γ-Radiolysis of 2′-Deoxyguanosine
The Structure of the Malondialdehyde-Like Product

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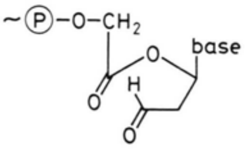
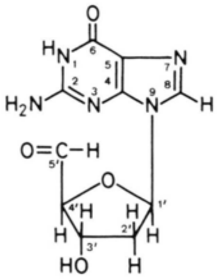
In the γ-radiolysis of N_2O/O_2-saturated solutions of 2′-deoxyguanosine, 2′-deoxyguanosine-5′-aldehyde has been identified as the major product ($G = 0.27$) which gives rise to a positive 2-thiobarbituric acid test usually associated with malondialdehyde or structurally closely related compounds. From experiments with tetranitromethane as oxidant instead of oxygen it is concluded that the major primary precursor is not the radical at C(5′) of the sugar moiety but a base radical. The relevance of these observations with respect to radiation-induced changes in DNA is discussed.

It is known that γ-irradiation of DNA in oxygenated solutions [1–3] or in the solid (but wet) state [4] leads to a lesion which gives a positive colour test with 2-thiobarbituric acid (TBA). A similar behaviour is shown by DNA after treatment with the antitumor drug bleomycin [5–7]. A positive TBA test is usually taken as evidence for the presence of malondialdehyde (MA). In the case of DNA most of the chromophore-yielding material remains bound to the macromolecule, hence it is clear that a product other than free MA must be formed. Structure **1** has been proposed to account for this lesion.



1

One expects MA to be set free from **1** under the severe conditions of the TBA test. Since it has been shown that many compounds which are not closely related to MA also give the TBA test (*cf.* Ref. [8, 9]), it appeared necessary to study low-molecular-weight compounds which are fully accessible to structural analysis. It will be shown that the major TBA-reactive lesion in the radiolysis of N_2O/O_2-saturated solutions of 2′-deoxyguanosine (dG) is the 5′-aldehyde **2**.



2

Aqueous solutions of dG (10^{-3} M) have been saturated with a 4:1 N_2O/O_2 mixture and subjected to 60-Co-γ-radiation at dose rates of 0.25 Gy s^{-1} and 3.7×10^{-3} Gy s^{-1}. The radiation-chemical yield (molecules/100 eV) of TBA-reactive material was independent of dose rate and has been found to be $G = 0.28 \pm 0.04$ (based on $\varepsilon(530\text{ nm}) = 1.5 \times 10^5$ $M^{-1}cm^{-1}$). Only a trace of such material ($G = 0.05$) is formed in the absence of oxygen.

The TBA test is carried out at elevated temperatures in a strongly acidic medium [8]. Kinetic measurements show that two components are responsible for the colour formation, a fast one with $k = (2.8 \pm 0.3) \times 10^{-3}$ s^{-1} at 85 °C which agrees with that of a MA standard and a much slower one ($k = (4.8 \pm 0.4) \times 10^{-4}$ s^{-1}). They are formed in a 5 to 95 ratio. Fig. 1 shows an HPL chromatogram of a γ-irradiated

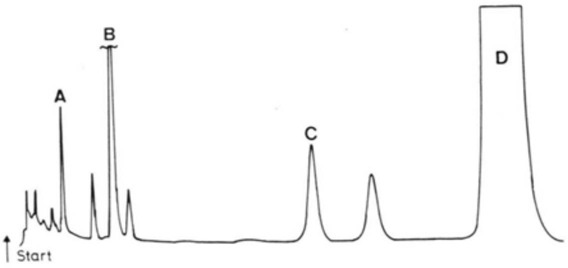


Fig. 1. HPLC (Nucleosil 5C18, 4.6 × 125 nm, H_2O 0.7 ml min^{-1}, λ = 260 nm) of a γ-irradiated N_2O/O_2 (4:1 v/v)-saturated aqueous solution of 2′-deoxyguanosine: A free malondialdehyde, B guanine, C 2′-deoxyguanosine-5′-aldehyde, D 2′-deoxyguanosine.

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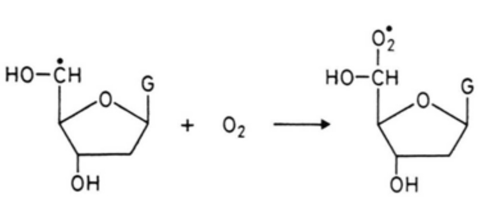
dG solution. Fractions A and C are TBA-reactive. The TBA-reactive material is eluted from the column quantitatively ($\geq 95\%$). Fraction A agrees in its retention time, pH dependence of its UV-spectral properties and TBA kinetics with free MA. Fraction C is hydrolysed into guanine and free MA and shows the slow TBA kinetics. It is reduced to dG by $NaBH_4$. It is sufficiently stable to be isolated by repetitive HPLC in amounts sufficient for obtaining an ^1H-NMR-spectrum on a 400 MHz Bruker instrument. This spectrum showed $\delta(D_2O)$ = 2.34 (ddd, 14.2 Hz, 6.1 Hz, 2.4 Hz; 2′-H$_a$), 2.66 (ddd, 14.2 Hz, 8.3 Hz, 5.9 Hz; 2′-H$_b$), 3.87 (dd, 4.3 Hz, 2.2 Hz; 4′-H), 4.55 (ddd, 5.9 Hz, 2.4 Hz, 2.2 Hz; 3′-H), 5.00 (d 4.3 Hz; 5′-H), 6.19 (dd, 8.3 Hz, 6.1 Hz; 1′-H), 7.87 (s; 8-H). This clearly indicates that the compound in question is 2′-deoxyguanosine-5′-aldehyde, **2**. The assignment was further substantiated by means of authentic material kindly supplied by Dr. J. Cadet, Grenoble. The δ value of 5′-H (5.00) shows that the carbonyl group at C(5′) is actually acetalised, either by hydration or intramolecularly involving the guanine moiety (for the sake of clarity, the carbonyl group is shown free in the structural formula given here for **2**).

The precursor of **2** is very likely the radical at C(5′), **3** which after addition of oxygen (*cf.* **4**) eliminates $HO_2^{\cdot}$ (reactions (3) and (4)) [10]. One might be tempted to assume that this radical is formed directly upon attack by the OH radicals generated in the radiolysis of N_2O/O_2-saturated solutions (reactions (1) and (2)).

$$H_2O \xrightarrow[\text{radiation}]{\text{ionizing}} {}^{\cdot}OH, e^-_{aq}, H^{\cdot}, H^+, H_2O_2, H_2 \quad (1)$$

$$e^-_{aq} + N_2O \rightarrow {}^{\cdot}OH + OH^- + N_2 \quad (2)$$



3 **4** (3)

$$\mathbf{4} \rightarrow HO_2^{\cdot} + \mathbf{2} \quad (4)$$

$$\mathbf{3} + C(NO_2)_4 \rightarrow \mathbf{2} + NO_2 + C(NO_2)_3^- + H^+ \quad (5)$$

If this interpretation were correct, $G(\mathbf{2})$ should have the same value when tetranitromethane is used as an oxidant instead of oxygen (reaction (5)). Under such conditions $G(\mathbf{2})$ is, however, only 0.06_7, *i.e.* one quarter of the value found in the presence of oxygen. This indicates that the attacking species are, for the most part, not OH but probably a base radical the structure of which is not yet known. In the presence of tetranitromethane the base radical is oxidised and hence deactivated, but in the presence of oxygen this radical could attack the sugar. It is tentativly suggested that this base radical is the N(3)-centered radical. Such N-centered radicals are expected to be formed in the reaction of OH with dG [11]. Their formation is the likely reason for the low radiation-induced oxygen uptake observed in this system [12]. If an N-centred OH-adduct radical attacks the sugar a base hydrate would be formed (*e.g.* OH at C(2), H at N(3)) which could revert to the base by water elimination. Such a reaction could account for the observation that in the product **2** the base is intact, despite the fact that a base OH-adduct radical has to be postulated as an intermediate.

If this interpretation is correct, then the present case provides a further example of sugar damage mediated by base radicals. Hitherto only in poly(U) has evidence for such reactions been obtained [13–15] where it has been concluded [14] that it is not the sugar that bears the base radical which is attacked, but a more distant one in contrast to the present, monomeric system, where base radical attack most likely occurs intramolecularly within the same nucleoside.

The formation of **2** in the radiolysis of dG suggests that, in principle, the same kind of lesion might occur in DNA besides lesion **1** and give rise to a positive TBA test. However, the kinetics of the TBA reaction using irradiated DNA indicates [16] no slow component and therefore no structure analogous to **2**. This would leave structure **1** as the most likely candidate. A similar precursor may be assumed for the free MA formed in the dG system. It is conceivable that with the guanine residue such a structure is unstable and hydrolyses prior to HPLC analysis.

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