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Modification of starch granules by hydrolysis with hydrochloric acid in various alcohols, and the formation of new kinds of limit dextrins*†

Jeffrey D. Fox‡ and John F. Robyt**
Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011 (U.S.A.)

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ABSTRACT

Potato starch was treated severally in methanol, ethanol, 2-propanol, and 1-butanol with 0.36% and 6.0% HCl at 25°. The average d.p. dropped rapidly for starch treated with 0.36% acid and reached a limiting value within 24 h. For the treatment with 6.0% acid, the limiting value was reached between 30 and 75 h. The limiting d.p. progressively decreased in the sequence methanol > ethanol > 2-propanol > 1-butanol, and also decreased with an increase in the concentration of the acid. The amylose and the amylopectin fractions were isolated from the modified starches, and their average d.p. and proportions were determined. The amylopectin fractions were analyzed by gel-permeation chromatography on Bio-Gel A-5m, before and after isoamylolysis, and the amylose fractions were analyzed on Bio-Gel A-0.5m. The above starch products represent a new type of limit dextrins that have different average d.p., chain-length distributions, and proportions of amylose and amylopectin. These differences represent variable susceptibilities of the glycosidic bonds to hydrolysis when the starch granules are placed in different alcohols with different concentrations of acid.

INTRODUCTION

In 1987, Ma and Robyt[1] reported the preparation and characterization of soluble starches that had different molecular sizes and different proportions of amylose and amylopectin by treatment with hydrochloric acid in various alcohols at 65°. Although the starch-granule morphology was retained, the molecular sizes progressively decreased in the order methanol > ethanol > 2-propanol > 1-butanol. The amylose component was completely absent from the starch treated in 2-propanol and 1-butanol. We now report further studies of the treatment of starch in the above alcohols with hydrochloric acid (0.36 and 6.0%) at 25°.

EXPERIMENTAL

Materials. — Potato starch was obtained from National Starch and Chemical Co. and contained 12% (w/w) of water. Bio-Gel A-5m and A-0.5m were obtained from

* Dedicated to Professor David Manners.
† Journal Paper No. J-14588 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 2416.
‡ Present address: Department of Biochemistry, University of Wisconsin, Madison, WI, U.S.A.
** Author for correspondence.

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Bio-Rad Laboratories (Richmond, CA) and isoamylase was from Sigma Chemical Co. (St. Louis, MO).

Treatment of the starch. — Potato starch (25 g) was suspended in anhydrous methanol, ethanol, 2-propanol, or 1-butanol (100 mL). Reaction was started by adding 1.0 or 20 mL of conc. (36% w/w) HCl to each sample at 25°. Each mixture was shaken occasionally, and the reaction was stopped at various times during a period of 165 h by filtering the starch from a 10-mL aliquot and washing with 200 mL of 1:1 methanol–water (2 × 200 mL).

Analysis of the modified starches. — Each treated starch was dissolved in hot water to a concentration of 25 mg/mL. The average degree of polymerization (d.p.) was determined[2] in triplicate by measuring the carbohydrate content by the phenol–sulfuric acid procedure[3] and the reducing value by the copper–bicinchoninate procedure[4] with a microsample plate reader[5].

Fractionation of the modified starches. — A solution of each modified starch (10 g) in hot water (100 mL) was cooled, 1-butanol (10 mL) was added, and the solution was stirred for 15 h at 24°. The resulting complex with 1-butanol was centrifuged, ethanol (2 vol.) was added to the supernatant liquor, and the amylopectin fraction was collected by centrifugation. Each precipitate was triturated four or five times with dry acetone, and once with dry ethanol, then dried under vacuum at 50°. The weight and d.p. of each fraction were determined.

Gel filtration of the fractions. — Each amylopectin fraction was dissolved in hot water to give a solution of 25 mg/mL. Samples of the solutions were each treated with isoamylase [1 unit/mg of amylopectin in 25mM acetate (pH 4.5) for 15 h]. Each amylopectin fraction (1 mL, 25 mg/mL), before and after hydrolysis with isoamylase, was eluted from a column (8 × 280 mm) of Bio-Gel A-5m with water and 1-mL fractions were collected. An iodine–iodide color was obtained by adding 25 μL of reagent (0.2 mg of I_2 and 2 mg of KI/mL) to 50 μL of each fraction, and the absorbance was measured at 600 nm.

Each amylose fraction (15 mg) was dissolved in 0.2 mL of methyl sulfoxide by stirring and gently heating, and 1 mL of water was added with stirring. Each amylose sample was added to a column (8 × 350 mm) of Bio-Gel A-0.5m and eluted with water; 1-mL fractions were collected. The iodine–iodide color was measured for each fraction as described above.

RESULTS AND DISCUSSION

The results of the modifications of potato starch at 25° with 0.36 and 6% HCl in each of the four alcohols are shown in Figs. 1 and 2, respectively. The lower concentration of acid yielded products with higher average d.p. but in each reaction the d.p. dropped rapidly. For the lower concentration of acid, the d.p. became constant after 24 h. For the higher concentration of acid, the d.p. became constant after 72 h in methanol and ethanol, and after 30 h with 2-propanol and 1-butanol. Although it had been expected that the d.p. values would continue to drop with time, they did not change after the limiting values were reached.

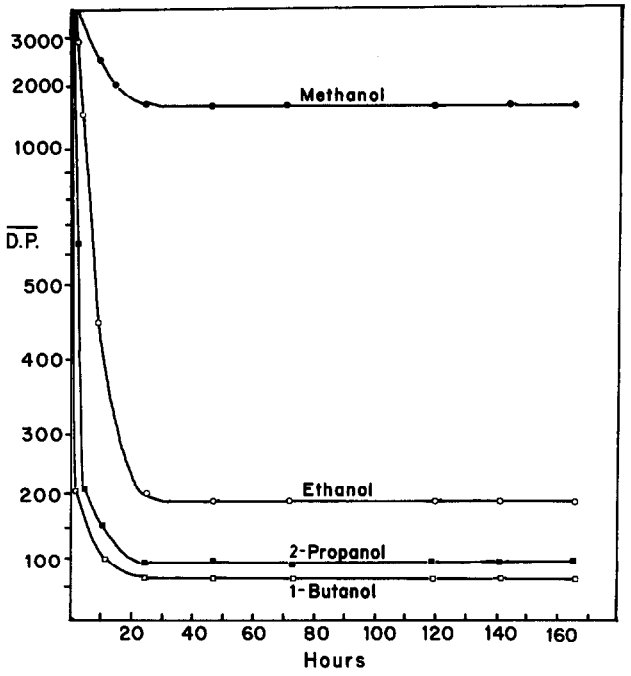

Fig. 1. Change of the average d.p. of potato starch as a function of the time of hydrolysis by 0.36% HCl at 25° in methanol (●), ethanol (○), 2-propanol (■), and 1-butanol (□).

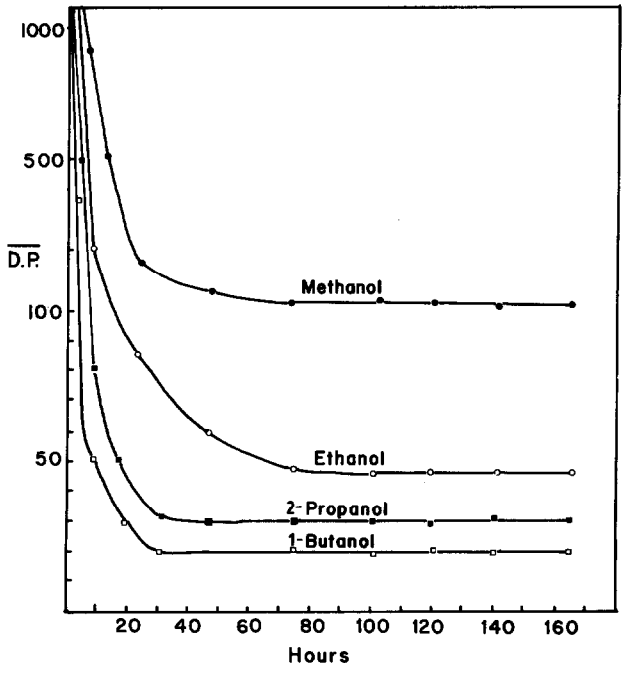

Fig. 2. Change of the average d.p. of potato starch as a function of the time of hydrolysis by 6.0% HCl at 25° in methanol (●), ethanol (○), 2-propanol (■), and 1-butanol (□).

TABLE I

Average d.p. of the limit dextrins obtained by treatment of potato starch with hydrochloric acid/alcohol at 25°

Alcohol	*D.p.*[a]	
	0.36% HCl	*6.0% HCl*
Methanol	1717 ±122	106 ±4
Ethanol	185 ±22	43 ±8
2-Propanol	90 ±4	30 ±1
1-Butanol	75 ±4	19 ±1

[a] Each d.p. was determined in triplicate.

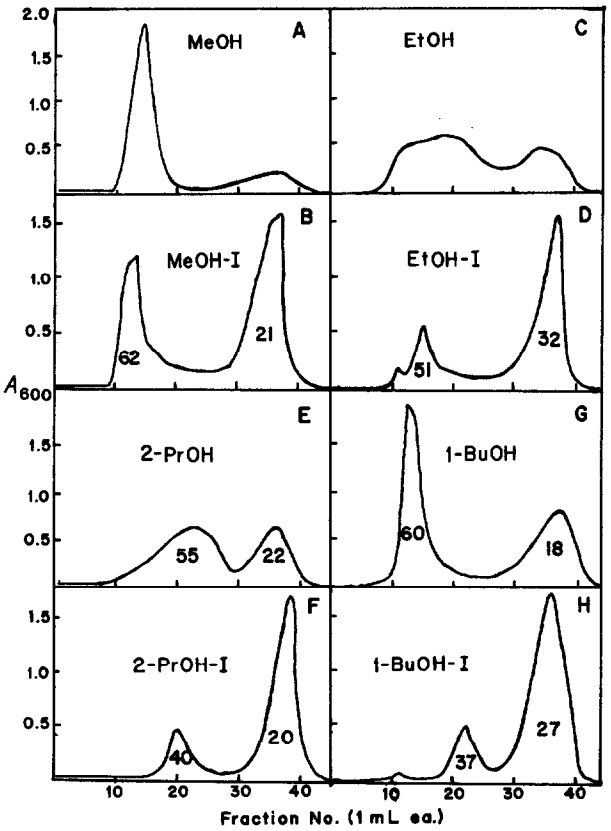

Fig. 3. Gel filtration on Bio-Gel A-5m of amylopectin fractions, isolated from potato starch modified by treatment with 0.36% HCl/alcohol at 25°, before (A, C, E, and G) and after (B, D, F, and H) treatment with isoamylase. The numbers under the peaks are the average d.p.

TABLE II

Average d.p. and proportions of amylopectin and amylose isolated from potato starch after treatment at 25° for 3 days with hydrochloric acid/alcohol

Alcohol	*0.36% HCl*				*6.0% HCl*			
	Amylopectin		*Amylose*		*Amyopectin*		*Amylose*	
	D.p.[a]	*Percentage*	*D.p.*	*Percentage*	*D.p.*	*Percentage*	*D.p.*	*Percentage*
Methanol	1855 ±81	75.6	1977 ±78	24.4	260 ±64	96	273 ±64	4
Ethanol	380 ±22	76.1	224 ±10	23.9	93 ±3	98	113 ±4	2
2-Propanol	178 ±9	91.7	120 ±10	8.3	66 ±3	99	70 ±5	1
1-Butanol	131 ±7	99.4	134 ±7	0.6	34 ±3	100	–	0

[a] D.p. determined in triplicate

The final d.p. values of the modified starches, given in Table I, depend on the concentration of the acid and the alcohol used. Thus, it is possible to obtain starch granules with relatively wide ranges of average d.p. by selecting the alcohol and the concentration of acid.

Table II gives the average d.p. and the proportions of the amylose and amylopectin fractions that were isolated from the different modified starch granules. The d.p.'s of the amylose and amylopectin fractions were relatively similar for the different types of modifications. As expected from the results with the whole starches, the two fractions showed a wide range of d.p., depending on the type of treatment of the starch. The d.p. for both amylopectin and amylose changed markedly on going from methanol to ethanol with 0.36% of HCl. The proportion of amylose in the 0.36% HCl/methanol- and 0.36% HCl/ethanol-modified starches, however, did not change significantly from that of the native starch, which contains 25% of amylose. The proportion of amylose was decreased markedly to 8.3% in the 0.36% HCl/2-propanol-modified starch. An

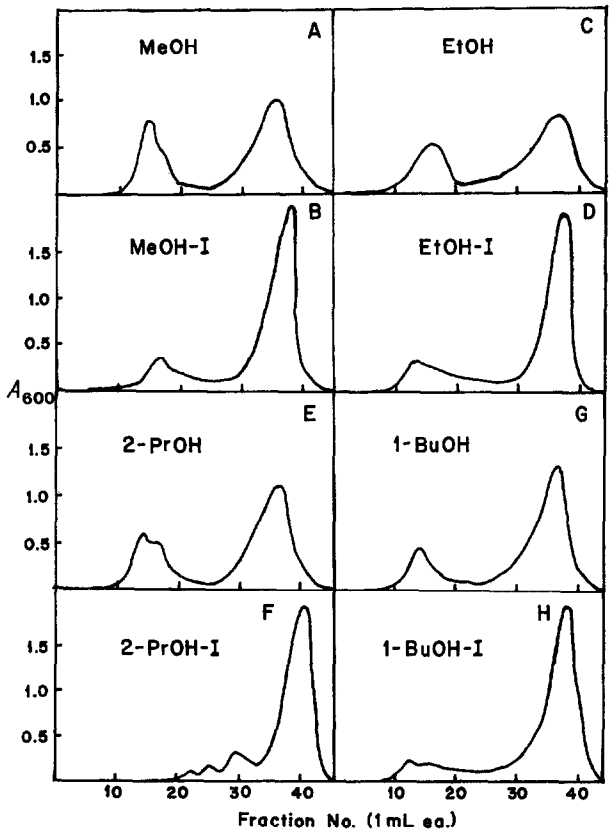

Fig. 4. Gel filtration on Bio-Gel A-5m of amylopectin fractions, isolated from potato starch modified by treatment with 6.0% HCl/alcohol at 25°, before (A, C, E, and G) and after (B, D, F, and H) treatment with isoamylase.

even more marked change occurred in the 0.36% HCl/1-butanol-modified starch in which the proportion of amylose was only 0.6%. An increase in the concentration of acid to 6% produced a decrease in the d.p. and a significant decrease in the proportion of amylose with each alcohol. The most marked change occurred in 6% HCl/methanol in which the d.p. dropped from ~1900 to ~270 and the proportion of amylose dropped from 24.4 to 4%. Amylose was completely absent from the 6% HCl/1-butanol-modified starch. The average d.p. values of the amylose and amylopectin fractions (Table II) were all higher than the average d.p. values of the mixture of the two in the modified starches (Table I), most probably due to losses of some smaller saccharide components in the fractionation procedure.

The amylopectin fractions were chromatographed on Bio-Gel A-5m before and after treatment with isoamylase, which hydrolyzes the (1→6)-branch linkages[6]. The amylopectin fractions obtained from starches treated with 0.36 and 6% HCl are shown in Figs. 3 and 4, respectively. The elution profiles of the products obtained with 6% HCl

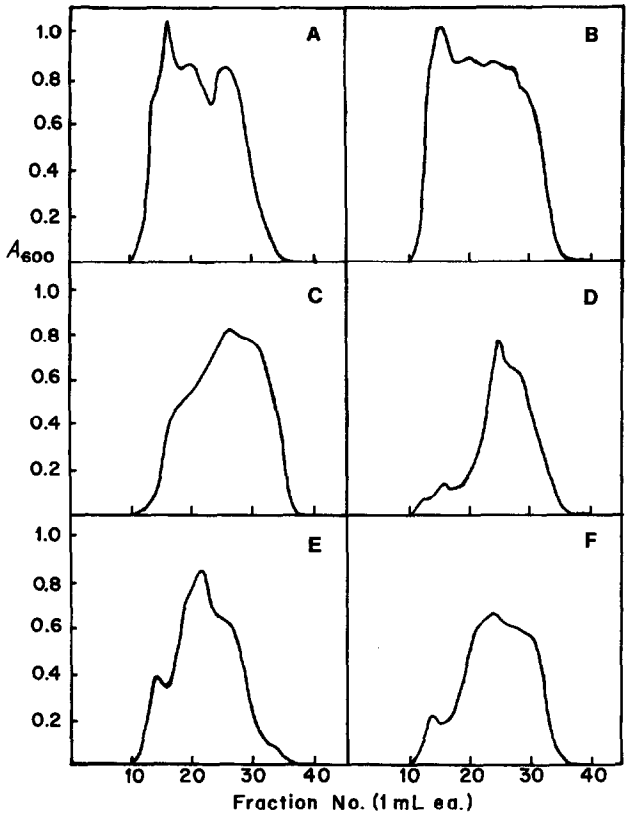

Fig. 5. Gel filtration on Bio-Gel A-0.5m of amylose fractions, isolated from potato starch modified by treatment with 0.36% (A–D) and 6.0% HCl (E and F) at 25°, in the alcohol indicated. A, Methanol; B, ethanol; C, 2-propanol; D, 1-butanol; E, methanol; and F, ethanol.

were relatively similar before and after treatment with isoamylase, with differences occurring primarily in the proportion of the lower molecular weight fraction obtained after treatment with the enzyme. However, the products obtained with 0.36% HCl gave elution profiles that were very different before and after treatment with isoamylase. An exception was the starch modified in 2-propanol with 6% HCl, which gave two fractions before treatment with isoamylase, and mainly one slow-moving fraction after the treatment (*cf.* Figs. 4E and 4F). The average d.p. values of the amylopectin fractions obtained by hydrolysis with 0.36% HCl in 2-propanol or 1-butanol (Table II) were higher than those of the amylopectin fractions obtained by chromatography on Bio-Gel A-5m (Figs. 3E and 3G). The amylose fractions were chromatographed on Bio-Gel A-0.5m and the elution profiles (Fig. 5) show relatively broad and heterogeneous distributions of chain lengths.

It has been shown previously[1] that the various alcohols produce different modifications of native starch granules because of different concentrations of acid *inside* the granule, which contains 10–12% of water. The present study shows that, in addition to hydrolysis occurring inside the granule, there is differential susceptibility to hydrolysis of the various glycosidic bonds in the granule, depending on the alcohol used and the concentration of the acid. This difference in glycosidic bond susceptibility is indicated by the variation in d.p. of the limit dextrins. A limiting average d.p. was obtained and hydrolysis ceased between 24 and 72 h, depending on the concentration of acid and the alcohol used. Thus, new types of limit dextrins can be produced by appropriate choice of alcohol and concentration of acid. The method may also be useful in studying and understanding the structure of the starch molecules in the granule.

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