Cleavage of Single-Stranded 4′-Oligonucleotide Radicals in the Presence of O_2

Bernd Giese,* Xenia Beyrich-Graf, Peter Erdmann, Luc Giraud, Petra Imwinkelried, Stephan N. Müller, and Urs Schwitter

Department of Chemistry, University of Basel
St. Johanns-Ring 19, CH-4056 Basel, Switzerland

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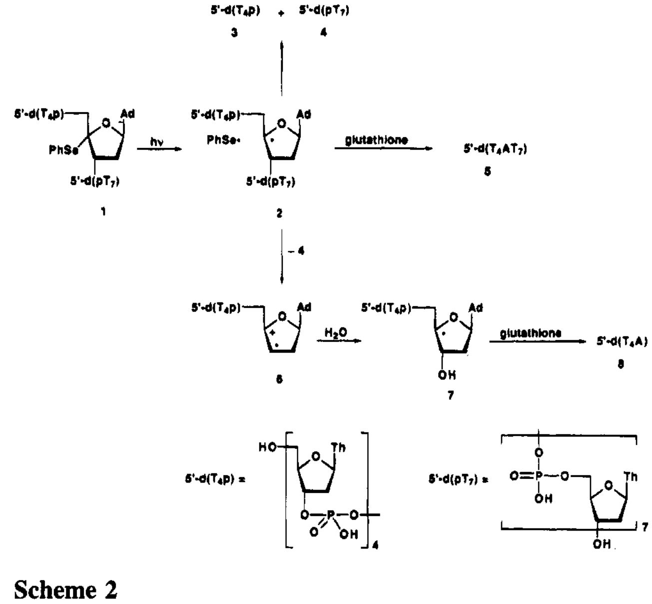
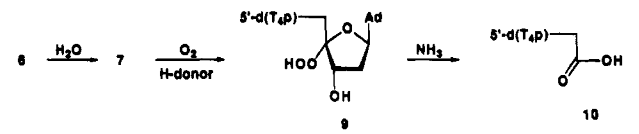
Antitumor antibiotics like bleomycin or neocarzinostatin induce damage of the DNA strand mainly by H-abstraction of the 4′- and/or 5′-position of a deoxyribose unit.[1] Recently we have shown that a single-stranded 4′-oligonucleotide radical like **2** can be generated by photoinduced C–Se bond cleavage of the modified oligonucleotide **1**.[2a] In the absence of added traps the 4′-deoxyribosyl radical **2** yields oligonucleotide phosphates **3** and **4** as main products. The reaction starts with a heterolytic cleavage of the secondary phosphate group at C-3′. This leads to oligonucleotide fragment **4** and radical cation **6**, which reacts with H_2O and generates radical **7**. Now the slower C–O bond cleavage of the primary phosphate group[2b] at C-5′ can occur, which leads to fragment **3**. The presence of the intermediate radicals **2** and **7** could be proven by trapping experiments with glutathione that yielded oligonucleotides **5** and **8** as further reaction products (Scheme 1).

With selenide **1** as radical precursor the C–O bond cleavage reaction takes place even in the presence of a 1000-fold excess of glutathione.[2a] One could therefore assume that, under aerobic conditions, phosphate elimination might also compete with the trapping by O_2. We therefore generated 4′-oligonucleotide radical **2** from selenide **1** in a saturated (about 1 mM) aqueous solution of O_2 and measured the products by MALDI-TOF mass spectrometry.[3,4] Figure 1 shows that, besides the cleavage products **3** and **4**, peaks at m/z = 1497 and 1292 appear. We assign these signals to peroxide **9** ($M - H^+ = 1499.1$) and glycolate **10** ($M - H^+ = 1291.8$). Although the peak at 1497 differs from the expected mass by about 0.2%, we assume that it corresponds to peroxide **9** because this signal is increased by 2, 4, and 6 mass units, respectively, if the reaction is carried out with $H_2{}^{18}O/O_2$, $H_2O/{}^{18}O_2$, and $H_2{}^{18}O/{}^{18}O_2$, respectively. This is in accordance with the formation of β-hydroxy peroxide **9** *via* addition of H_2O to radical cation **6** and trapping of radical **7** by O_2. The mass of glycolate **10** increases only by 2 units even if the reaction is carried out in $H_2{}^{18}O$ with ${}^{18}O_2$. Treatment

Scheme 1



Scheme 2



of a reaction mixture containing peroxide **9** and glycolate **10** with a diluted aqueous solution of NH_3 completely destroyed the peroxide **9** and led to an increase of glycolate **10** (Scheme 2).

In order to study the transformation of β-hydroxy peroxide **9** into glycolate **10**, we generated peroxides **13** and **14** by photolysis of selenide **11**[5a,b] and ketone **12**[5c] in the presence of O_2.[6] The intermediate peroxy radical could be detected by ESR spectroscopy ($g = 2.0155$).[7] *Ribo* isomers **13a,c** were enriched by chromatography. Treatment of an aqueous solution of peroxide **13a** or **13b** (crude product) with equimolar amounts of the disodium salt of phenyl phosphate at 20 °C yielded up to 90% of glycolate **15** and of base propenal **16**. In contrast, peroxide **13c** with acetylated OH groups at C-3′ and C-5′ yielded 45–60% of keto aldehyde **17c**, whereas glycolate **15c** or base

(1) Leading reviews: (a) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107. (b) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191. (c) Nicolaou, K. C. ; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387. (d) Pratviel, G.; Bernadou, J.; Meunier, B. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 746.

(2) (a) Giese, B.; Dussy, A.; Elie, C.; Erdmann, P.; Schwitter, U. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1861. (b) Giese, B.; Beyrich-Graf, X.; Burger, J.; Kesselheim, C.; Senn, M.; Schäfer, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1742.

(3) An aqueous solution of **1** (100–200 μL; A_{260} = 3–5 OD) was saturated with O_2 (about 1 mM) and irradiated (Osram Hg high-pressure lamp, 500 W, 320 nm filter) at 20 °C. Samples were taken after 5, 10, 20, 40, 60, and 90 min and analyzed by MALDI-TOF MS.

(4) (a) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299. (b) Nordhoff, E.; Ingendoh, A.; Cramer, R.; Overberg, A.; Stahl, B.; Karas, M.; Hillenkamp, F.; Crain, P. F. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 771. (c) Pieles, U.; Zürcher, W.; Schär, M.; Moser, H. E. *Nucleic Acid Res.* **1993**, *14*, 3191. Experiments were carried out with a Vestec Benchtop II instrument in the negative ion mode. Matrix solutions were either 2,4,6-trihydroxyacetophenone (0.3 M) or 2,4-dihydroxyacetophenone (0.3 M) in $CH_3CN/H_2O/EtOH$ = 50:45:5. The samples were prepared by mixing 1 μL of the reaction mixture with 1 μL of the matrix solution and 0.5 μL of a 0.3 M solution of ammonium tartrate in H_2O. After evaporation to dryness the spectra were recorded with a laser intensity of 0.2 μJ/pulse (about 20–50 pulses were accumulated per recording). The pulse time was 3 ns.

(5) The synthesis of radical precursors **11** and **12** uses methods described in the following: (a) Giese, B.; Erdmann, P.; Giraud, L.; Petretta, M.; Schäfer, T.; von Raumer, M. *Tetrahedron Lett.* **1994**, *25*, 2683. (b) Giese, B.; Erdmann, P.; Schäfer, T.; Schwitter, U. *Synthesis* **1994**, 1310. (c) Giese, B.; Imwinkelried, P.; Petretta, M. *Synlett* **1994**, 1003.

(6) Solutions of the radical precursors **11a** (0.25 mM in CH_3CN), **11c** (0.25 mM in CH_3CN), **12a** (4 mM in CH_3CN), and **12b** (4 mM in CH_3OH), respectively, were irradiated (Heraeus 150 W for **11a**, **11c**; Osram 500 W for **12a**, 295 nm filter), **12b**, 320 nm filter) at 20 °C while O_2 was bubbled through the solution. After the reaction of the radical precursor was completed (**11a**, **11c**, 1 h; **12a**, 2 h; **12b**, 0.5 h), the solvent was evaporated. The yields of the peroxide mixtures **13** + **14**, determined by ${}^1H$ NMR with C_2HCl_5 as standard, were 64% (**11a** as educt), 51% (**12a** as educt), 57% (**12b** as educt), and 76% (**11c** as educt). Thymidines **11a**, **12a**, and **12b** gave mainly the *ribo* peroxides **13a,b** whereas **11c** yielded *lyxo* peroxide **14c** as the main product (${}^1H$ NMR analysis). The *ribo* isomers **13a** and **13c** could be enriched by flash chromatography (Lichrospher RP-18, 15 μm, H_2O/CH_3CN = 1:1, −5 °C). The monophosphorylated peroxide **13b** decomposed during chromatography so that the crude product was used for the subsequent reactions. The assignment was done *via* the high-field shift of C-5′ (${}^{13}C$ NMR) and H-1′ (${}^1H$ NMR) of the *ribo* peroxides **13a–c** compared to the *lyxo* peroxides **14a–c**; see: Saito, I.; Morii, T.; Matsuura, T. *J. Org. Chem.* **1987**, *52*, 1008. The existence of the peroxide group was proven by chemical tests with Merckoquant 10011 and with *N,N*-dimethyl-*p*-phenylenediamine; see: Kappe, E.; Petri, P. *Z. Anal. Chem.* **1962**, *190*, 386.

(7) ESR measurements (Bruker ESP-300): A solution of the modified thymidine **11c** (10 mg in 1 mL of C_6H_6) was saturated with O_2 and irradiated in a Suprasil quartz tube (5.0 mm) with the filtered light (water-cooled Schott filter UG-5) of a Hanovia 977-B1, 1 kW, Hg–Xe high-pressure lamp. A singlet was observed with a g value of 2.0155, which is typical for peroxy radicals. See: Ingold, K. U. *Acc. Chem. Res.* **1969**, *2*, 1.

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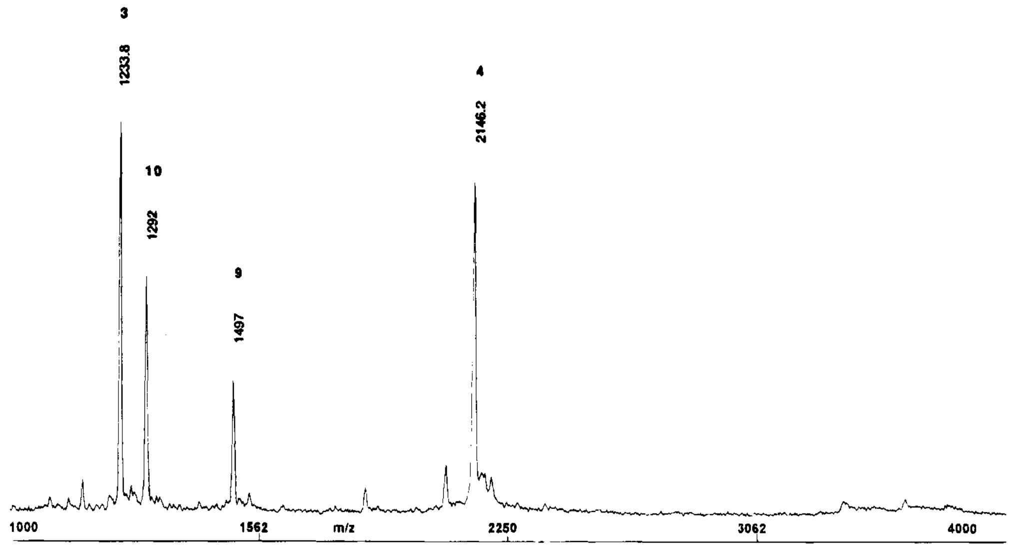
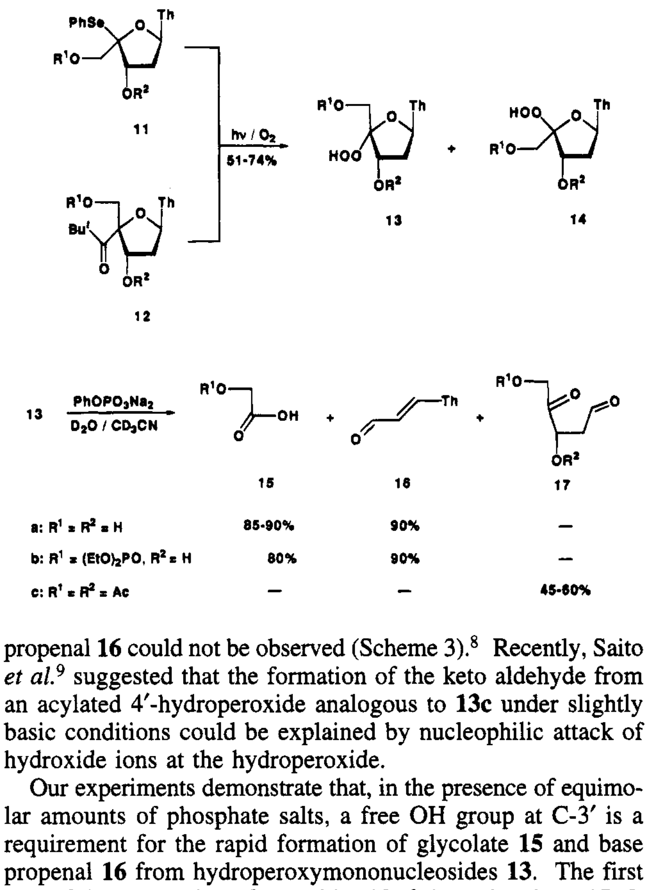


Figure 1. MALDI-TOF mass spectrum (negative-ion mode) of the reaction mixture after irradiation (100 min) of an aqueous solution of **1** in the presence of O_2.

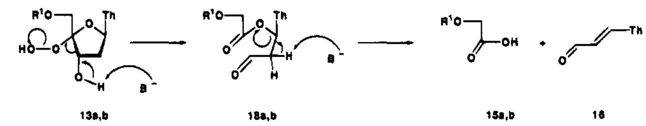
Scheme 3



propenal **16** could not be observed (Scheme 3).[8] Recently, Saito *et al.*[9] suggested that the formation of the keto aldehyde from an acylated 4′-hydroperoxide analogous to **13c** under slightly basic conditions could be explained by nucleophilic attack of hydroxide ions at the hydroperoxide.

Our experiments demonstrate that, in the presence of equimolar amounts of phosphate salts, a free OH group at C-3′ is a requirement for the rapid formation of glycolate **15** and base propenal **16** from hydroperoxymononucleosides **13**. The first step of the conversion of peroxides **13a,b** into glycolates **15a,b** and base propenal **16** can be rationalized as a Grob fragmentation. During this reaction the C-3′,C-4′ bond of the deoxyribose is cleaved and intermediate **18** is generated. A subsequent β-elimination which is facilitated by the acidity of the methylene group adjacent to the aldehyde function of **18** leads to products **15a,b** and **16** (Scheme 4). This mechanism explains the labeling experiments with 4′-oligonucleotide radical **2** and is in accordance with the known base-catalyzed cleavage of β-hydroxy peroxides.[10]

Scheme 4



Our experiments demonstrate that in reactions with the single-stranded 4′-oligonucleotide radical **2**, generated from selenide **1**, the 3-hydroxy 4-hydroperoxide **9** is a precursor of glycolate **10**. This is a surprising result, because it shows that the first strand cleavage step occurs before oxygen interacts with the DNA. In contrast to this, during the oxidative DNA damage with bleomycin or neocarzinostatin, the oxygen plays an important role already in the cleavage step.[1,11] It is conceivable that photolysis of selenide **1** generates a radical pair of oligonucleotide and phenylselenyl radicals in a solvent cage in which the intramolecular C−O bond cleavage could occur with a faster rate than the intermolecular attack. Further experiments with different radical precursors shall show how the conditions of radical generation influences the reactions of 4′-DNA radicals.

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Supplementary Material Available: Selected analytical data of radical precursors **11a,c**, **12b**, peroxides **13a−c**, products **14a,c**, **16**, **17**, and selected MALDI-TOF mass spectra and ESR spectra (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead for ordering information and Internet access instructions.

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(8) The deoxyribo peroxides **13a−c** (about 10 μmol) were solved in a 4:1 D_2O/CD_3CN mixture (0.5−1.0 mL), equimolar amounts of $Na_2(PhO)PO_3$ in D_2O (10 μL of a 1 M solution) were added, and the reaction was followed by NMR with C_2HCl_5 or CH_2Cl_2 as standard. The peroxides **13a** and **13b** were converted into glycolate **15a** (85−90%), **15b** (80%), and base propenal **16** (90%) at 20 °C with half-lifetimes of 10−30 min. Peroxide **13c** treated under similar conditions gave 45−60% of keto aldehyde **17c** and 50−65% of thymine.

(9) Saito, I.; Morii, T.; Matsuura, T. *J. Org. Chem.* **1987**, *52*, 1008.

(10) Richardson, W. H.; Heesen, T. C. *J. Org. Chem.* **1972**, *37*, 3416. See also: Schulz, M.; Boeden, H.-F., Berlin, P. *Justus Liebigs Ann. Chem.* **1967**, *703*, 190.

(11) (a) McGall, G. M.; Rabow, L. E.; Ashby, G. W.; Wu, S. H.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1992**, *114*, 4958. (b) Burger, R. M.; Drlica, K.; Birdsall, B. *J. Biol. Chem.* **1994**, *269*, 25978.