**γ-Radiolyses of DNA in Oxygenated Aqueous Solution. Structure of an Alkali-Labile Site**

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Erythritol-1-d has been isolated from γ-irradiated aqueous oxygenated solution of DNA after reduction with NaBD₄, alkali and phosphatase treatment. It is concluded that this product stems from a d-erythrose 2,4-diphosphate unit in the DNA which is formed via a sequence of reactions following H-abstraction at C-2' by OH radicals.

Irradiation of DNA with ionizing radiation leads to strand breaks. The treatment of irradiated DNA with alkali increases the yield of strand breaks. The effect of alkali is due to alkali-labile sites which are produced as a result of alterations of either the base or the sugar moiety. Recently we have isolated a sugar from γ-irradiated DNA after alkali and phosphatase treatment and determined its structure (2-deoxy-D-erythrose-pentonic acid). The latter is different from the sugars which have been isolated without alkali treatment.

In this paper we report the identification of meso-erythritol-1-d (7) from which we infer the structure of a further alkali-labile site (1) in DNA, γ-irradiated in oxygenated aqueous solution.

1 is a modified section of DNA structurally equivalent to the 2,4-diphosphate of d-erythrose. The free OH group next to the phosphate ester group causes alkali-lability.

In the γ-irradiated DNA 1 was identified by excision of the d-erythrose unit as follows:

Aqueous solutions of DNA from calf thymus (Merck; 0.25 mg/ml) were saturated with N₂O/O₂ (80/20; v/v) and irradiated with ⁶⁰Co-γ-rays (dose range: 10¹⁸ to 4 × 10¹⁸ eV·g⁻¹; dose rate: 3 × 10¹⁸ eV·g⁻¹·h⁻¹). After irradiation the samples were reduced with NaBD₄ leading to 6. The sodium ions were removed with an ion exchanger (Dowex 50 WX2, H⁺-form) and the boric acid evaporated as its methyl ester. The residue was dissolved in water (2 mg DNA/ml) and adjusted to pH 12 with NaOH. The solution was kept at 37 °C for 48 h, adjusted to pH 8 with formic acid, and incubated with alkaline phosphatase (0.4 U/ml; Boehringer) at 37 °C for 12 h. The freeze dried material was trimethylsilylated with BSTFA/TMCS (100/3) in pyridine at room temperature, concentrated in vacuo to remove the excess of the silylating agent, and analysed by GC-MS using a 123 m Dexsil 300 glass capillary column at 170 °C. From the GC-peak corresponding to the TMS ether of meso-erythritol a mass spectrum was taken. Its typical fragment ions were m/e 73 (100%), 103 (22%), 104 (10%), 205 (17%), 206 (14%), 217 (17%), 218 (10%), 232 (M-90-89; 1%), 307 (3%), 308 (4%) and 321.
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(M-90; 1%). This spectrum corresponds to the TMS ether of meso-erythritol-1-d₄ (7).

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\text{TMSOCHD} \rightarrow \text{CHOTMS} \rightarrow \text{CHOTMS} \rightarrow \text{CH}_2\text{OTMS}
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The reduction of D-erythrose to meso-erythritol was necessary to avoid the degradation of D-erythrose on alkaline treatment. The G value of meso-erythritol-1-d₄ is ca. 0.005. This small yield is likely due to both a low probability of H abstraction by OH at C-2' and to side reactions in the reaction sequence leading from radical at C-2' to the isolated product.

The formation of 7 from γ-irradiated DNA may be explained analogous to the mechanism proposed for the formation of D-erythrose in the γ-iradiolysis of 2-deoxy-D-ribose in oxygenated aqueous solution and for the formation of erythro-tetrodialdose in the γ-radiolysis of D-ribose-5-phosphate: OH radicals produced by γ-irradiation of N₂O saturated aqueous solutions abstract H atoms from the sugar moiety of DNA. Abstraction at C-2' gives rise to the formation of 2. Radical 2 is scavenged by molecular oxygen to give 3.

The peroxy radical 3 reacts with another peroxy radical leading to the oxyl radical 4, molecular oxygen and another oxyl radical. The oxyl radical 4 undergoes β-fragmentation to give 5. Radical 5 reacts with molecular oxygen and is expected to lead to product 1. Reduction of 1 with NaBD₄ yields 6. Treatment with alkali and phosphatase converts 6 into 7.

Köhlein and Hutchinson postulated that in the photolysis of bromouracil-containing DNA, radicals at C-2' are formed in high yields and are subsequently converted into strand breaks. In the present paper a mechanism is described which leads from the C-2' radical to strand breaks. We believe, however, that starting from bromouracil-containing DNA other reactions leading to strand breaks may occur in addition.