Structural Characterization of Starch Networks in the Solid State by Cross-Polarization Magic-Angle-Spinning ^{13}C NMR Spectroscopy and Wide Angle X-ray Diffraction

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ABSTRACT: The changes in structure and morphology following network formation of pregelatinized starch were studied in the solid state by cross-polarization magic-angle-spinning ^{13}C NMR spectroscopy and wide angle X-ray diffraction. Two types of mechanisms for network formation were employed, complexation with calcium and covalent cross-linking with epichlorohydrin. The spectra lines of the amylose network prepared by the calcium procedure were very narrow, and fine splitting was observed, indicating the formation of helical structure. The spectra lines of the network formed following the addition of epichlorohydrin were very broad and almost structureless, indicating that the resulting network is highly disordered and largely amorphous. These observations are supported by wide angle X-ray diffractograms. Changes in C-6 chemical shift was observed following starch complexation with calcium, whereas following the reaction with epichlorohydrin, only minor changes in spectra pattern were observed. The amylose network formed by the calcium procedure is of physical character, whereas a covalently cross-linked network was formed by the reaction with epichlorohydrin. These differences in structure have been observed to manifest themselves in the enzymatic degradation rates of these networks.

Introduction

Starch is a mixture of glucans, which is found mainly in the plant kingdom where it occurs as the principal food reserve polysaccharide utilized during plant growth. Starch forms the main source of carbohydrate in the human diet. Starch consists of two main polysaccharides, amylose and amylopectin, both of which are based on (1→4)-linked α-D-glucose chains. Whereas amylose is essentially a linear macromolecule, amylopectin is highly branched with branches linked at the 6 position.

The ability of polysaccharides to form a network structure (gel), even at very low concentrations, constitutes one of their most important functional properties. The formation of a three-dimensional network structure (gelation) offers an effective means to increase the system's mechanical and chemical stability. A wide range of modification mechanisms of starches are known.[1-3] These include self-association (induced by changes of pH, ionic strength, or physical and thermal means) and complexation with salts and covalent cross-linking (e.g., by epoxides). The characteristics of the network formed depends upon the pattern and type of mechanism used.

The gelation process, the formation of a three-dimensional nonsoluble network structure, is different than the gelatinization process, which is a means of dissolve starch.[4] Gelatinization is known to destroy the crystalline-like structure by opening starch tertiary and quaternary structures due to breakdown and rearrangement of hydrogen bonds. During this process the granular structure of starch is completely destroyed, but it is still in its macromolecular state.[1-3]

Starch, in its native or modified form, has been subjected to extensive study over the past 50 years. Early interest in starch was associated with the food and paper industry, textile manufacture, and pharmacology.[5,6] With the increased interest of biomedical and pharmaceutical research in biodegradable polymers as matrixes for controlled drug delivery systems, impressive activities on the modification of natural polymers to meet growing needs have been reported.[7-10] Cross-linked starch and starch networks have both the required biodegradability and a relatively high mechanical and chemical stability.

In this work we characterized the structure and morphology of starch networks formed by two distinct methods using cross-polarization magic-angle-spinning ^{13}C NMR spectroscopy (CP-MAS ^{13}C NMR) combined with wide angle X-ray diffraction measurements. The CP-MAS ^{13}C NMR technique is a convenient solid-state method for the characterization of these networks, which is very valuable when one considers that these networks are insoluble.

Amylose is a linear polysaccharide of relatively simple structure and was used as a model compound to study these processes. The networks were prepared by two different methods: complexation with calcium and covalent cross-linking with epichlorohydrin. The first step of network formation in both types of reactions involves the gelatinization of starch in order to ensure that the subsequent modifications are performed under homogeneous conditions.

The two methods of network formation studied in this work are being used to entrap drug molecules in the starch matrix for use in controlled drug delivery systems.[7-9] Since the enzymatic degradation of starch is very sensitive to both its structure and morphology, the degradation rates, thus drug release, will be affected and depend upon the structure of the network formed. Therefore, the characterization of the network structure is of great importance in understanding the entrapment and release mechanisms.

Experimental Section

Materials. Amylose (corn starch, S-4126, Sigma Chemicals), sodium hydroxide, calcium chloride (reagent grade, Sigma) and epichlorohydrin (Aldrich Chemicals) were used as received.

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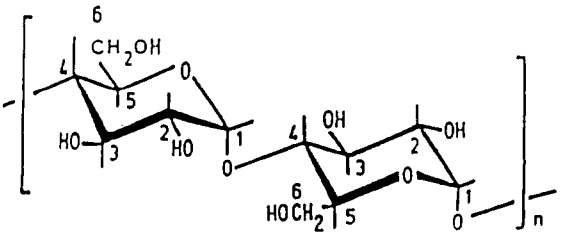

Figure 1. Amylose chemical structure.

Network Preparation. The networks were prepared at room temperature by a modification of the calcium procedure reported by Trimnell.[11]

The first stage of network formation in both calcium and epichlorohydrin procedures involves the gelatinization of starch by addition of sodium hydroxide solution (6.6% (w/v), 50 mL) to a suspension of water (30 mL) and amylose (12 g). The solution turns into a high viscosity paste after 1 h of continuous stirring at 600 rpm.

Solutions of calcium chloride (12.2 g, 24.5 mL of 50% (w/v) solution in water) or epichlorohydrin (20.5 mL), depending on the type of network formed, were added (in a ventilated hood) upon continuous slow mixing ending in coagulating the pastes. The particles formed were washed with 5 L of water followed by air-drying at room temperature for 5 days.

The cross-link densities of the networks formed were calculated as a percentage based on the stoichiometric amount of the cross-linking agent needed for reacting all three hydroxyl groups available (assuming all three hydroxyl group react) in amylose. In the preparation of the networks studied, either by the calcium method or by reaction with epichlorohydrin, we used stoichiometric amounts of cross-linker, thus forming networks having cross-link densities of 100%.

Wide-Angle X-ray Diffraction. Experimental X-ray powder diffraction patterns were obtained from a Phillips automatic powder diffractometer, equipped with a PW1050Q70 vertical goniometer. Diffraction patterns were obtained in the symmetrical reflection mode with copper Kα radiation (d = 1.54178 Å).

NMR Spectroscopy. The CP-MAS ^{13}C NMR spectra were recorded on a 200-MHz Bruker FT NMR (Fourier transform nuclear magnetic resonance) spectrometer equipped with CP-MAS (cross-polarization magic-angle-spinning) accessories. The dry samples were placed in a bullet type rotor and spun at a rate of ~2.5 kHz; the contact time was 4.5 ms. Spectra were accumulated at room temperature overnight in order to achieve a reasonable signal to noise ratio. The ^{13}C shifts were calibrated by substitution using external tetramethylsilane ($(CH_3)_4Si$) and hexamethylbenzene. The reproducibility of the chemical shifts was within 1 ppm.

^{1}H NMR spectra in the liquid state of amylose before and after the reaction with epichlorohydrin were recorded on a 200-MHz Bruker NMR spectrometer at room temperature in deuterated dimethyl sulfoxide.

Results and Discussion

The Native Amylose. The native amylose (Figure 1) has been examined in the solid state by CP-MAS ^{13}C NMR spectroscopy and a representative spectra is shown in Figures 3a and 9a. The fine splitting of the C-1 line at about 101 ppm into a triplet with almost identical intensity is characteristic of a highly crystalline A-type amylose[12] and suggests that the molecule attains a double helix structure of a relatively high fraction of the ordered region with a maltotrios as an asymmetric unit. The suggested structure is also confirmed by wide angle X-ray diffraction (Figure 2).

The broad resonance at 81.4 ppm is due to the noncrystalline component of the C-4 site. The C-6 resonance at 61.4 ppm is simple and relatively sharp. The resonances due to C-2, C-3, and C-5, having chemical shifts in the range of 72 ppm, could not be assigned due to extensive overlap.

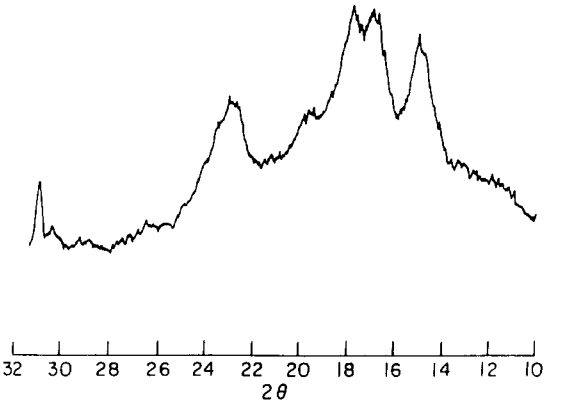

Figure 2. Wide angle X-ray powder diffraction pattern of native amylose.

The CP-MAS ^{13}C NMR spectroscopy technique yielded relatively high resolution spectra for the gelled samples studied. The chemical shifts obtained from these spectra are their "isotropic" values for the solid state. These shifts are similar in nature to those obtained in solution and may be used for elucidation of the molecular and crystalline structure.[13]

Previous solid-state ^{13}C NMR studies of crystalline carbohydrate structures have shown that resonances of the carbon atoms involved in the glycosidic linkage, and of the C-6 (bonded to the primary hydroxyl group), are particularly sensitive to the molecular conformation and lattice structure.[12,14]

The ^{13}C NMR chemical shifts of C-6 correlate with the torsional angle χ, which describes the orientation of the glycosidic linkage,[15] whereas the multiplicities of the resonances may be related to the symmetry and molecular conformation of the molecule.[2,16] The ^{13}C NMR spectra of the networks studied have been analyzed using correlations already established for amylose.[12]

Complexation with Calcium. One of the more familiar properties of amylose is its ability to complex with bivalent cations. Although the interaction between calcium and amylose has been the subject of many investigations,[17,18] the conformational changes taking place at the local level after complexation are not yet clear. CP-MAS ^{13}C NMR spectroscopy combined with the X-ray diffraction analysis provides a good probe for examining these processes as well as localizing the site of such interactions.

Comparison of the ^{13}C spectra of the native amylose to those after each stage of treatment leading to the final gelled system is shown in Figure 3.

The first step in the process leading to the final networks formed, either by the calcium procedure or by cross-linking with epichlorohydrin, involves the gelatinization of amylose using sodium hydroxide.

After the addition of sodium hydroxide the resonance lines of the amylose were broadened (Figure 3b). Broadening can be caused by distribution of isotropic chemical shifts due to the loss of crystallinity. The appearance of the noncrystalline spectra components upon the addition of sodium hydroxide and gelatinization may result from the unwinding or disordering of the glucose residues in the helical structure. The loss of crystallinity during this process is also observed in the wide angle X-ray diffractogram of a sample following treatment with sodium hydroxide (Figure 4). The observed lose of amylose crystallinity following gelatinization is similar to that reported in the literature.[3,4]

Upon addition of calcium chloride, the pattern of the spectra markedly changed and is very similar to that

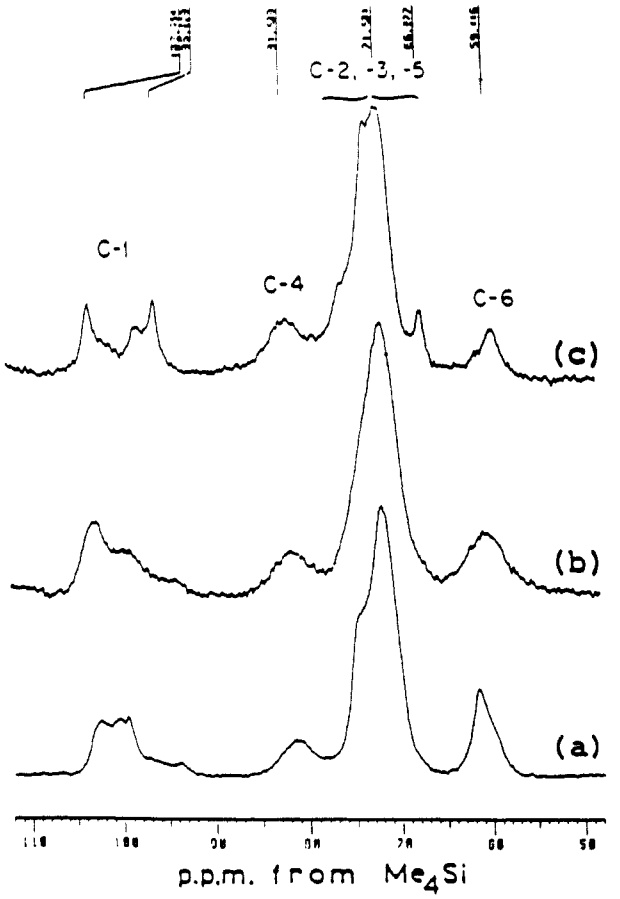

Figure 3. Comparison of the CP-MAS ^{13}C NMR spectra of native amylose (a), amylose treated with sodium hydroxide (b), and the network formed following the addition of calcium chloride (c).

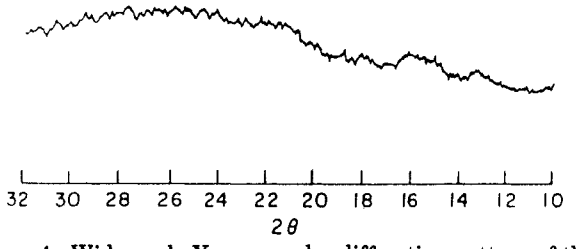

Figure 4. Wide angle X-ray powder diffraction pattern of the amylose following the reaction with sodium hydroxide.

observed for B-type amylose.[19] The resonance lines become much narrower, fine splitting is observed, and a new signal appears at 66.9 ppm (Figure 3c). The observed changes in spectral patterns clearly indicate chemical changes at the macromolecular level as calcium was bound. The network formed gave rise to a doublet for C-1 (101 ppm) characteristic of β-type amylose,[19] whereas the native amylose has been observed to give a triplet (Figure 5). The observed splitting of the C-1 line indicates the formation of a short range helical structure and can be interpreted in terms of an asymmetric unit.

Both amylose (Figure 5a) and the amylose network (Figure 5b) are crystalline double helices with parallel strands, but whereas the triplet splitting in the native amylose is correlated with a maltotrios asymmetric unit, the doublet splitting observed for the gelled amylose correlates with a maltose asymmetric unit.[14] The development of a B-type amylose structure following the incorporation of calcium has been also observed by Wing and co-workers[8] who studied the complexation of amylose with calcium by wide angle X-ray diffraction. Changes in the X-ray diffraction pattern indicating the retention of amylose crystallinity and an increase in chain order

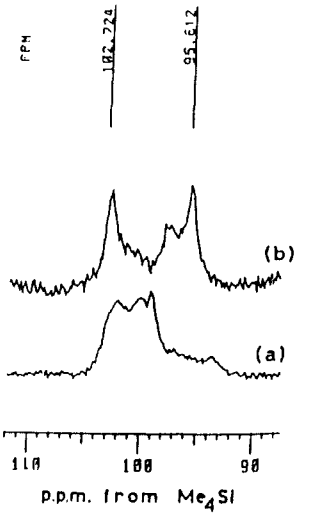

Figure 5. Comparison between the C-1 line of the native amylose (a) and the network formed after the treatment with calcium chloride (b).

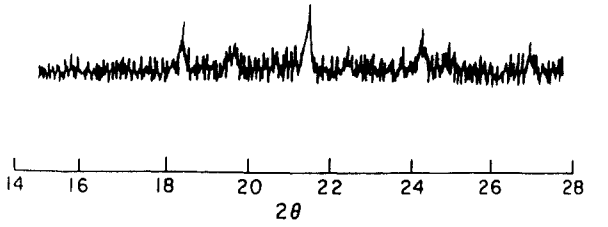

Figure 6. Wide angle X-ray powder diffraction pattern of the amylose network formed by the calcium procedure.

following the addition of calcium were also detected (Figure 6). These changes apparently manifest themselves in the appearance and sharpness of the diffraction lines.

The resonance of C-6 in the network formed is much broader relative to that observed for the original amylose molecule and is shifted to a lower field: from about 61.4 ppm in the original amylose molecule to 59.4 ppm in the network formed. According to CP-MAS ^{13}C NMR of small organic molecules,[20,21] changes in hydrogen bonding of O-6 may also result in a chemical shift of the attached C-6 carbon (Figure 3).

The retention of the helix structure after the addition of calcium chloride indicates that the ordered helix structure is thermodynamically favored for this type of ionic complex probably because a cooperative interaction can then operate between chains as well as within them. This rearrangement probably minimizes chain repulsion. The calcium bivalent cations may either fit inside the amylose hollow helix (Figure 7b) or rearrange between two helical chains (Figure 7a) creating either a physical or an ionic network structure, respectively. During the calcium complexation process the helix may include the calcium along the molecular axis, where the included calcium atom as well as hydrogen bonding stabilizes the helix structure. In this case the complex of amylose with calcium will have calcium atoms located in channels between the hexagonally placed double helix, and a physical network will be formed. The ability of amylose to form sufficiently extended hollow helixes (1000–2000 units) that twist around one another enables the formation of a strong physical network structure.[2]

In order to resolve whether the network formed is of a physical or an ionic character addition, experimental work has been conducted. The effect of molecular weight on the efficiency of network formation and the formation of an entangled network structure was studied by employing

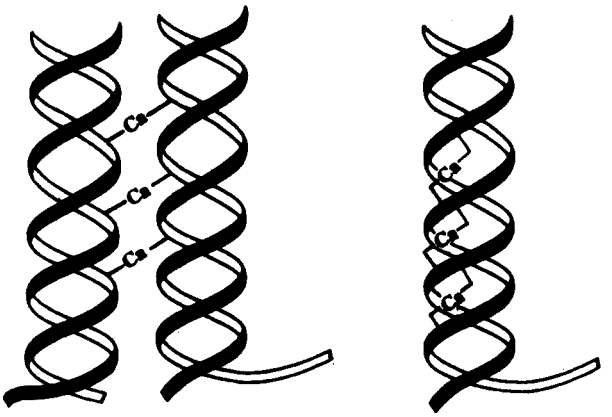

Figure 7. Possible mechanisms of calcium complexation with the amylose. The calcium bivalent cations may rearrange between two amylose helical chains (a) or fit inside the amylose hollow helix structure (b).

the calcium procedure (as described at the Experimental Section) to dextran molecules of various molecular weights. An insoluble network structure was observed to form only for dextran of molecular weight higher than 100 000 Da. Dextran molecules of low molecular weights (below 10 000 Da, glucose and maltose, did not form a network structure. The critical molecular weight essential for network formation, similar to the critical molecular weight for chain entanglement in polymers, suggests that the network is of physical character.

One of the important characteristics of the ionic bond is its ability to reversibly ionize in the presence of water, resulting in loosening of the matrix. When the amylose matrixes formed by the calcium procedure were immersed in water and base (sodium hydroxide, pH 9), the network kept its original shape for several days. This observation also supports the idea that the network formed is of physical character.

The calcium procedure has also been applied to polymeric dialdehyde starch, alginic acid, creatinine, and methyl cellulose, with little success. In these polysaccharides at least one hydroxyl group relative to amylose is absent. None of these polysaccharides formed a network structure with calcium under the conditions employed for amylose and starch, indicating that the hydroxyl groups have an important role in the complexation process.

The changes in spectral pattern following the complexation with calcium were observed to occur in the vicinity of the C-6 carbon (Figure 3c) indicating that the primary hydroxyl groups, rather than those on C-2 or C-3, complex with calcium. The new resonance at 66.9 ppm may be attributed to the C-5 carbon separating from the C-2 and C-3 resonances as a result of the of C-6 primary hydroxyl group complexation with calcium.

Reaction with Epichlorohydrin. Reaction of polysaccharides with epichlorohydrin usually leads to cross-linking, but monofunctional side reactions may also occur to form a series of byproducts with glycolic functional groups.[1] The condensation mechanism of epichlorohydrin with amylose has been extensively studied and it involves epoxide ring opening, mediated by the nucleophilic attack of the alkali amylose, and subsequent chlorine displacement and epoxidation[3] (see Figure 8).

The addition of epichlorohydrin following gelatinization does not markedly change the spectral patterns, and it remains very broad and almost structureless, indicating that the network formed is probably a random coil, highly disordered and largely amorphous (Figure 9).

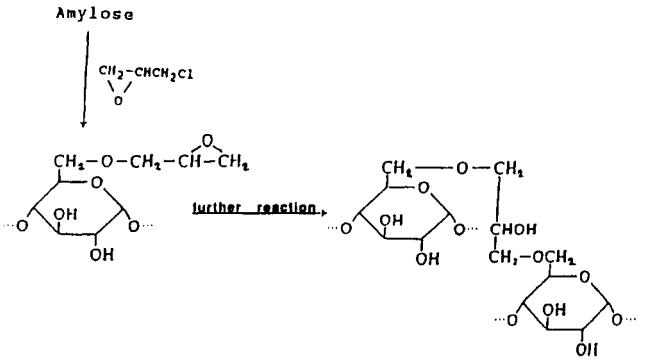

Figure 8. Reaction mechanism of amylose with epichlorohydrin.

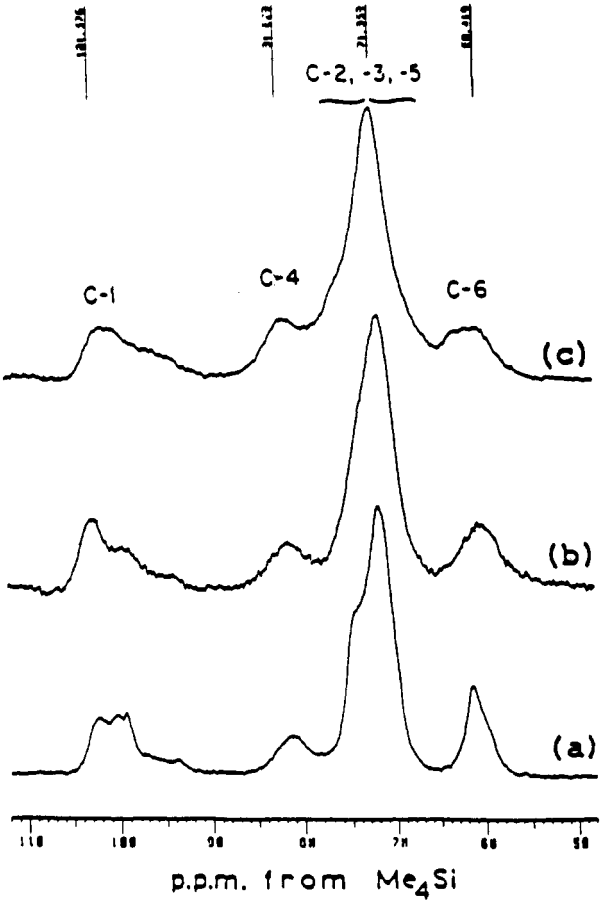

Figure 9. Comparison of the CP-MAS ^{13}C NMR spectra of the native amylose formed following the reaction with epichlorohydrin (c).

The amorphous nature of the networks formed following the reaction of amylose with epichlorohydrin is also supported by X-ray diffraction analysis (Figure 10).

Minor changes in both the chemical shift of C-6 and spectral pattern were observed following the reaction of amylose with epichlorohydrin (Figure 9c). The resonance of the C-6 carbon in the network formed is of weaker intensity and is shifted downfield by about 1 ppm from about 61.4 ppm in the native amylose molecule (Figure 9a) to 60.4 ppm in the cross-linked network. Some minor modifications of the spectra pattern were also observed as the disappearance of a shoulder on the left-hand side of the C-2, C-3, C-5 peak.

The liquid ^1H NMR spectra of amylose before and following cross-linking with epichlorohydrin are shown in Figure 11. The secondary hydroxyl groups, O-2 and O-3, of the native amylose at 5.0–5.4 ppm (Figure 11a) appear in the cross-linked network spectrum (Figure 11c). This indicates that both cross-link points as well as glycolic

Figure 10. Wide angle X-ray powder diffraction pattern of the amylose network formed by the reaction with epichlorohydrin.

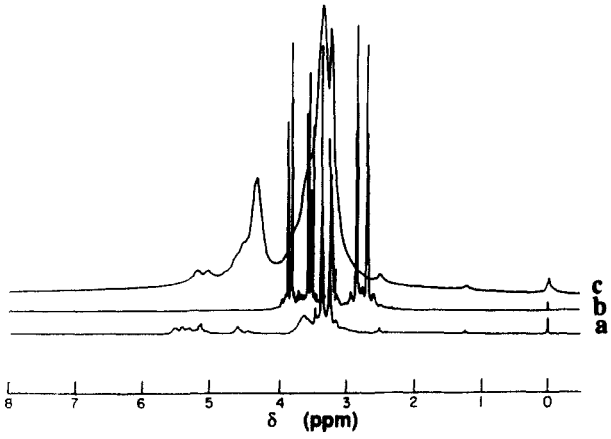

Figure 11. Comparison of the ^1H NMR liquid spectra of the native amylose (a) to epichlorohydrin (b) and the network formed following the reaction with epichlorohydrin (c).

functional groups (4.7 ppm) are formed. These observations suggest that epichlorohydrin cross-links and reacts monofunctionally at C-2, C-3, and C-6.

The enzymatic degradation rate of the amylose network formed by complexation with calcium was observed to be very similar to an unmodified amylose control and significantly higher than the network formed by the cross-linking reaction with epichlorohydrin.[23] These observations also suggest that the network formed via the calcium procedure is of physical character with the calcium included inside the amylose hollow helix structure.

Conclusions

The CP-MAS ^{13}C NMR spectroscopy technique yielded relatively high resolution spectra for the samples studied, and the spectra have been analyzed using correlations already established for amylose.

The changes in the spectral pattern following the complexation of amylose with calcium indicate the formation of a helical structure, whereas the spectra lines of the networks formed by the reaction with epichlorohydrin were very broad and almost structureless, indicating that the resulting network is highly disordered and largely amorphous. These observations are supported by the wide-angle X-ray diffractograms of the systems studied.

The changes in spectra pattern following the complexation with calcium were observed to occur in the vicinity of the C-6 carbon. The minor changes observed in both the chemical shift of C-6 and the overall spectra pattern, following the reaction with epichlorohydrin, indicate that epichlorohydrin cross-links and reacts monofunctionally at both primary and secondary hydroxyls.

The results also suggest that the cross-link density is not 100%, and although there might be some differences between the cross-link density of the amylose networks formed by calcium and epichlorohydrin, they cannot account for the observed differences in spectra pattern.

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Registry No. (Amylose)(epichlorohydrin) (copolymer), 66573-42-0.