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# Studies on the Retrogradation and Structural Properties of Waxy Corn Starch

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(Received June 18, 1997)

The protective effect of guanidine hydrochloride (Gu-HCl) on the retrogradation of waxy corn starch (WCS) gel was studied using differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FT-IR), and solid-state $^1$H nuclear magnetic resonance (NMR). The areas of the endothermic peaks on DSC curves attributed to re-gelatinization of WCS gels became small and shifted to lower temperature with increasing Gu-HCl contents; finally, the endothermic peak for WCS gel containing 40% Gu-HCl disappeared, indicating that the WCS gel remained in the gelatinized state.

The solid-state $^1$H NMR and FT-IR spectra for retrograded WCS gels showed significant changes compared with those for gelatinized WCS gels. In the process of retrogradation, these spectral changes should imply the formation of intra- and intermolecular hydrogen bonds of WCS and the increase of free water molecules. However, the $^1$H NMR and FT-IR spectra for a WCS gel containing 40% Gu-HCl did not show such changes along with the progress of retrogradation. From these results, we found that the retrogradation of starch can be protected by the addition of hydrogen bond-breaking materials, such as Gu-HCl.

Starch shows very low solubility in cold water and gives a suspension with water, even at a low starch concentration. However, the starch–water suspension becomes half-transparent and a viscous gel upon heating up to about 70 °C. This phenomenon is known as gelatinization. Gelatinization is also induced at room temperature by the addition of some anions, such as strong alkalines, halogens or dimethyl sulfoxide; finally, starch is gelatinized at −33 °C in liquid ammonia.[1] In a solution containing anions, the gelatinization of starch is promoted in the order $OH^- >$ salicylate ion $> SCN^- > I^- > Br^- > Cl^- > SO_4{}^{2-}$, and a starch suspension containing more effective anions than $I^-$ is gelatinized at room temperature. On the other hand, some cations also promote the gelatinization of starch in the order $Li^+ > Na^+ > K^+ > Rb^+$. Also, guanidine hydrochloride (Gu-HCl) and urea, hydrogen bond-breaking reagents, promote the gelatinization of starch.[2] A structural analysis of starch has been conducted using an X-ray powder-diffraction technique; it has been found that a crystal structure in starch transforms to an amorphous one during the gelatinization process.

A gelatinized starch in the amorphous state transforms partly to a crystal one upon storage at low temperature. This re-crystallization is known as retrogradation. The retrograded starch shows different properties from those of both native and gelatinized starch, such as an increase in the viscosity and a change of the crystal structure. These changes in the starch accompanied by retrogradation are significant problems in some industries using starch as a raw material, such as the food industry. A number of efforts to study the gelatinization and retrogradation of starch have been reported.[3—5] It has been found that a retrograded starch is transformed into a gelatinization state again by heating. Recently, we studied the retrogradation of waxy corn starch (WCS) comprising amylopectin, and reported that a B-form crystal structure was partly developed along with the progress of retrogradation.[6] This B-form structure is different from the A-form observed in native WCS. Thus, the transformation between the gelatinization and retrogradation states is related to a change in the crystal structure of starch.

In our previous work,[6] the effect of Gu-HCl on the retrogradation of WCS was investigated using a viscometer, X-ray powder-diffraction analysis, and FT-IR (Fourier-transform infrared spectroscopy); we demonstrated that Gu-HCl suppresses the retrogradation of WCS. Although the viscosity of WCS gel containing Gu-HCl increased with storage at −2 °C, the increasing rate of viscosity of the WCS gel was lower than that of WCS gel without Gu-HCl, indicating the suppression of retrogradation by Gu-HCl. The FT-IR spectra were also compared between gelatinized and retrograded WCS gels, and a difference in the range 3000—3800 cm$^{-1}$ was observed. This finding suggested that hydrogen bonds of starch were related to the gelatinization and retrogradation processes of starch.

To investigate the contribution of hydrogen bonds to the gelatinization and retrogradation of starch, in the present study we examined the effect of Gu-HCl on the gelatinization and retrogradation of WCS gel by using DSC (differential scanning calorimetry), FT-IR, and solid-state $^1$H NMR (nuclear magnetic resonance). From all of the results, Gu-HCl is considered to be a material which can promote the gelatinization of starch and to restrain the retrogradation of starch.

## Experimental

**Reagents.** WCS and Gu-HCl (analytical grade) were purchased from Honen Co. and Kanto Chemical Co., respectively.

**DSC Measurements.** DSC measurements were carried out using a Rigakudenki DSC 8230B, and the temperature for all samples sealed in aluminum pans was heated from 30 to 100 °C at a heating rate of 5 °C min$^{-1}$. The water sealed in the same pan was used as a reference.[7)]

In aluminum pans, 20% WCS gels (basis as dry state) containing different Gu-HCl contents (0, 20, 35 and 40%) were prepared with adequate amounts of water; these aluminum pans were sealed. These samples were used for analyzing the gelatinization of a WCS suspension containing different Gu-HCl contents.

On the other hand, in aluminum pans, 20% WCS gels (basis as dry sate) containing different Gu-HCl contents (0, 1, 3, 5, and 40%) were prepared with adequate amounts of water; these aluminum pans were sealed. The samples, except for WCS gel containing 40% Gu-HCl, were gelatinized in boiling water for 1 min and stored at $-2$ °C. A WCS gel containing 40% Gu-HCl was gelatinized at room temperature and stored at $-2$ °C. All of these samples were used to analyze the retrogradation of starch gels containing different Gu-HCl contents.

**FT-IR Spectrometry.** The FT-IR spectra were measured with a Horiba FT-710 recording spectrophotometer by the ATR (attenuated total reflection) method. An aqueous Gu-HCl solution consisting of the same Gu-HCl contents as the samples were used as references. The difference spectrum was obtained by subtracting the absorption spectrum of the reference from that of the sample. The scans were performed 100 times.

All samples used for the FT-IR measurements of starch gels were prepared as follows. In screw-cap bottles (1.6 mL), 10% WCS suspensions (basis as dry state) were prepared with adequate amounts of water and gelatinized by stirring with a hot stirrer for 15 min. On the other hand, in screw-cap bottles (1.6 mL), 10% WCS gels (basis as dry state) containing 40% Gu-HCl were prepared with adequate amounts of water; they were then gelatinized by stirring at room temperature for 15 min using a magnetic stirrer. All samples were stored at $-2$ °C.

**Solid-State NMR Spectrometry.** Solid-state $^1$H MASGNN (gated nondecoupling) NMR spectra were measured with a JEOL-JMN-A400 spectrometer operating at $^1$H frequency of 399.65 MHz. Adamantane (1.91 ppm) was used as an external reference. The number of scans performed were 4 times.

All samples for NMR measurements of starch gels were prepared as follows. In a round-bottom flask, a 10% WCS suspension (basis as dry state) was prepared with adequate amounts of water; this sample was gelatinized by stirring in a water bath at 90 °C for 30 min. On the other hand, a 10% WCS gel (basis as dry state) containing 40% Gu-HCl in a round-bottom flask was prepared with adequate amounts of water; this sample was gelatinized by stirring at room temperature for 30 min. Then, all samples were stored at $-2$ °C, and precipitated in ethanol; and the precipitates were dried under reduced pressure after washing with acetone.

## Results and Discussion

**DSC Measurements on the Gelatinization of Starch.** The effect of Gu-HCl on the gelatinization of WCS was examined using DSC measurements. DSC thermograms for WCS suspensions with different Gu-HCl contents are shown in Fig. 1. For WCS suspensions containing 0, 20, and 35% Gu-HCl, endothermic peaks for the gelatinization were observed at 71.9, 62.2, and 50.1 °C, respectively. The areas of these endothermic peaks decreased along with an increase in the Gu-HCl content, and the enthalpy values of the peaks were estimated to be 17.20, 10.63, and 3.34 J g$^{-1}$ for WCS suspensions containing 0, 20, and 35% Gu-HCl, respectively. Finally, the endothermic peak disappeared for WCS suspensions containing 40% Gu-HCl. These endothermic phenomena result from the gelatinization of WCS, because the gelatinization of starch is known to be accompanied by a transformation from a crystal state to an amorphous state. The decrease in the peak area shows a decrease in the content of the crystal state and an increase in that of the amorphous one in the WCS molecules. Since water molecules enter into starch particles, the crystal state is destroyed. In this process, guanidine molecules also enter into the starch particles and should replace the water molecules binding to starch in the amorphous state by hydrogen bonds. This replacement should result in a decrease in the temperature for gelatinization; finally, a WCS suspension containing 40% Gu-HCl was gelatinized at room temperature. In the presence of 40% Gu-HCl, the X-ray diffraction pattern demonstrated that no crystal state could be observed in the WCS molecule (data not shown), supporting destruction of the crystal structure. However, we showed that the A-form crystal structure remains in WCS containing 30% Gu-HCl using an X-ray diffraction analysis.[6)] These findings indicate a dependency of the Gu-HCl contents on the destruction of the starch crystal structure maintained by the intra- and intermolecular hydrogen bonding of starch molecules. These results indicate that the gelatinization of WCS is influenced by a change in the hydrogen-bonding state, which consequently Gu-HCl promotes gelatinization.

**DSC Measurements on the Retrogradation of Starch.** The effect of Gu-HCl on the retrogradation of WCS was examined using DSC measurements. A DSC thermogram for the retrograded WCS gel was compared with those of WCS gels containing different Gu-HCl contents (1, 3, 5, and 40%) stored at $-2$ °C after gelatinization, as shown in Fig. 2. The WCS gel containing 1% Gu-HCl stored for 3 weeks showed an endothermic peak due to re-gelatinization in the range 40—60 °C as well as a WCS gel without Gu-HCl. This endothermic peak became smaller with increasing the Gu-HCl content, and finally almost disappeared for WCS gel containing 5% Gu-HCl stored for 5 months. The values of the enthalpy for WCS gels with 0, 1, and 3% Gu-HCl stored at $-2$ °C for 3 weeks were 9.13, 8.97, and 4.23 J g$^{-1}$, respectively. These endothermic peaks seem to result from the re-gelatinization of WCS, suggesting the formation of a re-crystallized structure in gelatinized amorphous WCS

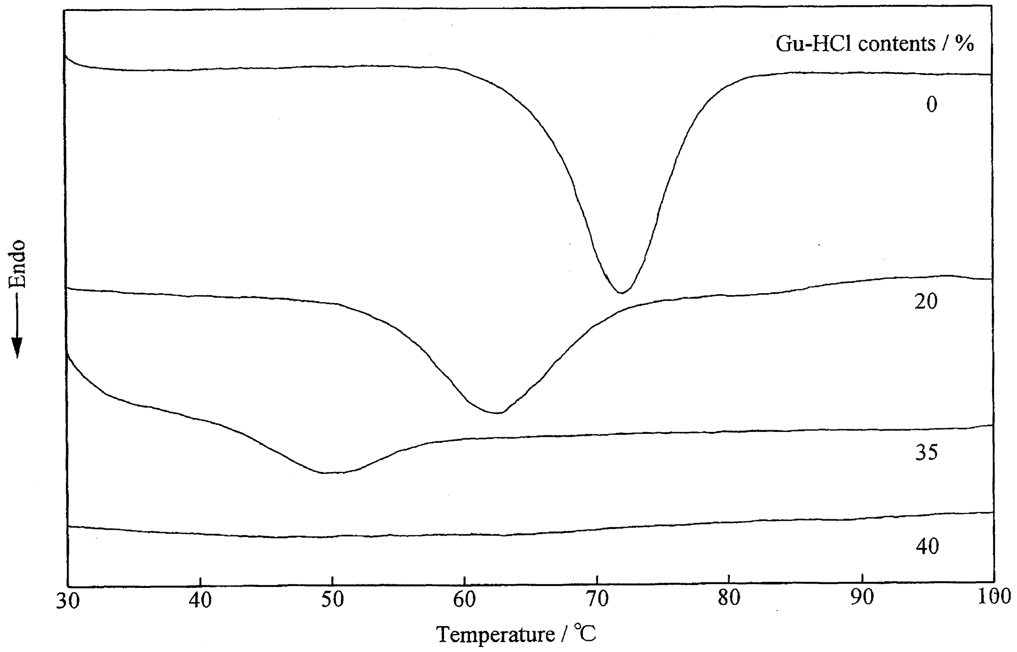

Fig. 1. DSC curves for WCS at different Gu-HCl contents.

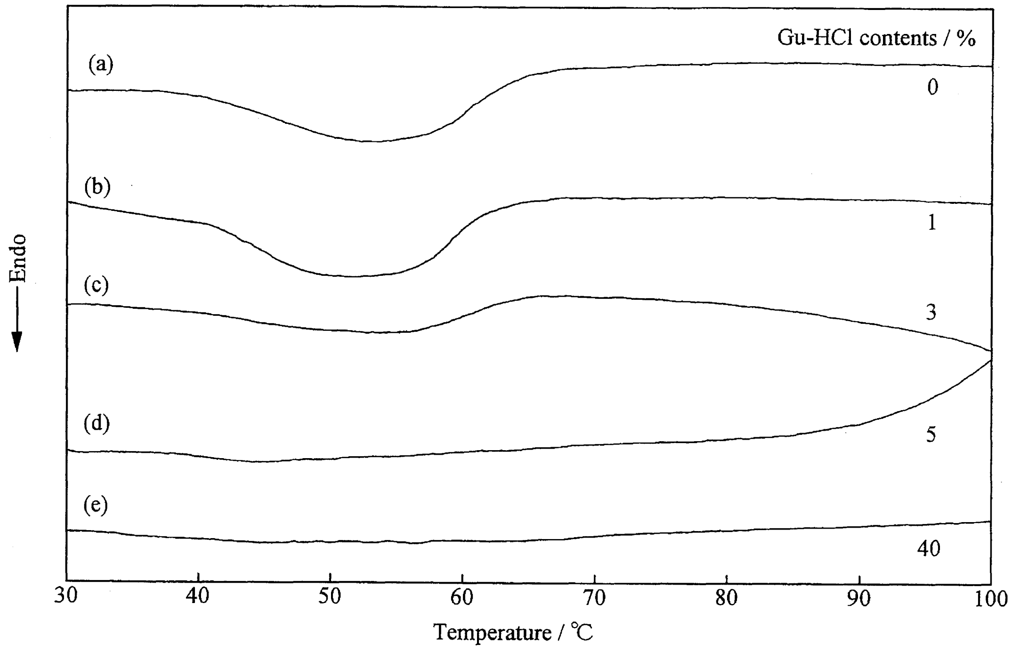

Fig. 2. DSC curves for WCS at different Gu-HCl contents during storage for (a) 3 weeks, (b) 3 weeks, (c) 3 weeks, (d) 3 months, (e) 5 months at −2 °C after the gelatinization.

during storage. This produced crystal structure was found to be a B-form by the X-ray diffraction pattern, as previously described.[6] WCS gel containing 40% Gu-HCl gave no endothermic peak in the DSC curve, even after 5 months of storage (Fig. 2d), and showed an X-ray diffraction pattern corresponding to an amorphous structure (data not shown). These findings show that WCS gel containing 40% Gu-HCl maintains the gelatinization state. It was thus demonstrated that Gu-HCl protects from the retrogradation of WCS due to an inhibition of the re-crystallization from amorphous to B-form structure.

**FT-IR Spectra.** FT-IR spectral changes accompanied by the gelatinization and retrogradation of WCS were examined. We previously studied the retrogradation of WCS by FT-IR using WCS powders precipitated again in ethanol after gelatinization as degraded samples.[6] However, there was a possibility that an accidental phenomenon of the retrograded WCS might occur during ethanol precipitation. In this work, to eliminate the effect of the ethanol precipitation, we measured FT-IR spectra for WCS gels by means of the

ATR method.

Figure 3 shows the FT-IR spectral changes for the WCS gels stored for 1 and 3 months at −2 °C after gelatinization. A significant spectral change upon storing for 3 months was observed in the range 3000—3700 cm$^{-1}$. The gelatinized WCS gel showed a broad unresolved absorption band (3000—3700 cm$^{-1}$), which should be assigned to the O–H stretching vibration (Fig. 3a). After storage for 3 months, this broad band was separated into two broad bands (3000—3250 and 3300—3700 cm$^{-1}$), as shown in Fig. 3c. This phenomenon indicates that a change in the state of O–H groups of water and WCS molecules takes place along with the progress of retrogradation. A reduced content of hydrogen-bonded O–H groups showing a band at around 3300 cm$^{-1}$ should result in this splitting pattern. Casu and Reggini[8] reported that the absorption band in the range 3300—3500 cm$^{-1}$ could be assigned to hydrated O–H groups in amorphous amylose. Also, the water molecule is known to show two absorption bands (3200—3700 and 1600—1700 cm$^{-1}$) assigned to O–H stretching and deformation vibrations, respectively. On the basis of these results, hydrogen-bonded O–H groups between water and WCS molecules in the amorphous WCS gel would be reduced during storage, resulting from dehydration in WCS particles. In addition, the increase in the peak areas of the O–H stretching (3300—3700 cm$^{-1}$) and deformation (1600—1700 cm$^{-1}$) vibration bands suggests an increase of water molecules with high motility, supporting induced dehydration. As a result of the dehydration, intra- and intermolecular hydrogen bonds (3000—3250 cm$^{-1}$) essential to re-crystallization should be formed in WCS particles along with retrogradation.

The effect of Gu-HCl on the retrogradation of WCS was examined. Figure 4 shows the FT-IR spectra for WCS gels with 40% Gu-HCl as a function of the storage time after gelatinization. No significant change among these spectra was observed. Therefore, it was also demonstrated that Gu-HCl can protect the gelatinized WCS gel from retrogradation. Since Gu-HCl is known as a hydrogen bond-breaking material, it must prevent the formation of intra- and intermolecular hydrogen bonds essential to the re-crystallization

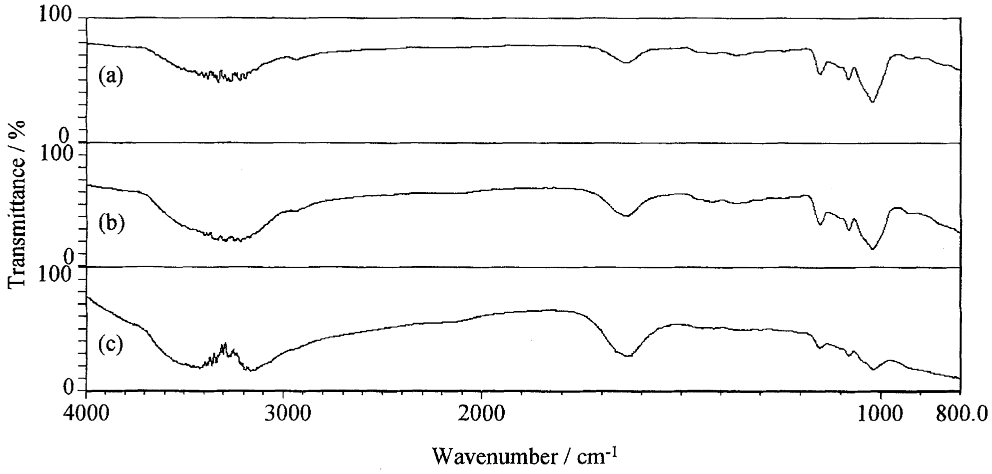

Fig. 3. FT-IR spectra for paste of WCS gel obtained (a) immediately after the gelatinization, and that stored for (b) 1 month, (c) 3 months at −2 °C after the gelatinization.

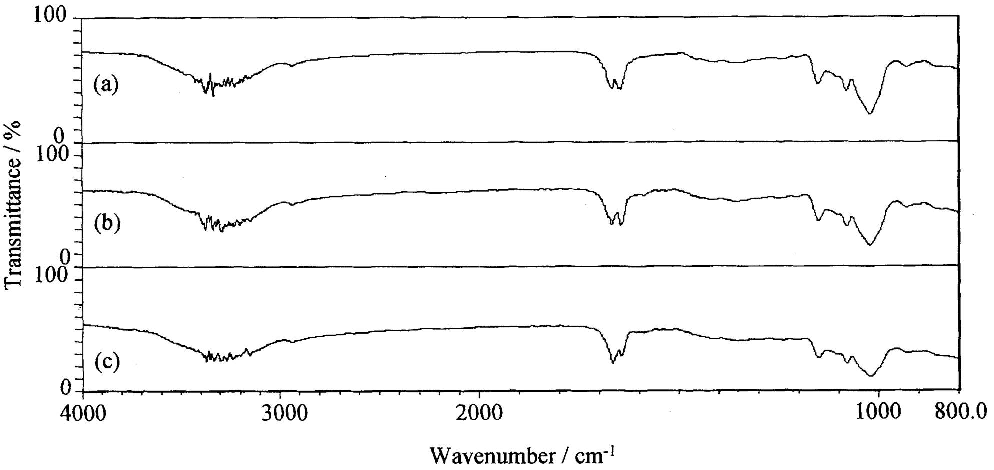

Fig. 4. FT-IR spectra for paste of WCS gel to, which an excess of Gu-HCl was added, obtained (a) immediately after the gelatinization at room temperature, and that stored for (b) 1 month, (c) 3 months at −2 °C after the gelatinization.

of WCS. It is thus thought that guanidine molecules bind more strongly to WCS than do water molecules and, consequently, prevent the formation of hydrogen bonds between the hydroxy groups of the gelatinized WCS in the amorphous state. This prevention of hydrogen-bond formation leads to the prevention of re-crystallization, i.e., retrogradation.

**NMR Spectra.** Solid-state $^1$H NMR spectral changes for WCS accompanied by gelatinization and retrogradation are shown in Figs. 5 and 6, respectively. All samples, except for native WCS, were obtained from precipitation with ethanol, as described in the experimental section. Native WCS before gelatinization gave two major broad-peak groups (the range of 0—5 ppm and 8.261 ppm) in the $^1$H NMR (Fig. 5). The $^1$H NMR spectrum for gelatinized WCS were separated into three major-peak groups (2.460, 6.322, and 8.353 ppm), as seen in Fig. 6a. Compared to the spectrum for native WCS, although a peak group in a lower magnetic field of around 8.353 ppm was also observed, other groups (2.460 and 6.322 ppm) changed. This solid-state $^1$H NMR change must reflect a transition from the A-form crystal state to an amorphous one in WCS. On the other hand, the WCS stored for 5 months at −2 °C after gelatinization showed three major-peak groups (2.261, 4.582, and 8.337 ppm) in the $^1$H NMR spectrum (Fig. 6b). Compared to the spectrum for the gelatinized WCS (Fig. 6a), the intensity of the peak group at around 2.460 ppm decreased, while that of the peak group at around 6.322 ppm increased. This NMR spectral change should result from partial re-crystallization in the WCS particle with retrogradation.

It is known that the $^1$H NMR signal of the O–H group shifts to a higher magnetic field along with the destruction of hydrogen bonds and to a lower magnetic field along with the formation of hydrogen bonds. Therefore, the solid-state $^1$H NMR spectral changes observed for the gelatinized and retrograded WCS gels should reflect the changes in the hydrogen-bonded states of water and WCS molecules. In the process of gelatinization, the shift to a higher magnetic field and the broadening of the peaks should imply the destruction of intra- and intermolecular hydrogen bonds of WCS and an increase of constrained water molecules by hydrogen bonding to WCS, respectively. However, in the process of retrogradation, the shift to lower magnetic field and sharpening of the peaks should imply the formation of intra- and intermolecular hydrogen bonds of WCS and an increase in the free water molecules, respectively.[9,10]

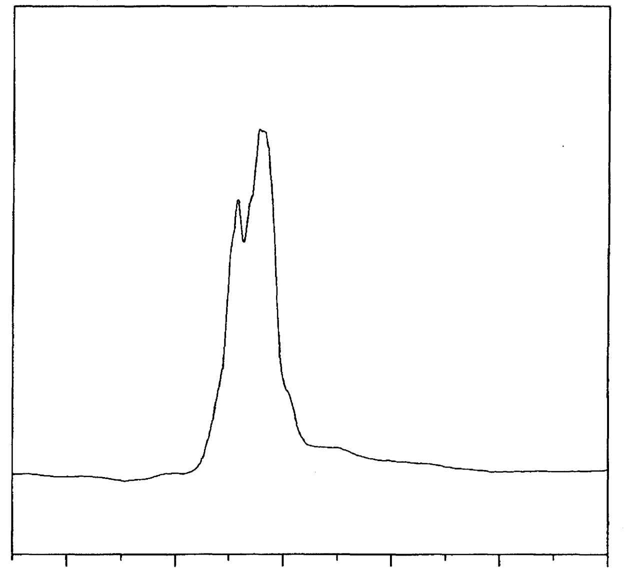

Fig. 5. Solid-state $^1$H NMR spectrum obtained from native WCS.

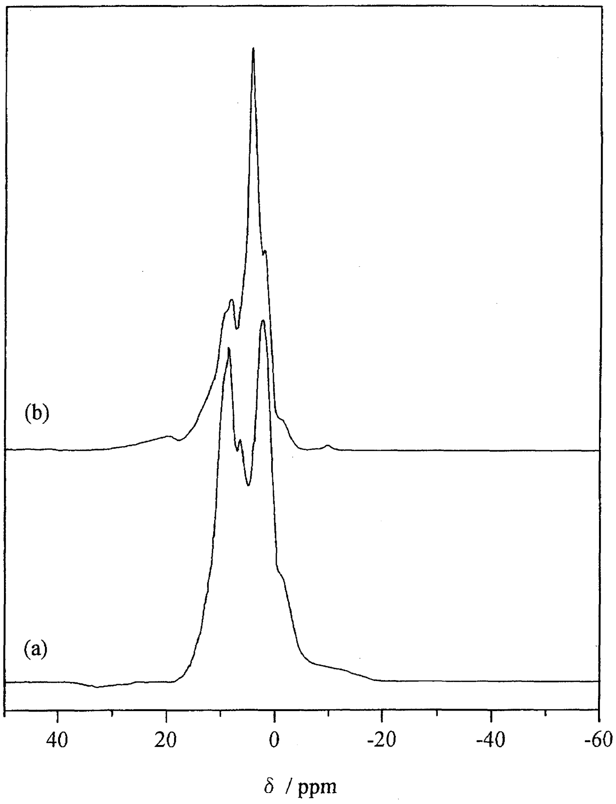

Fig. 6. Solid-state $^1$H NMR spectra for WCS gel obtained (a) immediately after the gelatinization, and (b) that stored for 5 months at −2 °C after the gelatinization.

The protective effect of Gu-HCl on the retrogradation of WCS was also observed in the solid-state $^1$H NMR spectra. The $^1$H NMR spectra of the gelatinized WCS containing 40% Gu-HCl and its 5-months stored sample were measured, as shown in Fig. 7a and b, respectively. No significant change was observed between these NMR spectra; moreover, both NMR spetcra are very similar to that for the gelatinized WCS (Fig. 6a). This finding strongly suggests that WCS containing 40% Gu-HCl stored for 5 months at −2 °C still remaines in the amorphous state. Although a detailed assignment for the NMR signals still can not be made, the change in the state of hydrogen bonds mediated by O–H groups of WCS is thought to be closely related to $^1$H NMR changes accompanied by the transition between the gelatinization and retrogradation of WCS.

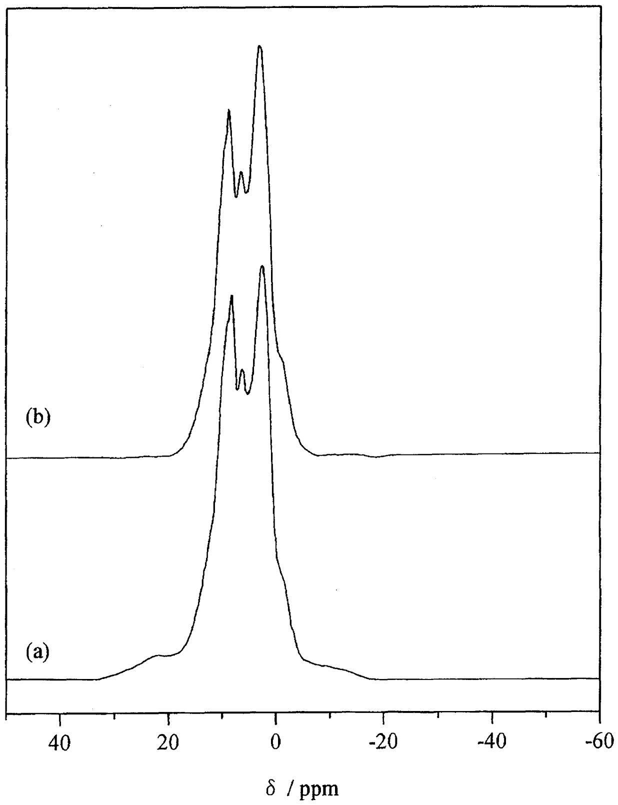

Fig. 7. Solid-state $^1$H NMR spectra for WCS gel to, which an excess of Gu-HCl was added, obtained (a) immediately after the gelatinization at room temperature, and (b) that stored for 5 months at $-2$ °C after the gelatinization.

## Conclusion

We reported here on the changes in the structural property of WCS accompanied by gelatinization and retrogradation and the effect of Gu-HCl on the changes using DSC, FT-IR (ATR method) and solid-state $^1$H NMR techniques. The DSC results indicated that re-crystallization takes place along with the progress of the retrogradation of WCS. From our previous X-ray powder-diffraction study,$^{6)}$ this re-crystallized structure is found to be a B-form. The FT-IR results showed that the dehydration and formation of intra- and intermolecular hydrogen bonds essential to re-crystallization take place in the retrogradation process. This increase of the hydrogen-bonded state in O–H groups of WCS was also suggested by the solid-state $^1$H NMR results. These results demonstrate that the retrogradation of WCS means re-crystallization from amorphous WCS. On the other hand, the existence of Gu-HCl allowed the maintenance of the gelatinization state of WCS, as can be seen in the DSC, FT-IR, and solid-state $^1$H NMR results. In this re-crystallization, Gu-HCl must inhibit hydrogen-bond formation among the O–H groups of WCS molecules, and consequently protect the gelatinized WCS from retrogradation. Therefore, the retrogradation of starch can be protected by the addition of hydrogen bond-breaking materials, such as Gu-HCl.

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