Radical-Induced Dephosphorylation of Fructose Phosphates in Aqueous Solution

Henryk Zegota++
and
Clemens von Sonntag*+

Max-Planck-Institut für Strahlenchemie, Stiftstr. 34-36, D-4330 Mülheim 1
Z. Naturforsch. 36b, 1331–1337 (1981); received May 29, 1981

D-Fructose-1-phosphate, D-Fructose-6-phosphate, γ-Irradiation, Radical Reactions, Mass Spectrometry

Oxygen free N$_2$O-saturated aqueous solutions of D-fructose-1-phosphate and D-fructose-6-phosphate were γ-irradiated. Inorganic phosphate and phosphate free sugars (containing four to six carbon atoms) were identified and their G-values measured. D-Fructose-1-phosphate yields (G-values in parentheses) inorganic phosphate (1.6), hexos-2-ulos (0.12), 6-deoxy-2,5-hexodiulose (0.16), tetrulose (0.05) and 3-deoxytetritulose (0.15). D-Fructose-6-phosphate yields inorganic phosphate (1.7), hexos-5-ulos (0.1), 6-deoxy-2,5-hexodiulose (0.36), 3-deoxy-2,5-hexodiulose and 2-deoxyhexos-5-ulos (together 0.18). On treatment with alkaline phosphatase further deoxy sugars were recognized and in fructose-1-phosphate G(6-deoxy-2,5-hexodiulose) was increased to a G-value of 0.4. Dephosphorylation is considered to occur mainly after OH attack at C-5 and C-1 in fructose-1-phosphate and at C-5 and C-6 in fructose-6-phosphate. Reaction mechanisms are discussed.

Introduction

The radiation chemistry of sugar phosphates in aqueous solutions has found already considerable attention [1-10]. Most of the work has been carried out with D-ribose-5-phosphate. The same processes that govern phosphate elimination in D-ribose-5-phosphate have been shown also to operate in DNA where they lead to strand breaks [11, 12]. In 10$^{-2}$ M aqueous solutions of the sugar phosphates, practically all the radiation energy is absorbed by the solvent water. In the radiolysis of water the following species are formed (reaction (1)):

$$
H_2O \rightarrow \cdot OH, H, e_{aq}^-, H_2O_2, H_2O^+, OH^- \quad (1)
$$

In the presence of N$_2$O the hydrated electrons (e$_{aq}^-$) are converted into OH radicals (reaction (2)):

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

The OH radicals and H atoms (G(OH + H)≈6)**

---

* Reprints requests to Prof. Dr. C. von Sonntag. 0340/5087/81/1000–1331/$ 01.00/0
++ Permanent address: Institute of Applied Radiation Chemistry, Technical University, Lodz, Poland.
** The G value is defined as molecules formed per 100 eV absorbed energy.

---

Results

Dilute solutions (10$^{-2}$ M, pH 8.2) of the sugar phosphates were saturated with oxygen-free N$_2$O for 45 min and γ-irradiated at a dose rate of 3 × 10$^{11}$ eV · g$^{-1}$ · h$^{-1}$ at doses of 1.2 × 10$^{19}$ eV · g$^{-1}$ and 2.4 × 10$^{19}$ eV · g$^{-1}$ at room temperature. On irradiation the pH drops by one unit. The phosphate free carbohydrate products were either reduced with NaBD$_4$ and trimethylsilylated (TMS) [14] or react with the solute molecules by abstracting carbon-bound hydrogen atoms. Because of the high reactivity of the OH radicals hydrogen abstraction occurs nearly at random [13]. Therefore, D-fructose-1-phosphate (1) and D-fructose-6-phosphate (2) will both give rise to a set of five different primary radicals. Some of these radicals will eliminate organic phosphate in the subsequent free radical reactions. In the present paper resulting phosphate free products (and some products retaining the phosphate group) will be presented and mechanistic aspects will be discussed.

---

D-Fructose-1-phosphate D-Fructose-6-phosphate

1 2
Table I. Products and their G values from the radiolysis of fructose-phosphates in aqueous solutions saturated with oxygen-free N$_2$O.

<table>
<thead>
<tr>
<th>No.</th>
<th>Product</th>
<th>D-Fructose-1-phosphate (1)</th>
<th>D-Fructose-6-phosphate (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Inorganic phosphate</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>D-arabino-Hexos-2-ulose</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>D-lyxo-Hexos-5-ulose</td>
<td>—</td>
<td>0.10</td>
</tr>
<tr>
<td>6</td>
<td>6-Deoxy-D-threo-2,5-hexodiulose</td>
<td>0.16 0.40$^b$</td>
<td>0.36 0.39$^b$</td>
</tr>
<tr>
<td>7</td>
<td>3-Deoxy-2,5-hexodiulose</td>
<td>—</td>
<td>0.18 0.36$^{b,c}$</td>
</tr>
<tr>
<td>8</td>
<td>2-Deoxyhexos-5-ulose</td>
<td>0.01$^c$</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4-Deoxy-D-glycer-2,3-hexodiulose</td>
<td></td>
<td>0.21$^b$</td>
</tr>
<tr>
<td>10</td>
<td>D-Arabinonic acid</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>D-glycer- Tetrulose</td>
<td>0.05</td>
<td>— 0.01$^b$</td>
</tr>
<tr>
<td>12</td>
<td>3-Deoxytetrulose</td>
<td>0.15</td>
<td>0.03</td>
</tr>
</tbody>
</table>

$^a$ From ref. [10];
$^b$ After treatment with alkaline phosphatase;
$^c$ Deoxy compounds with the deoxy group in the middle of the molecule.

converted into the methoxime-TMS derivative [9, 15, 16]. Identification was by GC–MS. For the interpretation of the mass spectra of the deoxy compounds and lactones the NaBD$_4$-reduced TMS-derivatives are most suitable [14] whereas osuloses and diuloses are often better characterized by the methoxime-TMS derivative [16].

Quantitative measurements were done by GC using 2-deoxy-D-erythro-pentitol or erythritol as internal standards and appropriate response factors [9]. Some results were also obtained by treating the irradiated samples with alkaline phosphatase prior to derivation. Only the carbohydrates with six and less carbon atoms, but not the dimer fraction, have been analyzed. The results are compiled in Table I.

**D-Fructose-1-phosphate**

The major phosphate free product is 6-deoxy-D-threo-2,5-hexodiulose (6). Reference material was available [17]. The other major product is D-arabinohexos-2-ulose (glucosone) (4). It was identified as its methoxime-TMS derivative. Reference material was available [16].

Very early in the gas chromatogram of a NaBD$_4$ reduced and trimethylsilylated sample appears a compound showing typical fragment ions (intensities in parentheses) at m/e (%) 73(100), 103(92), 104(30), 130(11), 147(27), 190(7), 220(26), 233 (M-90, 1%).

\[
\begin{align*}
103 & \quad 220 \quad 103 \\
\text{CH}_2\text{OTMS-CDOTMS-CH}_2-\text{CH}_2\text{OTMS}
\end{align*}
\]

The mass spectrum is similar to that of its isotopic isomer described earlier [18, 20]. The precursor is 3-deoxy-tetrulose (12).

After NaBD$_4$ reduction the threitol and erythritol TMS ethers show identical mass spectra with typical fragment ions at m/e (%) 73(100), 103(20), 104(11), 117(15), 118(9), 130(4), 133(4), 147(31), 205(15), 206(14), 218(15), 232(<1), 308(4).

\[
\begin{align*}
103 & \quad 308 \quad 206 \quad 205 \quad 308 \quad 103 \\
(218) & \quad (218)
\end{align*}
\]

The precursor is D-glycer-tetrulose (11).

The material from the arabinitol TMS ether peak shows the fragment ions at m/e (%) 73(100) 103(29), 105(11), 147(20), 205(9), 207(5), 217(8), 219(7), 307(3), 309(4), 319(2), 321(2).

\[
\begin{align*}
105 & \quad 409 \quad 207 \quad 307 \quad 309 \quad 205 \quad 411 \quad 103 \\
(319) & \quad (217) \quad (219) \quad (321)
\end{align*}
\]

The precursor is arabinonic acid (10).

After NaBD$_4$ reduction, low yields of deoxyhexitols with the deoxy group in the middle of the molecule were also observed but have not been characterized.

On treatment with alkaline phosphatase G(6-deoxy-D-threo-2,5-hexodiulose) increases and deoxy compounds with the deoxy group in the middle of the molecule become more noticeable and two more compounds have been identified:

The most prominent fragment ions of the first compound are: m/e (%) 73(100), 103(13), 104(70), 130(5), 147(19), 206(5), 218(7), 220(23), 232(9), 308(3), 322(3), 335(1).
CDHOTMS-CH₂-CHOTMS-CHOTMS-CDOTMS-CH₂OTMS

The precursor is 2-deoxyhexos-5-ulose (8).

The other compound shows typical fragment ions at m/e (%): 73(100), 103(9), 131(17), 147(23), 205(12), 206(7), 218(3), 232(28), 244(2), 245(3), 335(1).

CH₂OTMS-CDOTMS-CDOTMS-CH₂-CHOTMS-CH₂OTMS

The precursor is 4-deoxy-D-glycero-2,3-hexodiulose (9).

**D-Fructose-6-phosphate**

Again, the most prominent product is 6-deoxy-D-threo-2,5-hexodiulose (6). Its yield is markedly higher than that from fructose-1-phosphate but does not increase significantly on phosphatase treatment. Two further deoxy compounds have been identified: 2-deoxyhexos-5-ulose (8), (described above) and 3-deoxy-2,5-hexodiulose (7). The latter is characterized by the mass spectrum of the TMS ethers of a NaBD₄-reduced sample. Typical fragment ions are m/e (%): 73(100), 103(9), 131(18), 147(25), 206(15), 218(5), 232(35), 244(2), 245(4), 308(12), 335(2).

On phosphatase treatment, the yields of the deoxy compounds carrying the deoxy group in the middle of the molecule is doubled. GC-analysis revealed five peaks already present prior to phosphatase treatment. No MS analysis has been done with this fraction. It is believed that 4-deoxy-2,3-hexodiulose (9) might also be present in this fraction (see Discussion). This compound would not give rise to new GC peaks.

In the fraction of phosphate free products after NaBD₄-TMS treatment mannitol and glucitol-TMS ethers give rise to identical mass spectra with typical fragment ions at m/e (%): 73(100), 103(15), 104(13), 117(5), 147(19), 206(21), 218(9), 308(6), 320(16), 332(1).

CDHOTMS-CH₂OTMS-CHOTMS-CHOTMS-CDOTMS-CH₂OTMS

Their common precursor is D-lyxo-hexos-5-ulose (5), 3-Deoxytetrulose (12, see above) has also been found.

**Discussion**

**Reactions from the radicals at C-5**

It has been shown in a number of cases that phosphate is readily eliminated if the radical site is at β position to the phosphate group [3, 7, 9, 11, 12, 19-21]. Phosphate elimination (reaction (3)) is expected to occur from radical 13 which is generated by hydrogen abstraction from C-5 of D-fructose-6-phosphate. There is good evidence that radical cations (cf. refs. [22, 23]) are intermediates in these elimination processes. The reaction of the radical cation 14 with water gives rise to radical 15 (reaction (4)).

On the basis of results from ESR studies on similar radicals [24] radical 15 should be unstable and readily transform into radical 16 (reaction (5)) which is reduced in a disproportionation reaction by other radicals (-RH) yielding 6-deoxy-D-threo-2,5-hexodiulose (6) (reaction (6)).

In D-fructose-1-phosphate the corresponding radical at C-5, 17 will lead to the same product however retaining the phosphate group at C-1. The elimination of the OH group at C-6 of radical 17 (reaction (7)) is a process which has its analogs in
the reactions of similar radicals [25, 26] and the subsequent reactions (reaction (9)) will be analogous to those discussed above (reactions (4–6)).

In competition to reaction (7) one would expect a rearrangement of radical 17 to radical 19 (reaction (8)), a process which is well established [25, 27–29]. The rearranged radical 19 is of the same type as radical 13, rapidly eliminates phosphate and in a reaction sequence similar to reactions 4–6 ultimately ends up as 6 (reactions (11)).

As is shown in Table I, G (6) from D-fructose-6-phosphate (2) is not affected by phosphatase treatment but G (6) from D-fructose-1-phosphate (1) strongly increases on phosphatase treatment indicating the presence of 20. It is also noted that the combined yields of 6 as expressed by the phosphatase treated samples are equal for both 1 and 2. This is to be expected because the OH radicals will attack the 5-position in both cases with similar probabilities. The low yield of 6 (G(6) = 0.4) does not entirely reflect the OH attack at this position because of side reactions, e.g. dimer formation [9].

Without phosphatase treatment 3-deoxy-D-glycer-2,5-hexodiulose (7) is formed from D-fructose-6-phosphate (see Table I). This product may also have the radical at C-4 as its precursor. Reactions (12)–(18) present a scheme according to which product 7 might be formed. Reactions (12) appears to be well established in the light of results from DNA [11]. The subsequent water elimination reactions (13) and (14) have their analogs in reactions observed with other sugars [9, 30–32]. The driving force of these reactions may be the gain in energy by the formation of an allyl radical and water. An allyl radical is also formed by loss of water from the
2,3-dihydroxypropyl radical as shown by ESR spectroscopy [33]. The disproportionation reactions (15) and (17) yield enol ethers. In a previous study on D-ribose-5-phosphate [9] such intermediates have been characterized by deuterium labelling experiments. Similar experiments have not been carried out in the present study and conclusions with respect to the enol ether intermediates are drawn by analogy.

Reactions from the radicals at C-1 in 1 and at C-6 in 2

Another dephosphorylation process is the oxidation of the radicals $a$ to the phosphate group (26 and 27) [9]. Other radicals or radiolytically formed $\mathrm{H}_2\mathrm{O}_2$ may serve as oxidants. In general, such a reaction is more efficient in the presence of good oxidizing agents, e.g. $\mathrm{Fe}^{3+}$ ions [9]. Carbonium ions are likely intermediates.

The product 2-deoxyhexos-5-ulose (8) observed in the radiolysis of fructose-6-phosphate might also have the radical at C-6 as a precursor. A process similar to that formulated for 7 might be considered (reactions (21–23)). The intermediate 28 could also be reached by starting from the radical at C-4.

Reactions from other radical sites

4-Deoxy-D-glycero-2,3-hexululose (9) is only observed after phosphatase treatment, i.e. it is formed as its 1-phosphate in the case of fructose-1-phosphate (1) and as its 6-phosphate in the case of fructose-6-phosphate (2). It is a very much expected product which arises from the well documented water elimination of 1,2-dihydroxyalkyl radicals [25, 34] followed by the reduction of the acylalkyl radical by other radicals (RH) present (cf. reactions (24) and (25)). Most likely the isomers of 9 carrying the deoxy function at C-3 and the carbonyl functions at C-2 and C-4 are also formed but their polyhydric alcohol derivatives were not sufficiently separated by GC from other isomers and no mass spectrum could taken from an isolated stereoisomer.

Carbon-carbon bond scission

There is some material with less than six carbon atoms, especially erythulose and 3-deoxytetulose from D-fructose-1-phosphate.

Carbon-carbon bond fragmentation can occur after H abstraction only by a $\beta$-fragmentation process. For these two compounds one might consider radical 32 as the precursor (reaction (26–29)).
The C-4 radical from the isomer, D-fructose-6-phosphate, might react in a different reaction path, eliminating phosphoric acid whereby forming an allyl radical. Such a type of reaction is observed with 2'-deoxy-5'-cytidylic acid [35]. A possible product is 6-deoxy-2,4,5-hexotriulose which might have escaped detection, 6-deoxy-D-threo-2,5-diulose (6) being such a prominent product. Both would give on NaBD₄ reduction the same polyhydric alcohols albeit the former with one more D atom incorporated. If the 6-deoxy-2,4,6-hexotriulose would be only a minor contributor, it would not be easily recognisable in the mass spectrum. There is a very small probability that OH radical abstract oxygen bound H atoms from carbohydrates [36]. Therefore it has to be expected that the yield of oxyl radicals which we known to fragment quite readily [34] is quite small. The likely precursor of D-arabinonic acid (10) is the oxyl radical at O-2 which fragments by splitting the bond between C-1 and C-2. In accordance with such a mechanism is the observation that 10 is found with 1 but with 2 only after phosphatase treatment.

Material balance

In the present study only the disproportionation products of the radicals have been identified, but not the combination products. For this reason no complete material balance can be reached. This can only be achieved if dimerization processes are inhibited, e.g. by O₂ [6, 13]. Olefinic intermediates such as enol ethers etc. further reduces the product yields by scavenging radicals. Evidence for this has recently been obtained from a study on the photolysis of D-fructose in aqueous solutions [32]. The importance of the sum of the phosphate eliminating processes is therefore reflected by G((inorganic phosphate) which are around 1.6 for both 1 and 2. This indicates that more than one quarter of the radicals generated eliminate phosphate or their reactions lead to products with highly labile phosphate.

Experimental

D-Fructose-1-phosphate, Ba salt A grade (Calbiochem), D-fructose-6-phosphate, Ca salt (Merck) and alkaline phosphatase in suspension (1 mg/1 ml) (Boehringer) were commercially available. Irradiations and work up were as described previously [9, 25].

One of us, H. Z., would like to thank the Max-Planck-Gesellschaft for a stipend. We are very grateful for the help by the staff of the institutes GC- and MS-departments, especially Ms. U. Rauhut, Mrs. K. Sageb, Mr. H. Damen, and Mr. M. Scheppat.