Radiation Chemistry of DNA-Model Compounds, VIII
Dephosphorylation Products from Reactions of OH Radicals with Ribose-5-phosphate in Aqueous Solution. The Effect of Oxygen

LOTHAR STELTER, CLEMENS VON SONNTAG, and DIETRICH SCHULTE-FROHLINDE
Institut für Strahlenchemie im Max-Planck-Institut für Kohlenforschung, Mülheim a. d. Ruhr

DNA Model Compound, Ribose-5-phosphate, γ-Rayolysis, Dephosphorylation, Ribosyl-5-phosphate Radicals, Oxygen Effect

The reaction of hydroxyl radicals with ribose-5-phosphate in oxygenated aqueous solution leads to the following phosphate free products (G-values): Erythrodialdose (0.4), formic acid (0.4) and ribodialdose (0.1). In addition, inorganic phosphate (0.6) and some minor phosphate free products (total G ~ 0.08) are formed.

Within the limits of experimental error material balance between dephosphorylation products and inorganic phosphate is obtained. A mechanism is proposed which can account for the main products. The primary steps of the dephosphorylation are H atom abstraction from C-5 of ribose-5-phosphate by OH radicals and subsequent addition of O2. The peroxy radicals formed decompose to ~ 80% by C-C bond cleavage followed by elimination of inorganic phosphate and only to ~ 20% by direct dephosphorylation. The relevance of these results with respect to the radiolysis of DNA is discussed.

Introduction

Interaction of ionizing radiation with living cells may lead to the formation of breaks in the DNA chain. These breaks may be either caused by scission of a phosphate ester linkage or of a carbon-carbon bond in the sugar moiety. In a preceding communication we proposed a mechanism for the former type of scission, using ribose-5-phosphate as a model compound for DNA in deoxygenated aqueous solution. Carbon-carbon bond breakage played a minor role. The dephosphorylation product described was indentified as 5-deoxy-D-erythro-pentos-4-ulose (CH3-CO-CHOH-CHOH-CHO). Recently, the analogous product 2,5-dideoxy-ribose-4-ulose (CH2-CO-CHOH-CH2-CHO) was isolated from aqueous solutions of DNA irradiated under similar conditions. This strongly supports the relevance of ribose-5-phosphate as an appropriate model compound for studying the mechanisms involved in the formation of strand breaks in DNA and encouraged us to undertake further investigations on this system. In this paper we want to report on some results obtained from the irradiation of aqueous solutions of ribose-5-phosphate in the presence of oxygen. As will be shown, in the presence of oxygen, the fragmentation mechanism is greatly changed, C-C bond breakage becoming an important process.

When a 10^-2 M aqueous solution of ribose-5-phosphate is irradiated with γ-rays, practically all the radiation energy is absorbed by the water molecules, giving rise to the formation of hydroxyl radicals, hydrated electrons and hydrogen atoms as the primary radical products (reaction (1)).

\[ \text{H}_2\text{O} \rightarrow \cdot \text{OH}, \text{e}^-_{\text{aq}}, \cdot \text{H} \]  

By saturating the solution with N2O/O2 (4/1 p.b.v.) the hydrated electrons are converted into hydroxyl radicals (reaction (2)) the total G-value = number of particles formed per 100 eV absorbed.

** G-value = number of particles formed per 100 eV absorbed.
of which then amounts to approximately 5.5.

\[ e_{\text{aq}} + N_2O \to \cdot OH + OH^- + N_2 \]  

(2)

The hydrogen atoms which are formed only in small amounts \((G = 0.55)\) are mostly scavenged by \(O_2\). The OH radicals react with the solute molecules primarily by abstracting hydrogen from either one of the five carbon atoms. Abstraction of hydrogen from the hydroxyl groups is less likely. The resulting ribosyl phosphate radicals add oxygen to give peroxy radicals. These then undergo further reactions and, finally, end up as stable products.

One would expect two types of products: One in which only the carbohydrate residue is altered while the phosphate linkage remains intact, and another which results from the rupture of the phosphate bond. Here we will be concerned only with the dephosphorylation products.

**Results**

**Identification of the products**

Aqueous solutions of ribose-5-phosphate \((10^{-2} \text{ M})\) were saturated with \(N_2O/O_2 (4/1 \text{ p. b. v.})\) and irradiated with \(\gamma\)-rays. For GC analysis, the irradiated samples were derivatized by reduction with \(\text{NaBH}_4\) and by subsequent trimethylsilylation. A typical gas chromatogram is shown in Fig. 1. Comparison of the retention times of the most prominent peaks with those of authentic standards by spiking experiments indicated inorganic phosphate, erythritol and ribitol as main products (peaks a, c and e, respectively). The absence of the enantiomers of erythritol and ribitol in comparable amounts shows that any carbonyl function present in the product molecules before reduction must be located at an outer carbon atom. The less intense peaks b and d correspond to glycerol and arabitol, respectively. The nature of the minor peaks which are, in part, due to impurities was not determined.

For the mass spectrometric identification of the peaks the samples were reduced with \(\text{NaBD}_4\). On conversion of the carbonyl into alcohol groups deuterium atoms were thus incorporated in the product molecules at these positions. The mass spectrum of peak a corresponds to the trimethylsilyl (TMS) ester of phosphoric acid\(^5\).\(^6\).

The spectra of peaks c and e (Figs. 2A and 2B, respectively) are very similar, reflecting a close similarity in the structure of the two products. The fragment ions at \(m/e\) 104, 206, 218 and 308 which are present in both spectra, are typical of deuterated polyalcohols\(^7\). The two spectra differ in that the one (Fig. 2A) contains an ion at \(m/e\) 322, whereas the other (Fig. 2B) shows one at \(m/e\) 320. The former fragment results from the elimination of 90 m. u. (trimethyl silanol) from the molecular ion of the tetritol TMS ether, while the latter is formed by elimination of 90 m. u. from the \(m/e\) 410 fragment ion of the pentitol derivative. The lack of significant amounts of \(m/e\) 205 (besides 206) and of \(m/e\) 307 (besides 308) in both spectra shows that the two products are symmetrically deuterated at both ends. The ions 308 in Fig. 2A and 320 in Fig. 2B finally indicate that the exact positions of the deuterium atoms are at the outermost carbons.

Treatment of the irradiated samples with methoxylamin instead of sodium borodeuteride and subsequent silylation leads to two derivatives the mass spectra of which are shown in Figs. 3A and 3B. Except for the formation of the fragment ion \(m/e\) 73 (TMS) which gives the base peak in all four spectra, these derivatives preferentially fragmentate between the second and third carbon atom from either end of the molecule, with the charge localized predominantly at the larger fragment. Rupture of the C-C bonds adjacent to the methoxime groups is not observed. As a consequence of the structural symmetry \(m/e\) 160 appears as the most predominant peak (besides the base peak) in the mass spectrum of the smaller derivative, whereas the most intense peak of the larger is at \(m/e\) 262, the fragment \(m/e\) 160 being present in a smaller amount.

From the gas chromatographic and mass spectrometric results, it is concluded that the three products

---

**Fig. 1.** Gas chromatogram of reduced and trimethylsilylated products from \(\gamma\)-irradiated \(N_2O/O_2 (4/1)\) saturated aqueous solutions of ribose-5-phosphate \((10^{-2} \text{ M})\). a = phosphoric acid, b = glycerol, c = erythritol, d = arabitol, e = ribitol. OV-101, 50 m glass capillary column, 110 to 170 °C, 1°/min.
Fig. 2. A. Mass spectrum of GC-peak c (Fig. 1). TMS derivative of erythritol-1,4-d$_2$. 70 eV ionizing voltage. B. Mass spectrum of GC-peak e (Fig. 1). TMS derivative of ribitol-1,5-d$_2$. 70 eV ionizing voltage.

Fig. 3. A. Mass spectrum of methoximated and trimethylsilylated erythrodialdose. 70 eV ionizing voltage. B. Mass spectrum of methoximated and trimethylsilylated ribodialdose. 70 eV ionizing voltage.
in question are inorganic phosphate, erythrodialdose and ribodialdose.

To check whether the aldehyde groups are formed via enols, mass spectra were also recorded from samples irradiated in D₂O. No mass shifts as compared to the samples irradiated in H₂O were observed.

The mass spectrum of the glycerol peak (peak b in Fig. 1) indicates the presence of two products with the structures CHO-CHOH-CHO and CHO-CO-CHO which appear to be formed in about equal amounts. The arabitol peak (peak d in Fig. 1) is thought to have erythro-pentos-4-ulose as the precursor. However, the spectrum does not allow to discriminate between this product and arabodialdose as the possible precursor. Attempts to obtain mass spectra of the methoxymated and silylated products were unsuccessful.

Erythrodialdose which contains four carbon atoms was found as a main product of the radiolysis of ribose-5-phosphate. The other fragment, containing one carbon atom, was identified as formic acid. Its determination is described in the experimental section.

Quantitative results

Plots of products concentration against dose were approximately linear in the dose range of \(4 \times 10^{18}\) to \(1.5 \times 10^{19}\) eV/g which corresponds to a total conversion of about 4 to 15%. From these plots the G-values of the products were determined. They are listed in Table I. The minor products (peaks b and d in Fig. 1) are given as the sum. Errors are estimated to be \(\pm 10\%\) for the phosphate free products and \(\pm 15\%\) for the inorganic phosphate.

Table I. Products from \(\gamma\)-irradiated aqueous solutions of ribose-5-phosphate (10⁻³ M) saturated with NaO/O₂ (4/1).

<table>
<thead>
<tr>
<th>Product</th>
<th>G-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorg. Phosphate</td>
<td>0.61</td>
</tr>
<tr>
<td>Formic acid</td>
<td>0.41</td>
</tr>
<tr>
<td>Erythrodialdose</td>
<td>0.40</td>
</tr>
<tr>
<td>Ribodialdose</td>
<td>0.10</td>
</tr>
<tr>
<td>Minor phosphate free products</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Discussion

In comparison with the results obtained from the irradiation of ribose-5-phosphate in deoxygenated solution⁵,⁶, in the presence of oxygen a relatively small number of phosphate free products are formed. Among these, erythrodialdose and formic acid are the main products (Table I). This shows that dephosphorylation of the substrate molecule is accompanied to a large extent by the rupture of a carbon-carbon bond. In deoxygenated solution C-C bond rupture did not play a significant role⁸.

As can be seen from Table I, material balance between the phosphate free carbohydrate products and inorganic phosphate is obtained within the limits of experimental error. The yield of formic acid equals that of erythrodialdose, indicating that the two products have the same precursor.

Other than in oxygen free solution, no products containing deoxy-keto groupings are formed. Thus, the 5-deoxy-pentos-4-ulose, for example, a product described in earlier papers⁴,⁵, is not observed. The mechanisms for the radiation-induced phosphate ester cleavage proposed there are, therefore, not operative in the presence of oxygen.

The formation of the main products, shown in Table I, can be explained, if one assumes that the first step in the reaction sequence is hydrogen atom abstraction from C-5 of the substrate molecule 1 (reaction (3)). The resulting ribosyl phosphate radical (2) adds oxygen to give the peroxy radical (3) (reaction (4)). Peroxy radicals are known to decay in different ways⁹, one of which is the bimolecular decomposition into oxy radicals and molecular oxygen⁹. Thus, radical 3 may react with another peroxy radical, leading to 4 (reaction (5)). Oxy radicals can undergo β-fragmentation⁹,¹⁰. Since formic acid and erythrodialdose are formed only in the presence of molecular oxygen (the extremely small amounts of erythrodialdose detected in irradiated oxygen free solutions of ribose-5-phosphate can be ignored), it is most likely that they are formed via such a process. β-Fragmentation of the oxy radical (4) yields formyl phosphate (5) and the radical 6 (reaction (6)). Formyl phosphate is unstable in aqueous solution and hydrolyses yielding phosphoric acid (7) and formic acid (8) (reaction (7)). Radical 6 is a typical carbohydrate radical which is known to give the dicarbonyl compound (9) in the presence of oxygen¹¹ (reaction (8)). The chemical steps involved are uncertain. Erythrodialdose (9) has also been isolated as a radiolysis product of ribose irradiated under similar conditions¹¹.
The formation of ribodialdose corresponds to an oxidation of the esterified alcohol group at C-5 of the ribose-5-phosphate molecule to an aldehyde group and inorganic phosphate. The rest of the molecule remains unchanged. It is reasonable to assume that the first two steps will be the same as in reactions (3) and (4), that is, abstraction of a hydrogen atom from C-5 of the ribose-5-phosphate molecule and formation of the peroxy radical (3). The mechanism by which the peroxy radical decays to give ribodialdose is still uncertain. One possibility can, however, be excluded: Elimination of HO₂⁻ from 3 would lead to an enol phosphate which is unstable in water and would hydrolyse to give ribodialdose via the enol. If the conversion of the enol to the aldehyde takes place in D₂O, a deuterium atom will be incorporated in the product molecule at C-4. This is, however, not the case, as has been shown by mass spectrometry. If the mechanism proposed for the formation of the main products is
correct, about 10% of the OH radicals (G(OH)=5.5) should attack the ribose-5-phosphate molecule at C-5. This value is the same as that found for the attack by OH radicals at C-5 of 2-deoxyribose under similar conditions\textsuperscript{12}.

**Conclusion:** One may assume that the same mechanism as described for the C-C fragmentation of the model compound ribose-5-phosphate could also operate in the DNA macromolecule. Then, one would expect that, in the presence of oxygen, hydrogen atom abstraction at C-5 of a sugar component in the DNA should give rise to a strand break by scission of the carbon-carbon bond between C-4 and C-5. Analogous to the formation of erythrodialdose, this reaction may lead to a labile phosphate\textsuperscript{13} with a carbonyl group at C-1 and C-4, which on hydrolysis should give 2-deoxy-tetrodialdose. This product has in fact been isolated from \(\gamma\)-irradiated oxygenated aqueous solutions of DNA\textsuperscript{14}.

**Experimental**

**Irradiation**

Solutions of 10\(^{-2}\) M ribose-5-phosphate sodium salt (Calbiochem) in tripoly distilled water or 99.7\% D\(_2\)O were saturated with N\(_2\)O/O\(_2\) (4/1 p.b.v.) and irradiated in a \(\gamma\)-Co-\(\gamma\)-source (dose rate 2.4 \(\times\) \(10^3\) eV/\(\text{ml min}\)) at room temperature. A stream of N\(_2\)O/O\(_2\) (4/1 p.b.v.) was maintained through the solutions during irradiation.

**Reduction and methoximation**

After irradiation, 20 ml aliquots were reduced with NaBH\(_4\) or NaBD\(_4\). Alternatively, the samples were brought to dryness and treated with methoxyamine hydrochloride\textsuperscript{15} in dry pyridine to give the methoxime derivatives of the products.

**Trimethylsilylation**

The reduced or methoximated samples were brought to dryness, taken up in 0.2 ml of dry pyridine and trimethylsilylated with 0.8 ml of N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA, Serva) containing 3\% trimethylchlorosilane (TMCS, Serva). The mixtures were heated at 50 °C for about 15 min, left overnight at room temperature and then analyzed by GC. Under these conditions, all the radiolysis products including phosphoric acid or its sodium salt were completely silylated.

**GC-analyses**

The GC-analyses were carried out using a Varian 1400 gas chromatograph equipped with an OV-101 glass capillary column, 50 m \(\times\) 0.35 mm i.d.\textsuperscript{16}, and a flame ionization detector.

The column oven temperature was programmed from 110 °C to 170 °C at a rate of 1 °C/min. All higher boiling products and the starting material were removed from the column by backflushing at elevated temperatures\textsuperscript{18}.

**Combined GC-MS**

The gas chromatographic peaks were identified by comparing their retention times with those of authentic standards and by mass spectrometry. For the combined GC-MS, the samples were evaporated in vacuo over P\(_2\)O\(_5\) to remove the solvent and excess silylating agents, and redisolved in dry \(n\)-hexane, containing 1\% BSTFA. The gas chromatographic conditions were the same as described above. The mass spectra were recorded with a Varian Atlas CH-4 mass spectrometer combined with a computer\textsuperscript{17}.

**Quantitative determinations**

The quantitative determination of the carbohydrate products and inorganic phosphate was carried out by GC making use of the internal standard method\textsuperscript{18}. 6-Deoxy-L-mannose (Serva) served as the standard. The samples were reduced with NaBH\(_4\) and trimethylsilylated, as described, and the peak areas measured with the help of a computer. The correction factor\textsuperscript{19} for the polyalcohols was taken as 1.0 (±5\%). For inorganic phosphate (Na\(_2\)HPO\(_4\), 2 H\(_2\)O), it was determined to be 2.3 (±5\%).

Formic acid was reduced to formaldehyde with Mg/HCl, as described by W. M. Grant\textsuperscript{20}. The formaldehyde was determined spectrophotometrically by means of the Hantzsche reaction\textsuperscript{21}. All determinations were made against unirradiated samples.

The authors are grateful to the Drs. Dizdaroglu, Henneberg and Schomburg for their assistance in the GC and MS analyses.

11 C. von Sonntag and M. Dizdaroglu, to be published.
13 G. Scholes and J. Weiss, Nature 171, 920 [1953].
19 C. von Sonntag and M. Dizdaroglu, unpublished results.
20 W. M. Grant, Anal. Chem. 20, 267 [1948].