Molecular Characteristics Influencing Retrogradation Kinetics of Rice Amylopectins

Vivian M.-F. Lai,[1] Shin Lu,[2] and Cheng-yi Lii[3]

ABSTRACT

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The enthalpy changes (Δ*H*) for melting of crystallites formed during retrogradation of 60% (w/w) amylopectins (AP) aged at 4°C were investigated using AP from 13 rice cultivars with well-known structural properties. According to the Avrami equation, the resultant kinetic parameters for AP retrogradation were obtained in relation to structural factors. Generally, the AP systems studied showed two stages of retrogradation behavior during early (≤7 days) and late (≥7 days) storage. The Avrami exponent for early-stage kinetics (n_1, 1.04–5.54) was greater than the corresponding value for late-stage kinetics (n_2, 0.28–1.52). While the Avrami *K* constant of the early-stage kinetics (K_1, 1.0×10^{-5} to 2.3×10^{-1} day^{-n}) was lower than the corresponding value of late-stage kinetics (K_2, 4.4×10^{-2} to 1.4 day^{-n}). The Δ*H* values for late and infinite retrogradation stages showed a significantly positive correlation with the proportions of short chain (chain length [CL] ≤ 15 glucose units) and long chain (CL = 16–100 glucose units) fractions, respectively. Retrogradation of AP with a higher number-average degree of polymerization, greater proportion of short chain fractions, and shorter average chain lengths revealed significantly greater n_1 values and smaller K_1 values. Values for n_2 and K_2 showed little influence from the molecular properties except for the proportion of extra long (CL >100 glucose units) and long chain fractions on K_2. The negatively linear relationships between log *K* and *n* suggest the importance of some nonstructural factors for AP retrogradation mechanisms in various starch systems.

Extensive association of starch molecules occurs during storage of gelatinized starch materials at below the melting temperature of starch crystallites, resulting in viscosity increase, gel firming, and textural staling of predominantly starch-containing systems. This phenomenon is called retrogradation and is of considerable importance to the food industry (Atwell et al 1988). It is generally regarded as a crystallization or recrystallization (i.e., formation and subsequent aggregation of double helices) process of amylopectin (AP) and amylose (AM) molecules (Miles et al 1985, Atwell et al 1988). Because the amount of AP in most starches is greater than AM, most of the crystallites formed during starch retrogradation are related to the association of AP chains (Miles et al 1985). The melting of these crystallites is thermoreversible (<100°C) and can be easily detected by calorimetry (Miles et al 1985, Ring et al 1987). Unlike AM retrogradation, AP retrogradation proceeds slowly over several weeks of storage (Miles et al 1985, Biliaderis and Zawistowiski 1990) and contributes to the long-term physical properties of starch systems (Miles et al 1985, Orford et al 1987, Russell 1987) or to the eating qualities of starch-containing products (Juliano 1985, Lii et al 1998).

The changes in degree of crystallization of AP on retrogradation have been investigated frequently using different techniques such as differential scanning calorimetry (DSC) (Russell 1983, Fan and Marks 1998, Jouppila et al 1998, Mua and Jackson 1998), X-ray diffraction (Gidley and Bulpin 1987), rheometry (Doublier and Choplin 1989, Mita 1992), and Fourier transform infrared spectroscopy (FTIR) (van Soest et al 1994) or Raman spectroscopy (Bulkin et al 1987). The overall crystallization process of macromolecules includes at least three stages: nucleation, crystal growth, and perfection (Wunderlich 1990). The growth stage can be kinetically described using the Avrami equation (Wunderlich 1976, 1990, 1997). This equation is also used to describe the retrogradation or recrystallization kinetics of AP (Mua and Jackson 1997, 1998), starches (van Soest et al 1994, Bulkin et al 1987, Fan and Marks 1998, Jouppila et al 1998, Mua and Jackson 1998), flours (Jouppila et al 1998), and breads (Russell 1983, Armero and Collar 1998). Based on the experimentally obtained Avrami exponent $n \approx 1$, it is generally concluded that the crystallization mechanism during AP retrogradation is instantaneous nucleation followed by growth of rod-like crystals (Wong and Lelievre 1982, Mita 1992, Zhang and Jackson 1992). However, retrogradation kinetics with values of *n* far different from unity were also found in literature (Armero and Collar 1998, Jouppila et al 1998), suggesting that potentially diversified crystallization mechanisms in various starch-containing systems are still not clarified. It is conceivable that AP retrogradation kinetics in starch-containing systems are concomitantly governed by several factors such as molecular properties (Shi and Seib 1992; Mua and Jackson 1997, 1998), sample concentration (Jouppila et al 1998, Roulet et al 1988), AM content in starch (Russell 1987, Fan and Marks 1998), storage conditions (Lu et al 1997a, Mua and Jackson 1997, Jouppila et al 1998), and the presence of nonstarch components (Fredriksson et al 1998). Different methods may lead to different kinetic characteristics of retrogradation (Roulet et al 1988, 1990). Among these factors, the role of AP structure in the retrogradation kinetics of pure starch systems and complicated products remains ambiguous.

The present report deals with the elucidation of retrogradation kinetics in pure AP systems in terms of molecular properties using AP from 13 rice cultivars with well-known molecular properties (Lu et al 1997b). The resultant kinetic parameters were also compared with those already reported for other starch-containing systems to try to conclude the concomitant effects from nonstructural factors present in various starch-containing systems. In addition, linear relationships between Avrami *K* constant and exponent *n* not yet clarified in literature for a series of starch-containing systems are discussed also.

MATERIALS AND METHODS

Materials

AP was isolated from starches of 13 rice cultivars: five indica (Kaoshiung Sen 7 [KSS7], Taichung Sen 10 [TCS10], Taichung Sen 17 [TCS17], Taichung Native 1 [TCN1], and Taisen 1 [TS1]), five japonica (Kaoshiung 142 [KS142], Taichung 189 [TC189], Tainan 9 [TN9], Tainung 67 [TNu67], and Tainung 70 [TNu70]), two indica waxy cultivars (Taichung Sen Waxy 1 [TCSW1] and Hong Xiao Waxy [HKW]), and one japonica waxy cultivar (Taichung Waxy 70 [TCW70]). Fractionation procedures and structural characteristics of the AP were investigated previously (Lu et al 1997b).

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[1] Department of Food and Nutrition, Providence University, Shalu, Taichung 43301, Taiwan.

[2] Department of Food Science, National Chung-Hsing University, Taichung 40227, Taiwan.

[3] Institute of Chemistry, Academia Sinica, Nankang, Taipei 11529, Taiwan. Graduate Institute of Food Science and Technology, National Taiwan University, Taipei 10764, Taiwan. Corresponding author. E-mail: cylii@chem.sinica.edu.tw. Fax: 886-2-2783-1237.

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Retrogradation Properties

A portion of 60% (w/w) AP suspension (≈120 mg) was hermetically sealed in a stainless steel crucible with a stainless steel stopper and aluminum O-ring, followed by heating at 100°C for 30 min, cooling at room temperature for 1 hr, and storage at 4°C for 0–35 days. The degree of recrystallization of retrograded AP samples was examined by heating from 5 to 120°C at a heating rate of 5°C/min using differential scanning calorimetry (DSC) (Setaram DSC 121, Caluire Cedex, France). An empty sealed crucible was used as the reference. The enthalpy changes (J/g of AP) were determined as means of three replicate measurements

Calculation of Retrogradation Kinetics

By assuming that the enthalpy change (ΔH) associated with melting of crystals is proportional to the total volume of crystals present, the increase in ΔH during AP retrogradation can be described by the Avrami equation for spherical crystals (Avrami 1940, Wunderlich 1997):

$$\theta = (\Delta H_\infty - \Delta H_t)/(\Delta H_\infty - \Delta H_0) = \exp(-K\,t^n) \qquad (1)$$

where θ is the fraction of crystallization resting to take place; ΔH_0 and ΔH_t are values (J/g of dry AP) at storage times of 0 and t days, respectively (where $\Delta H_0 = 0$ in this study); ΔH_∞ is the limiting enthalpy change at infinite time ($t \rightarrow \infty$) obtained from the plot of $1/\Delta H_t$ against $1/t$ (Mita 1992); K is the constant (day^{-n}) concerning nucleation, linear crystal growth rate, and crystal geometry; and n is the Avrami exponent relating to geometry and nucleation type and provides qualitative information on the nature of crystal growth (Wunderlich 1990). Equation 1 can be alternatively expressed as:

$$\log(-\ln\theta) = n \log t + \log K \qquad (2)$$

Accordingly, the n and K values, respectively, can be obtained from the slope and intercept of log (–ln θ) versus log t plot.

Statistical Analysis

Pearson's correlation analysis was computed using Statistical Analysis System software (vers. 6.12, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Retrogradation Kinetics of Rice AP

After storage for an induction time (t_{ind}) during which the average enthalpy changes (ΔH) were undetectable, the ΔH values for melting of AP crystals (60%, w/w, 4°C) increased logarithmically with storage time (Fig. 1). The increasing ΔH values for 13 AP were roughly classified into three types of kinetic retrogradation behavior. Rapid retrogradation behavior, exemplified by KSS7 and TCS17 (Fig. 1A), showed a rapid increase in ΔH commonly at the 4th day of storage and maximized ΔH by the 14th day. Intermediate retrogradation rates, exemplified by TCN1, KS142, and TN9 (Fig. 1B), generally showed an increase in ΔH beginning on the 4th to 6th days and leveled off at about the 21st day. Slow retrogradation rates, exemplified by HKW and TCW70 (Fig. 1C), showed increased ΔH values detectable on the 7th day and gradually increased ΔH during storage. The retrogradation of the other AP, including TCS10, TS1, TC189, TNu67, TNu70, and TCSW1, showed intermediate retrogradation rates.

According to Equation 2, the double logarithmic fraction of AP crystallization resting [log (–ln θ), where $\theta = (\Delta H_\infty - \Delta H_t)/(\Delta H_\infty - \Delta H_0)$] appeared to be linearly correlated with logarithmic time (log t) (Fig. 2). A two-stage relationship with a slope varying on the 7th day of storage (log t = 0.85) was observed for each AP except HKW and TCW70. In Fig. 1, rapid retrogradation systems of KSS7 and TCS17 (Fig. 2A) displayed log (–ln θ) values ≥0, with a slope changing at log (–ln θ) ≈ 0.3. Intermediate retrogradation systems, including TCN1, TCS10, TS1 (Fig. 2B); KS142, TC189 (Fig. 2C); TN9, TNu67, TNu70 (Fig. 2D); and TCSW1 (Fig. 2E), generally revealed a slope deviating at log (–ln θ) = –0.3 to 0.1. Slow retrogradation systems HKW and TCW70 (Fig. 2E) exhibited only one stage of retrogradation during late storage. These results suggest that for the retrogradation of 60% rice AP at 4°C there were probably two different crystallization mechanisms

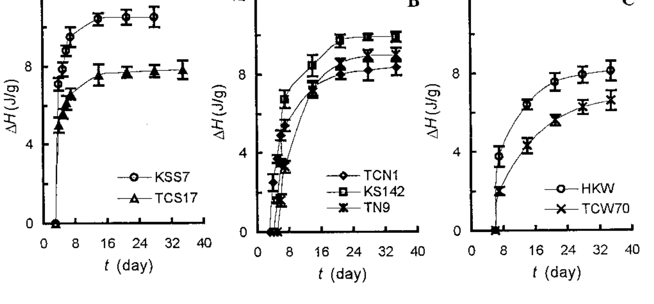

Fig. 1. Typical increases in enthalpy change of 60% (w/w) amylopectins from rice cultivars KSS7, TCS17 (**A**), TCN1, KS142, TN9 (**B**), and HKW, TCW70 (**C**) at 4°C as a function of storage time.

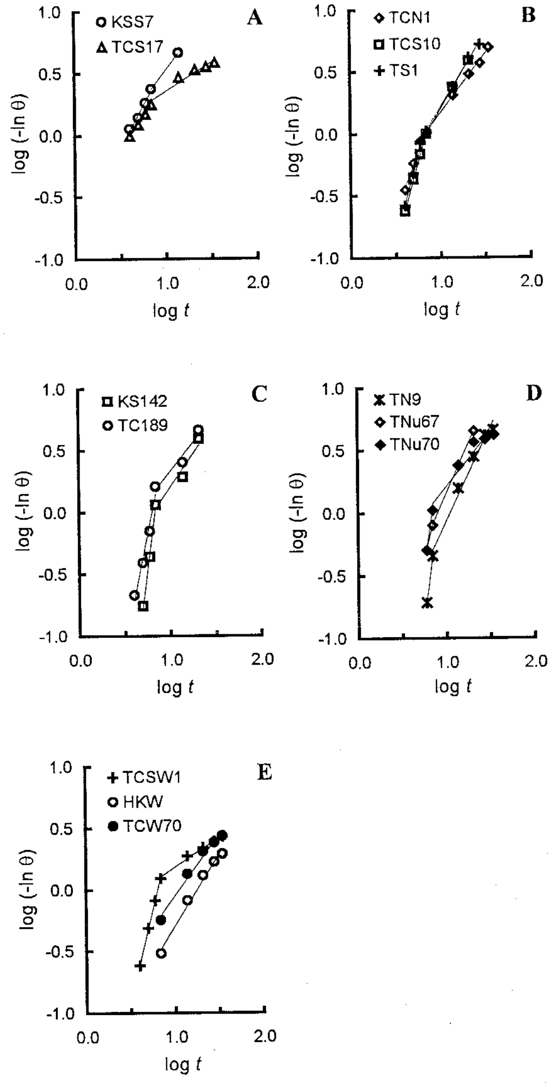

Fig. 2. Plots of double logarithmic fraction of crystallization resting to take place [log (–ln θ)] against logarithmic time (log t) during retrogradation of 60% (w/w) amylopectins from rice cultivars KSS7, TCS17 (**A**); TCN1, TCS10, TS1 (**B**); KS142, TC189 (**C**); TN9, TNu67, TNu70 (**D**); and TCSW1, HKW, TCW70 (**E**) at 4°C. [$\theta = (\Delta H_\infty - \Delta H_t)/(\Delta H_\infty - \Delta H_0)$, t in day].

during early (≤7 days) and late (≥7 days) storage. Such two-stage retrogradation phenomena are different from those found in potato starch pastes (Mita 1992) and wheat starch pastes (Wong and Lelievre 1982), where the slope changed in the first 2–30 hr of storage and involved both AM and AP crystallization.

The ΔH values developed during storage, together with retrogradation kinetic parameters from the results of Fig. 2, are compared in Table I, where the subscripts 1 and 2 represent data of early (≤ 7 days) and late (≥ 7 days) storage stages, respectively. The induction time (t_{ind}) ranged from three to six days. The average ΔH values yielded during early, late, and infinite storage stages (ΔH_1, ΔH_2, and ΔH_∞) had ranges of 2.0–9.5, 1.0–5.8, and 7.7–10.5 J/g, respectively, almost regardless of rice cultivar. The Avrami exponent (n_1) for early-stage retrogradation kinetics had a range of 1.04–5.54, which was greater than the corresponding values (n_2) for late-stage kinetics (0.28–1.52). The K_1 constant of early-stage kinetics generally followed the decreasing order of KSS7, TCS17 (1.7×10^{-1} to 2.3×10^{-1} day^{-n}) > TCN1, TCS10, TS1 (6.7×10^{-3} to 2.4×10^{-2} day^{-n}) > five japonica cultivars and TCSW1 (1.0×10^{-5} to 4.4×10^{-3} day^{-n}). These K_1 values were lower than the corresponding K_2 values of late-stage kinetics and those for HKW and TCW70 (4.4×10^{-2} to 1.4 day^{-n}), contrary to the tendency of increasing rate of ΔH. The kinetic equations accounted for 97.5–99.9% and 88.5–99.9% of the data deviations for early and late retrogradation stages (R^2 = 0.975–0.999 and 0.885–0.999, respectively). A higher n value accompanied by lower K value was reported for 20% corn AP (Mua and Jackson 1998), 40–50% wheat starches (Longton and LeGrys 1981, Zhang and Jackson 1992), 60-80% corn starches (Jouppila et al 1998), and 28.6% rice flour (Fan and Marks 1998). The n value for each AP system decreased as the crystallization proceeded, which is in agreement with the findings for two-stage spherulitic crystallization (Wunderlich 1976, Sperling 1993).

Correlation Between Retrogradation and Molecular Properties

In our previous studies (Lu et al 1997b), the number-average degree of polymerization (DP_n) was 2,743–7,850, 7,327–11,931, and 7,721-9,101 glucose units (gu), respectively, for indica, japonica, and waxy AP. The average numbers of chain (NC) of these AP were 128–424, 424–760, and 389–517, respectively; the average chain lengths (CL) were 18.5–22.1, 15.4–17.5, and 17.6–19.8 gu, respectively; the average exterior chain lengths (ECL), were 13.2–15.8, 11.3–12.6, and 12.2–13.2 gu, respectively; and the average interior chain lengths (ICL) were 4.2–5.3, 3.2–4.1, and 4.4–5.7 gu, respectively. The weight percentages of short chains (*s*) (CL ≤ 15 gu) were 58.5–65.1, 64.6–65.7, and 64.3–65.8%, respectively, for the indica, japonica, and waxy cultivars. The weight percentages of long chains (*l*) (CL = 16–100 gu) were 29.2–36.2, 34.3–35.4, and 34.2–35.7%, respectively. Only the AP of indica TCN1, TCS17, and KSS7 possessed 3.7–10.9% of extra-long chains (*el*) (CL > 100 gu). Generally, the higher the DP_n, the lower the chain length and the greater the *s* value (Lu et al 1997b). Correlation coefficients between the retrogradation characteristics in Table I and the molecular properties of AP were investigated and listed in Table II. The induction time t_{ind} was significantly ($P < 0.05$) correlated positively with DP_n and *s* values. The ΔH_1 showed no significant

TABLE I

Retrogradation Properties and Kinetic Parameters During Early (≤7 days) and Late (≥7 days) Storage Stages of Amylopectins from Taiwan Rice Cultivars (60%, w/w, at 4°C)

		Retrogradation Properties (J/g)			Early			Late		
Cultivar	**t_{ind}[a] (days)**	**ΔH_1[b]**	**ΔH_2[c]**	**ΔH_∞**	**n_1**	**K_1 (day^{-n})**	**R^2**	**n_2**	**K_2 (day^{-n})**	**R^2**
KSS7	3	9.5	1.0	10.5	1.32	1.7×10^{-1}	0.980	0.98	3.5×10^{-1}	0.999
TCS17	3	6.6	1.4	8.0	1.04	2.3×10^{-1}	0.998	0.28	1.4	0.990
TCN1	3	5.4	3.0	8.4	1.96	2.4×10^{-2}	0.983	0.96	1.6×10^{-1}	0.997
TCS10	3	6.0	3.5	9.5	2.58	6.7×10^{-3}	0.999	1.23	9.1×10^{-2}	0.999
TS1	3	6.1	3.2	9.3	2.55	7.9×10^{-3}	0.988	1.18	1.1×10^{-1}	0.996
KS142	4	6.8	3.1	9.9	5.54	2.3×10^{-3}	0.996	1.08	1.3×10^{-1}	0.944
TC189	3	7.9	2.0	9.9	3.52	1.5×10^{-3}	0.975	0.93	2.5×10^{-1}	0.947
TN9	5	3.3	5.8	9.1	5.49	1.0×10^{-3}	0.999	1.22	6.6×10^{-2}	0.973
TNu67	5	5.0	4.1	9.1	2.98	2.4×10^{-3}	0.999	1.52	4.4×10^{-2}	0.999
TNu70	5	6.3	3.3	9.6	4.78	9.6×10^{-3}	0.999	0.61	5.1×10^{-1}	0.885
TCSW1	3	6.6	2.7	9.3	2.91	4.4×10^{-3}	0.999	0.40	6.5×10^{-1}	0.999
HKW	6	2.0	5.7	7.7	. . .[d]	. . .	. . .	0.96	6.8×10^{-2}	0.989
TCW70	6	3.8	4.9	8.7	. . .	. . .	. . .	0.78	1.8×10^{-1}	0.972

[a] Induction time (t_{ind}) during which no detectable enthalpy changes (ΔH) were obtained.
[b] Subscripts 1 and 2 represent early and late retrogradation stages.
[c] $\Delta H_2 = \Delta H_\infty - \Delta H_1$.
[d] Insufficient data.

TABLE II

Correlation Coefficients Between Retrogradation Characteristics and Molecular Properties of Amylopectins from Taiwan Rice Cultivars (60%, w/w, at 4°C)[a]

Molecular Property[b]	**t_{ind}**	**ΔH_1**	**ΔH_2**	**ΔH_∞**	**n_1**	**K_1**	**n_2**	**K_2**
DP_n	0.55*[c]	–0.34	0.53	0.15	0.75**	–0.72*	0.16	–0.36
NC	0.52	–0.26	0.46	0.21	0.76**	–0.67*	0.16	–0.33
CL	–0.44	0.10	–0.37	–0.43	–0.79**	0.61*	–0.32	0.42
ECL	–0.48	0.18	–0.43	–0.37	–0.82**	0.66*	–0.24	0.40
ICL	–0.31	–0.02	–0.22	–0.45	–0.64*	0.44	–0.45	0.40
el	–0.43	0.20	–0.48	–0.40	–0.64*	0.70*	–0.40	0.59*
l	0.13	0.06	0.24	0.61*	0.41	–0.58	0.46	–0.65*
s	0.58*	–0.37	0.59*	0.18	0.72*	–0.68*	0.29	–0.44

[a] Retrogradation characteristics as in Table I.
[b] DP_n, NC, CL, ECL, and ICL are number-average degree of polymerization, average numbers of chain, average chain length, average exterior chain length, and average interior chain length in glucose units (gu), respectively; *el*, *l* and *s* are wt% of extra long (>100 gu), long (16–100 gu), and short (≤15 gu) chain fractions, respectively.
[c] * , ** = $P \le 0.05$ and ≤ 0.01, respectively.

correlation with any of the molecular properties; the ΔH_2 and ΔH_∞ exhibited significantly positive correlation with *s* and *l* values ($P < 0.05$), respectively. For early-stage retrogradation kinetics, the n_1 significantly increased with increasing DP_n, NC ($P < 0.01$), and *s* value ($P < 0.05$), and with decreasing CL, ECL ($P < 0.01$), ICL, and *el* ($P < 0.05$). These molecular parameters, except ICL, also appeared to significantly affect K_1 ($P < 0.05$) in ways contrary to those for n_1. For the n_2 and K_2 values for late-stage retrogradation, only *el* and *l* values appeared to play a significant role in changing K_2.

Retrogradation Kinetics of Various Starch-Containing Systems

A comparison of retrogradation kinetics of AP in this study with those of other starch-containing systems reported may allow the identification of various AP retrogradation mechanisms in practical starch systems. As indicated in Table III, the *n* and *K* values for retrogradation of 20% AP (1) isolated from regular corn starches at 4°C were 1.17–1.73 and 9.7×10^{-2} to 3.7×10^{-1} day^{-1}, respectively (Mua and Jackson 1998). Both ranges were narrower than those of 60% rice AP in this study (2 and 3). The retrogradation of regular corn starches (4–7) showed *n* and *K* value ranges of 0.34–5.60 and 1.0×10^{-5} to 3.0 day^{-1}, respectively, varying with starch concentration and storage temperature (Jouppila et al 1998, Mua and Jackson 1998). The waxy corn starch (8) exhibited an *n* value of 2.19, which was greater than those of regular corn starch (4) and its AP fractions (1) at the same experimental conditions (20%, w/w, 4°C for 14 days) (Mua and Jackson 1998). And, the *K* value of the waxy starch (8) (2.5×10^{-1} day^{-1}) was less than that of the nonwaxy starch (4). By using Raman spectroscopy and an assumed value of $n = 1$, the *K* value of 52% waxy corn starch (9) appeared to be 2.2×10^{1} day^{-1} (Bulkin et al 1987). This is much higher than *K* value of pure AP and starch systems by other techniques. The results of FTIR indicated that the retrogradation of 10% potato starch (10) at 5°C followed a kinetic equation with $K = 2.3\times10^{-1}$ day^{-1} with an assumed value of $n = 1$ (van Soest et al 1994). The potato starch systems at 4.2–16.7% and 22°C (11) or 52% and 1°C (12), of which only retrogradation properties within one day were measured, showed AM retrogradation with $n = 0.64$–0.66 and $K = 1.5\times10^{1}$ to 2.5×10^{1} day^{-1} (11) or $n = 1$ (assumed) and $K = 6.0\times10^{2}$ day^{-1} (12). For 28.6% commercial rice starch during storage at 4°C (13), Fan and Marks (1998) found $n = 0.63$ and $K = 9.2\times10^{-1}$ day^{-n}. Both parameters were different from those of 40% wheat starches at 23°C (14) ($n = 0.78$–1.26, $K = 1.6\times10^{-1}$ to 5.8×10^{-1} day^{-n}) (Zhang and Jackson 1992) and 28.6% rice flours at 4°C (15) ($n = 0.76$–1.53, $K = 1.8\times10^{-1}$ to 4.6×10^{-1} day^{-n}) (Fan and Marks 1998). By DSC and static rheometry (SR), the values for staling kinetics of breads appeared to be $n = 0.7$ and $K = 1.7$–2.1 day^{-n} at 21°C (16) (Russell 1983) or $n = 0.80$–3.37

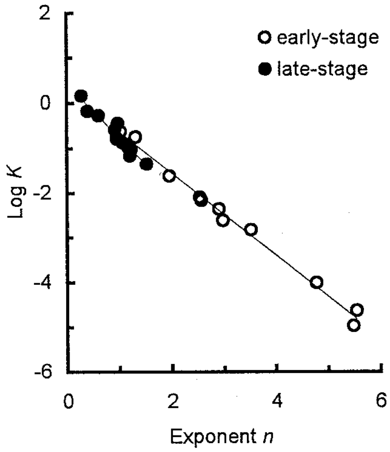

Fig. 3. Relationship between logarithmic *K* constant and Avrami exponent (*n*) for early- and late-stage retrogradation kinetics of 60% (w/w) rice amylopectins at 4°C (*K* in day^{-n}).

TABLE III
Avrami Kinetic Parameters (*K* and *n* values) for Retrogradation and Staling of Starch-Containing Systems

Starches and System No.	Experimental Condition/Method[a]	*n*	*K*	Literature Cited
AP from corn starches				
1	20%, 4°C, 0–14 days/DSC	1.17–1.73	9.7×10^{-2}–3.7×10^{-1} day^{-1}	Mua and Jackson 1998
AP from rice starches				
2	60%, 4°C, 0–7 days/DSC	1.03–5.54	1.0×10^{-5}–2.3×10^{-1} day^{-n}	This study
3	60%, 4°C, 7–35 days/DSC	0.28–1.52	4.4×10^{-2}–1.4 day^{-n}	This study
Corn starch (regular)				
4	20%, 4°C, 0–14 days/DSC	0.59	3.7×10^{-1} day^{-1}	Mua and Jackson 1998
5	60%, 10–60°C, 0–27 days/DSC	0.44–2.00	2.1×10^{-2}–9.0×10^{-1} day^{-1}	Jouppila et al 1998
6	70%, 10–60°C, 0–27 days/DSC	0.62–5.60	1.0×10^{-5}–7.5×10^{-1} day^{-1}	Jouppila et al 1998
7	80%, 50–80°C, 0–19 days/DSC	0.34–1.70	4.1×10^{-2}–3.0 day^{-1}	Jouppila et al 1998
Corn starch (waxy)				
8	20%, 4°C, 0–14 days/DSC	2.19	2.5×10^{-1} day^{-1}	Mua and Jackson 1998
9	52%, 1°C, 0–14 days/Raman	1[b]	2.2×10^{1} day^{-1}	Bulkin et al 1987
Potato starch				
10	10%, 5°C, 0–15 days/FTIR	1[b]	2.3×10^{-1} day^{-1}	van Soest et al 1994
11	4.2–16.7%, 22°C, 0–5 hr/DR	0.64–0.66[c]	1.5×10^{1}–2.5×10^{1} day^{-1} [c]	Mita 1992
12	52%, 1°C, 0–1 days/Raman	1[b,c]	6.0×10^{2} day^{-1} [c]	Bulkin et al 1987
Rice starch				
13	28.6%, 4°C, 0–35 days/DSC	0.63	9.2×10^{-1} day^{-n}	Fan and Marks 1998
Wheat starch[d]				
14	40%, 23°C, 0–11 days/DSC	0.78–1.26	1.6×10^{-1}–5.8×10^{-1} day^{-n}	Zhang and Jackson 1992
Rice flours				
15	28.6%, 4°C, 0–35 days/DSC	0.76–1.53	1.8×10^{-1}–4.6×10^{-1} day^{-n}	Fan and Marks 1998
Bread				
16	21°C, 0–15 days/DSC	0.7	1.7–2.1 day^{-n}	Russell 1983
17	24°C, 0–15 days/SR	0.80–3.37	1.2×10^{-3}–1.6×10^{-1} day^{-1}	Armero and Collar 1998

[a] Indices obtained from DSC(differential scanning calorimetry), DR (dynamic rheometry), FTIR (Fourier transform infrared spectroscopy), Raman spectroscopy, and SR (static rheometry) are enthalpy change (ΔH), storage modulus (G'), absorbance ratio of bands 1,053 to 1,035 cm^{-1}, half width of 480 cm^{-1} band, and gel firmness, respectively.

[b] Presumed value.

[c] Data involved mainly amylose gelation.

[d] Native and acid-treated wheat starches.

and $K = 1.2 \times 10^{-3}$ to 1.6×10^{-1} day^{-1} at 24°C (17) (Armero and Collar 1998). Using DSC, the n values of AP retrogradation in pure AP or starch systems had a range of 0.28–5.60 and the K values were 1.0×10^{-5} to 9.2×10^{-1} day^{-n} (or day^{-1}). The exponents of the units for K in the original reports were inconsistent, but such inconsistency caused minor discrepancy in comparisons because most of the n values were ≈ 1.

There were negative linear relationships between log K and n (Fig. 3) for early and late retrogradation stages of the AP studied. Such relationships can also be discovered in the data extracted from several reports (Zhang and Jackson 1992, Fan and Marks 1998, Jouppila et al 1998). The constants of the relationships obtained by least-squares method are given in Table IV. Evidently, the 60% rice AP retrograded at 4°C (2 and 3 of this study), 60 and 70% corn starches retrograded at 10–60°C (5 and 6 of Jouppila et al 1998), and 40% wheat starch retrograded at 23°C (14 of Zhang and Jackson 1992) exhibited similar slopes ($a = -0.923$ to -1.180). The 80% corn starch at 50–80°C (7 of Jouppila et al 1998) and 28.6% rice flours at 4°C (15 of Fan and Marks 1998) showed a slope of –1.353 and –0.497, respectively. The intercept b values were 0.055–0.895, increasing as the absolute magnitude of a increased ($R^2 = 0.903$–0.998). Log K-n relationships were not observed in the following systems: those containing complicated compositions (Armero and Collar 1998); those at very low starch concentrations such as 1% (del Rosario and Pontiveros 1983); or for AM retrogradation occurring on very short-term storage (0–5 hr) (Mita 1992). Accordingly, it may be suggested that a low n value, narrow range in n or K, and great n-dependence of K tended to appear under diffusion-controlled conditions including prolonged storage, starch concentrations as high as 80%, and the presence of nonstarch components in flours. Because both n and K are constants concerning nucleation type and crystal geometry (Wunderlich 1997), their relationship can facilitate the clarification of the differences in AP retrogradation mechanisms between various systems. In addition, the n value was reported to be highly correlated with amylogram parameters (Armero and Collar 1998). This index may reveal important information about the texture or eating quality of starchy products.

Influence of Molecular Factors on AP Retrogradation Mechanisms

Events during crystallization process. By considering nucleation, crystal growth, and perfection stages for the overall recrystallization process (Wunderlich 1990), the nucleation rate, which should be inversely proportional to induction time t_{ind}, tends to decrease with increasing DP$_n$ and s values. The subsequent crystal growth is nucleation-controlled under isothermal conditions (Biliaderis and Zawistowiski 1990, Wunderlich 1997, Fredriksson et al 1998). Because smaller and less perfect crystalline regions are prone to be present at lower storage temperatures such as 4°C (Longton and LeGrys 1981, Jouppila et al 1998), crystal perfection after recrystallization would proceed slowly for several weeks of storage (Shi and Seib 1995). Consequently, the early and late kinetic stages for pure AP (Fig. 2) can be linked to the crystal growth stage and to both crystal growth and perfection stages, respectively. These stages may be relevant to two major mechanisms of AP retrogradation in 30% wheat and potato starches at 4–20°C: the formation of crystalline clusters along AP chains and the subsequent formation of cross-links between adjacent clusters (Keetels et al 1996). Multistage retrogradation behaviors are also found in 52% waxy corn and potato starches (Bulkin et al 1987), 10% potato starch gels (van Soest et al 1994), and 0.6–2.8% AM (Doublier and Choplin 1989). The retrogradation of nonwaxy starches appears to involve more complicated crystallization mechanisms caused by AM and AP molecules (Miles et al 1985, Orford et al 1987, Mita 1992, van Soest et al 1994).

It is reasonable that ordered helices of AP chains should appear during cooling or early storage at 4°C and act as nuclei for subsequent crystal growth (Wong and Lelievre 1982, Bulkin et al 1987, van Soest et al 1994, Keetels et al 1996). Hence, heterogeneous nucleation where nuclei are already present (Wunderlich 1990) may adequately describe what occurs in the retrograded AP and starch systems. For macromolecular crystallization, heterogeneous nucleation may be athermal or thermal. Athermal nucleation, where all crystals started growing at the same time, seems to be more possible than thermal nucleation, where new crystals started growing throughout the crystallization (Wunderlich 1976). If athermal nucleation is considered as the primary mechanism for AP crystallization, an instantaneous and predetermined nucleation would be followed by fibrillar or rod-like ($n \leq 1$), disk-like or diffusion-controlled spherical ($1 < n \leq 2$), spherical ($2 < n \leq 3$), or solid-sheaf ($n \geq 5$) crystal forms (Wunderlich 1997). Therefore, the results of Table II demonstrate that the crystallization types during early storage of 60% AP may be disk-like (KSS7, TCS17, and TCN1), spherical (TCS10, TS1, TNu67, and TCSW1), and solid-sheaf (KS142, TN9). TC189 and TNu70 showed crystallization types between spherical and solid-sheaf. As to the late-stage retrogradation, all rice AP exhibited similar mechanisms of instantaneous nucleation followed by rod- or disk-like growth of crystals. This result agrees with Wunderlich (1976) in that for two-stage spherulitic crystallization, the interior of a macromolecular spherulite is often fibrillar. Thermal nucleation may be partially present during the retrogradation as well.

Effects of molecular properties on extent of retrogradation. The molecular properties of AP in this study are DP$_n$ = 2,743–11,931 gu, CL = 15.4–22.1 gu, ECL = 11.3–15.8 gu, and ICL = 3.2–5.7 gu. The short-chain fractions composed of exterior and interior chains are comparable to the A chains and parts of B1 chains, and the long chain fractions are comparable to the B1–B4 chains in the revised cluster model of Hizukuri (1986). The data of Table II suggest the incorporation of short chains in crystal growth and perfection stages through crystallization and cocrystallization processes. Similarly, the retrogradation extent of 36% waxy maize starch at 20°C was closely related to that of ECL (11.3–13.9 gu) (Würsch and Gumy 1994). For the systems of linear maltooligosaccharides at 30–50% w/w (at 15 and 30°C), the minimum CL required was 10 gu for crystallization and 6–9 gu for short chains cocrystallized with longer chains (Gidley and Bulpin 1987). However, the minimal CL for formation of stable crystallites between branching chains in AP

TABLE IV

Relationships Between K and n Values for Retrogradation of Various Starchy Systems[a]

		log $K = a \times n + b$			
Starch	**System No.**	a	b	R^2	**Data Source**
60% rice AP	2	–0.923	0.296	0.991	This study
	3	–1.180	0.422	0.934	This study
60% corn starch	5	–1.007	0.451	0.903	Jouppila et al 1998
70% corn starch	6	–0.989	0.473	0.990	Jouppila et al 1998
80% corn starch	7	–1.353	0.895	0.998	Jouppila et al 1998
40% wheat starch	14	–1.089	0.584	0.949	Zhang and Jackson 1992
28.6% rice flour	15	–0.497	0.055	0.972	Fan and Marks 1998

[a] Detailed experimental conditions and the unit of K are shown in Table III.

molecules should be greater than that of maltooligosaccharides because of the effects of steric hindrance. For the 36% waxy maize starch aged at 20°C, Würsch and Gumy (1994) discovered that when ECL ≤11, no retrogradation enthalpy is observed. The presence of short chains with CL = 6–11 gu is probably detrimental to the association of long external chains in 25–50% waxy maize starch systems (Würsch and Gumy 1994, Shi and Seib 1995). Accordingly, the minimal CL for crystallization and cocrystallization of AP chains may be 12 gu but is probably dependent on the sample concentration and storage condition used.

The ΔH_1, ΔH_2, and ΔH_∞ for AP retrogradation reflect the amount of crystallites produced during early crystal growth, late crystal growth with perfection, and the overall recrystallization process, respectively. The results in Table II demonstrate that ΔH_1 was not closely related to AP molecular properties. Because the minimal CL for crystallization and cocrystallization of AP chains may be 12 gu, all short, long, and extra-long chain fractions probably contribute equally to the early-stage crystallization. However, ΔH_2 and ΔH_∞ increased significantly with increasing proportion of short (CL ≤ 15 gu) and long chains (CL = 16–100 gu), respectively. For the diffusion-controlled late-stage crystallization, the influence of short chains appears to exceed that of longer chains, probably because of greater mobility (Zhang and Jackson 1992). Self-crystallization of short chains and partial cocrystallization of short chains with long chains are probable (Gidley and Bulpin 1987). The effects of long chains on ΔH_∞ are also observed in the results of maize starches (25–50%, 4°C), where the degree of retrogradation is proportional to the amount of branching chains with CL = 16–30 gu (Shi and Seib 1995). However, the proportion of short chains with CL < 17.8 may also have a notable role in increasing the final ΔH as it does for high-amylose barley starch (Fredriksson et al 1998). On the other hand, Mua and Jackson (1998) concluded that intermediate AP fractions developed more crystallization and gel firmness than did high and low M_w fractions, similar to the results of AM fractions. These effects from molecular properties may be further governed by storage period (Mua and Jackson 1997).

Effects of molecular properties on retrogradation mechanisms. Table II indicates that for 60% rice AP, n_1 increased and K_1 decreased with increasing DP_n, NC, and proportion of short chains (*s*) or with decreasing CL, ECL, and proportion of extra-long chains (*el*). The effects of DP and NC on n_1 agree with the results of others on 20% AP gels aged at 4°C in that the *n* value was significantly proportional to M_w and inversely proportional to branching (Mua and Jackson 1998). The roles of chain lengths, *s*, and *el* values in changing K_1 partially agree with the finding that cereal AP showed lower retrogradation rates (25%, in 0.05*M* NaCl at 1°C) than did noncereal AP because the former has lower CL values (Orford et al 1987, Kalichevsky et al 1990). Similarly, the AP fragments (DP = 586–1,235 gu) resulted in higher initial rates of recrystallization than did higher molecular weight counterparts (Zhang and Jackson 1992), in agreement with the concept of macromolecular crystallization (Wunderlich 1976).

Other influencing factors on AP retrogradation kinetics. Amylose content in starch, sample concentration, storage conditions, and analysis method appear to influence the resultant kinetic parameters (Table III). First, the data of the literature for 11 and 12 shows that *n* tended to increase and *K* tended to decrease with the decrease in AM content of starch materials. This is in agreement with the work of Fan and Marks (1998) and was also the case when the crystallized species in wheat starch gels changed from AM (short-term process) to AP (long-term process) (Wong and Lelievre 1982). Second, higher sample concentrations may give more nuclei and allow a decreased *n* and increased *K* (Wunderlich 1976), as in the retrogradation of 40–50% wheat starches (Roulet et al 1988) and 60–70% corn starches (Jouppila et al 1998). However, the concentration effects on retrogradation rate could not be ruled out from the data in Table III due to the variation in storage temperature and analysis method used. Third, when the storage temperature was far below melting temperatures of crystallites formed by AP (40–60°C), a lower storage temperature would cause a higher *n* value (del Rosario and Pontiveros 1983, Wu and Eads 1993, Jouppila et al 1998); increased nucleation rate or retrogradation rate (Roulet et al 1988, Fredriksson et al 1998); and a greater proportion of smaller and less perfect crystallites (Longton and LeGrys 1981, Lu et al 1997a, Jouppila et al 1998). These effects may account for some deviations in the data of Table III, except for systems 5–7 retrograding at temperatures close to melting temperatures of crystallites. For those, the crystallization rate in starch appeared to increase with increasing temperatures (Jouppila et al 1998). Comparisons of systems 9 (for AP retrogradation) and 12 (for AM retrogradation) with the other systems of Table III suggest that the data by Raman spectroscopy probably gave a greater *K* value than those by DSC, X-ray diffraction, FTIR, or static rheometry (SR). This can be attributed to the fact that Raman spectroscopy responds to small-range molecular ordering but the other methods reflect long-range and notable recrystallization (Longton and LeGrys 1981, Roulet et al 1988). The adoption of a fixed *n*, instead of a varying *n*, in fitting the experimental data with the Avrami equations may cause some discrepancies in the kinetic data of systems 9, 10, or 12 (Longton and LeGrys 1981, Zhang and Jackson 1992).

The results in this study suggest that the Avrami approach allows insight into the type of AP recrystallization mechanisms in various starch-containing systems, although this approach can be successful only with microscopic data (e.g., crystallite size) rather than with thermal analysis data (Wunderlich 1997). However, with thermal analysis, the resultant *K* constant of the Avrami equation does not reflect the rate constant of recrystallization during starch retrogradation. Some complications in the Avrami approach of retrogradation kinetics remain to be considered, including the potential changes in volume of individual crystallite, linear growth rate, number of nuclei, and crystal morphology (Wunderlich 1976).

CONCLUSIONS

By using the Avrami equation in modeling the changes in ΔH during retrogradation, the 60% rice AP aged at 4°C indicated a two-stage recrystallization process. Correlation analysis suggested that the kinetic parameters of early-stage retrogradation (≤7 days) were more significantly correlated than late-stage retrogradation (≥7 days) with the number-average molecular weight and chain lengths of the AP molecules. The proportion of short, long, and extra-long chain fractions appeared to have greater influences on the enthalpy changes and late-stage kinetics than the other structural factors. The negatively linear relationship between log *K* and *n* for a series of AP or starch systems may be potentially governed by some nonstructural factors such as sample concentration, storage condition, and the presence of nonstarch components in flours. Knowledge about the events and kinetic properties of AP recrystallization would be helpful for controlling starch retrogradation in food industry.

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