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Homolytic Cleavage of the O-glycoside Bond in Carbohydrates: A Steady-state Radiolysis Study

I. P. EDIMECHEVA[1], R. M. KISEL[1], O. I. SHADYRO[1]*, K. KAZEM[1], H. MURASE[2] and T. KAGIYA[3]

Carbohydrates/Radiolysis/O-glycoside bond/Cleavage.

The formation of products resulting from the O-glycoside bond cleavage following radiolysis of aqueous solutions of methyl-α-D-glucopyranoside (I), 3-O-methyl-α-D-glucopyranose (II), maltose, lactose, gentiobiose and cellobiose were studied. Radiation-induced destruction yields were also determined for dextran, laminarin and trimethylcelulose upon irradiation of their aqueous solutions. Oxygen, quinones and compounds capable of forming quinoid structures were found to inhibit radiation-induced homolytic destruction processes taking place in glycosides, di- and polysaccharides. The data obtained in this study enabled the authors to demonstrate an important role played by the fragmentation reaction of C-2 radicals which were generated from the starting substances in the formation of final radiolysis products.

INTRODUCTION

There are many factors determining the interest towards the action of ionizing radiation on carbohydrates. Among these, the need for carbohydrate radiolysis data should be noted, which are necessary for solving some applied tasks associated e.g. with the use of radiation for sterilization and preservation of carbohydrate-containing foodstuffs and drug products.[1)] Along with this, the radiobiological trend in carbohydrate studies is not in the least of minor importance, in view of wide prevalence and biological significance of these substances.[2)] This is why a large number of studies have been performed with a view to investigate, under various conditions, the radiolysis of monosugars and their derivatives, as well as di- and polysaccharides. The data obtained in these studies are summarized in monographs[2,3)] and reviews.[4,5)]

The facts established in the course of these studies show that the O-glycoside bond cleavage is one of the main processes occurring on irradiation of glycosides, di- and polysaccharides.[3,6,7)] This process results in formation of products with lower molecular masses as compared with those of the starting compounds, and this causes significant changes in physicochemical properties of the systems being irradiated. Furthermore, the radiation-induced destruction of carbohydrates and carbohydrate-containing compounds leads to formation of substances having mutagenic properties[4)] and, as shown in our recent study,[8)] signal molecules playing a key role in apoptosis.

The investigations carried out on radiation–chemical[2–4)] and homolytic[9–11)] transformations of di- and polysaccharides made it possible to formulate conclusions about regularities observed in the course of reactions leading to rupture of the O-glycoside bond in carbohydrates. At the same time, a number of experimental facts established in carbohydrate radiolysis studies remain yet unexplained. Among those are such important issues as: 1) why is the radiation-induced destruction of polysaccharides, unlike other biopolymers, effectively inhibited by oxygen,[2)] and 2) which are the substances that are able to influence this process?

This study has been performed with a view to obtain information that would allow reasonable answers to these questions to be found.

MATERIALS AND METHODS

Methyl-α-D-glucopyranoside (I), 3-O-methyl-α-D-glucopyranose (II), maltose, lactose, cellobiose, gentiobiose, coenzyme Q_0 and 1,4-benzoquinone used in this study were purchased from *Aldrich* (Germany). Dextrans of various molecular masses, as well as laminarin,were obtained from *Sigma* (Germany). The water-soluble Vitamin E-analogue 2-(α-D-glucopyranosyl)-6-hydroxy-2,5,7,8-tetramethylchroman (TMG) was provided by *CCI Company* (Japan). Trim-

*Corresponding author: Phone: +375(17)206-6146,
Fax: +375(17)209-5464,
E-mail: shadyro@open.by
[1]Department of Chemistry, Belarusian State University, 220050 Minsk, Belarus; [2]CCI Corporation, Gifu, Japan; [3]Health Research Foundation, Kinki Invention Center, 14 Yoshida Kawahara-cho, 606-8305 Sakyo-ku, Kyoto, Japan.

ethylcellulose was prepared from cellulose as described in.[12] 2,3,5-Trimethyl-1,4-benzoquinone was prepared by oxidation of 2,3,5-trimethylhydroquinone according to.[13] The synthesized compounds were purified and tested for compliance with the standard characteristics.

Sample preparation and irradiation

The starting aqueous solutions were prepared using doubly-distilled water by dissolving appropriate amounts of the respective carbohydrates (10^{-1} M), accurately weighed. Before irradiation, the ampoules containing the carbohydrate solutions were bubbled through with argon gas for 1 h, or saturated with O_2 or N_2O. Solutions containing quinones and TMG (10^{-3} M) were prepared by dissolving the appropriate amounts of these substances, accurately weighed, in the starting solutions of the substances under study, and deaerated with Ar prior to irradiation. pH values of the starting solutions were about 6.5. The ampoules were sealed and irradiated in a γ-unit with a ^{137}Cs source. The radiation dose rate was 0.33 Gy/s. Doses absorbed were varied in the range between 0 and 0.5 kGy in the case of polysaccharides, and between 0 and 7 kGy in the case of glycoside or disaccharide solutions.

Analysis of radiolysis products

Methanol formed on radiolysis of aqueous solutions of methylglycosides was determined by GLC using a GC-17 "Shimadzu" (Japan) chromatograph equipped with a silica capillary column RTX/RESTEK 9l = 30 m, 0.32 mm ID, 0.5 μm df). Chromatographic conditions: carrier gas: nitrogen; flow rate: 1.5 ml/min; starting temperature: 40°C; injector temperature: 220°C; detector: FID; detector temperature: 240°C.

Prior to performing determinations of glucose and galactose formed on radiolysis of aqueous solutions of lactose, the irradiated solutions were evaporated to obtain a 5 x the initial concentration.

The analyses of glucose and galactose were made by HPLC method using a LC-10 (Shimadzu) chromatograph equipped with a column EC 250/4 Nucleosil Carbohydrate (Maherey-Nagel), 250/4 mm. Chromatographic conditions: temperature: 40°C; eluent: acetonitrile/water 86/14 v/v; flow rate 1 ml/min; detector: RID.

Concentration of glucose formed on maltose radiolysis was determined using the same method, except for the component ratio of the eluent, which was 80/20.

Molecular mass distribution in aqueous solutions of dextrans and laminarin was studied using the gel penetration chromatography method. The separation was achieved on a column with dimensions 0.5 × 50 cm packed with TOYOPERL HW-55 gel (Japan). Eluent was 0.15 N NaCl solution containing 0.05% of sodium azide; the eluent flow rate was 8 ml/h. Registration was carried out with a pulsed refractometer RI-03. The column calibration was performed using standard dextran solutions of T series (T-10, T-20, T-40 and T-70) (Farmacia LKB, Sweden) with known weight-average (M_w) and number-average (M_n) molecular mass values.

RESULTS

Radiolysis of aqueous methylglycoside solutions

The main radiolysis product found in aqueous solutions of substances (I) and (II) is methanol formed as a result of the O-glycoside bond rupture. The graphs reflecting dependencies of methanol accumulation on dose absorbed were linear within the dose range studied (see Fig.). From these relationships, radiation-chemical yields of CH_3OH were calculated. As seen from the obtained data, the CH_3OH yields depend on structures of the starting glycosides and on the presence of additives (see Table 1).

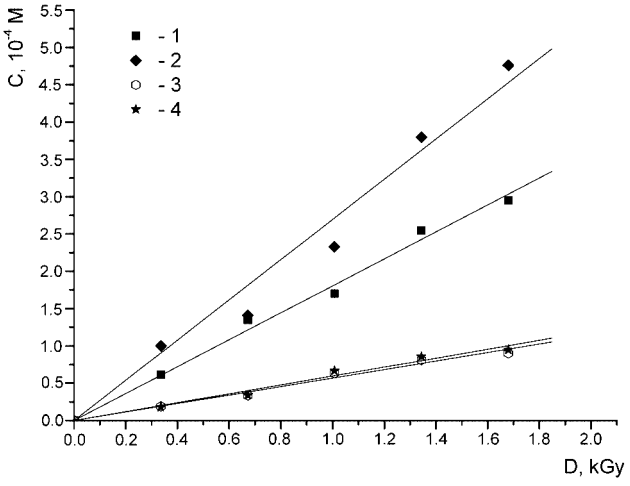
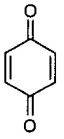
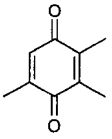
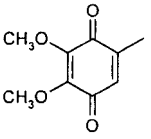
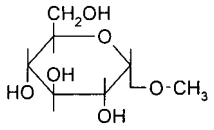
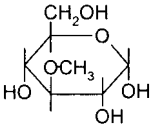


Fig. Relationships between accumulation of CH_3OH and the dose absorbed on radiolysis of aqueous 0.1 M methyl-α-D-glucopyranoside solutions in the presence of: 1) N_2O; 2) argon; 3) oxygen; 4) 1,4-benzoquinone (10^{-3} M).

In the case of aqueous solutions of substance (I), higher methanol yields were obtained as compared to those for aqueous solutions of substance (II). Saturation of the starting solutions of (I) and (II) with N_2O increased the yields of methanol on irradiation. Oxygen and quinones totally inhibited the formation of CH_3OH on irradiation of compound (II) solutions, and significantly, in the case of solutions of compound (I). TMG was found to inhibit the formation of methanol more effectively on radiolysis of compound (II), as compared to compound (I). Among radiolysis products of methylglycosides, we have also identified glucose. Its yields in cases of deaerated solutions of (I) and (II) were 0.16 and $0.04 \cdot 10^7$ mol/J, respectively.

Table 1. Radiation-chemical yields (G) of methanol formation on radiolysis of 0.1 M aqueous solutions of methylglycosides under various conditions.

Starting systems	G·10^7, mol/J						
	Ar	N_2O	O_2				TMG
Methyl-α-D-glucopyranoside 	1.72 ± 0.17	2.60 ± 0.03	0.55 ± 0.06	0.58 ± 0.05	0.59 ± 0.07	0.69 ± 0.09	1.26 ± 0.13
3-O-Methyl-α-D-glucopyranose 	0.62 ± 0.06	1.10 ± 0.01	0.06 ± 0.006	0.01 ± 0.005	0.01 ± 0.005	–	0.03 ± 0.01

± – Standard error (SE)

Radiolysis of aqueous disaccharide solutions

The main product of the O-glycoside bond cleavage during radiolysis of aqueous 0.1 M solutions of maltose, lactose and cellobiose is glucose. In the case of lactose, galactose is also formed. The yield values for these products are given in Table 2.

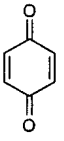
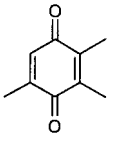
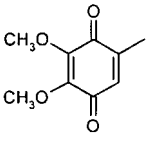
It follows from the obtained data that the type of glycoside bond has no influence on yield of the main product of its cleavage. During radiolysis of lactose, glucose is formed with greater probability than galactose. Both glucose and galactose yields increase on addition of N_2O into the starting solutions, and are suppressed by such oxidants as oxygen and benzoquinones.

Radiolysis of aqueous solutions of maltose in the presence of TMG is characterized by glucose yield values reduced by about 30%.

Radiolysis of aqueous polysaccharide solutions

To obtain data on radiation-induced destruction of polysaccharides in aqueous solutions, effects of dose absorbed on changes observed in the degree of polymerization and molecular mass distribution of the starting solutions of dextran, laminarin and trimethylcellulose (TMC) after irradiation were studied. From these data, destruction yields (G) under various irradiation conditions of the polysaccharides were calculated.

Table 2. Radiation-chemical yields (G) of glucose and galactose formation on radiolysis of 0.1 M aqueous solutions of a number of disaccharides in the presence of various additives.

Starting systems	Products	G·10^7, mol/J						
		Ar	N_2O	O_2				TMG
Maltose	glucose	1.10 ± 0.10	1.80 ± 0.10	0.41 ± 0.05	0.21 ± 0.07	0.40 ± 0.09	0.11 ± 0.06	0.80 ± 0.09
Lactose	glucose	1.06 ± 0.12	1.40 ± 0.10	0.31 ± 0.01	0.10 ± 0.03	0.13 ± 0.03	–	–
	galactose	0.25 ± 0.03	0.39 ± 0.08	0.13 ± 0.02	0.05 ± 0.03	0.05 ± 0.02	–	–
Cellobiose	glucose	1.14 ± 0.15	1.85 ± 0.20	0.42 ± 0.04	–	–	–	–
Gentiobiose	glucose	1.16 ± 0.12	1.90 ± 0.19	0.44 ± 0.04	–	–	–	-

± – Standard error (SE)

$$G = \frac{(1/Mn - 1/Mn_0)C \cdot A \cdot 10^2}{D}$$

where Mn and Mn_o are average molecular masses of the polysaccharides before and after irradiation, respectively; A = the Avogadro's number; D = dose absorbed; C = polysaccharide concentration (g/l).

The values for polysaccharide destruction yields obtained in this study are given in Table 3.

Table 3. Radiation-chemical destruction yields (G) for various polysaccharides on irradiation of the respective 0.6% aqueous solutions.

Starting systems	Gs, ruptures/100eV		
	Ar	N_2O	O_2
Laminarin M = 5600	2.20 ± 0.20	2.80 ± 0.30	0.90 ± 0.12
Dextran M = 35000	1.00 ± 0.06	1.91 ± 0.20	0.60 ± 0.05
M = 115000	1.10 ± 0.08	1.60 ± 0.20	0.40 ± 0.20
TMC	0.22 ± 0.02	0.50 ± 0.04	0.22 ± 0.02

± – Standard error (SE)

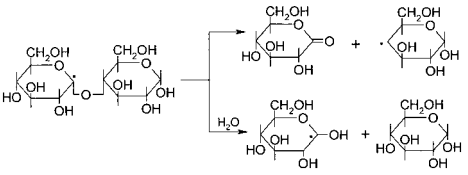
In radiolysis of dextrans, molecular mass of the starting substance was found to have no effect on destruction yields, which increased in the presence of N_2O and decreased in the presence of oxygen. Comparison of results obtained in radiolysis studies of aqueous polysaccharide solutions shows that a complete etherification of polysaccharide macromolecules makes them more resistant towards radiation-induced destruction, with oxygen having no effect on its realization probability. It should be noted that we have observed a similar tendency[7,14] when studying radiolysis of solid samples of cellulose and its derivatives.

DISCUSSION

The obtained results show that the process of destruction with the O-glycoside bond rupture is the main one observed in radiolysis of glycosides, di- and polysaccharides. A notable increase in destruction yield values in the presence of N_2O (Tables 1, 2 and 3) indicates that the reaction of $e^-_{aq.}$ yielding $^{\bullet}OH$ radicals:

$$e^-_{aq} + N_2O \rightarrow N_2 + OH^- + {}^{\bullet}OH$$

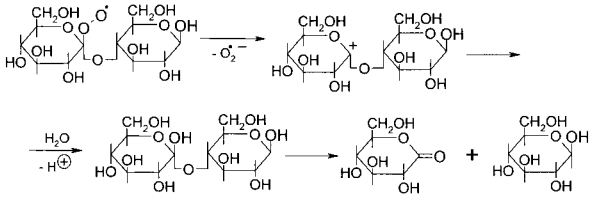
intensifies the process of O-glycoside bond cleavage. This fact demonstrates the importance of the role played by $^{\bullet}OH$ radicals as destruction initiators. Interaction of $^{\bullet}OH$ radicals, which are highly reactive species, with carbohydrates gives radicals of various types.[2,9,10] According to data reported in a review,[4] the O-glycoside bond cleavage on radiolysis of carbohydrates occurs mainly due to β-scission and hydrolysis of radicals with an unpaired electron at C-1, formed from the starting compound. For disaccharides, according to,[4,6] this will be described by the following scheme:



(1)

(2)

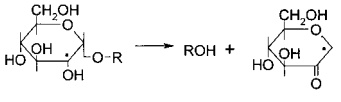
Taking into account the reactions (1) and (2) only, it is difficult to explain the facts established in our study. Thus, methanol and glucose yields in radiolysis of methylglycosides and disaccharides attain the values amounting to about 70% of G_{OH} = 2.7. Based on this fact and bearing in mind that the glycoside bond cleavage is not the sole process that can be initiated by $^{\bullet}OH$ species on radiolysis of methylglycosides and disaccharides,[2–4] one can assume that the probability of its realization is high. However, a reaction of type (1) involving glycoside bond rupture does not lead to formation of methanol and glucose in a straightforward way; on the other hand, such high yields of the named products cannot be explained by realization of a type (2) reaction. Considering reactions of types (1) and (2) only, one can hardly explain the inhibitory effects of oxygen of formation yields of methanol and glucose, as well as on destruction of polysaccharides, since it has been shown that reaction of oxygen with C-1 radicals of carbohydrates will not lead to suppression of destruction, because, according to,[4] the following reaction then takes place:



(3)

Other schemes of radiation-induced cleavage of the glycoside bond in carbohydrates were proposed, which involved a pyranose ring opening step, but realization probability of such reactions is not high.[2,3,6]

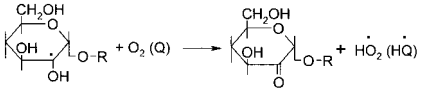
The concept developed in our studies[15–19] is based on the assumption that fragmentation reactions involving rupture of two β-bonds in radicals formed from the starting molecules can play a key role in radiation-induced damage to biologically important molecules. On irradiation of aqueous solutions of carbohydrates, this will be the following reaction:



(4)

Taking α-diols[20] and glycerides[21] as examples, the decomposition of radicals involving rupture of two bonds was shown to proceed via a cyclic transition state. Replacement of a hydroxyl group by a methoxy group at the carbon atom carrying an unpaired electron does not favour the necessary cyclic transition state, and this leads to a drastic fall in probability of decomposition for the corresponding substrates.[20,21] We observed this phenomenon in the present study. Thus, low yields of destruction of TMC macromolecules are due to the absence of free hydroxyl groups in their structure, which makes fragmentation reaction of type (4) impossible to proceed. This fact may serve as a confirmation of a substantial contribution of fragmentation reaction (4) into the total polysaccharide destruction process.

Oxidants such as oxygen[20] and quinones,[22] as well as phenolic antioxidants (TMG)[23] capable of forming quinoid structures, inhibit the fragmentation process (4) due to oxidation of α-hydroxyalkyl radicals of the starting substances. Hence, the inhibitory effects of oxygen and quinones on destruction of carbohydrates may be explained by realization of the following process:



(5)

The absence of hydroxyl groups in TMC molecules precludes the possibility of their destruction by reaction (4), and consequently oxygen has no effect on the values of TMC destruction yields (Table 3).

Data obtained in studies[9,10] using the ESR method and concerning homolytic transformations of carbohydrates in aqueous acid and alkaline solutions upon the action of hydrogen peroxide in the presence of metal ions of variable valency also provide evidence for the possibility of reaction (4).

Hence, homolytic rupture of the O-glycoside bond in carbohydrates upon action of radiation and other initiators is possible not only due to the reactions of β-scission (1) and hydrolysis of C-1 radicals of the starting substances (2), but also due to fragmentation reaction of C-2 radicals (4), proceeding via rupture of two bonds. Taking into consideration of all these mechanisms makes it possible to find a more adequate explanation of facts accumulated hitherto on radiolysis of carbohydrates and to propose regulation pathways for these mechanisms by means of radiation-chemical transformations.

The ability of quinones and compounds capable of forming quinoid structures to inhibit reactions of type (4) opens up outlooks to the search for substances decreasing destruction yields of polysaccharides and polysaccharide-containing substances on irradiation. This appears to be an important issue, since the free-radical destruction of polysaccharides and polysaccharide-containing substances alters their physical and chemical properties to a substantial extent, and can lead to generation of signaling molecules playing an important role in biosystem functioning.

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