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RADIATION-INDUCED DEPHOSPHORYLATION OF SUGAR PHOSPHATES

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ABSTRACT

γ-Radiolysis of 10 and 50mM D-glucitol 6-phosphate gave $G(H_3PO_4)$ values 1.8 and 1.2 for solutions saturated with nitrous oxide and argon, respectively. The main, neutral products were L-gulose and 6-deoxy-5-hexulose. Radiolysis of solutions of D-glucose 6-phosphate and D-glucitol 6-phosphate in D_2O afforded 6-deoxyhexos-5-ulose-*6-d*, together with D-*gluco*-hexodialdose from the former, and 6-deoxy-5-hexulose-*6-d* with L-gulose from the latter. A scheme for the dephosphorylation process is proposed.

INTRODUCTION

Radiation-induced scission of phosphate ester bonds is an important transformation of biologically significant phosphates[1-3] and is mainly responsible for radiation-induced cleavage of DNA chains[4]. The role of other reactions, *e.g.*, destruction of deoxyribose moieties, is not essential[5]. Thus, a study of the mechanism of radiation-induced dephosphorylation may throw some light on the chemical nature of the radiobiological processes.

Recently, we have demonstrated[3] that dialdoses and deoxyhexosuloses are the main, neutral products of the radiolysis of aqueous solutions of sugar phosphates. We now report on the behaviour of D-glucitol 6-phosphate, which was selected in order to avoid the influence of a hemiacetal group and to simplify the structure of the neutral products..

RESULTS AND DISCUSSION

The $G(H_3PO_4)$ values for 10 and 50mM D-glucitol 6-phosphate were 1.8 and 1.2 for solutions saturated with nitrous oxide and argon, respectively, when measured immediately after irradiation. The irradiation of D-glucose 6-phosphate, under analogous conditions, gave $G(H_3PO_4)$ values of 1.7 and 1.1, respectively. These results indicate that the stability of the phosphate ester bond for alditol phosphates does not depend on the concentration and is almost equal to that of the corresponding sugar phosphate.

Liberation of H_3PO_4 may occur[5] slowly (labile phosphates) immediately after irradiation, and rapidly in the presence of alkali. The yields of the labile phosphates were measured after treatment with M NaOH and found to be 0.4 and 0.6 for solutions saturated with argon and nitrous oxide, respectively.

Carbohydrates undergo an oxidation process during irradiation; for D-glucitol 6-phosphate, the formation of carbonyl compounds, measurable as reducing sugars[7], would be expected. The total yields of these products (as D-glucose) were 1.4 and 0.9 for solutions saturated with nitrous oxide and argon, respectively. In the former reaction, G values of 0.6 and 0.8 were found for neutral and phosphate-containing sugars, respectively, as revealed from electrophoretic data. D-Glucose 6-phosphate (G 0.15) was identified amongst the latter products by reaction with D-glucose 6-phosphate dehydrogenase. Aldophosphates with a shortened carbon chain[8] and/or aldosulose phosphates[2] may also contribute to the total yield of reducing phosphates.

The phosphate-free, neutral products were quantified by the phenol–sulphuric acid method[9] and by formaldehyde determination[10] after periodate oxidation. Neutral products were isolated either by preparative paper electrophoresis or by using ion-exchange resins[11]. For the radiolysis of 50mM D-glucitol 6-phosphate under nitrous oxide, G(neutral product) values of $\sim$0.8 (phenol–sulphuric acid method, based on D-glucose) and $\sim$0.7 (periodate oxidation, D-mannitol as standard) were obtained for each method of isolation. The latter method will not detect compounds lacking $-CHOH-CH_2OH$ or $-CO-CH_2OH$ groups. However, a comparison of the values of G(neutral product) with that (1.8) for $G(H_3PO_4)$ shows that the amount of these products is much lower than it should be if both groups of substances arise by one process. The same difference was obtained on radiolysis[3] of D-glucose 6-phosphate. It is possible that the release of inorganic phosphate leads to products of very low and/or rather high molecular weight, that are not detected by the analytical methods used. Similar observations were made upon investigation of the neutral products of the radiolysis of aqueous solutions of alkyl phosphates[12] and nucleotides[4].

The nitrous oxide-saturated solution of D-glucitol 6-phosphate, after radiolysis, was treated with ion-exchange resins to give the neutral products. P.c. of these products and g.l.c. of the corresponding alditol acetates revealed two main products *A* (50%) and *B* (35%); the amount of glucitol was $\leq$5%. Thus, radiation-induced scission of a phosphate ester bond, analogous to chemical hydrolysis, represents only a small part of the total dephosphorylation process.

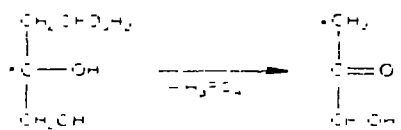
Product *A*, isolated by preparative paper chromatography, was reducing, and the mass spectrum of the acetylated derivative contained peaks (*m/e* 331, 317, 289, 242, 202, 157, 146, *etc.*) characteristic of hexose penta-acetates[13]. The mass spectrum of the corresponding alditol acetate contained peaks (*m/e* 375, 361, 289, 259, 217, 187, *etc.*) characteristic of hexitol hexa-acetates[13]. Reduction of *A* with sodium borodeuteride, followed by acetylation, gave an alditol acetate having one deuterium atom at a terminal position, as revealed by mass spectrometry (see below). Thus, *A* contains one aldehyde group and must be a hexose, and it was identified (p.c., solvents *1*, *2*, and *3*) as gulose. It was also indistinguishable from D-gulose by ion-exchange

chromatography (retention time, 180 min) The $[\alpha]_D^{20}$ value ($+14^{\circ}$, water) is similar to that of L-gulose[14] The acetate of *A* was identical (g.l.c., columns *1* and *2*) with D-gulose penta-acetate. The alditol {$[\alpha]_D$ -2° (*c* 2, water): lit.[14] $[\alpha]_D$ -2° for D-glucitol} derived from *A* and its hexa-acetate were indistinguishable [p.c. (solvents *1* and *3*), g.l.c. (columns *1* and *2*)] from D-glucitol and D-glucitol hexa-acetate, respectively. Therefore, *A* must be L-gulose; no other hexose was detected on radiolysis of D-glucitol 6-phosphate.

The product *B* (R_{GLC} 1.8, solvent *1*), isolated as for *A*, afforded, on reduction with sodium borohydride, two deoxyalditols[15], the acetates of which were identical (g.l.c., column *2*) with those obtained from 6-deoxyhexos-5-ulose by reduction and acetylation. The acetates gave mass spectra containing fragment ions (*m/e* 317, 303, 289, 231, 217, 201, 187, 145, *etc.*) characteristic[13] of 6-deoxyhexitol penta-acetates. Reduction of *B* with sodium borodeuteride and subsequent acetylation gave two products, the mass spectra of which contained several ions (*m/e* 289, 217, 187, 170, 157, and 145) in common with the foregoing penta-acetate, and ions (*m/e* 318, 304, 232, 202, 160, 130, and 88) shifted by one mass-unit. These results proved the presence of one deuterium atom in the position adjacent to the methyl group Hence, *B* is a 6-deoxy-5-hexulose. The similarity of the structure of the main, neutral products formed on radiolysis of D-glucitol 6-phosphate to those from D-glucose 6-phosphate and D-ribose 5-phosphate suggests a similar mechanism for the scission of the phosphate group, and shows that this process does not depend on the presence of the hemiacetal group

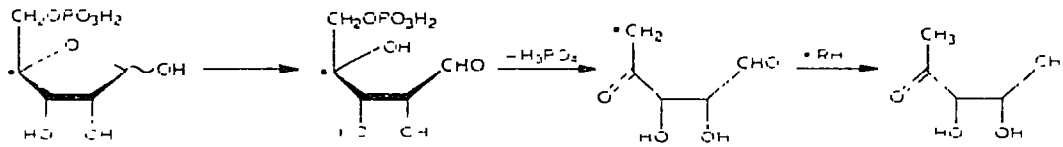
Mechanism of the radiation-induced scission of the sugar–phosphate bond[5,16,17]

Irradiation of glycerol monophosphates affords[16] radicals having the odd electron in α, β, and γ-positions, which can eliminate H_3PO_4: radical **1** is the least stable and dephosphorylation gives **2**. Radicals of type **2** are possible precursors of deoxyketo sugars.



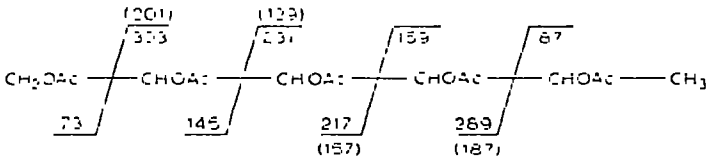
1 **2**

Recently[5], a mechanism of dephosphorylation was proposed (**3** → **6**) for the formation of 5-deoxy-D-*erythro*-pentos-4-ulose from D-ribose 5-phosphate. We have independently observed[3] the formation of this product. However, the relative probabilities for radical **3** to undergo a disproportionation reaction or furanose-ring opening have not been determined and the role of the solvent in the elimination of the phosphate group is not clear. We have reported[3] on the low probability of other schemes for radiation-induced dephosphorylation[18,19].



3 4 5 6

A comparison of the structure of the phosphate-free products obtained on radiolysis of 50mM D-glucose 6-phosphate and D-glucitol 6-phosphate in H_2O and D_2O was made. Radiolysis of D-glucose 6-phosphate in D_2O followed by removal of phosphates gave 6-deoxyhexos-5-ulose-*6-d*. The presence of one deuterium atom at position 6 in this product was demonstrated by reduction (sodium borohydride) followed by acetylation and g.l.c.–m.s. of the product. The mass spectrum contained peaks at *m/e* 318, 304, 232, 202, 160, and 130, shifted by one mass-unit in comparison with those of the corresponding product obtained after radiolysis in H_2O, whereas the fragments without a labelled methyl group (*m/e* 289, 217, 187, 170, 157, 145, *etc.*) were not shifted. The fragment ions are shown in **7** and **8**; secondary fragments obtained by elimination of Ac_2O or AcOH are shown in parentheses next to the corresponding primary ions.



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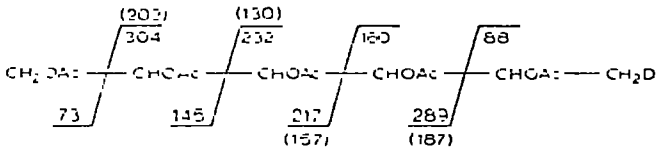

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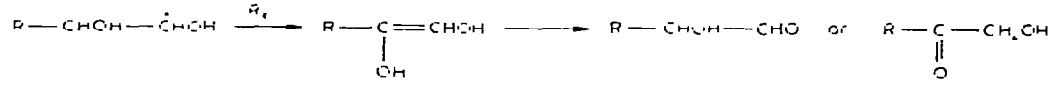
Deuterium was not incorporated into D-*gluco*-hexodialdose, as the mass spectra of the derived D-glucitol hexa-acetates associated with radiolysis in H_2O and D_2O were identical. Likewise, on radiolysis of a solution of D-glucitol 6-phosphate in D_2O, deuterium was incorporated into the resulting 6-deoxy-5-hexulose (at position 6) but not into the L-gulose.

The foregoing data indicate that D-*gluco*-hexodialdose cannot be formed from D-glucose 6-phosphate *via* an enol phosphate as earlier suggested[3], since this should

involve deuterium incorporation ot C-5; deuterium incorporation was not observed. A similar finding was associated with the formation of L-gulose from D-glucitol 6-phosphate.

One possible scheme for oxidation *via* a disproportionation reaction is hydrogen transfer with subsequent formation of a double bond, as follows[20]:

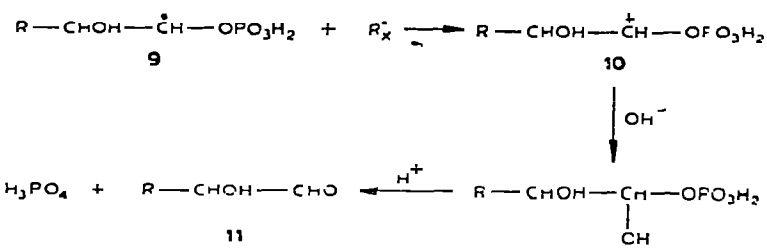


This reaction may also proceed[16] by the electron transfer

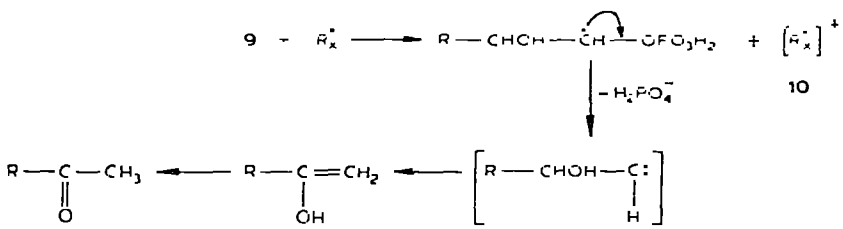
$$R_1^{\bullet} + R_2^{\bullet} \rightarrow A_1^{+} + A_2^{-},$$

where R_1 and R_2 are primary radicals, and A_1^{+} and A_2^{-} the ions. This suggestion is in good agreement with the results of a study of the radiolysis of different phosphates in the presence of radiosensitizers[17]. Thus, $G(H_3PO_4)$ is increased by the electron affinity of the added sensitizers, which facilitate the electron-transfer reaction.

It is possible that the primary radical, with an unpaired electron at C-6, formed from D-glucose 6-phosphate and D-glucitol 6-phosphate undergoes a disproportionation reaction with electron transfer, *e.g.* [for R = -(CHOH)$_3$-CH$_2$OH],



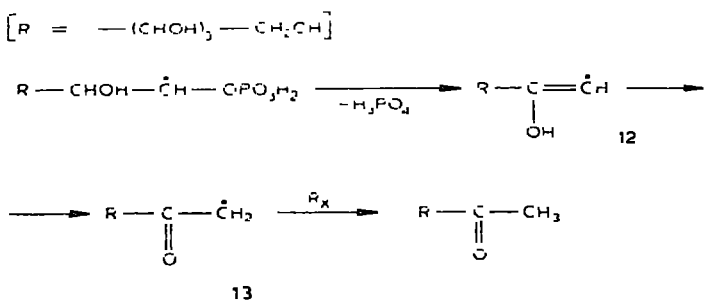
On losing an electron, radical **9** gives the ion **10** which, after addition of OH^- and hydrolysis, leads to the product **11** (*A*). According to this scheme, deuterium incorporation into **11** would not occur. If the radical $R_1^{\bullet}$ is of type **9**, then it might react as follows: [R = -(CHOH)$_3$-CH$_2$OH]



These types of reaction are known in carbene chemistry and would allow the deuterium incorporation into the CH_3 group that is observed experimentally.

Based on the previous paper[2], one might expect the formation of the primary radical with the free valency at C-5. However, this seems unlikely because the formation of a hexose with inverted stereochemistry at C-5 was not observed.

The scheme[3] for the formation of deoxyketo compounds by radiolysis of D-glucose 6-phosphate and ribose 5-phosphate can be rewritten for D-glucitol 6-phosphate as follows:



This scheme would allow deuterium incorporation in the stage **12** → **13**. Both radical and ionic schemes for the formation of deoxycarbonyl compounds explain the incorporation of deuterium, and additional experiments are necessary to distinguish between them.

Recently, Stelter *et al.*[21] proposed a mechanism for the formation of 5-deoxy-D-*erythro*-pentos-4-ulose on radiolysis of D-ribose 5-phosphate, where deuterium incorporation cannot occur. We have observed deuterium incorporation into the 6-deoxyhexos-5-ulose and 6-deoxy-5-hexulose formed on radiolysis of solutions of D-glucose 6-phosphate and D-glucitol 6-phosphate in D_2O, so that a different mechanism must occur with these compounds.

Thus, primary phosphates undergo radiation-induced dephosphorylation and oxidation to aldehydes. L-Gulose is the main, neutral product from D-glucitol 6-phosphate, and 6-deoxy-5-hexulose is also formed as a result of elimination of H_3PO_4. The process which is analogous to chemical hydrolysis does not take place to a significant extent.

EXPERIMENTAL

Sugar phosphates were irradiated in oxygen-free, aqueous solutions (50 and 10mM) saturated with argon or nitrous oxide, using a ^{60}Co γ-source (dose rate, 4.2×10^{16} eV.ml^{-1}.sec^{-1}). A commercial preparation of the disodium salt of D-glucose 6-phosphate, which contained < 1% of inorganic phosphate, was used. D-Glucitol 6-phosphate obtained by reduction of D-glucose 6-phosphate with sodium borohydride, followed by neutralisation of the desalted solution with $NaHCO_3$, was homogeneous on chromatography and electrophoresis, and contained < 1.2% of

inorganic phosphate. The heavy water contained 80% of D_2O and HOD, as revealed by mass-spectral data.

Paper chromatography and electrophoresis were carried out on Whatman No 2 and 3 papers, using (*1*) 1-butanol–pyridine–water (6:4:3), (*2*) ethanol–water (95.5), and (*3*) butanone–acetic acid–saturated aqueous boric acid (9:1:1), and a pyridine acetate buffer (pH 4.5). G.l.c. was performed on Varian Aerograph series 1700 and Pye Unicam 104 instruments with 2-m columns of (*1*) 3% of SE-30 on Chromosorb W and (*2*) 3% of ECNSS-M on Gas Chrom Q. Mass spectra were measured with a CH-6 Varian MAT instrument at 70 eV. G.l.c.–m.s. was carried out on Varian MAT-III GC/MS (GNOM) and LKB model 900 instruments, with column *1*.

Reducing sugars were determined by the Park–Johnson method[7], and inorganic phosphate by the Marsch method[22]. The alkaline treatment and the isolation of the products of radiolysis was carried out as previously described[6]. For identification of *4*, a liquid chromatograph type 71-100A (CSSR) was used with DA X-4 resins and 0.5M borate buffer (pH 8.5). D-Glucose 6-phosphate was determined by the dehydrogenase (Calbiochem) procedure[23]; the irradiated solutions do not inhibit the enzyme action. Reduction and acetylation of neutral sugars were effected by the usual procedures[11].

D-Gulose was obtained by the modified procedure[24]: to a solution of D-gulono-1,4-lactone (100 mg) in pyridine–water (1:1, 2 ml), a solution of sodium borohydride (100 mg) in 1 ml of this mixture was added with cooling. The solution was stirred at 0° for 2 h and at ambient temperature for 2 h, then deionized, and concentrated with methanol. Final purification was carried out by preparative p.c. (solvent *1*) to give chromatographically pure D-gulose (28 mg), $[\alpha]_D^{20} -12°$ (*c* 1.2, water)[14].

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