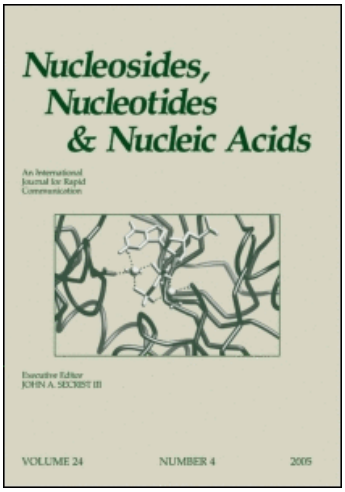

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Epoxide Adducts at the Guanine Residue Within Single-Stranded DNA Chains: Reactivity and Stability Studies

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EPOXIDE ADDUCTS AT THE GUANINE RESIDUE WITHIN SINGLE-STRANDED DNA CHAINS: REACTIVITY AND STABILITY STUDIES

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▫ *Emphasis was placed in this work on the assessment of structural and biological features of nucleobase adducts that result from the reaction of DNA with epoxide derivatives. Thus we have prepared and characterized a set of site-specifically modified oligonucleotides at N^7-position of a guanine residue, upon reaction with diepoxibutane, with the purpose of further investigating some of their biochemical features. The stability of the lesion-containing DNA fragments has also been investigated and clearly shows that the latter modified oligomers may be used as substrates for in vitro enzymatic assays, aimed at determining the biological effects within cell of these chemically induced DNA damage.*

Keywords Epoxide-Mediated DNA Adducts, Guanine Lesion, Modified Oligonucleotides, Chemical Stability

INTRODUCTION

Epoxide derivatives (Figure 1) are known as environmental pollutants and important reagents in chemical industries. The latter compounds can also be formed metabolically from parent alkenes, used as industrial chemicals and present in vehicle gases and tobacco smoke.[1–5] These compounds may be toxic, mutagenic, and carcinogenic by virtue of their ability to modify DNA.[6–12] Several types of lesions arising from the reaction of these alkylating agents with the four DNA bases have been reported. These include monosubstituted *O* and *N*-hydroxyalkylated bases, DNA–DNA, and DNA–protein cross-links. The two latter alteration families are formed by the reaction with bimnctional epoxide derivatives.[13–20]

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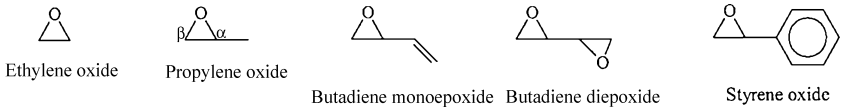

FIGURE 1 Structure of several epoxides of interest, involved in reactivity studies toward DNA.

The carbon atoms at α (*asymmetric center in substituted epoxides that is less reactive due to steric hindrance*) and β positions of oxygen are electrophile species that then react with nucleophilic centers. The nitrogen at position 7 of guanine is the most nucleophilic atom in DNA and sterically available to large molecules, and reaction of epoxides was found to predominately give rise to N^7-guanine adducts. In addition, N^1-adenine, N^3-adenine, N^6-adenine, N^7-adenine, N^3-cytosine, O^2-cytosine, N^3-thymine, O^2-thymine, N^1-guanine, N^2-guanine, and O^6-guanine derivatives are formed in significant yields.[14,21–24]

Nonetheless, the chemical and molecular bases of the various biological effects of epoxides are not fully understood. Thus, despite many studies reported in the literature, the exact role of epoxide-mediated DNA lesions toward the cell machinery is still largely unknown. This lack of information may be mainly attributed to the instability of the major part of these nucleobase adducts, making it difficult to obtain of pure modified lesion-containing oligonucleotides (ODNs) used at probing biological processes. In the goal to further delineate the biological significance of adducts that result from the reaction of DNA with epoxide derivatives, we have recently initiated a research program aimed at preparing several base lesion-containing DNA oligomers. In the present work we report the preparation and the characterization of a set of site-specific modified oligonucleotides at the N^7 position of the guanine, upon reaction with diepoxibutane (DEB). In addition, the stability of the lesion containing DNA fragments has been investigated.

RESULTS AND DISCUSSION

As mentioned above, the DNA sites targeted for alkylation by epoxide derivatives mainly include the N^7 position of guanine, due to good accessibility and high electrophilic.character of this nitrogen atom within the biopolymer. The resulting N^7-hydroxyalkylated guanine adducts, as the different N^7-guanine lesions generate a positive charge at the substituted nitrogen, lead to a destabilization of the modified nucleobase. This instability may give rise to spontaneous degradation of the N^7-modified guanine moieties by two well-known processes: one involves hydrolysis of the *N*-glycosidic linkage to yield the corresponding free modified bases and apurinic sites (depurination reaction); the second one deals with ring opening of the imidazole cycle to give rise to 5-*N*-alkyl-2,6-diamino-4-hydroxyformamidopyrimidine derivatives (alkyl-Fapy-G lesions) (Ref. [25] and references cited, herein).

The major objective of the present research project was to prepare oligonucleotides (ODNs) that contain N^7-hydroxyalkylated guanine adducts. The site-specific incorporation of such base adducts in oligonucleotides by chemical assembling is precluded due to the high lability of the latter alkylated nucleoside. This particularly concerns the alkaline conditions of deprotection used at the final step of solid-phase synthesis.

Preparation and Characterization of the Oligonucleotides Containing N^7-Hydroxyalkylated Guanine Adducts

As an alternative to the usual phase condensation method, which involves acid and alkali deprotection steps incompatible with the instability of the N^7-guanine

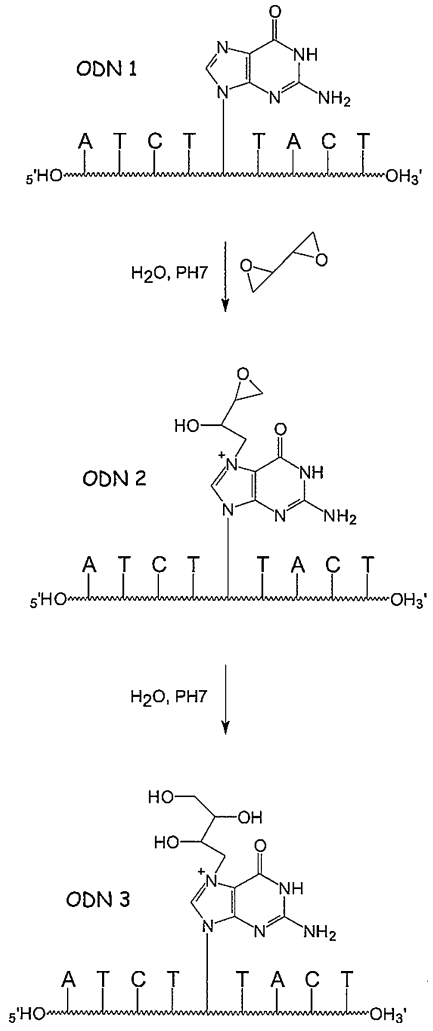

FIGURE 2 Structure of the studied 9-mer oligonucleotide and of the main alkylation products.

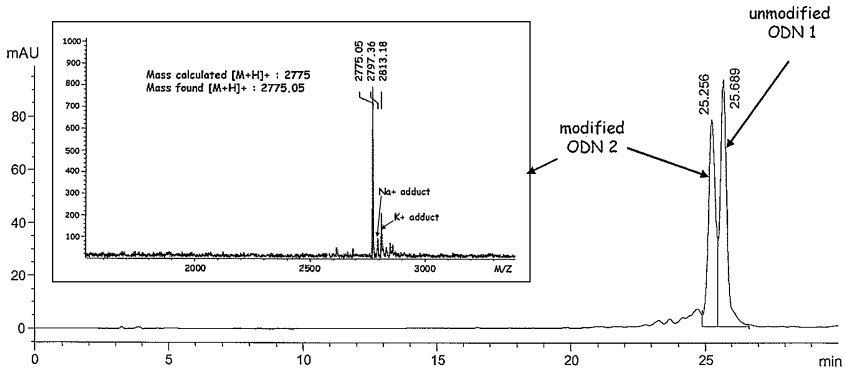

FIGURE 3 RP-HPLC analysis of the reaction mixture of DEB with the 9-mer ODN 1, after 16 h at 20°C (*detection λ = 260 nm*). The inset shows the MALDI-TOF mass spectrum (*positive mode*) of the collected modified ODN **2**, eluted at 25.25 min.

adducts, specific alkylation of guanine within short DNA fragments was carried out. Typically, unmodified synthetic ODNs that exhibit one guanine residue in their sequence were treated with epoxide derivatives (Figure 2).* The reverse-phase HPLC profile obtained after the overnight incubation of the nonamer **1** (5′ATCTGTACT-3′) with diepoxybutane is shown in Figure 3A. This shows the formation of a major product (in approximately 50% yield), which eluted at 25.2 min, corresponding to the N^7-hydroxyalkylguanine containing ODN **2**. Relevant structural information on the latter modified oligonucleotide was inferred from MALDI TOF and electrospray ionization mass spectrometry (MALDI TOF-MS and ESI-MS, respectively) analyses. Interestingly, the MALDI mass spectrum of ODN **2** obtained in the positive mode (Figure 3B) revealed the presence of a pseudo-molecular ion at $[M + H]^+ = 2775.05$. This is indicative of a molecular weight of 2774 for **2**, which was in agreement with the expected value (MW = 2774).

Partial conversion of ODN **2** (*monoepoxide derivative*) into ODN **3** (*trihydroxyluted derivative*) by spontaneous hydrolysis in neutral aqueous solution after incubation for 96 h at room temperature[26,27] was observed. The modified oligonucleotide **3** is formed as the sole significant product, in an approximate 30% yield as detected by MALDI TOF-MS analyses (Figure 4). It is worth mentioning that no detectable depurinated and imidazole ring-opening products were detected in this incubation experiment. This is of major interest since the stability of the studied adducts in single-stranded DNA fragments (stability that is generally significantly increased in double-stranded sequences[25] appears totally compatible

*Typically 4 AU_{260nm} of ssDNA fragments were incubated in 250 pL of water (PH 6.5) and 500 mM diepoxybutane (10 pL, Aldrich) for 16 h at room temperature. Then, the reaction mixture was purified by RP-HPLC, by using a Cl8 column and a linear gradient of acetonitrile (from 3 to 15% 30 min) in 10 mM TEAA. The N^7-modified guanine-containing ODNs are obtained in an approximately 50% yield.

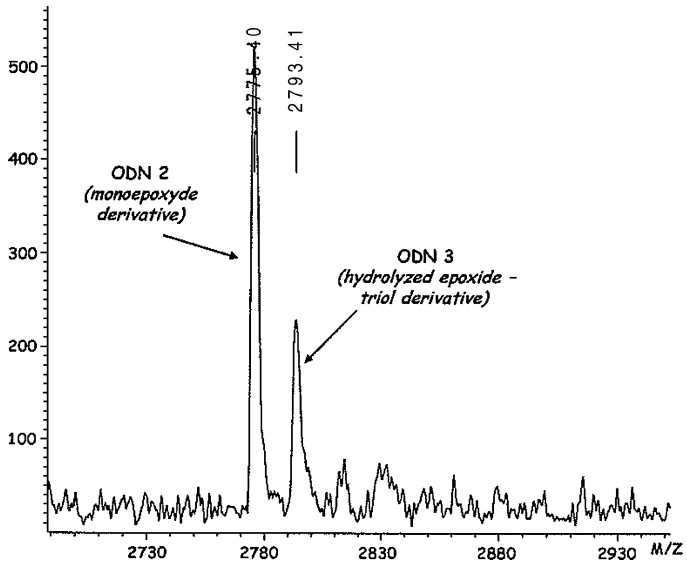

FIGURE 4 MALDI-TOF mass, spectrum (*positive mode*) of ODN, 2 after partial hydrolysis (incubation in H_20, 96 h at room temperature).

with the in vivo replication and repair processes (*whose timescales are of few hours*), and should allow in vitro biochemical assays aimed at evaluating their cellular consequences.

Stability Study of the N^7-Hydroxyalkylated Guanine Containing DNA Fragment

The isolation of modified ODN **2** has allowed the determination of the stability of the latter N^7-hydroxyalkylated guanine containing DNA fragment under the hot piperidine conditions used to reveal alkali-labile DNA damage in modified oligonucleotides.[28] A comparative study of the stability of ODNs **1** and **2** was performed by treating both 5′-^{32}P-labeled oligonucleotides with 1 M piperidine at 90°C for 30 min. In a subsequent step, the DNA fragments were analyzed by denaturing polyacrylamide gel electrophoresis (PAGE) and the results are shown in Figure 5A. Interestingly, the unmodified guanine containing ODN **1** was stable under piperidine treatment while the cleavage was quantitative after 30 min at the N^7-hydroxyalkylated guanine site of modified ODN **2**. It should be added that the PAGE mobility of the released labeled DNA fragment was consistent with the cleavage of ODN **2** at the modified site. To confirm the latter alkali-induced cleavage mechanism at the alterated site,.the piperidine reaction mixture was analyzed by MALDI-TOF mass spectrometry. The spectrum obtained (Figure 5B) clearly shows that the cleavage of the DNA fragment occurs at the central modified guanine by a β,δ-elimination mechanism that generates a single peak at $[M + H]^+ = 1229$ uma. The latter data provide further support to the site-specific

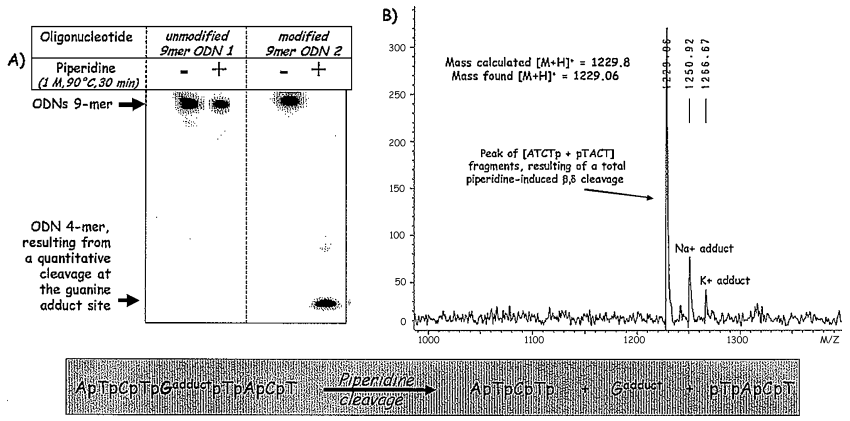

FIGURE 5 A) Denaturating 20% PAGE analysis of the 5′-end labeled ODN 1 and ODN 2 submitted to piperidine treatments, revealing alcali labile sites. B) MALDI-TOF mass spectrum (*positif mode*) of the modified ODN **2** after 1 M piperidine treatment-induced cleavage.

formation of N^7-hydroxyalkylated guanine upon diepoxibutane-mediated alkylation of the nonanucleotide.

CONCLUSION AND OUTLOOK

The successful incorporation of labile N^7-hydroxyalkylated guanine moieties into several single-stranded DNA fragments (*from trinucleotides up to 15-mer DNA fragments, data not shown*) has been achieved, upon treatment with diepoxibutane of oligonucleotides that contain one guanine base, in the conditions described herein. The resulting modified oligomers have been characterized by mass spectrometry analyses and then successfully used to confirm the high lability of these N^7-hydroxyalkylated guanine derivatives to hot piperidine treatment. It should be added that this approach, which was used for the synthesis of diepoxybutane-mediated guanine lesions, is currently extended in our laboratory to the preparation of various N^7-alkylated guanine containing oligonucleotides by using other epoxide derivatives. The availability of such site-specific modified DNA probes would facilitate the determination of biological features (mutagenicity, repair)[29–33] of these important epoxide-induced DNA base lesions. This will be achieved by using in vitro enzymatic assays compatible with the stability of the present adducts.

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