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Review

# Diverse effects of naturally occurring base lesions on the structure and stability of the human telomere DNA quadruplex

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ABSTRACT

Various base lesions continuously form in cellular nucleic acids and the unrepaired lesions are promutagenic and procarcinogenic. Though natural base lesions have been extensively studied in double-stranded DNA models, these studies are only less than a decade old for non-canonical DNA models, such as quadruplexes. Here we present a report on the effects of three frequently occurring natural lesions that can form in the TTA loops on the structure of the human telomere quadruplex d[$AG_3(TTAG_3)_3$]. We compared the effect of the abasic site and 8-oxoadenine replacing adenine and 5-hydroxymethyluracil substituting for thymine. The results showed that the three lesions impacted the stability and quadruplex folding in markedly different ways. The effects depended on the type of lesion and the position in the sequence. Analogous lesions of guanine in the G-tetrads extensively destabilized the quadruplex and the effects depended more on the position than on the type of lesion. The distinct effects of the loop substitutions as well as comparison of the modifications of the loops and the quadruplex tetrads are discussed in this communication.



## 1. Introduction

Nucleic acids in living organisms are continuously attacked by exogenous and endogenous agents including UV light, ionizing radiation, and reactive compounds. DNA bases are susceptible to chemical modification via different type of damage, such as oxidation, alkylation, radiation damage, and hydrolysis. These modifications are widespread and play important roles in altering physiological states and they can lead to diseases. The majority of genetic impairments are widely believed to originate in oxidative processes that can be the basis of mutation, aging, cell death and carcinogenesis [1–3]. Reactive oxygen species that arise endogenously originate in cell aerobic metabolism and also contribute to age-dependent diseases [4,5]. Damage to DNA can arise directly from ionizing energy or indirectly from hydroxyl radicals through the ionization of the DNA solvation shell [6]. The direct type of damage contributes to about 50% of overall DNA damage [7]. Indirect-type damage occurs when hydroxyl radicals remove a hydrogen atom from a CH of the sugar moiety in the presence of oxygen [8]. The radical cation (electron ionization hole) can then travel hundreds of Angstroms by hopping before being entrapped, preferentially by purine bases [9,10]. Oxidation of purines initially leads to 8-oxo-7,8-dihydroguanosine (8-oxoG), 8-oxo-7,8-dihydroadenosine (8-oxoA), and 8-oxo-7,8-dihydroinosine (8-oxoI), which can occur both within RNA and DNA strands, as well as in the nucleotide pools. Of these, the oxidation products of guanine have been studied most extensively [11]. 8-oxoA is thought to be less mutagenic than 8-oxoG according to Tan et al. [12], while a frequency of their occurrence in mammalian DNA was reported to be similar [13–15]. Increased levels of 8-oxoA were detected in some human tumor tissues and this suggests that 8-oxoA could also be carcinogenic [16]. Its role in the development of type II diabetes has also been reported [17]. Ionizing radiation can lead to the formation of 5-hydroxycytosine, 5-hydroxyuracil, 5-

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hydroxymethyluracil and thymine glycol, which may then cause chronic inflammatory disease, prostate, breast and colorectal cancer development [18]. Despite the various cellular defense mechanisms against oxidative damage [19] the human cell genome receives *ca.* $10^4$ oxidative hits a day [20].

Base damage can arise anywhere in chromosomal DNA including the telomeres, both in the double-stranded part of the telomere DNA, composed of TTAGGG/CCCTAA repeats in humans, and in the 100–200 nucleotide-long 3′ single-stranded overhang of TTAGGG repeats. The latter can form non-canonical secondary structures, including D-loop, T-loop, and the four-stranded G-quadruplexes. The overhang structures are protected by the protein complex, Shelterin *in vivo* [21]. The extent of shielding by Shelterin from direct and indirect-types of oxidation is high but not perfect. Furthermore, the reactive oxygen species reacting with the binding proteins results in protein radicals, which can in turn, oxidize the nucleic acid, forming C4'-radicals that can lead to abasic (AP) sites and initiate the charge hopping processes [22].

Human telomere (htel) quadruplex shows considerable conformational polymorphism [23–25] with a number of quadruplex topologies, depending primarily on the type of counter ions, ionic strength, oligonucleotide sequence, and DNA concentration. The effect of natural base lesions of G-tetrads on htel quadruplex structural properties has been studied by us and other laboratories [26–40]. Much less is known however about the effect on the quadruplex structure of such lesions of the loops [40–42]. Current knowledge is that the sequence and length of loop determine primarily the quadruplex folding, which of course, influences its stability [43,44]. A recent study on single-molecule mechanical unfolding of a three-tetrad telomeric G-quadruplex however concluded that the loop interactions contributed more to G-quadruplex stability than the stacking of G-tetrads [45]. Given the critical consequences of unrepaired oxidative DNA damage [46] in promoters and chromosome-end G-quadruplexes we continued our studies on natural base damages with lesions that can form in the TTA loops of the human telomere G-quadruplex. The effects of three such lesions, an abasic (AP) site in the place of adenine, 8-oxoadenine (8-oxoA) and a thymine lesion, the 5-hydroxymethyluracil (5-hmU) were selected for this review (Fig. 1). The lesions were site-specifically incorporated into the sequences corresponding to the TTA loops of the htel G-quadruplex, the 22mer d[$AG_3(TTAG_3)_3$]. The sequences used for the study are shown in Table 1.

According to the original report of Wang and Patel [47] the human telomere 22 nucleotide long $AG_3(TTAG_3)_3$ sequence forms an antiparallel three-tetrad quadruplex with edgewise, diagonal and edgewise loops in $Na^+$ ions containing solution. Its folding does not principally change in the presence of potassium ions [25,48,49]. The schematic folding and the numbering used in this communication of the $AG_3(TTAG_3)_3$ are illustrated in Fig. 2. UV absorption, CD, and $^1H$ NMR spectroscopies and gel electrophoresis were the methods used for elucidating the stability and folding topologies.

**Table 1**
The sequences of the human telomere 22mer and its analogs used in this work.

| Abbreviation | 5′-to-3′ sequence |
|---|---|
| wt (wild-type) | AGGG TTA GGG TTA GGG TTA GGG |
| apA7 | AGGG TT***AP*** GGG TTA GGG TTA GGG |
| apA13 | AGGG TTA GGG TT***AP*** GGG TTA GGG |
| apA19 | AGGG TTA GGG TTA GGG TT***AP*** GGG |
| 8-oxoA1 | ***O***GGG TTA GGG TTA GGG TTA GGG |
| 8-oxoA7 | AGGG TT***O*** GGG TTA GGG TTA GGG |
| 8-oxoA13 | AGGG TTA GGG TT***O*** GGG TTA GGG |
| 8-oxoA19 | AGGG TTA GGG TTA GGG TT***O*** GGG |
| 5-hmU5 | AGGG ***hU***TA GGG TTA GGG TTA GGG |
| 5-hmU6 | AGGG T***hU***A GGG TTA GGG TTA GGG |
| 5-hmU11 | AGGG TTA GGG ***hU***TA GGG TTA GGG |
| 5-hmU12 | AGGG TTA GGG T***hU***A GGG TTA GGG |
| 5-hmU17 | AGGG TTA GGG TTA GGG ***hU***TA GGG |
| 5-hmU18 | AGGG TTA GGG TTA GGG T***hU***A GGG |

The abbreviations $apA_x$, 8-$oxoA_x$ and 5-$hmU_x$ stand for the studied sequences containing abasic site (*AP*) or 8-oxoadenine (*O*) substituted for adenine, and 5-hydroxymethyluracil (*hU*) for thymine, respectively. The number x corresponds to the order of the base in the $AG_3(TTAG_3)_3$ 22mer.

## 2. Abasic (AP) sites replacing 2'-deoxyadenosine in TTA loops of the htel quadruplex

### 2.1. Structural properties of the adenine AP site-containing quadruplexes in $Na^+$ solutions

The CD spectrum of the $Na^+$-stabilized quadruplex is characterized by positive bands at 295, 240 and 215 nm and a deep negative band at 260 nm [25,49–51]. It is generally accepted [49,50] that this spectrum corresponds to an antiparallel quadruplex of basket-type [47] (Fig. 2). The AP site-modified apA7 and apA13 oligonucleotides also folded into antiparallel scaffolds in sodium solutions similar to the non-modified wt sequence, based on their CD spectra [40] (Fig. 3A). The presence of the AP site caused substantial destabilization of their quadruplexes as compared to the $T_m$ value of the wt. The apA19 displayed a very unusual spectrum containing two separated positive maxima close to 295 and 255 nm. The same CD spectrum has been recently observed in $Na^+$ solution with a htel 27mer and its derivative substituted by 8-bromoguanine in position 22 (corresponding to G20 in our 21mer). The structure was interpreted by NMR as a novel antiparallel (2 + 2) quadruplex with edgewise-propeller-edgewise loops [52]. The loss of adenine in apA19 affected quadruplex thermostability less than with apA7 and apA13 [40] (Fig. 3, insert a1 and Table 2).

### 2.2. Structural properties of the adenine AP site-containing quadruplexes in $K^+$ solutions

In $K^+$ solution the CD spectrum of the wt quadruplex distinctly differed from that observed in $Na^+$ solution [25,40]. Namely, the negative 260 nm band, characteristic of $Na^+$-stabilized quadruplex

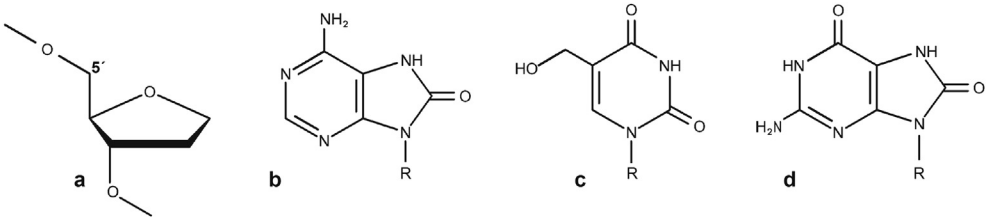


**Fig. 1.** (a) Tetrahydrofuranyl abasic (AP) site (dSpacer), incorporated; (b) 7,8-Dihydro-8-oxoadenine (8-oxoA); (c) 5-hydroxymethyluracil (5-hmU); (d) 7,8-Dihydro-8-oxoguanine (8-oxoG); The R stands for the 2'-deoxyribose residue.