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Slowly Digestible State of Starch: Mechanism of Slow Digestion Property of Gelatinized Maize Starch

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The mechanism underlying the previously reported parabolic relationship between amylopectin fine structure, represented by the weight ratio of linear short chains [degree of polymerization (DP < 13) to long chains (DP ≥ 13], and slowly digestible starch (SDS) content was investigated from the viewpoint of starch retrogradation and substrate susceptibility to enzyme hydrolysis. A maize mutant sample, termed "highest long-chain starch" (HLCS) representing group I samples with a higher proportion of long chains, showed a bell-shaped SDS pattern with retrogradation time, whereas insignificant changes in SDS were found for the sample termed "highest short-chain starch" (HSCS) representing group II samples with a higher proportion of short chains. This corresponded to results from X-ray powder diffraction and differential scanning calorimetry that showed a rapid increase of crystallinity and enthalpy for HLCS during retrogradation, but negligible changes for sample HSCS. Therefore, retrogradation was associated with SDS content for group I samples, but not for group II samples. Analysis of amylopectin fine structure, SDS content, retrogradation enthalpy, SDS material debranching profile, and hydrolysis pattern demonstrated, for group I samples, that linear branched chains of DP 9−30 of amylopectin may act as anchor points to slow the digestion of branced-chain fractions of DP > 30, which constitute the major slowly digestible portion, whereas for group II samples, it is the inherent molecular structure of amylopectin with a higher amount of branches and shorter chains that is not favorable for rapid enzyme digestion. The concept of a slowly digestible starch state (SDS state) that could be a chemical or physical entity is proposed to better describe the mechanistic underpinning of the slow digestion property of starches.

KEYWORDS: Slowly digestible starch (SDS); SDS state; resistant starch (RS); nutritional property of starch; starch retrogradation; amylopectin fine structure; maize mutants

INTRODUCTION

Carbohydrates are one of the important energy-providing macronutrients, and the glucose generated from glycemic carbohydrate digestion plays an important role in energy metabolism and glucose homeostasis (*1*). The continued increase in incidence of obesity and related metabolic diseases such as diabetes and cardiovascular disease has been related to an increase in the consumption of refined carbohydrates. Starch, composed of essentially linear amylose and highly branched amylopectin with α-D-glucopyranose as the structural unit, is the main glycemic carbohydrate material in cereal- and tuber-based food products. Starch has been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (*2*) to specify its nutritional quality related to physiological response and health effect. RDS leads to a rapid increase of blood glucose and insulin levels (*3*), whereas SDS has moderate glycemic and insulinemic responses. Chronic postprandial hyperglycemia, characteristic of a diet containing a high amount of RDS, causes a substantial fluctuation of postprandial blood glucose and not only generates a high stress to the glucose homeostasis regulatory system (*4*) but also produces superoxide free radicals associated with cell damage and a series of health complications (*5*). Therefore, improving food quality with a higher amount of SDS, as well as RS, is of increasing interest to academic researchers, the food industry, and consumers. Due to the scarcity of SDS in regular food products, a fundamental understanding of the slow digestion property of starch at a mechanistic level is important to increase SDS content in food products.

Our previous study (*6*) using a variety of maize mutant samples showed that the content of SDS is parabolically related to the weight ratio of short chains (DP < 13) to long chains (DP ≥ 13) of amylopectin. More specifically, amylopectin with a high proportion of either long chains (group I) or short chains (group II) was more slowly digestible, and the slow digestion property of the starch material could be predicted and manipulated on the basis of its amylopectin fine structure. However,

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Table 1. Ratio of SF/LF [Weight Ratio of Short Linear Chains (DP < 13) to Long Linear Chains (DP ≥ 13) of Amylopectin], SDS Content of Cooked Maize Flour, and Retrogradation Enthalpy (ΔH) for Two Groups of Maize Starch Samples Divided by SF/LF Ratio with the Lowest SDS Content[a]

		sample								
group		6, HLCS	17	23	26	33	45	46	47	36
I	SF/LF	0.251	0.166	0.431	0.303	0.525	0.392	0.400	0.387	0.548
	Δ*H* (J/g)	12.38	1.63	5.68	4.26	7.63	5.76	7.25	2.42	6.58
	SD[b]	0.95	0.17	0.42	0.30	0.45	0.13	0.18	0.27	0.57
	SDS%	20.28	16.20	7.17	14.49	0.17	9.69	7.52	11.08	9.38
	SD[c]	3.96	1.31	2.52	4.02	2.52	3.42	4.02	2.70	4.02

		sample								
group		11, HSCS	12	13	14	15	16	19	20	36
II	SF/LF	0.782	0.700	0.647	0.669	0.552	0.580	0.645	0.612	0.548
	Δ*H* (J/g)	0.215	4.78	5.30	6.10	3.03	4.84	5.95	3.39	6.58
	SD[b]	0.05	0.06	0.20	0.99	0.21	0.46	0.99	0.38	0.57
	SDS%	20.35	16.33	12.38	16.88	11.58	10.69	6.71	12.18	9.38
	SD[c]	4.59	2.61	3.73	2.18	2.89	3.97	2.89	3.57	4.02

[a] Group I includes samples with lower SF/LF ratios, whereas group II includes samples with higher SF/LF ratios. HLCS, highest long-chain starch; HSCS, highest short-chain starch. [b] Standard deviation for enthalpy. [c] Standard deviation for SDS content.

these opposing types of amylopectin fine structures suggested that the creation of SDS is supported by different mechanistic paths. Their elucidation would provide a knowledge base to create slowly digestible starch and theoretical guidance for further increasing the SDS content through either genetic means or chemical/physical modification technologies.

During starch gelatinization, starch granular structure is disrupted and the slow digestion property of the native starch granules, which is determined by the supramolecular semicrystalline structure of amylopectin, is lost (*7*). Thereafter, the SDS property is predominantly related to the inherent molecular structure of amylopectin, as we have shown in the previous study (*6*). It is known that starch molecular structure varies depending on botanical sources and genetic backgrounds, and the molecular structure also determines the possible changes of its physical states after starch gelatinization. Researchers (*8, 9*) have shown that starch retrogradation, that is, the reassociation of amylose and amylopectin molecules to form double helices and possibly crystalline structures, decreases starch's susceptibility to enzyme hydrolysis. In this paper, the mechanisms of achieving a slow digestion property of cooked starch were investigated from the viewpoint of amylopectin fine structure and its retrogradation process after starch gelatinization.

MATERIALS AND METHODS

Materials. Pure starch samples were isolated from 18 maize mutant flour samples provided by Tate & Lyle (Decatur, IL) following the traditional procedure; additionally, toluene was used to remove the proteins. α-Amylase (EC 3.2.1.1) type VI-B from porcine pancreas (19.6 units/mg), amyloglucosidase (EC 3.2.1.3) from *Rhizopus* mold (21100 units/g), pepsin (EC 3.4.23.1) from porcine stomach mucosa (1:2500, 51 units/mg), pancreatin from porcine pancreas, invertase (EC 3.2.1.26) grade VII from baker's yeast (355 units/mg), and guar gum were purchased from Sigma Chemical Co. (St. Louis, MO). Glucose assay reagents and isoamylase (EC 3.2.1.68, 250 units/mL) were from Megazyme International Ireland Ltd. (Wicklow, Ireland).

For the convenience of discussion, the waxy maize mutant 6 with the highest amount of long chains is termed "highest long-chain starch" (HLCS), representing the group I samples with a lower weight ratio of short chains (DP < 13) to long chains (DP ≥ 13) based on amylopectin debranching profiles, whereas the sample 11 is termed "highest short-chain starch" (HSCS) to represent the group II samples with a higher weight ratio of short to long chains (see **Table 1**).

Methods. *Enzymatic Starch Hydrolysis and Chromatographic Analysis.* RDS, SDS, and RS fractions of starches cooked using a pressure cooker (121 °C) were measured according to the Englyst test (*2*), and their values are expressed on a dry starch weight basis. The extraction and purification of starch residuals after 20 and 120 min of digestion in the Englyst digestion, the analysis of chain length distribution, and debranching profiles of these residuals were based on the methods of Zhang et al. (*6*).

Differential Scanning Calorimetry (DSC). A differential scanning calorimeter (DSC 2920, TA Instruments, New Cartle, DE) was used to determine the thermal properties of native and retrograded starch samples. Native starch samples (3−3.5 mg) were mixed with distilled water (1:3 w/w) and hermetically sealed in aluminum pans and then scanned from 25 to 120 °C at a heating rate of 10 °C/min after equilibration for 1 h at room temperature. For retrogradation studies, native starch samples (with distilled water 1:3 w/w) were first scanned from 25 to 120 °C to gelatinize the starch, and then the gelatinized HLCS and HSCS samples in DSC pans were kept at 4 °C for 1, 3, 5, and 7 days to examine time course retrogradation, whereas other samples were analyzed after 7 days to represent the relatively stabilized outcome of retrogradation. The thermal parameters of amylopectin retrogradation were measured after the samples were equilibrated at room temperature for 1 h by scanning the samples 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 as described by Zhang et al. (*7*). For retrograded starch sample preparation, 2.0 g of starch (10%) was first cooked using a Rapid Visco-Analyzer (Newport Scientific Inc.), standard method I, and then the paste was transferred into a plastic tube (50 mL). After the samples were retrograded at 4 °C for different times (0, 16, 48, 72, 120, and 168 h), the samples were freeze-dried and equilibrated at room temperature for 3 weeks in a desiccator containing a saturated salt solution of $MgCl_2 \cdot 6H_2O$ (33% relative humidity) to prevent further progress of starch retrogradation (*10*).

Starch Hydrolysis in a Dilute System. A dilute system was used to examine the effect of amylopectin structure on enzyme hydrolysis susceptibility. HLCS and HSCS starch samples (100 mg, 2.5%, w/v) were cooked in a boiling water bath for 20 min to produce starch solution samples and cooled in a water bath at 37 °C. A buffer of 16 mL of sodium glycerophosphate (100 mM $C_3H_7O_8PNa_2$, 25 mM NaCl, 5 mM $CaCl_2$, pH 6.9) containing porcine pancreatic α-amylase (1 unit/100 mg of starch) was added into the starch solution to start the enzyme reaction at 37 °C with a shaking speed of 160 rpm. Aliquots of 0.5 mL of reaction mixture were taken at 0, 5, 20, 60, and 120 min, and the reaction was stopped by adding 50 μL of H_2SO_4 (1 M). The pH of the reaction mixture (200 μL) was adjusted to pH 4.0 by adding 200 μL of sodium acetate buffer (300 mM NaOAc, 0.02% NaN_3, pH 4.0). Debranching was carried out by adding 3 μL of isoamylase and

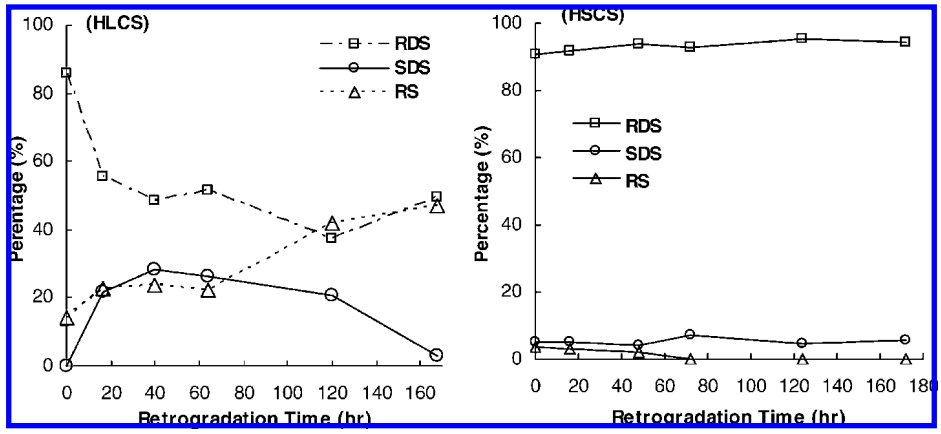

Figure 1. RDS, SDS, and RS content changes of 10% cooked starch pastes over a 7 day retrogradation period at 4 °C. HLCS, highest long-chain starch; HSCS, highest short-chain starch (see **Table 1** for sample information).

incubated at 40 °C for 24 h. Debranching profiles of the samples were performed using size exclusion chromatography according to the procedure of Zhang et al. (*7*).

Statistical Analysis. Common statistical analysis was carried out using statistical tools from Microsoft Excel, and the *P* value was obtained by comparing the calculated *t* value with two tails probability from the table of percentage points of *t* distribution

$$t = \frac{R \times \sqrt{\mathrm{df}}}{\sqrt{1 - R^2}}$$

where *R* is the correlation coefficient and df is the degree of freedom (sample − 2).

RESULTS AND DISCUSSION

SDS Contents during the Time Course of Retrogradation. Pure starch samples of HLCS and HSCS, both waxy mutant starch types and having the highest SDS contents in the tested samples, were chosen as representatives of group I and group II samples (**Table 1**) to investigate the effect of amylopectin retrogradation on their susceptibility to enzyme hydrolysis. Starch retrogradation is a physical process in which starch molecules (both amylose and amylopectin) reassociate to form ordered structures of double helices and crystallites during the time period of cooling and storage after starch gelatinization (*11*). Amylose recrystallization occurs rapidly and is called short-term retrogradation, whereas amylopectin molecules need a longer time to associate, a process called long-term retrogradation. Amylopectin retrogradation rate is related to its fine structure (*12, 13*) and is thermally reversible at a temperature of <100 °C (*14*). Starch retrogradation, particularly amylopectin retrogradation, is a dynamic process driven by thermodynamic forces to reach equilibrium, and there are a series of meta-states during the process (*15*).

Different patterns of SDS changes were shown between HLCS and HSCS during a 7 day time course retrogradation. The SDS content increased rapidly for the HLCS (**Figure 1**, left) during the initial time period of retrogradation. After SDS content reached the peak point at ∼40 h, it decreased until disappearance at 7 days of retrogradation, showing a bell-shaped profile. During the retrogradation period, RS content continuously increased, whereas RDS was essentially stabilized after 40 h of retrogradation. Contrarily, no significant changes were found for HSCS regarding the contents of SDS, RDS, and RS during the 7 day period of retrogradation (**Figure 1**, right). The bell-shaped SDS pattern of HLCS indicates retrogradation is associated with its slow digestion property and that its SDS content is a changeable value determined by the stage of retrogradation. However, the basis for the slow digestion property of HSCS is an inherent property related to its amylopectin fine structure with highly branched shorter chains as described in the previous paper (*6*).

Trends similar to that of the digestibility data were found for X-ray powder diffraction (**Figure 2**) and DSC (**Figure 3**) data. For sample HLCS, X-ray diffraction patterns showed that crystallinity increased quickly in the early stage of retrogradation and remained constant to 7 days. Consistently, amylopectin retrogradation enthalpy also increased rapidly to day 3 of retrogradation, at which time it remained constant over the 7 day period. On the other hand, both X-ray diffraction and DSC data for gelatinized sample of HSCS resembled pure amorphous material. This supports a conclusion that amylopectin retrogradation of HLCS is associated with its slow digestion property and that the amylopectin fine structure of HSCS is responsible for its slow digestion property. Additionally, for the study of typical starches with various amylopectin structures that retrograde, it appears that a 7 day retrogradation period is sufficient to clarify the relationship between starch retrogradation and its slow digestion property.

Starch retrogradation generally decreases starch digestibility (*8*) due to formed B-type crystalline structures that are resistant to enzyme digestion. However, its association with the slow digestion property of HLCS indicates the dynamics of starch retrogradation is critical to understand this association. Cooke and Gidley (*16*) showed X-ray diffraction patterns represent crystalline structure, and DSC thermograms show formation of double helices. Further observation of the DSC data of sample HLCS showed a wide melting endotherm spreading from ∼40 to ∼95 °C, suggesting a range of double-helical structures formed with different DPs of the linear chains. An intermediate physical state during retrogradation with certain orders of crystalline structure is likely the mechanism for its slow digestion property. Therefore, the slow digestion property of HLCS is dependent on the physical state of amylopectin association during retrogradation and can be defined as a physical entity to specify the dynamic relationship between the slow digestion property and amylopectin retrogradation with early stage retrogradation favoring SDS and later stage retrogradation favoring RS.

SDS Content and Amylopectin Retrogradation. Amylopectin fine structure is the determinant of SDS content as shown in our previous investigation (*6*) and is also a causative factor for retrogradation dynamics as shown above and by Shi and Seib (*12, 13*). In this section, all group I (low SF/LF ratio) and II (high SF/LF ratio) samples, as defined in **Table 1** and

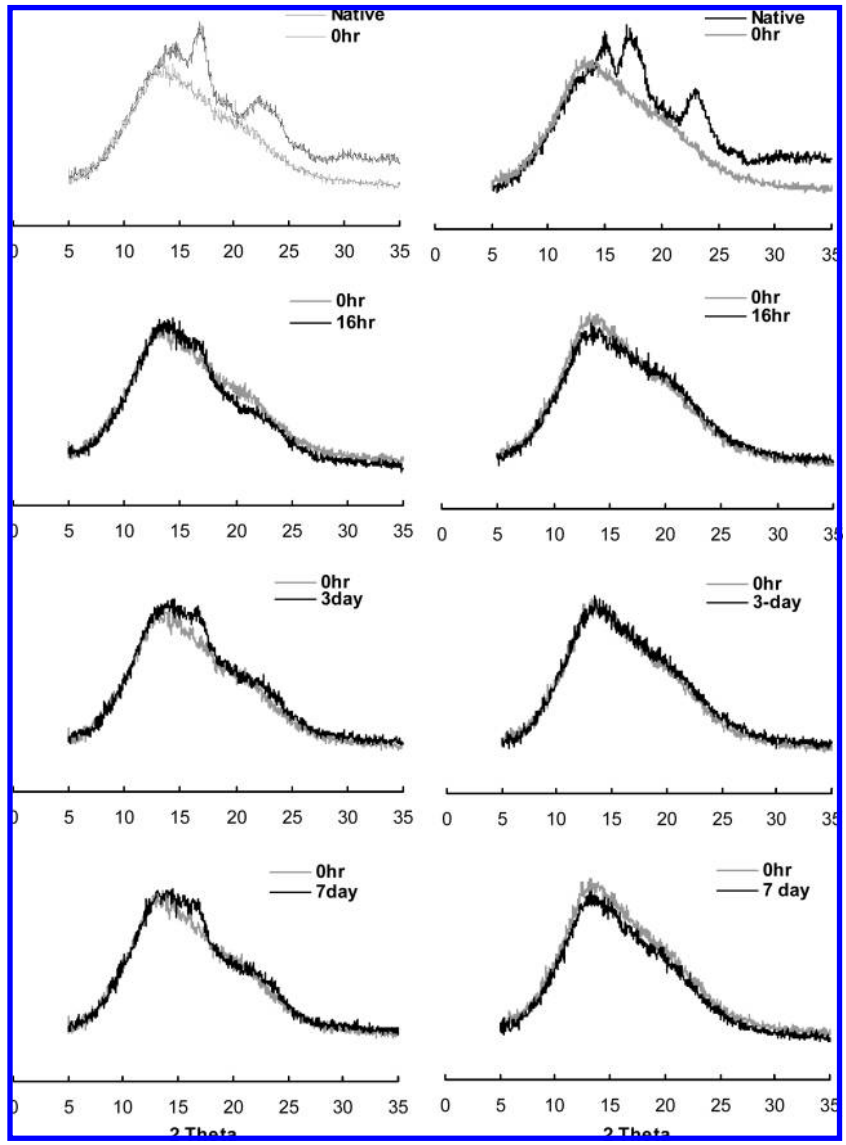

Figure 2. X-ray powder diffraction patterns of freeze-dried cooked starch pastes (10%) after retrogradation at 4 °C for different times. Native is uncooked starch; the left panel is for sample HLCS, and the right panel is for sample HSCS. HLCS, highest long-chain starch; HSCS, highest short-chain starch (see **Table 1** for sample information).

our previous paper, are discussed in terms of their amylopectin fine structure, retrogradation enthalpies, and SDS contents. The correlative relationship between these parameters is shown in **Table 2**. For group I samples, chain fractions of DP 9−30, which are mostly A and B_1 chains from the cluster model of amylopectin (*17*), were significantly and positively correlated with retrogradation enthalpy, but longer chains of DP > 30, particularly DP < 69, were negatively correlated. For group II samples, only the shortest chains of DP 5−9, which cannot form double helices (*18*), were significantly and negatively correlated with retrogradation. Comparatively, the long-chain fractions of DP > 30 for group I samples were highly correlated to SDS, wheras the short-chain fraction for group II samples, particularly the DP 5−9 fraction, were highly correlated to SDS. Therefore, for both groups of samples, the fine structure of amylopectin is associated with both SDS content and amylopectin retrogradation, although in different ways.

For group II samples, no correlations between retrogradation enthalpy and SDS content as well as with the SF/LF ratio were found (**Figure 4C**,**D**). This result indicates retrogradation is not associated with the SDS contents for the group II samples and extends the above conclusion obtained from the representative HSCS sample that the SDS property in this group is inherent to its amylopectin fine structure. Contrarily for group I samples (after sample HLCS was excluded as an outlier), a significant positive correlation was shown between enthalpy and the weight ratio of SF/LF (**Figure 4B**), which would result in a significant negative correlation ($r = -0.84$, $p < 0.01$) between SDS content and enthalpy (**Figure 4A**) based on the negative relationship between SDS content and SF/LF weight ratio (*6*). Thus, a long-term (7 days) retrogradation is negatively correlated to the slow digestion property of group I samples measured just after overnight cooling. This conclusion seems to be counterintuitive to the conclusion obtained from the single HLCS sample that retrogradation is associated with SDS content, but it further enhances the dynamic relationship between SDS content and starch retrogradation, as represented by the bell-shaped SDS profile of HLCS. In other words, starch retrogradation is the basis for the appearance of a transient state that has the highest SDS formation for each group I sample, and amylopectin structure is the fundamental basis of the transient state formed through retrogradation.

Although amylopectin retrogradation is not related to the digestion property of group II samples as discussed above, most

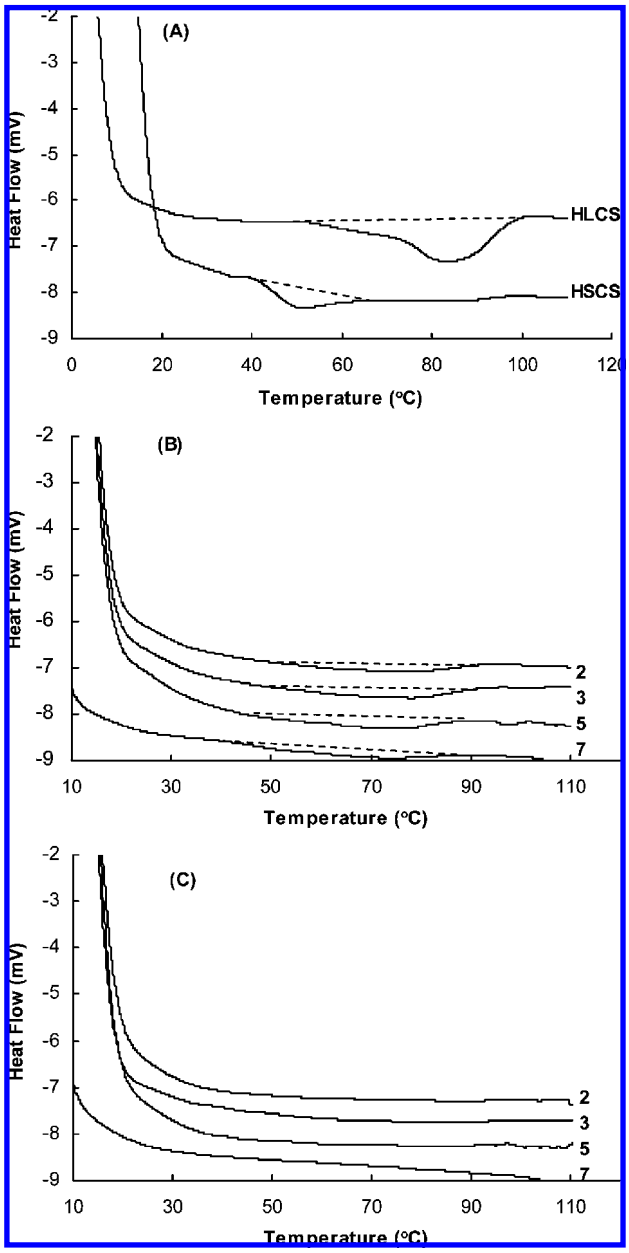

Figure 3. Differential scanning calorimetry (DSC) thermograms of samples HLCS (**B**) and HSCS (**C**) after retrogradation for different days at 4 °C. DSC thermograms of native (ungelatinized) starches are shown in panel **A**. HLCS, highest long-chain starch; HSCS, highest short-chain starch.

Table 2. Correlations between Amylopectin Branch Chain Fractions and SDS and Retrogradation for Group I and II Samples (Group I Includes Samples with Lower SF/LF Ratios, whereas Group II Includes Samples with Higher SF/LF Ratios; See **Table 1**)

chain fraction	group I				group II			
	SDS	*P* value	enthalpy	*P* value	SDS	*P* value	enthalpy	*P* value
DP5−9	−0.779	0.013	0.621	X[a]	0.689	0.028	−0.697	0.025
DP9−13	−0.855	0.003	0.729	0.039	0.279	X	0.281	X
total SF	−0.830	0.006	0.690	0.055	0.803	0.005	−0.535	X
DP13−30	−0.637	X	0.695	0.027	0.123	X	0.306	X
DP30−69	0.752	0.019	−0.518	X	−0.715	0.020	0.273	X
<DP69	0.721	0.028	−0.740	0.019	−0.563	X	0.280	X
total LF	0.830	0.006	−0.690	X	−0.803	0.005	0.535	X
SF/LF	−0.795	0.010	0.631	X	0.801	0.005	−0.553	X

[a] Not statistically significant.

samples did show a relatively high value of retrogradation enthalpy. Comparison of the DSC curves from two groups (**Figure 5**) reveals that most of the endothermic peaks of group II samples were relatively sharper than those of group I samples, indicating there is a narrower range of crystalline structures in group II samples, which may not contribute much to their slow digestion property. Alternatively, group I samples showed variable degrees of crystallite perfection indicated from the longer range of melting temperatures, and a certain state of retrogradation would likely produce more SDS.

Debranching Profile of SDS Material. The SDS material is the starch portion digested between 20 and 120 min on the basis of the Englyst assay (*2*). Maize mutant samples with intermediate SDS content were chosen to examine the structure of the SDS material from each of the two groups of samples. Sample 26 was selected to represent group I samples, and sample 15 was selected to represent group II samples (**Table 1**). For sample 26, the SDS material was the digested portion between 20 and 120 min (the residuals after digestion for 20 and 120 min were individually collected to examine the SDS material by difference); for sample 15, the SDS material was prepared by collecting the undigested material at 20 min (it had negligible RS).

Molecular weight distributions of samples 26 and 15 and their debranching profiles are shown in **Figure 6**. For sample 26, there were two major peaks of DP 69/65 and 36/34 after it was digested for 20 min (**Figure 6B**) and 120 min, respectively. The SDS material (difference between 20 and 120 min digestion) is found in a decrease of the first peak (DP 69) in both quantity and chain length, and a decrease of chain length from the second peak (DP 36). Although sample 26 has a high proportion of amylose, which mostly would become the RS of the sample, amylopectin retrogradation apparently also contributes to the RS as seen from its debranched profile after digestion for 120 min (**Figure 6C**). For sample 15, the chain length profile of the SDS material includes undigested or partially digested amylopectin molecules (**Figure 6E**) and several small peaks with a DP range of 15−175. Multiple peaks are shown after isoamylase debranching of the residues from Englyst digestion for 20 min (**Figure 6F**), indicating there are more branches with shorter chains that cannot be cleaved by isoamylase, which needs longer branch chains (DP > 3) for its action.

The debranching profiles of sample 26 from digestion for 20 and 120 min showed similar chain length distributions with a peak DP of 20−21, although with a range of DP 6−50 (**Figure 6C**). This result is consistent with the DSC result showing a range of crystalline structures with different degrees of perfection. The less perfect region is likely the SDS portion. This is also consistent with the finding that chains of DP 13−30 were the major chains correlated with retrogradation (**Table 2**), and the crystallites formed by association of these chains might act as the anchor points for the long chains of DP > 30, making them less accessible and slowly digestible. On the other hand, at early stages of retrogradation, prior to the formation of such anchor points, there was almost no SDS (data not shown). This might be the real meaning of the statement "retrogradation is associated with the slow digestion property" of the group I samples.

For sample 15, the origin of the multiple peaks of the debranching profile (**Figure 6E**) are likely from the inherent structure of the amylopectin molecules with higher branches and more and shorter short chains that are difficult for the enzymes to digest. The remaining material after the 20 min Englyst digestion is likely enriched with branches and shorter chains that are also difficult for isoamylase to digest.

These results provide additional evidence of the mechanism for the slow digestion properties of the group I and II samples:

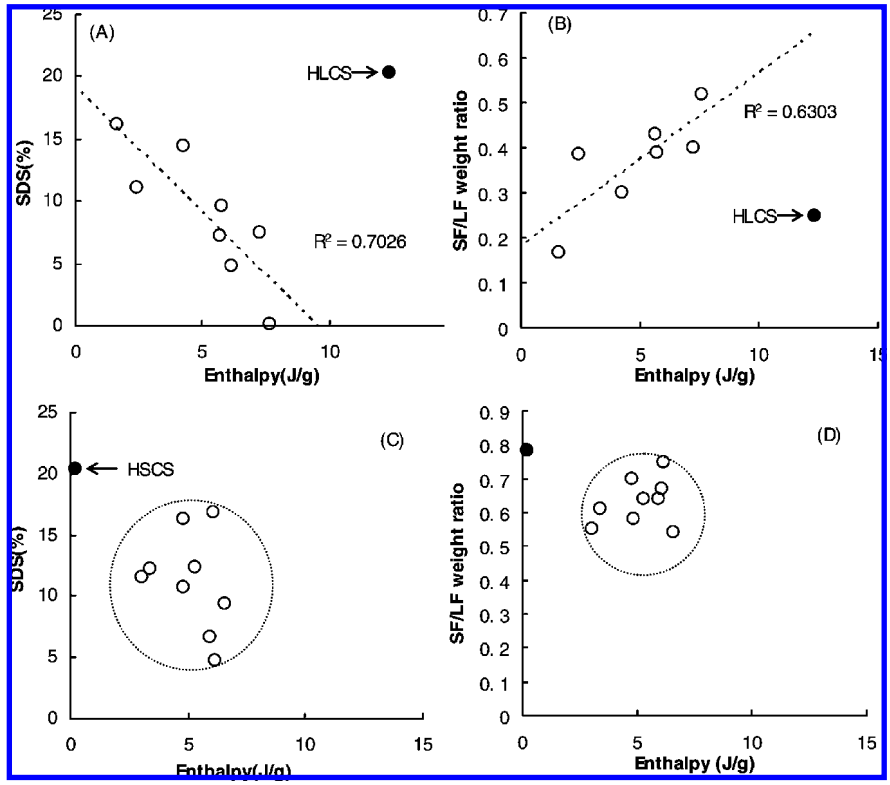

Figure 4. Relationship between maize starch retrogradation and SDS content, and weight ratio of the short linear chain fraction (SF, DP < 13) to long linear chain fraction (LF, DP ≥ 13) representing amylopectin fine structure: (**A**, **B**) group I samples; (**C**, **D**) group II samples (see **Table 1** for information on groups). HLCS, highest long-chain starch; HSCS, highest short-chain starch.

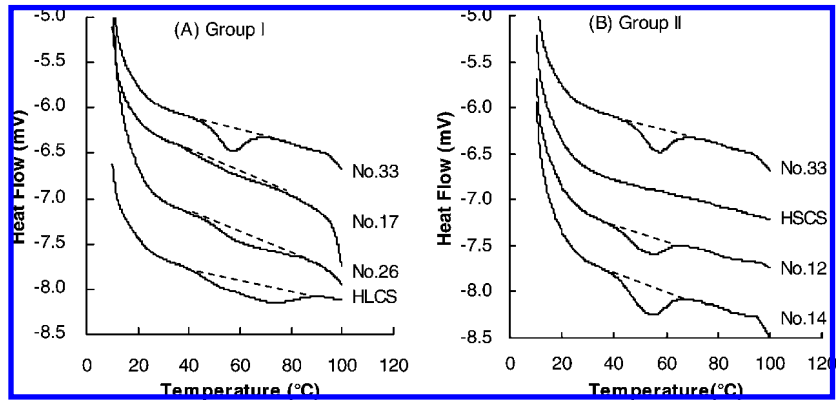

Figure 5. Differential scanning calorimetry (DSC) thermograms of group I and II maize mutant starch samples after retrogradation for a week at 4 °C. HLCS, highest long-chain starch; HSCS, highest short-chain starch (see **Table 1** for samples).

retrogradation is essential for SDS of group I, but amylopectin chemical structure itself is important for the slow digestion property of group II.

Chain Length Distribution in a Dilute Enzymatic Reaction System. To directly address the mechanism of the slow digestion property of the group II samples with high proportions of short chains, a dilute system (low substrate concentration to prevent chain association and low enzyme concentration to facilitate the observation of the effect of different substrate structures on the reaction rate) was used to examine the enzymatic reaction rate based solely on amylopectin fine structure. Again, waxy mutant-based starches HLCS and HSCS were chosen for this purpose, and size exclusion chromatographic tracings of debranched profiles were observed over the α-amylase digestion process. For sample HLCS, linear chain length decreased rapidly with reaction time, whereas the decreasing rate for HSCS was much slower (**Figure 7**). This result shows that different amylopectin fine structures affect hydrolysis rate and that the inherent high branching of HSCS leads to its slow digesting character. In lieu of retrogradation, HLCS amylopectin was easily digested in its molecularly dispersed, and non-retrograded, form. This is consistent with the finding of Ao et al. (*19*) that normal maize starch, enzymatically modified to have shorter A and B_1 chains and more branches, had less susceptibility to enzyme digestion compared to the original starch sample.

Slowly Digestible State of Starches. There have been extensive studies on RS including its structure, formation mechanism, and physiological effects (*20*), but much less is known about SDS in terms of its structure and mechanism when starch is dispersed in thermal processing. This study suggests for the first time the mechanism of formation of slowly digestible starch after gelatinization in the context of amylopectin fine structure. For amylopectin with a high proportion of long B chains, starch retrogradation is related to its slow digestion property through a speculated anchoring effect of crystallites formed by outer A chains and some short B chains (mainly B_1

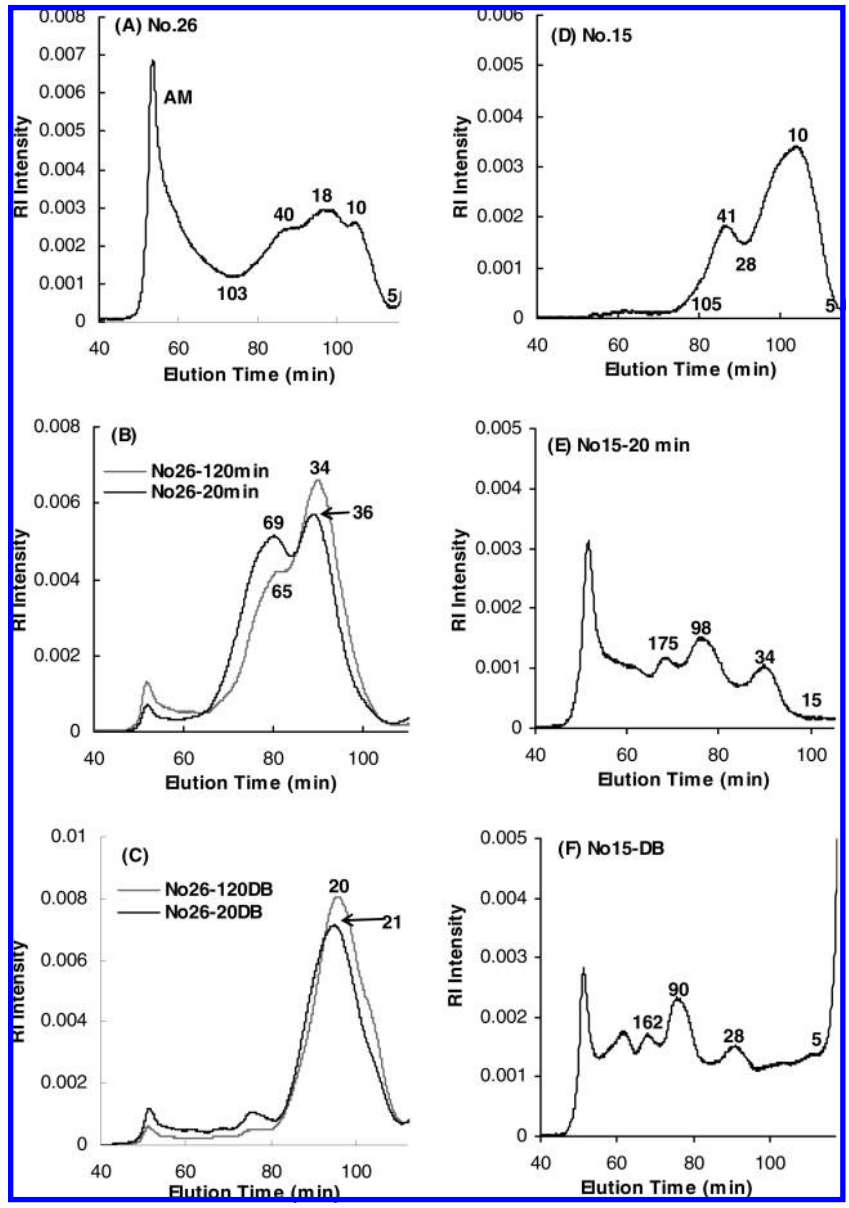

Figure 6. High-performance size exclusion chromatograms of samples 26 and 15, which represent group I and II maize starches. "20 min" and "120 min" mean digested for 20 and 120 min, respectively, using the Englyst assay procedure, and "DB" indicates debranched digested residues with isoamylase.

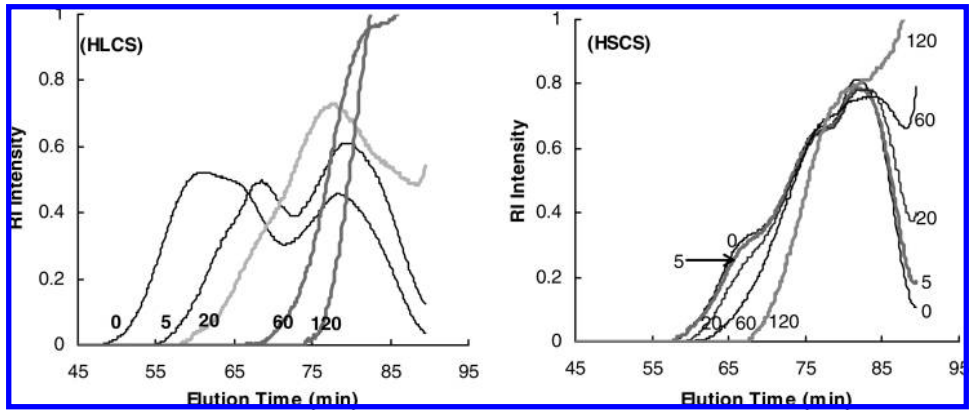

Figure 7. Starch debranched profiles for HLCS and HSCS after digestion for different times (0, 5, 20, 60, and 120 min) by α-amylase (see **Table 1** for information on HLCS and HSCS).

chains) on the longer chains (B_2-B_4) that comprise the main portion of SDS. This type of SDS is a physical entity that is time-dependent as longer time retrogradation will lead to RS, which is consistent with the finding of Shin et al. (*21*) that the amount of slowly digestible starch made from partially debranched amylopectin is dependent on retrogradation time.

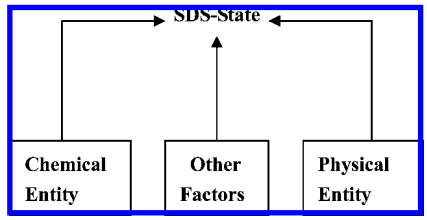

Figure 8. Slowly digestible starch state (SDS state). Starch molecular structure is the basis for either physical or chemical entity SDS. "Other factors" include food matrix and form, various treatments to starch, and enzyme inhibitors.

Additionally, this type of SDS is not stable to heat treatment as amylopectin retrogradation crystallites melt at <100 °C.

For amylopectin with a higher proportion of short chains, especially short A chains (DP < 13), the amylopectin structure itself slows enzyme digestion due to its higher branch density and short chain length, which are more difficult for the amyloytic enzymes to digest (*19*). It is known that α-amylase prefers at least five subsites (five glucose units) to cleave α-1,4 glycosidic linkages and that the rate of cleaving α-1,6 linkages by amyloglucosidase is much slower than cleaving α-1,4 linkages (*22*). Therefore, amylopectin with a branch pattern of a high branch density, short chains, and an appropriate distribution of these branches will be more slowly digestible. Just like a truly slowly digestible pullulan (*23*) with a high density of α-1,6 glycosidic linkage (25−33%) and uniformly distributed shorter linear chains as its basic structure unit of maltotriose or maltotetraose joined through these linkages, SDS made from this type of amylopectin structure is heat stable and is a chemical entity.

In conclusion, there is not one, but two, common structural bases for starch to be slowly digestible. Group I samples represent a physical entity that produces a slow digestion property, and group II samples represent a chemical entity of slow digestion property. Therefore, for the convenience of continued investigation of SDS, a concept of a SDS state (**Figure 8**), as a starting point for further improvement, can be used to describe the structure requirements and mechanisms of slowly digestible starches. In vivo digestibility and physiological relevance must be determined, and, if of value, SDS as a chemical entity could be used as an ingredient due to its heat stability for food product development. The physical entity SDS would require special attention to retain or produce its retrograded form in food processing.

ABBREVIATIONS USED

HLCS, highest long-chain starch; HSCS, highest short-chain starch; DP, degree of polymerization; SDS, slowly digestible starch; RDS, rapidly digestible starch; RS, resistant starch; DSC, differential scanning calorimetry; SF, short-chain fraction; LF, long-chain fraction.

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