Influence of Acyl Chain Lengths in Mono- and Diacyl-*sn*-glycerophosphatidylcholine on Gelatinization and Retrogradation of Starch

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The influence of starch with 1- or 2-monoacyl-*sn*-glycerophosphatidylcholine (GPC) having various chain lengths of fatty acids on gelatinization and retrogradation of starch was studied by the measurement of starch−GPC complex formation, complexing index, and differential scanning calorimetry. The addition of GPC to the starch sample slightly increased the blue value and λ_{max} with increasing chain length of GPC but decreased the phosphorus content and complexing index. The gelatinization onset and peak temperatures of starch complexes increased significantly with increasing chain length, but the enthalpies were statistically lower, except for the treatment with 1,2-distearoyl-*sn*-GPC when compared with that of the control. Among GPC (di and mono), 1- and 2-monomyristoyl-*sn*-GPC showed the highest complexing ability, whereas the complexing ability of the GPC decreased with the increasing chain length. According to the Avrami equation, the retrogradation rate (k, day^{-1}) of starch was slower than that of the control, whereas the retrogradation rates of 1- and 2-monomyristoyl-*sn*-GPC were slowest among the GPCs. The positive linear relationship between k and the number of acyl groups of GPC suggests that a GPC with a shorter chain length could retard the retrogradation of starch during storage.

Keywords: *Glycerophosphatidylcholine; Avrami equation; phospholipase A_2; lipase; gelatinization; retrogradation*

INTRODUCTION

Changes in starch during cereal processing contribute to product texture, and the most important changes are gelatinization and retrogradation. Retrogradation of gelatinized starch occurs in many starch-based food systems (*1*). Retrogradation is considered to be a process in which the molecules of gelatinized starch reassociate to form crystallites. Retrogradation contains two crystallization stages: in the first stage, the rigidity and crystallinity of starch gels develop quickly by amylose recrystallization. In the second stage, the crystallinity develops slowly by amylopectin (*2*). Starch retrogradation and the nature of the formed crystals depend on the starch source, amylose content, storage temperature, and the presence of other food ingredients (*3*−*6*). The extent of retrogradation can significantly influence the texture and quality of starch-containing food products (*1*).

In food systems, complexes with monoglycerides, free fatty acids, lysophospholipids, and surfactants are of interest because they can affect the functional properties of food products (*7*). Lipids or surfactants are used as modifiers in starch-containing foods. For example, monoacyl lipids are used in bread-making to retard staling (*8*). These complexes are readily formed as insoluble precipitates in neutral aqueous media and decrease the susceptibility of amylose to amylolysis (*9*, *10*). Therefore, it has been suggested that there is a competitive mechanism between amylose retrogradation and the formation of amylose−lipid complexes, in which crystallization of amylose−lipid complexes is favored (*11*). Eliasson et al. (*12*) has reported that formation of complexes prevents leaching of amylose during gelatinization, inhibits swelling of starch granules heated in water, and reduces the water-binding capacity of starch. Complex formation of starch−lipids has been used to explain the positive effect of emulsifier on the staling of starch, either directly by preventing the amylose molecules from crystallizing or indirectly by changing the water distribution in starch.

The objectives of this study were to (1) determine the influence of starch inclusion complexes with different chain lengths of 1,2-diacyl-, 1-monoacyl-, and 2-monoacyl-*sn*-GPC on the gelatinization and retrogradation of starch, using differential scanning calorimetry (DSC), and (2) evaluate whether the Avrami model of starch crystallization (*13*) fits the kinetics of starch retrogradation in gelatinized starch.

MATERIALS AND METHODS

Materials. 1,2-Dimyristoyl-*sn*-glycerophosphatidylcholine (1,2-M-*sn*-GPC), 1,2-dipalmitoyl-*sn*-glycerophosphatidylcholine (1,2-P-*sn*-GPC), 1,2-distearoyl-*sn*-glycerophosphatidylcholine (1,2-S-*sn*-GPC), and lysophosphatidylcholine (LPC) were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and used as received after their purity had been checked by TLC. Phospholipase A_2 (phosphatidylcholine 2-acylhydrolase, EC 3.1.1.4) from bee venom was obtained from Sigma Chemical Co. (St. Louis, MO). Lipase (triaclyglycerol acylhydrolase, EC 3.1.1.3) from *Aspergillus niger* was provided by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan). Wheat starch (type "Hermes", provided by Okumoto Flour Milling Co.,

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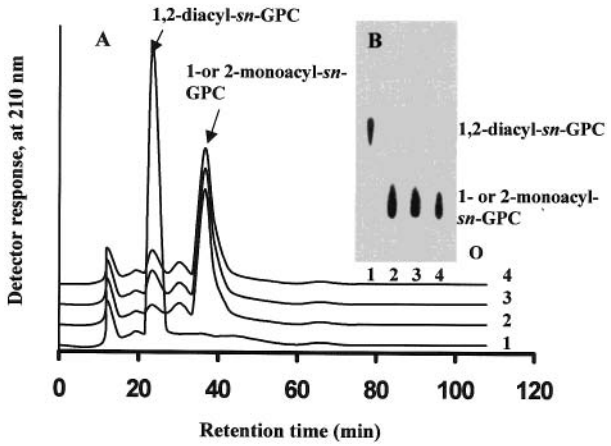

Figure 1. (A) HPLC chromatograms for the quantitative determination of 1- and 2-monoacyl-*sn*-GPC isolated from TLC. Conditions: column, IRICA-NH_2 (8 × 250 mm); mobile phase, acetonitrile/methanol/0.2% triethylamine, pH 4.0 (60:30:10, v/v); flow rate, 1 mL/min; detection, 210 nm. (B) TLC chromatogram of isolated 1- and 2-monoacyl-*sn*-GPC. Conditions: developing solvent, chloroform/methanol/water (64:25:4 v/v); spray reagent, molybdenum blue. 1, 1,2-dimyristoyl-*sn*-GPC; 2, LPC; 3, 2-monomyristoyl-*sn*-GPC; 4, 1-monomyristoyl-*sn*-GPC.

Ltd., Osaka, Japan) was defatted with a hot butanol/water (3:1, v/v) mixture and re-extracted three times in a screw-capped tube. To remove the remaining butanol, the starch sample was washed repeatedly with deionized water and then freeze-dried.

Preparation and Isolation of 1- and 2-Monoacyl-*sn*-GPC. The two types of 1- and 2-monoacyl-*sn*-GPC were prepared by enzymatic hydrolysis as follows. The incubations were carried out in 10-mL glass vials with screw caps. For the preparation of 1-monoacyl-*sn*-GPC, 1,2-diacyl-*sn*-GPC was hydrolyzed by bee venom phospholipase A_2, as follows (*14*). The reaction mixture contained 2% GPC (w/v), chloroform (4 mL), and 50 μg of phospholipase A_2 (1225 units/mg) in 36 μL of 50 mM Tris-HCl, pH 8.9, 5 mM $CaCl_2$ buffer, and was incubated at 37 °C for 3 h. For 2-monoacyl-*sn*-GPC, *A. niger* lipase (1500 units) was used as a source of phospholipase A_1, acting on 1,2-diacyl-*sn*-GPC. The same conditions as above were used, except that 4 mL of hexane was added to the reaction mixtures and the reactions were extended for 48 h at 45 °C. One unit of the lipase activity was defined as the quantity of enzyme that liberates 1 μmol of *p*-nitrophenyl/min under the routine assay conditions (*15*). After concentration, the hydrolyzed GPC was isolated by a silica gel TLC and quantitatively analyzed by HPLC in comparison with standard lysophosphatidylcholine as described by Virto and Adlercreutz (*16*). HPLC data showed that the GPC (monomyristoyl-*sn*-GPC) was a major product (>98%) by phospholipases A_1 and A_2, and DGPC was formed in a small amount, as shown in Figure 1.

Preparation of Starch—Lipid Complex. Complexes of GPC with starch were prepared according to the method of Eliasson et al. (*12*). Di- and monoacyl-*sn*-GPC do not readily disperse in water at room temperature. They were mixed at the ratio of 1:10, heated at 70 °C to obtain the lamellar liquid crystalline phase, then allowed to cool to 60 °C, and kept at that temperature before addition of starch. Defatted starch (2 g) was dispersed in 3.4 mL of water, to which various amounts of GPCs dispersed in water were added. These mixtures were stirred at 360 rpm for 1 h at room temperature, completed with heating for 1 h at 60 °C while stirring at 360 rpm, and then immediately cooled to room temperature in a water bath. It is considered that the starch−GPC complex preparation obtained by heating at 60 °C for 1 h involves the gelatinized granules, the partially gelatinized granules, and also the considerably annealed granules. All lipids were added at a concentration of 2% (w/w) calculated on dry starch basis. The control had the same amount of water in place of the lipid suspension.

Measurement of Phosphate Content, Blue Value, and Complexing Index (CI). The CI determines the degree of starch−lipid complex formation. Measurement of the index is based on the method of Gilbert and Spragg (*17*) and involves the formation of a starch−iodine complex. The iodine solution used for this assay was prepared by dissolving 0.1 g of potassium iodide and 0.02 g of I_2 in 50 mL of distilled water and allowing it to dissolve overnight. Triplicate 0.01 g samples of the starch−lipid were placed in 10-mL tubes. A control contained defatted starch only. To each tube was added up to 5 mL of distilled water. The sample suspension was vortexed vigorously for 2 min and heated at 60 °C for 15 min, and then the mixture was centrifuged at 2000 rpm for 20 min. The supernatant (100 μL) was added to 2 mL of iodine solution and made up to 5 mL with deionized water. The tube was inverted several times to mix, and then the absorbance was measured at 680 nm. The phosphorus content in each sample complex was converted into inorganic phosphate by wet ashing of the whole complex with 0.3 mL of sulfuric acid and three drops of peroxide. The phosphate was determined as reported by Morrison (*18*).

Differential Scanning Calorimetry (DSC). The DSC measurements were done using a Shimadzu DSC apparatus (model DSC-60, Kyoto, Japan), controlled by TA-60 WS software and connected to a thermal analysis. The calorimeter was calibrated with indium (melting point = 156.7 °C, ΔH = 27.6 J/g), and the reference used was liquid paraffin as reported by Morita et al. (*19*). Starch−lipid complexes (4−5 mg) in aluminum DSC pans were weighed, and deionized water was added to the starch sample (dry solid) to make the ratio of water to starch 2:1. After sealing, the pan was left for 1 h to allow the sample to mix and equilibrate at room temperature before heating. Then the sample was scanned (first scan) at a rate of 5 °C/min from 30 to 125 °C under nitrogen gas. To evaluate whether the starch−lipid complex samples were completely gelatinized during the first DSC scan, the samples were rescanned immediately after being cooled to room temperature after the first scan. Over the range of gelatinization temperature, no endothermic transition was observed in these rescan thermograms, indicating that the starch−lipid complexes had been fully gelatinized.

The sample pans of gelatinized starch−lipid complexes were stored for 0−12 days at 22 °C. Subsequently, the samples were equilibrated at room temperature for 1 h and then rescanned (second scan) in the calorimeter from room temperature to 125 °C at a rate of 5 °C/min to measure the retrogradation temperature and enthalpy. The onset temperature (T_o), peak temperature (T_p) of the starch or starch−lipid complex, and enthalpy for starch (ΔH_g) and the starch−lipid complex (ΔH_{s-l}) were measured to characterize the thermal properties of starch.

Calculation of Retrogradation Kinetics. Retrogradation of starch during storage was estimated from a DSC thermogram. The Avrami model was employed to describe the kinetics of starch retrogradation in the starch. The model (*13*) can be expressed as

$$\theta = \frac{\Delta H_\infty - \Delta H_t}{\Delta H_\infty - \Delta H_0} = \exp(-kt^n) \qquad (1)$$

where θ is the fraction of uncrystallized starch at time t, ΔH_0 and ΔH_t are the enthalpy changes at time 0 and time t, respectively, ΔH_∞ is the limiting enthalpy change, k is the rate constant, and n is the Avrami exponent. ΔH_∞ was taken to be the limiting enthalpy change at infinite time ($t \rightarrow \infty$) obtained from the plot of $1/\Delta H_t$ against $1/t$ (*20*). The rate constants (k) and exponents (n) for the starch (where ΔH_0 was zero in this study) were obtained from linear regression of the retrogradation enthalpy data as

$$\log\left\{-\ln\left(\frac{\Delta H_\infty - \Delta H_t}{\Delta H_\infty}\right)\right\} = \log k + n \log t \qquad (2)$$

Table 1. Effects of 1,2-Diacyl- and 1- or 2-Monoacyl-*sn*-GPC on Phosphorus Content, Blue Value, λ_{max}, and Complexing Index of Starch−Lipid Complex

complex	phosphorus content (μg/100 mg)	blue value (680 nm)	λ_{max}(abs)	CI[a] (%)
starch	44.3 ± 0.7[b]	0.408 ± 0.011	614.0 (0.461)	
starch−1,2-M-*sn*-GPC[c]	67.0 ± 4.9	0.121 ± 0.007	602.5 (0.155)	70.4 ± 1.1
starch−1,2-P-*sn*-GPC	52.5 ± 4.5	0.125 ± 0.006	604.0 (0.157)	69.3 ± 1.4
starch−1,2-S-*sn*-GPC	49.8 ± 4.3	0.203 ± 0.028	608.8 (0.230)	50.3 ± 6.9
starch−1-M-*sn*-GPC	112.6 ± 1.5	0.020 ± 0.002	550.0 (0.027)	95.0 ± 0.5
starch−1-P-*sn*-GPC	103.2 ± 2.8	0.028 ± 0.003	560.1 (0.039)	93.1 ± 0.8
starch−1-S-*sn*-GPC	77.1 ± 2.4	0.034 ± 0.002	599.2 (0.046)	91.6 ± 0.5
starch−2-M-*sn*-GPC	109.4 ± 3.4	0.020 ± 0.004	551.2 (0.024)	95.0 ± 1.0
starch−2-P-*sn*-GPC	97.6 ± 4.9	0.031 ± 0.002	559.2 (0.042)	92.5 ± 0.5
starch−2-S-*sn*-GPC	71.9 ± 2.7	0.037 ± 0.003	604.3 (0.043)	91.0 ± 0.6

[a] Complexing index. Calculated as {(Abs_{680nm} starch − Abs_{680nm} starch-GPC)/Abs_{680nm} starch} × 100. [b] Mean ± standard deviation (three determinations). [c] GPC, glycerophosphatidylcholine; M, myristoyl; P, palmitoyl; S, stearoyl.

Statistical Analysis. Values were obtained as the means ± standard deviation of three determinations, following ANOVA and analyzed by Duncan's multiple-range test. Differences among samples were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Complex Formation Ability. The complexing abilities of 1,2-diacyl-*sn*-GPC and phosphatidylcholine hydrolyzed [1- or 2-monomyristoyl(M)-*sn*-GPC, 1- or 2-monopalmitoyl(P)-*sn*-GPC, 1- or 2-monostearoyl(S)-*sn*-GPC] with starch in the blue value, λ_{max}, phosphate content, and complexing index (CI) are shown in Table 1. The blue value and λ_{max} of the starch−lipid complex increased with an increase in GPC fatty acid chain length, M < P < S for 1,2-diacyl-*sn*-GPC and 1- or 2-monoacyl-*sn*-GPC, and the phosphate content and CI decreased with an increase in GPC fatty acid chain length, M > P > S for 1,2-diacyl-*sn*-GPC and 1- or 2-monoacyl-*sn*-GPC. The 1- or 2-monoacyl-*sn*-GPC showed the highest complexing ability when compared with that of the 1,2-diacyl-*sn*-GPC. This result suggests that the presence of GPCs with 1- or 2-monoacyl-*sn*-GPC caused a great reduction in iodine affinity for starch. This view is supported by a considerable change in the color of solutions after exposure to iodine, the weak brown color indicating the absence of an amylose−iodine complex (*21*). Schoch and Williams (*22*) observed that fatty acids interfered with the formation of amylose−iodine complexes. An X-ray study also revealed that the helix of the amylose−lipid complex is similar to that of the amylose−iodine complex (*23*).

Thermal Properties of Starch—Lipid Complexes. The complexing abilities of different chain lengths of GPC with starch were investigated by analyzing the gelatinization properties of the lipid−starch samples using DSC. Representative thermograms are shown in Figure 2. The heating DSC curve for di- or monoacyl-*sn*-GPC dispersion, the main endothermic peak accompanying gelatinization (*G*), was observed around 60−78 °C, and another endothermic peak (L_2) was observed around 95−115 °C. The endothermic peak at the higher temperature is attributed to the disintegration of the amylose−lipid complex (*2*). When 1,2-M-*sn*-GPC, 1,2-P-*sn*-GPC, and 1,2-S-*sn*-GPC were added, a small endothermic peak (L_1) was observed near the melting point of those (40−50 °C), suggesting that the excess of 1,2-diacyl-*sn*-GPCs was outside the helix of the complex, probably in the interstices of the folded helices as reported by Huang and White (*24*). Furthermore, the melting peak of the starch−lipid complexes disappeared completely in the control. The enthalpies of starch with

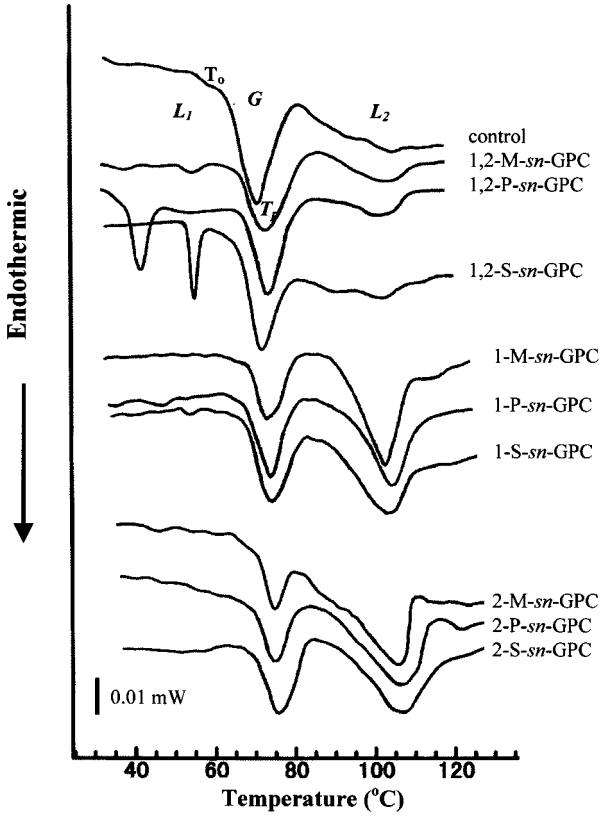

Figure 2. DSC curves showing gelatinization properties of starch and starch−lipid (*G*, gelatinization endotherm; L_1, endotherm of free lipid melting; L_2, endotherm of amylose−lipid complex melting; T_o, onset temperature; T_p, peak temperature; M, myristoyl; P, palmitoyl; S, stearoyl).

different chain lengths of GPCs were compared (Table 2). Additions of GPC with increasing fatty acid chain lengths caused a significant increase in gelatinization enthalpies (ΔH_g). As the chain length of 1- or 2-monoacyl-*sn*-GPC added to starch became shorter, the area of the first endothermic peak in DSC curves tended to decrease due to the enthalpy of gelatinization of starch granules, and the area of the second peak tended to increase due to the enthalpy of the starch−lipid complexes (ΔH_{s-l}). The shortening of the chain length of 1- or 2-monoacyl-*sn*-GPC is considered to accelerate the formation of starch−lipid complexes because the lipid is more easily accommodated into the amylose helix and then the lipids suppressed starch gelatinization (*24*). Furthermore, addition of 2% monoacyl-*sn*-GPC increased the ΔH_{s-l} by 1.52−2.00 J/g for 1-monoacyl-*sn*-GPC and by 1.48−1.93 J/g for 2-monoacyl-*sn*-GPC over

Table 2. Thermal Properties of Gelatinization and Starch−Lipid Complex[a]

	gelatinization			starch−lipid		
complex	T_o (°C)	T_p (°C)	ΔH_g (J/g)	T_o (°C)	T_p (°C)	ΔH_{s-l} (J/g)
starch	65.1a[a]	68.9a	7.8f	ND	ND	ND
starch−1,2-M-*sn*-GPC	68.3b	72.0b	6.0cd	99.1d	103.8ab	5.2c
starch−1,2-P-*sn*-GPC	69.5b	73.4cd	6.6de	99.4d	103.7a	4.4b
starch−1,2-S-*sn*-GPC	68.3b	72.1bc	7.6ef	99.6d	103.7a	2.9a
starch−1-M-*sn*-GPC	69.9c	73.5d	3.0a	94.7a	105.3bc	8.2e
starch−1-P-*sn*-GPC	70.1cd	73.9de	3.5a	95.6ab	106.0c	6.7cd
starch−1-S-*sn*-GPC	69.9c	74.0de	4.7b	95.4ab	105.3c	5.8c
starch−2-M-*sn*-GPC	72.0e	76.1f	2.5a	96.6abc	106.3c	7.9de
starch−2-P-*sn*-GPC	71.1de	75.6f	2.6a	97.0bc	106.1c	6.5c
starch−2-S-*sn*-GPC	69.9c	75.0ef	5.4bc	98.1cd	106.4c	5.6c

[a] Values followed by the same letter are not significantly different according to Duncan's multiple range test ($p < 0.05$). ND, not detected. GPC, glycerophosphatidylcholine; M, myristoyl; P, palmitoyl; S, stearoyl.

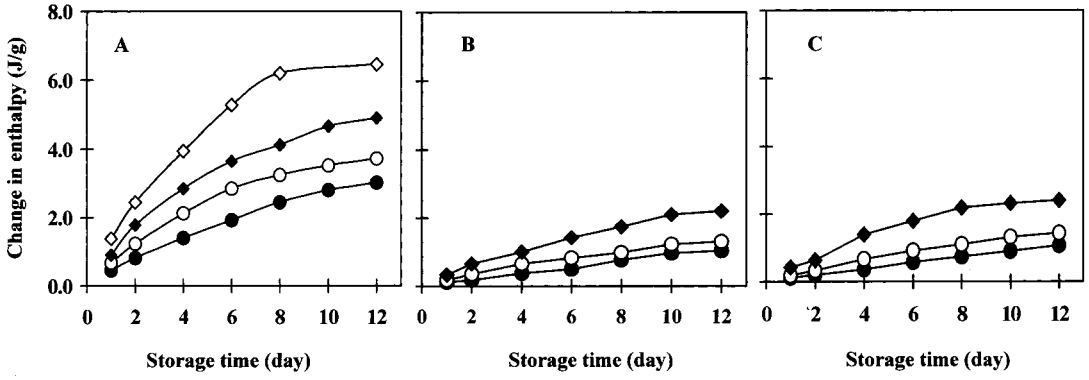

Figure 3. Gelatinization enthalpy of retrogradated starch−GPC as a function of storage at 22 °C: (A) 1,2-diacyl-*sn*-GPC; (B) 1-monoacyl-*sn*-GPC; (C) 2-monoacyl-*sn*-GPC; (◇) control; (●) myristoyl; (○) palmitoyl; (◆) stearoyl.

that obtained with the 1,2-diacyl-*sn*-GPC. This suggests that monoacyl-*sn*-GPCs promote the formation of starch−lipid complexes more than do diacyl-*sn*-GPCs. Presumably, a monoacyl-*sn*-GPC with a short fatty acid chain has a more favorable configuration for forming starch−lipid complexes than does a diacyl-*sn*-GPC with a long fatty acid chain. The gelatinization enthalpies of starch−GPCs were significantly lower than 7.8 J/g of the starch control (ΔH_g), as follows: 6.0 (M), 6.6 (P), and 7.6 (S) J/g for 1,2-diacyl-*sn*-GPC; 3.0 (M), 3.5 (P), and 4.7 (S) J/g for 1-monoacyl-*sn*-GPC; 2.5 (M), 2.6 (P), and 5.4 (S) J/g for 2-monoacyl-*sn*-GPC. The exotherm due to the complex formation should be experimentally probed. Morrison et al. (*25*) reported that if lipids capable of forming complexes with amylose are present during starch gelatinization, the exothermic heat of complex formation partially offsets the endothermic heat of starch gelatinization.

Similar changes in the behavior of starch gelatinization were reported to be caused by the presence of monoglyceride or LPC (*12*, *24*). Such phenomena may be caused by structural changes of starch granules when complexed with lipids. The length of a monoglyceride molecule is ~22 Å; thus, an amylose helix will require 16.5 glucose units to accommodate it (*24*). In contrast, smaller monoacyl-*sn*-GPC molecules are more easily accommodated into the amylose helix. The melting enthalpy of an amylose−monoacyl-*sn*-GPC complex is therefore influenced by the ease with which the monoacyl-*sn*-GPC can be accommodated into the helix. The significant difference in melting behaviors of amylose−monoacyl-*sn*-GPC and amylose−diacyl-*sn*-GPC is probably due to structural differences. Monoacyl-*sn*-GPC has a single chain with both hydrophobic and hydrophilic portions and, as such, it can pass through the surface of granules more easily than the diacyl-*sn*-GPC molecules. In this study, 1- or 2-monoacyl-*sn*-GPC formed complexes with greater ease compared to diacyl-*sn*-GPC with starch amylose.

Retrogradation of Starch—Lipid Complex. The enthalpy value of regelatinization of starch stored at 22 °C during 0−12 days was observed in the range from 50 to 75 °C. The peak is identified as the melting peak of amylopectin crystallites (*26*, *27*). The ΔH_t values at 0 days of all the samples tested were undetectable after heating, because starch was completely gelatinized during heating. The enthalpy for melting of starch increased logarithmically with storage time (Figure 3). The increasing ΔH of starch-containing GPC was roughly classified into two types of kinetic retrogradation behavior. Rapid retrogradation behavior, as exemplified by the control (defatted starch) and starch−diacyl-*sn*-GPC complex (Figure 3A), showed a rapid increase in ΔH commonly at the second day of storage, resulting in maximal ΔH by the 12th day. Slow retrogradation rates, as exemplified by 1-monoacyl-*sn*-GPC and 2-monoacyl-*sn*-GPC starch complexes (Figure 3B,C), showed an increase in ΔH that was detectable on the fourth to seventh days and ΔH gradually increased during storage. The complex with starch and GPC showed lower ΔH than the control after 12 days of storage. In the course of storage, 1- or 2-monoacyl-*sn*-GPC reduced greatly the value of ΔH compared with that of the control or 1,2-diacyl-*sn*-GPC. This result suggests that the retrogradation of starch was retarded by the coexistence of 1- or 2-monoacyl-*sn*-GPC.

The Avrami model was found to give a reasonable description of starch retrogradation during starch gelatinization, with a regression coefficient of 0.98−0.99 (Figure 4), and ΔH_∞ values were in the ranges of 5.64−9.00 (diacyl-*sn*-GPC) and 2.05−5.00 (monoacyl-*sn*-GPC) J/g (Table 3). The Avrami exponent (n) for retrograda-

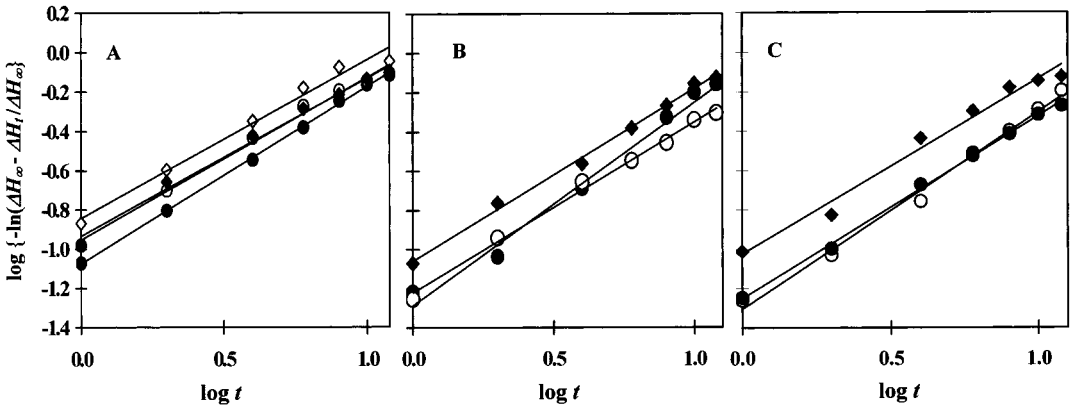

Figure 4. Plots of logarithmic fraction of crystallization [log(−ln($\Delta H_\infty - \Delta H_t/\Delta H_\infty$))] against logarithmic time (log t) during retrogradation of starch−GPC: (A) 1,2-diacyl-*sn*-GPC; (B) 1-monoacyl-*sn*-GPC; (C) 2-monoacyl-*sn*-GPC; (◇) control; (●) myristoyl; (○) palmitoyl; (◆) stearoyl.

Table 3. Temperature and Enthalpy of Retrogradation and Avrami Parameters for Starch−GPC Complex with Various Chain Lengths of Fatty Acids

	retrogradation (22 °C, 12 days)			Avrami parameter[a]			
complex	T_o (°C)	T_p (°C)	ΔH_t (J/g)	ΔH_∞ (J/g)	n	k (day^{-1})	r^2
starch	47.5	60.1	6.5	10.9	0.80	0.141	0.98
starch−1,2-M-*sn*-GPC	47.9	54.6	3.0	5.6	0.90	0.084	0.99
starch−1,2-P-*sn*-GPC	47.0	54.2	3.7	6.9	0.83	0.110	0.99
starch−1,2-S-*sn*-GPC	47.7	55.7	4.9	9.0	0.80	0.117	0.99
starch−1-M-*sn*-GPC	47.0	57.9	1.0	2.1	1.03	0.052	0.98
starch−1-P-*sn*-GPC	47.0	58.3	1.3	3.3	0.87	0.059	0.99
starch−1-S-*sn*-GPC	47.1	58.1	2.2	4.2	0.88	0.087	0.99
starch−2-M-*sn*-GPC	47.1	58.1	1.1	2.3	1.00	0.050	0.99
starch−2-P-*sn*-GPC	46.8	58.1	1.4	3.5	0.93	0.056	0.99
starch−2-S-*sn*-GPC	47.0	57.8	2.4	5.0	0.88	0.085	0.98

[a] Avrami exponent (n), rate constant (k), and regression coefficient (r^2) were obtained from linear regression of experimental data, as shown in eq 2. GPC, glycerolphosphatidylcholine; M, myristoyl; P, palmitoyl; S, stearoyl.

tion kinetics had a range of 0.80−0.90 (diacyl-*sn*-GPC), which was smaller than that of monoacyl-*sn*-GPC (0.87−1.03). The difference in values between the diacyl-*sn*-GPC and monoacyl-*sn*-GPC in starch suggests that the mechanism for recrystallization of starch might be different (*13*), but the n value coincided perfectly with the n value of wheat starch, which is in the range of 0.78−1.26 (*26*). For the rate constant (k), the addition of diacyl-GPC slowed the retrogradation rate by factors of 1.68 ($k_c/k_{1,2-m}$), 1.28 ($k_c/k_{1,2-p}$), and 1.21 ($k_c/k_{1,2-s}$), respectively, with respect to the control (k_c). The retrogradation rate of monoacyl-GPC ($k_{1/2-m,p,s}$) was less than the retrogradation rates of the control and diacyl-GPC ($k_{1,2-m,p,s}$), for which the ratios of retrogradation rate were 2.71 (k_c/k_{1-m}), 2.39 (k_c/k_{1-p}), and 1.62 (k_c/k_{1-s}); 2.82 (k_c/k_{2-m}), 2.52 (k_c/k_{2-p}), and 1.66 (k_c/k_{2-s}); 1.62 ($k_{1,2-m}/k_{1-m}$), 1.86 ($k_{1,2-p}/k_{1-p}$), and 1.34 ($k_{1,2-s}/k_{1-s}$); 1.68 ($k_{1,2-m}/k_{2-m}$), 1.96 ($k_{1,2-p}/k_{2-p}$), and 1.37 ($k_{1,2-s}/k_{2-s}$), respectively. This result suggests that the starch−monoacyl-GPC complex is better at retarding the retrogradation of starch during storage than is the starch−diacyl-GPC complex. The interaction between GPCs with the amylose and amylopectin might prevent the reorganization of amylopectin molecules during storage.

Correlation between the Retrogradation Rate and the Number of Acyl Groups of GPC. The positive linear relationships between the rate constant (k) and the number of acyl groups of GPC obtained by the linear regression analysis are shown in Table 4. The starch−1,2-diacyl-, −1-monoacyl-, and −2-monoacyl-*sn*-GPC complexes during retrogradation at 22 °C had slopes (a) of 9.3 × 10^{-3}, 8.7 × 10^{-3}, and 8.8 × 10^{-3}, respectively, whereas the intercept (b) values were −4.6 × 10^{-2}, −7.4 × 10^{-2}, and −7.6 × 10^{-2} (r^2 = 0.88−0.89), respectively, and each equation was assumed to be the number of acyl groups at k = 0, which indicates the minimum number of acyl groups to have an influence on the rate constant. These results suggested that the differences of rates for retrogradation of starch are due to the difference in the number of acyl groups involved in the GPC−starch interaction and strongly confirm that a shorter chain length reduces the retrogradation rate of starch.

Table 4. Relationship between k Constant and Number of Acyl Groups (C) of GPC for Retrogradation of Starch[a]

	$k = (aC) + b$		
GPC	a	b	r^2
1,2-diacyl-*sn*-	9.3 × 10^{-3}	−4.6 × 10^{-2}	0.89
1-monoacyl-*sn*-	8.7 × 10^{-3}	−7.4 × 10^{-2}	0.89
2-monoacyl-*sn*-	8.8 × 10^{-3}	−7.6 × 10^{-2}	0.88

[a] Retrogradation characteristic as in Table 3.

Conclusion. This study investigated the effects of GPC with different chain lengths of acyl groups on starch gelatinization and retrogradation. The results indicate that GPC retards the retrogradation of starch and that 1- or 2-monoacyl-*sn*-GPC with short chain length is more effective in retarding the retrogradation; between 1- and 2-monoacyl-*sn*-GPCs there was no significant difference in gelatinization and starch−GPC complexes. The positively linear relationship between k and the chain length of a GPC suggests that a GPC with a shorter chain length could retard the retrogradation of starch during storage.

ABBREVIATIONS USED

GPC, glycerophosphatidylcholine; M, myristoyl; P, palmitoyl; S, stearoyl; DSC, differential scanning calorimetry; ΔH_g, gelatinization enthalpy; ΔH_{s-l}, enthalpy of the melting of starch−lipid complexes; ΔH_∞, enthalpy at the limiting change; ΔH_t, enthalpy at time t; T_o, onset temperature; T_p, peak temperature; t, time; n, Avrami exponents; k, rate constants; CI, complexing index; r^2, regression coefficient.

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