

《Original》 Aldehydes in γ -irradiated Solid Starch

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Abstract

A study of the radiation-induced formation of carbonyl compounds in solid starch by means of precipitation with 2,4-dinitrophenylhydrazine and subsequent analysis of the hydrazones by gas chromatograph has been performed. Formation of carbonyl groups was found to increase linearly with increasing doses up to about 2×10^{21} eV/g. Generally, at least 5 different peaks can be detected including formaldehyde and acetaldehyde.

요 약

고체전분이 방사선에 조사될때 생성되는 carbonyl 물질이 gas chromatograph에 의하여 검파되었는데 2,4-dinitrophenylhydrazine으로 침전시켜 얻은 hydrazones을 분석하였다.

Carbonyl groups의 생성은 선량이 2×10^{21} eV/g 될때까지 직선으로 증가하며 formaldehyde와 acetaldehyde가 검파되었다. 이외에 최소한 미상의 3개의 peak가 발견되었다.

1. Introduction

During recent years information has been accumulated on the radiation chemistry of solid simple carbohydrates. Studies have been devoted to various aspects of the degradation when the irradiated compounds were dissolved in water, such as yield of acids¹⁻³), formation of gases⁴), and separation of degradation products^{5, 6}). There are relatively few radiation chemistry studies on polysaccharides. However, the reported data give some general insight. The most noticeable effect of radiation on polysaccharides is chain degradation. This holds for all conditions of radiation as shown

by decrease in viscosity, changes in light-scattering, electrophoretic and ultra-centrifuge patterns.⁷⁻¹⁰)

The radiation induced formation of carbonyl compounds in several solid carbohydrates has been studied by means of precipitation with 2,4-dinitrophenylhydrazine and subsequent analysis of the hydrazones by thin layer chromatography. On irradiation of polysaccharide, *e. g.* cellulose, with increasing doses there was an increasing formation of carbonyl groups⁷). This method is, however, severely hampered by an instability of many of the hydrazones both during storage in solution and as solid dry material.

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With the aid of a gas chromatographic technique it is possible to identify 2,4-dinitrophenylhydrazones of certain carbonyl compounds formed by irradiation of solid carbohydrates¹³⁾, *i.e.* irradiation of D-fructose and subsequent dissolution in water yields formaldehyde, acetaldehyde and acetone. Apart from the radiation chemistry viewpoint, it seems to be of interest to isolate and identify these degradation products as some of them might have reactive properties causing indirect chemical and biological effects.

2. Materials and Methods

Chemical

The following chemical as test sample has been used in its purest commercially available form: potato starch(drying loss 18%), Showa Chem. Co., Ltd., Japan.

Equipments

1. Irradiator: 25,000 curie ⁶⁰Co γ -rays source BNL Shipboard Irradiator, U. S. A.
2. Gas Chromatograph: Varian Aerograph Gas Chromatograph with Flame Ionization Detector, Series 2100, U. S. A.

Irradiation

The solid sample was irradiated with ⁶⁰Co γ -rays in a sealed glass tube in the presence of air. The dose rate varied between 2.8 and 6.3×10^{19} eV/g·h.

Preparation of References

0.4 g of 2,4-dinitrophenylhydrazine (DNPH) was dissolved in 20 ml of methanol, with a few drops of conc. HCl (ca 1%). Then, formaldehyde, which is slightly excess to DNPH, was added to DNPH. The same procedure was done for acetaldehyde. The precipitation was carried out at room temperature.

The precipitate was filtered off, washed with 0.2 N HCl and distilled water, vacuum dried, and weighed. A weighed amount of the precipitate was mixed with a measured

volume of CHCl₃(0.1-0.25% w/v). Sample size of 3 μ l was used for injection.

Preparation of Samples

30 g of the irradiated solid starch was dissolved in 150 ml of distilled water. After shaking vigorously, 100 ml of aqueous solution (starch solution) was obtained through filter paper for the precipitation.

0.8 g of DNPH, dissolved in 100 ml of a 1:1 2N aqueous HCl-ethanol solution, was added to the starch solution. The precipitation was carried out at room temperature. After 2 hours storage the solution was filtered off. The precipitate was washed with 2 N HCl and distilled water, vacuum dried, and weighed. A weighed amount of the precipitate was mixed with a measured volume of CHCl₃, generally in the range of 10-20% w/v. The slurr was thoroughly crushed and stirred after which the undissolved precipitate was separated from the supernatant. Sample size of 10 μ l was used for injection.

3. Results and Discussion

The interaction of radiation with the starch molecule caused an oxidative degradation(Fig. 1).

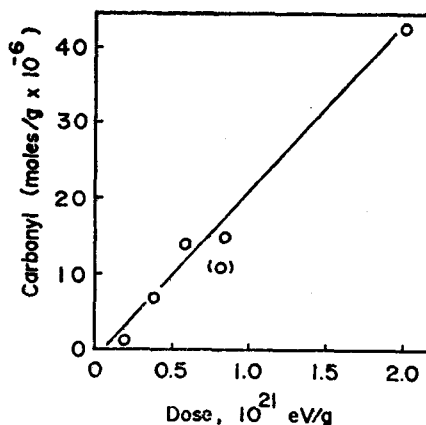


Fig. 1. Yield-dose curve for carbonyl groups from γ -irradiated solid starch

Calculated on the basis of the reaction between 2,4-dinitrophenylhydrazine and carbonyl.

With increasing doses there was an increasing formation of carbonyl groups. Since one mole of phenylhydrazine will react with one mole of an aldehyde or a ketone, the yield of carbonyl groups can be calculated. Based on the determination at doses of 0.2 and 2×10^{21} eV/g, G-value of carbonyl groups is about 0.001.

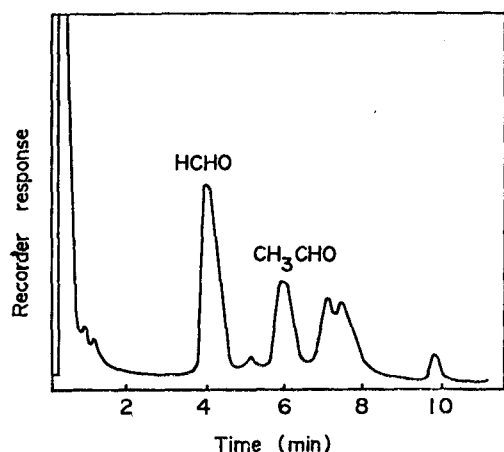


Fig. 2. Gas chromatogram of 2,4-dinitrophenylhydrazones of formaldehyde and acetaldehyde obtained from γ -irradiated solid starch

Conditions: 150×0.2 cm stainless steel column packed with Silicone GE SE-30, 5% on 70-80 mesh Chromosorb W Regular (Hexamethyldisilazane treated); column temperature, 175°C ; detector temperature, 180°C ; injection port temperature, 275°C ; nitrogen flow rate, 20 ml/min; range, 10^{-10} amps/mv; attenuation, $\times 1$; chart speed, 0.25 in/min.

Fig. 2 shows that irradiation of starch and subsequent dissolution in water yields formaldehyde and acetaldehyde. The identification of aldehydes relies on the identical retention time of reference and starch sample.

In the presence of oxygen, irradiation of D-glucose in aqueous solution undergoes a complex series of reactions, including oxidation, degradation, and polymerization. It has been revealed already that attack at either of those terminal groups by free radicals, which are radiolytic products of water, leads to oxidation products, *i.e.* $-\text{CHO}$ or $-\text{COOH}$,

whereas attack at a secondary alcohol group leads to fission of the carbon chain or gives a keto-group.¹⁴⁾

Irradiation of solid simple carbohydrates produces a large amount of stable radicals which when dissolved may react with radicals, oxygen, water or carbohydrates.^{12, 19)}

Upon dissolving the crystals, the compounds trapped in the crystal lattice react with water and dissolved oxygen to form a great variety of degradation products. While the effects of irradiation on polysaccharides in solution and in dry state are much the same, cellulose and pectin, when irradiated in a dry state show an after-effect, but only stored dry in the presence of oxygen.²⁰⁾ This is probably due to long-lived radicals formed with oxygen. Potato starch, in which glucose units are joined, contains 18% of water. Therefore it seems logical to assume that the formation of carbonyl groups is to be, partly, caused by the vulnerability of alcohol groups to free radical attack.

Degradation is the most predominant feature observed when starch and starch fractions are irradiated in the solid state. Since the intensity of colour produced between amylose and iodine is directly related to the chain length, the change in Blue Value with dose is convenient and sensitive method of following the degradation which accompanies γ -irradiation. The decrease in Blue Value with dose follows an exponential relationship²⁾. This indicates that random chain breaks occur, with initial breaks thus exerting a great effect on the molecular weight decrease than do subsequent breaks on the shorter polysaccharide chains, and on the formation of degradation products. According to the resonance energy transfer curves for amylose complexes and cycloamylose complexes¹⁹⁾ in relation to random chain breaks, an excitation would appear to be produced in the starch which can be efficiently transferred to

a suitable acceptor molecule. No definite information has been available about possible excitation states for starch, however, one may draw this phenomena from the published data,^{16, 18, 21)} *i. e.* as a result of intersystem crossing as well as internal conversion of the excited molecules.

It was known that the number of breaks produced in the polysaccharide chains of the molecules per energy absorbed decreases with increasing water content. Chemical changes involving covalent bonds do follow the degree of degradation is indicated by the ability of irradiated starch to reduce iodine in solution, as discussed above, the ionic yield for this ability decreasing from about 0.5 at 6% water to 0.2 at 20% water.⁸⁾ In starch water may exert its maximum "protection" at higher water contents (about 18-20% H₂O), even though the radiolysis products hydrogen and hydrogen peroxide are considered the most important intermediates in radiation-induced degradation. This strongly indicates that irradiation of starch gives rise to free radicals and their stability are dependent on the water content and on the oxygen availability. Under the conditions used in this experiment, starch might not changed up to about 0.1×10^{21} eV/g.

Although these discussion on the role of excitation and free radical are mostly based on the previously published reports, and more or less theoretical, it might be an aid to a better understanding of why irradiation gives rise to complicated effects in some solid system. Because of the complexity of the living system, no definite conclusions can be drawn at the present time concerning the relation between the radiation induced carbonyl compounds and the biological effects.

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