Lintnerized Starches. Gel Filtration and Enzymatic Studies of Insoluble Residues from Prolonged Acid Treatment of Potato Starch

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ABSTRACT

Granular potato starch was hydrolyzed with 2.2N hydrochloric acid (HCl) at 35°C for periods of time up to 40 days. After removal of the acid and soluble carbohydrates, the residues (lintnerized potato starches) were washed with deionized water and dried to 10% moisture. When the percentage of solubilized carbohydrate was plotted vs. time, the curve showed two different steps. The first step, with a high rate, was attributed to hydrolysis of the amorphous part of starch granules, and the second step, with a lower rate, to hydrolysis of the more organized areas. X-ray patterns, apparent amylose content, and iodine absorption spectra of the HCl residues were determined. Sephadex G-50 gel-permeation chromatography of the dissolved residues was performed directly and after successive enzymatic digests with pullulanase and \( \beta \)-amylase. The results show the progressive appearance of two major chain populations: II, DP 25, and III, DP 15. II was identified enzymatically as being singly branched, and III, primarily as linear chains.

III appears as the more acid-resistant and crystalline part of the starch granules, as confirmed by X-ray diffraction. From the molecular size of III, the calculated dimension of the starch crystallites would be about 60 Å in the chain axis direction. The amylose fraction is rapidly degraded, and there is no evidence for amylose participation in the crystalline areas. Amylopectin chains are primarily responsible for the crystallinity of starch, imposing the crystallite dimension. The chains of DP 15 which constitute these crystallites could originate from degraded amylose chains, or from the external A-chains or internal chains of amylopectin. These results lead to a new model of amylopectin containing clusters of highly ordered DP 15 chains.

During the past 10 years, acid hydrolysis of starch according to Lintner (1) has been used mainly to study, by electron microscopy, the structure of the granule (2-6). There have been few studies on the chemical characteristics of the residues obtained after lintnerization, except the very recent investigations of Kainuma and French (7,8) on Nágeli amylopectins. The present study was undertaken to investigate the composition and the nature of the residues of potato starch during lintnerization. Results obtained by gel chromatography and enzymatic treatment lead to a new model for amylopectin structure.

MATERIALS

The starch used was commercial potato starch, with a moisture content of 18.9% and apparent amylose content of 20%, determined by the amperometric method (9). Sephadex G-50 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Pullulanase of \textit{Enterobacter aerogenes} (\textit{Aerobacter aerogenes}), a specific enzyme for \( \alpha-1,6 \) linkages, was purified according to Mercier et al. (10). Crystallized sweet potato \( \beta \)-amylase, purified \textit{Aspergillus niger} glucose oxidase (grade II), and horse radish peroxidase (grade I) were obtained from Sigma Chemical Co., St. Louis, Mo. \textit{A. niger} glucoamylase was prepared according to Qureshi (11).

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METHODS

Lintnerization of Potato Starch

Ungelatinized potato starch was hydrolyzed at 35°C. t a concentration of 1.67 g. of dry starch in 100 ml. of 2.2N hydrochloric acid. After various times up to 40 days, the insoluble residue was obtained by vacuum filtration on a porous glass filter (5 to 15 μ). The residue was washed with deionized water to remove chloride ions, residual acid, and soluble carbohydrate. Each residue was dried to 10% moisture under vacuum, at 20°C., over phosphorus pentoxide. The residue was called "lintnerized starch" although the acid treatment was more drastic and more extensive than described by Lintner (1).

Extent of Starch Hydrolysis

The extent of starch hydrolysis with time was followed by measuring the carbohydrate in the filtrate using the anthrone method (12). Results are expressed as percent of the initial starch. For example, "15% lintnerized starch" represents degradation of 15% of the original polysaccharide to soluble form.

Fine Structure of Starch Residues

The fine structure of native and 15, 70, and 85% lintnerized potato starches was studied with a combination of enzymatic action (pullulanase and β-amylase) and chromatography as illustrated in Fig. 1.

Enzymatic Hydrolysis of Native and Lintnerized Starch

Native and lintnerized starches were dispersed in water at 100°C. Debranching with pullulanase and β-amylolysis were carried out as described by Mercier and Whelan (13). Before chromatography and between successive enzymatic actions, the enzyme was inactivated by heating the digest at 100°C. for 20 min. Debranching and β-amylolysis were followed by determining the increase in reducing power (14). Enzymatic action was stopped when the reducing power became constant (24 to 48 hr.).

Fig. 1. Enzymatic method for the study of α-glucan structure.
Chromatographic Analysis

Solutions of native and lintnerized starch before and after enzymatic actions were chromatographed on a Sephadex G-50 column (2.5 × 100 cm.) at 20°C. The elution was carried out with an upward flow using a 10mM phosphate buffer, pH 7.0, containing 0.02% sodium azide to prevent bacterial growth. The column was calibrated with debranched waxy maize starch by obtaining the DP (ratio of the total carbohydrate to reducing value) of fractions at various elution volumes (13).

For chromatography of solutions of native and lintnerized starch before any enzymatic action was performed, 2-ml aliquots containing 1 to 2 mg. of polysaccharide were introduced at the lower part of the column with a peristaltic pump. Elution flow was adjusted to 20 ml. per hr. The elution patterns were recorded directly with a Technicon AutoAnalyzer (transmittance at 420 nm., orcinol-sulfuric acid reagent) (15).

Chromatography of enzymatic digests was performed at 25 ml. per hr. with 2-ml. aliquots containing 5 to 15 mg. of polysaccharides. To avoid artifacts in the elution patterns from nonstarch carbohydrates in the enzyme preparations, the starch-type polysaccharide in each 30-min. fraction was determined by hydrolysis with fungal amylglucosidase as described by Lee and Whelan (16).

Liberated glucose was measured with the glucose oxidase-peroxidase system as by Lloyd and Whelan (17). The elution patterns so obtained represent the weight of polysaccharide expressed as mg. of glucose per 100 mg. of polysaccharide recovered vs. elution volume. Thus these patterns were comparable since each corresponded to 100 mg. of polysaccharide. In all cases, the recovery was 90 to 100%.

Other Determinations

Apparent amylose was determined by an amperometric method (9). The wavelength of the maximum absorbance (λmax) of the lintnerized starch-iodine complex was determined by a modification of the method of Bailey and Whelan (18). The X-ray patterns were recorded with a spectrodiffraclometer (Compagnie Générale de Radiologie, France) as by Charbonniere et al. (19).

Fig. 2. Hydrolysis of potato starch in 2.2N HCl at 35° C.: curve 1) percentage (X) of hydrolyzed starch vs. time of hydrolysis; curve 2) log10 [100/(100–X)] vs. time of hydrolysis.
RESULTS

Hydrolysis Kinetics and Some Characteristics of Lintnerized Starch

The extent of hydrolysis (X) vs. time is shown in Fig. 2, curve 1. Curve 2 in this figure shows the change of $\log_{10} \left[ \frac{100}{(100-X)} \right]$ vs. hydrolysis time.

Figure 3 shows the decrease in apparent amylose content of starch during lintnerization.

The variation of $\lambda_{\text{max}}$ of the iodine complexes of lintnerized starches vs. $\log_{10}$ of hydrolysis time in hours is plotted in Fig. 4, curve 1. The average chain length of the residues, calculated according to Bailey and Whelan (18) and Lee$^2$, is shown in Fig. 4, curve 2.

In Fig. 5 are compared the X-ray diffractograms of native potato starch (pattern 1), and 85% lintnerized starch (pattern 2), corresponding to 40 days of treatment.$^3$

Gel Filtration of Native and Lintnerized Starch Before Enzymatic Hydrolysis

The aqueous, dispersed native starch is excluded from the Sephadex G-50 (Fig. 6, pattern 1, peak I) since, according to Pharmacia, the maximum molecular weight which will permeate the gel particle is about 10,000 (DP about 60). With increasing hydrolysis the elution patterns show the progressive appearance of two peaks II and

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$^3$The complete results of X-ray study of various lintnerized starches, including lintnerized potato starch, will be published elsewhere.
Fig. 4. Lintnerized potato starch iodine complex: curve 1) change of $\lambda_{\text{max}}$ with time (logarithmic scale); curve 2) change of chain length with time as calculated from $\lambda_{\text{max}}$.

Fig. 5. X-ray diffractograms recorded at 27% $H_2O$ (dry basis): curve 1) native potato starch; curve 2) 85% lintnerized potato starch.

III (Fig. 6) which represent two distributions of chain populations with $DP$ independent of the extent of hydrolysis. Calibration of the Sephadex column (13) allows us to assign $DP$ 25 to the peak II population and $DP$ 15 to peak III. These populations arise from the initial population I and from its “associated fraction” or low-molecular-weight trail located between peaks I and II. Peaks II and III are
already visible at 2.6% hydrolysis, obtained after 10 hr. of treatment (Fig. 6, pattern 2). Then peak I gradually disappears, its "associated fraction" and peaks II and III becoming larger. At 52% hydrolysis, obtained after 8 days of treatment, peak I has completely vanished (Fig. 6, pattern 5). At greater extents of hydrolysis, the components of peak II seem to be more rapidly hydrolyzed than those of peak III. Population III finally dominates the elution pattern.

Gel Filtration of Native and Lintnerized Starch after Pullulanase Debranching

Debranching of native potato starch with pullulanase (P₁) gives three main components (Fig. 7, pattern 2) designated A (45%), B (22%), and C (33%). The ratio of population A to population B is approximately 2 on a weight basis and 5.4 on a molecular basis. The presence of A and B populations after debranching of starch is in agreement with previous results obtained on waxy maize starch (13,20).

Consecutive action of pullulanase and β-amylase (P₁β₁) transforms the chains of all three populations into glucose, maltose, and maltotriose (Fig. 7, pattern 3, peak b), these products being identified by TLC. This result proves the linearity of A, B, and C. The A-population, which has the same position as the peak III population in lintnerized starch, consists of linear chains of DP 15, and the intermediate B-population contains linear chains of DP 45. Peak C consists of linear chains of DP > 60 excluded from the gel matrix. The difference between the proportion of C (33%) and the apparent amylose content in native starch (20%) indicates that C contains 13% of long linear chains liberated during the pullulanase debranching of the branched fraction.

The elution pattern of debranched 15% lintnerized starch (2 days of treatment) shows only A and B populations (Fig. 7, pattern 5). Population C, observed in elution pattern of debranched native starch (Fig. 7, pattern 2) has disappeared completely. Compared with pattern 4, obtained before debranching, pattern 5
Fig. 7. Elution patterns from a column (2.5 × 100 cm.) of Sephadex G-50 of native and 15, 70, and 85% linterized potato starch after treatment with pullulanase (P₁) or consecutive treatment with pullulanase and β-amylase (P₁, β₁). For each pattern, the enzyme treatment and percent hydrolysis are: 1) native; 2) P₁, native; 3) P₁, β₁, native; 4) 15%; 5) P₁, 15%; 6) P₁, β₁, 15%; 7) 70%; 8) P₁, 70%; 9) P₁, β₁, 70%; 10) 85%; 11) P₁, 85%; 12) P₁, β₁, 85%. Populations I, II, and III as in Fig. 6: populations A, B, and C are resistant to pullulanase action; populations a and b are products of pullulanase and β-amylase action.

shows that peak B is located as the “associated fraction” between peaks I and II and that peak A is larger than the initial peak III. This increase results from the contribution of linear chains coming from the debranching of certain chains from population I, the “trail”, and population II. The linearity of these chains is verified, as in the case of native starch, by β-amylolysis (P₁β₁) yielding glucose, maltose, and maltotriose (Fig. 7, pattern 6).

The elution pattern of debranched 70% linterized starch (20 days of treatment) shows the complete disappearance of peak B (Fig. 7, pattern 8), only peak A remaining. Comparison with pattern 7 proves that peak A was produced in part by debranching peak II.

The apparent degree of hydrolysis (T₁)⁴ of the 70% linterized starch, after

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4The apparent degree of β-amylolysis, T₁, is the ratio of carbohydrate in peak b to the total carbohydrate. This ratio is also used when the last digest is a debranching to estimate the yield, T₃, of oligosaccharides of DP 2 through 10 which are included in peak b along with glucose, maltose, and maltotriose produced by β-amylolysis.

The real β-amylolysis ratio, T₂, is calculated from the reducing power of a β-amylolysis digest after 20 hr., and expressed as percent maltose. It indicates more exactly the proportion of polysaccharide material transformed into glucose, maltose, and maltotriose.

The β-amylolysis ratios T₁ and T₂, the oligosaccharide yields, or the proportions of polysaccharides contained in peaks a and b, are calculated as percent of the weight (C₆H₁₂O₆ basis) of polysaccharide material contained in the digests or recovered after chromatography.

The amounts of starting material and product are reported on a C₆H₁₂O₆ basis.
consecutive action of pullulanase and β-amylase (P₁β₁), is 98.2%. An
enzyme-resistant fraction is revealed on the elution pattern (Fig. 7, pattern 9) as a
broad, weak peak a, DP between 30 and 10. Under our experimental conditions
peak a is not observed for native starch or for the 15% lintnerized starch after P₁β₁.
The elution pattern of debranched 85% lintnerized starch (40 days of
treatment) shows only peak A (Fig. 7, pattern 11). The complete disappearance of
peak II, still faintly present on the elution pattern of the lintnerized starch before
debranching (Fig. 7, pattern 10), indicates its branched nature. The ratio T₁ after
β-amylolysis (P₁β₁) is 98.1%. As with the 70% residue, there is also an
enzyme-resistant population a (Fig. 7, pattern 12).

**Gel Filtration of Native and Lintnerized Starch after β-Amylolyis**

The real limit T₂₄ of direct β-amylolysis (β₁) of native starch is 57%. The β-limit
dextrin is completely excluded from the gel matrix (peak I) and the reaction
products, essentially maltose and maltotriose, appear in peak b (Fig. 8, pattern 2).
After removal of maltose and maltotriose by dialysis, the β-limit dextrin was
debranched by pullulanase (β₁P₁) and the elution pattern shows three main peaks
(Fig. 8, pattern 3). Linearity after β₁P₁ (pattern 3) was confirmed by further
β-amylolysis (β₁P₁β₂) which gave only peak b material (pattern 4). The first peak

![Figure 8](image-url)

*Fig. 8. Elution patterns from a column (2.5 x 100 cm.) of Sephadex G-50 of native and 15, 70,
and 85% lintnerized potato starch after β₁, β₁P₁, and β₁P₁β₂ treatments (see text for
explanation of enzyme treatments). For each pattern, the enzyme treatment and substrate are:
1) native; 2) and 2₁) β₁, native; 3) β₁P₁ of native β-limit dextrins under 2₁; 4) β₁P₁β₂ of
native debranched β-limit dextrins; 5) 15%; 6) β₁, 15%; 7) β₁P₁, 15%; 8) β₁P₁β₂, 15%; 9) 70%;
10) β₁, 70%; 11) β₁P₁, 70%; 12) 85%; 13) β₁, 85%.*
Table 1. Apparent and Real β-Amylolysis Limits (T2 and T3) After β1 and
β1, P1, β2 Treatments, Yield (T3) After β, P1 Treatment,
and Percentages of β-Limit Dextrins Obtained From
15% Lintnerized Starch

<table>
<thead>
<tr>
<th>Enzyme treatment</th>
<th>T2 %</th>
<th>T1 or T3 %</th>
<th>DP &gt; 10b %</th>
<th>DP 2 to 10c %</th>
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</thead>
<tbody>
<tr>
<td>β1</td>
<td>63.5</td>
<td>73.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β1, P1</td>
<td>...</td>
<td>73.5 + 19.5 = 93.0</td>
<td>26.5d</td>
<td>10.0</td>
</tr>
<tr>
<td>β1, P1, β2</td>
<td>96.0</td>
<td>93.0 + 3.0 = 96.0</td>
<td>7.0e</td>
<td>...</td>
</tr>
</tbody>
</table>

aT2 was obtained by measurement of total reducing value. T1 and T3 were obtained by measuring the area under peak b. See footnote 4.
bObtained from 100−T1 (or 100−T3).
cFrom T1−T3; represents the short β-limit dextrins contained in peak b.
dAmount of β-limit dextrin with DP > 10, from Fig. 8, pattern 6.
eUndebranched β-limit dextrins and debranched chains of DP > 10 (Fig. 8, pattern 7, peak a).
fUndebranched β-limit dextrins of DP 10 to 30 (Fig. 8, pattern 8, peak a).

of pattern 3 (peak I, 6%) consists of long linear chains excluded from the gel matrix. The intermediate second peak (50%) contains essentially linear chains of DP 30 through 35. The third peak (44%) contains maltose and maltotriose derived from the external stub branches of the β-limit dextrin and smaller β-amylase-treated internal chains of branched fraction of starch (Fig. 8, pattern 3, peak b). The second and third peaks of pattern 3 corroborate the profile obtained by Lee et al. (20) and Akai et al. (21) from the pullulanase-debranched waxy maize starch β-limit dextrins.

The main results obtained from 15% linterized starch after β-amylolysis, pullulanase debranching, and a second β-amylolysis are summarized in Table I. The limit T2 of direct β-amylolysis (β1) of the 15% linterized starch is 63.5%. However, the apparent limit T1, obtained from the Sephadex fractionation, is 73.5%, indicating that peak b contains short β-limit dextrins with DP < 10 (Fig. 8, pattern 6). Debranching (β1, P1) of the β-limit dextrin increases to 93% the yield T3 of oligosaccharides of DP 2 through 10, and a resistant fraction appears as peak a (Fig. 8, pattern 7) already described (Fig. 7, pattern 9). After further β-amylolysis (β1, P1, β2), both T1 and T2 finally rise to 96%. The enzyme-resistant fraction a is seen in Fig. 8, pattern 8, as in Fig. 7, patterns 9 and 12, and is equal to 4.0% of the total carbohydrate.

The ratios T1 and T2 of direct β-amylolysis (β1) of 70% linterized starch are 98.0 and 86.4%, respectively. But in contrast to 15% linterized starch, debranching (β1, P1) after β-amylolysis does not increase the reducing power or the yield T3 of oligosaccharides of DP 2 through 10. The remaining a-fraction is only 2.0% of the total polysaccharide in β1 or β1, P1 digests (Fig. 8, patterns 10 and 11). A further β-amylolysis (β1, P1, β2) neither modifies the quantity of this resistant fraction nor the real or apparent β-amylolysis ratios, respectively equal to 86.7 and 97.8%.

The ratios T1 and T2 of direct β-amylolysis (β1) of 85% linterized starch are 97.8 and 87.5%, respectively. The remaining a-fraction is 2.2% (Fig. 8, pattern 13). As with 70% linterized starch, a further debranching (β1, P1) and a final β-amylolysis (β1, P1, β2) do not modify the β-amylase limits.

The differences observed between T1 and T2 after direct β-amylolysis (β1) are 10.0, 11.6, and 10.3%, respectively, after 15, 70, and 85% linternerization. This
difference is explained by the presence of short $\beta$-limit dextrans of DP 2 through 10 included in peak $b$, as shown by paper chromatography. These short $\beta$-limit dextrans come from branched chains of population II. For 15% lintnerized starch, these dextrans corresponding to 10% of peak $b$ are further debranched, with final limits $T_1$ and $T_2$ near 96% after $\beta_1 P_1 \beta_2$ treatments. In the case of the 70 and 85% lintnerized starch, approximately 10% of peak $b$ consists of pullulanase-resistant dextrans.

The $T_1$ and $T_2$ $\beta$-amylase limits after $\beta_1, \beta_1 P_1 \beta_2$, and $P_1 \beta_1$ treatments, and the percentages of residual enzyme-resistant fractions of DP 10 through 30 (peak $a$) and of DP 2 through 10 (peak $b$) are summarized in Table II.

**DISCUSSION**

The results show that, from the beginning of acid hydrolysis, potato starch is transformed into a branched chain population, II, of apparent DP 25 and into an essentially linear population, III, of DP 15. These fractions are progressively produced from amylase and amyllopectin present in peak I and from the “trailing” chains eluted between peaks I and II. Then, as the extent of hydrolysis increases, the proportion of residual linear chains increases as shown by the $\beta$-amylolysis limit (Table II). Simultaneously, the entire chain population is degraded to DP 15, which corresponds to the acid-resistant population III.

Pullulanase-debranching of native and lintnerized starch transforms the branched material of population I, the “trail,” and population II into linear chains.

![Diagram](image_url)

Fig. 9. Three possible modes for acid-catalyzed rupture on an $\alpha$-1,4 linkage (position 1, 2, or 3) around an $\alpha$-1,6 branching point located at two different levels (a or b) on the same amylopectin chain: A) A-chain; B) B- (or C-) chain.
<table>
<thead>
<tr>
<th>Starch Substrate</th>
<th>β-Amylolyis Limits, %</th>
<th>Enzyme-Resistant β-Limit Dextrans, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>after β₁ T₁&lt;sup&gt;a&lt;/sup&gt; T₂&lt;sup&gt;a&lt;/sup&gt;</td>
<td>after β₁ P₁ β₂ T₁&lt;sup&gt;a&lt;/sup&gt; T₂&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>DP 10 to 30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DP 2 to 10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Native</td>
<td>... 57.0</td>
<td>100 100</td>
</tr>
<tr>
<td>15% Lintnerized</td>
<td>73.5 63.5</td>
<td>96.0 96.0</td>
</tr>
<tr>
<td>70% Lintnerized</td>
<td>98.0 86.4</td>
<td>97.8 86.7</td>
</tr>
<tr>
<td>85% Lintnerized</td>
<td>97.8 87.5</td>
<td>97.8 87.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>See footnote 4.
<sup>b</sup>Obtained from 100-T₁; represent the undebranched long β-limit dextrans contained in peak a.
<sup>c</sup>Obtained from T₁-T₂; represent the undebranched short β-limit dextrans contained in peak b.
<sup>d</sup>Nonsignificant result.
The linear chains obtained with native starch are distributed into two populations: A (DP 15) and B (DP 45). Remarkably, population A, formed by pullulanase debranching, has the same DP as population III, formed by direct acid hydrolysis. The chains of peak A, being the sum of the initial population III plus the linear chains formed by debranching populations I, “trail,” and II, are more and more numerous as hydrolysis progresses (Fig. 7, pattern 5). This fact indicates that the material of DP 45 (population B) is preferentially hydrolyzed to the linear chains of population A. Furthermore, the debranching of population II (Fig. 7, patterns 7 and 8) into a linear material of DP 15 (peak A) suggests the singly branched nature of this population, where both the main chains and branches would be of DP 15.

Acid hydrolysis of the singly branched chains of population II seems to occur in preference to hydrolysis of the chains of population III (Fig. 6). If acid rupture of population II occurs near the branching point, it could give two chains of DP 15, increasing population III, plus some acid-soluble material.

The undebranched, short β-limit dextrins (USβ-LD) shown in Figs. 7 and 8, peak b (DP 2 through 10) and the undebranched, long β-limit dextrins (ULβ-LD) in peak a (DP 10 through 30), mainly appearing after a further β-amylolysis of the singly branched chains of population II, indicate that the α-1,6 branching points can be located rather near the reducing group for the USβ-LD, and spaced by about 15 glucose units from the reducing group of the carrier chains for the ULβ-LD.

Formation and Nature of Undebranched Limit Dextrins

Resistance to pullulanase action could result from the presence of a pullulanase-stable branching linkage, such as α-1,2 or α-1,3, from a steric obstacle caused by the presence of two adjacent branches linked α-1,6 on the same main chain, or from the presence of a single glucose unit stub (22). Although the presence in starch of α-1,3 linkages was suggested by Wolfrom and Thompson (23), there has been no further evidence for such an origin of pullulanase-resistant limit dextrans. Recently Kainuma and French (24) showed the absence of two adjacent α-1,6 linkages in starch. Therefore the suggestion of single glucose unit stubs seems to be most likely.

Under our experimental conditions, the ULβ-LDs are not observed after debranching of native starch or after β₁P₁β₂ treatment of waxy maize starch (13). These results suggest that the two types of limit dextrans originate directly from lintnerization or indirectly after β-amylolysis of the lintnerized residue. In the latter case, the acid treatment could produce a structure in the residual chains which would prevent the debranching after the action of β-amylase.

The enzyme-resistant structure could be explained by considering Figs. 9 and 10. In Fig. 9, we show the three different positions for possible acid cleavage of an α-1,4 link near the α-1,6 branching point, at two different levels (a and b) separated by 15 glucose units. In Fig. 10 we show the three initial structures, resulting from the acid rupture at positions 1, 2, or 3 indicated in Fig. 9, which could lead to the formation of USβ-LD and ULβ-LD.

Whenever cleavage occurs in position 1, the resulting “stubbed” dextrin would be resistant to pullulanase action. Hydrolysis in position 2 results in resistance only with odd-membered A-chains after action of β-amylase (25, 26). When acid hydrolysis occurs at position 3, the β-limit dextrans are eventually debranched by pullulanase giving maltose and maltotriose (27).
The USβ-LD and ULβ-LD with glucosyl stubs are analogous to stubbed oligosaccharides obtained from starch after successive actions of acid, glucoamylase and pullulanase (24).

The schemes proposed in Figs. 9 and 10 account for the higher percentage of resistant dextrans when the enzymatic treatment begins with β-amylolysis rather than with debranching (Table II). Thus, after P1β1 of the 15% linterized starch, the ULβ-LDs are absent, but represent 4% after β1P1β2. This also suggests that at this period of acid hydrolysis, α-1,4 cleavages occur more frequently in position 2 than in position 1, position 1 rupture being observed more with 70% linterized starch. Also it is suggested that for 15% linterized starch, the ruptures in position 2 preferentially occur at level a, since short β-limit dextrans obtained after β1 treatment of this residue (10% of peak b) are further debranched, whereas the short β-limit dextrans obtained with 70 and 85% linterized starches are pullulanase resistant. Indeed if a rupture in position 2 at level b occurs, the USβ-LD should be obtained after β1 in peak b, which is not the case, since the T1 and T2 ratios after β1P1β2 are identical (96%).

Origin and Nature of Acid-Resistant Fraction

The X-ray study of linterized starch (Fig. 5) clearly shows that the acid hydrolysis preferentially acted on the amorphous part of starch, and progressively produced an increasingly crystalline material represented by populations II and III. This result corroborates the findings of Kainuma and French (7) for sulfuric acid-treated starches (Nageli amylopectins). Thus it was evident that the first stage of hydrolysis shown in Fig. 2 (0 to 8 days) corresponds to the hydrolysis of the more amorphous part of the starch granules, while during the second stage (8 to 40 days) the more crystalline material is slowly hydrolyzed. The constant molecular size of populations II and III during the course of linterization can be explained by the absence of degradation within the crystallites, suggesting that water or H3O+...
ion could not penetrate into the crystalline phase. Very slow hydrolysis could proceed by the progressive erosion of external crystallite surfaces.

Our data indicate that the starch crystallites consist of closely packed chains of about 15 glucose units. According to Senti and Witnauer (28) the distance between glucose units in a fully extended amylose helix is about 4 Å. Thus the calculated dimension of a starch crystallite, consisting of a compact association of chains of DP 15, is close to 60 Å along the chain axis. This figure agrees with the one estimated by Meyer and Bernfeld (29) and calculated from electron micrographs by Sterling and Pangborn (30) for the diameter of microfibrils observed in lintnerized

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**Fig. 11.** Proposed structure for potato amylopectin. 1 = compact area; 2 = less compact area, rich in branching points; \( \phi \) = reducing unit.
potato starch, but it is lower than the figure of 146 Å for globoid amylopectin micelles estimated from X-ray diffraction data by Hizukuri and Nikuni (31).

The complete disappearance of peak C (Fig. 7, pattern 5) after P₁ of 15% lintnerized starch indicates that amyllose, which constitutes the main part of this peak in native starch (Fig. 7, pattern 2) is strongly acid-hydrolyzed. Thus the peak I in Fig. 7, pattern 4, corresponds to residual amylopectin. Figures 3 and 4 indicate the rapid hydrolysis of amylose, the apparent amylose content becoming almost nil for 15% lintnerized starch (λ<sub>max</sub> 555 nm. and CL 38). The curve in Fig. 4 can be attributed only to the hydrolysis of the amylose fraction of starch since the λ<sub>max</sub> of the purple amylopectin iodine complex is not greatly affected by hydrolysis<sup>5</sup> or by debranching (32). Figure 4 also corroborates the decrease of DP with time as obtained by gel chromatography.

It is difficult to account for the preferential hydrolysis of amylose and its complete elimination in residual polysaccharide after 15% lintnerization. Our results only indicate the rapid splitting of amylose into chains of DP < 60, too short to form a blue iodine complex. It is possible that some of the degraded amylose chains could eventually appear in population III. They might also associate with residual amylopectin chains to constitute the “crystallites.”

Irrespective of whether the amylose participates in crystallites, two important facts are evident:

1. The external chains of amylopectin with DP 15 (20,33) could be considered as precursors of population III and thus as crystallite constituents.

2. Amylopectin chains are primarily responsible for the crystallinity of starch (29), the crystallite dimension being the length of the amylopectin external chains. The amylose chains, being initially at least in part outside the crystallite regions, are rapidly hydrolyzed and the resistant fragments would have the same length as the crystallite itself.

**Hypothesis for the Structure of Potato Amylopectin**

The gel filtration and enzymatic studies of lintnerized potato starch confirm one of the structural schemes of amylopectin, specifically the “cluster” model proposed by French (8). Such an organized structure is shown in Fig. 11. This structure explains most of the results obtained in this study. Particularly, it accounts for the following observations:

1. The linear populations A and B which appeared after debranching of native starch. The B chains would form the backbone of the amylopectin molecule and should extend over two or more clusters, each cluster resulting from the association of two or three A-chains (A/B = 5.4). The long chains obtained in the excluded volume (peak C or I) after debranching or after β₁P₁ treatment of native starch could come from the slightly branched amylose chains as previously suggested (34, 35, 36).

2. The debranching of the β-limit dextrins of native potato starch. During β-amylolysis, B-chains are reduced by about 10 glucose units up to the first branching point of an A-chain. An analogous result was obtained by Mercier et al. (13,20) and Akai et al. (21) for waxy maize starch.

3. The formation of the USβ-LD and ULβ-LD during lintnerization.

<sup>5</sup>Unpublished result.
4. The formation of the acid-resistant populations II and III.

In such a crystalline model of amylopectin structure, it is conceivable that associated clusters of A-chains, 60 Å in length, could constitute a crystalline layer 60 Å in thickness in the chain axis direction. Such associated clusters then comprise the acid-resistant fraction in starch granules. The intercrystalline areas between successive clusters or crystalline layers would contain the majority of α-1,6 linkages (A → A, A → B, B → B, or B → A) and would be much more acid susceptible. Effectively, the acid acting in the intercrystalline areas could promote the formation of USβ-LD and ULβ-LD and allow the release of singly branched chain population II and linear chain population III, after aqueous dispersion. The presence of the singly branched chains in lintnerized starch could be explained by the presence of any α-1,6 linkages less acid susceptible and more involved in crystalline areas than the others.

As waxy maize starch consists only of amylopectin, and nevertheless contains an amorphous fraction, amylopectin chains in potato starch also contribute to the amorphous parts. The structure of the amylopectin chains in these areas can be essentially the same as the one previously described but possibly more “opened” and more or less mixed with amyllose chains.

The proposed model is different from the classical Meyer model (37) and from the more recent model of Gunja-Smith et al. (38). In our model, the chains A of DP 15 and B of DP 45 are not entirely comparable with the external “A” chains and with the internal “B” chains of the Meyer model. Our A-chains represent the external “A” chains and any internal “B” chains of DP 15 of the Meyer model. Our B-chains only represent the internal “B” chains of DP 45. This distinction in long and short internal “B” chains of the Meyer model is in agreement with the assumption of Akai et al. (21).

In a starch granule, such amylopectin molecules could be radially oriented as the crystalline fiber shown from microscopic studies by Sterling (6) and by Hess et al. (39). Acid-treated starch granules often show a separation into concentric layers (3,5,40) owing to the hydrolysis of tangentially oriented amorphous layers. With our hypothesis, such hydrolysis would also occur at a submicroscopic scale from the hydrolysis of the layers rich in α-1,6 linkages located between successive, concentric, 60-Å layers of associated clusters in the amylopectin molecule.

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