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Efficient conversion of thymine glycol into the formamide lesion in oligonucleotides

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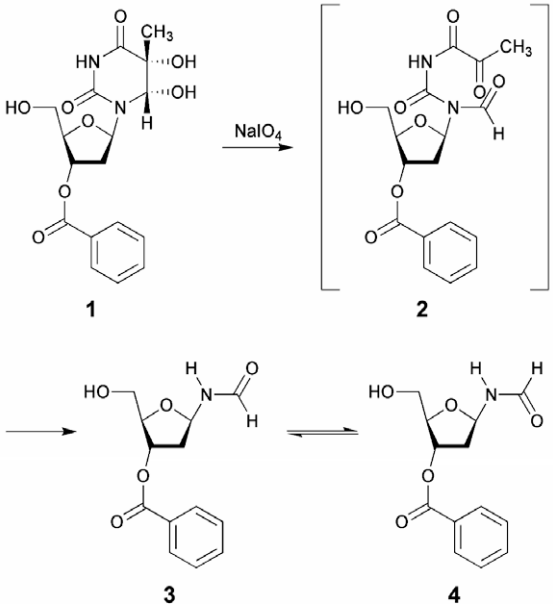
ABSTRACT

Oxidation of (5*R*,6*S*)-5,6-dihydro-5,6-dihydroxythymidine (thymidine glycol) with sodium periodate efficiently produced *N*-(2-deoxy-β-D-*erythro*-pentofuranosyl)formamide, a hydroxyl radical-induced decomposition product of pyrimidine bases in DNA, and this method was successfully applied to the conversion of thymine glycol in oligonucleotides into the formamide lesion.

Ionizing radiation produces various lesions in DNA by direct absorption of the radiation energy or by reactions with radiation-induced reactive species. The major factor that causes the latter reactions is the hydroxyl radical, and oxidatively-damaged bases, as well as ring-opened or fragmented products of further reactions, are formed at all four types of nucleobases.[1] Among these lesions, we previously studied thymine glycol (5,6-dihydro-5,6-dihydroxythymine). Oligonucleotides containing 5*R*- or 5*S*-thymine glycol were synthesized by incorporating the building blocks of the two isomers separately,[2] and base excision repair and translesion synthesis were analyzed using these oligonucleotides.[3] In the present study, a fragmentation lesion was prepared using thymine glycol as a starting material. We expected that a radiation-induced fragmentation lesion might be formed quantitatively by a simple reaction of thymine glycol, because this oxidized base is not stable, and that DNA containing such a lesion could be obtained easily by the conversion of thymine glycol incorporated site-specifically into oligonucleotides. While alkali hydrolysis of thymidine glycol is known to produce deoxyribosylurea,[4] redox reactions were tested in this study, to obtain other types of lesions.

For facile detection of the materials by UV absorption and their longer retention on a reversed-phase HPLC column, 3′-*O*-benzoyl-(5*R*,6*S*)-5,6-dihydro-5,6-dihydroxythymidine (**1** in Scheme 1) was used in the analysis of the reactions of thymine glycol. Since epimerization at the C6 position of thymine glycol had been reported,[5] we thought that the aldehyde intermediate might be reduced to an alcohol, in which the N1–C6 bond is cleaved, and that oxidative cleavage of the C5–C6 bond of this product with sodium periodate would result in the formation of 5-hydroxy-5-methylhydantoin, which is one of the important lesions derived from the thymine base.[6] Therefore, the reduction of **1** with sodium borohydride



Scheme 1. Oxidation of 3′-*O*-benzoyl-(5*R*,6*S*)-thymidine glycol with sodium periodate.

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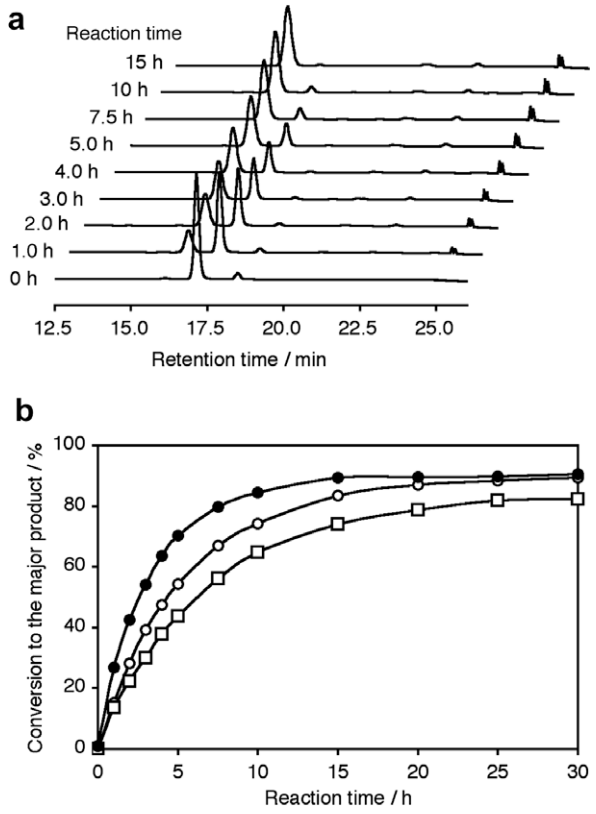


Figure 1. (a) HPLC analysis of the periodate oxidation. Compound **1** (1.0 mg) was mixed with 150 mM $NaIO_4$ in 250 mM sodium acetate buffer (pH 6.0), and after the addition of ethylene glycol, the mixtures were analyzed by HPLC, using a Waters μBondasphere 5 μm C18 300 Å column (3.9 × 150 mm) at a flow rate of 1.0 mL/min, with a linear gradient of acetonitrile (10–23% for 20 min plus 50% for 5 min) in 0.1 M triethylammonium acetate (pH 7.0). Chromatograms monitored at 230 nm are shown. (b) Conversion of thymidine glycol into the major product by the reactions with 75 mM (open circles) or 150 mM (closed circles) $NaIO_4$ at pH 6.0, and with 75 mM $NaIO_4$ at pH 8.0 (squares). The ratios of the product peak area to the total peak area are plotted.

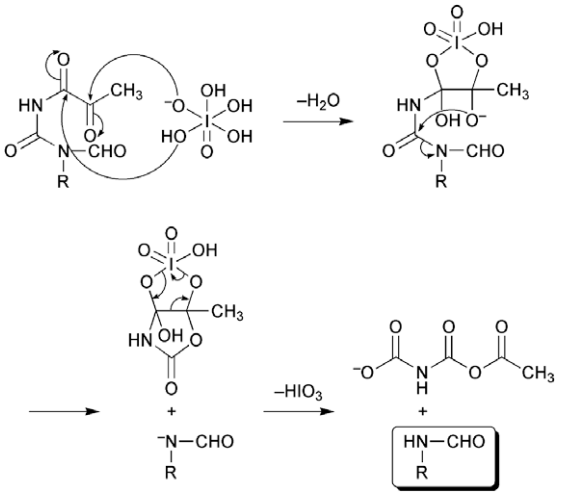
was tested first. Although the reaction proceeded almost quantitatively, NMR analysis of the product revealed that the carbonyl group at the C4 position, not the transient formyl group at the C6, was reduced to an alcohol (data not shown).

Subsequently, direct periodate oxidation of the vicinal diol at the base moiety of **1** was investigated, expecting that the second oxidation of the resultant α-diketone at the C4 and C5 positions with periodate would occur. Compound **1** was treated with sodium periodate at a concentration of 75 or 150 mM in sodium acetate buffer at pH 6.0 or 8.0, and the reaction was monitored by reversed-phase HPLC. As shown in Figure 1, a single major product with a retention time shorter than that of the starting material was detected, and the reaction proceeded faster at pH 6.0 than at pH 8.0. A small amount of hydrophobic by-products, which had UV absorption spectra similar to those of the starting material and the product, was detected (Fig. 1a), and the increase of the major product reached a plateau after 15 h at pH 6.0 when the sodium periodate concentration was 150 mM (Fig. 1b).

After purification on a reversed-phase column, the product was analyzed by NMR spectroscopy and mass spectrometry. The ^1H NMR spectra revealed that the methyl group at the base moiety was lost and that the obtained compound was a 3:2 mixture of two isomers. There were two sets of unidentified proton signals in the low field, which were not present in the thymidine glycol spectra, and one of these protons was exchangeable with deuterium upon the addition of D_2O. In the COSY spectra, cross-peaks were detected between these two signals and between the exchangeable signal and the H1′. In ^{13}C NMR spectroscopy, the DEPT and HMQC experiments revealed that the base moiety contained only one carbon, as a methine. In the positive-ion-mode high-resolution mass spectra, the obtained *m*/*z* value was 266.1033. From these results, the product was identified as *N*-(2-deoxy-β-D-*erythro*-pentofuranosyl)formamide, bearing the benzoyl group at the 3′ position (**3** and **4**). This compound reportedly exists in equilibrium between the cis (**3**) and trans (**4**) forms, which yield separate NMR spectra.[7] Antiphase NOESY cross-peaks were observed between the H1′ and formamide proton-signal pairs, and this result indicated that these two molecules were in a chemical exchange process.[8] In our study, the major form was trans, in agreement with the previous report,[7] because a cross-peak between the two protons of the formamide moiety in the NOESY experiments was detected only for the major isomer.

N-(2-Deoxy-β-D-*erythro*-pentofuranosyl)formamide is one of the radiation-induced pyrimidine degradation products,[9] and is also formed by ozonolysis,[10] iron-mediated Fenton reactions,[11] and UVC irradiation.[12] It was shown that the cis isomer could form two hydrogen bonds with the adenine base in the complementary strand,[13] and the application of this modified nucleoside to the formation of an artificial base pair with an expanded-size base has been studied.[14] The synthesis of this nucleoside from thymidine and its incorporation into oligonucleotides have been reported.[15] In this method, the thymine base was oxidized with potassium permanganate, and without purification, the resultant thymine glycol was oxidized further with lead tetraacetate to form *N*-formyl-*N*′-pyruvoylurea (compound **2** in Scheme 1). The formamide lesion is produced by the hydrolysis of the N1–C2 bond in this intermediate, whereas the hydrolysis on the C6 side results in the formation of 5-hydroxy-5-methylhydantoin. Actually, this hydantoin derivative was formed simultaneously in the reaction to obtain formamide,[16] and this side reaction lowered the yield of the formamide product.[15] In our present study, formamide was formed almost exclusively from thymine glycol by its oxidation with sodium periodate. From this difference, we propose a mechanism in which the cyclic periodate ester, formed in the oxidation reaction of the α-diketone intermediate (**2**), is involved in the N1–C2 cleavage, as shown in Scheme 2.

To develop a convenient method to obtain oligonucleotides containing the formamide lesion, we applied the above reaction to the conversion of thymine glycol into the formamide lesion at the oligonucleotide level. Although the periodate oxidation was



Scheme 2. Proposed mechanism of formamide formation.

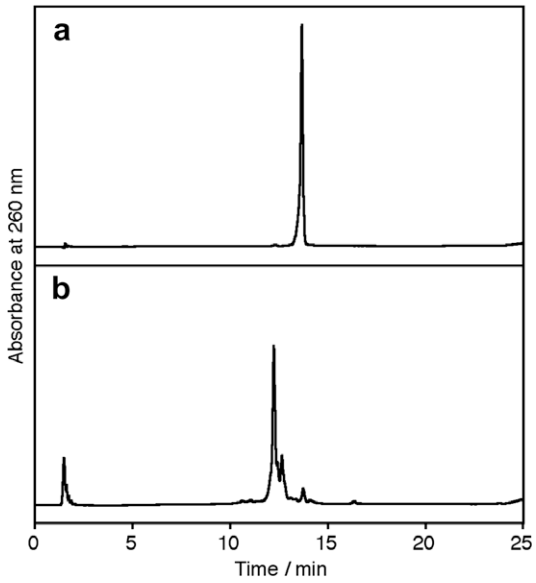


Figure 2. HPLC analysis of the thymine glycol-containing 11-mer before (a) and after (b) the periodate oxidation. The conditions were the same as those described in the legend to Figure 1a, except that the acetonitrile gradient was 5–11% for 20 min plus 25% for 5 min.

used in the synthesis of modified oligonucleotides,[17] we confirmed that unmodified oligonucleotides remained intact after an overnight treatment with $NaIO_4$, prior to the following experiments. In addition, it should be noted that the aforementioned reaction with lead tetraacetate cannot be used for oligonucleotides, because this reagent is promptly hydrolyzed by water. An oligonucleotide containing 5*R*-thymine glycol, d(CGTACT_gCATGC), in which T_g represents 5*R*-thymine glycol, was prepared by using the (5*R*,6*S*)-thymine glycol building block, as described previously,[2] and this oligonucleotide was treated with 150 mM $NaIO_4$ in 250 mM sodium acetate (pH 6.0) at room temperature for 15 h. After desalting on a gel-filtration column, the reaction mixture was analyzed by HPLC. As shown in Figure 2, the thymine glycol-containing 11-mer was converted to a single major product, and small amounts of several by-products with slightly longer retention times were detected. The flow-through peak detected for the reaction mixture was probably due to contaminants from the reagent or the buffer, because its UV absorption spectrum was different from that of the nucleic acids. The major product was purified by HPLC in an isolation yield of 43% (2.3 nmol from 5.4 nmol), and was analyzed by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. The observed *m*/*z* value of the obtained product in the negative-ion mode was 3208.5 (calculated *m*/*z*, 3208.6), whereas that of the starting material, that is, the thymine glycol-containing 11-mer, was 3323.8 (calculated *m*/*z*, 3323.6). This result demonstrated that the thymine glycol in the 11-mer oligonucleotide was successfully converted into the formamide lesion. To verify that the same conversion can be performed in longer oligonucleotides, a 30-mer, d(CTCGTCAGCATCTT_gCATCATACAGTCAGTG), was treated with $NaIO_4$ in the same manner. HPLC analysis revealed that a single major product, which could be separated from the starting material, was obtained, as shown in Figure 3.

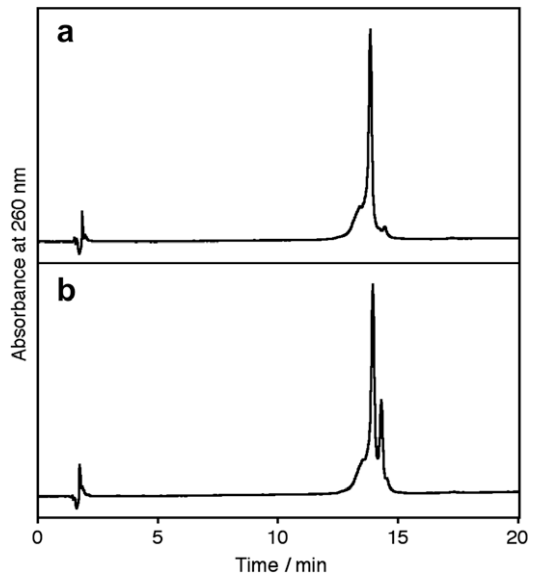


Figure 3. HPLC analysis of the 30-mer oligonucleotide. (a) The crude sample after the $NaIO_4$ treatment. (b) Co-injection with the thymine glycol-containing 30-mer. The conditions were the same as those described in the legend to Figure 1a, except that the acetonitrile gradient was 7–13% for 20 min.

In this study, we found that thymine glycol could be converted into the formamide lesion quantitatively by the $NaIO_4$ oxidation, and applied this procedure to the lesion-containing oligonucleotides. Since oligonucleotides containing thymine glycol are now commercially available, by using its building block,[2] the post-synthetic conversion method described in this Letter will enable biologists, who may find chemical synthesis difficult to accomplish, to obtain oligonucleotides containing the formamide lesion, and thus will facilitate biochemical studies on this type of DNA damage.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.12.001.

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