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Chemistry of the 2-Deoxyribonolactone Lesion in Oligonucleotides: Cleavage Kinetics and Products Analysis

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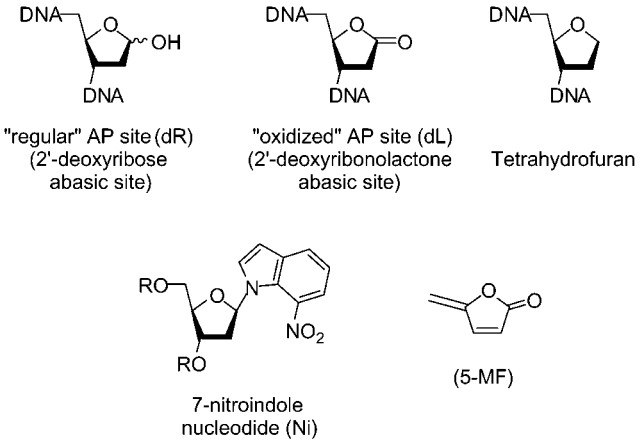
Abstract: Deoxyribonolactone in DNA is an oxidized abasic site damage that is produced by a variety of physical and chemical agents such as γ-irradiation and ene−diyne antibiotics. The extent and biological significance of the lesion are poorly documented due to the high lability of the damaged DNA. The chemistry of degradation of deoxyribonolactone-containing DNA was investigated using oligonucleotides of different length (5-, 11-, 23-, 34-mers) in which the lactone was photochemically generated, as already reported, from oligonucleotide precursors containing a photoactive nitroindole residue. The procedure was successfully extended to double-strand synthesis by irradiation of the preformed duplex in which one strand contained the nitroindole residue. The degradation kinetics were investigated as a function of pH, temperature, length, and ionic strength. The cleavage fragments resulting from β- and δ-eliminations were isolated and identified by ^{1}H NMR. It was found that the lesion is extremely sensitive to pH and temperature while slightly dependent upon ionic strength, length, and sequence. The cleavage rates for the β- and δ-elimination steps are of the same order of magnitude. The deoxyribonolactone site leads to greater instability of DNA than the "regular" deoxyribose abasic site.

Introduction

Loss of a base in DNA leaving a deoxyribose residue is probably the most frequent DNA lesion,[1−3] and a number of reports have been devoted to the chemistry and biology of this "abasic site" damage.[4] Closely related is the deoxyribonolactone (dL) site that structurally corresponds to the oxidized form of the former (see Chart 1). This oxidative damage has been shown to be formed under the action of a diversity of physical and chemical agents that share the property of abstracting hydrogen at the anomeric carbon of nucleotide residues through a radical process. Evolution of this C-1′ radical in water in the presence of oxygen leads to formation of deoxyribonolactone. Ionizing radiations,[5−7] anticancer antibiotics of the ene−diyne family,[8−11] heterocyclic *N*-oxides of the tirapazamine family,[12,13] artificial nucleases based on manganese porphyrin,[14] or copper phenanthroline[15−17] have been reported to create such deoxyribonolactone damage. In a number of cases, formation of deoxyribonolactone has been evidenced by identification of the degradation products evolving from this damaged DNA through cleavage by successive β- and δ-eliminations of the 3′- and 5′-

Chart 1. Structures of the 2′-Deoxyribose and 2′-Deoxyribonolactone Lesions and of the Tetrahydrofuran Stable Analogue, 7-Nitroindole Nucleoside (Ni), and 5-Methylene Furanone (5-MF)



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phosphates, with ultimate formation of 5-methylene furanone (5-MF).[14−18] The high lability of deoxyribonolactone in DNA constitutes a strong limitation to knowledge of the generality and extent of its formation, as well as to the biological consequences of the lesion. Indeed, little is known about the chemistry and biology of the lesion.[19] It has been reported to be mutagenic and to be resistant to repair enzymes.[20,21] Recently it has been proposed to form cross-links with the base excision repair enzyme endonuclease III.[22] This instability also limits the possibility of introducing the lesion at any preselected position in a DNA fragment. Several reports have appeared concerning creation of the damage in short oligomers in very specific conditions or in poor yields.[23−31] More recently, however, new approaches of a more general character have appeared.[32]

We recently reported an efficient synthesis based upon photochemical conversion of a nitroindole nucleoside, selectively incorporated in an oligonucleotide, into deoxyribonolactone with simultaneous expulsion of nitrosoindole.[33−35] The procedure was applied to synthesis of an undecamer containing the lesion in the middle of the sequence, and the solution structure was determined by high-field NMR spectroscopy.[36] Longer oligomers were prepared to study the translesional synthesis by polymerases.[37] In the present report, we describe the chemistry of oligonucleotides containing the lesion. We determine the structure of the products that result from cleavage of the DNA strands at the site of the damage through β- and δ-eliminations. We determine the kinetics of the reaction steps. We also study the effects of different factors such as pH, temperature, and ionic strength on the cleavage and evaluate the influence of the length of the oligonucleotides on the cleavage and the possible effect of the nature of the bases that flank the lactone moiety.

Results and Discussion

Synthesis of Single-Stranded Oligonucleotides Containing Deoxyribonolactone. Fragments of different lengths were required for the various studies, i.e., relatively small fragments for the chemical and structural studies (5-, 11-mers) and longer fragments (23-, 34-mers) for the enzymology studies (see Table 1). All oligonucleotides were prepared using essentially the same procedure as reported previously.[33,34]

Table 1. Oligodeoxyribonucleotide Sequences (5′ → 3′)

1	GC**dL**TA
2	CA**dL**GT
3	CGCAC**dL**CACGC
4	CGCGTACGCAC**dL**CACGCATGCGC
5	CGCGTACGCAC**dR**CACGCATGCGC
6	AGCGATGAGAGGCCA**dL**GAGGAATCGCTGGTACCG

The nitroindode phosphoramidite was incorporated site specifically into oligonucleotides using the solid-phase procedure. Typical coupling yields for the modified nucleoside were of the order 80−90%. After HPLC or PAGE purification, the oligonucleotides were irradiated using a high-pressure mercury lamp giving the deoxyribonolactone containing oligonucleotides and nitrosoindole as a side product. Synthesis of longer fragments (23-, 34-mers) required slightly longer irradiation times than the shorter fragments to reach total transformation (∼60 min compared to 30 min in identical irradiation conditions). In all cases, the oligonucleotides were obtained essentially in a pure state. This was ascertained by the presence of one single peak in the HPLC analysis for the smaller oligonucleotides (5-, 11-mers). Analysis of the longer fragments was monitored by capillary gel electrophoresis (CGE) showing essentially one single peak in the electropherogram.

However, in the CGE and PAGE analysis, the lactone-containing DNAs and the corresponding precursor nitroindole-containing oligonucleotides appeared as one common single peak or spot. A base treatment assay was thus developed to estimate the purity of the samples. Warming the irradiated samples in 50 mM NaOH for 15 min at 70 °C led to total cleavage of the lactone-containing oligonucleotides as indicated by disappearance of the corresponding CGE peak or PAGE spot (see for example, Figures 1 and 2) while the precursor nitroindole oligonucleotides remained unchanged in these conditions.

Synthesis of Duplex DNA Containing Deoxyribonolactone. Due to the poor stability to temperature (vide infra) of the lactone fragment in the oligonucleotides, it was of high interest to design synthetic conditions to directly introduce the lesion into the duplex. Duplex **A** (see Table 2) was thus prepared in which the complementary strand contains a thymine residue opposite the lactone. The single-stranded oligonucleotide precursor containing the nitroindole fragment was prepared as described and hybridized to the complementary strand. The resulting duplex **B** was then irradiated under the same conditions as above except for the temperature, which was adjusted to 4 °C. Total conversion to the lactone required longer irradiation time than for the single-stranded oligonucleotide (120 min compared to 30 min). Again the conversion was quantitative, yielding pure duplex **A** as indicated by electropherograms monitored in the course of the irradiation and ascertained by the base treatment assay (Figure 1). The 4 °C temperature was selected for the irradiation to make sure that conversion of the nitroindole nucleoside into the deoxyribonolactone occurred at the duplex level as testified by the melting temperatures of the starting and final oligonucleotides (Table 2). It can be noted

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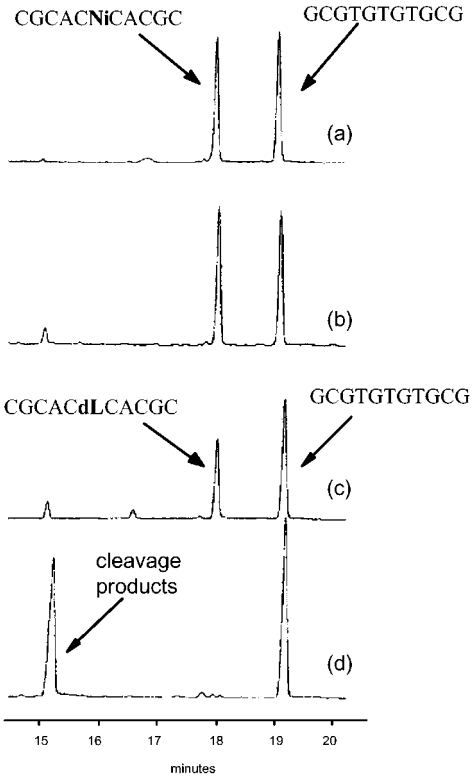

Figure 1. Irradiation of duplex undecamer **B** (CGCACNiCACGC/GCGTGTGTGCG). Base treatment assay (50 mM, NaOH, 70 °C, 15 min). Capillary gel electrophoresis analysis (a) before irradiation, (b) before irradiation after basic treatment, (c) after irradiation without basic treatment, and (d) after irradiation and basic treatment. The two pentameric cleavage products appear as one single peak.

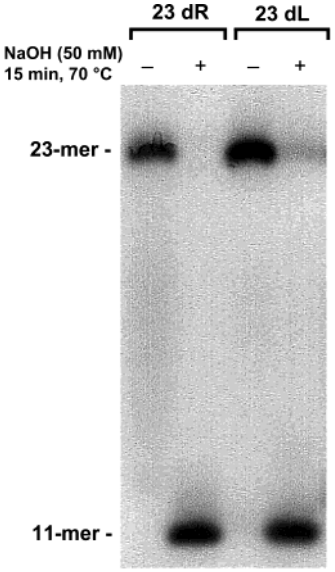

Figure 2. Analysis by polyacrylamide gel electrophoresis of the 23-mer oligonucleotides containing deoxyribonolactone **4** (23dL) and deoxyribose **5** (23dR) without (−) and with (+) basic treatment (50 mM, NaOH, 70 °C, 15 min).

that the presence of the nitroindole ⟨pseudobase⟩ in the duplex leads to weaker destabilization of the DNA duplex structure than the deoxyribonolactone fragment. As already mentioned, abasic sites lead to a considerable decrease in duplex stability.[38]

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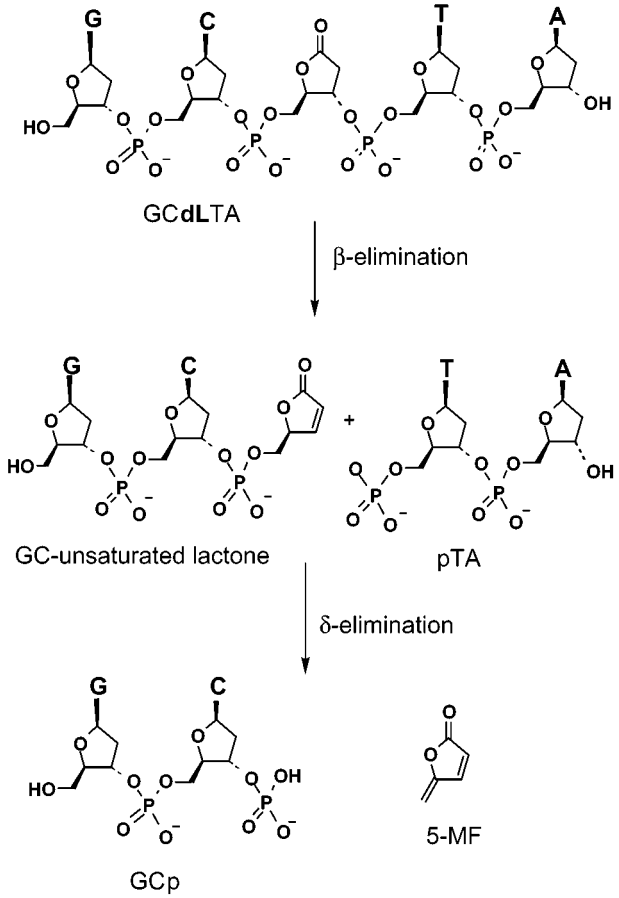
Table 2. Melting Temperatures

	duplex (5′ → 3′)	T_m (°C)
A	CGCAC**dL**CACGC/GCGTGTGTGCG	35.6
B	CGCAC**Ni**CACGC/GCGTGTGTGCG	44.5
C	CGCAC**A**CACGC/GCGTGTGTGCG	56.9

Scheme 1. Cleavage Products Resulting from *β*- and *δ*-Eliminations of Pentamer **1** GCdLTA



Identification of the Cleavage Products. For unambiguous identification of the cleavage products, the pentamer GCdLTA **1** was examined as a typical oligonucleotide-containing deoxyribonolactone in the middle of the sequence. The irradiated sample, prepared in (relatively large) quantities, was subjected to conventional workup including HPLC purification with ammonium acetate buffer and subsequent lyophilization. The treatment resulted in partial (∼20%) degradation of the oligonucleotide with formation of a mixture of three nucleotidic fragments: GC-unsaturated lactone, GCp, and pTA in the ratios (0.13/0.16/0.3) in addition to unreacted GCdLTA **1** (Scheme 1). The three cleavage fragments were isolated and purified by HPLC using a phosphate buffer (pH 6) in which conditions all compounds exhibited enough stability to allow ^{1}H NMR and CGE analysis. As represented in Figure 3, the cleavage products could be unambiguously identified as resulting from *β*- and *δ*-elimination reactions. Fragment GCp-lactone showed the characteristic peaks of the guanine and cytosine aromatic protons and the H2′, H3′, and H4′ signals at 6.29, 7.77, and 5.45 ppm, indicating the presence of the unsaturated lactone.

The two other dinucleotides pTA and GCp were identified by their characteristic aromatic base signals respectively at 8.46, 7.50, 8.21 and 7.98, 7.81, 5.87 ppm. The simultaneous presence

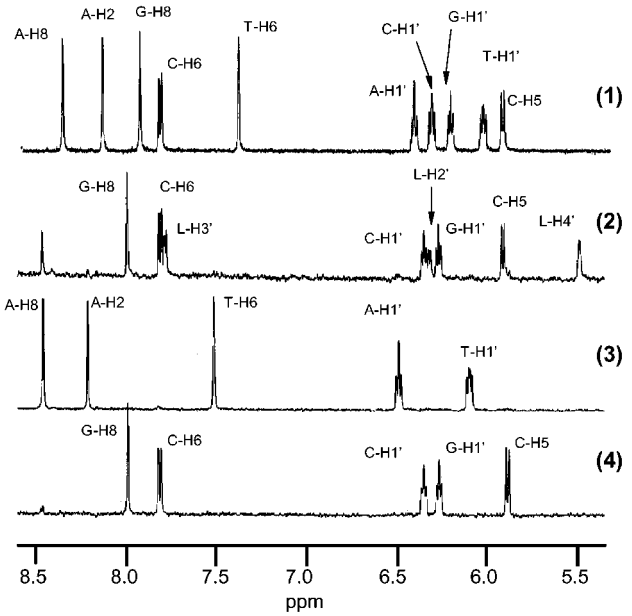


Figure 3. 500-MHz ^{1}H NMR spectra of pentamer GCdLTA (1) and cleavage products, (2) GC-unsaturated lactone, (3) pTA, and (4) GCp.

in the degradation mixture of the β- and δ-elimination products, respectively pTA and GCp indicated that the kinetics of the two elimination steps is of the same order of magnitude.

Relative Kinetics of the β- and δ-Elimination Reaction Steps. Identification of the β- and δ-elimination products resulting from cleavage of the pentamer GCdLTA **1**, simultaneously by their retention time in CGE and by their high-field ^{1}H NMR spectrum, allowed evaluation of the relative kinetics of the two steps of the degradation process, i.e., the β- and δ-elimination reactions. Cleavage of the pentamer GCdLTA was studied at pH 9.0 (borate buffer) at 37 °C. Figure 4 shows the electropherograms registered as a function of time for the reaction mixture. All peaks were identified as indicated and integrated with an internal reference. Using the integrated values of the peaks corresponding to the elimination products, the k_β (β-elimination) and k_δ (δ-elimination) were calculated (see Experimental Section). The values $k_\beta = 1.3 \times 10^{-4}$ s^{-1} and $k_\delta = 1.4 \times 10^{-4}$ s^{-1} were thus obtained. The two steps proceed at comparable velocities, which accounts for the nonaccumulation of the intermediate α,β-unsaturated lactone. k_β therefore measures the kinetics for degradation of DNA containing the deoxyribonolactone damage.

Influence of Structure and Medium on the Stability of Deoxyribonolactone-Containing Oligonucleotides. It was of interest to determine the different factors that contribute to degradation through β- and δ-elimination of DNA strands containing deoxyribonolactone. Better knowledge of the lability induced by the presence of the lesion is of fundamental interest in the general context of DNA damage. In addition, such determinations were also directed toward gathering information of practical importance for further chemical and biological studies. CGE appeared as the method of choice to analyze the degradation of DNA strands of different lengths in different conditions. The procedures were essentially the same for all experiments. The oligonucleotides containing the lactone were incubated in defined media. Aliquots were collected at different time intervals, and the reactions were quenched by addition of a Tris-borate−urea buffer solution (pH 8.2) prior to CGE analysis. Plotting the deoxribonolactone fragment peak area as a function of time indicated pseudo-first-order disappearance of the oligonucleotides with good correlation coefficients in all cases.

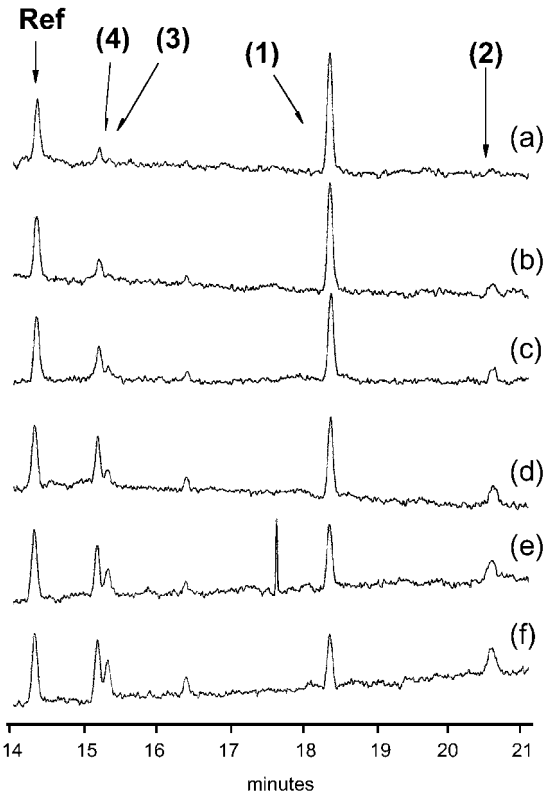


Figure 4. Cleavage of the pentamer GCdLTA **1** at pH 9.0 (borate buffer), 37 °C, as a function of time. Electropherograms after (a) 15, (b) 30, (c) 60, (d) 90, (e) 120, and (f) 150 min; (1) GCdLTA, (2) GC-unsaturated lactone, (3) pTA, and (4) GCp; reference, dAMP.

Table 3. Half-Lives (min) of the Lactone-Containing Oligonucleotides. Influence of pH and of Length

	pH					
	10.0	9.5	9.0	8.5	8.0	7.5
5-mer **2**[a]	12					
5-mer **1**[a]	12	20	93			
11-mer **3**[b]	15	39	139	258	1140	5960
23-mer **4**[b]	34					
34-mer **6**[c]	35					

[a] Borate−NaOH buffer, 37 °C, 100 mM NaCl (buffer) concentration, 13 μM. [b] Buffer concentration, 5 μM. [c] Buffer concentration 3 μM.

(1) Effect of pH. The pentamer GCdLTA **1** that had been extensively analyzed along with its degradation products by high-field NMR and CGE (Figures 3 and 4) was first studied as a function of pH. At 37 °C, pH 9, a half-life $t_{1/2} = 93$ min was determined from examination of the disappearance of the oligonucleotide (Table 3). The value fits well with the k_β value determined, as previously quoted, from the appearance of the cleavage fragments (Figure 4). At pH's 9.5 and 10.0, a strong acceleration of the reaction was observed, as reflected by the half-lives $t_{1/2} = 20$ and 12 min determined, respectively. A similar dramatic effect of the pH was measured for cleavage of the 11-mer **3**, with the half-life of the oligomer decreasing from 139 min at pH 9.0 to 15 min at pH 10. 0 (Table 3). The base-catalyzed character of the cleavage was further supported by the plot log $k = f$(pH) that underlined the linear character of

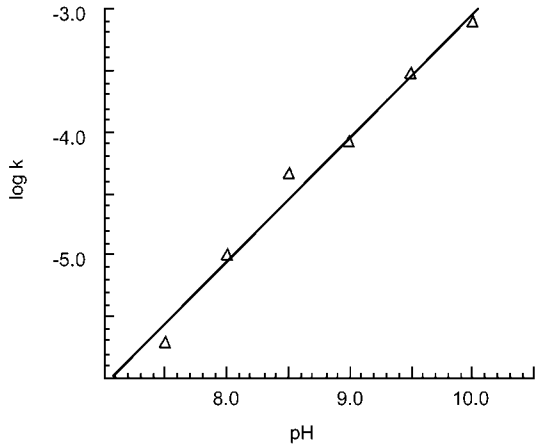


Figure 5. Effect of pH on the cleavage kinetics of the 11-mer oligonucleotide **3**, log $k = f$ (pH).

Table 4. Half-Lives (min) of the 11-Mer **3**. Influence of the Ionic Strength[a]

ionic strength (mM)	$t_{1/2}$ (min)
0.1	14
0.2	14
0.3	13
0.6	17

[a] pH 10, borate−NaOH buffer, 37 °C.

Table 5. Half-Lives (min) of the 11-Mer **3**. Influence of Temperature

T °C	pH	$t_{1/2}$ (min)
70[a]	8	20
50[a]	8	100
37[a]	7.5	5960

[a] 50 mM borate − NaOH buffer.

the kinetics of disappearance of the undecamer as a function of pH, with a slope equal to 1.0 (Figure 5).[39]

(2) Effect of Ionic Strength. As ionic strength is an important parameter that has to be adjusted in DNA studies, its influence was analyzed. The 11-mer oligonucleotide **3** was incubated at 37 °C, pH 10, with different concentrations of added NaCl. As indicated in Table 4, the kinetics of disappearance of the DNA strand are only weakly sensitive to the ionic strength, the highest salt concentrations leading to small increase of the reaction velocity.

(3) Effect of Temperature. The strong influence of temperature on the cleavage kinetics was evidenced by examination of the 11-mer oligonucleotide **3** at higher temperatures at pH close to neutral. Rapid decomposition occurred when the temperature was increased, as outlined in Table 5. These observations are of fundamental importance in studies involving DNAs containing the deoxyribonolactone damage. Great care must be exerted in all protocols involving temperature increase, such as hybridization or conventional polyacrylamide gel electrophoresis.

(4) Effect of the Nature of the Lactone Flanking Bases. The possible influence of the nature of the bases flanking the lactone site was evaluated by comparing the behavior of the two pentamers GCdLTA **1** and CAdLGT **2**, in which the lactone is flanked respectively by two pyrimidines or two purines. The same half-life $t_{1/2} = 12$ min was determined at pH 10, 37 °C, indicating small, if any, influence of the neighboring bases (Table 3).

(5) Effect of the Length of the DNA Fragment. The 5-, 11-mer, 23-, and 34-mers were examined in identical conditions of pH, temperature, and ionic strength (pH 10, 37 °C, 100 mM NaCl). It appears (Table 3) that the stability to cleavage increases slightly for the longer fragments (compare the 12-min half-life of the 5-mers to 34 min determined for the 23-mer). However, for longer fragments, the stability does not seem to be sensitive to the number of constituting nucleotides.

Comparison of the Deoxyribonolactone Site with the Deoxyribose Abasic Site. As noted previously, the "abasic site" resulting from removal of a nucleic base and leaving of a deoxyribose residue is probably the most frequent lesion that occurs spontaneously in DNA. This has been abundantly documented. This "regular" abasic site (Chart 1) also leads to strand cleavage through β- and δ-eliminations occurring on the aldehydic form of deoxyribose.[40] Due to this lability, most structural and biological studies have indeed been performed on the stable tetrahydrofuranyl analogue (Chart 1).[41] It seemed of interest to compare those two forms of abasic sites, the deoxyribose and the deoxyribonolactone, in a DNA strand. The comparison was performed with 23-mer oligonucleotides. For practical reasons, the cleavage study was monitored using polyacrylamide gel electrophoresis that requires smaller amounts of material than CGE. The labeled ribonolactone-containing strand was prepared by irradiation of the nitroindole oligonucleotide precursor that had been preliminarily ^{32}P labeled at the 5′-end following standard protocol. The 23-mer fragment containing the deoxyribose site was obtained according to a published procedure from an oligonucleotide precursor containing a uracil base.[42] After ^{32}P labeling at the 5′-end, the precursor was treated with uracil DNA glycosylase, which removed the uracil abnormal base leaving a deoxyribose residue. The purity of the 23-mers was verified by the base treatment assay (Figure 2). The two labeled oligonucleotides were incubated at 37 °C, pH 9.0, and aliquots were collected at different time intervals for PAGE analysis. The relative amounts of starting oligonucleotide and labeled cleavage fragment were quantified by radioactivity counting. Although the precision of the determination was relatively poor as compared to the previously described CGE analysis, the half-life of the ribonolactone-containing strand was evaluated $t_{1/2} = 150$ min, a value that agrees with all previous data. The half-life of the deoxyribose-containing oligonucleotide was $t_{1/2} = 3200$ min in the same incubation conditions; i.e., the "regular abasic site" is ∼20 times more stable in our experimental conditions than the deoxyribonolactone.

Conclusion

Deoxyribonolactone formation in DNA has been identified as arising under the action of a number of different physical and chemical agents of significance as environmental factors or in cancer therapy. However, very little is known about the real importance of this lesion in the cell. Questions remain to

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be answered as to the generality of its formation, as well as to its biological consequences in terms of lethality or mutagenicity, for example. This is essentially due to the instability of damaged DNA at the site of the lesion and, as a consequence, to the absence, until very recently, of general methods to introduce the lesion in DNA fragments and to study their chemistry. The present results, based on an efficient synthetic procedure, elucidate some aspects of the chemistry of the lesion. The cleavage of damaged DNA strands through β- and δ-elimination at the site of the lesion has been clearly established with identification of the reaction products and determination of the kinetics. The dramatic influence of pH and temperature has been evaluated, and the lifetime of the lesion in conditions close to those found in the cell has been evaluated, which sheds some idea about its persistence in the cell as compared to the well-known "regular" abasic site damage. It was shown that the deoxyribonolactone site creates greater instability in DNA than its "regular" abasic site counterpart. From a practical point of view, the data presented here allowed us to define experimental conditions to handle, analyze, and hybridize DNA strands containing the lesion, perform a structural high-field NMR analysis of a damaged duplex,[36] and conduct a preliminary study of the enzymology of the lesion.[37] More generally the present data should contribute to opening the route to extensive examination of the biological consequences of the lesion in vitro and possibly in vivo.

Experimental Section

Materials. All commercially available chemical reagents were used without purification. Reagents used for automated DNA synthesis were obtained from PE Biosystem. Reagents for capillary electrophoresis were from Beckman Instruments Inc. (Fullerton, CA). Oligonucleotides were labeled using [γ-^{32}P]ATP (specific activity 3000 Ci/mmol) from NEN Life Science, and T4 polynucleotide kinase was purchased either from Boehringer Mannheim or MBI Fermentas. All NMR data were recorded on a Varian Unity Plus 500-MHz spectrometer.

Oligonucleotide Synthesis. Oligodeoxyribonucleotides containing the nitroindole moiety were synthesized using standard solid-phase cyanoethyl phosphoramidite chemistry on a Expedite DNA synthesizer. The nitroindole nucleoside phosphoramidite was prepared as described.[33,34] During the automated synthesis, the standard 1- and 15-μmol cycle was used except for incorporation of the nitroindole nucleoside, which was done with prolonged coupling time (15 min). The oligomers were purified by HPLC on a reversed-phase nucleosil C-18 column (Macherey-Nagel 10 $\times$ 250 mm) with a linear gradient of acetonitrile (5−35% in 20 min) in 20 mM ammonium acetate solution (pH 7). The following ϵ (260 nm, mol^{-1}dm^3 cm^{-1}) were used for the determination of the concentration of single-stranded oligonucleotides in aqueous solution: $\epsilon = (15.4N_A + 11.5N_G + 7.4\ N_C + 8.7N_T + 5.7N_{Ni}) \times 0.9 \times 10^3$.

Analysis of Oligodeoxyribonucleotides Using Capillary Gel Electrophoresis. Electropherograms were monitored on a P/ACE 5000 (Beckman) apparatus, samples were injected under 10 kV for 2−5 s in the capillary (DNA eCap, 100 μm i.d., 27 cm in length filled with ssDNA 100-R gel and ssDNA 100 buffer), and separation of the products (0.2−1 $OD_{260\ nm}$) proceeded under 8.1 kV at 30 °C for 40 min. The products were detected by UV $_{254\ nm}$ absorbance.

Labeling of Oligonucleotides and Denaturing PAGE. Oligonucleotides were labeled using [γ-^{32}P]ATP (specific activity 3000 Ci/mmol) from NEN Life Science and T4 polynucleotide kinase purchased either from Boehringer Mannheim or MBI Fermentas. A 20-μL aliquot of a dye mixture (95% formamide, 20 mM EDTA, pH 7.0) was added to 5 μL of the samples. Labeled oligonucleotides were subjected to 20% denaturing polyacrylamide gel electrophoresis (PAGE) at neutral pH (100 mM HEPES, pH 7.0) and moderate temperature (40−50 °C).

Irradiation of the Single-Stranded Oligodeoxyribonucleotides Containing the Nitroindole Moiety. Preparation of 1−4 and 6. Aqueous solutions of the oligonucleotides were prepared (100−150 μL, $OD_{260\ nm}$ varying between 1 for the pentamers, 11-mer, 23-mer, and 3 for the 34-mer) and illuminated using a 200-W Hg/Xe lamp (Oriel Instrument, Stratford, CT) and a KNO_3 (2 M) filter. The temperature of the solutions was maintained at 4 °C. Complete conversion of the nitroindole-containing precursors was reached after 30 (11- and 23-mer) or 60 min (34-mer).

Irradiation of Double-Stranded Oligodeoxyribonucleotides. Synthesis of Duplex A. An aqueous solution (100 μL) of the11-mer containing the nitroindole residue (11 μM) and the complementary strand (11 μM) was heated at 70 °C for 15 min and slowly cooled at room temperature for 30 min to give duplex **B** (Table 2). The temperature of the solution was maintained at 4 °C during irradiation. The illumination, using the lamp as above, was completed within 120 min.

Analysis of the Irradiated Oligonucleotides. Basic Treatment Assay. Total conversion of the oligonucleotides containing the nitroindole precursor was checked by basic heat treatment of the illuminated solutions. The solutions (20-μL aliquots) were added to 20 μL of piperidine (2 M) or NaOH (100 mM) and heated at 70 °C for 15 min. The solutions were evaporated to dryness and solubilized in 20 μL of water three times. Total cleavage of the oligonucleotides containing the deoxyribonolactone was checked by CGE and PAGE.

Melting Temperature Experiments. Equimolar solutions (7.7 μM) of unmodified or nitroindole- or deoxyribonolactone-containing 11-mer and its complementary strand were mixed in 0.20 mM NaCl, 1 mM EDTA, and phosphate buffer (10 mM, pH 7.0). UV absorption spectra (at 260 nm) and melting experiments were recorded using a Lambda 5 UV/visible Perkin-Elmer spectrophotometer equipped with a Perkin-Elmer C570-070 temperature controller and interfaced with a Epson AX2e computer. Before each melting experiment, samples were heated at 80 °C for 5 min and cooled slowly to ensure that the oligonucleotides were in the duplex state. For the deoxyribonolactone-containing 11-mer, the same T_m was observed when the determination was performed without preliminary annealing at 80 °C.

Identification of the Oligonucleotide Cleavage Products. After irradiation of the nitroindole-containing pentamer $^{5'}$GC−Ni-TA$^{3'}$ (1.6 μmol, 40 μM) for 30 min (100-W mercury lamp Hanovia, Pyrex filter), the nitrosoindole was removed by extraction with diethyl ether (3 $\times$ 10 mL). The aqueous solution containing the pentamer $^{5'}$GC-dL-TA$^{3'}$ was concentrated. Partial cleavage (~20%) of the oligodeoxyribonucleotide occurred during solubilization in ammonium acetate solution (200 mM, pH 7) and evaporation to dryness under vacuum. The cleavage products (GC-unsaturated lactone 0.13 μmol, pTA 0.30 μmol, and GCp 0.16 μmol) were separated by HPLC on a reversed-phase nucleosil C-18 column (Macherey-Nagel 10 $\times$ 250 mm) with a linear gradient of methanol (4 mL/min, 5−25% in 20 min) in 5 mM phosphate solution (pH 6). ^{1}H NMR spectra of each product in D_2O was recorded Table 6).

Kinetic Studies of Cleavage. The oligodeoxyribonucleotides containing the lactone were incubated under different experimental conditions, and aliquots of the solutions were injected in CGE at 10 different reaction times. The cleavage reaction was quenched before injection by adding 20 μL of Tris-borate−urea buffer (Beckman) to an aliquot of the solution (5 μL) and by cooling the samples at room temperature. Each experiment was done at least in duplicate. The amounts of starting material and of an internal reference (dAMP or dGMP) were followed by UV_{254nm} absorbance. Areas of the signals detected by CGE were quantified using the P/ACE Station software (Beckman). The variation of the concentration of oligodeoxyribonucleotides was given by the ratio between the area of this product and the area corresponding to the internal reference.

Table 6. ^{1}H NMR Chemical Shift Assignments for the Oligonucleotides GCNiTA, GCdLTA, GC-lactone, GCp, pTA (by 2D COSY and 1D TOCSY Experiments)

	residue	H8	H6	H5/Me/H2	H1′	H2′/H2″	H3′	H4′	H5′/H5″
GCdLTA	G	7.94			6.19	2.75/2.68	4.89	4.28	3.82/3.80
	C		7.83	5.90	6.30	2.55/2.31	4.87	4.35	4.20/4.14
	T		7.39	1.86	6.01	2.20/1.78	4.74	4.18	3.96/3.94
	A	8.37		8.15	6.40	2.88/2.60	4.77	4.24	4.07
	dL					3.14/2.71	4.95	4.87	4.17/4.14
GC-lactone	G	7.99			6.24	2.79/2.71	4.91	4.29	3.84/3.81
	C		7.80	5.85	6.32	2.56/2.32	4.81	4.34	4.20/4.11
	lactone					6.29	7.77	5.45	4.13
GCp	G	7.98			6.24	2.78/2.73	4.90	4.29	3.84/3.81
	C		7.81	5.87	6.32	2.56/2.33	4.86	4.34	4.21/4.15
pTA	T		7.50	1.91	6.06	2.18/1.73	4.77	4.22	3.99/3.95
	A	8.46		8.21	6.46	2.92/2.61	4.80	4.26	4.09

(1) Effect of pH. Both pentamers (13 μM) and the 11-mer (5 μM) were incubated at 37 °C in three 50 mM borate−NaOH solutions (200 μL), pH's 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0. The ionic strength was adjusted to 100 mM by adding NaCl.

(2) Effect of Ionic Strength. The 11-mer (0.5−120 μM) was incubated at 37 °C in borate−NaOH solutions (50 mM, 200 μL, pH 10.0) with an ionic strength of 0.1, 0.2, 0.3, and 0.6 mM.

(3) Effect of Temperature. The 11-mer (0.5 μM) was incubated at 50 °C and 70 °C in a borate−NaOH buffer (50 mM, 200 μL, pH 8.0). Aliquots of these solutions were collected and directly injected in CGE.

(4) Effect of the Length of the Oligonucleotide Containing the Deoxyribonolactone. Two borate−NaOH buffers (50 mM, 200 μL, pH 10.0) containing either the 23- (5 μM) or the 34-mer (3 μM) were prepared. These solutions were incubated at 37 °C; aliquots were collected at differents times and treated as decribed above.

Kinetics of the β- and δ-Elimination Reactions. The kinetic constants for β- and δ-eliminations were determined for cleavage of the pentamer GCdLTA **1** at pH 9.0, 37 °C.

The concentrations of the starting oligonucleotide GCdLTA **1** and of the β-elimination fragment, GC-lactone, were determined at different time intervals by CGE using dAMP as an internal references. The β- and δ-elimination reaction constants k_β and k_δ were calculated using equation

$$\frac{\mathrm{d[GC - lactone]}}{\mathrm{d}t} = -k\delta[\mathrm{GC - lactone}] + k\beta[\mathrm{GCdLTA}]$$

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Supporting Information Available: PAGE analysis showing comparison of stabilities of the aldehydic and the lactonic abasic sites. This material is available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

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