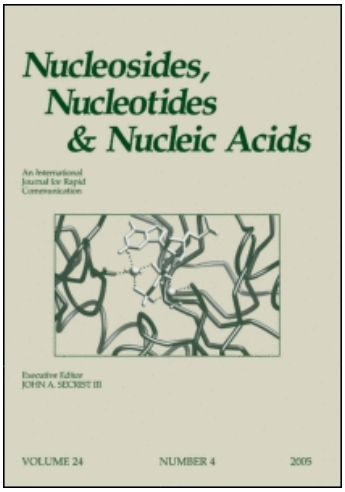

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EFFECTS OF ACROLEIN ON THE QUADRUPLEX FORMING d(TTAGGG)$_4$ TELOMERIC REPEAT SEQUENCE

Nicola Borbone, Giuliana D'Isa, Giorgia Oliviero, Gennaro Piccialli, Michela Varra, and Luciano Mayol ▫ *Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II," Napoli, Italy*

▫ *HPLC and ESI-MS analysis have been used to investigate the effect of acrolein exposure on d(TTAGGG)$_4$ human telomeric repeat. Preliminary results disclosed a novel relationship between the structure assumed by oligodeoxynucleotides (ODNs) and the capability of their nucleobase residues to react with acrolein.*

Keywords Oligonucleotide, Acrolein, Quadruplex Structure

INTRODUCTION

Acrolein, one of the products of incomplete combustion of organic substrates (including cigarettes smoke)[1] and the principal citotoxic metabolite of the anticancer and antiarthritis drug cyclophosphamide,[2] is biologically important at a number of levels. It is endogenously produced during lipid peroxidation,[3] and much 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.[4] One of the mechanisms of this mutagenicity is founded on the ability of these molecules to react with the nucleophylic sites on DNA bases to form exocyclic adducts.[5,6] The most abundant adducts, formed with dG residues, are γ-hydroxypropano-deoxyguanosine (γ-OH-PdG, **1**) and α-hydroxypropano-deoxyguanosine (α-OH–PdG, **2**), in equilibrium with the corresponding open ring form (Figure 1).[7] It has been demonstrated that in B-DNA the γ-OH-PdG derivative is prevalently present in the open-ring form, and the canonical G–C base pairing is preserved at neutral pH values. In contrast,

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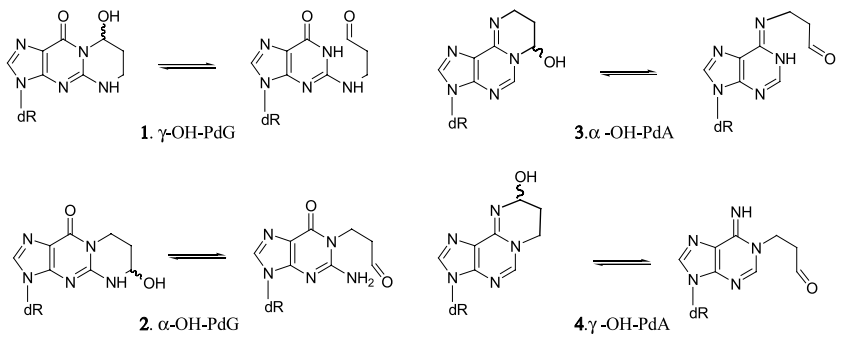

FIGURE 1

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 and could explain the major mutagenic effect due to the formation of **2**. Recently, we have undertaken a study concerning the effect of **1**, the major acrolein-dG adduct, on monomolecular quadruplex structure of telomeric repeat $d(TTAGGG)_4$.[8] The results indicate that the presence of γ-hydroxypropano-dG adducts into $d(TTAGGG)_4$ may influence the stability of its quadruplex structure in Na^+ buffer.

On pursuing this project we have exposed to acrolein folded and unfolded $d(TTAGGG)_4$ in order to verify if acrolein reacts with quadruplex folded $d(TTAGGG)_4$ to form N-2 acrolein-dG adducts.

Reaction of Folded $d(TTAGGG)_4$ with Acrolein

50 OD of $d(TTAGGG)_4$ were dissolved into 500 mL of 140 mM NaCl, 5 mM $MgCl_2$, and 5 mM NaH_2PO_4 buffer, and then submitted to annealing procedure. After 24 h at room temperature, 0.1 mL of acrolein were added and the mixture was heated at 37°C for 72 h, under stirring. The excess of acrolein was then removed by extraction with chloroform. The water solution was lyophilized, dissolved into 20 mM of Tris-HCl buffer containing 10 mM of $MgCl_2$ at pH 8, and treated with phosphodiesterase I and alkaline phosphatase. In order to identify the nucleoside adducts, the products of enzymatic digestion were purified by HPLC and analyzed by ESI-MS. The HPLC profile showed four peaks **a**–**d** (Figure 2). Products **b**, **c**, and **d** resulted to be the unmodified nucleosides dG, dT and dA, respectively, while the mass value of product **a** (m/z 308.2 $[M + H]^+$) indicated it to be a dA-acrolein adduct. However, the retention time of **a** did not correspond to the value reported in the literature for the principal dA adducts (**3** and **4**, Figure 1). No significative peaks corresponding to dG-acrolein adducts **1** and **2** were detected in the analysis of reaction mixture. To confirm that compound **a** was a dA-acrolein adduct, pure dA was submitted to reaction with acrolein in the same experimental

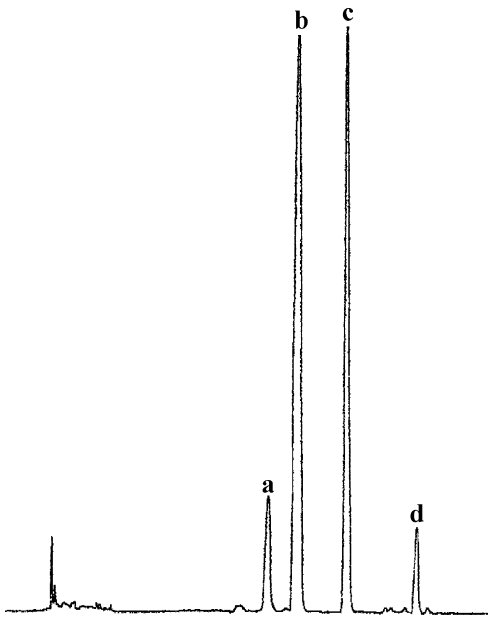

FIGURE 2

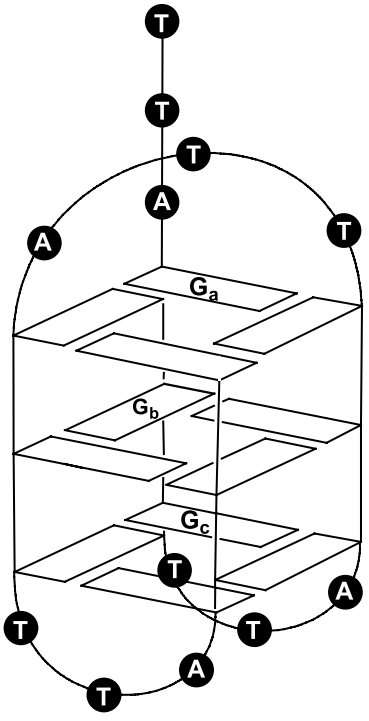

FIGURE 3

conditions. As expected, the reaction mixture analyzed by HPLC and ESI-MS afforded the compounds **3** and **4** as main products along with a small amount of adduct **a**. These qualitative preliminary results showed that the folding of ODN into a quadruplex structure may prevent the reaction of acrolein with dG residues involved in G-quartets, exposing at the same time dA residues present in the loops and in the 5′ tail (Figure 3). This hypothesis was confirmed by reaction of acrolein with unfolded $d(TTAGGG)_4$ followed by enzymatic digestion, HPLC purification, and mass analysis. In this case, together with the unmodified nucleosides dG, dT and dA, dG-acrolein adducts were detected and identified in agreement with HPLC and mass data present in literature.[9]

These results focused on a further possible factor associated to acrolein toxicity. In fact, these preliminary data pointed to a relationship between the structure assumed by ODNs and the capability of their nucleobase residues to react with acrolein.

REFERENCES

1. Yang, Q.; Hergenhahn, M.; Weninger, A.; Bartsch, H. Cigarette smoke induces direct DNA damage in the human B-lymphoid cell line Raji. Carcinogenesis **1999**, *20*(9), 1769–1775.
2. Anderson, M.M.; Hazen, S.L.; Hsu, F.F.; Heinecke, J.W. Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanal, and acrolein. A mechanism for the generation of highly reactive alpha-hydroxy and α,β-unsaturated aldehydes by phagocytes at sites of inflammation. J.Clin. Invest. **1997**, *99*(3), 424–432.
3. Luczaj, W.; Skrzydlewska, E. DNA damage caused by lipid peroxidation products. Cell. Mol. Biol. Lett. **2003**, *8*(2), 391–414.
4. Marnett, L.J.; Riggins, J.N.; West, J.D. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. J.Clin. Invest. **2003**, *111*(5), 583–593.
5. Yang, I.Y.; Miller, H.; Wang, Z.; Frank, E.G.; Ohmori, H.; Hanoaka, F.; Moriya, M. Mammalian translesion DNA synthesis across an acrolein-derived deoxyguanosine adduct: participation of pol η in error-prone synthesis in human cells. J. Biol. Chem. **2003**, *278*(16), 13989–13994.
6. Minko, I.G.; Washington, M.T.; Kanuri, M.; Prakash, L.; Prakash, S.; Lloyd, R.S. Translesion synthesis past acrolein-derived DNA adduct, γ-hydroxypropanodeoxyguanosine, by yeast and human DNA polymerase η. J. Biol. Chem. **2003**, *278*(2), 784–790.
7. Wilson, V.L.; Foiles, P.G.; Chung, F.L.; Povey, A.C.; Frank, A.A.; Harris, C.C. Detection of acrolein and crotonaldehyde DNA adducts in cultured human cells and canine peripheral blood lymphocytes by 32P-postlabeling and nucleotide chromatography. Carcinogenesis **1991**, *12*(8), 1483–1490.
8. D'Isa, G.; Galeone, A.; Oliviero, G.; Piccialli, G.; Varra, M.; Mayol, L. Effect of γ-hydroxypropano deoxyguanosine, the major acrolein-derived adduct, on monomolecular quadruplex structure of telomeric repeat $d(TTAGGG)_4$. Bioorg. Med. Chem. Lett. **2004**, *in press*.
9. Nechev, L.V.; Harris, C.M.; Harris, T.M. Synthesis of nucleosides and oligonucleotides containing adducts of acrolein and vinyl chloride. Chem. Res. Toxicol. **2000**, *13*(5), 421–429.