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RADIOLYSIS OF AQUEOUS SOLUTIONS OF SUGAR PHOSPHATES*

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ABSTRACT

The mechanism of the radiation-induced dephosphorylation reaction was investigated by studying the γ-radiolysis of 10mM solutions of D-glucopyranosyl phosphate, D-glucose 6-phosphate (Glc-6-P), and D-ribose 5-phosphate (Rib-5-P). Dephosphorylation occurred with OH-radical participation, since $G(H_3PO_4)$ values for nitrous oxide-saturated solutions were 4.1, 1.7, and 2.3, and for nitrogen-saturated solutions 2.6, 1.1, and 1.6, respectively. The formation of phosphate-free compounds accompanied the release of inorganic phosphate. The main, neutral products of the radiolysis of Glc-6-P were 6-deoxyhexos-5-ulose ($G = 0.2$) and D-*gluco*-hexodialdose ($G = 0.3$). Irradiation of Rib-5-P gave *ribo*-pentodialdose and 5-deoxypentos-4-ulose as the main, neutral products. A scheme for the dephosphorylation process is proposed.

INTRODUCTION

Radiation-induced cleavage of phosphate ester is one of the most important transformations of biologically significant phosphates. For example, this process is mainly responsible for the radiation-induced rupture of DNA and RNA chains. The phenomenon has been studied by using alkyl phosphates[1,2] and nucleotides[3,4] as model compounds, attention being drawn to the liberation of inorganic phosphate and the formation of "labile" phosphate. In the present study of the radiation-induced formation of inorganic phosphate from sugar phosphates, we have investigated the fate of the carbohydrate moieties.

Dephosphorylation of the dipotassium salt of D-glucopyranosyl phosphate (Glc-1-P) and the disodium salts of D-glucose 6-phosphate (Glc-6-P) and D-ribose 5-phosphate (Rib-5-P) proceeded to different extents upon radiation. The $G(H_3PO_4)$ value for nitrogen-saturated solutions was found to be 2.6 molecules/100 eV for Glc-1-P, 1.1 for Glc-6-P, and 1.6 for Rib-5-P. The yields increased to 4.1, 1.7, and 2.3, respectively, when the irradiation was carried out in nitrous oxide-saturated solutions, thus indicating the participation of the OH radical in the dephosphorylation process.

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*Dedicated to Dr. Horace S. Isbell, in honour of his 75th birthday.

RESULTS AND DISCUSSION

Radiolysis of Glc-6-P. The irradiated solution was treated with ion-exchange resins to give the neutral, radiolysis products. Paper chromatography of this mixture and g.l.c. of the corresponding alditol acetates revealed the presence of two main products (Table I) and a small amount of glucose; three minor components were also found but their structures were not studied owing to the small amounts available. The yield of D-glucose was not more than 0.05, as revealed by g.l.c. of the acetylated, neutral fraction and by determination with D-glucose oxidase[5]. Thus, the radiation-induced scission of a phosphate-ester bond, which is analogous to a chemical hydrolysis, represents only a small part of the total dephosphorylation process.

TABLE I

G.L.C. DATA (COLUMN *1*) FOR THE COMPOSITION OF ALDITOL ACETATE MIXTURES OBTAINED FROM THE NEUTRAL PRODUCTS OF THE IRRADIATION OF GLC-6-P AND RIB-5-P IN NITROUS OXIDE-SATURATED SOLUTIONS

	Glc-6-P					*Rib-5-P*			
G.l.c. conditions	180 → 240°, 4°/min					160 → 220°, 4°/min			
Alditol acetates	*1*[a]	B_1-H	*2*[a]	*3*[a]	A_1-H	*1*[a]	*C*-H	*2*[a]	*D*-H
Retention times (min.)	6	8	10	11	13	3	7	10	13
Peak areas (%)	7	66	12	22	100	6	49	16	100

[a] *1*, *2*, and *3* are the minor products of radiolysis; for other symbols, see text.

It has been reported[6] that the main dephosphorylation product (*A*), which was isolated by preparative paper chromatography (R_{GLC} 1.8, solvent *1*), was the reducing sugar without deoxy units[7] or a lactone group[8]. Reduction of *A* with sodium borohydride gave A_1-H which was indistinguishable from D-glucitol by paper chromatography (solvents *1* and *2*), and its acetate was different (g.l.c., columns *2* and *3*) from those of D-mannitol and galactitol but identical with D-glucitol hexa-acetate. The mass spectrum of the acetate of A_1-H contained peaks for fragment ions (*m/e* 375, 361, 289, 217, *etc.*) characteristic of hexitol hexa-acetates[9].

The number and position of the reducing groups in *A* could be determined after reduction (to A_1-D) with sodium borodeuteride and subsequent acetylation. The mass spectrum of the acetate revealed the presence of two deuterium atoms, as indicated by the ion with *m/e* 377 (M − 59) and those of the secondary fragments of the VI series[9] (*m/e* 316, 274, *etc.*; Table II). As there were also ions with *m/e* 362, 290, and 218, and the fragments of the series V, IV, and III, the symmetrical 1,6-positioning of the deuterium atoms in A_1-D is indicated. Thus, *A* has two aldehydo groups and must be D-*gluco*-hexodialdose.

TABLE II

MASS-SPECTRAL DATA FOR ALDITOL ACETATE DERIVATIVES OF THE RADIOLYSIS PRODUCTS OF GLC-6-P AND RIB-5-P

m/e	*Intensity* (%) *Glc-6-P*				m/e	*Intensity* (%) *Rib-5-P*		m/e	*Intensity* (%) *Rib-5-P*	
	A$_1$-*H*	A$_1$-*D*	B$_1$-*H*	B$_1$-*D*		C-*H*	C-*D*		D-*H*	D-*D*
377	—	5	—	—	305	—	2	247	—	2
375	3	—	—	—	303	2	—	245	2	—
362	—	29	—	—	290	—	2	232	—	5
361	23	—	—	—	289	2	0.2	231	4	—
319	—	—	—	5	218	—	3	218	—	10
317	—	—	4	—	217	25	—	217	10	2
316	—	2	—	—	202	—	8	176	—	10
314	6	—	—	—	200	8	—	175	8	—
304	—	—	—	9	188	—	19	160	—	25
303	—	—	11	—	187	20	3	159	16	60
290	15	67	—	20	176	—	13	158	50	15
289	61	9	26	—	175	11	3	146	—	45
274	—	14	—	—	159	—	22	145	50	60
272	11	—	—	—	158	21	9	130	7	50
260	11	50	—	—	146	—	65	129	30	12
259	49	6	—	—	145	71	22	117	20	60
258	—	—	—	9	140	10	8	116	50	100
256	—	—	18	—	128	11	28	115	100	8
232	—	—	—	40	127	23	3	104	—	45
231	—	—	50	29	116	32	100	103	80	70
218	22	86	—	46	115	100	26	101	—	40
217	85	15	68	8	104	—	38	100	30	10
202	—	—	—	16	103	70	55	88	—	35
201	—	—	26	—	98	18	—	87	15	17
188	23	95	—	28						
187	90	16	44	4						
171	25	100	—	100						
170	100	19	100	25						

The second, main, neutral product (*B*) had higher chromatographic mobility (R_{GLC} ~2.8, solvent *1*) and afforded, after reduction with sodium borohydride, two alditols, each of which gave a positive reaction for deoxy sugars[7]. The alditols were separated after acetylation by preparative g.l.c. (column *2*). One of the products (from B_1-H) was identical with 6-deoxy-D-glucitol penta-acetate (**1**), and both products gave mass spectra which were identical with that of **1** (fragments *m/e* 317, 303, 289, 231, 217, *etc.*); the products therefore are epimers.

After reduction of *B* with sodium borodeuteride (to B_1-D and B_2-D) and acetylation, two compounds were isolated by preparative g.l.c., and these gave identical mass spectra. The presence of a peak (Table II) at *m/e* 319 (M−59) and other fragments of this series (*e.g.*, *m/e* 258), which are 2 mass units larger than those from the undeuterated acetate, indicated the presence of two deuterium atoms, while the main fragments at *m/e* 304, 290, 232, 218, *etc.* proved their location at positions 1 and

5. Hence *B* is a 6-deoxyhexos-5-ulose (the D-*xylo* configuration is suggested but not established).

The quantitation of *A* and *B* was effected[6] by g.l.c. (column *3*), using D-galactose and galactitol acetate as internal standards. The yields were 0.3 and 0.2, respectively, for nitrous oxide-saturated solutions. Taking into account the total yield of neutral sugars[5] (1.1 based on D-glucose as standard by the phenol–sulphuric acid method), the compounds detected represent ~50% of the phosphate-free sugars obtained on radiation.

Radiolysis of Rib-5-P. The isolation of neutral, radiolysis products of Rib-5-P was carried out by using ion-exchange resins, as for Glc-6-P. The composition of the mixture of neutral products isolated by preparative electrophoresis was the same as that (Table I) determined by g.l.c. of the corresponding alditol acetates. Hence, the possibility is excluded of the specific, irreversible absorption of some of the products by the ion-exchange resins. G.l.c.(column *1*)–m.s. analysis of the mixture of alditol acetates allowed structures for the main components (*C* and *D*) to be proposed. The retention times (g.l.c., columns *2* and *3*) and mass spectrum of the pentitol acetate derived from *C* were identical with those of ribitol penta-acetate. The characteristic shift of the peaks in the mass spectrum of the deuterated pentitol acetate, obtained by reduction of *C* with sodium borodeuteride followed by acetylation, indicated the presence of two deuterium atoms (*m/e* 305 and the fragments of the C-5 series, *m/e* 202, shifted by 2 mass units in comparison with the undeuterated sample). The occurrence of peaks at *m/e* 290, 218, 188, 146, 116, *etc.*, shifted by 1 mass unit, indicated symmetrical deuteration, *i.e.*, in positions 1 and 5. Therefore, *C* is *ribo*-pentodialdose. The ribose present in the radiolysis products of Rib-5-P was derived by ≯20% from the dialdose, as determined by comparison of the intensity of the peaks at *m/e* 289 and 290 in the mass spectrum of the deuterated pentitol acetate.

The structure of *D* was also elucidated by comparison of the mass spectra of the deuterated and undeuterated pentitol acetates (Table II). The presence of peaks at *m/e* 245, 231, 217, 145, *etc.* indicated a molecular weight 304 and a terminal deoxy group. The peak at *m/e* 247 (M−59), shifted by 2 mass units for the deuterated pentitol acetate, indicated the presence of two deuterium atoms, and the fragments with *m/e* 232, 218, 146, *etc.* suggested that the deuterium atoms were located in positions 1 and 4. Therefore, *D* appears to be a 5-deoxypentos-4-ulose.

Mechanism of the radiation-induced scission of the sugar phosphate bond. The similarity of the structure of the main, neutral products formed on radiolysis of Glc-6-P and Rib-5-P suggests a similar mechanism for the scission of the phosphate group.

Wilkinson and Williams[1], who studied the radiolysis of aqueous solutions of alkyl phosphates, suggested the reactions *1* and *2* (R = alkyl) for the oxidation of the carbon atom bearing the phosphate group to the corresponding aldehyde.

$$\text{R–CH}_2\text{OPO}_3^{2-} + \text{OH} \longrightarrow \text{R–}\dot{\text{C}}\text{HOPO}_3^{2-} + \text{H}_2\text{O} \qquad (1)$$

$$\text{R–}\dot{\text{C}}\text{HOPO}_3^{2-} + \text{OH} \longrightarrow \text{R–CH(OH)OPO}_3^{2-} \xrightarrow{\text{H}^+} \text{R–CHO} + \text{HPO}_4^{2-} \qquad (2)$$

The comparison of $G(H_3PO_4)$ after irradiation of nitrogen- and nitrous oxide-saturated solutions of sugar phosphate indicated the role of the OH radical in the dephosphorylation process. Hence, reaction *1* probably occurs with Glc-6-P and Rib-5-P, but reaction *2* is unlikely on the basis of kinetics, since the rate constant of the reaction of Glc-6-P with the OH radical is rather high[10], namely $k \sim 7 \times 10^8$ l.mole^{-1}.sec^{-1}.

von Sonntag *et al.*[2], who studied the radiolysis of aqueous solutions of methyl phosphates, showed that reaction *2* can be disregarded. It was suggested that reaction *1* was followed by a hydrolytic step (reaction *3*), since the yield of acid products increased with increasing pH.

$$\cdot CH_2O(CH_3O)_2PO + H_2O \longrightarrow \cdot CH_2OH + (CH_3O)_2PO_2^- + H^+ \qquad (3)$$

For the sugar monophosphates, reaction *3* can be rewritten as reaction *4* (R = sugar residue).

$$R\text{-}\dot{C}HOPO_3^{2-} + H_2O \longrightarrow R\text{-}\dot{C}HOH + PO_4^{3-} + H^+ \qquad (4)$$

We have found that $G(H_3PO_4)$ did not increase with increase in pH for radiolysis of the nitrous oxide-saturated solutions of sugar phosphate (Table III). Our results accord with the data[11] on maximum $G(H_3PO_4)$ values at pH 8 for radiolysis of alkyl phosphates. Hence, it is improbable that reaction *4* can be used to account for dephosphorylations of sugar phosphates.

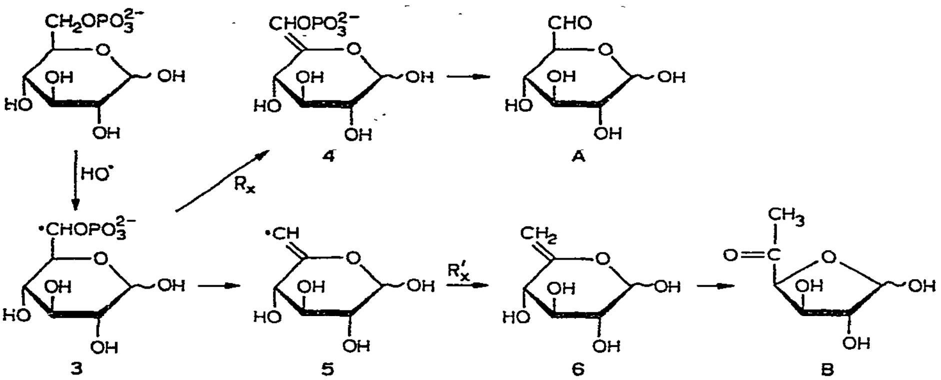
TABLE III

YIELDS OF INORGANIC PHOSPHATE, $G(H_3PO_4)$, FOR SUGAR PHOSPHATES IRRADIATED AT DIFFERENT pH VALUES (DOSE 6×10^{19} eV.ml^{-1})

	Glc-6-P			*Rib-5-P*	
pH	11	7	3	11	7
$G(H_3PO_4)$	1.7	1.7	1.4	1.9	2.2

On the basis of the structures of the main, neutral products of radiolysis, the following scheme for the radiation-induced dephosphorylation is suggested, using Glc-6-P as an example, R_x is the oxidizing radical, and R'_x the reducing radical.

Attack by OH radical on Glc-6-P leads to the radical **3** with unpaired-electron localisation, probably at C-6. The location of the odd electron at carbon instead of at phosphorus was proved by pulse-radiolysis data[10]. The suggestion that the position of the free valency is at the carbon atom bearing the phosphate group is based on data for the radiolysis of alkyl phosphates[1,2] and nucleotides[3,12], and also on the structure of the final products of radiolysis of sugar phosphates. The position of the free valency at C-5 cannot be excluded. The primary radical **3** appears to undergo two transformations: (*1*) disproportionation to give the enol phosphate **4**, which is hydrolysed to D-*gluco*-hexodialdose (*A*) (*cf.* ref. 13); (*2*) elimination of the phosphoric



acid residue to give the unsaturated radical **5**. Disproportionation of **5** affords the unsaturated sugar **6**, which is transformed into 6-deoxyhexos-5-ulose (*B*), or, possibly, into some other minor components. The expected increase in the yield of *B* (g.l.c. data) on irradiation of Glc-6-P in alkaline medium is in favour of the transformation **3**→**5**, since elimination of HPO_4^{2-} should be facilitated under such conditions.

An analogous scheme can be suggested for the dephosphorylation of Rib-5-P.

Although the results presented herein are preliminary and the need for additional substantiation is recognised, the proposed mechanism adequatelv explains the available data.

EXPERIMENTAL

The sugar phosphates were irradiated in oxygen-free, and nitrogen- and nitrous oxide-saturated, aqueous solutions (10mM) by a ^{60}Co γ-source (dose rate, 5×10^{16} eV. $ml^{-1}.sec^{-1}$). The pH of solutions was adjusted as appropriate with NaOH or H_2SO_4. The commercial preparations of the dipotassium salt of Glc-1-P and disodium salt of Glc-6-P were recrystallized from aqueous ethanol and then contained less than 0.2 and 1%, respectively, of inorganic phosphate. The disodium salt of Rib-5-P (1.5% of inorganic phosphate) was obtained after treatment with Amberlite IR-120(H$^+$) resin followed by neutralisation with sodium hydrogen carbonate.

Paper chromatography and electrophoresis were carried out on Whatman No. 2 and 3 papers, using (*1*) 1-butanol–pyridine–water (6:4:3) and (*2*) ethanol–water (95:5), and a pyridine acetate buffer (pH 4.5). G.l.c. was performed on a Varian Aerograph series 1700 instrument with 1.5-m columns of (*1*) 3% SE-30 and (*2*) 3% OV-225 on Chromosorb W, and (*3*) 3% ECNSS-M on GasChrom Q. Mass spectra were measured with a CH-6 Varian MAT instrument at 70 eV, with an ion-source temperature of 180–190° and the inlet system at 120°. G.l.c.–m.s. was carried out on a Varian MAT-III GC/MS (GNOM) instrument, using column *1*.

The isolation of the products of radiolysis of Glc-6-P and Rib-5-P was carried out by the successive treatment of the irradiated solutions with Dowex-1 x8(HCO_3^-) and Amberlite IR-120(H$^+$) resins. Inorganic phosphate was determined in the presence of labile phosphates by the Marsch method[14]. This determination, when performed for solutions at pH 3 and 11, involved neutralisation and a blank treated in a similar fashion. Reduction and acetylation of neutral sugars were effected by the usual procedures[6].

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