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In situ observation of pressure-induced gelation of starches studied with FTIR in the diamond anvil cell

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Abstract

Pressure-induced gelation of six different starch types has been followed by Fourier-transform infrared (FTIR) spectroscopy. The diamond anvil cell allowed us to follow changes in situ at high pressure. Changes in the infrared spectrum due to conformational modifications and solvation changes in the starch granule were analysed. The spectra of these starches showed a similar behaviour at increasing pressure. Bands in the region 900–1300 cm^{-1} increased in intensity and changed position at high pressure. A correlation was found between the stability and the structure of the different starch types. B-type starches tend to be more pressure resistant than A- and C-type starches. In situ observation of the gelation was also performed with a microscope. At the pressure at which gelation occurred, a swelling of the starch granules was observed. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Starch; Gelation; High pressure; Infrared spectroscopy

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1. Introduction

Starch is widely used in industrial and food applications. The diversity in use depends on differences in physical and functional properties of starches from different sources (Lineback, 1986). The native structure of starch granules has been reported recently. Studies with X-ray microfocus diffraction show that the granules grow in rings, alternating between amorphous amylose and crystalline amylopectin. The amylopectin crystals are built of double helices which are arranged radially in the granule (Waigh, Hopkinson, Donald, Butler, Heidelbach & Riekel, 1997). When starch granules are dispersed in cold water, they undergo a limited reversible swelling because of the absorption of small amounts of water into the amorphous regions. On heating, the optical properties of starch and its X-ray diffraction pattern are retained, until the gelation temperature is reached. The gelation of starch has been the subject of many investigations. The temperature-induced starch gelation is an endothermic transition which is well identified with differential scanning calorimetry (Muhr and Blanshard, 1982; Svensson and Eliasson, 1995; Stute, Klinger, Boguslawski, Eshtiaghi & Knorr, 1996; Matveev, Elankin, Kalistrova, Danilenko, Nieman & Yuryev, 1998). Other techniques, such as X-ray diffraction and microscopic observations, showed that structural changes in the starch granules occur at high temperatures (Zobel, 1988; Kudta and Tomasik, 1992). When a starch–water suspension is heated, irreversible physical changes occur with the loss of birefringence and crystallinity. Granular swelling and viscosity changes can also be observed with the microscope (Douzals, Marechal, Coquille & Gervais, 1996).

Since 1980, several papers have described the influence of high pressure on the gelation of starch (Thevelein, Van Assche, Heremans & Gerlsma, 1981; Muhr, Wetton & Blanshard, 1982; Kudta and Tomasik, 1992). Thevelein et al. (1981) found that the temperature of gelation increased with increasing pressure. At pressures higher than 200 MPa, the gelation temperature was not further influenced. Muhr and Blanshard (1982) found that at pressures above 200 MPa the gelation temperature decreased with increasing pressure.

Studies by Hayashi and Hayashida (1989) showed that pressure treatment at about 500 MPa increased the amylase digestibility. This has been correlated with the pressure-induced gelation for a number of starches (Ezaki and Hayashi, 1992). Improved cooking properties of rice grains were observed after pressurization (Wantanabe, Arai, Honma & Fuke, 1991). This suggests possible applications for the pressure treatment of rice in the food industry.

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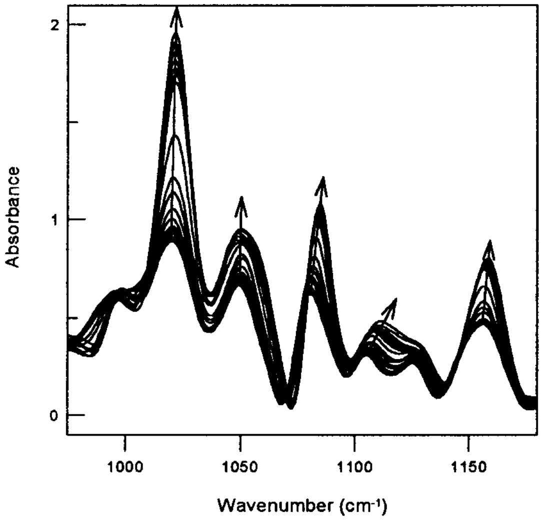

Fig. 1. Changes in the deconvoluted spectra of rice starch suspension at pressures going up from 0.1 MPa to 1 GPa. The arrows indicate changes that occur at increasing pressure.

The mechanism of gelation of starch at high temperature has been described in many articles (Zobel, 1988; van Soest, de Wit, Tournois & Vliengenthart, 1994; Lelièvre and Liu, 1994). The first step in the gelation process is the penetration of water in the amorphous regions initiating swelling of the granules and the loss of birefringence as the temperature is increased. At high temperatures, there is a decrease in order and rupture of the crystalline regions with uncoiling of the helical structures until the granule structure is disrupted and a sol is obtained. The viscosity of this sol is the result of the re-association of the amylose and amylopectin that were solubilized during the gelation process.

Stute et al. (1996) found that differences in properties between pressure- and temperature-induced starch gels can be observed. Some starch samples did not show any extensive swelling after pressure treatment leading to a weaker gel compared to the temperature-treated starch. This behaviour can be explained by assuming that the starch granule will be hydrated as a result of the formation of hydrogen bonds between water and starch which are enhanced at high pressure (Mozhaev, Heremans, Frank, Masson & Balny, 1996).

The gelation of starch and starch components has been studied previously by using FTIR spectroscopy and making use of the bands in the region 900–1300 cm^{-1} that are sensitive to polymer conformation (Wilson and Belton, 1988; Goodfellow and Wilson, 1990; van Soest, 1995). FTIR coupled with the diamond anvil cell gives us the opportunity to follow the pressure-induced gelation in situ. In a previous communication we suggested an elliptical phase diagram for the temperature- and pressure-induced gelation similar to that observed for proteins (Rubens, Goossens & Heremans, 1997). This study is focused on the spectral changes upon pressurization of aqueous suspensions of six different starch types.

2. Materials and methods

Starch samples from rice, potato, corn, pea, tapioca and waxy corn were suspended in deionized water. Each suspension was mounted in the stainless steel gasket of a diamond anvil cell (Diacell Products, Leicester, UK). Pressure was determined from the shift of the ruby fluorescence with a Spex Raman spectrometer. The ruby technique has the advantage that it allows easier inspection of the sample under the microscope.

Visual observation of gelation was performed with an Olympus BH2 microscope equipped with a long focus objective and a JVC CCD-camera.

Infrared spectra were obtained with a Bruker IFS66 FTIR spectrometer equipped with a broadband MCT detector. The infrared light was focused on the sample by a sodium chloride lens. 350 interferograms were coadded after registration at a resolution of 2 cm^{-1}. The time to take one data point at each pressure is about 10 min. Pressure was increased at a rate of about 100 MPa per hour. Resolution enhancement was applied to all experimental spectra using Fourier self deconvolution. The deconvolution assumes that the experimental spectrum may be expressed as a spectrum of sharp lines being convoluted by a common line-broadening function. If the shape and width of this line-broadening function is known, its effect can be mathematically removed from the spectrum. The assumed line shape was Lorentzian with a half width of 21 cm^{-1}. A resolution enhancement factor (K) of 1.7 was used. This factor is defined as the ratio of the widths of the original and the deconvoluted spectrum (Arrondo, Muga, Castresana & Goni, 1993). Absorbance ratios were calculated from the resolution enhanced spectra.

3. Results and discussion

The spectra of rice starch at different pressures are shown in Fig. 1. All the spectra show absorption bands in the 900–1300 cm^{-1} region which are sensitive to the gelation of starch. The bands in this region of the infrared spectrum result mainly from CO and CC vibrational modes that are highly coupled. This coupling makes the assignment of individual bands difficult. The assignment of the bands in this region was based on model calculations and deuterium exchange methods for glucose (Hineno, 1977). As the absorptions in this region arise largely from C–O stretchings of the ring, linkages (C–O–C) and COH groups, the positions of these bands are similar in all carbohydrates as the associated vibrational modes are unlikely to be radically affected by polymer formation (Belton, Wilson & Chenery, 1986). As most vibrations arise within the isolated d-glucose residues and the contribution of inter-residue vibrations is very small, polymerization does not largely affect these vibrations (Vasko, Blackwell & Koenig, 1972; Cael, Koenig & Blackwell, 1974). Previous studies have shown that some bands are highly sensitive to polymer

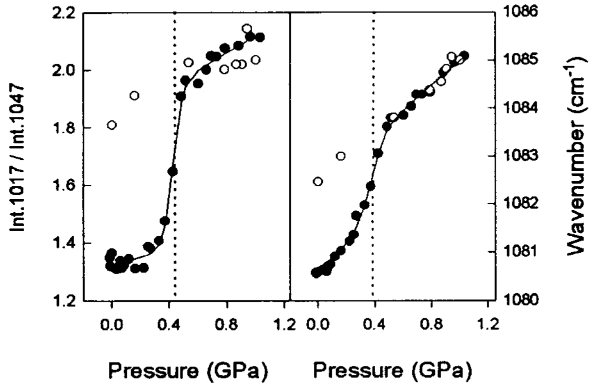

Fig. 2. Spectral changes in intensity ratio (left) and peak frequency (right) of rice starch at different pressures. (●) increasing pressure; (○) decreasing pressure. The dotted line represents the midpoint of transition as determined with curve fitting (solid line).

conformation (Wilson, Kalichevsky, Ring & Belton, 1987; Wilson and Belton, 1988). The bands at 1047 and 1022 cm^{-1} are sensitive to the amount of ordered or crystalline and amorphous starch, respectively (van Soest, 1995; Rinlav, Hulleman & Gatenholm, 1997).

The pressure-induced gelation of starch granules can be studied via the following changes in the infrared spectra: intensity changes, changes in bandwidth and frequency shifts.

Changes in intensity of specific bands are found to be the major spectral change during gelation. All the absorption peaks in the region between 900 and 1200 cm^{-1} increase with pressure. This overall enhancement can mainly be attributed to a broadening of the adjacent librational water band at 840 cm^{-1} at increasing pressure. The changes in intensity for the bands at 1017 and 1047 cm^{-1} can be seen in Fig. 1. They have been assigned to alteration in the ratio of amorphous/crystalline material (van Soest et al., 1994). The midpoint of pressure-induced gelation ($p_{1/2}$), was determined from the absorbance ratio 1017/1047 cm^{-1} as shown in Fig. 2. For all the starches investigated, the ratio increases with increasing pressure suggesting an increase in the amorphous material concomitant with a decrease in the amount of crystalline material. In Table 1 the gelation pressures of the different starch types as determined from the changes in absorbance ratios are summarized. One can see that the B-type starches are more pressure resistant than A- and C-type starches. This is in agreement with previous observation of starch gelation obtained using DSC (Ezaki and Hayashi, 1992; Stute et al., 1996).

Table 1
Midpoint of gelation ($p_{1/2}$) as determined from absorbance ratio and frequency shift. Structure was determined from X-ray diffraction (Stute et al., 1996)

Starch type	$p_{1/2}$ (Mpa) from Absorbance ratio[a]	Frequency shift[b]	Structure
Rice	430 ± 6	390 ± 10	A
Waxy corn	440 ± 10	420 ± 20	A
Corn	520 ± 2	510 ± 4	A
Tapioca	440 ± 6	410 ± 9	C
Pea	460 ± 20	370 ± 10	C
Potato	650 ± 9	480 ± 30	B

[a] From the absorbance ratio 1017/1047 cm^{-1}.

[b] From the frequency shift of the 1080 cm^{-1} band.

Changes in bandwidth are the second major effect that can be observed during pressurization. The absorption bands at 1155, 1080 and 1017 cm^{-1} show a tendency to decrease in bandwidth during gelation while the band at 1047 cm^{-1} shows a broadening with increasing pressure. It should be noted that the changes in bandwidths are not as sharp and well defined as the changes in intensity and frequency. Following the assignment of the 1047 cm^{-1} band to the crystalline phase (van Soest, 1995), the band broadening may be caused by the disordering of the polymers in the crystalline phase. A wider distribution of vibrational energies results in an increase in bandwidth in the infrared spectrum. The broadening of the 1047 cm^{-1} band can be interpreted as a decrease of molecular order during gelation in the crystalline region. It can then be assumed that during gelation, the crystalline order is lost by the pressure-induced penetration of the solvent. The narrowing of the bands at 1155, 1080 and 1017 cm^{-1} can be interpreted as an evolution to a more uniform state of the amorphous phase. The polymers in the amorphous phase experience similar pressure-induced interactions with the solvent. These interactions give rise to a narrowing of some bands in the infrared spectrum resulting from a smaller distribution in polymer conformations.

A more detailed analysis of the spectra reveals pressure-induced frequency shifts. One can see from Fig. 1 that the bands at 1080 and 1110 cm^{-1} show the greatest shifts. These bands can be assigned to C–O and C–C stretching and the C–C–O bending (Hineno, 1977). As it is apparent from Fig. 1 that the band at 1110 cm^{-1} is a convolution of bands at 1103 and 1125 cm^{-1}, the band at 1080 cm^{-1} was used to follow frequency shifts. The shift of the 1080 cm^{-1} band upon increasing pressure is shown in Fig. 2. The determination of the transition points from frequency shifts of the 1080 cm^{-1} band and the intensity ratio at 1017/1047 cm^{-1} reveals systematic differences, the transitions obtained from the intensity ratios being higher than those obtained from the shift of the 1080 cm^{-1} band. Table 1 shows that significant differences can be found for all starch samples except for starch from waxy corn. Starches from potato and pea show the largest differences in transition pressures while these differences are smaller for tapioca, rice and corn. The differences are less significant for starch of waxy corn. As spectral changes in the 900–1300 cm^{-1} region show the same tendency at increasing pressure for all samples, one general gelation mechanism is suggested. A two-step mechanism similar to the temperature-induced gelation can be proposed. In the first step the amorphous parts of the starch granule are hydrated. This hydration induces a swelling of the granule leading to a distortion of the crystalline regions what can lead to a destruction of the granule structure. In a second step, the crystalline regions become more accessible for water (Cameron and Donald, 1993; Svensson and Eliasson, 1995). In waxy corn the two steps may not be very well separated.

Using the diamond anvil cell, it was also possible to observe the changes in granule structure at high pressure under the microscope. Before gelation a swelling of the starch granules was observed. One should bear in mind that not all starch types show a similar degree of swelling during high pressure application. Starch of rice, waxy corn and tapioca tend to show a complete disintegration of the granules while starch of potato, corn and pea show the typical limited swelling. These differences were not reflected in the spectral changes.

As discussed in previous articles, water plays an important role in the stability of starch granules (Biliaderis, 1990; Lelièvre and Liu, 1994). DSC experiments have shown that the melting temperature of rice starch increases with decreasing water content. When starch granules are dispersed in cold water, they will absorb about 35% water. At this moisture level, water exerts its maximum plasticizing effect on both the glass transition and the melting point. It is said that the amorphous region plays an important role in the gelation process as it destabilizes the crystalline regions upon swelling (Bogracheva, Morris, Ring & Hedley, 1998). This swelling, caused by hydration of amorphous regions has also been observed with SAXS (Cameron and Donald, 1993). A detailed study of the microscopic changes in the starch granule during pressure induced gelation is in progress in our laboratory. This will allow the establishment of a correlation between the changes observed in the infrared spectra and the degree of swelling.

4. Conclusions

It has been demonstrated that it is possible to follow in situ the gelation of starch granules under high pressure by FTIR. Spectral changes were used to determine the midpoint of gelation. A- and C-type starches proved to be more sensitive to pressure-induced gelation compared with B-type starches. These results are in good agreement with previous work using DSC. Analysis of the spectra showed changes in bandwidth, increasing absorption intensities and frequency shifts in the 900–1300 cm^{-1} region during gelation. The frequency shift of the absorption band at 1080 cm^{-1} precedes the former two changes. This can be explained by a hydration of the polymers in the starch granule that occurs before changes in crystallinity during gelation. Changes in the starch granule structure were simultaneously followed in the infrared spectrum and under the microscope. The swelling of the starch granule was observed, in situ, at high pressures.

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