Radiation Chemistry of DNA, II *

Strand Breaks and Sugar Release by γ-Irradiation of DNA in Aqueous Solution. The Effect of Oxygen

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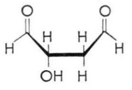
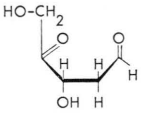
DNA, Strand Break, Oxygen Effect

From γ-irradiated oxygenated aqueous solutions of DNA, 2-deoxy-tetrodialdose (**1**) and 2-deoxy-pentos-4-ulose (**2**) have been isolated as free sugars. The formation of **1** indicates that in the presence of oxygen the DNA strand is not only broken by mere phosphate ester cleavage but also by C–C bond rupture in the sugar moiety. Such a process has not been encountered in deoxygenated solutions so far. The mechanism for the formation of **1** is discussed.

Irradiation of living cells with ionizing radiation causes strand breaks in the DNA[1]. These strand breaks may either be due to the scission of a phosphate ester linkage or of a carbon-carbon bond in the sugar moiety. Recently, three products, 2,5-dideoxy-pentos-4-ulose, 2,3-dideoxy-pentos-4-ulose and 2-deoxy-pentos-4-ulose have been isolated from deoxygenated N_2O saturated aqueous solutions of DNA after γ-irradiation[2]. In this paper we wish to report on the formation of the two sugars 2-deoxy-tetrodialdose **1** and 2-deoxy-pentos-4-ulose **2** in irradiated aqueous solutions of DNA in the presence of oxygen.



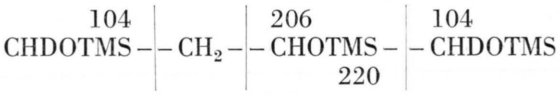
1



2

Aqueous solutions of DNA (from calf thymus, Serva, 250 mg/l) were saturated with N_2O/O_2 (4:1) and irradiated with γ-rays (dose: $4 \times 10^{18} - 1.6 \times 10^{19}$ eV/g; dose rate: 4.3×10^{18} eV/g·h). After irradiation the samples were freeze dried, reduced with $NaBD_4$, trimethylsilylated and analysed by GC–MS. On reduction with $NaBD_4$ the carbonyl groups are converted into hydroxyl groups, and deuterium atoms are incorporated in the product molecules at these positions[3]. The mass spectra of TMS-ethers of the reduced products were interpreted on the basis of typical fragmentation patterns of this class of compounds[3].

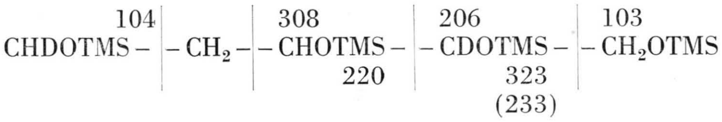
Three TMS-ethers of deuterated polyalcohols were identified. The first corresponded to butanetriol-(1,2,4)-1,4-d_2. Typical fragmentations are m/e 104 (100%), 206 (2%), 220 (40%), 234 (M-90; 12%) and 309 (M-15; 5%).



$$\overset{104}{\text{CHDOTMS}}-\Big|-\text{CH}_2-\Big|-\overset{206}{\text{CHOTMS}}-\underset{220}{\Big|}-\overset{104}{\text{CHDOTMS}}$$

The precursor is **1**.

The other two TMS-ethers had the retention times of TMS-ethers of 2-deoxy-xylitol and 2-deoxy-ribitol, respectively. Deuteration has been recognized to be at the positions 1 and 4 since typical fragment ions are m/e 103 (20%), 104 (100%), 206 (5%), 220 (30%), 233 (30%), 246 (M-90-90; 10%), 308 (20%), 321 (M-15-90; 5%) and 323 (10%).



$$\overset{104}{\text{CHDOTMS}}-\Big|-\text{CH}_2-\Big|-\overset{308}{\underset{220}{\text{CHOTMS}}}-\Big|-\overset{206}{\underset{\substack{323\\(233)}}{\text{CDOTMS}}}-\Big|-\overset{103}{\text{CH}_2\text{OTMS}}$$

The precursor is **2**.

The mass spectra of the undeuterated polyalcohol TMS-ethers are identical with those obtained from authentical material.

2,5-dideoxy-pentos-4-ulose and 2,3-dideoxy-pentos-4-ulose which had been identified in deoxygenated N_2O saturated aqueous solutions of DNA after γ-irradiation[2] are absent in the presence of oxygen.

When DNA is irradiated with γ-rays in N_2O/O_2 saturated aqueous solutions the OH radicals produced in the system will attack the DNA. At the lowest dose given approximately every second nucleotide unit is attacked by an OH radical. The major part of the OH radicals are expected to react with the bases as has been shown with nucleotides[4, 5]. Hence, we estimate that only every eighth or tenth sugar unit is attacked by an OH radical. Initial yields are approximately $G(\mathbf{1}) = 0.005$ and $G(\mathbf{2}) = 0.004$.

The formation of 2-deoxy-tetrodialdose **1** can be explained in a way similar to that proposed for the

* Part I is ref. [2].
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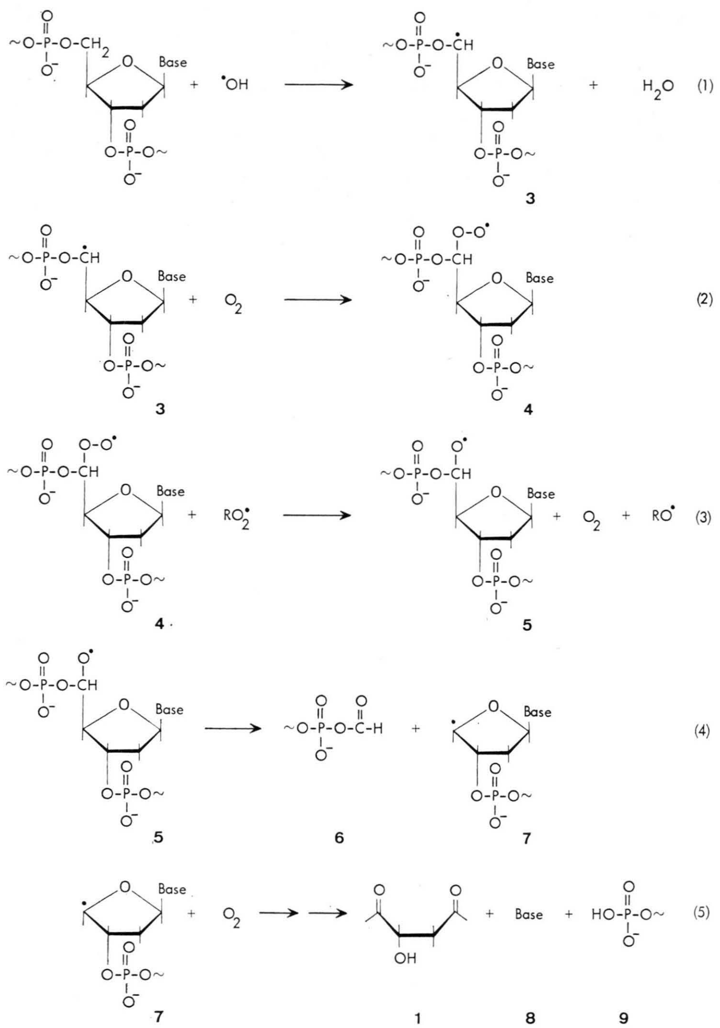

formation of erythro-tetrodialdose in the γ-radiolysis of oxygenated aqueous solutions of ribose-5-phosphate[6]. It is assumed that some of the OH radicals abstract a hydrogen atom from C-5′ of the 2-deoxy-ribose in the DNA chain to give the radical **3** (reaction 1). The hydrogen atom abstraction is followed by the addition of oxygen to form the peroxyl radical **4** (reaction 2). Radical **4** can react with another peroxyl radical leading to the oxyl radical **5**, molecular oxygen[7, 8] and another oxyl radical (reaction 3). The oxyl radical **5** can undergo β-fragmentation[7, 8] to give a DNA fragment with a formyl phosphate end group **6** and radical **7** (reaction 4). In this step the DNA chain is broken between the carbon atoms 4′ and 5′ of the sugar moiety.

Radical **7** is expected to loose the other phosphate bound part of the DNA chain in a process discussed previously[9, 2]. The possible mechanism of the liberation of the unaltered base **8** has been discussed in the context of the radical induced scission of the N-glycosidic linkage in thymidine[10]. Detailed mechanistic investigations, especially on the formation of **2** are being undertaken.

The formation of 2-deoxy-pentos-4-ulose **2** shows that the DNA strand is broken but the carbon skeleton of the sugar is preserved. This means that the strand break in this case is caused by mere phosphate ester cleavage. Strand breaks by phosphate ester cleavage while the carbon skeleton of the sugar remains intact have already been observed in the absence of oxygen[2]. The formation of strand breaks caused by C – C-bond rupture as shown in this paper (formation of product **1**) appears to be a special effect of oxygen.

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