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Effect of γ-hydroxypropano deoxyguanosine, the major acrolein-derived adduct, on monomolecular quadruplex structure of telomeric repeat d(TTAGGG)$_4$

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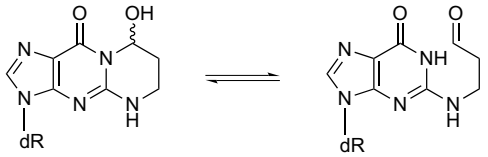
Abstract—The three oligodeoxyribonucleotides (ODNs) **a**–**c**, having the telomeric repeat d(TTAGGG)$_4$ sequence and incorporating γ-hydroxypropano deoxyguanosine at different positions, were synthesized. Gel electrophoresis and CD analyses indicated that the ODNs assume monomolecular quadruplex structures in Na^+ and in K^+ buffers. The T_m values, obtained by CD melting experiments, showed that the presence of the acrolein-dG adduct into the ODN **b** decreases the thermal stability of the monomolecular quadruplex structure in Na^+ solution, whereas for **a** and **c** no significant effect could be detected in the same experimental conditions. On the contrary, all ODNs **a**–**d** show the same behaviour in K^+ buffer. These findings are briefly discussed.

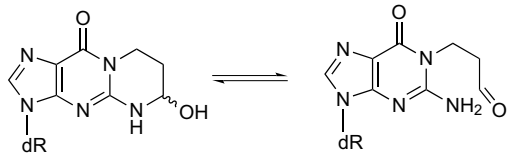
1. Introduction

Acrolein, one of the products of incomplete combustion of organic substrates (including cigarette smoke)[1,2] and the principal cytotoxic metabolite of anticancer and arthritis drug cyclophosphamide,[3–6] is biologically important at a number of levels. First of all, it is endogenously produced during lipid peroxidation.[7–11] Furthermore, evidence has been provided that acrolein, as well as other members of the α,β-unsaturated aldehydes family, is highly mutagenic to bacterial and mammalian cells and exhibits tumour-initiating activity.[7,12] One of the mechanisms of this mutagenicity is founded on the ability of these molecules to react with DNA.[13–20] In fact, acrolein is a bifunctional electrophilic agent able to form exocyclic adducts with nucleophilic sites present in DNA bases.[21,22] The most abundant adducts, formed with dG residues,[21,22] are γ-hydroxypropano deoxyguanosine (γ-OH-PdG, **1**) and α-hydroxypropano deoxyguanosine (α-OH-PdG, **2**), in equilibrium with the corresponding open ring form (Fig. 1).

Several structural studies have been carried out by a number of authors to investigate the relationship



1. γ-OH-PdG



2. α-OH-PdG

Figure 1.

Keywords: Acrolein; Telomeric repeat; Quadruplex.
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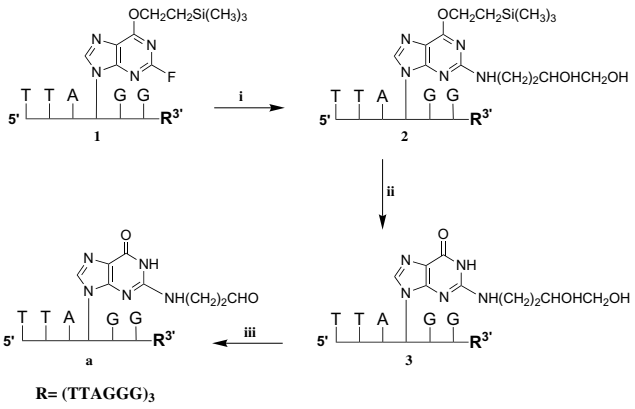
Table 1. Specific position of modified dG residue into the telomeric sequence $^{5'}$TTAG$_a$G$_b$G$_c$TTAGGGTTAGGGTTAGGG$^{3'}$

ODNs	G_a	G_b	G_c
a	γ-OH-PdG	dG	dG
b	dG	γ-OH-PdG	dG
c	dG	dG	γ-OH-PdG
d	dG	dG	dG

between the lesion product on B-DNA structure and the mutagenic activity of α,β-unsaturated aldehydes.[23–28] It has been demonstrated that in B-DNA the γ-OH-PdG derivative is prevalently present in the open-ring form[23,24] and the canonical G–C base pairing is preserved at neutral pH values. In contrast, the presence of N-1 alkyl dG derivatives, such as α-OH-PdG, prevents the Watson–Crick base pairing and alters the B motif of the double helix both in open and in closed ring forms. This structural event could interfere with cellular replication processes[23–25] and could explain the major mutagenic effect due to the formation of α-OH-PdG derivative. Nevertheless, to the best of our knowledge, no structural studies concerning potential alteration on noncanonical DNA conformations,[29–31] such as left handed Z-DNA, cruciforms, triplex and quadruplex structures, induced by the presence of dG-acrolein derivatives have been reported so far. On the other hand, much experimental evidence indicates that these noncanonical conformations do exist in vivo and may play important roles in regulating replication and transcriptional processes as well as in recombination events.[29–31] One of the most important G-rich regions of the genome able to assume a noncanonical DNA conformation (quadruple helix structure) is the telomeric repeat, occurring at the ends of eukaryotic chromosome.[32,33] Telomeres are essential for gene integrity and exert a crucial role in cellular senescence and carcinogenesis.[34] Several in vivo experiments showed that changes in the composition of the telomeric complex, due to mutated DNA-binding site, lead to telomere dysfunction.[35] Consequently, it is reasonable to hypothesize that acrolein, by reacting with dG residues, could interfere with normal functions of telomeric regions. In this frame, we have undertaken a preliminary structural investigation concerning the effects produced on the monomolecular quadruplex conformation assumed by ODNs containing a γ-OH-PdG derivative. For this purpose, we have synthesized three ODNs, **a**, **b** and **c** (Table 1), having the sequence of the telomeric repeat **$^{5'}$TTAG$_a$G$_b$G$_c$TTAGGGTTAGGGTTAGGG$^{3'}$**, in which the modified dG residue γ-OH-PdG, has been introduced at different positions. The ODN **d** corresponds to the unmodified sequence, able to form monomolecular quadruplex structures, as shown by spectroscopic[36] and crystallographic techniques.[37]

2. Results and discussion

The ODNs have been prepared through the synthetic procedure developed by Harris and co-workers[38,39] with minor modifications. The general procedure, reported in Scheme 1 for **a**, is based on the solid-phase synthesis



Scheme 1. Post-synthetic modification of the fluorinated ODN. Reagents and conditions: (i) 4-amino-1,2-butanediol in DMSO, 50 °C, 5 h; (ii) 0.1 M acetic acid, rt, 2.5 h; (iii) $NaIO_4$, rt, 10 min.

(using N-PAC phosphoramidite building block) of the ODN **1** containing a 2-fluoro 2′-deoxyinosine residue which, in turn, is converted in the intermediate **3** by treatment with 4-amino-1,2-butanediol and successive removal of O^6-TMSE group from modified dG residue.[38] The oxidation reaction on **3**, with $NaIO_4$, leads to the target ODN **a**.

In particular, after solid phase synthesis, the fluorinated ODN was detached from solid support and deprotected by treatment with NaOH (0.1 M, rt, 12 h). The purified ODN **1**[40] was reacted with 4-amino-1,2-butanediol[41] (DMSO, 50 °C, 5 h) and then isolated by precipitation in diethyl ether. Dried solid residue was dissolved in water and further deprotected at O^6 function by treatment with acetic acid (0.1 M, rt, 2.5 h) to obtain **3**. The pH of the solution was adjusted at 7.0 and the mixture was purified by HPLC using a RP C_{18} column. This further purification step was necessary since we observed that the reaction of **1** with the amine causes a partial degradation of **2**, thus generating side products with shorter sequences, whose amount can be reduced using a lower excess of amine than that reported in the literature.[42] Finally, **3** was converted into the target product **a** by oxidation with $NaIO_4$ followed by standard purification. Using this procedure, we obtained 15 OD_{260} units of the final product **a** from 30 OD_{260} units of fluorinated ODN **1**. The introduction of the modified dG residue in ODNs **a**–**c** was confirmed by MALDI-TOF spectrometry (positive mode).[43] The ability of ODNs **a**–**c** to fold into a intramolecular quadruplex structure, in Na^+ buffer, was tested by CD and electrophoresis analyses. Figure 2 shows CD spectra of **a**–**d** at 20 °C and neutral pH, characterized by a positive and negative Cotton effect at 295 and 265 nm, respectively. These data strongly suggest that **a**–**d** assume a very similar monomolecular quadruplex structure and they are corroborated by the electrophoretic analyses performed in non-denaturing conditions. The result of a typical experiment is reported in Figure 3, which shows for the ODNs (**a**–**d**) almost identical electrophoretic mobility. Furthermore, in agreement with previously reported observations, **a**–**d** being folded in monomolecular quadruplex structures,

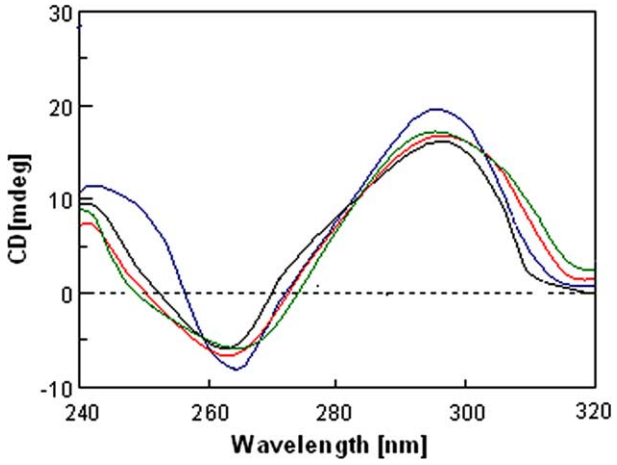


Figure 2. CD profiles for ODNs **a**–**d**, **a** (red); **b** (blue); **c** (green); **d** (black) in 5 mM phosphate buffer containing 140 mM NaCl and 5 mM $MgCl_2$ at 20 °C.

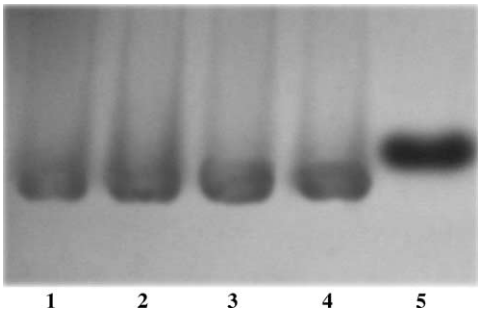


Figure 3. Analysis on 12% nondenaturing polyacrylamide gel containing 70 mM NaCl at 25 °C: lane 1 unmodified 24-mer ODN **d**; lane 2 ODN **a**; lane 3 ODN **b**; lane 4 ODN **c**; lane 5 12-mer with sequence $d(T)_{12}$.

migrate much faster than a shorter (12-mer) ODN, used as a mobility reference.[44]

Thermal stabilities of **a**–**c**, valued by CD melting denaturation experiments at 295 nm, were performed increasing the temperature at a rate of 0.5 °C/min (Fig. 4). T_m

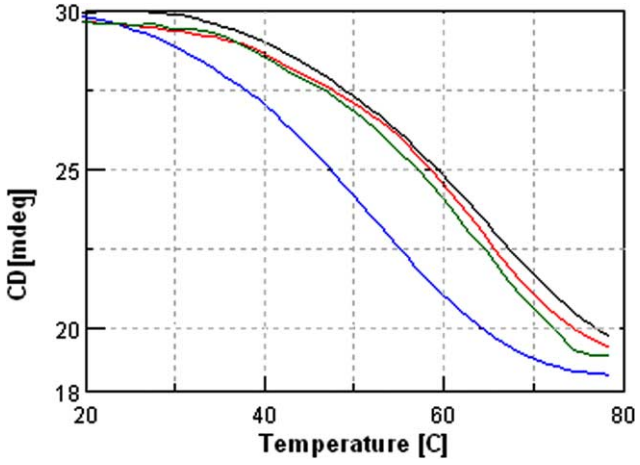


Figure 4. Melting profiles of ODNs **a**–**d**; **a** (red) T_m = 65 °C; **b** (blue) T_m = 54 °C; **c** (green) T_m = 63 °C; **d** (black) T_m = 67 °C.

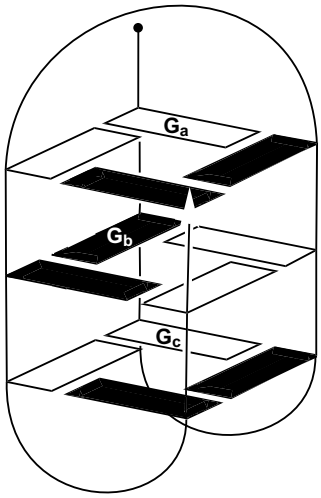


Figure 5. Schematic representation of quadruplex structure: the black and white rectangles represent G-*syn* and G-*anti*, respectively. The position of modified dG residue into ODNs **a**–**c** are indicated.

values for **a** and **c** were found to be similar to that of the unmodified **d** (T_m = 67 °C), while a significant reduction of T_m was observed for **b** (T_m = 54 °C).

These results can be tentatively explained assuming that the modified ODNs **a**–**c** adopt, in Na^+ buffer, a very similar monomolecular quadruplex structure (Fig. 5) described for the four-repeat human telomer d[AGG-G(TTAGGG)$_3$] sequence,[36] with four GGG segments involved in the formation of three stacked G-tetrads and three TTA connecting loops in successive edgewise, diagonal and edgewise alignments. In this structure adjacent strands result to be antiparallel to each other with a *syn–syn–anti–anti* orientation for dG residues in each tetrad.

In this structure, G3, G4 and G5 should adopt *anti*, *syn*, *anti* conformations, respectively. On the other hand, it is reasonable to suppose that the presence of a bulky group at N2-position of the modified nucleobase, should favour an *anti*-orientation around the N-glycosidic bond. Thus, while in ODNs **a** and **c**, where the modified nucleoside is expected to adopt an *anti* N-glycosidic conformation, the presence of the propanal group at N2 should produce a slightly positive, if any, effect on the stability of the quadruplex structure, in the case of **b** it leads to a destabilization of the conformation, since the modified nucleobase is required to assume a *syn*-orientation.

A more physiologically relevant study of the four repeat human telomere sequence (ODN **d**) in K^+ solution is complicated by the absence of a definitely predominant conformation. In fact, in these conditions, parallel quadruplex structure found in a K^+ containing crystal[37] is likely to coexist with additional forms, including the Na^+ solution structure, in those conditions as suggested by Phan and Patel.[45]

Nevertheless, some remarks of purely qualitative nature can be drawn by CD studies. First of all, CD profiles of

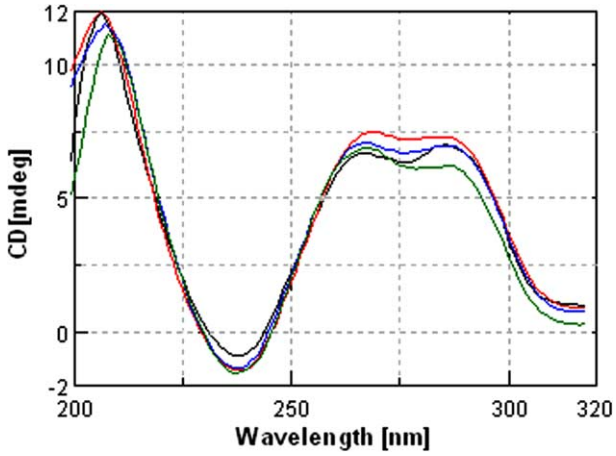


Figure 6. CD profiles for ODNs **a**–**d**, **a** (red); **b** (blue); **c** (green); **d** (black) in 5 mM phosphate buffer containing 140 mM KCl and 5 mM $MgCl_2$ at 20 °C.

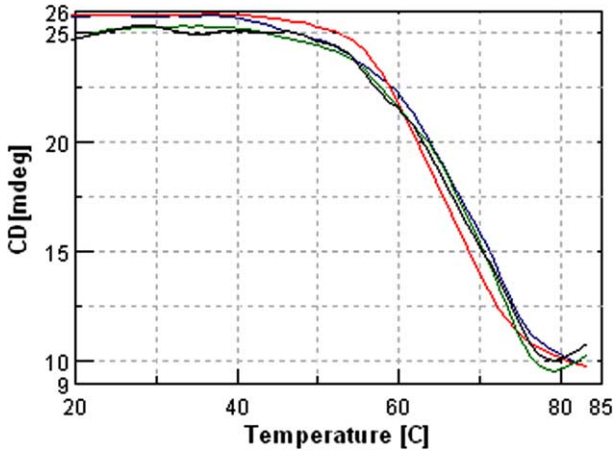


Figure 7. Melting profiles of ODNs **a**–**d**; **a** (red); **b** (blue); **c** (green); **d** (black); all T_m values are about 72 °C.

ODNs **a**–**c**, almost completely superimposable to that of **d**, indicate that a similar equilibrium among various conformations in K^+ solution should exist for the modified ODNs, as well (Fig. 6). Secondly, the T_m values of ODNs **a**–**c** are, with no exception, very close to that of the unmodified counterpart (Fig. 7). This result, although not conclusive, is consistent with our hypothesis for Na^+ solution structures, considering that the crystal structure, significantly present in K^+ solution, is characterized by all the guanosines in an *anti*-conformation.

3. Conclusion

In conclusion, in this preliminary study we focused on a further possible factor associated to acrolein toxicity. In fact, we found that the presence of N-2 acrolein-dG into the four repeat human telomere sequence ODN may, at least in principle, affect the stability of the monomolecular quadruplex structure, which it assumes in solution. Possible production of γ-hydroxypropano deoxyguanosine adducts into the synthetic sequence $d(TTAGGG)_4$ by exposure to acrolein, is currently under investigation in our laboratory with the aim of evaluating the sensitivity of this ODN at different experimental conditions. In fact, as reported by Marnett and co-workers,[46] the adduct formation by reaction with α,β unsaturated aldehydes, may be critically influenced by the DNA secondary structure, as a consequence of the steric access to the target nucleophile located in the minor groove. The ascertainment of the effective formation in vivo of the N-2 acrolein-dG derivative and, in the case, its influence on telomeres maintenance, will be the next target of our researches. As a matter of fact, the reaction of acrolein with nucleophilic sites of DNA bases might be prevented in vivo, since the formation of protein-DNA complexes supply the ends of linear eukaryotic chromosomes with a protective cap, which favours the conservation of their integrity.[34] On the other hand, recent studies suggest that telomeres exist in two different architectures, namely an 'open' and a 'closed' form, depending on the cell-cycle.[47–52] The switch from one state to the other, through a not completely clear mechanism, might produce a temporary increase of the exposure of telomeric DNA region, thus allowing the nucleophilic attack of guanine N-2 to the α,β-unsaturated aldehyde to occur. A detailed NMR study of the solution structures of ODNs **a**, **b** and **c** is currently in progress in our laboratory, as well.

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