Investigations of Sugar-Starch Interactions
Using Carbon-13 Nuclear Magnetic Resonance. I. Sucrose[1]

L. M. HANSEN,[2] C. S. SETSER,[2] and J. V. PAUKSTELIS[3]

ABSTRACT

Carbon-13 nuclear magnetic resonance techniques were used to investigate sugar starch systems. The results suggested that sugar-starch interactions occurred during the heating of starch just before the onset temperature of starch gelatinization, measured by differential scanning calorimetry. Differences in chemical shifts (>1 Hz) of sugars in a sugar-starch system compared with chemical shifts in a sugar solution alone represented potential interactions for the G1, G6, F1, and F3 carbon atoms of the sucrose molecule. As molar concentrations of sucrose increased, the number of carbon atoms involved and the strength of interactions appeared to decrease. Results of solubilized starch investigations indicated that the starch granule likely was necessary for these interactions to occur.

Sugars increase the onset temperature of starch gelatinization, according to several reported studies (Bean and Osman 1959, Miller and Trimbo 1965, Bean and Yamazaki 1978, Koepsel and Hoseney 1980, Chungcharoen and Lund 1987). In general, disaccharides increase the gelatinization temperature more than monosaccharides (Savage and Osman 1978, Spies and Hoseney 1982). The mechanism through which the increase occurs remains unclear. Suggested mechanisms include 1) competition between the sugars and starch for available water and associated changes in free volume (Derby et al 1975, Hoseney et al 1977, Slade and Levine 1987); 2) the ability of sugars in a water system to retard gelatinization by inhibiting the swelling of the starch granules (D'Appolonia 1972, Bean and Yamazaki 1978, Savage and Osman 1978, Wooton and Bamunuarachchi 1980); and 3) the ability of sugars to penetrate the starch granule and interact in the amorphous areas, thus stabilizing this region and increasing onset temperature (Spies and Hoseney 1982).

Many techniques are used to study starch gelatinization and effects of different sugars on thermal properties of starch. Methods include amylograph measurements of viscosity changes (Lund 1984), microscopic observation of loss of birefringence and changes in the swelling of the granule (Bean and Osman 1959, Watson 1977, Bean and Yamazaki 1978, Bean et al 1978), and differential scanning calorimetry (DSC) to determine melting temperatures and enthalpies of melting as modified by sugar solution (Wooton and Bamunuarachchi 1980, Spies and Hoseney 1982). These methods to investigate starch-sugar systems measure physical changes of the starch. If sugars actually enter the granule and interact with the starch, this needs to be determined at the molecular level. Recently, our laboratory developed ^{13}C nuclear magnetic resonance (NMR) techniques to investigate sugar-starch interactions at the molecular level (Hansen et al 1987). This methodology can be used only to determine changes in interactions between sucrose and starch as a function of temperature, and not to determine initial interactions. The objective of this research was to investigate changes in sucrose-starch interactions as a function of increasing temperature using ^{13}C-NMR techniques and to determine whether or not these interactions are altered at different concentrations of sucrose.

MATERIALS AND METHODS

Solutions

Wheat starch (Aytex P, prime wheat starch, Ogilvie Mills, Inc.) was used in all investigations. Reagent grade sucrose (Mallinckrodt, Inc.) was used to study sugar-starch interactions.

Starch and sugar dispersions were prepared substituting 0.15% xanthan gum (Keltrol T, Kelco) solution for water as the solvent to prevent the settling of starch. For all sugar-starch investigations, except solubilized starch, sugar-starch dispersions were prepared on a 30% starch, 70% sugar solution (w/w) basis. Sugar solutions of 0.5, 1.0, 1.5, and 2.0 M were used. Solubilized starch suspensions were prepared to investigate the importance of the granular structure in sugar-starch interactions. For these studies, suspensions of 11.1% sucrose, 15% solubilized starch, and 73.9% water and of 20% sucrose, 15% solubilized starch, and 65% water were prepared.

Solubilized starch was prepared by suspending 15 g of starch in 20 ml of cold water and adding the mixture slowly to 65 ml of boiling water with continuous stirring. Excessive clumping of the starch was avoided by this procedure. The starch was transferred to an Erlenmeyer flask, covered with aluminum foil, and autoclaved at 121°C for 8-12 hr. In order to determine if the starch was solubilized, unautoclaved starch and autoclaved starch were compared for intact granules and remnants, using a Zeiss Universal research microscope with Olympus trinocular head, operating in bright field and polarized modes. Few granules were apparent in the autoclaved starch, whereas almost all of the unautoclaved starch appeared as swollen granules. Ungelatinized wheat starch granules at 1, 3, and 5% (weight basis) were added to autoclaved starch to estimate the percentage of solubilized starch. In comparison to prepared suspensions, 90-95% of the autoclaved starch was solubilized.

Sufficient carbon atoms (^{13}C) of both sucrose and starch were needed for strong NMR signals; this was the basis for the sugar-starch ratio used in all investigations. In addition, the dispersions had to remain sufficiently fluid to be injected into a 5-mm (i.d.) NMR sample tube. Gelatinization of starch, as evidenced by use of DSC techniques, was apparent during the temperature range used at different molar sugar concentrations studied. For the sugar-solubilized starch experiments, approximately 1 hr before measurement on the NMR, the 15% starch dispersion was removed from the autoclave, and either 2.5 or 5.0 g of sucrose was added to 20 g of solubilized starch to give the ratios previously listed. After the sucrose was well blended by vigorous hand stirring, the sample was injected into the NMR sample tube. All other sucrose-starch dispersions were prepared and allowed to equilibrate at room temperature for approximately 8 hr before measurement in the NMR spectrometer.

DSC

DSC was utilized to verify starch gelatinization. Measurements were made using the Perkin Elmer DSC-2, FTS Systems Flexicooler with temperature controller, and the method of Kim et al (1986). The instrument was calibrated with indium.

NMR Measurements

NMR measurements were made following the procedure of Hansen et al (1987). The following instrument settings were used:

512 pulses; pulse width, 6.0 μsec (60°); 0.49-sec delay between pulses; line broadened 5.0 Hz; data size 16K zero filled to 32K; sweep width, 10,000 Hz; digital frequency resolution error approximately 0.3 Hz with standard deviation of duplicate measurements averaging <0.1 Hz. Chemical shift differences are reported in hertz; 100.61 Hz equal 1 ppm. Chemical shifts of sucrose alone and starch alone were determined as a function of temperature before determining chemical shifts of sucrose and starch in combination. In order to obtain a stronger lock signal and better resolution with the solubilized starch experiments, the external reference used in other NMR experiments was changed from 50% D_2O/50% Me_2SO to 75% D_2O/25% Me_2SO.

Sugar-Starch Interactions

All NMR chemical shifts were determined relative to the external Me_2SO standard. The standard was not affected by the solutions, so the same standard was used for every sample. An external reference was necessary because an internal reference would interact with either starch or sucrose. If bulk effects were observed as a function of the external reference, all observed slope changes would have similar directional and magnitude changes. The external reference did not influence our interpretation of the chemical shift data because observed changes varied in magnitude and were random rather than directional.

At each temperature, differences were noted between the chemical shifts of the carbon atoms of the sugar in solution alone and the chemical shifts of the carbon atoms of the sugar in the sugar-starch dispersions. If an interaction between the sugar and

the starch was occurring, the environment near the sugar carbon atoms would be altered, and the chemical shift of the carbon atoms of the sugar-starch dispersions would differ from that of the carbon atoms in the sugar solution alone. We suggest a plot of the differences in chemical shifts as a function of temperature should produce a line with approximately zero, or constant slope, if no interactions occurred other than bulk effects because of diamagnetic susceptibility changes. Chemical shift changes resulting in slope deviations for a particular carbon would indicate possible interaction sites; the larger the deviation, the stronger the interaction. Deviations of less than 1 Hz (measurements were made to ± 0.1 Hz) were not thought to be significant and were considered within the experimental error of the measurements.

Labeling of Chemical Shifts

Chemical shifts of carbon atoms of the sucrose molecule were assigned previously (Bock and Lemieux 1982, Hull 1982). To clarify whether a carbon atom was associated with the fructose or glucose moiety of the sucrose molecule, in all discussions, carbon atoms have been labeled F1 through F6 and G1 through G6. That is, F1 represents the C1 carbon of the fructose moiety and G1 represents the C1 carbon of the glucose moiety.

RESULTS AND DISCUSSION

Sucrose-Starch Interactions

The effects of different molar concentrations of sucrose on chemical shifts in sucrose-starch dispersions are illustrated in Figures 1 through 4. In general, as the concentration of sucrose increased, the temperature at which major chemical shift differences occurred increased. The graphs illustrate the temperature regions in which deviations occurred. Deviations greater than 1 Hz were considered as significant. Interestingly, major deviations occurred prior to and at the onset temperature of starch gelatinization. After the onset of starch gelatinization, few changes in slope were noted. Since changes in starch granule
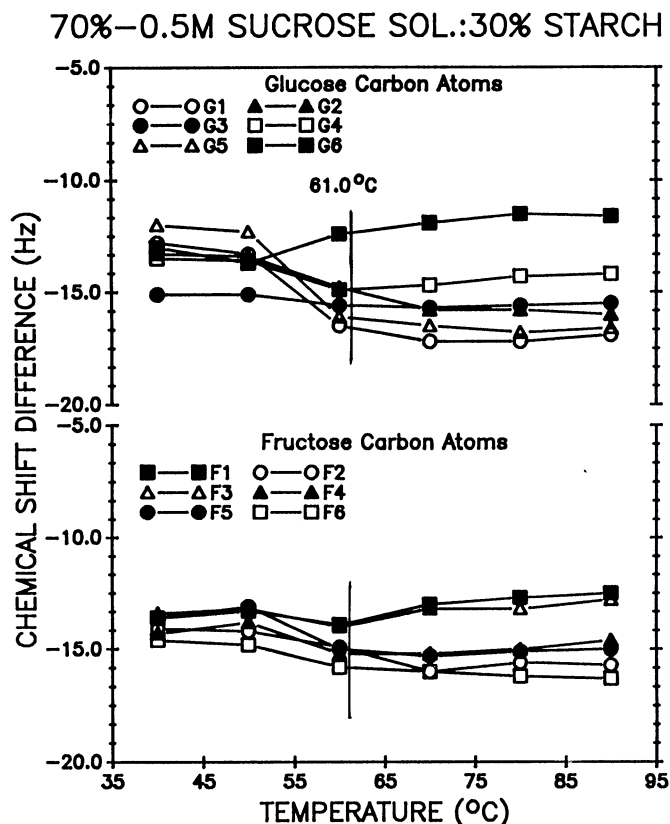

Fig. 1. Chemical shift differences between sucrose carbon atoms in a 70% 0.5M sucrose solution with 30% starch (w/w) dispersion and in a 0.5M sucrose solution as a function of increasing temperature. Spectra and chemical shift data were acquired using the following nuclear magnetic resonance instrument settings: 512 pulses, pulse width 6.0 μsec (60°), 0.49-sec delay between pulses, line broadened 5.0 Hz. Chemical shifts are all upfield relative to reference sucrose. The upper portion of the figure shows the glucose carbon atoms, and the bottom portion illustrates the fructose carbon atoms of the sucrose molecule. Vertical line represents gelatinization onset temperature of the system as measured by differential scanning calorimetry; onset temperature of a 70% distilled water/30% starch system is 55.2°C.
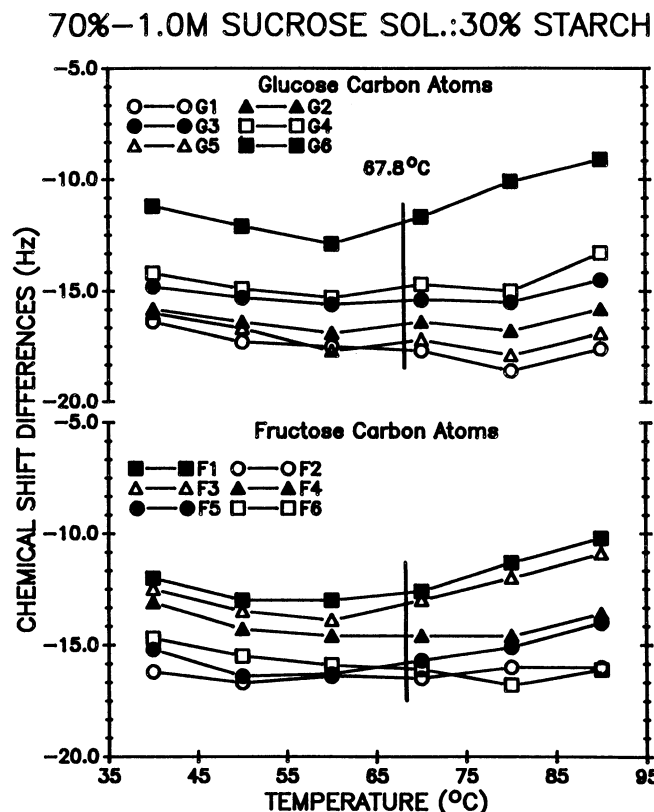

Fig. 2. Chemical shift differences between sucrose carbon atoms in a 70% 1.0M sucrose solution with 30% starch (w/w) dispersion and in a 1.0M sucrose solution as a function of increasing temperature. All other conditions are the same as for Fig. 1.

structure occur during gelatinization, major deviations in chemical shifts also might occur during this temperature range because the environment near the sucrose carbon atoms had been altered. Onset temperatures of starch, measured with the DSC, as altered by 0.5, 1.0, 1.5, and 2.0M sucrose solutions, were 61.0 ± 0.1, 67.8 ± 0.3, 77.5 ± 0.7, and 88.4 ± 0.2 °C, respectively.

Temperature regions in which changes in slope occurred were compared with onset temperature of starch gelatinization for each system. A comparison of systems with differing molar concentration of sucrose indicated that G1, G6, F1, and possibly F3 had significant chemical shift differences at all concentrations investigated. At all concentrations, chemical shift differences of G1 decreased, whereas the chemical shift differences of F1, F3, and G6 increased as a function of increasing temperature. The differences in chemical shifts of these carbon atoms were large enough to be considered possible sites of interaction with starch.

An assumption was made that the difference in chemical shift between the sucrose atom in solution alone and the sucrose atom in sucrose-starch dispersions was a result of an interaction between the sucrose and starch. However, a three-way interaction among sucrose, starch, and water cannot be ruled out. Research findings of Bock and Lemieux (1982) support the idea that the chemical shift differences result from sucrose-starch interactions and not conformational changes in the sucrose molecule with increasing temperature. They state that sucrose in solution is a rigid molecule, stabilized by a hydrogen bond between OH–1[f] and O–2[g], which exists in the crystal lattice, with only slight flexing of the furanoid ring and different degrees of freedom of rotation around the hydroxymethyl to carbon bonds. If this is the case, differences in chemical shifts of sucrose between sucrose solution and sucrose-starch dispersions would not result from conformational changes in the sucrose molecules but likely from an interaction between the sucrose and starch.

Interestingly, as the molar concentration of sucrose solutions increased, the differences or changes in slope decreased. Stronger interactions and more carbon atoms (i.e., G1, G2, G3, etc.) were

involved with the lowest sucrose concentration investigated. One possible explanation might be that only a limited number of interaction or exchange sites on the starch granules was available and, at higher sucrose concentrations, more sucrose atoms existed than sites for interaction or exchange. Thus, only a portion of the sucrose interacted with the starch, and chemical shift changes appeared to be fewer.

Related research by Richardson et al (1987), using ^{17}O-NMR techniques, found that as sucrose concentration increases, relaxation rates of water increase, indicating decreased water mobility. They attributed this decrease in water mobility to: 1) formation of intermolecular hydrogen bonds between sucrose and water, 2) formation of water bridges between sucrose molecules, and 3) extensive sucrose-to-sucrose hydrogen bonding. If extensive hydrogen bonding were occurring between sucrose and water or sucrose and sucrose, then possibly less sucrose would actually bind or interact with starch.

TABLE I
Theoretical and Calculated Mole Ratios of Sucrose and Starch and Amount of Starch Contributing to NMR[a] Peaks at 90°C

Solution	Theoretical Mole Ratio[b]	NMR Mole Ratio[c]	Starch (%) NMR Peaks[d]
70% 0.5M Sucrose/ 30% starch	0.189:1	0.230:1	82.0
70% 1.0M Sucrose/ 30% starch	0.378:1	0.614:1	61.5
70% 1.5M Sucrose/ 30% starch	0.567:1	0.877:1	60.0
70% 2.0M Sucrose/ 30% starch	0.757:1	1.249:1	60.6

[a]Nuclear magnetic resonance.
[b]Theoretical mole ratio = moles sucrose/moles starch (i.e., glucose units.)
[c]Mole ratio determined by NMR = (area sucrose peak/sucrose NOE) divided by (area starch peak/starch NOE).
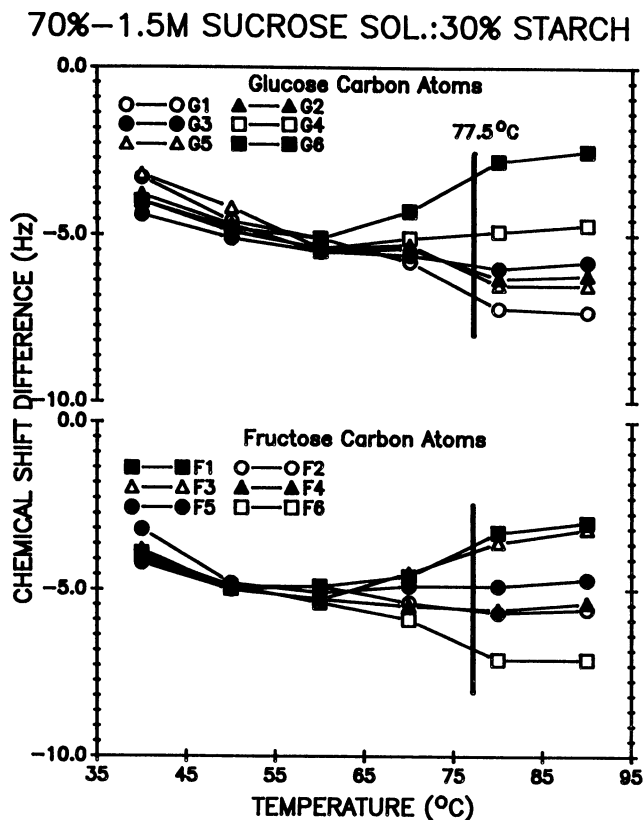[d]Percentage of starch contributing to NMR signal = a/b × 100.

70%—1.5M SUCROSE SOL.:30% STARCH

Fig. 3. Chemical shift differences between sucrose carbon atoms in a 70% 1.5M sucrose solution with 30% starch (w/w) dispersion and in a 1.5M sucrose solution as a function of increasing temperature. All other conditions are the same as for Fig. 1.
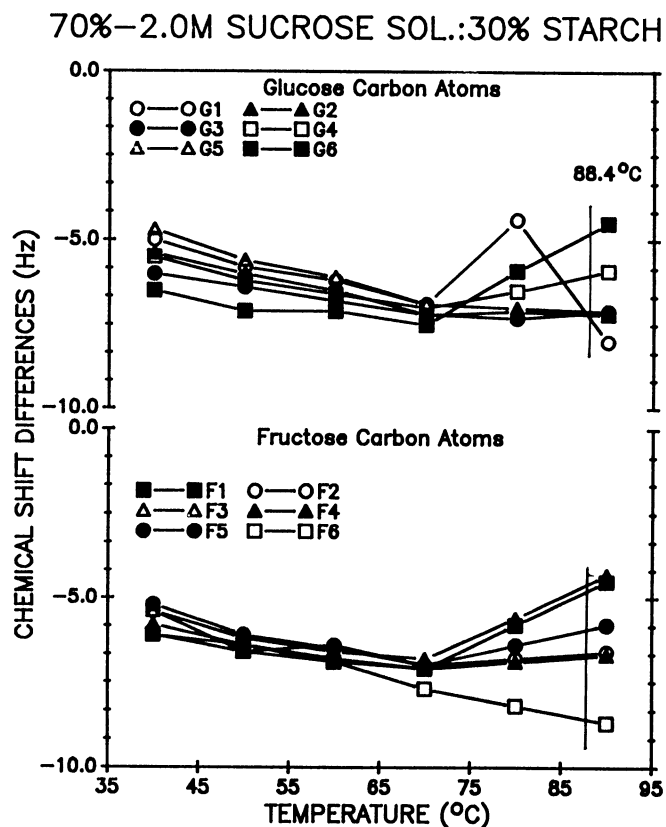
70%—2.0M SUCROSE SOL.:30% STARCH

Fig. 4. Chemical shift differences between sucrose carbon atoms in a 70% 2.0M sucrose solution with 30% starch (w/w) dispersion and in a 2.0M sucrose solution as a function of increasing temperature. All other conditions are the same as given in Fig. 1.

TABLE II
Average Chemical Shift Differences of Sucrose Carbon Atoms in Dispersions of Sucrose, Solubilized Starch, and Water

Temperature (°C)	Chemical Shift Differences (Hz)											
	11% Sucrose/15% Starch/74% Water						20% Sucrose/15% Starch/65% Water					
	Glucose Carbon Atoms											
	G1	G2	G3	G4	G5	G6	G1	G2	G3	G4	G5	G6
40	−6.9	−5.7	−5.8	−5.5	−6.4	−3.5	−3.6	−3.4	−3.5	−3.5	−3.5	−2.3
50	−6.1	−4.9	−5.5	−4.8	−5.6	−3.3	−4.2	−3.5	−3.8	−3.7	−3.9	−2.5
60	−5.7	−4.0	−5.0	−4.3	−5.2	−2.9	−4.3	−4.1	−4.4	−4.2	−4.4	−3.3
70	−5.0	−2.5	−4.5	−4.2	−5.2	−2.9	−5.2	−4.5	−4.8	−4.7	−4.9	−3.7
80	−6.1	−1.9	−4.6	−4.3	−4.4	−0.6	−4.8	−4.4	−4.7	−4.5	−4.9	−3.5
	Fructose Carbon Atoms											
	F1	F2	F3	F4	F5	F6	F1	F2	F3	F4	F5	F6
40	−4.2	−6.7	−4.2	−5.2	−6.0	−6.0	−3.3	−3.6	−3.7	−3.5	−3.1	−2.9
50	−3.9	−5.7	−4.1	−4.9	−5.3	−5.2	−3.4	−4.5	−4.0	−3.8	−3.5	−3.3
60	−4.1	−3.8	−4.1	−4.7	−4.8	−4.8	−4.1	−4.3	−4.3	−4.4	−3.9	−4.1
70	−3.6	−4.7	−4.2	−4.5	−5.0	−4.6	−4.6	−4.8	−4.9	−5.0	−4.5	−4.5
80	−4.1	−6.7	−3.9	−5.2	−4.9	−4.1	−4.3	−4.9	−4.4	−4.8	−4.3	−4.5

Table I gives the theoretical mole ratio, the mole ratios determined by NMR procedures, and the percentage of starch contributing to the peaks. The highest temperature (90°C) measured in NMR studies was used in the calculations, because at this temperature the greatest amount of starch should have gelatinized. Only after onset temperature of starch gelatinization can the NMR ratios be determined, because prior to gelatinization starch molecules are not mobile enough to show NMR peaks. Starch does not have a specific molecular weight, however, the moles of starch can be determined by calculating the theoretical value for moles of glucose that would be present in the amount of starch used. The area under a sucrose carbon atom peak and starch carbon peak from the NMR spectra can be calculated from an integration procedure. These areas then can be used to determine the ratio of sugar to starch contributing to the starch peaks. Dividing the ratio determined from the NMR peaks by the theoretical ratio will give the percentage of starch that becomes mobile enough to be measured by NMR and result in a peak. In proton NMR experiments, the area of the peak is proportional to the number of hydrogen atoms contributing signal. However, in ^{13}C experiments, the relative area of the peak may be altered by the mobility of the molecules being observed (nuclear Overhauser effect, NOE). Thus, the NOEs of the starch and sugar must be determined, and the area of the starch and sugar peaks must be divided by their respective NOEs. The NOEs for starch ranged from 1.35 to 1.45 and for sucrose from 1.80 to 1.95. In these calculations, all of the sucrose added was assumed to contribute to the signals.

Results of this study indicated that, with the $0.5M$ sucrose-starch dispersion, 82% of the starch in the system is mobile enough to contribute to NMR signals. This amount decreases to about 82% for the 1.0, 1.5, and $2.0M$ sucrose-starch dispersions. Callaghan and co-workers (1983) reported that 60% of starch was mobile enough to give NMR signals. These researchers compared intensities of ^{13}C-NMR signal per unit mass of equivalent maltose and starch solutions and found that the starch signal was only 60% that of an equivalent maltose solution. A $0.5M$ sucrose solution would have more water than solutions of greater molar concentrations, and possibly this additional water increased the mobility of starch, enabling it to contribute more to peaks. Also, at lower molar concentrations more soluble starch would be leached from the granule. Solubles leached from the granule are more mobile (presumably larger NOE) than intact starch and would contribute more to the signals. In addition, as the molar concentration of sugar solutions increases (i.e., correlation time decreases) water mobility decreases (Richardson et al 1987); this also could limit the rate at which starch could reorient with larger NOE and contribute to the NMR signals. Whether or not the amount of starch contributing to the signals affected the chemical shifts of the sucrose has not been determined.

Solubilized Starch Investigations

To determine if NMR measurements involved starch within the granule (amylose and amylopectin) or amylose that had leached from the granule, limited investigations on solubilized starch were undertaken. Shown in Table II are the chemical shift differences between carbon atoms of sucrose solution alone and sucrose solution dispersed in soluble starch. Sucrose solutions of 11 and 20% were used. Changes in chemical shift differed slightly, depending upon the concentration of sucrose added. With the 20% sucrose/15% solubilized starch dispersion, changes were small and not considered to be significant. With the 11.1% sucrose/15% solubilized starch dispersion, changes involving G2, G6, and F2 were greater than 1 Hz. Because F2 was comparatively difficult to measure accurately, the changes associated with this peak were interpreted cautiously. Because significant changes in chemical shifts were few, amylopectin in the granule structure appeared to be necessary for significant interactions or changes in interaction to occur, however, further studies are needed to verify this observation. Possibly in the solubilized starch system, sucrose-starch interactions occurred immediately and did not change as a function of temperature. Our methods do not determine if initial interactions are occurring but only if there was a change in the interactions.

Conclusions

Our results suggest that sugar-starch interactions occurred during the heating of starch. Major changes in chemical shifts of sucrose carbon atoms believed to be potential interaction sites, occurred with the G1, G5, G6, F1, F3, and F6 carbon atoms. The chemical shift changes occurred just before and within the temperature range of starch gelatinization, as measured by the DSC. As molar concentration of sucrose increased, the number of carbon atoms involved (i.e., G1, G2, G3, etc.) and the strength of the interactions appeared to decrease. In addition, results of solubilized starch investigations indicated that the starch granule probably was necessary for interactions or changes in interactions as a function of temperature to occur.

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