Complexes of Starch Polysaccharides and Poly(ethylene *co*-acrylic acid): Structural Characterization in the Solid State

R. L. SHOGREN,*[1] A. R. THOMPSON,[2] R. V. GREENE,[1] S. H. GORDON,[1] and G. COTE[1]

[1]Biopolymer Research and [2]Analytical Chemistry Support Units, Northern Regional Research Center, USDA, Agricultural Research Service, Peoria, Illinois 61604

SYNOPSIS

The structural characterization of model complexes of amylose (AM) and amylopectin (AP) with poly(ethylene-*co*-acrylic acid) (EAA) was undertaken in order to better understand the interactions that occur between the polysaccharides and EAA in starch-EAA-polyethylene films. X-ray diffraction and CP/MAS ^{13}C-NMR studies showed that precipitates from solution mixtures of AM and EAA form crystalline, helical *V*-type inclusion complexes. The proportion of AM forming the *V*-type complex in the EAA/AM blends, estimated from shifts in the C1 resonance of AM, increased with increasing EAA/AM ratio, reaching a value of about 80% at EAA/AM = 0.5 (w/w). Similar measurements for EAA/AP complexes showed < 10% *V* structure. Approximately 80% of the AM and 4% of the AP in these blends was resistant to amylase digestion, in good agreement with their *V*-structure contents as determined above. Resonances at 184 and 181 ppm were observed for the carboxyl carbon of EAA in the EAA/AM and EAA/AP complexes. The resonance at 181 ppm, which was not observed in pure EAA, probably reflects greater shielding of the carboxyl inside the polysaccharide helix as well as changes in hydrogen bonding. The intensity of this peak was 2–3 times larger for the EAA/AM than for the EAA/AP complexes. FTIR experiments suggest that most (> 50%) of the EAA carboxyl groups were hydrogen bonded to polysaccharide hydroxyl groups in both AM and AP complexes when EAA/polysaccharide < 1.

INTRODUCTION

Plastics containing starch are of interest since starch is an abundant, inexpensive, renewable resource[1,2] and starch confers partial biodegradability to the plastics.[3] Biodegradable films would be especially useful in applications such as crop mulch films and planting pots where later removal and disposal of the plastic can be difficult and costly.[1] Otey et al.[1,2] and Swanson et al.[4] have developed methods for producing blown films from moist blends of starch, EAA, and polyethylene. These films are semitransparent and have fair mechanical properties. Little is known, however, regarding the molecular basis of the interaction between components of the films.

Fanta et al.[5] have postulated that starch and EAA may form *V*-type, helical inclusion complexes similar to those formed from starch and iodine or fatty acids.[6] It is known that such helices have a hydrophobic cavity about 6 Å in diameter[7] and strongly bind many organic compounds.[8] Starch is composed predominantly of amylose and amylopectin. Amylose, which consists of linear α-1,4-linked glucose residues, readily forms insoluble, crystalline *V*-type inclusion complexes.[8] Amylopectin, a highly branched polymer of short α-1,4-linked chains connected by α-1,6-linked branches, does not form insoluble complexes with low molecular weight compounds such as butanol.[9] Fanta et al.[5] have, however, found that both starch amylose and amylopectin form insoluble complexes with EAA when these were mixed at 2% concentration in 4*M* NH_4OH. Chiroptical studies of model dilute solution mixtures of EAA with AM strongly suggested that these indeed form helical *V*-type inclusion complexes.[10] Less stable *V* structures were formed in solution mixtures of EAA and AP.[10]

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* To whom correspondence should be addressed.

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X-ray diffraction, CP/MAS ^{13}C-NMR, and FTIR techniques have been used successfully to probe the structure and interactions of many biopolymers in the solid state. X-ray diffraction techniques have been applied previously to determine the structures of starch and amylose in the *A, B, C,* and *V* crystalline forms.[8] Large differences in the CP/MAS ^{13}C-NMR spectra of these biopolymers have also been observed.[11-15] We describe here X-ray diffraction, CP/MAS ^{13}C-NMR, and FTIR studies of solid complexes derived from dilute solution mixtures of EAA with amylose and amylopectin.

EXPERIMENTAL

Materials

Amylose (AM) from potato was purchased from Sigma Chemical and was further purified as described by Karkalas and Raphaelides.[16] Amylopectin (AP) from potato was purified from potato starch as described by Schoch.[9] Intrinsic viscosities of AM and AP in 20 m*M* NaCl, pH 7, were 110 and 120 mL/g, respectively. These values correspond to molecular weights of approximately 9×10^5 for AM using the empirical equation of Banks and Greenwood (eq. 4.80, Ref. 17) and about 500×10^6 for AP using data from table 2.15, Ref. 17.

The poly(ethylene-*co*-acrylic acid) (EAA) was Dow Primacor 5981. It has a reported composition of 20 wt % acrylic acid and values of M_w and M_n of 18,000 and 7000, respectively.[18] The copolymer sequence was reported to be random.[18]

Preparation of Solid Complexes

Solutions of 1.6–1.8 mg/mL AM in 20 m*M* NaOH were prepared as described by Shogren et al.[10] Solutions of 0.5% AP in 0.5*M* NaOH were prepared by slowly adding 1 g AP to 200 mL 0.5*M* NaOH with rapid magnetic stirring. Stirring was continued for 1–2 h. The AP solution was then titrated to pH 7 with 1*M* HCl and dialyzed extensively against water. The AP solution was finally diluted to approximately 2 mg/mL, adjusted to 10 m*M* NaOH with 1 *M* NaOH, and centrifuged at 12,000*g* for 2 h to remove undissolved gels. The AP concentration in the supernatant was 1.3–1.5 mg/mL as determined by phenol sulfuric acid assay.[19]

Aqueous suspensions of EAA in 10 m*M* NaOH were prepared by heating 1 g of EAA and 100 mL of 36 m*M* NaOH to above 90°C (the melting point of EAA) with stirring (26 mmol/L of NaOH were required to neutralize the acrylic acid). These suspensions were then cooled to room temperature and remained stable for months.

The required volume of 1% EAA suspension in 10 m*M* NaOH was added to solutions of AM or AP in 20 m*M* NaOH with stirring to obtain the desired ratio of EAA to polysaccharide. This mixture was then aged 1–2 days at room temperature since previous optical rotation studies indicated that this amount of time was required for maximal interaction to occur.[10] The EAA/AM complex was then precipitated by addition of 1 *M* HCl (3 mL per 100 mL complex solution) to prepare the acid form of EAA/AM complex. The precipitates were then collected by centrifugation, washed with water, and air-dried. They were then pulverized by shaking in a stainless-steel vial with two steel balls using a Wig-L-Bug amalgamator (Crescent Dental Mfg. Co., Lyons, IL). The vial was cooled with liquid nitrogen prior to shaking. No precipitate formed on neutralization of the EAA/AP mixtures, so these solutions were titrated to pH 5 with 1*M* HCl, dialyzed extensively against water, and lyophilized. Specimens of amorphous AM and AP were prepared by dialyzing and lyophilizing solutions of AM and AP prepared as described above.

Amylase Digestion of Complexes

A weighed sample of complex (200–400 mg) was suspended in 40 mL of 0.1 *M* sodium 3-(*N*-morpholino)propanesulfonate buffer, pH 7.2, containing 0.01% sodium azide to prevent microbial growth. This suspension was mixed by rocking to disperse the complex particles. To the suspension was added 0.040 mL of a suspension of twice-crystallized porcine pancreatic α-amylase (PPA) (Sigma type I-A, 29×10^3 U mL^{-1}). Digestion was carried out at room temperature (25°C). At timed intervals, aliquots were removed, filtered through a 0.45 micron pore-size polysulfone syringe filter, and analyzed for total carbohydrate by the phenol-H_2SO_4 method.[19] Digestions were terminated after the concentrations of soluble carbohydrate in the reaction media reached steady-state values (8 days for EAA/AM and 1 day for EAA/AP). The insoluble residues were washed with water and air-dried.

Analytical Methods

X-ray diffraction experiments were performed by Philips Electronic Instruments Co., Mahwah, NJ, using an APD1700 diffractometer with CuK_α radiation and a scintillation detector.

CP/MAS ^{13}C-NMR experiments were performed using a Bruker MSL-300 spectrometer. Proton pulse widths were 5 μs and 2 ms contact times were used unless otherwise noted. Delay times between acquisitions were 1 or 5 s. Samples were spun in zirconia rotors at a rate of approximately 3500 or 4500 Hz. Spectra were normalized so as to make the most intense peak the same height in each spectrum.

High-power decoupling (HPDEC)/MAS ^{13}C-NMR experiments were performed on the same spectrometer at temperatures from 20 to 97°C. Recycle times (2 s) were long enough to allow almost complete relaxation at the upper end of this temperature range. Rotation rates were 2500 Hz.

FTIR experiments were performed using an Analect RFX-75 spectrometer. Spectra were obtained at 4 cm^{-1} resolution and were an average of 32 scans. Samples (1 mg) were pulverized by vigorous shaking in a stainless-steel vial with two steel balls using a Wig-L-Bug amalgamator (Crescent Dental Manufacturing, Lyons, IL). KBr, 300 mg, was added to the vial, and the shaking was continued. Vials were cooled with liquid nitrogen prior to shaking. Pellets were pressed at 24,000 psi for 1–2 min.

The compositions of amylase-digested complexes were estimated from the ratio of the EAA C—H symmetric stretch at 2854 cm^{-1} to the AM or AP C—O stretch at 1026 cm^{-1}. AM alone had a small absorbance at 2854, and so the ratio of A_{2854}/A_{1026} for AM was subtracted from the same ratio for the EAA/AM complexes. A calibration curve of A_{2854}/A_{1026} vs. EAA/AM was constructed from FTIR spectra of complexes having known compositions prepared as described above.

RESULTS AND DISCUSSION

Solid-State Characterization

A wide-angle X-ray diffraction scan of a precipitate of EAA/AM 0.5/1 is shown in Figure 1. One weak and two strong diffraction maxima were observed at 8.0, 12.8, and 20.3 degrees 2θ, respectively. D spacings, calculated using Bragg's law,[20] were 11 ± 0.5, 6.8 ± 0.3, and 4.4 ± 0.2 Å. These diffraction peaks correspond closely to the strong or very strong reflections found for the X-ray pattern of the hydrated, left-handed, amylose 6_1 V-type helix at d = 11.80, 6.82, and 4.46 Å.[21,22] In contrast, the native B crystalline structure found in potato starch exhibits strong diffraction peaks at values of d = 15.9, 5.9, 5.2, 4.0, and 3.7 Å,[8] whereas gelatinized starch exhibits a single broad amorphous band at 2θ = 18 degrees.[23] The peaks at d = 11.80, 6.82, and 4.46 Å for V amylose represent reflections from the 110, 200, and 310 planes, respectively, of an orthorhombic unit cell having a = 13.6, b = 23.7, and c = 8.2 Å.[21,22] There are two cylindrical amylose chains per unit cell, one in the center of the ab plane and one at each of the four corners of the ab plane, all of which are parallel to the c axis. This is essentially a hexagonal close-packed array of helical cylinders, but is assigned to an orthorhombic unit cell because adjacent helices have opposite chain directions and are therefore nonequivalent.[22] Six glucose residues form one complete turn of the helix every 8.2 Å along the helix axis.[22] The value of a = 13.6 Å represents the effective helix diameter.

The position of the EAA in this crystal structure cannot be determined directly because of the poor resolution of the diffraction pattern. If EAA occupied the space between helices, the dimensions of the unit cell for the EAA/AM complex would be expected to be somewhat larger than for V-amylose alone since the spaces between helices of the latter would probably need to expand to accommodate the EAA. Our X-ray pattern is, however, nearly identical to the pattern obtained for V-amylose alone,[22] suggesting that EAA occupies the interior of the helix. Similarly, Mikus et al.[24] and Takeo et al.[25] found identical diffraction patterns for palmitic acid/amylose complexes and V-amylose. Amylose also forms a 7_1 V-type helix having a larger diameter

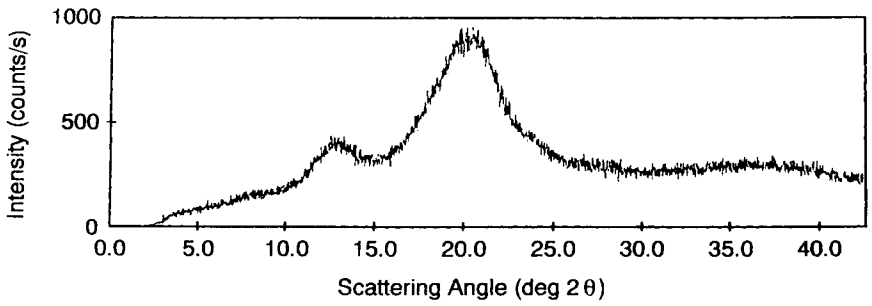

Figure 1 X-ray diffractometer scan of EAA/AM 0.5/1.

(14.7 Å) when complexed with bulky organic molecules such as *tert*-butanol.[26] Since EAA/AM complexes adopt the 6_1 conformation, the EAA inside the helix is probably in an extended configuration rather than folded into a more bulky shape.

A lower limit of the crystallite size, L_{hkl}, in a direction perpendicular to the *hkl* plane can be estimated using the Scherrer equation[27] $L_{hkl} = K\lambda/(\beta_0 \cos\theta)$, where $K \simeq 1$, β_0 is the peak width at half-height in radians, λ is the wavelength (1.541 Å), and θ is the scattering angle. Values of $L(310) = 21$ Å and $L(200) = 35$ Å were calculated for EAA/AM 0.5/1. Annealing in water at 80°C for 2 days did not change the diffraction pattern significantly. These rather small crystallite sizes may reflect the limited lengths or mobilities of EAA molecules present on the surface of the EAA micelle.[10] Short-chain hydrocarbon branches on the EAA molecule (see NMR data below) might also interrupt the crystalline helix domains.

Solid-state NMR spectra of amorphous amylose, EAA, and complexes containing EAA and AM in w/w ratios of 0.16/1, 0.3/1, 0.5/1, and 1.5/1 are shown in Figure 2. One difference between the amylose and EAA/AM complex spectra was the sharpness of the C1 resonance around 100 ppm in the latter spectrum. Gidley and Bociek[11] have shown that the chemical shift of this carbon is approximately linearly related to the absolute value of the glycosidic angle ψ. The large peak at 103 ppm represents the V_L conformation, whereas the smaller peaks at 98 and 95 ppm reflect disordered states. The relative abundance of these latter peaks in the EAA/AM complexes decreased, and the proportion of *V* structure increased as the ratio of EAA/AM increased. For EAA/AM 0.5/1, most of the amylose has been converted into the *V* form, in agreement with the chiroptical studies of the same complexes in dilute suspension.[10] Specifically, the ratio of the peak heights: $h(95)/h(103)$ for EAA/AM 0.5/1 divided by the same ratio for amylose, is approximately 0.2, leading to an estimate of *V* structure content of EAA/AM 0.5/1 of 80%. The remaining 20% of the AM is in the disordered, amorphous form.

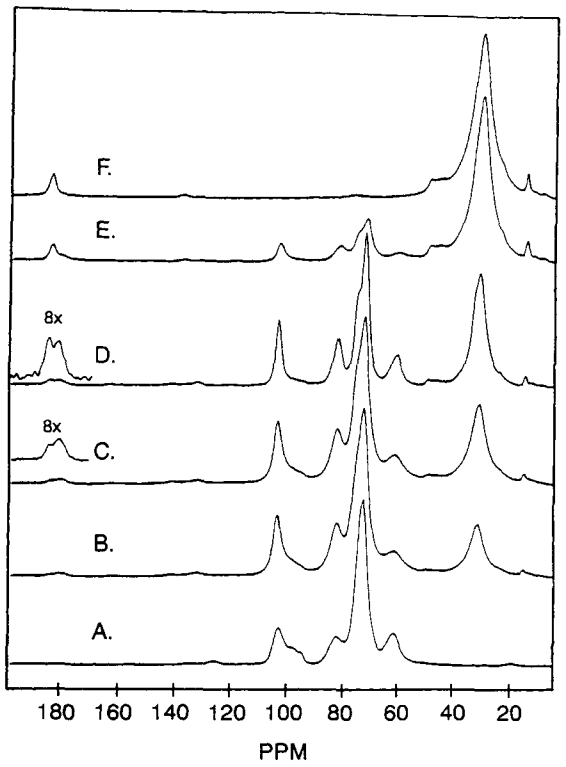

Figure 2 CP/MAS ^{13}C-NMR spectra of amorphous AM (A), EAA/AM 0.16/1 (B), EAA/AM 0.3/1 (C), EAA/AM 0.5/1 (D), EAA/AM 1.5/1 (E), and EAA (F).

It is also evident that the carboxyl carbon resonance at 184 ppm for EAA split into two separate peaks at 184 and 181 ppm when complexed with amylose. Carboxyl groups in EAA copolymers are largely hydrogen bonded to each other.[28] This causes a deshielding of the carboxyl carbon and a downfield shift. The carboxyl carbon of an isolated (not H-bonded) acrylic acid residue in EAA is expected to resonate at approximately 181 ppm,[29] and, thus, the resonance at 184 ppm in EAA reflects the hydrogen-bonded state of the carboxyl group. Therefore, the new resonance at 181 ppm in the EAA/AM complex would appear to represent EAA carboxyl carbons that are complexed within the amylose helix and are not hydrogen bonded or only weakly so. This may be due to the rather hydrophobic character of the interior surface of the amylose helix that consists mainly of hydrogen atoms attached to carbon atoms.[6] Upfield shifts could also conceivably result from a different magnetic susceptibility inside the helix or intermolecular steric crowding of the carboxyl carbon by amylose hydrogen atoms in analogy to the upfield shifts caused by intramolecular steric interactions.[29] Similar (2–3 ppm) upfield shifts of the carboxyl carbon have also been seen for solution spectra of formic and acetic acid complexed with cyclodextrins.[30]

The relative areas of the carboxyl resonances at about 184 and 181 ppm were estimated by adding together two Gaussian bands and varying the peak widths and heights until the best visual fit was obtained. For small values of EAA/AM, most of the carboxyl carbons resonated at 181 ppm, indicating that most of the EAA was complexed inside the AM helix. As the ratio EAA/AM increased, the fraction

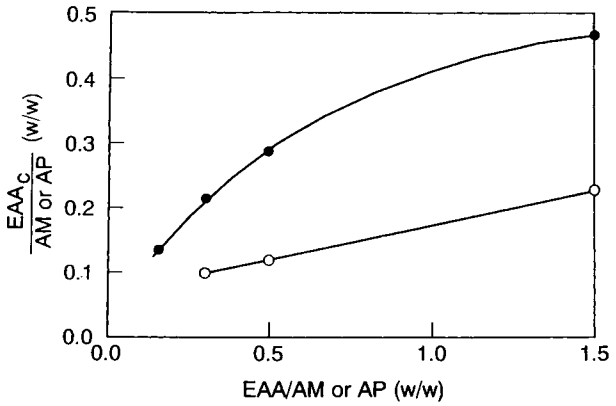

Figure 3 Effect of composition on the weight fraction of EAA carboxyl groups complexed relative to the weight of AM (●) or AP (○). This quantity is given by $A_{181}/(A_{181} + A_{184}) \times [\text{EAA/AM(P)}] = \text{EAA}_c/\text{AM(P)}$, where A denotes the areas of the respective carboxyl NMR peaks.

of carboxyl carbons complexed, $A_{181}/(A_{181} + A_{185})$, decreased. The product, $(\text{EAA/AM}) \times A_{181}/(A_{181} + A_{185}) = \text{EAA}_c/\text{AM}$, reflects the weight of EAA complexed relative to the weight of AM. A plot of EAA_c/AM vs. EAA/AM is shown in Figure 3. Since AM was nearly fully complexed by EAA when EAA/AM > 0.5 (see Fig. 2), we would expect, however, EAA_c/AM to reach an asymptotic value $\simeq 0.5$ when EAA/AM > 0.5. EAA_c/AM continued to increase as EAA/AM increased. This could be explained if EAA contained regions rich in acrylic acid residues and AM interacted preferentially with these regions when an excess of EAA was present.

Solid-state NMR spectra for AP and complexes of AP and EAA are shown in Figure 4. The resonances at 95 and 98 ppm, representing the disordered portion of AP, were only slightly less intense for the EAA/AP complexes than for the AP. This suggests that the AP in the complexes possessed < 10% *V* structure. Values of the ratio $A_{181}/(A_{181} + A_{185})$, shown in Figure 3, were about 40% of same ratio for EAA/AM complexes. This suggests that a smaller proportion of the carboxyl groups of EAA were complexed by AP than by AM. The branches at C6 in AP probably disrupt *V*-helix formation and inhibit helix crystallization. Chiroptical solution studies have shown that the change in specific rotation of AP upon addition of EAA was about 40–50% that for AM.[10] Thus, the upfield shift of the carboxyl group of EAA in the solid complex correlates well with the formation of *V* structure in the EAA/AP complex in solution. The reason why the C1 resonance of AP changes little while the carboxyl resonance of EAA shows appreciable splitting is unknown. It is possible that the C1 chemical shift of AP and AM may be affected by intermolecular order as well as conformation. Upfield shifts of the carboxyl carbon due to weaker hydrogen bonding may also occur independent of *V*-type complex formation.

Some HPDEC/MAS ^{13}C-NMR experiments (data not shown) were performed at temperatures from 20–97°C in order to learn more about the mobilities of the EAA and polysaccharide components of the EAA/AM and EAA/AP complexes. HPDEC methods were chosen since more mobile carbons will have narrow peaks and, at the higher end of this temperature range, spin lattice relaxation rates were fast enough to allow nearly complete relaxation in only 1–2 s. In both complexes and pure EAA, the line widths of the aliphatic carbon resonance at 29 ppm decreased markedly with increasing temperature, indicating large increases in mobility with increasing temperature. The line widths of the AM and AP resonances in their respective complexes with EAA did not, however, change significantly with increasing temperature. The EAA carboxyl carbon peak at 181 ppm in the EAA/AM complex remained broad even at 97°C. Conversely, the major peak at 184 ppm for EAA/AP and pure EAA sharp-

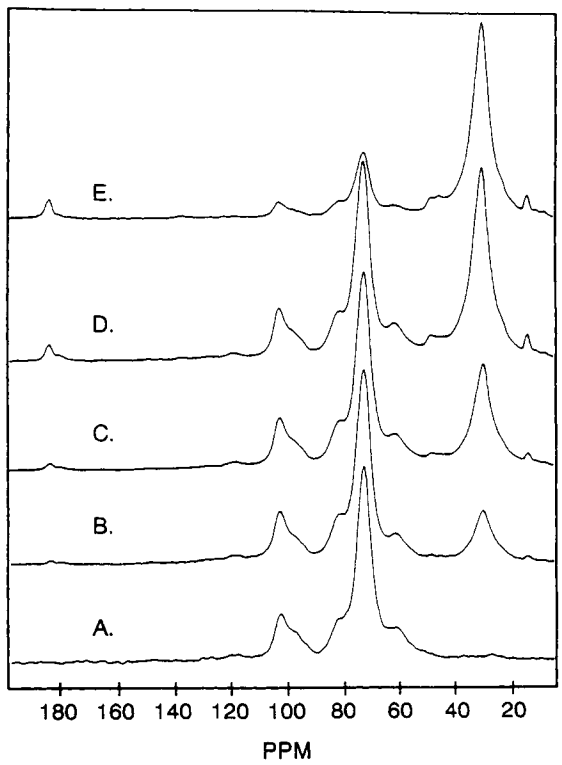

Figure 4 CP/MAS ^{13}C-NMR spectra of amorphous AP (A), EAA/AP 0.15/1 (B), EAA/AP 0.3/1 (C), EAA/AP 0.5/1 (D), and EAA/AP 1.5/1 (E).

ened and shifted to 182 ppm at 97°C. These data suggest a "tight fit" and/or unique hydrogen-bonding interaction that persists at high temperatures for the EAA carboxyl group inside the AM *V*-helix.

Complexes of EAA/AM 0.5/1 and EAA/AP 0.5/1 were digested with porcine pancreatic amylase as described in the Experimental section. Approximately 80% of the AM and 4% of the AP remained insoluble after digestion as determined from the FTIR spectra (see Fig. 6). The former value corresponds closely with an estimate of the *V*-structure content of 80% for EAA/AM 0.5/1 (see above). The NMR spectrum of the solid EAA/AM 0.5/1 complex after amylase digestion is shown in Figure 5. Comparing Figures 2 and 5, it appears that the intensity of the resonances at 95 and 98 ppm decreased after amylase digestion. These results suggest that the disordered, amorphous portions of the EAA/AM complex were digested, leaving the more crystalline *V*-type complex intact. Since nearly all of the EAA/AP complex was digested, the *V*-type structure present is probably rather disordered and very limited in size. The small quantity of polysaccharide resistant to digestion may represent contaminating AM since typical AP preparations normally contain 3–4% AM.[9]

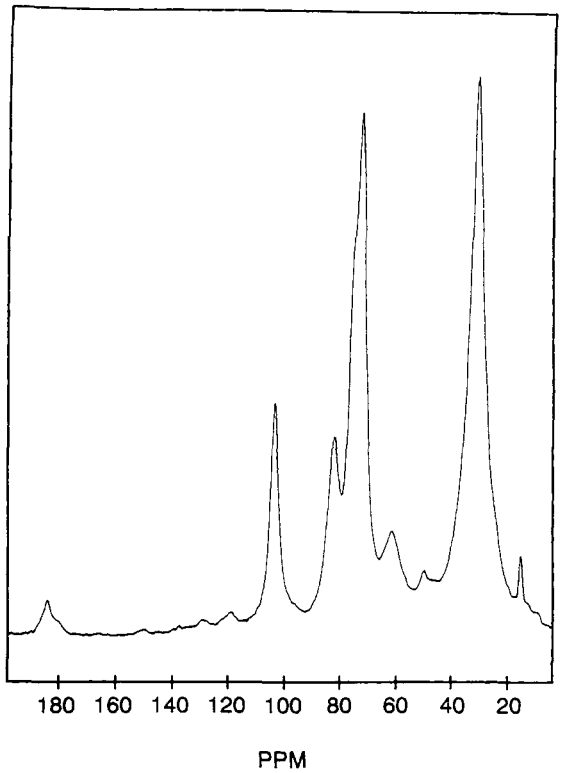

Figure 5 CP/MAS ^{13}C-NMR spectrum of insoluble residue remaining after digestion of EAA/AM 0.5/1 with porcine pancreatic amylase.

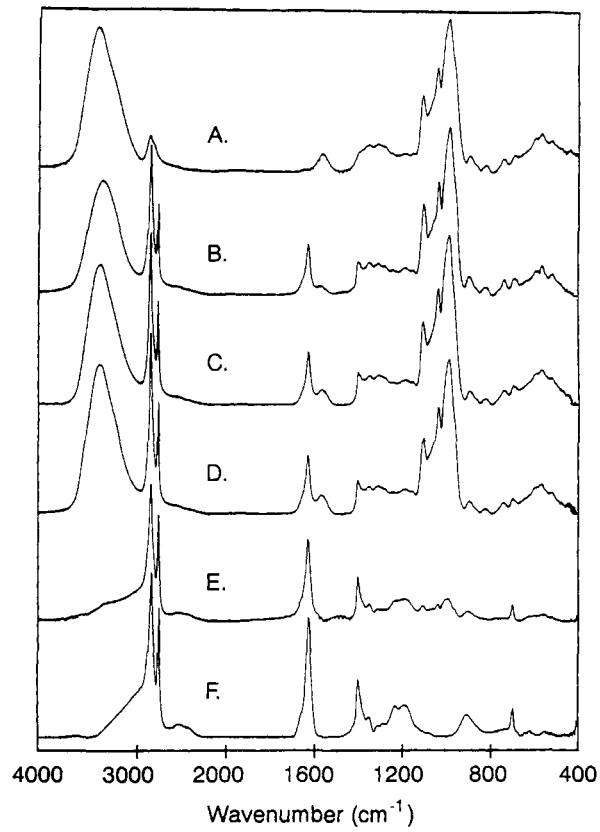

Figure 6 FTIR spectra of AM (A), EAA/AM 0.5/1 (B), EAA/AP 0.5/1 (C), sample B after amylase digestion (D), sample C after amylase digestion (E), and EAA (F).

FTIR spectra of EAA, AM, EAA/AM 0.5/1, EAA/AP 0.5/1, and residues from the amylase digests of the latter two samples are shown in Figure 6. The absorption bands at 1704.5, 2674, and 940 cm^{-1} for EAA arise from the stretching vibration of the C=O, stretching of the O—H, and out-of-plane deformation of the O—H, respectively, in a hydrogen-bonded carboxyl dimer.[28,31] The shoulder at 1735 cm^{-1} may reflect weakly hydrogen-bonded states of the carboxyl. For example, monomeric (not H-bonded) carboxylic acids in nonpolar solvents have a C=O vibration at about 1760 cm^{-1}. This decreases to about 1735 and 1720 cm^{-1} in ether and alcohol solutions, respectively, where hydrogen bonding to the solvent occurs.[32] This decrease in carboxyl stretching frequency correlates well with an increase in the enthalpy of formation (ΔH) or strength of the hydrogen bond.[33] Similarly, the hydroxyl stretching frequency decreases as the strength of the hydrogen bond increases. For example, O—H

stretching vibrations in alcohols and carboxylic acids appear at 3200–3500 cm^{-1} and 2600–3100 cm^{-1}, respectively, and have $\Delta H = 4.5 \pm 1$ and 7 ± 0.5 kcal/mol, respectively.[33]

Figure 7 shows changes in the intensity of the bands at 1735, 2674, and 940 cm^{-1} for complexes of EAA with AM and AP as a function of the ratio of these components. The ratio of I_{1735}/I_{1705} indicates the amount of weakly hydrogen-bonded carboxyl groups relative to the amount of stronger such-bonded groups. The intensities of the 2674 and 940 cm^{-1} bands have been normalized to the intensity of the symmetric C—H stretch vibration at 2852 cm^{-1}. The latter peak is proportional to the total amount of EAA present in the sample. The symbol Δ indicates that the contribution of AM or AP to each of these intensities has been subtracted. Further, the ratios $\Delta I_{2674}/\Delta I_{2852}$ and $\Delta I_{940}/\Delta I_{2852}$ were normalized to values of the same ratios for pure EAA (0.109 and 0.175, respectively). Thus, the plotted ratios $\Delta I_{2674}/\Delta I_{2852}$ and $\Delta I_{940}/\Delta I_{2852}$ indicate the proportion of carboxyl groups that have formed the strongly hydrogen-bonded dimers. The ratio $\Delta I_{1735}/\Delta I_{1705}$ increased as the ratio of EAA to AM or AP decreased. This suggests that the EAA carboxyl groups form weaker hydrogen bonds as the ratio of EAA/AM decreased. Presumably, the hydroxyl groups of AM or AP and the carboxyl of EAA form these weaker bonds. Thus, as the proportion of EAA complexed within the AM or AP helix increased (see NMR data), the more weakly hydrogen-bonded carboxyl content increased. The ratio $\Delta I_{2674}/\Delta I_{2852}$, initially about equal to zero, increased rapidly at ratios of EAA to AM and AP of about 0.3 and then more slowly thereafter. The latter value corresponds to the composition at which most of the AM has formed the *V*-type complex with EAA.[10] Therefore, for EAA/AM(P) < 0.3, most of the EAA carboxyls are probably hydrogen bonded to AM or AP hydroxyls. EAA added in excess of this begins to form dimeric hydrogen-bonded carboxyl groups. Even at EAA/AM(P) = 10, however, some of the weaker hydrogen bonds persist. This suggests that EAA carboxyl groups not involved in *V*-type complex formation may still hydrogen bond with AM or AP. This would seem to be a reasonable conclusion since, for a ratio of EAA/AM of 10/1, there are about 0.7 hydroxyl groups from the polysaccharide for each carboxyl group from the EAA. Changes in $\Delta I_{940}/\Delta I_{2852}$ with changing composition were similar but somewhat more gradual than those observed for $\Delta I_{2674}/\Delta I_{2852}$. Taken together, the FTIR spectra and hydrogen-bonding characteristics of the carboxyl in EAA/AM and EAA/AP complexes appear similar,

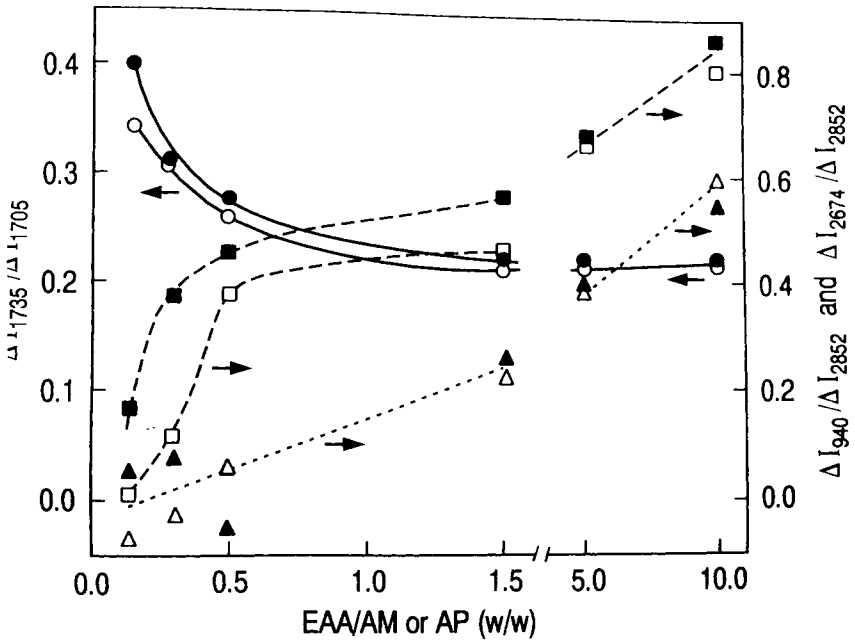

Figure 7 Effect of composition on the infrared absorbance ratios $\Delta I_{1735}/\Delta I_{1705}$ (●,○), $\Delta I_{2674}/\Delta I_{2852}$ (■,□), and $\Delta I_{940}/\Delta I_{2852}$ (▲,△) for EAA/AM (closed symbols) and EAA/AP (open symbols). Δ indicates that the contribution of AM or AP to the absorbance has been subtracted. The latter two ratios have been normalized to the value of the same ratio for pure EAA.

within experimental error. This suggests that part of the ^{13}C-NMR carboxyl resonance at 181 ppm observed for the EAA/AM complex is likely due to changes in magnetic environment other than H-bonding.

CONCLUSIONS

X-ray diffraction and solid-state NMR studies have shown that EAA and AM form crystalline 6_1 helical *V*-type inclusion complexes. Similar complexes prepared from EAA and AP had smaller amounts of poorly organized *V* structure. These results are consistent with chiroptical studies of solution mixtures from which the complexes were prepared. The EAA/AM complexes were resistant to amylase digestion, whereas the EAA/AP complexes were not. This implies that the ordered, crystalline state of the EAA/AM complex was necessary for resistance to enzyme attack. FTIR studies of complexes of EAA with both AM and AP suggest that the carboxyl groups of EAA are weakly hydrogen bonded to the polysaccharide hydroxyls rather than them forming more stable hydrogen-bonded carboxyl dimers. The former hydrogen bonds are not crucial for the formation of *V*-type inclusion complexes, however, since complexes of AM and AP with the sodium carboxylate form of EAA are quite stable (see chiroptical paper). Hydrogen bonding between EAA carboxyl and polysaccharide hydroxyl groups may increase the stability of the complexes but hydrophobic interactions are probably more important.[34] The methods described here should be applicable to the structural characterization of plastic films currently being made from starch, EAA, and polyethylene.

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