MECHANISM OF STARCH DEPOLYMERIZATION WITH CHLORINE[1]

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ABSTRACT

It is proposed that in the chlorinolysis of glycosidic bonds, cleavage occurs between carbon C1 and the glycosidic oxygen with elimination of the aglycone as the hypochlorite ester, which may undergo hydrolysis or dehydrochlorination to a carbonyl function; and the formation of a glycosyl chloride which solvolyses and also forms new glycosidic bonds. Chlorinolysis of methyl alpha-D-glycopyranoside and maltose gives rise to small amounts of levoglucosan, and hydrolysis of the reduction products of the chlorinolyzed amylose gives D-galactose.

The improvement of flour by chlorine treatment is well recognized, but the action of chlorine on the various flour components is not well understood. To obtain a clearer picture of the action of chlorine on the starch components, the two reactants have been investigated in an isolated system. Initial reactions conducted in an aqueous slurry showed that extensive depolymerization occurred, along with extensive oxidation of the D-glucosidic carbons C2 and C3, inducing ring opening and transformation of carbons C2 and C3 to carboxyl groups (1). The optimum pH for this attack was slightly greater than 7, where the solution contains its largest combined concentrations of hypochlorite ions and undissociated hypochlorous acid. Under near neutral or alkaline conditions no appreciable attack occurred at carbon C6, since practically no uronic acid was obtained. Some free D-glucose and much D-gluconic acid were observed. Since flour is normally bleached in the presence of a limited amount of water, subsequent chlorine oxidations (2,3) were conducted on starch of 13% moisture content. Under these semidry conditions practically no oxidative attack on carbon atoms C2 or C3 occurred, and very little uronic acid was formed. Rather, an extensive depolymerization ensued because of the cleavage of glycosidic bonds, which also led to the formation of large amounts of D-glucose and D-gluconic acids, both as chain end units and free molecules. The extensive depolymerization was shown not to be due to acidity resulting from developed hydrogen chloride, but due mainly to oxidative depolymerization. Though strongly photoactivated, the depolymerization in the light was similar to that in the dark.

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To obtain still further insight into the depolymerization mechanism by which chlorine cleaves the glycosidic bonds, investigations have now been made of the action of chlorine on vacuum-oven-dried starch on corn amylose, and on the model compounds maltose and methyl alpha-D-glucopyranoside. The results lead us to propose, here and elsewhere (4), a new depolymerization mechanism applicable to either dry or wet reactions. The mechanism, with slight modification, may also explain glycosidic bond cleavage in cellulose subjected to chlorine bleaching action.

Materials and Methods

Wheat starch, supplied by Ogilvie Flour Mills Co., Ltd., Montreal, Canada, was defatted by extraction with 85% methanol (5) and dried in a vacuum oven at 105°C. for 4 hr.

Chlorine, obtained from Matheson Co., Inc., was dried by passage through sulfuric acid. Acetic acid was Baker's analyzed reagent.

Commercial methyl alpha-D-glucopyranoside was purified by heating with 0.1*N* sodium hydroxide at 100°C. for 30 min. It was then deionized, concentrated to a syrup, taken up in ethanol, and crystallized from ethanol, m.p. 164°C. It was dried in an oven at 105°C. for 12 hr. before use.

Anhydrous maltose was prepared from chromatographically pure maltose monohydrate by vacuum drying in an Abderhalden apparatus at 100°C. for 24 hr. with phosphorus pentoxide as desiccant.

Corn amylose was prepared according to the procedure described by Schoch (6). It was dried in a vacuum oven at 105°C. for 4 hr. with phosphorus pentoxide as desiccant.

Oxidations of dry wheat starch with gaseous chlorine were conducted at 18±2°C. in 1-liter Pyrex bottles in the dark as well as in the presence of 500 ft.-candles of incandescent light, according to the procedure described earlier (3). The chlorine consumed during the oxidation was iodometrically determined, as previously described by Whistler and Schweiger (1).

Viscosity of aqueous solutions of the oxidized starch, number of carboxyl groups per 100 D-glucose units, and the number of aldehyde groups per 100 D-glucose units in the oxidized starch were measured as previously described (3). The combined number of aldehyde and ketonic groups per 100 D-glucose units in the oxidized starch was determined by the sodium cyanide method of Schmorak and Lewin (7).

Oxidation of methyl alpha-D-glucopyranoside, maltose, and corn amylose with chlorine was conducted in glacial acetic acid. The carbohydrate (0.01 mole) was added to glacial acetic acid containing 0.01

mole of chlorine. The Pyrex glass tube containing the above reaction mixture was sealed and heated at 55°–60°C. with occasional shaking until almost all of the chlorine was consumed. After cooling, the tube was opened cautiously and the reaction mixture was concentrated to a syrup under reduced pressure. The syrup was dissolved in 50 ml. of water and again concentrated to a syrup. Two additional concentrations from water served to remove the major part of the acetic acid, and the products were obtained as a syrup (A).

The syrup (A) was treated with 1*M* aqueous potassium carbonate at 25°C. for 12 hr. with stirring, at a pH greater than 10, to remove any residual acetyl groups. The mixture after dilution with 50 ml. of water was treated with IR-120(H^+) to lower the pH to 4. The mixture was filtered and the resin was washed thoroughly with water. The combined filtrate and washings were treated with IRA-400(OH^-) until the pH increased to 6–7. The resin was removed by filtration, and the filtrate and washings were concentrated to a syrup (B), which contained oxidation products along with unreacted material.

Oxidized starch was hydrolyzed to component sugars in 5% sulfuric acid solution at 90°C. for 8 hr.

Chromatographic examination of products after the oxidation of methyl alpha-D-glucopyranoside, maltose, and amylose, as well as after the hydrolysis of oxidized starch, was made on Whatman paper No. 1. The irrigants were: (a) 1-butanol:acetic acid:water (4:1:1 v./v.), (b) 1-butanol:acetic acid:water (4:1:5 v./v.), and (c) 1-butanol:ethanol:water (40:11:19 (v./v.). The spray reagents employed were (d) an acetone solution containing 0.5% silver nitrate followed by an ethanolic solution of sodium hydroxide (8), and (e) a solution of resorcinol in 1-butanol containing hydrochloric acid (9).

The neutral products of the oxidation of methyl alpha-D-glucopyranoside and maltose were separated on cellulose columns or on Whatman 3MM paper using the irrigant (C).

The acetates were examined by thin-layer chromatography on silica gel G. The plates were dipped for 4 min. in 5% DMSO (dimethylsulfoxide) in benzene and dried at 80°C. for 10 min. before use. Plates were irrigated with a mixture of isopropyl ether:benzene (4:1 v./v.), and the components were detected by spraying the plates with 5% sulfuric acid in methanol and charring until permanent spots were obtained.

Results

Oxidation of dry starch is conducted with a starch:chlorine molar ratio of 1:3. Parallel oxidations are conducted in the dark and with

bottles exposed on two sides to 500 ft.-candles of incandescent light. The consumption of chlorine during these oxidations is shown in Fig. 1.

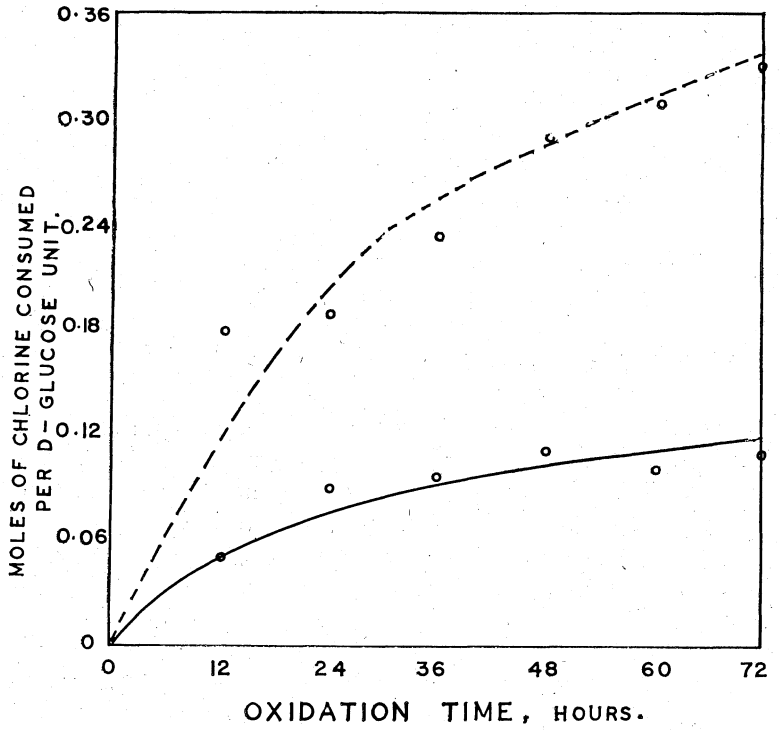

Fig. 1. Chlorine consumption for oven-dried wheat starch treated with 3 moles of chlorine for each mole of D-glucose unit in light (A) and in dark (B).

Yields of oxidized starches after 72 hr. in the dark as well as in the presence of light are 98–100%. No detectable amounts of carbon dioxide are produced during the oxidations (1). The rates of formation of carboxyl and aldehyde, and the combined number of aldehyde and ketonic groups per 100 D-glucose units of oxidized starch, are determined by different analytical methods. The number of ketonic groups formed per 100 D-glucose units in the oxidized starch is found by taking the difference between the determinations of the combined aldehyde and ketonic functions and the number of separately determined aldehyde functions. The reduced viscosities of the oxidized starches are measured also (see Table I).

It is evident from the values in Table I that very few aldehyde end

TABLE I

FORMATION OF OXIDIZED GROUPS IN OVEN-DRIED STARCH OXIDIZED BY CHLORINE GAS (1:3 RATIO) AT 18° ± 2°C.

TIME	GROUPS PER 100 D-GLUCOSE UNITS				
	Carboxyl	Aldehyde	Aldehyde and Ketonic	Ketonic	Reduced Viscosity
hr.					
In dark					
12	0	0	1.58	1.58	1.16
24	0	0	3.50	3.50	0.96
36	0.2	0	5.20	5.20	0.86
48	0.2	0.1	6.63	6.62	0.55
60	0.2	0.1	6.76	6.75	0.46
72	0.2	0.1	6.75	6.74	0.40
In light					
12	0.64	1.56	3.20	1.64	0.83
24	0.77	1.83	4.87	3.04	0.68
36	1.01	2.11	6.12	4.01	0.50
48	1.05	2.37	7.07	4.70	0.42
60	1.05	2.40	7.50	5.10	0.34
72	1.0	2.40	7.60	5.20	0.28

groups are formed during the dry oxidation in the dark, and that after 36 hr. there is no increase in oxidized functional groups in either dark or light reactions. The sum of the aldehyde and keto groups remains constant over the period 36–72 hr. However, over this period chlorine is still slowly consumed, as shown in Fig. 1, and the continued decrease in reduced viscosity indicates further depolymerization of starch molecules. This evidence could be interpreted to suggest that the oxidation in both dark and light reactions occurs in two distinct stages. Photoactivation markedly increases the extent of the initial oxidation. It is entirely possible that the initial fast reaction may be due to trace amounts of moisture and that the second slower oxidation stage may result from purely anhydrous chlorinolysis. It has already been suggested (3) that the extensive depolymerization encountered in dry starch is not due to hydrolytic cleavage of the glycosidic bonds. Such cleavage is significant in semidry starch (13% moisture), but even then it is not the most important depolymerization mechanism (3).

To elucidate the mechanisms of this oxidative cleavage of glycosidic bonds in the absence of light and under anhydrous conditions, the neutral products of chlorinolysis are examined from methyl alpha-D-glucopyranoside, maltose, and amylose in dry acetic acid. From maltose an 82% yield of neutral products is obtained; from amylose after the reaction sequence of chlorinolysis, borohydride reduction, hydrolysis, and acetylation, the neutral products were obtained in a 60% yield. Acidic products are apparently produced in relatively minor amounts and were not investigated.

The alkaline-treated syrup (B) obtained from the chlorine oxidation of methyl alpha-D-glucopyranoside, on quantitative chromatographic separation, is found to contain unreacted methyl alpha-D-glucopyranoside (22%), D-glucose (52%), and a third fraction ((26%). In addition, trace amounts of four different compounds having $R_{glucose}$ (R_{gl}) values similar to those of disaccharides are observed. The third fraction is a mixture of two components with R_{gl} values of approximately 1.7 and 1.8, the latter nearly identical with the R_{gl} value of levoglucosan. The mixture is isolated from Whatman 3MM papers. On acetylation, two acetylated products are obtained in equal proportion, as observed by thin-layer chromatography. One of the products is 1,6-anhydro-2,3,4-tri-*O*-acetyl-β-D-glucopyranose, m.p. 109°C., $[\alpha]_D^{25}$ − 45° (c, 2 in ethanol). It does not depress the melting point of authentic levoglucosan triacetate. The second component is an unidentified syrup which will be subjected to further investigation.

The alkaline-treated syrup (B) resulting from the chlorine oxidation of maltose contains unreacted maltose (55%), D-glucose (15%), and a third fraction (12%) also having an R_{gl} value 1.7–1.8. After acetylation, 1,6-anhydro-2,3,4-tri-*O*-acetyl-β-D-glucopyranose and an unknown acetylated product are present in the proportion of approximately 1:10, as visually indicated by thin-layer chromatography.

The syrup (A) obtained on chlorinolysis of amylose is reduced with an excess of sodium borohydride in water. After decomposition of the excess sodium borohydride, the reduced carbohydrate is hydrolyzed with 1*N* sulfuric acid at 90°C. for 8 hr. and neutralized with Amberlite IR-45. Paper chromatography indicates the presence of galactose, glucose, and sorbitol. As this mixture is difficult to separate by usual chromatographic techniques, the hydrolysate is again reduced with sodium borohydride and the excess of borohydride removed; the solution is concentrated to dryness and acetylated. Thin-layer chromatography separates two acetates. The major acetate is hexaacetyl-D-glucitol, m.p. 99°–100°C. (mixed m.p. 99°–100°C.). The second acetate obtained in small amount is hexaacetylgalactitol, m.p. 169°C. (mixed m.p. 168°C.). Yields of D-glucose and D-galactose, estimated chromatographically, are 55 and 5% respectively based on the amylose.

Discussion

Information on the mechanism of the depolymerization is obtained from the results of the chlorinolysis of amylose, and of the model compounds methyl alpha-D-glucopyranoside and maltose in glacial acetic acid. Initially, methyl alpha-D-glucopyranoside was reacted with chlorine in glacial acetic acid. After mild alkaline treatment of the

products to saponify any acetyl groups, the principal chlorinolysis products are D-glucose in 52% yield and levoglucosan in roughly 13% yield. An approximate 13% of another nonreducing component of undetermined structure is also found, along with small amounts of disaccharides. These results can be explained by the reaction mechanism shown in Fig. 2. This mechanism carries the assumption that a

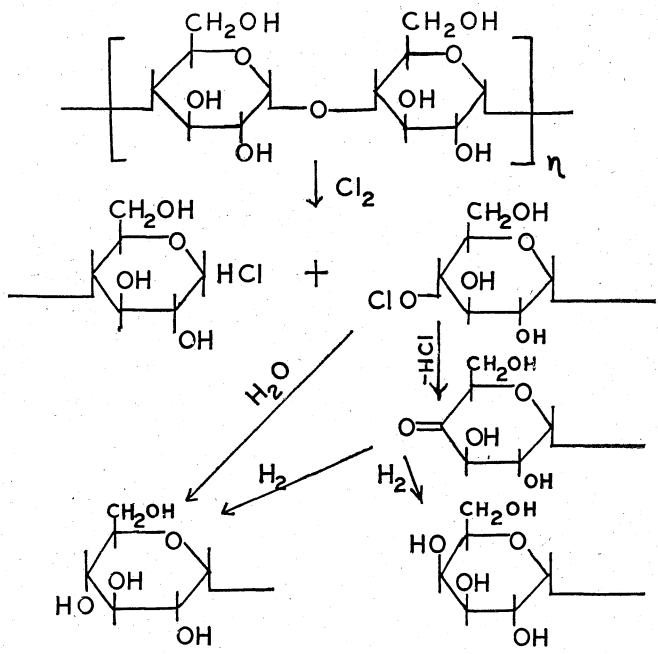

Fig. 2.

chlorine molecule coordinates with the glycosidic oxygen atom leading to ionization and attack on carbon C1 by an acetate anion, a chloride anion, or the hydroxyl group at C6 with the elimination of the aglycone conjugate as a hypochlorite ester. An aldose 1-acetate could also be formed by acetolysis of the glycosyl chloride (10). In the case of methyl alpha-D-glycopyranoside, the D-glucose 1-acetate formed would be relatively resistant to further oxidation and must be the major reaction product, since on saponification it would give rise to the large quantities of D-glucose observed and accounts for the absence of free D-glucose in the mixture prior to saponification. Any water produced from acetylation of the carbohydrate would compete with acetic acid in solvolysis of the D-glucopyranosyl chloride units and would thereby produce D-glucose units. These probably would be oxidized further to

D-gluconic acid. Since acidic oxidation products were not investigated, the extent of this secondary reaction is undetermined. The D-glucosyl chloride intermediate can also undergo intramolecular dehydrohalogenation to produce levoglucosan, or it may react with a hydroxyl group of another sugar unit to produce a disaccharide (Fig. 3).

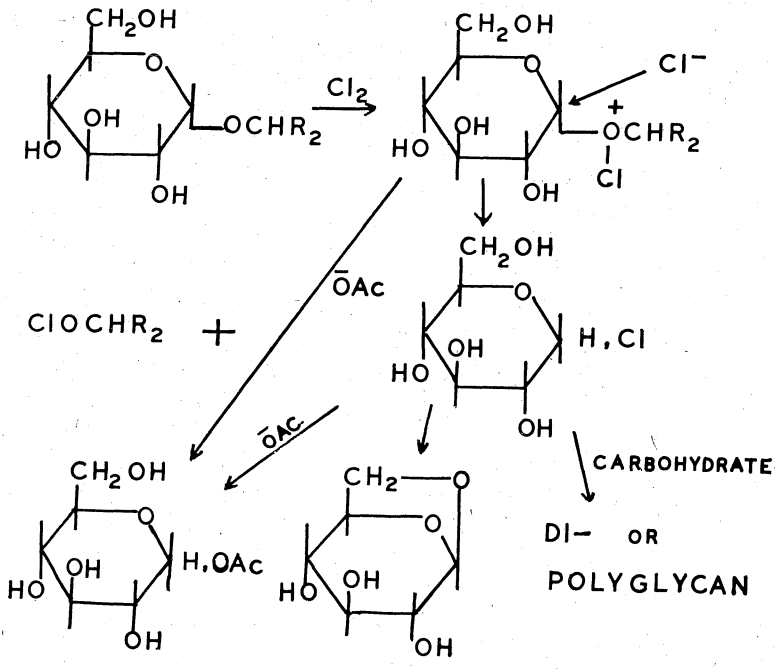

Fig. 3.

Further confirmation of this reaction mechanism is obtained by examining the chlorinolysis of maltose. Here again, free D-glucose and levoglucosan are obtained after mild saponification of the reaction products. In this reaction, levoglucosan and the unidentified nonreducing component are obtained in the proportion 1:10, as evidenced by thin-layer chromatography. When maltose is oxidized with chlorine in a molar ratio 1:1, about 50% of the maltose is recovered. This is surprising, as it indicates that chlorinolysis of the glycosidic bond occurs at a rate equal to, or greater than, the oxidation of the reducing end. Thus, depolymerization by chlorinolysis of the glycosidic bond appears to be a major mode of degradation of oligo- or polysaccharides during chlorine treatment.

The mechanism proposes that the aglycone portion of the glycoside

is eliminated as a hypochlorite ester. The hypochlorite ester can undergo hydrolysis to the alcohol or dehydrohalogenation to a carbonyl compound. In the case of 1→ 4-linked D-glucose units, dehydrohalogenation of the intermediate D-glucopyranose 4-hypochlorite units would produce a 4-keto-D-glucopyranose unit which on reduction would give some D-galactopyranose units (Fig. 2). This postulate is supported by observations with corn amylose. When amylose undergoes chlorinolysis in glacial acetic acid and the oxidized products are reduced and hydrolyzed, small quantities of D-galactose are observed. Further reduction and acetylation leads to the isolation of galactitol hexaacetate.

Previous mechanisms have suggested that chlorinolysis of glycosidic bond occurs between the oxygen and the aglycone carbon (11). Such a mechanism would predict that in aqueous chlorinolysis of oligo- or polysaccharides the glycosidic oxygen is either displaced from the aglycone carbon of the attached sugar by a hydroxyl ion which would lead to inversion, or a carbonium ion is involved, which would lead to racemization. However, no new sugars have been detected during chlorinolysis of oligo- or polysaccharides. Specifically, D-galactose is not found among the aqueous chlorinolysis products of starch or cellulose.

The proposed mechanism, along with other oxidative mechanisms, probably operates with glycosidic bonds under semidry and aqueous conditions. Here, however, the chloro-oxonium chloride complex, formed by coordination of chlorine with the glycosidic oxygen or the further glycosyl chloride intermediate, is hydrolyzed to the aldose. This may possibly explain the initial fast oxidation observed with "dry" starch. After moisture or bound water is consumed, the reaction slows down. This fast reaction, therefore, may be some measure of the water content of the starch (3) In aqueous oxidations the aldose can be further oxidized to the lactone, which is a major product of aqueous chlorine oxidations. The reaction mechanism proposed also explains the presence of small amounts of previously inexplicable aldose observed in aqueous chlorine oxidations of glycosides and polysaccharides.

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