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The abasic site lesions in the human telomeric sequence d[TA(G_3T_2A)$_3G_3$]: A thermodynamic point of view

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ABSTRACT

Background: The abasic sites represent one of the most frequent lesions of DNA and most of the events able to generate such modifications involve guanine bases. G-rich sequences are able to form quadruplex structures that have been proved to be involved in several important biological processes.
Methods: In this paper, we report investigations, based on calorimetric, UV, CD and electrophoretic techniques, on 12 oligodeoxynucleotides analogues of the quadruplex forming human telomere sequence d[TA(G_3T_2A)$_3G_3$], in which each guanine has been replaced, one at a time, by an abasic site mimic.
Results: Although all data show that the modified sequences preserve their ability to form quadruplex structures, the thermodynamic parameters clearly indicate that the presence of an abasic site decreases their thermal stability compared to the parent unmodified sequence, particularly if the replacement concerns one of the guanosines involved in the formation of the central G-tetrad.
Conclusions: The collected data indicate that the effects of the presence of abasic site lesions in telomeric quadruplex structures are site-specific. The most dramatic consequences come out when this lesion involves a guanosine in the centre of a G-run.
General significance: Abasic sites, by facilitating the G-quadruplex disruption, could favour the formation of the telomerase primer. Furthermore they could have implications in the pharmacological approach targeting telomere.

1. Introduction

The ability of DNA to form higher order secondary structures and, in several cases, the occurrence of proteins able to specifically recognize and interact with them make these structures one of the most attractive research fields in molecular biology. In recent years, amongst the various unusual DNA structures discovered to date, G-quadruplexes (based on the stacking of two or more cyclic arrangements of four guanines, called G-tetrads) have been the focus of several disciplines ranging from structural chemistry to medicinal chemistry [1] and, recently, to nanotechnology [2]. Their spread is probably due to some main peculiar features: 1) an outstanding thermal stability if compared to other DNA structures [3]; 2) the consensus sequence required for their formation (at least four runs of guanines, with at least two guanines per run) that is particularly prone to be identified in genomes by suitable algorithms [4]; 3) their tremendous structural variability [5] and 4) the presence of more than 370,000 G-rich sequences in the human genome potentially able to fold in G-quadruplex structures [4].

Particularly, G-rich sequences have been observed in critical segments of eukaryotic and prokaryotic genomes, promoter regions of ribosomal DNAs, as well as telomeres in eukaryotes and immunoglobulin heavy chain switch regions of higher vertebrates [6]. The potential of these sequences to form G-quadruplex structures is related to transient duplex destabilization, a process that accompanies several significant biological events such as transcription, replication and recombination. Taking into account the potential roles that G-quadruplexes could play in many biological processes the sequence integrity of the G-tract is a key factor for their functionality. It is well known that a broad variety of causes renders DNA susceptible to damages and mutations [7,8]. One of the most frequent lesions of DNA is the presence of abasic sites that can arise from spontaneous (such as depurination) [9] and enzymatic processes [10] or chemical damage, for example due to free radicals and alkylating agents promoting the release of bases, often by introducing modifications that destabilize the *N*-glycosidic bond [11,12]. Since many of the events generating abasic sites involve guanine bases, the investigation of the effects of their presence on G-quadruplex structures for which a biological role has been ascertained or is strongly suspected (such as the structures formed by telomeric sequences) [13], represents

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an important and quite unexplored research topic. Recently we investigated the structural, thermodynamic and kinetic properties of parallel G-quadruplex formed by modified sequences d(TG_5T) containing an abasic site replacing, one at a time, a guanosine in the sequence [14]. Although all oligonucleotides investigated preserve their ability to form quadruplexes, both spectroscopic and kinetic experiments point to sequence-dependent different effects on the structural flexibility and stability. Afterwards, CD and PAGE investigations concerning the human telomere sequences d[$G_3(TTAG_3)_3$] [15] and d[$A(G_3T_2A)_3G_3$] [16] containing abasic sites have been reported. In the first study authors found that none of the 12 possible abasic sites hinders the formation of the quadruplex, but all provide the structure with a reduced thermodynamic stability compared to the parent sequence [15]. However, since this study is based on a quadruplex formed by a natural human telomeric sequence never appropriately characterized by specific structural methods of investigation as NMR or X-ray, it is rather difficult to discuss the effects of abasic sites in this sequence without having the unmodified quadruplex structure as a reference. The second study focusses on the effects of molecular crowding on the structure and stability of the quadruplex formed by the human telomere sequences d[$A(G_3T_2A)_3G_3$] [16]. In this case, although structural studies have clearly ascertained [17] that this sequence forms an antiparallel quadruplex in Na^+ solution, a detailed characterization of this structure in K^+ solution is lacking, probably as it does not form a single G-quadruplex structure in this condition [18]. Taking into account that the K^+ solution is usually accepted as a good model of intracellular environments (in which the potassium is much more abundant than sodium) this point represents the major drawback of the study. Even though the structure of the single-stranded telomeric overhang is not known, some important investigations showed that the most plausible structure in solution involves quadruplexes in an alternating hybrid 1–hybrid 2 arrangement [19,20]. For these reasons, investigations concerning the effects of abasic sites on the telomeric structure should mainly involve sequences able to form hybrid 1 or hybrid 2 quadruplex types.

The present study is based on the human telomere sequence d[$TA(G_3T_2A)_3G_3$], that has proven to form a major monomolecular quadruplex in potassium ions solutions (hybrid 1), whose structure has been extensively investigated by NMR techniques [21]. Each of the sequences in which a guanine residue has been replaced, one at a time, by an abasic site mimic (Fig. 1, Table 1), has been studied for the first time by calorimetric (DSC: differential scanning calorimetry) and specific UV (TDS: thermal difference spectra) [22] techniques, besides CD and electrophoretic techniques. Our data suggest that the presence of the abasic site destabilizes the 3 + 1 hybrid 1 structure and favours the formation of an antiparallel quadruplex with two G-tetrads. A short discussion concerning the biological and pharmacological impact of the presence of abasic sites in the telomere architecture is also included.

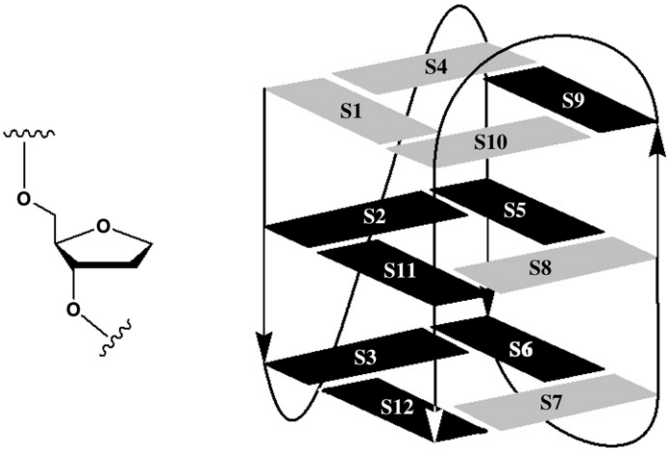

Fig. 1. Schematic representation of dSpacer and d[$TA(G_3T_2A)_3G_3$] quadruplex structure. Left: structure of the tetrahydrofuranyl analogue, dSpacer (dS), introduced in ODNs in Table 1 as an abasic site mimic. Right: quadruplex structure adopted by the sequence d[$TA(G_3T_2A)_3G_3$] (Nat). Non-G residues have been omitted for clarity. *Anti* and *syn* residues are in black and grey, respectively. Guanosines have been labelled according the residues replaced by a dS in ODNs listed in Table 1.

Table 1
Sequences of the modified oligonucleotides containing the abasic site (dS); the unmodified sequence is listed first.

Sequence name	Sequences
Nat	TAGGGTTAGGGTTAGGGTTAGGG
S1	TAdSGGTTAGGGTTAGGGTTAGGG
S2	TAGdSGTTAGGGTTAGGGTTAGGG
S3	TAGGdSTTAGGGTTAGGGTTAGGG
S4	TAGGGTTAdSGGTTAGGGTTAGGG
S5	TAGGGTTAGdSGTTAGGGTTAGGG
S6	TAGGGTTAGGdSTTAGGGTTAGGG
S7	TAGGGTTAGGGTTAdSGGTTAGGG
S8	TAGGGTTAGGGTTAGdSGTTAGGG
S9	TAGGGTTAGGGTTAGGdSTTAGGG
S10	TAGGGTTAGGGTTAGGGTTAdSGG
S11	TAGGGTTAGGGTTAGGGTTAGdSG
S12	TAGGGTTAGGGTTAGGGTTAGGdS

2. Materials and methods

2.1. Oligonucleotides synthesis and purification

Oligonucleotides reported in Table 1 were synthesized on a Millipore Cyclone Plus DNA synthesizer using solid phase β-cyanoethyl phosphoramidite chemistry at 15 μmol scale. The synthesis was performed by using normal 3′-phosphoramidites and a 5′-dimethoxytrityl-3′-phosphoramidite-1′,2′-dideoxyribose (dSpacer, dS, Link Technologies) for the introduction of an abasic site mimic moiety in each sequence. For ODN S12 a universal support was also used. The oligomers were detached from the support and deprotected by treatment with concentrated aqueous ammonia at 55 °C overnight. The combined filtrates and washings were concentrated under reduced pressure, redissolved in H_2O, analyzed and purified by high-performance liquid chromatography on a Nucleogel SAX column (Macherey–Nagel, 1000-8/46), using buffer A: 20 mM KH_2PO_4/K_2HPO_4 aqueous solution (pH 7.0) containing 20% (v/v) CH_3CN and buffer B: 1 M KCl, 20 mM KH_2PO_4/K_2HPO_4 aqueous solution (pH 7.0) containing 20% (v/v) CH_3CN; a linear gradient from 0 to 100% B for 45 min and flow rate at 1 ml/min were used. The fractions of the oligomers were collected and successively desalted by Sep-pak cartridges (C-18). The isolated oligomers proved to be > 98% pure by NMR.

2.2. Circular dichroism

CD (circular dichroism) spectra and CD melting curves were registered on a Jasco 715 circular dichroism spectrophotometer in a 0.1 cm pathlength cuvette and the wavelength was varied from 220 to 320 nm. The spectra were recorded with a response of 16 s at 2.0 nm bandwidth and normalized by subtraction of the background scan with buffer. The temperature was kept constant at 20 °C with a thermoelectrically controlled cell holder (Jasco PTC-348). For all the sequences the strand concentration was 25 μM.

CD melting curves were registered as a function of temperature from 20 to 80 °C at 294 nm with a scan rate of 1 °C min^{-1}. The CD melting curves were modelled by a two-state transition according to the van't Hoff analysis [23]. The melting temperature (T_m) and the enthalpy change (ΔH°) values (Table 2) provide the best fit of the experimental melting data.