Branch-structure difference in starches of A- and B-type X-ray patterns revealed by their Naegeli dextrins [1]

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Abstract

Naegeli dextrins and debranched Naegeli dextrins were prepared from native starches that display A- and B-type X-ray diffraction patterns. In comparison with their debranched counterparts, Naegeli dextrins prepared from the A-type starches consistently possessed substantially more singly branched molecules than those prepared from the B-type starches. The results indicated that the A-type starches had branch points scattered in both amorphous and crystalline regions. The branch linkages present in the crystalline region might be protected during the exhaustive acid hydrolysis. The B-type starch had most branch points clustered in the amorphous region, making them more susceptible to the acid hydrolysis. These models are consistent with the previously reported amylopectin structures that the A-type starch has more short A-chains (dp 6–12) than the B-type starch. The short A-chain is likely attached to a B-chain with the branch linkage located in the crystalline region. The branch linkages present in the crystalline region and the short double helices derived from the short A-chains provide the 'weak points', which are more susceptible to enzymatic hydrolysis and to generate pinholes and pits to the A-type starches. Banana starch, a C-type starch resistant to enzymatic hydrolysis, produced Naegeli dextrins with substantially less singly branched chains than the A-type starches. © 1997 Elsevier Science Ltd.

Keywords: Starch; Branch-structure of amylopectin; X-ray pattern; Naegeli dextrins

1. Introduction

Starches with amylopectins of relatively short average branch chain lengths (dp 23–29), such as waxy maize, maize, rice, wheat, taro, tapioca, and sweet potato, display the A-type X-ray diffraction pattern. Other starches that have amylopectins of long branch chains (dp 30–44), such as potato, canna, high-amylose maize, ae waxy maize, and tulip, display the B-type X-ray pattern. Those starches with amylopectins of branch-chain length in between the two (dp 26–30) display the C-type X-ray pattern [1,2]. The difference in the average chain length between the A- and B-type starches can be as little as 1 glucose unit. Hanashiro et al. [3] investigated the

branch-chain length distribution by using high-performance anion-exchange chromatography with pulsed amperometric detection. The authors divide and assign the chain-length distributions as A-chains, dp 6–12; B_1-chains, dp 13–24; B_2-chains, dp 25–36; and B_3-chains, dp > 37. A-type starches contain amylopectin of more A-chains, whereas B-type starches contain amylopectin with fewer A-chains. Sweet potato starch, however, is an A-type starch with fewer A-chains [3].

Studies have also shown that the polymorphic forms of starch crystalline structures depend on chain length [1–5], concentration [5,6], temperature [6], and the presence of other solutes and solvents [7–9]. Pure malto-oligosaccharides were isolated and investigated for their X-ray diffraction patterns [4]. Retrograded pure oligosaccharides of dp 10, 11, and 12 prepared from an aqueous solution at a concentration between 8% and 10% display the A-type diffraction pattern, whereas pure oligosaccharides of dp 13–18, prepared at a concentration between 2.8% and 3.3%, display the B-type pattern [4]. At 50% concentration, pure oligosaccharides of dp 10–14 display the A-pattern, whereas at 35%, dp 10–12 display the A-pattern; dp 13, the C_a-pattern; and dp 14, the C_c-pattern [5]. Pure oligosaccharides of dp < 10 do not crystallize [4,5]. The crystalline structures of starch were reviewed by Imberty et al. [10].

Native granular starches differ in their susceptibilities to enzyme hydrolysis, depending on the sources of the starch and the amylase [11]. Susceptibilities and hydrolytic patterns of various native granular starches have been investigated by using pancreatic α-amylase [11–14], bacterial α-amylase [11,13], fungal α-amylase [11,15–18], barley malt α-amylase [19], amyloglucosidase [11,20,21], and isoamylase [22]. The results show that A-type starches, except ginkgo starch, are more susceptible to pancreatic α-amylase than are the B- and C-type starches. Taro, rice, waxy rice, and wheat starches are among the most susceptible, whereas potato, high-amylose maize, yam, and banana starches are the least susceptible to enzyme hydrolysis [11,13,16,18,21]. The pattern of enzymatic hydrolysis of these starches also differs. Most enzymes hydrolyze maize, wheat, rice, and other A-type starches by boring holes into the granule and then hydrolyzing the starch from the inside out, but they attack potato, yam, lily, and other B-type starches and banana starch (C-type) mainly by hydrolyzing the lamella on the surface of the starch granule [11–13,18–21]. After heat and moisture treatment, potato starch (B-type) is changed to a B-

and A-type X-ray pattern, but yam starch remains the B-type. Enzymatic hydrolyses of the two starches increase about 4 times, from 5.0 to 21% for potato and from 1.5 to 6.0% for yam after the heat-moisture treatment [23].

A- and B-type crystalline polymorphic spherulites of starch have been made from amylodextrin solutions (dp 22, at 5–20%, w/w, and 2 °C) with and without 30% ethanol [9]. Both the A- and B-type spherulites have 10- to 15-μm diameters and display Maltese-cross birefringence. The A-type spherulite has a rough surface, whereas the B-type has a smooth surface. The spherulites have been subjected to various amylase hydrolyses, i.e., glucoamylase 1, β-amylase, and α-amylase [24]. Results show that the A-type spherulites are hydrolyzed significantly faster than are the B-type. After enzyme hydrolysis, A-type spherulites lose the spherulitic structure but retain the A-type X-ray pattern, whereas B-type spherulites retain the spherical structure but lose part of their crystallinity [24].

In this study, we investigated the structures of Naegeli dextrins prepared from A- and B-type granular starches. Naegeli dextrins are produced by heterogeneous acid hydrolysis of starch granules. After an extensive acid hydrolysis, the amorphous regions of the granule are removed and leave crystalline residues which consist of amylodextrins of linear, and singly- and multiply-branched structures [25]. The differences in the Naegeli dextrin structure, observed by the high-performance anion-exchange chromatography equipped with an enzyme reactor and a pulsed amperometric detector, revealed the difference in the branch structure between the A- and B-type starches. The branch-structure difference may, in turn, explain the difference in the branch-chain length distribution and enzyme susceptibility between the A- and B-type starches.

2. Experimental

Materials.—Waxy maize and normal maize starches were gifts from American Maize Co. (Hammond, IN, USA). Tapioca and Hylon VII (high-amylose maize starch with about 70% apparent amylose) starches were gifts from National Starch and Chemical Company (Bridgewater, NJ, USA). Potato and rice starches were products of Sigma Chemical

Co. (St. Louis, MO, USA). Starch isolated from ae waxy maize was a gift from A. E. Staley Co. (Decatur, IL, USA). Banana starch was provided by Professor A.R. Bonilla, University of Costa Rica.

Amyloglucosidase (EC 3.2.1.3, from Rhizopus mold), NaN$_3$, glucose, and maltose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Nucleosil 300-10 silica gels were purchased from Alltech (Deerfield, IL, USA). Isoamylase (EC 3.2.1.68, crystal line, from *Pseudomonas amyloderamosa*) and maltopentaose were purchased from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Maltotriose, maltotetraose, maltohexaose, and maltoheptaose were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Water (18 MΩ cm) used in all eluents and sample preparations was obtained from the Milli-Q Reagent Water System (Millipore, Bedford, MA, USA).

X-ray diffraction patterns of starch.—The X-ray patterns of the starches were obtained with copper nickel foil-filtered, K$_\alpha$ radiation using a diffractometer (D-500, Siemens, Madison, WI, USA). The diffractometry was operated at 27 mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 4° to 40° at 0.05° step size with a count time of 2 s. Starch samples were equilibrated in a 100% relative humidity chamber for 24 h at 25 °C .

Preparation of Naegeli dextrins.—Naegeli dextrins were prepared from native maize, waxy maize, Hylon VII, and potato starches following the method of Kainuma and French [26]. The starch (6.25 g, dry-starch basis, dsb) was suspended in 15.3% (v/v) H$_2$SO$_4$ (250 mL) and held at 22–25 °C. For each starch variety, a series of three samples of different degrees of hydrolysis was prepared. The starch suspension was gently shaken daily by hand. At 30-day intervals, the supernatant was siphoned off and was analyzed for total carbohydrate hydrolyzed [27]. The starch residues, except for one of each variety, were resuspended in freshly prepared 15.3% H$_2$SO$_4$. The remaining acid-resistant starch residues were washed with deionized water until the washings reached pH 7. All residues were then dried at 38 °C in an air-draft oven.

Rapid preparation of Naegeli dextrins.—A rapid method of preparation of Naegeli dextrin was also used following the method reported by Umeki and Kainuma [25]. The starch (20 g, dsb) was suspended in 15.3% H$_2$SO$_4$ (400 mL) and held at 38 °C in an incubator. For each starch variety, a series of five samples of different degrees of hydrolysis was prepared. Starch suspensions were gently shaken daily

by hand. Samples were taken on day 3, 6, 9, 12, and 30, and the supernatant was siphoned off. An aliquot of the supernatant was analyzed for total carbohydrate content to calculate the starch hydrolyzed [27]. The starch residues were washed until the washings reached pH 7. The samples were then dried at 38 °C.

Enzymatic hydrolysis.—All native starches, except the ae waxy maize starch, were dispersed in aq 90% Me$_2$SO by heating in a boiling-water bath for 1 h and continuously stirred for another 24 h at room temperature; the dispersed starches in Me$_2$SO were then precipitated by using EtOH. The starch precipitates were redissolved in hot water and stirred in a boiling-water bath for 30 min. All Naegeli dextrins, except the ae waxy maize and the Hylon VII Naegeli dextrins, were dissolved in hot water and stirred in a boiling-water bath for 15 min. The ae waxy maize and Hylon VII Naegeli dextrins were dissolved in 0.7 M NaOH for 30 min at 4 °C and then neutralized by using 1 M HCl. The starch solutions and Naegeli dextrin solutions were subjected to enzymatic debranching by using isoamylase according to Jane and Chen [28].

Branched chain-length distribution analysis.—The branch chain-length distributions of the starches after isoamylase debranching and Naegeli dextrins before and after isoamylase debranching were analyzed by using high-performance anion-exchange chromatography with a postcolumn amyloglucosidase reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) system [29]. The on-line postcolumn amyloglucosidase reactor was inserted between the anion-exchange column and the PAD detector to convert each fraction of amylodextrin of different chain length to glucose. Consequently, the PAD detector gave quantitative response to each fraction of varying chain length. A mixture of homologous maltodextrins (dp 1–7), containing the same concentration (0.1 mg/mL) of each sugar, was used to monitor the activity of the enzyme reactor and to calibrate the eluting volumes of dextrins. The separation of a sample (25 μL, 1 mg/mL) with the system employed a PA-100 anion-exchange analytical column and a PA-100 anion-exchange guard column (Dionex, Sunnyvale, CA, USA) with a gradient composed of eluent A (100 mM NaOH) and eluent B (100 mM NaOH, 300 mM NaNO$_3$) with a flow rate of 0.5 mL/min. The separation gradient was: 0–5 min, 99% A and 1% B; 5–30 min, linear gradient to 8% B; 30–150 min, linear gradient to 30% B; 150–200 min, linear gradient to 45% B.
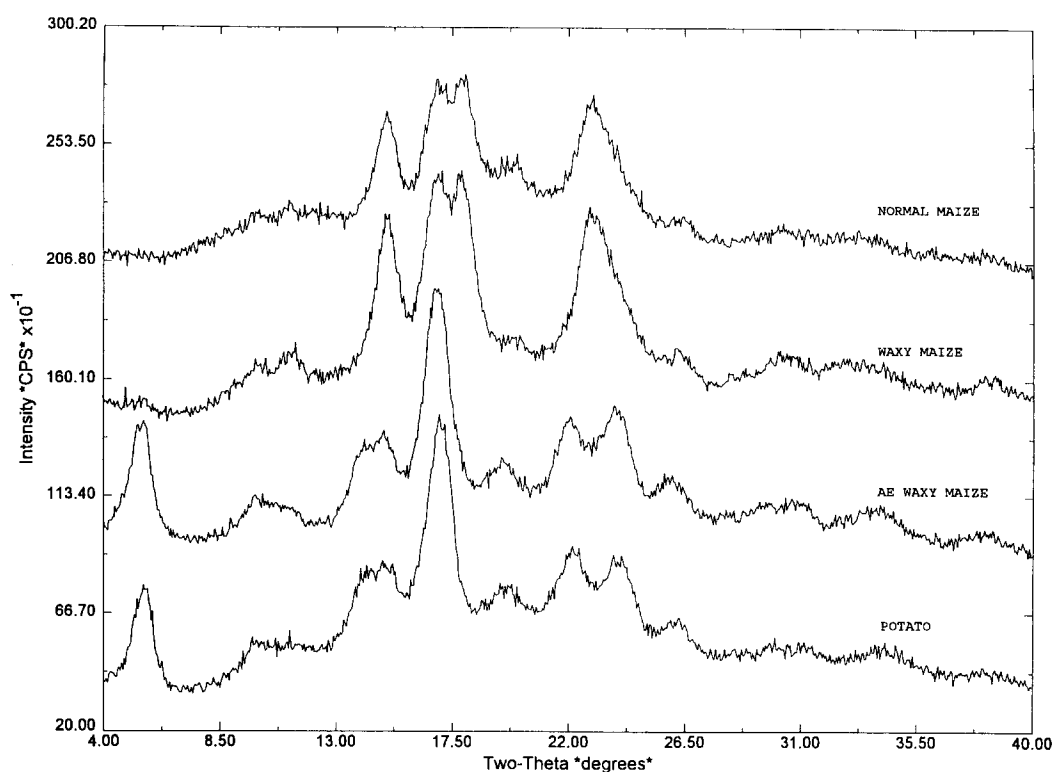
Fig. 1. X-ray patterns of normal maize, waxy normal maize, ae waxy maize, and potato starches.

3. Results and discussion

Waxy maize and normal maize starches both displayed the typical A-type X-ray diffraction pattern, whereas ae waxy maize and potato starches displayed the B-type X-ray pattern (Fig. 1) [30]. Acid hydrolysis rates of starches differed with the starch variety, and the temperature effect on the acid hydrolysis rate of starch also varied with the starch variety (Fig. 2). At both 25 and 38 °C, waxy maize starch had the greatest hydrolysis rate and Hylon VII the least. At 25 °C, potato starch had a slightly slower hydrolysis rate than normal maize starch (Fig. 2a), which was consistent with that reported by Kainuma and French [26]. At 38 °C, however, the acid hydrolysis rate of potato starch was substantially more enhanced than that of other starches. This difference may be attributed to the large granule size and the high phosphate-derivative content of potato starch, which results in more highly swollen granules at 38 °C. Increasing the hydrolysis temperature from 25 to 38 °C, significantly shortened time required for the Naegeli dextrin preparation. The structures of the Naegeli dextrins of the same degree of hydrolysis prepared at the two selected temperatures showed no detectable differences.

Anion-exchange chromatograms of the Naegeli dextrins and the debranched Naegeli dextrins of waxy maize and normal maize starches (A-type) at different percentages of total acid hydrolysis are shown in Figs. 3 and 4, respectively. Without the acid treatment, both debranched native waxy maize and normal maize starches displayed the peak chain-length at dp 13. The chromatograms of the Naegeli dextrins and the debranched Naegeli dextrins of ae waxy maize and potato starches (B-type) are shown in Figs. 5 and 6, respectively. The peak chain-lengths of debranched native ae waxy maize and potato starches were dp 16 and dp 14, respectively. At a comparable degree of hydrolysis, the A-type starch Naegeli dextrin displayed different structural features from the B-type counterparts. Waxy and normal maize starches, after various degrees (up to 70%) of hydrolysis, consistently retained a substantial proportion of singly branched molecules with the peak chain length of about dp 25 (Figs. 3 and 4). After isoamylase debranching reactions, the singly branched molecules were hydrolyzed and produced two linear molecules (Figs. 3 and 4). Ae waxy and potato starches (B-type), however, produced Naegeli dextrins with much smaller proportions of the singly branched molecules (dp $\sim$ 31 for ae waxy maize starch and dp 25 at the
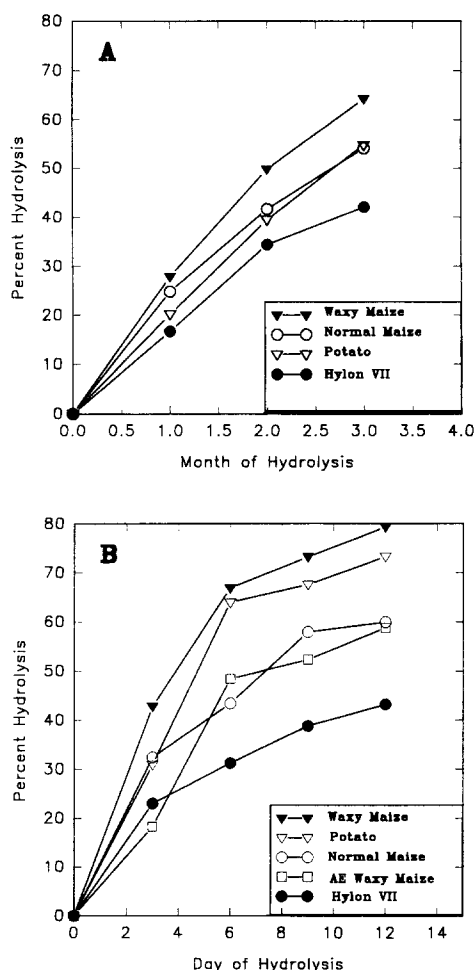
Fig. 2. Acid hydrolysis (15% H_2SO_4, v/v) of various starches at different temperatures for various periods of time: (A) 22–25 °C; (B) 38 °C.
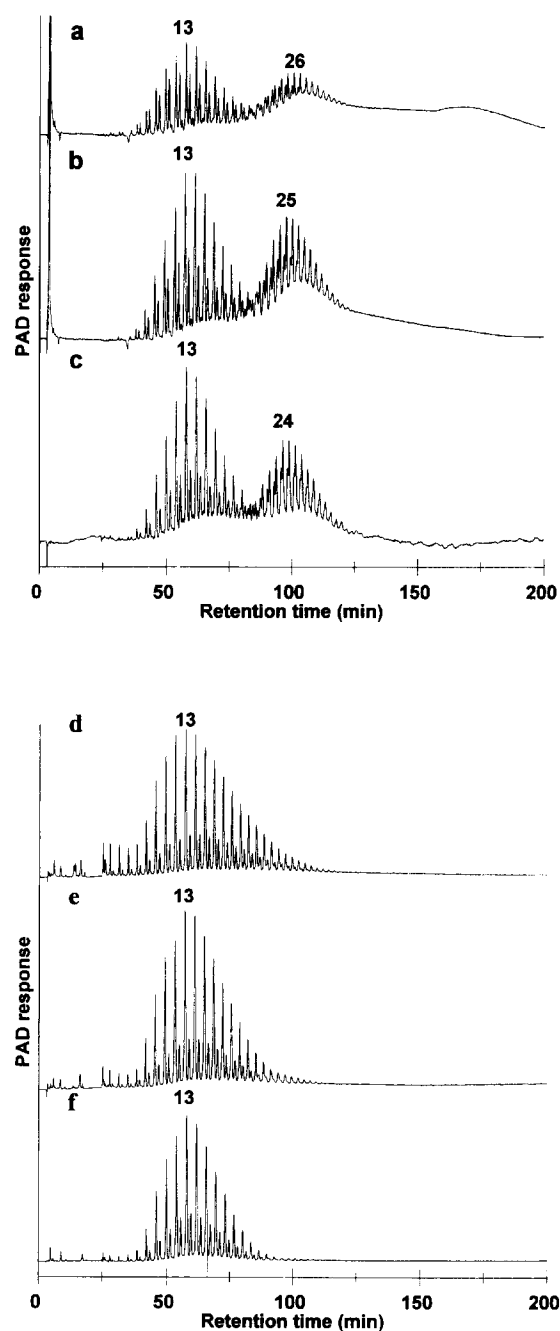

Fig. 3. HPAEC-ENZ-PAD chromatograms of waxy maize Naegeli dextrins (a–c) and debranched waxy maize Naegeli dextrins (d–f) with 47% (a,d), 63% (b,e), and 73% (c,f) of total acid hydrolysis.

shoulder for potato starch), which also disappeared after debranching reactions (Figs. 5 and 6).

The chromatograms of the debranched Naegeli dextrins of less percentage of acid hydrolysis contained more short branch chains (dp 2–8) (Fig. 3d, Fig. 4d, Fig. 5d, and Fig. 6d) than the Naegeli dextrins prepared with higher percentage hydrolysis (60–70%) (Fig. 3f, Fig. 4f, Fig. 5f, and Fig. 6f). Because branch chains shorter than dp 6 are not found in native starch amylopectins, these short chains are likely produced by partial hydrolysis of branch chains on the surface of crystallites or at defect structures (weak points) of the crystallites. After extensive hydrolysis, the short branch chains of dp 2–8 diminished and the chain-length distribution of the debranched Naegeli dextrins also narrowed (Fig. 3f, Fig. 4f, Fig. 5f, and Fig. 6f), indicating improved crystallinity. This coincided with the increasing sharpness of the X-ray diffraction patterns of the

Naegeli dextrins after the acid treatment conducted in our laboratory and as previously reported by Kainuma and French [26].

The structures of the Naegeli dextrins suggested that A-type and B-type starches had different branching patterns. The branch α-(1 → 6)-linkages of the A-type starch were likely more scattered; substantial
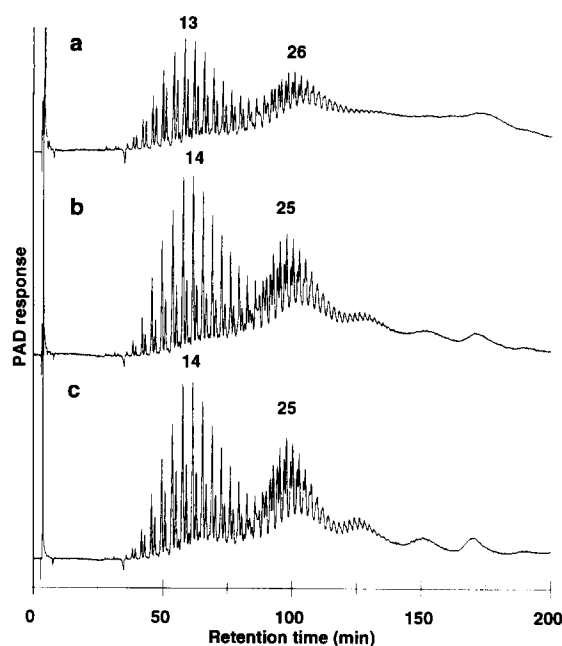
Fig. 4. HPAEC-ENZ-PAD chromatograms of normal maize Naegeli dextrins (a–c) and debranched normal maize Naegeli dextrins (d–f) with 45% (a,d), 58% (b,e), and 66% (c,f) of total acid hydrolysis, respectively.

Fig. 5. HPAEC-ENZ-PAD chromatograms of ae waxy maize Naegeli dextrins (a–c) and debranched ae waxy maize Naegeli dextrins (d–f) with 14% (a,c), 49% (b,e) and 69% (c,f) of total acid hydrolysis, respectively.

branch-linkages were located within the crystalline region, whereas others were in the amorphous region. Those branch linkages located within the crystalline region were protected from acid hydrolysis. For the B-type starch, most of the branch linkages were clustered in the amorphous region, and they were more susceptible to the acid hydrolysis. Models of the amylopectin structures of the A- and B-type
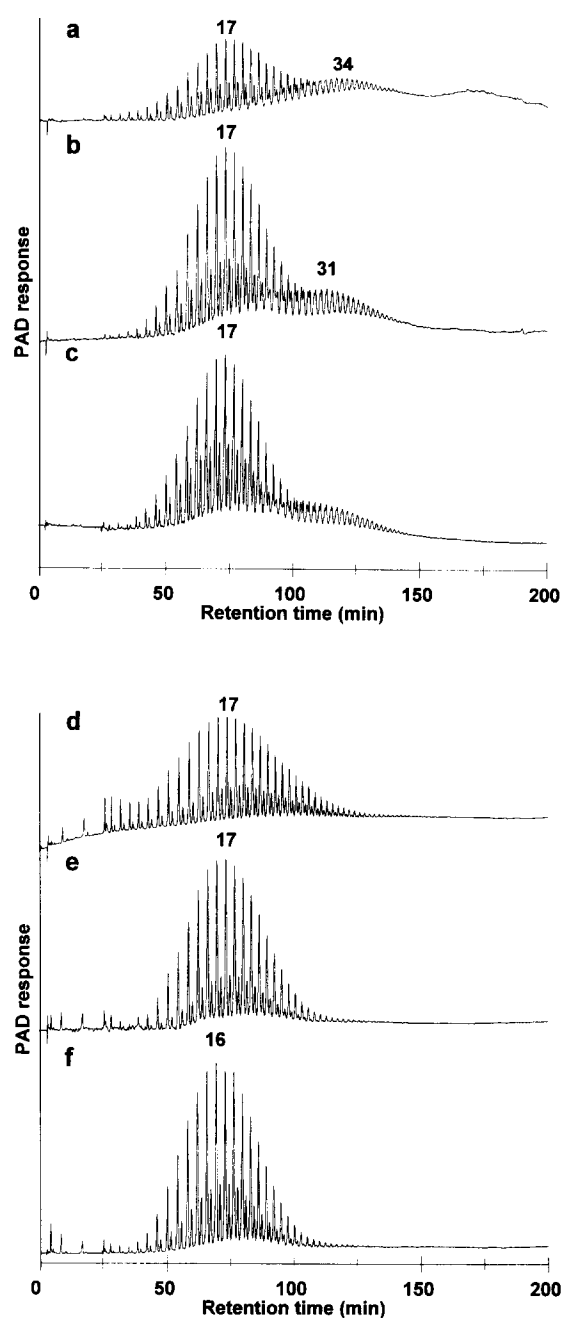
starches are proposed in Fig. 7. The models were constructed using repeating distances of 9.0 nm and 9.2 nm [31,32] and the short to long-chain ratios of 11 and 6 [2] for waxy maize and potato amylopectins, respectively. The repeating distances reported earlier by Zobel [33] are 10.1 nm and 9.7 nm for waxy maize and potato starches, respectively. The structural models are consistent with the differences in the
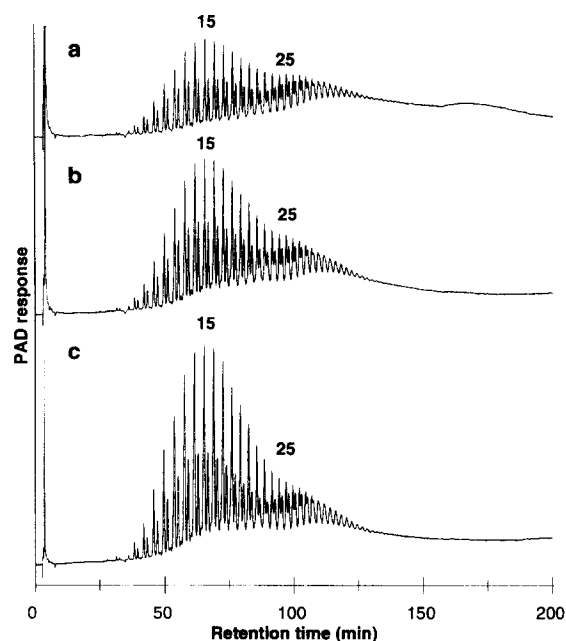
Fig. 6. HPAEC-ENZ-PAD chromatograms of potato Naegeli dextrins (a–c) and debranched potato Naegeli dextrins (d–f) with 37% (a,d), 53% (b,e), and 63% (c,f) of total acid hydrolysis, respectively.
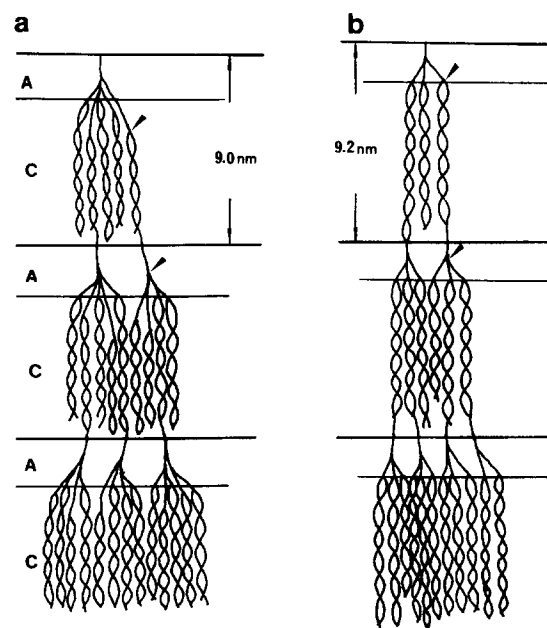

Fig. 7. Proposed models for branching patterns of (a) waxy maize starch, which displays the A-type X-ray pattern, and (b) potato starch, which displays the B-type X-ray pattern. 'A' and 'C' stand for the amorphous and crystalline regions, respectively; 9.0 nm and 9.2 nm are the repeating distances of waxy maize and potato starches, respectively. The chain length between the arrows stands for the internal long-B chain.

branch chain-length distributions between the A- and the B-type starches reported by Hizukuri and coworkers: the A-type starch has more short A-chains (dp 6–12) than do the B-type counterparts [3]. Because of the presence of the short branch chains, the A-type starches consistently have shorter average chain lengths than the B- and the C-type starches [1].

The proposed structures are also consistent with the results of α-limit dextrins reported by Bertoft and co-workers [34,35]. The α-limit dextrins of waxy maize starch produced by *Bacillus* α-amylase hydrolysis display the largest clusters of dp 150–200 [34], whereas that of potato amylopectin display the smallest clusters of dp 30–70 [35]. The authors attributed the small cluster of potato amylopectin to the large proportion of long branch chains and to the structurally different domains [35]. The internal long-B chains of potato amylopectin are hydrolyzed by *B. amyloliquefaciens* α-amylase, and a single broad distribution of internal B-chains is observed on the chromatogram of debranched Φ,β-limit dextrins of potato amylopectin [35]. In contrast, poly-modal distributions of the debranched internal chain length are obtained from cereal starch counterparts, indicating the internal long-B chains of cereal amylopectin are less susceptible to the α-amylase hydrolysis [34,36]. The susceptibility difference of the internal long-B chains of potato and waxy maize amylopectin can be attributed to the difference in the branch-point locations on those internal chains (i.e., the clustered versus scattered branch structures). With the scattered branch structure, waxy maize amylopectin has shorter

distances between branch points than potato as shown by the arrows in Fig. 7. The short internal chains between branch points are less effective to interact with and fill up the nine sub-binding sites of the *Bacillus* α-amylases in order to be hydrolyzed by the enzyme [37].

The proposed models also suggested a mechanism whereby the A-type starch granules display a greater susceptibility to enzyme hydrolysis than do the B-type starch granules. With the scattered branch points, there are likely more short A-chains derived from branch linkages located inside the crystalline region, which produces an inferior crystalline structure. This inferior crystalline structure, containing α-(1 → 6)-linked branch points and the short double helices, is more susceptible to enzyme hydrolysis leading to 'weak points' in the A-type starch granules. These weak points are promptly attacked by enzymes to generate pinholes [38] and pits [11,12,20]. The B-type starch, with clustered branching points and relatively fewer short branch chains, may develop a superior crystalline structure, resembling that derived from retrograded amylose molecules. Retrograded amylose crystallites are resistant to enzymatic and acidic hydrolysis [39,40]. Thus, the B-type starch crystallites are more resistant to enzyme hydrolysis and display a nonporous, smooth surface. The scattered branch points in the A-type starch may also be a result of compromising the large density of branches. With the large ratio of short to long branch-chains of 11 [2] in the waxy maize starches, if all the branch points are clustered within a close distance, it would cause a great space constraint to the branch structure. By scattering the branch structure, the space constraint is decreased. With a relatively small ratio of short to long branch-chain of 6 [2], potato amylopectin can have a clustered branch structure without a severe space constraint. The scattered branch structure in the A-type starches also provides more flexibility for double helix packing and a lower entropy barrier for the double helices to arrange themselves into the closely packed hexagonal unit of the A-type diffraction pattern [41].

Naegeli dextrins of other starches have also been studied. Three examples, representing the A-, B-, and C-type starches, are shown in Fig. 8. Tapioca starch is a highly digestible root starch and displays the A-type X-ray pattern [2]. After 76% hydrolysis, tapioca Naegeli dextrin showed a substantial proportion of singly branched chains (Fig. 8a), which was similar to other A-type starches shown in Figs. 3 and 4. Hylon VII starch is a B-type starch containing amy-
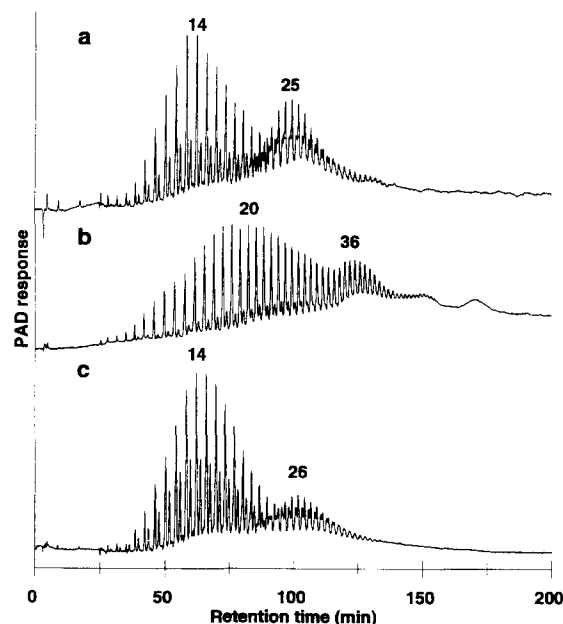

Fig. 8. HPAEC-ENZ-PAD chromatograms of (a) tapioca Naegeli dextrins with 67% of total acid hydrolysis, (b) hylon VII Naegeli dextrin with 49% of total acid hydrolysis, and (c) banana Naegeli dextrin with 65% of total acid hydrolysis, respectively.

lopectin with extended branch chain-lengths. Hylon VII starch is very resistant to enzymatic as well as acidic hydrolysis. After 49% acid hydrolysis, the profile of Hylon VII Naegeli dextrin (Fig. 8b) resembled that of potato and ae waxy starches (Figs. 5 and 6) except for the baseline shifting caused by a large quantity of acid-hydrolyzed resistant amylose residues. Banana starch, a C-type starch, is also known to be enzyme resistant. After about 65% acid hydrolysis, banana Naegeli dextrin showed a relatively low concentration of singly branched chains (Fig. 8c), indicating a more closely clustered branching pattern of the amylopectin. In conclusion, the branching patterns of amylopectins may play a key role in the determination of the type of unit packing and X-ray diffraction pattern as well as the susceptibility to enzymatic hydrolysis.

Acknowledgements

References

[1] S. Hizukuri, T. Kaneko, and Y. Takeda, *Biochim. Biophys. Acta*, 760 (1983) 188–191.

[2] S. Hizukuri, *Carbohydr. Res.*, 141 (1985) 295–306.
[3] I. Hanashiro, J. Abe, and S. Hizukuri, *Carbohydr. Res.*, 283 (1996) 151–159.
[4] B. Pfannemuller, *Int. J. Biol. Macromol.*, 9 (1987) 105–108.
[5] M.J. Gidley and P.V. Bulpin, *Carbohydr. Res.*, 161 (1987) 291–300.
[6] S. Hizukuri, *Agric. Biol. Chem.*, 25 (1961) 45–49.
[7] S. Hizukuri, M. Fujii, and Z. Nikuni, *Biochim. Biophys. Acta*, 40 (1960) 346–348.
[8] S. Hizukuri, Y. Takeda, S. Usami, and Y. Takase, *Carbohydr. Res.*, 83 (1980) 193–199.
[9] S.G. Ring, M.J. Miles, V.J. Morris, R. Turner, and P. Colonna, *Int. J. Biol. Macromol.*, 9 (1987) 158–160.
[10] A. Imberty, A. Buleon, V. Tran, and S. Pérez, *Starch / Staerke*, 43 (1991) 375–384.
[11] H. Fuwa, T. Takaya, and Y. Sugimoto, in J.J. Marshall (Ed.), *Mechanisms of Saccharide Polymerization and Depolymerization*, Academic Press, 1980, New York, pp 73–100.
[12] D.J. Gallant, B. Bouchet, A. Buleon, and S. Pérez, *Eur. J. Clin. Nutr.*, 46 (1992) S3–S16.
[13] J.-C. Valetudie, P. Colonna, B. Bouchet, and D.J. Gallant, *Starch / Staerke*, 45 (1993) 270–276.
[14] S. Fujita, D.V. Glover, K. Okuno, and H. Fuwa, *Starch / Staerke*, 41 (1989) 221–224.
[15] T. Takaya, Y. Sugimoto, E. Imo, Y. Tominage, N. Nakatani, and H. Fuwa, *Starch / Staerke*, 30 (1978) 289–293.
[16] T. Takaya, Y. Sugimoto, K. Wako, and H. Fuwa, *Starch / Staerke*, 31 (1979) 205–208.
[17] K. Kainuma, H. Ishigami, and S. Kobayashi, *J. Jpn. Soc. Starch Sci.*, 32 (1985) 136–141.
[18] V. Planchot, P. Colonna, D.J. Gallant, and B. Bouchet, *J. Cereal Sci.*, 21 (1995) 163–171.
[19] A.W. MacGregor and D.L. Ballance, *Cereal Chem.*, 57 (1980) 397–402.
[20] J.S. Smith and D.R. Lineback, *Staerke*, 28 (1976) 243–249.
[21] A. Kimura and J.F. Robyt, *Carbohydr. Res.*, 277 (1995) 87–107.

[22] A. Kimura and J.F. Robyt, *Carbohydr. Res.*, 287 (1996) 255–261.
[23] R. Hoover and T. Vasanthan, *Carbohydr. Res.*, 252 (1994) 33–53.
[24] G. Williamson, N.J. Belshaw, D.J. Self, T.R. Noel, S.G. Ring, P. Cairus, V.J. Morris, S.A. Clark, and M.L. Parker, *Carbohydr. Polym.*, 18 (1992) 179–187.
[25] K. Umeki and K. Kainuma, *Carbohydr. Res.*, 96 (1981) 143–159.
[26] K. Kainuma and D. French, *Biopolymers*, 10 (1971) 1673–1680.
[27] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
[28] J. Jane and J.-F. Chen, *Cereal Chem.*, 69 (1992) 60–65.
[29] K.-S. Wong and J. Jane, *J. Liq. Chromatogr.*, 20 (1997) 297–310.
[30] H. Zobel, in R.L. Whistler, J.N. BeMiller, and E.F. Paschall (Eds.), *Starch: Chemistry and Technology*, 2nd ed., Academic Press, Orlando, FL, 1984, pp 285–309.
[31] P. Jenkins, R.E. Cameron, and A.M. Donald, *Starch / Staerke*, 45 (1993) 417–420.
[32] P.J. Jenkins and A.M. Donald, *Int. J. Biol. Macromol.*, 17 (1995) 15–20.
[33] H.F. Zobel, *Starch / Staerke*, 40 (1988) 44–50.
[34] E. Bertoft, *Carbohydr. Res.*, 212 (1991) 229–244.
[35] Q. Zhu and E. Bertoft, *Carbohydr. Res.*, 288 (1996) 155–174.
[36] A.W. MacGregor and J.E. Morgan, *Cereal Chem.*, 61 (1984) 222–228.
[37] J.F. Robyt and D. French, *Arch. Biochem. Biophys.*, 100 (1963) 451–467.
[38] J.E. Fannon, R.J. Hauber, and J.N. BeMiller, *Cereal Chem.*, 69 (1992) 284–288.
[39] J. Jane and J.F. Robyt, *Carbohydr. Res.*, 132 (1984) 105–118.
[40] V.M. Leloup, P. Colonna, S.G. Ring, K. Roberts, and B. Wells, *Carbohydr. Polym.*, 18 (1992) 189–197.
[41] M.J. Gidley, *Carbohydr. Res.*, 161 (1987) 301–304.