Slow Digestion Property of Native Cereal Starches

Genyi Zhang,[†] Zihua Ao,[‡] and Bruce R. Hamaker*[,‡]

School of Food Science and Technology, Southern Yangtze University, 170 HuiHe Road, Wuxi, Jiangsu Province 214036, People's Republic of China, and Whistler Center for Carbohydrate Research and Department of Food Science, Purdue University, Food Science Building, West Lafayette, Indiana 47907-1160

Received April 7, 2006; Revised Manuscript Received August 3, 2006

The slow digestion property of native cereal starches, represented by normal maize starch, was investigated. The in vitro Englyst test showed that 53.0% of the maize starch is slowly digestible starch (SDS), and scanning electron microscopy (SEM) revealed that SDS starts from an increase of pore size until almost complete fragmentation of starch granules. However, similar amounts of SDS (∼50%) were shown for partially digested fragmented starch residuals, which would normally be considered resistant to digestion based on the Englyst assay. Molecularly, both amylopectin (AP) and amylose (AM) contributed to the amount of SDS as evidenced by a similar ratio of AP to AM at different digestion times. Consistently, similar degrees of crystallinity, comparable gelatinization behavior, and similar debranched profiles of starch residuals following different digestion times indicated that the crystalline and amorphous regions of starch granules were evenly digested through a mechanism of side-by-side digestion of concentric layers of semicrystalline shells of native starch granules.

Introduction

Public awareness of the effects of diet on human health has increased in the past decades with considerable emphasis on dietary carbohydrates. Recently, a great deal of focus has been given to glycemic index (GI),[1] which describes the level of the postprandial glucose rise in blood as compared to a reference food or glucose. Long-term intake of foods with a high GI has been shown to be associated with obesity and related chronic diseases of diabetes and cardiovascular disease.[2] Based on the Dietary Guidelines for Americans 2005,[3] dietary carbohydrates should provide 45−65% of the total caloric intake, indicating that starchy foods derived from cereals, tubers, and roots are important for healthy diets, and that those less refined and less processed (generally with low GI value) should be increased in the diet. Studies[4,5] have shown that GI's of food products are positively correlated with the amount of rapidly digestible starch (RDS) and that the content of RDS and slowly digestible starch (SDS) can be used to predict the GI of cereal-based food products.

Starch is classified into RDS, SDS, and resistant starch (RS)[6] according to the rate of glucose release and its absorption in the gastrointestinal tract. SDS, which leads to a slower entry of glucose into the blood stream and a lower glycemic response, is digested completely in the small intestine at a lower rate as compared to RDS, while RS is the starch portion that cannot be digested in the small intestine, but is fermented in the large intestine. Experimentally, each starch fraction can be quantified on the basis of the in vitro Englyst method:[6,7] starch digested within 20 min belongs to RDS, whereas SDS represents the digested starch between 20 and 120 min, and the remaining fraction is RS. A moderate postprandial glycemic and insulinemic response of SDS implies that SDS-rich foods may provide wide health benefits in reducing common chronic diseases such as obesity, diabetes, and cardiovascular disease through lessening the stress on regulatory systems related to glucose homeostasis.[8]

The digestion of starch is a process catalyzed by amylolytic enzymes mainly comprised of pancreatic α-amylase and the intestinal brush border glucoamylases, maltase-glucoamylase, and sucrase-isomaltase.[9] Both activities of the amylolytic enzymes and starch substrate properties affect the rate of starch digestion, and as far as SDS is concerned, the main focus is on the substrate property. Up to now, there are limited reports on SDS preparation,[10−12] and mechanisms of slow digestion and structural properties of SDS are not well understood. To apply this concept in food production processes and provide applicable ways to utilize SDS, fundamental researches are needed to understand the mechanism and structural basis of a SDS to bring health benefits to consumers.

Some native cereal starches with semicrystalline A-type structure contain documented high levels of SDS; for example, in native normal maize starch, more than 50% of the starch is SDS based on an in vitro measurement.[13] A recent report by Seal et al.[14] showed that raw normal maize starch produced lower glycemic and insulinemic responses in both healthy subjects and those with type II diabetes. There have been extensive studies on raw cereal starches regarding their biosynthesis,[15] structure,[16,17] physicochemical properties, and enzymatic hydrolysis.[18,19] However, the mechanism of their slow digestion property has not been reported. The purpose of this study is to investigate this mechanism of native cereal starches represented by normal maize starch to improve the knowledge base on the fundamentals of SDS, and provide a theoretical basis for applications related to SDS preparation.

Experimental Section

Materials. Normal maize, potato, and wheat starches were obtained from Tate & Lyle Ltd. (Decatur, IL), Penford Food Ingredients Co. (Englewood, MO), and Midwest Grain Products Inc. (Atchison, KS),

* Corresponding author. E-mail: hamakerb@purdue.edu.
[†] Southern Yangtze University.
[‡] Purdue University.

10.1021/bm060342i CCC: $33.50 © 2006 American Chemical Society
Published on Web 10/25/2006

respectively. Rice and waxy maize starches were purchased from Sigma Chemical Co. (St. Louis, MO). α-Amylase (EC 3.2.1.1, type VI-B from porcine pancreas, 19.6 U/mg), amyloglucosidase (EC 3.2.1.3, from Rhizopus mold, 21 100 U/g), pepsin (EC 3.4.23.1 from porcine stomach mucosa, 1:2500, 51 U/mg), pancreatin from porcine pancreas, invertase (EC 3.2.1.26, grade VII from Bakers yeast, 355 U/mg), and guar gum were purchased from Sigma Chemical Co. (St. Louis, MO). Glucose assay reagents and isoamylase (EC 3.2.1.68, 250 U/mL) were from Megazyme International Ireland Ltd. (Wicklow, Ireland).

Methods. *Enzymatic Starch Hydrolysis.* Starch fractions of RDS, SDS, and RS were measured on the basis of the Englyst test,[6,7] and the values were expressed on a dry weight basis.

Starch hydrolysis kinetics was measured using an in vitro enzymatic hydrolysis method developed for this study. Specifically, 290 U/mL α-amylase and 6 U/mL amyloglucosidase were dissolved into 10 mL of NaOAC buffer (0.1 M, 4 mM $CaCl_2$, pH 5.2, made with benzoic acid saturated distilled water) to hydrolyze 200 mg of starch (dwb) in a water bath held at 37 °C with a shaking speed of 160 rpm. Aliquot samples (100 μL) were taken at different time intervals, and the reaction was stopped with 900 μL of absolute ethanol in a 1.5 mL microcentrifuge tube. After centrifugation (10 000 rpm, 2 min), glucose concentration of the supernatant was measured using a glucose oxidase/peroxidase (GOPOD) kit. Percentage of hydrolyzed starch was calculated by multiplying a factor of 0.9 to the glucose content.

The above procedure was also used to prepare prehydrolyzed native normal maize starch samples by stopping reaction at 20, 40, 60, and 120 min using ethanol (to 80% concentration). After centrifugation at 5000 rpm for 10 min and vacuum-drying, the precipitates were treated with pepsin (5% w/v, pH 2.0 water by adding HCl) for 30 min at 37 °C in a water bath. Starch residues were centrifuged and washed with distilled water until pH was neutralized. The residues were then dehydrated with ethanol and dried in a hot air oven (50 °C). These prehydrolyzed samples were named D20, D40, D60, and D120, respectively.

Differential Scanning Calorimetry. A differential scanning calorimeter (DSC 2920, TA Instruments, New Cartle, DE) was used to examine the thermal properties of starch samples. Native starch samples (normal maize, rice, wheat, potato, waxy maize starch) and prehydrolyzed normal maize starch (3−3.5 mg) were mixed with distilled water (1:3 w/w) and hermetically sealed in aluminum pans. After equilibrating for 1 h at room temperature, samples were scanned at a heating rate of 10 °C/min from 25 to 130 °C.

X-ray Powder Diffraction. A Kristalloflex diffractometer (Siemens Corp., Munich, Germany) was used to examine the crystalline property of starch samples (native starches, prehydrolyzed starches). Cu Kα radiation of $\lambda = 1.5406$ Å was generated at 40 kV and 20 mA. Starch samples were mounted on a sample carrier with amorphous background, and then scanned at a rate of 2°/min from 2θ 5° to 35° at room temperature.

Chromatographic Analysis: Molecular Weight Distribution Profiles. Starch samples were dissolved in 90% dimethyl sulfoxide (DMSO) in a boiling water bath with continuous stirring for 1 h, and the dissolved samples were stirred overnight at room temperature to completely disrupt the starch granules. Starch molecules were then precipitated with 80% ethanol. After centrifugation to remove the supernatant, the precipitates were vacuum-dried and dissolved in distilled hot water at a concentration of 2 mg/mL and subjected to continuous heating for 30 min in a boiling water bath to completely dissolve the samples.

The dissolved samples, filtered through a 5 μm filter, were injected into a high-performance intermediate-pressure size exclusion chromatograph system with multi-laser scattering and refractive index detectors (HPSEC-MALLS-RI), a pump (model LC-10AT vp, Shimadzu Corp., Columbia, MD), and a model 7125 syringe sample loading injector (Rheodyne Inc., Catati, CA) with a 200 μL sample loop. A HR 16/50 column containing Sephacryl S-500 HR gel (Amersham Biosciences, Piscataway, NJ), a DAWN DSP-F laser photometer fitted with argon laser ($\lambda = 488.0$ nm) with a K-5-129 flow cell (Wyatt Technology, Santa Barbara, CA), and an Optilab 903 interferometric refractometer (Waytt Technology) were used. The flow rate was set at 1.3 mL/min with a mobile phase of 0.02% NaN_3 (w/v). A d*n*/d*c* value of 0.146 was used in molecular weight calculation, and data processing was performed using ASTRA software (Version 4.9, Wyatt Technology).

Pure amylopectin from prehydrolyzed and control normal maize starches was isolated using the above chromatography system based on RI signals. The collected amylopectin eluent was then concentrated, desalted, and freeze-dried for debranching analysis.

Chromatographic Analysis: Isoamylase Debranched Profiles. Total starch or isolated amylopectin (10 mg) was dissolved in 2.5 mL of NaOAc buffer (4 mM, pH 4.0) containing 0.02% NaN_3. Debranching was carried out for 24 h at 40 °C after addition of 2.5 μL of isoamylase.

The debranched samples, filtered through a 0.45 μm filter, were injected into a high performance size exclusion chromatograph system (HPSEC) consisting of a Varian 9012 pump (Varian Associates Inc., Sugar Land, TX), a model 7125 syringe sample loading injector (Rheodyne Inc.) with a 50 μL sample loop, two series HR 10/30 tandem columns with the first containing Superdex 200 prep grade gel and the second containing Superdex 30 prep grade gel (Amersham Biosciences, Piscataway, NJ), and a Varian Star 9040 refractive index detector (Varian Associates). The flow rate was set at 0.4 mL/min with a mobile phase of 6.5 mM $(NH_4)_2SO_4$ adjusted to pH 3.0 using acetic acid to avoid the interference of salt signals. The mobile phase was deaerated and filtered through a 0.1 μm membrane filter. Experimental data were processed using Varian's Star Chromatography Workstation (Version 4.51, Varian Associates), and the degree of polymerization (DP) of debranched samples was calculated on the basis of pullulan standards.

Amylose Content and Iodine Binding Reaction. Amylose contents of native and prehydrolyzed starches were determined by the Megazyme amylose kit using con-A (Megazyme, Wicklow, Ireland).

Iodine solution (0.2% I_2 and 2% KI) was prepared with 100 mM phosphate buffer (pH 7.0) and stored in a brown bottle at room temperature. Chromatography eluent (2.6 mL/tube) was collected, and 50 μL of iodine solution was added to each tube. Blue value (A_{620}) was measured using a Bechman Du530 UV/vis spectrophotometer (Bechman Coulter Inc., Fullerton, CA) at a wavelength of 620 nm.

Scanning Electronic Microscopy (SEM). Starch samples with different degrees of hydrolysis were dehydrated and dried in a hot air oven (50 °C, overnight), and then were mounted on aluminum stubs using double-sided tape and sputter coated with gold to a thickness of 10 nm. Digital images of starch granules were obtained using an SEM (JSM-840, JEOL, Tokyo) at an accelerating voltage of 10 kV.

Table 1. Englyst Test Results of Native Cereal Starches

native starch	RDS (%)	SDS (%)	RS (%)	crystallinity (%)
maize	24.4 ± 0.3	53.0 ± 4.9	22.6 ± 4.9	31.0
waxy maize	34.5 ± 2.6	47.6 ± 3.9	17.8 ± 2.9	30.3
wheat	40.1 ± 0.4	50.0 ± 1.4	9.6 ± 1.8	31.0
rice	32.4 ± 1.1	43.8 ± 2.5	23.8 ± 3.6	29.5
potato	8.7 ± 0.2	15.2 ± 1.8	76.0 ± 2.1	29.8

Results

Starch Fractionation and Enzymatic Hydrolysis. Based on the Englyst test, percentages of RDS, SDS, and RS in normal maize starch were 22.4%, 53.0%, and 22.6%, respectively. Although there were noticeable differences between normal maize, waxy maize, rice, and wheat starches regarding their contents of RDS and RS, comparable SDS amounts were found (Table 1). X-ray powder diffraction (Figure 1) showed that maize (normal and waxy), rice, and wheat starches were A-type starches with similar degrees of crystallinity, while potato starch

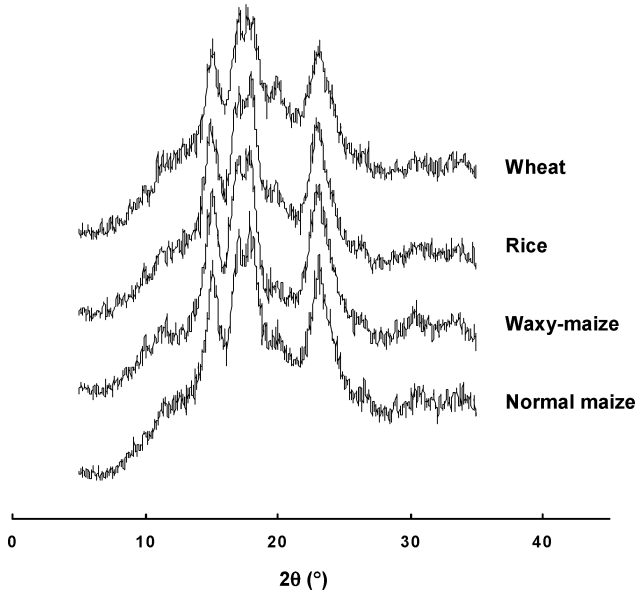

Figure 1. X-ray power diffraction patterns of different cereal starches.

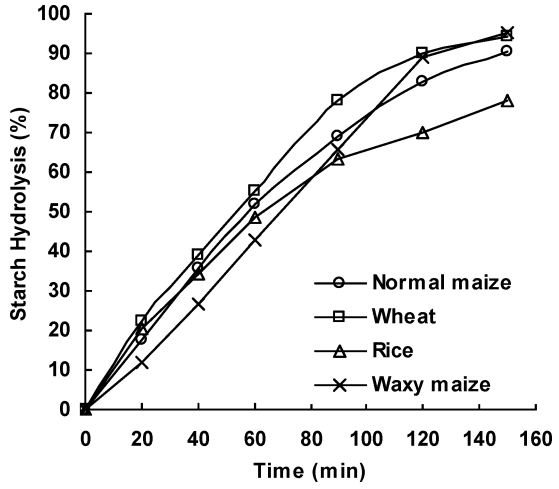

Figure 2. Kinetics of enzymatic hydrolysis of native cereal starches.

was a typical B-type starch[20] with only ~15% SDS. These results support previous findings by Englyst et al.[6]

Regarding the progression of enzymatic digestion, the hydrolysis kinetics of cereal starches (Figure 2) showed similar and typical SDS hydrolysis patterns in which the percentage of digested starch increased gradually. Although there was a statistical difference among different cereal starches, the trend of slow digestion was the same. Additionally, hydrolysis almost reached 100% after a longer time of digestion (Figure 2), indicating that there is a negligible portion of RS in native cereal starches, which is not consistent with the result of ~20% RS from the Englyst assay. This inconsistency suggests that the RS measured by the Englyst assay, in this case, is not truly resistant to digestion. Therefore, the two time points to measure SDS (20 and 120 min digestion) based on the Englyst method might not be suitable to accurately quantify SDS content of native cereal starches. Digestion rates obtained from hydrolysis kinetics may be more appropriate to characterize their slow digestion property.

Sustainable Slow Digestion Property of Native Cereal Starches. Normally, the digestion properties of RDS, SDS, and RS are maintained as consecutive fractions that are rapidly digestible, slowly digestible, and resistant to digestion. However, further Englyst testing on the prehydrolyzed maize starch

Table 2. Englyst Test of Enzyme-Pretreated Native Maize Starches[a]

starch	RDS (%)	SDS (%)	RS (%)
control	24.4 ± 0.3	53.0 ± 4.9	22.6 ± 4.9
D20	35.6 ± 0.9	50.6 ± 1.8	13.7 ± 1.5
D40	37.8 ± 1.5	49.5 ± 2.9	12.7 ± 2.5
D60	41.7 ± 0.3	46.9 ± 0.3	11.4 ± 0.1
D120	39.5 ± 0.9	50.4 ± 2.6	10.1 ± 2.5

[a] D20, D40, D60, and D120 represent prehydrolysis for 20, 40, 50, and 120 min, respectively.

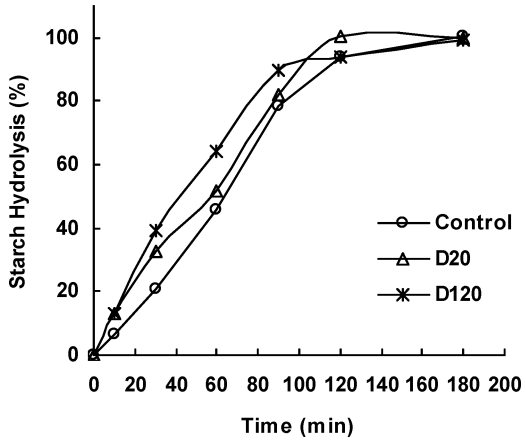

Figure 3. Enzymatic hydrolysis profiles of normal maize starch residuals after prehydrolysis for different time periods. D20 and D120 represent prehydrolysis for 20 and 120 min; control is native maize starch.

residuals after different times of digestion showed an almost constant amount of SDS (Table 2), although an increase of RDS accompanied a reduction of RS with increasing time of prehydrolysis treatment. This observation further demonstrates that there is negligible RS in native maize starch. Hydrolysis profiles of residuals showed similar and typical SDS kinetics (Figure 3). Therefore, native cereal starch can be considered as an ideal SDS material with little RDS and RS from the point of view of digestion rate. The slow digestion property of native A-type cereal starches is likely controlled by their inherent structure and not granular size and morphology as the latter did not affect the amount of SDS; longer time of prehydrolysis reduced the particle size and morphology without affecting the SDS content (Figure 4, Table 2).

Both the Englyst test and the enzymatic hydrolysis kinetics showed that native cereal starches with an A-type semicrystalline structure belong to the category of slowly digestible starches and that the slow digestion property was sustained throughout the entire digestion process.

Digestion Process − Chromatographic Analysis and SEM Examination. Size-exclusion chromatographic analysis of native normal maize starch hydrolyzed by amylolytic enzymes (α-amylase and amyloglucosidase) showed that amylopectin (AP, eluted from 35 to 45 min) was likely the major component digested (Figure 5, same amount of prehydrolyzed starch residuals was analyzed). As hydrolysis progressed, the content of the AP fraction decreased while an intermediate AP-like material (non-iodine binding fraction eluted from 45 to 65 min) produced from hydrolysis of AP increased. AP was the major component of SDS as shown by the difference in chromatographic profiles between D40 and D120 that represents the major portion of SDS based on hydrolysis kinetics. Molecular weight distribution analysis (Table 3) of the prehydrolyzed samples

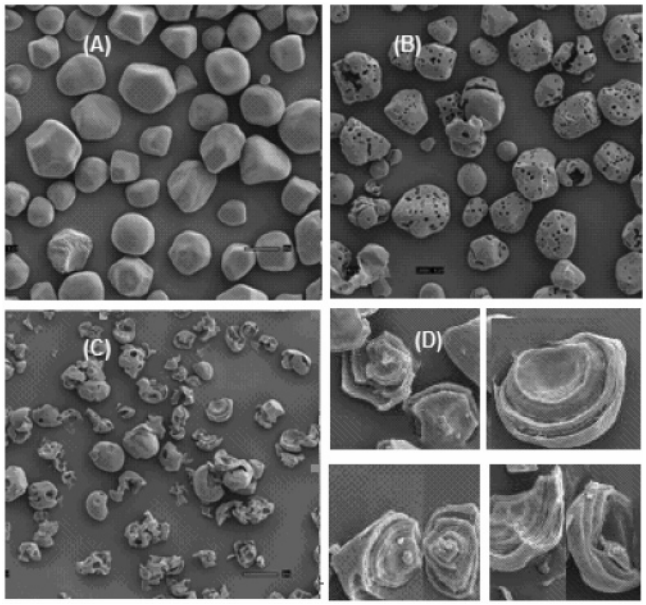

Figure 4. SEM images of normal maize starch hydrolyzed for 0 (control, A), 40 min (B), and 120 min (C). Different pyramid shape residuals after digestion are shown in panel D.

additionally showed gradual decreases in molecular weights of both AP and intermediate AP-like materials as hydrolysis proceeded.

Amylose (eluted from 65 to 90 min) was also digested during the SDS period shown by the iodine binding profile (Figure 5, right panel) in which the molecular weight of amylose (with a λ_{max} of 650 nm) decreased (right shift in the chromatographic profiles) and the amylose peak narrowed. The relatively similar amylose contents between prehydrolyzed samples and the native maize starch control sample (range of 22−24%) indicate that both AP and amylose were digested at a similar rate. Thus, amylose also contributed quantitatively to SDS, although in lesser amount than AP. Amylose might have little impact on the inherent slow digestion property of native cereal starches, because waxy maize starch with negligible amylose content (1.2%) showed a slow digestion property similar to that of normal maize starch.

Considering that SDS remains in undigested starch residues, the SDS portion of native maize starch is found in the layered materials formed by crystalline (the bright layers) and amorphous (the dark layers) shells in starch granules as shown in Figure 4. Although there were a few undigested small granules in D40 and even D120 digested residuals, most SDS appears to start from an enlargement of the pore sizes of starch granules (D40) and hydrolyzed interior regions (Figure 4), until almost complete digestion and fragmentation of starch granules occurs (D120). The remaining outer shell residuals at D120 with a pyramidal shape (Figure 4D) had a limited number of layers of crystalline and amorphous shells, yet they still held the slow digestion property as shown from the above Englyst test and hydrolysis kinetic results (Table 2, Figure 3).

There were some small granules with smooth surfaces (without noticeable surface pores) that remained even after 120 min digestion. Although only a few of these undigested starch granules were observed, their presence supports a view that the surface pores leading to interior channels are important for enzymatic hydrolysis of native A-type starch granules. The Englyst test results of channel-free potato starch (with 76% RS) also support this view.

Evenly Digested Crystalline and Amorphous Regions of Starch Granules. Both the crystalline and the amorphous regions of native starch granules were digested at nearly the same rate, as shown by similar degrees of crystallinity after hydrolysis at different time intervals (Figure 6). The even digestion of crystalline and amorphous regions is supported by DSC data of the starch residuals after different digestion periods that showed similar values for enthalpy and gelatinization temperature (Table 4), although there was a slight increase in T_p with increasing digestion times. Debranching analysis of hydrolyzed starch residuals at different times showed similar chromatographic profiles as compared to the control (Figure 7). Because there are crystalline and amorphous lamellae in the semicrystalline amylopectin structure, the unchanged debranching profiles imply a similar digestion progression through the crystalline and amorphous lamellae.

Discussion

Slow Digestion Property of Native Cereal Starches. Native cereal starches with A-type crystalline structure have been shown to be more susceptible to enzymatic hydrolysis as compared to B-type starches.[21] Further studies also classified them into the category containing a large portion of SDS.[6,13] Our results are consistent with these studies and additionally show that the slow digestion property of native A-type cereal starches is sustainable beyond the 2-h limit used in the Englyst test, implying that there is negligible real RS in these starches. Therefore, native A-type cereal starches are ideal SDS materials. There have been two patents for use of raw starch as a medical food for diabetic patients.[22,23]

Side-by-Side Digestion Mechanism and Layer-by-Layer Digestion Pattern. In the Englyst assay, both α-amylase and amyloglucosidase are used to simulate the in vivo starch digestion process.[6,7] The rate of starch hydrolysis is mainly determined by porcine pancreatic α-amylase,[6] while the function of amyloglucosidase in the assay is mainly to convert amylase degradation products to glucose and to prevent inhibition of α-amylase activity. Thus, the digestion pattern of raw starch granules is principally controlled by porcine pancreatic α-amylase, and the direct hydrolysis of raw starch granules of amyloglucosidase[24] is considerably limited.

Enzymatic hydrolysis requires the binding of amylolytic enzymes to starch molecules. Porcine pancreatic α-amylase has five subsites to bind substrate (each subsite binds a glucose unit),[25] and from its hydrolysis product profile (maltose, maltotriose, and other dextrins with DP 2−7), it requires binding to at least three glucose units before cleaving an α-(1,4)-glycosidic linkage. Therefore, α-amylase needs to bind substrate from the side of starch molecules (versus a head-on orientation). For native starch granules, the alignment of double helices formed by AP in the crystalline regions is perpendicular to the starch granule surface (Figure 8A), and side-binding means α-amylase needs to bind starch molecules in a direction parallel to double helices. The enlargement of the pores and resulting pyramidal-shaped residuals (Figures 4) supports this view, as have other investigations. Atomic force microscopy[26] analysis revealed that digestion starts at the surface of pores and then goes deep into the granule where a pyramidal tip is reflected from the square-sided appearance of the hole. Pohu et al.[27] also showed that hydrolysis of resistant A-type crystallites occurs on the sides of crystalline lamellae. These experimental results indicate that, initially, the internal sides of the pores and channels of native maize starch granules are the binding and digestion sites for α-amylase. Such hydrolysis from the sides of crystalline lamellae would produce an enlargement of channels, fragmenta-

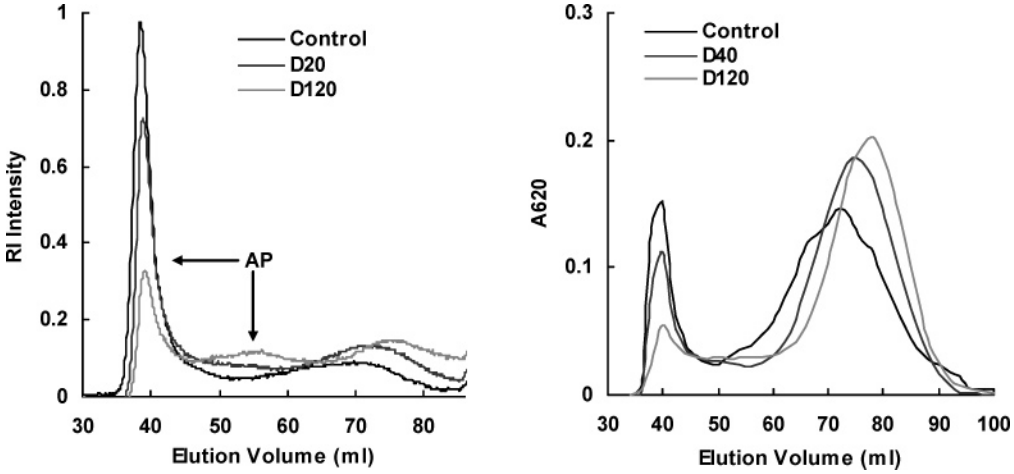

Figure 5. HPSEC profiles of native and prehydrolyzed normal maize starch. The left graph represents starch concentration (RI intensity); the right graph represents absorption at 620 nm after iodine reaction. D40 and D120 represent digestion for 40 and 120 min; control is native maize starch.

Table 3. Molecular Weight Distribution of Prehydrolyzed Normal Maize Starches for Different Time Intervals[a]

	amylopectin		amylose		intermediate AP	
sample	M_w	R_g	M_w	R_g	M_w	R_g
control	1.08×10^8	220.7	5.62×10^6	129.3	none	none
	(1.9%)	(0.6%)	(0.6%)	(0.27%)		
D20	6.18×10^7	146.4	1.69×10^6	59.2	1.21×10^7	81.0
	(1.0%)	(0.27%)	(0.4%)	(0.17%)	(0.3%)	(0.17%)
D40	4.78×10^7	128.4	9.70×10^5	78.8	6.76×10^6	63.7
	(0.6%)	(0.20%)	(0.5%)	(0.5%)	(0.22%)	(0.18%)
D60	3.86×10^7	101.7	9.29×10^5	56.4	7.28×10^6	51.7
	(0.4%)	(0.13%)	(0.4%)	(0.27%)	(0.25%)	(0.12%)
D120	2.92×10^7	93.6	5.94×10^5	80.8	5.06×10^6	48.4
	(0.3%)	(0.17%)	(0.8%)	(1.0%)	(0.23%)	(0.2%)

[a] The percentage value is the standard error in percent (SE%).

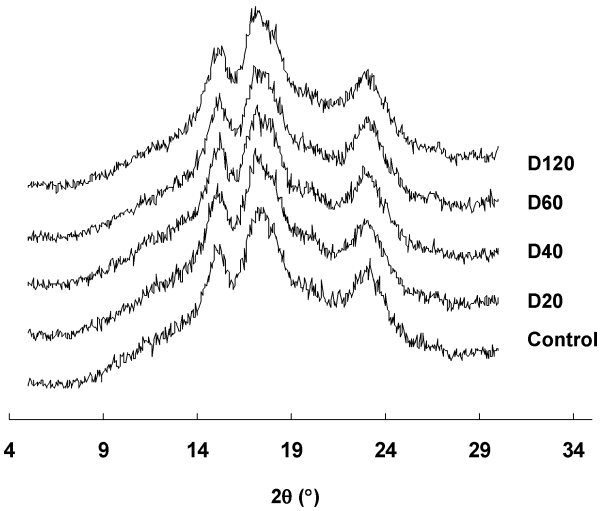

Figure 6. X-ray power diffraction of native maize starch residuals after pretreatment with enzymes (α-amylase and amyloglucosidase) for 20 (D20), 40 (D40), 60 (D60), and 120 (D120) min; control is native maize starch.

Table 4. DSC Parameters of Starch Residuals Prehydrolyzed for Different Times

sample	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
control	61.1 ± 0.14	66.8 ± 0.36	77.4 ± 0.13	10.3 ± 0.28
D20	60.7 ± 0.03	66.0 ± 0.20	76.2 ± 0.03	10.5 ± 0.64
D40	61.5 ± 0.02	67.0 ± 0.10	75.4 ± 0.11	10.4 ± 0.06
D60	62.6 ± 0.29	67.8 ± 0.09	76.8 ± 0.06	9.7 ± 0.07
D120	62.6 ± 0.04	68.2 ± 0.07	77.6 ± 0.16	10.9 ± 0.60

tion of granules, and pyramidal residuals (Figures 4D and 8C). This digestion mechanism could be called side-by-side digestion.

It is well known that native cereal starch hydrolysis begins with enlargement of the surface pores and channels with concurrent hydrolysis from the hilum region toward the outside of the granules.[28] This is the so-called "inside-out" digestion pattern, and it is consistent with the outcome of enzymatic hydrolysis of native maize starch observed by SEM. However, this "simple" concept implies that the hydrolysis pattern would require α-amylase to bind starch molecules from the head or end points of double helices, which is contradictory to principles of α-amylase-catalyzed reaction. Actually, this "inside-out" pattern (Figure 8B) is the observed static result of a side-by-side digestion mechanism under an uneven distribution of substrates from the less dense center to denser periphery of starch granules (the less amount substrate in the center of starch granule requires less time to be digested, while the periphery requires a longer time to be digested).

This observed "inside-out" digestion pattern only fits A-type cereal starches with pores and channels. For B-type starches without pores, the side-by-side digestion mechanism produces a different hydrolysis pattern observed as "exo-pitting",[28] because the enzyme begins digestion from the resistant surface. This is one reason B-type starches and some of the small starch granules with smooth surfaces in maize starch (Figure 4) are more resistant to digestion.

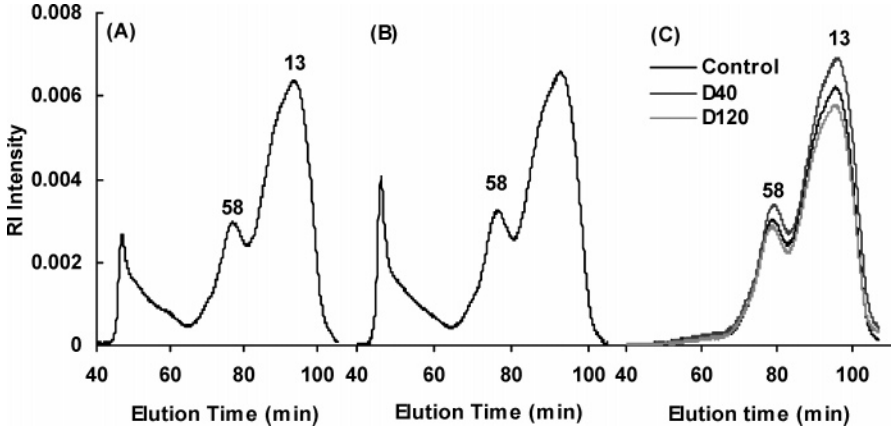

Figure 7. Debranched profiles of normal maize starch (A), residual after prehydrolysis for 120 min (B), and isolated amylopectin (C). D40 and D120 represent prehydrolysis for 40 and 120 min, respectively, followed by isolation and debranching of amylopectin.

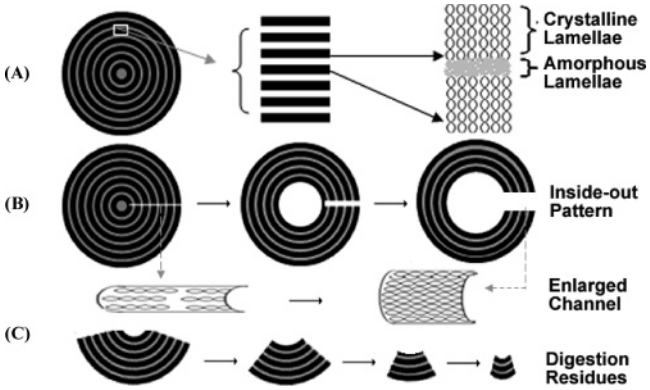

Figure 8. Schematic diagram to show the structure of cereal starch granule and the process of enzymatic digestion. (A) Crystalline and amorphous layer structure, (B) the inside-out layer-by-layer digestion starts at the channel and always ends at the crystalline layer (black), (C) side-by-side digestion to enlarge the internal channel (top) and the resulting pyramidal shape residuals with less and less layers after fragmentation of starch granules (bottom).

Even Digestion of Crystalline and Amorphous Regions. Native starch granules have a semicrystalline structure composed of ordered crystalline regions formed by AP and less ordered amorphous regions (Figure 8A). It had been assumed that amorphous regions are digested easier than the crystalline regions.[28,29] However, our results show that the crystalline and amorphous regions are evenly digested. This is supported by Gérard et al.,[30] who showed the same crystallinity before and after digestion of native maize mutant starches.

Amylopectin and amylose are packed in alternating concentric regions of dense crystalline layers (formed by AP) and amorphous background (major location for amylose) in starch granules.[31−33] Based on the above-described side-by-side digestion mechanism, there is no selectivity between crystalline and amorphous regions. Thus, both crystalline and amorphous regions in native cereal starches are digested evenly. This even digestion of amorphous and crystalline regions suggests that the amorphous regions of the starch granule may not be so "free and mobile" to be digested, as suggested by Planchot et al.[21] The fact that the relative ratio of amylose and amylopectin remained constant as hydrolysis proceeded supports the concept of even digestion of crystalline and amorphous regions.

Mechanism of Slow Digestion Property of Native Cereal Starches. Our results show that native cereal starches are truly slow digesting, and the slow digestion property is sustained even after prehydrolysis to various times. It is well known that starch digestion is affected by both intrinsic structure including macrostructure and molecular structure, and extrinsic factors such as food matrixes and food processing.[18,34,35] In the current study, only the intrinsic property of native cereal starch granules was considered as the first step to reveal the mechanism for its slow digestion property.

The inside-out enzymatic hydrolysis pattern of native maize starch is due to the presence of surface pores (diameter 0.1−0.3 μm) and interior channels (diameter 0.07−0.1 μm), which are large enough for enzymes to enter into the starch granules and start their action in a side-by-side digestion manner. The inside-out digestion pattern is consistent with the resistant surface where amylopectin and higher concentration of amylose molecules are tightly packed and are impenetrable to amylolytic enzymes.[18] Therefore, the pores and channels allow enzymes to digest starch granules in a side-by-side mechanism because enzymes can bind the sides of double helices of starch molecules within the internal channels. However, pores and channels are not maintained after longer time amylase treatment, while the SDS property is retained. Therefore, the slow digestion property cannot be solely explained on the basis of changes in granular structures including surface pores and channels, or granule size and morphology. Other subgranule structural features are associated with the slow digestion property of cereal starches.

For native cereal starch granules, the layered structure of crystalline and amorphous regions is likely the fundamental structural basis for their slow digestion property. This property is realized by a layer-by-layer inside-out digestion process resulted from an even digestion of crystalline and amorphous regions using the side-by-side digestion mechanism. SEM observation did not reveal any enzyme-treated starch residuals with amorphous layers on the surface (Figure 4). Therefore, the amorphous region would be more susceptible to digestion than crystalline regions, if these two regions were separate from each other. Yet, because they are contiguous, digestion proceeds evenly leading to a constant slow digestion profile. This study indicates that a SDS material can be made by encapsulating easily digestible material, for example, gelatinized starch, between layers of films that are resistant to enzyme digestion, and food products with multiple layers of this structure would likely have a slow digestion property.

References and Notes

(1) Jenkins, D. J.; Wolever, T. M.; Taylor, R. H. *Am. J. Clin. Nutr*. **1981**, *34*, 362−366.
(2) Ludwig, D. S. *J. Nutr*. **2000**, *130*, 280S−283S.
(3) Dietary Guidelines for Americans, 2005; http://www.health.gov/dietaryguidelines/dga2005/document/html/chapter7.htm.

(4) Englyst, K. N.; Englyst, H. N.; Hudson, G. J.; Cole, T. J.; Cummings, J. H. *Am. J. Clin. Nutr*. **1999**, *69*, 448−54.
(5) Englyst, K. N.; Vinoy, S.; Englyst, H. N.; Lang, V. *Br. J. Nutr*. **2003**, *89*, 329−339.
(6) Englyst, H. N.; Kingman, S. M.; Cummings, J. H. *Eur. J. Clin. Nutr*. **1992**, *46*, S33−50.
(7) Englyst, H. N.; Hudson, G. J. *Food Chem*. **1996**, *57*, 15−21.
(8) Ludwig, D. S. *J. Am. Med. Assoc*. **2002**, *287*, 2414−2423.
(9) Nichols, B. L.; Avery, S.; Sen, P.; Swallow, D. M.; Hahn, D.; Sterchi, E. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 1432−1437.
(10) Shin, S. I.; Choi, H. J.; Chuang, K. M.; Hamaker, B. R.; Park, K. H.; Moon, T. W. *Cereal Chem.* **2004**, *81*, 404−408.
(11) Guraya, H. S.; James, C.; Champagne, E. T. *Starch/Stärke* **2001**, *53*, 131−139.
(12) Shi, Y. C.; Cui, X.; Birkett, A. M.; Thatcher, M. G. U.S. Patent Application 20030219520, 20030215562, 2003.
(13) Ferguson, L. R.; Tasman-Jones, C.; Englyst, H. N.; Harris, P. J. *Nutr. Cancer* **2000**, *36*, 230−237.
(14) Seal, C. J.; Daly, M. E.; Thomas, L. C.; Bal, W.; Birkett, A. M.; Jeffcoat, R.; Mathers, J. C. *Br. J. Nutr*. **2003**, *90*, 853−864.
(15) James, M. G.; Denyer, K.; Myers, A. M. *Curr. Opin. Plant Biol*. **2003**, *6*, 215−222.
(16) Buléon, A.; Colonna, P.; Planchot, V.; Ball, S. *Int. J. Biol. Macromol*. **1998**, *23*, 85−112.
(17) Tester, R. F.; Karkalas, J.; Qi, X. *J. Cereal Sci*. **2004**, *39*, 151−165.
(18) Oates, C. G. *Trends Food Sci. Technol*. **1997**, *8*, 375−382.
(19) Tetlow, I. J.; Morell, M. K.; Emes, M. J. *J. Exp. Bot*. **2004**, *55*, 2131−2145.
(20) Zhang, G.; Venkatachalam, M.; Hamaker, B. R. *Biomacromolecules* **2006**, *7*, 3259−3266.
(21) Planchot, V.; Colonna, P.; Buléon, A. *Carbohydr. Res*. **1997**, *298*, 319−326.
(22) Axelsen, M.; Smith, U. U.S. Patent 6,316,427, 2000.
(23) Qi, X.; Tester, R. WO 2005/044284 A1, 2005.
(24) Kimura, A.; Robyt, J. F. *Carbohydr. Res*. **1995**, *277*, 87−107.
(25) Gilles, C.; Astier, J. P.; Marchis-Mouren, G.; Cambillau, C.; Payan, F. *Eur. J. Biochem*. **1996**, *238*, 561−569.
(26) Thomson, N. H.; Miles, M. J.; Ring, S. G.; Shewry, P. R.; Tatham, A. S. *J. Vac. Sci. Technol., B* **1994**, *12*, 1565−1568.
(27) Pohu, A.; Putaux, J.; Planchot, V.; Colonna, P.; Buléon, A. *Biomacromolecules* **2004**, *5*, 119−125.
(28) Gallant, D. J.; Bouchet, B.; Buléon, A.; Pérez, S. *Eur. J. Clin. Nutr*. **1992**, *46*, S3−S16.
(29) French, D. In *Starch Chemistry and Technology*; Whistler, R. L., BeMiller, J. N., Paschall, J. F., Eds.; Academic Press: San Diego, CA, 1984; pp 183−247.
(30) Gérard, C.; Colonna, P.; Buléon, A.; Planchot, V. *J. Sci. Food Agric*. **2001**, *81*, 1281−1287.
(31) Atkin, N. J.; Cheng, S. L.; Abeysekera, R. M.; Robards, A. W. *Starch/Stärke* **1999**, *51*, 163−172.
(32) Kainuma, K.; French, D. *Biopolymers* **1972**, *11*, 2241−2250.
(33) Robin, J. P.; Mercier, C.; Charbonniere, R.; Guilbot, A. *Cereal Chem.* **1974**, *51*, 389−406.
(34) Colonna, P.; Leloup, V.; Buléon, A. *Eur. J. Clin. Nutr*. **1992**, *46*, S17−S32.
(35) Slaughter, S. L.; Ellis, P. R.; Butterworth, P. J. *Biochim. Biophys. Acta* **2001**, *1525*, 29−36.

BM060342I