unusual structures the effect of AP lesions is less known. This was a rather unexplored area until recently. Studies on the energetic effects and repair of synthetic AP sites in a loop structure formed by a CAG triplet repeat sequence were published not long ago [11,12], and also on the effect of a stabilized AP site on the structure of model parallel quadruplexes composed of four 5′-$dT(G)_nT$ strands [13]. The frequently occurring natural base lesion, the AP site can form in any other non-canonical DNA structures as well, e.g. in the non-coding telomeric regions of the human chromosomes, including the G-rich, potentially quadruplex forming 3′-overhang structures. Non-repaired lesions in the overhang structures can have critical consequences *in vivo*, considering the important role of telomeres in the maintenance of genome integrity and cell survival [14]. We present here a study on the effect of the loss of particular guanine bases from the 21 nucleotide-long basic human telomere sequence unit $dG_3(TTAG_3)$ on the formation of G-quadruplexes and their stability.

2. Materials and methods

The deoxyoligonucleotides used in this study were prepared in an Expedite 8900 apparatus by the Department of Functional Genomics and Proteomics, Masaryk University, Brno, on 50 nmol scales using chemicals from Glen Research. For the abasic sites the dSpacer CE Phosphoramidite was used. Deprotection and DMT removal were carried out on Poly-Pak cartridges (Glen), and the eluted oligomers were purified and desalted by Centri.Spin-10 Sephadex G-25 (Princeton Separations) size exclusion chromatography. Purity was checked by denatured gel electrophoresis. Solution concentrations were determined at 260 nm, 90 °C using molar absorbancies of 10,640 M^{-1} cm^{-1} for all sequences, based on [15]. The coefficients for the AP-quadruplexes, related to nucleosides, differed within 1% of 10,640. CD spectra were

Abbreviations: AP site, abasic site; AP-quadruplex, abasic site-containing quadruplex.

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Table 1
The DNA sequences studied.

Number[a]	5′–3′ sequence[b]
0	GGGTTAGGGTTAGGGTTAGGG
1	**x**GGTTAGGGTTAGGGTTAGGG
2	G**x**GTTAGGGTTAGGGTTAGGG
3	GG**x**TTAGGGTTAGGGTTAGGG
7	GGGTTA**x**GGTTAGGGTTAGGG
8	GGGTTAG**x**GTTAGGGTTAGGG
9	GGGTTAGG**x**TTAGGGTTAGGG
13	GGGTTAGGGTTA**x**GGTTAGGG
14	GGGTTAGGGTTAG**x**GTTAGGG
15	GGGTTAGGGTTAGG**x**TTAGGG
19	GGGTTAGGGTTAGGGTTA**x**GG
20	GGGTTAGGGTTAGGGTTAG**x**G
21T	GGGTTAGGGTTAGGGTTAGG**xT**

[a] The number is used to refer to this sequence in the text, and is also indicative of the position of the AP substitution.
[b] The **x** stands for the abasic site.

measured in a Jobin–Yvon Mark VI dichrograph in 1 cm pathlength cells at oligonucleotide concentrations of 65 μM in nucleosides (~3 μM in strand) at 21 °C. Before the measurements the samples were held at 90 °C for 5 min, then left to slowly cool to room temperature. Melting curves (0–95 °C, 95–0 °C and again 0–95 °C) were measured by ramp rate of 0.2 °C/min in a Varian Cary 4000 spectrophotometer, and analyzed at 296 nm [16]. Melting of each quadruplex was reversible and the melting and annealing curves were superimposable, indicating true equilibria, which are the basis for two-state melting. Thermodynamic parameters were extracted from the curves with the use of MeltWin program, version 3.0 [17], as described earlier [18].

CD and absorption spectra were determined in Robinson–Britton (R–B) buffer plus 0.1 M NaCl or KCl. The resulting Na^+ or K^+ concentration was 0.169 M.

Native polyacrylamide gel electrophoreses were run in a temperature-controlled apparatus (SE-600; Hoefer Scientific). Gel concentration was 16% (29:1 monomer to bis ratio). Two micrograms

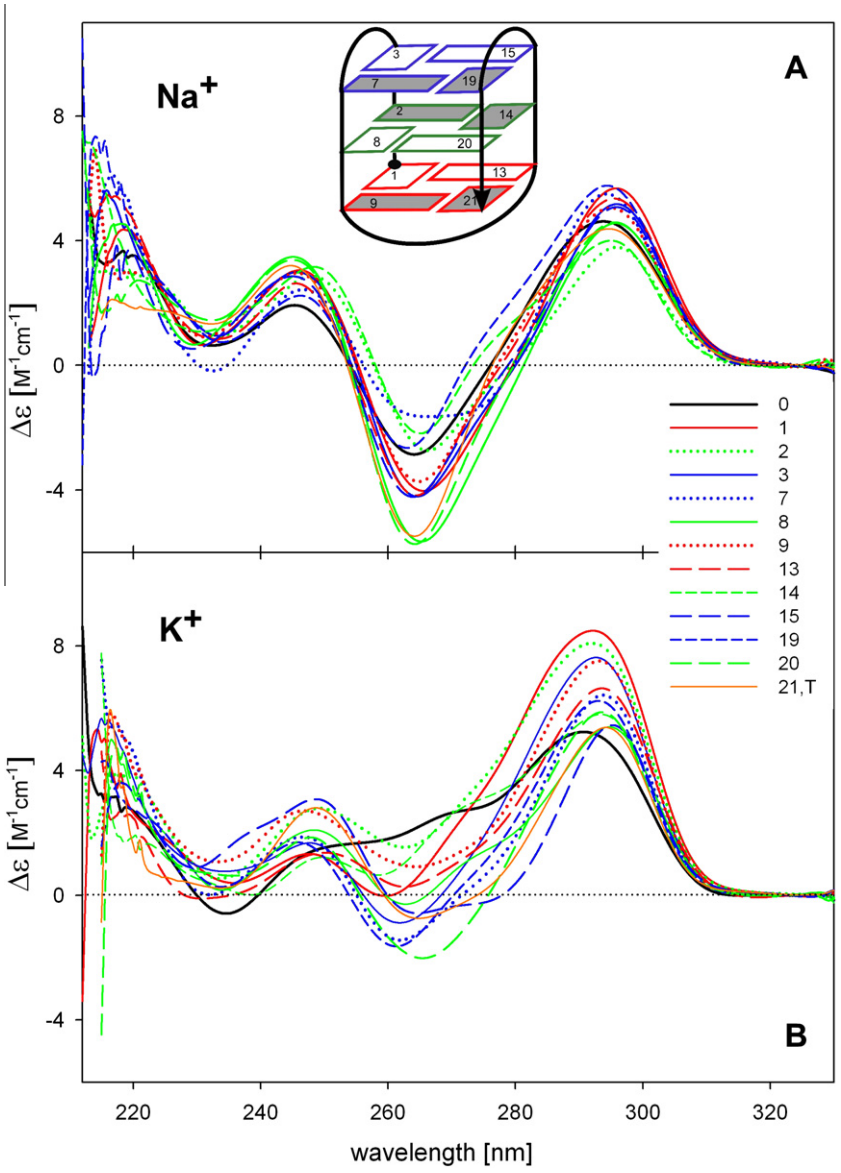

Fig. 1. CD spectra of $dG_3(TTAG_3)_3$ and its analogs containing abasic sites measured in R-B buffer, pH 7 and 100 mM NaCl (top) or KCl (bottom) at 0 °C. DNA concentrations were 65 μM in nucleosides. Inset: Quadruplex of $dAG_3(TTAG_3)_3$ formed in the presence of Na^+ (*syn* geometries are shadowed) [20] .