Yields in the Radiation Degradation of Solid Carbohydrates

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The degradation induced by γ-irradiation of various simple poly-
crystalline carbohydrates has been studied by ESR measurements
of the solid samples, and by analyses of product formation and
reactions occurring when the samples are dissolved in water. The
area under the ESR absorption curve is not inversely proportional
to the absolute temperature at which the ESR measurements are
made, between 160 and 300°K, indicating a possible interaction
between the radicals. When the irradiated carbohydrates are dissolved
in water, they react consuming oxygen that is dissolved in the water.
It is postulated that radical-oxygen and radical-radical reactions are
competitive. The consumption of dissolved oxygen during the reaction
was fit to an approximate equation from which the ratio of reaction
velocities of radical-oxygen and radical-radical reactions was found
to be in the range of 10—15. The G-value of oxygen consumption at
high oxygen concentrations of the water is 4 or higher. The aqueous
solutions of the irradiated carbohydrates contain compounds, possibly
organic peroxides, that oxidize ferrous ions of the ferrous sulfate
reagent. The G-value of ferrous ion oxidation is 4—10 for various
carbohydrates. The yield of acids and the total degradation of the
carbohydrates are strongly dependent on the crystalline form of the
carbohydrate.

During recent years information has accumulated on the radiation chemistry
of solid simple carbohydrates.[1-12] Studies have been devoted to various
aspects of the degradation when the irradiated compounds were dissolved
in water, such as total amount of destruction,[6,7,10,12] yield of acids,[3,10-12]
formation of gases,[8] and separation of degradation products.[4,5,7,9,12]

Parallel with and preceding these chemical studies were a number of
electron spin resonance (ESR) investigations of irradiated carbohydrates.[13-22]
Some of these studies concerned yields of radicals and/or comparisons of
spectra of different polycrystalline samples,[13-18,22] and some concerned struc-
tures of radicals with measurements on single crystals.[18-21]

The eventual goal of the ESR and the chemical investigations is an under-
standing of the reactions linking the structures of the radicals in the solid
state with the degradation patterns after dissolving the irradiated compound.
This goal has not yet been reached, mainly because of two reasons. First, the

rather simple radical structure suggested by Ueda et al.[18] in their exploratory single crystal studies does not seem to hold in a more detailed analysis.[23] Second, the degradation pattern is rather complicated; a great number of neutral and acidic components are formed.[4,7,12] Indeed, it seems as if the degradation is caused by a random "explosion" of the molecules. Phillips and Baugh [3] found no differences between polycrystalline and freeze-dried α-D-glucose when they compared the ESR spectra and radical concentrations. When dissolved in water, the polycrystalline sample gave about three times more acid than the freeze-dried sample. Thus the possibility of linking radical structure with the degradation products seems to be slight with the presently available data.

It was also shown previously that the type of crystal structure of a carbo-hydrate strongly influences the radiation degradation pattern.[2,11,12]

This communication further relates variations in radiation degradation of different crystal modifications of carbohydrates. In addition, it also shows the strong influence exerted by the presence or absence of oxygen in the water used for dissolving the compounds. Additional ESR data provide some evidence on the distribution of the induced radicals within the crystal and on a possible complex structure of these radicals.

EXPERIMENTAL

Chemicals. α-D-Glucose, α-D-glucose·H$_2$O, D-fructose, sucrose, maltose·H$_2$O, α-lac-tose·H$_2$O, and α,α-trehalose·2H$_2$O were purchased commercially in their purest available grades. β-D-Glucose was prepared according to Hudson and Dale [24] and β-lactose accord-ing to Tanret.[25] The anhydrous forms, (αβ)-maltose, α-lactose, and α,α-trehalose, were prepared by heating the hydrates in vacuum. Measurements of specific rotation showed that the β-D-glucose and the β-lactose were at least 98 % in their proper forms, and that the preparations made by heat-drying were at least 95 % pure.

Irradiation. The solid carbohydrates were irradiated with ^{60}Co γ-rays at room temper-ature in sealed polyethylene or glass tubes in the presence of air. During the course of these studies, the dose rate varied between 15 and 50 × 10^{18} eV/g h.

Preparations of solutions. The irradiated carbohydrates were generally dissolved in air-saturated water, unless stated otherwise. Water with other oxygen concentrations was prepared by bubbling with oxygen or nitrogen.

Determination of total degradation of D-glucose by direct isotope dilution. The irradiated sample of D-glucose (0.5 g) was dissolved in 10 ml water and a specified amount of [^{14}C]-D-glucose in water was added. The solution was freeze-dried and after further rigorous drying the penta-O-acetyl-β-D-glucose was prepared [26] and recrystallized to constant specific activity. The acetyl derivative was dissolved in a PPO liquid scintillator solution and radioactivity subsequently measured.[27] For each of the three glucose forms the degradation was measured at five radiation doses between 6 and 83 × 10^{20} eV/g.

Potentiometric titration. The solutions were titrated with carbonate-free NaOH using a Radiometer pH meter, model PHM 22.

Separation of acids from neutral components and chromatography of the acids. Separa-tion of the acids from the bulk of unchanged glucose and other neutral compounds was performed on ion-exchange resins as described previously.[12] Both paper and cellulose thin layer chromatography were employed in the further investigations of the acid fraction.

Peroxide determination. Different methods sensitive for various peroxides were used. Hydrogen peroxide was determined with the titanium sulfate reagent,[28] H$_2$O$_2$ and hydro-peroxides (ROOH) were determined with the potassium iodide reagent,[29,30] and the total amount of peroxides (H$_2$O$_2$, ROOH, and ROOR') was determined with the ferrous sulfate

reagent.[31],[32] The determinations were made on the solutions that were obtained after the oxygen consumption was measured.

Oxygen measurements. Samples were dissolved under vigorous agitation in a 100 ml volume in which oxygen concentrations before and after dissolving the irradiated carbohydrates were measured with a Beckman Oxygen Analyzer, model 777. Gas-exchange with the surrounding atmosphere through the narrow glass inlet tube was negligible during these procedures.

ESR. The details of the measurements of absorption derivative spectra and concentrations of free radicals have been given previously.[17],[33]

RESULTS AND DISCUSSION

Total degradation. The G($-$glucose)-values and their standard errors for the different forms of glucose are given in Table 1. The lowest dose in the

Table 1. Yields in the radiation degradation of solid carbohydrates expressed as G-values. (No. of changes per 100 eV).

	Total degradation	Acid formation	Oxygen consumption	Fe²⁺ oxidation
Dose range eV/g × 10⁻²⁰	6 — 83	0.2 — 10	(0) — 1	(0) — 1
Carbohydrate				
α-D-Glucose	9.3 ± 0.3	13	> 4	6
β-D-Glucose	10.6 ± 0.7	9		
α-D-Glucose·H₂O	5.3 ± 0.1	2.7	5	> 6
D-Fructose			> 4	> 4
Sucrose		1.0	4	4
α-Lactose·H₂O		> 40	4	10
β-Lactose		4.0		
α-Lactose anh.		4.8		
Maltose·H₂O		3.5		
Maltose anh.		3.0		
α,α-Trehalose·2H₂O		0.9		
α,α-Trehalose anh.		1.0		

present investigation was 6×10^{20} eV/g. Degradation was found to increase linearly with the dose up to the highest dose employed, 83×10^{20} eV/g. The standard errors are those calculated from the regression lines. The present data are not in complete agreement with our earlier results.[12] The lowest doses in the earlier investigation were 3.7 and 10.8×10^{20} eV/g. The initial G-values were about 20 for α- and β-D-glucose and about 11 for α-D-glucose·H_2O, and they decreased continuously to about one-half of these values at doses of $40-50 \times 10^{20}$ eV/g. In the present investigation, the G-values were 9.3 ± 0.3 for α-D-glucose, 10.6 ± 0.7 for β-D-glucose, and 5.3 ± 0.1 for α-D-glucose·H_2O (Table 1). The discrepancies between the present and the previous results are not fully understood. They may be partly due to differences in the crystalline uniformity of the samples, which influence enormously the degradation

yield and pattern.[3,12] However, when we calculated the standard errors of the previous investigation we found that they are about 25 % for each individual measurement of the G-value at the low doses, and the discrepancies between the results may therefore not be significant.

From the data given by Zagradski and Zaorska,[10] we calculated a G(−sucrose) of about 6 at a dose of 3×10^{20} eV/g. This value is of the same order as those found for D-glucose in the present investigation.

From the data reported by Geissler,[6] we calculated a very high G(−glucose), about 50, at a dose of 3.5×10^{20} eV/g and similarly high G-values for fructose and sucrose. The reliability of these results cannot be evaluated as Geissler used enzymatic systems for the determination of unchanged sugar. A small effect on the enzyme system by any of the degradation products would cause a considerable error in the final results.

Acid formation. The data in Table 1 show the G-values of total acid formation at low doses for a number of carbohydrates. These values are in agreement with various previously published results.[2−4,10,11] The acid yields are markedly influenced by the crystal form, and also vary with the oxygen concentration of the water for dissolving the samples. A 20 % higher yield of acids results when irradiated sucrose is dissolved in O_2-saturated water compared with O_2-free water. The corresponding difference for α-D-glucose is about 10 %, whereas there is no difference for α-lactose·H_2O. The very high yield from the last substance (G > 40) indicates that a chain reaction is operating.

Acid products. The mixtures of acids from the three forms of D-glucose were separated by paper and cellulose thin layer chromatography. Several spots occurred in common for the different glucoses; this indicates that several acids formed by irradiation of the three forms are the same. Some spots, however, were obtained only from one or two of the three forms. A total of about 15 different acids were revealed with $AgNO_3$ and aniline-phthalic acid as developing reagents.

Peroxide formation. After dissolving the irradiated carbohydrates, neither hydrogen peroxide nor hydroperoxides could be found in the solution.

There is a high yield of compounds that are able to oxidize ferrous ions in the ferrous sulfate reagent. The yield of ferric ions at a fixed radiation dose is dependent on the oxygen concentration of the water. At relatively low doses (Fig. 1 left) the yields reach a saturation level in air-saturated water for some carbohydrates, whereas at higher doses (Fig. 1 right) some carbohydrates give increasing yields of ferric ions at higher oxygen concentrations.

Yields of ferric ions for a number of carbohydrates measured at low radiation doses and high oxygen concentrations are shown in Table 1. In some cases only a lower limit of the yield can be given since no saturation level was reached.

These compounds that are able to oxidize ferrous ions are not stable. Storage of the solution in darkness at room temperature for a few days decreases the amount by about 50 %.

The oxidation of ferrous ions in the ferrous sulfate reagent is not definite proof for the presence of organic peroxides of the type ROOR′; it merely shows that some oxidizing agents have been formed. The designation of these agents as peroxides, however, is supported by the evidence that the formation
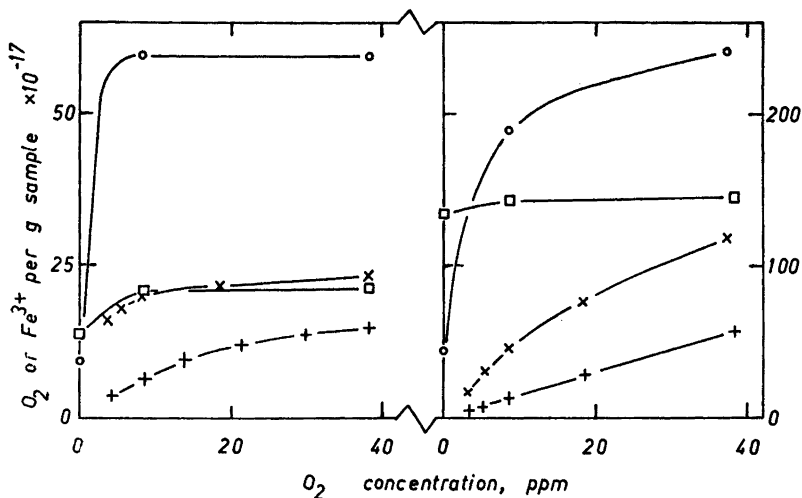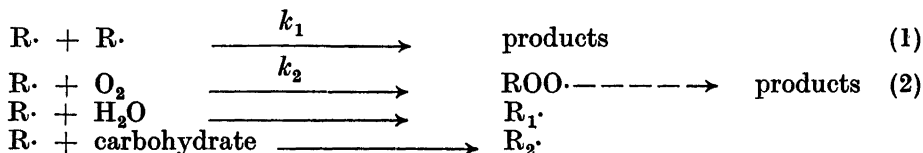
Fig. 1. Oxygen consumption and ferrous ion oxidation by γ-irradiated carbohydrates dissolved in water containing different oxygen concentrations. Number of O_2 molecules consumed and number of Fe^{3+} ions formed per g carbohydrate vs. oxygen concentration, in ppm, of the water. To the left: ○ Fe^{3+} and × O_2 for α-lactose·H_2O irradiated with 68.8×10^{18} eV/g; □ Fe^{3+} and + O_2 for sucrose irradiated with 55.1×10^{18} eV/g. To the right: same compounds and notations but with a dose of 826×10^{18} eV/g.

of these compounds, in most cases, is enhanced when the oxygen concentration of the water is increased and that at the same time oxygen is consumed.

Oxygen consumption. The dependence of the oxygen consumption on the oxygen concentration in the water when the crystals were dissolved and on the irradiation dose is very much the same as for the dependence of the ferrous ion oxidation (Fig. 1) on these parameters. At low radiation doses, oxygen consumption levels off at high oxygen concentrations, whereas at higher radiation doses the yield increases continuously as oxygen concentration increases. In Table 1, data are given for yields of oxygen consumption measured at low doses and extrapolated to infinite oxygen concentrations.

No direct correspondence between the yield of ferric ions and the oxygen consumption was found (Fig. 1 and Table 1).

Irradiation of solid carbohydrates produces a large amount of stable radicals (R·) which when dissolved may react with water, oxygen, carbohydrate, and other radicals according to the following schematic formulas:

$$R\cdot + R\cdot \xrightarrow{\quad k_1 \quad} \text{products} \tag{1}$$

$$R\cdot + O_2 \xrightarrow{\quad k_2 \quad} ROO\cdot ----\rightarrow \text{products} \tag{2}$$

$$R\cdot + H_2O \longrightarrow R_1\cdot$$

$$R\cdot + \text{carbohydrate} \longrightarrow R_2\cdot$$

where $R_1\cdot$ and $R_2\cdot$ may re-enter into any of the reactions or react with ROO·.

Regarding reactions (1) and (2) as the principal causes for radical disappearance and reaction (2) as the principal cause for O_2 disappearance, the following equation was deduced:

$$\frac{1}{\Delta[O_2]} = \frac{k_1}{k_2[O_2]} + \frac{1}{[R_0]} \tag{3}$$

where,

k_1 and k_2	are rate constants for reactions (1) and (2), respectively
$\Delta[O_2]$	is change in O_2 concentration when the irradiated sample is dissolved to make a saturated solution of the carbohydrate
$[O_2]$ and $[R_0]$	are O_2 and radical concentrations, respectively, in the vicinity of the carbohydrate crystal at the moment it dissolves

Estimating $[O_2]$ from the bulk oxygen concentration must take into account the decreased oxygen concentration of the solution near the crystal surface. In the present approximate approach this was done by dividing the bulk oxygen concentration with the volume of a saturated aqueous carbohydrate solution prepared from a unit volume of water.

From plots based on eqn. (3), one can calculate k_2/k_1 and $[R_0]$ (Fig. 2). The latter, $[R_0]$, can be considered an estimate of the maximum oxygen consumption at an infinite oxygen concentration (Table 1). From plots similar to Fig. 2, the ratio of radical-oxygen to radical-radical velocity constants (k_2/k_1) are in the range 10—15 for the investigated carbohydrates. Thus, the rate constant for the reaction between radicals and oxygen is one order of magnitude greater than that for the reaction between radicals.
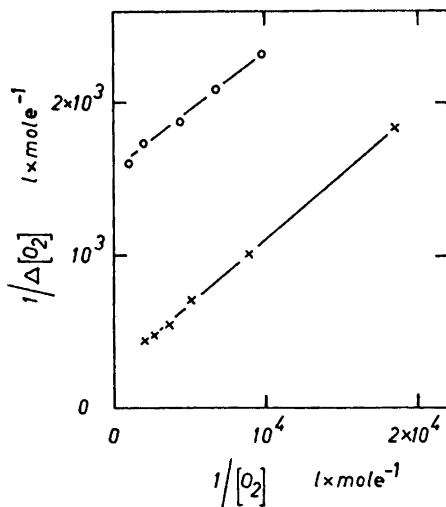

Fig. 2. The reciprocal of the change in oxygen concentration when the sample is dissolved vs. the reciprocal of the oxygen concentration of the water. α-Lactose·H$_2$O irradiated with 68.8 × 10^{18} eV/g (O), and sucrose irradiated with 55.1 × 10^{18} eV/g (×). The experimentally measured concentrations have been corrected for the volume change valid for saturated carbohydrate solutions. From the slope a ratio of k_2/k_1 of 12 was calculated for sucrose, and 13 for α-lactose·H$_2$O. From the intercept with the ordinate, values for R_0 of 2.5 × 10^{18} and 2.4 × 10^{18} molecules/g carbohydrate were obtained for sucrose and α-lactose·H$_2$O, respectively.

When no activation energy is needed,[34-36] reaction rates between radicals are dependent almost entirely on diffusion but with some additional dependence on steric factors. The diffusion coefficient of O_2 is 3—5 times greater than those of simple carbohydrates in dilute aqueous solutions,[37] which partly explains the ratio between the two rate constants. The other part may possibly be explained by a steric factor.

Irradiation of starch gives rise to free radicals, the stability of which are dependent on the water content of the sample and on the oxygen availability around the sample.[38] Radiation-induced radicals in amylopectin with 4.7 % water have a half-life of 6.7 h in air, whereas those in amylopectin with 17.8 % water have a half-life of about 20 min.[38] This differential behaviour of radicals in amylopectin was used in the present investigation in order to obtain more conclusive evidence for radical-oxygen reactions.

When freshly irradiated amylopectin containing 4.3 % water was dissolved, it consumed an amount of oxygen comparable to that consumed by irradiated simple carbohydrates. After a few days storage of the irradiated amylopectin sample in air, during which the radicals decayed nearly completely, no oxygen consumption could be detected under similar conditions. In addition, neither freshly irradiated nor stored irradiated amylopectin with 20 % water consumed oxygen when it was dissolved. Correspondingly, only freshly irradiated amylopectin with 4.3 % water gave any oxidation of ferrous ions with the ferrous sulfate reagent.

These results strongly indicate that the formation of compounds that are able to oxidize ferrous ions and the consumption of oxygen require the presence of free radicals.

ESR. The radical yield has previously been measured for some carbohydrates over a wide range of doses.[2,17,39] The present investigation has been extended to cover nearly all carbohydrates mentioned under Experimental. All give, at low doses (2 $\times$ 10^{19} eV/g), radical yields with G-values in the range 3—5. The G-value decreases rapidly with increasing dose, similar to the behaviour of sucrose.[17] At high doses the radical concentration reaches a plateau and further irradiation may even cause a decrease of the radical concentration.[39] For sucrose the plateau level is reached at 5—6 $\times$ 10^{21} eV/g with a concentration of about 2 $\times$ 10^{19} radicals/g, *i.e.* the average radical-radical distance is just above 20 A.u.

The area under the ESR absorption curve of ordinary free radicals should be inversely proportional to the absolute temperature at which the measurements are made, because a population difference between two energy levels is being measured.[40] Experimental investigations with radiation-induced radicals in sucrose and in α-D-glucose show that the intensity and shape of the ESR absorption curve is almost independent of the temperature between 160 and 300 °K under conditions when there is no power saturation. Other radiation-induced free radicals, *e.g.* in cholesterol, follow the expected temperature dependance under similar conditions.[41] Radicals formed in polyethylene and polymethylmethacrylate also seem to follow the expected temperature dependance.[42] Experimental conditions have unfortunately limited the investigations to the above-mentioned temperature interval. The behaviour at still lower temperatures is necessary for a better understanding of the

phenomenon. One may speculate that the effect arises from solid charge-transfer complexes of biradicals,[43-45] radical pairs,[46-50] or radicals within small-sized clusters, the radicals being of types such that the exchange interaction is of the order of kT (0.02—0.04 eV).[44] The above-mentioned temperature phenomenon casts some doubt on the significance of the radical yields reported here if, in fact, these measurements give information only about the amount of some radical complexes which vary with temperature.

Irradiated sucrose samples have been used for an international exchange of standards of radicals for comparing absolute radical concentrations.[51] The results obtained, however, are not affected by the temperature behaviour as long as the measurements are performed at the same temperature.

Yoshida et al.[42] studied the ESR saturation effects on radiation-induced free radicals in polymers. They calculated the spin-lattice relaxation time, T_1, and its dependence on the bulk radical concentration, and concluded that the radicals most probably are formed in clusters. The concentration that was seen in their ESR relaxation studies was the local concentration in the clusters, which remains independent of the dose and of the macroscopic radical concentration.

In a number of ESR investigations, it has been revealed that irradiation can cause the formation of radical pairs, i.e. radicals formed in close proximity to one another. Such radical pairs have been found and studied in persulfates,[46] dimethylglyoxime,[47,48] hydrogen peroxide,[48] diaryl carbonates,[49] and in polymers.[50] The electronic spin-spin interaction is usually rather small in these radical pairs and might be confused with hyperfine interactions.[48] So far only the radical pair trapped in dimethylglyoxime has been measured over a wide range of temperatures and the ESR absorption follows the expected inverse dependence on temperature.[52] It was concluded that the exchange interaction between radicals was less than kT at 140°K (the lowest temperature at which they were studied). In the cases of hydrogen peroxide,[48] dimethylglyoxime,[47] and polymers,[50] the transition corresponding to $\Delta M = 2$ found at half-field magnetic strength ($g \approx 4$) has been observed. In the present investigation neither α-D-glucose nor sucrose gave any evidence of a $\Delta M = 2$ transition with a gain on the instrument 1000 times that with which the $\Delta M = 1$ transition was observed. However, since the probability of the $\Delta M = 2$ transition is low, the absorption at half-field may be difficult to observe experimentally.[48]

CONCLUSIONS

Irradiation of solid crystalline simple carbohydrates gives rise to relatively stable free radicals, the types of which are to some extent still unknown. Non-radical entities are possibly also formed. The radicals may be trapped in clusters. During storage at room temperature, the radicals change to new types of radicals or to non-radical compounds.[17,22]

Upon dissolving the crystals, the compounds trapped in the crystal lattice react with water and dissolved oxygen to form a great variety of neutral and acidic compounds. The types formed are dependent on such factors as the carbohydrate used, the oxygen concentration, and also the procedure used

to dissolve the crystal. This warrants certain precautions in future studies of radiation degradation of solid compounds.

More than 10 acidic compounds and more than 15 volatile compounds [8] were detected after dissolving irradiated D-glucose. Under certain conditions, the amount of a few of these compounds accounted for about 25 % of the total D-glucose degradation [12] (D-gluconic and D-glucuronic acid from freeze-dried D-glucose).

Recent theoretical and experimental investigations on the role of multiple ionizations in radiation action (Ref. 53 and quoted references) might be an aid to a better understanding of why irradiation gives rise to complicated degradation patterns in some solid systems. As much as 10 % of the total energy input can give multiple ionizations in some systems and these multiple ionizations cause considerable damage in their vicinity, which may explain why the degradation of irradiated solid compounds seems to involve a non-specific "explosion" forming a great variety of products.

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