Complexes of Starch Polysaccharides and Poly(Ethylene *co*-Acrylic Acid): Structure and Stability in Solution

R. L. SHOGREN,* R. V. GREENE, and Y. V. WU

U.S. Department of Agriculture, Agricultural Research Service, Northern Regional Research Center, 1815 N. University St., Peoria, Illinois 61604

SYNOPSIS

Chiroptical methods have been used to study the conformation and interactions of amylose and amylopectin with poly(ethylene *co*-acrylic acid) (EAA) in aqueous solution. These studies, along with X-ray diffraction and solid-state NMR data, show that amylose and EAA, as well as amylopectin and EAA, form helical *V*-type inclusion complexes when mixed in aqueous suspension. This structure apparently accounts for the partial compatibility observed in films containing starch and EAA. About $\frac{2}{3}$ by weight of EAA does not interact with amylose and probably represents the ethylene-rich central core of the EAA micelle. EAA/amylose complexes in 10 m*M* NaOH were stable to temperatures > 90°C, whereas EAA/amylopectin complexes in the same solvent were largely disrupted at this temperature. Urea, at a concentration of 8 *M*, further destabilized both EAA/amylopectin and EAA/amylose complexes. Solutions with an alkaline pH (> 9.5) dispersed EAA optimally and allowed maximum complexing with amylose. At pH values > 13, the EAA/amylose complexes were weaker, most likely due to electrostatic repulsion between ionized hydroxyl groups of amylose and carboxyl groups of EAA.

INTRODUCTION

Starch is predominantly composed of amylose, a linear polymer of α-1,4-linked glucose units, and amylopectin, a highly branched polymer of short α-1,4-linked chains connected by α-1,6 linkages.[1] The use of starch in plastics would be advantageous because (1) starch from corn is an abundant, inexpensive, renewable resource produced in the United States and (2) starch is biodegradable and, hence, may be useful in applications where recovery or recycling would be difficult.[2,3] The main difficulties of incorporating starch into plastics are its hydrophilicity and the brittle nature of solid starch, due to its tendency to form crystals with extensive hydrogen bonding.[1] One way to improve the mechanical properties of starch is by blending with a flexible vinyl polymer. Otey et al.[2,3] developed formulations containing starch and poly(ethylene *co*-acrylic acid) (EAA) for potential use as biodegradable agricultural mulch films. In their experiments, blends of starch and EAA containing alkali and 5%–10% water were successfully blown into films at 120–140°C.[2] These films have tensile strengths and elongations at breaks intermediate between those of the pure polymeric components,[2-4] suggesting at least partial compatibility. Fanta et al.[5] found that extraction of these films with solvents in which starch and EAA are readily soluble leaves a small residue that contains both components.[5] These authors suggested that this residue may be the *V*-type starch inclusion complex in which the EAA occupies the center of the starch 6_1 helix.

This type of crystalline structure has been identified by X-ray diffraction in complexes of amylose with carboxylic acids and other organic molecules.[6,7] An increase in entropy,[8] due to stripping of ordered water molecules from the hydrophobic guest molecule on complexing, as well as dispersive forces between hydrophobic guest molecules and the rather hydrophobic helix interior[1] probably contribute

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* To whom correspondence should be addressed.

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much of the stability of the complex. In addition, intramolecular hydrogen bonds and intermolecular dispersive interactions within crystalline domains further stabilize the helix.[9]

Chiroptical techniques such as optical rotation and circular dichroism have long been used to study secondary and tertiary structure in proteins, nucleic acids, and polysaccharides in solution. The application of these methods toward characterizing polysaccharides was reviewed by Rees et al.[10] Bulpin et al.[11] applied optical rotation to study the formation and stability of the *V*-type structure in amylose/fatty acid complexes in solution. To test the hypothesis that starch and EAA indeed form the *V*-type structure and to assess the stability of the complex to temperature, urea and alkali concentration, we performed chiroptical studies of solution mixtures of EAA with amylose or amylopectin. Cross polarization/magic angle sample spinning (CP/MAS) C-13 NMR and X-ray diffraction studies of precipitates from these solutions are presented in the accompanying manuscript.[12]

EXPERIMENTAL

Materials

Amylose (AM) and amylopectin (AP) from potato were purchased from Sigma Chemical Co. AM was further purified by a procedure similar to that described by Karkalas and Raphaelides.[13] AM, 3 g, was dissolved in 100 mL of dimethyl sulfoxide by stirring overnight. The solution was centrifuged at 12,000*g* for 30 min to remove insoluble particulate matter. Two volumes of 1-butanol were added to the supernatant to precipitate the amylose. The AM was finally washed extensively with methanol and dried in a vacuum oven at 50°C for 1 day. AP was further purified by first dissolving 5 g in 100 mL of boiling water. Insoluble debris was removed by centrifugation at 6000*g* for 20 min. Three volumes of cold methanol were added to the supernatant, and precipitated amylopectin was allowed to settle out overnight. The precipitate was finally washed with cold methanol and dried in a vacuum oven at 50°C for 1 day. Intrinsic viscosities of solutions of AM and AP in 20 m*M* NaCl, pH 7, were 110 and 45 mL/g, respectively. These values correspond to molecular weights of approximately 9×10^5 for AM as calculated from the empirical equation of Banks and Greenwood (equation 4.80, Ref. 14) and $> 10^7$ for AP (table 2.15, Ref. 14).

Poly(ethylene *co*-acrylic acid) (EAA) was Dow Primacor 5981. It has a reported composition of 20 wt. % acrylic acid and weight average and number average molecular weights of 18,000 and 7000, respectively.[15] Palmitic acid (PA) and behenic acid (BA) were obtained from Sigma Chemical Co.

Solution Preparation

Solutions of 5% amylose in 0.5 *M* NaOH were prepared by slowly adding 0.5 g AM to 10 mL of 0.5 *M* NaOH with rapid magnetic stirring. Stirring was continued for 1–2 h. This solution was then diluted with distilled water to give 2 mg/mL AM in 20 m*M* NaOH. The solution was centrifuged at 30,000*g* for 1 h or 15,000*g* for 2 h to remove undissolved gels. The amylose concentration in the supernatant was 1.6–1.8 mg/mL as determined by optical rotation (see below) or by phenol-sulfuric acid assay[16] with the 2 mg/mL solution as a standard. Most of the amylose gels were removed by centrifugation because further clarification by filtration through a 0.22 μ Millipore filter did not reduce the amylose concentration measurably. Solutions of amylose in 1 m*M* NaOH, 1 *M* NH_4OH, or water were prepared by dialysis of the above solution against the appropriate solvent in 12–14 $\times 10^3$ molecular weight cutoff tubing. Solutions of AP were prepared identically.

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; they remained stable for months. Solvent conditions were changed by dialysis against the appropriate solvent in 12–14 $\times 10^3$ molecular weight cutoff tubing. No EAA passed through the dialysis bag as judged by measurement of carboxylate absorbance at 210 nm and dialysate volume before and after dialysis. Solutions of palmitic acid (1 mg/mL) or behenic acid (0.5 mg/mL) in 10 m*M* NaOH were prepared in a similar manner and kept hot to avoid precipitation upon cooling.

For optical measurements, amylose or amylopectin solutions were diluted with the required solvent to 0.5–1.5 mg/mL and enough 4 mg/mL EAA solution was added to achieve the desired ratio of EAA/AM (w/w). To prepare solutions containing 8 *M* urea, solid urea was added to amylose solutions in 20 m*M* NaOH followed by addition of EAA in 10 m*M* NaOH. When varying the EAA/AM ratio, the concentration of amylose was kept constant. Gen-

erally, measurements were carried out 1 day after mixing except when time-dependence studies were performed.

Analytical Methods

Optical rotations were measured at 589, 578, 546, 436, and 365 nm with a Perkin-Elmer 241 polarimeter in a 10 cm-path-length jacketed cell and a Haake circulating water bath. Measurements were made at room temperature unless otherwise noted. Temperature dependencies of optical rotation were measured by increasing the temperature in 5 or 10 degree increments and then waiting 5–10 min until the rotation stabilized. The specific rotation was calculated[17] from $[\alpha] = 100\alpha/lc$ and the molecular rotation from $[\phi] = M[\alpha]/100$, where α is rotation in degrees, l is pathlength in dm, c is concentration in g/dL, and M is the molecular weight of a glucose residue. The transition wavelength, L_k, and the area of the ellipticity band, A, were estimated with the Drude equation: $[\phi] = AL_k/(L^2 - L_k^2)$, assuming a Gaussian band shape for wavelengths of $L \gg L_k$. This equation is derived from eq. 10 of Ref. 17 where $A = \pi[\theta_k]\Delta_k$ and $[\theta_k]$ and Δ_k are the height and half-width of the ellipticity band. Circular dichroism spectra were obtained in a Jasco J-600 spectropolarimeter and a 1 cm cell. Ellipticities were calculated from $[\theta] = \theta M/cl$, where θ is ellipticity in degrees and c, l, and M are as defined above.[18] Turbidity measurements were performed with a Bausch & Lomb Spectronic 2000 spectrophotometer and a 1 cm cell.

RESULTS AND DISCUSSION

To interpret the chiroptical data below, some background information regarding the solution and optical behavior of EAA and amylose is needed. EAA forms micellar particles containing many EAA molecules when dispersed in hot aqueous alkali.[15] The structure of these particles and the relationship between the particle structure and primary structure of EAA or other ionomers, however, is poorly understood.[19] Ethylenic portions of many EAA molecules probably segregate in the dense interior of the micelle, whereas most acrylate groups likely occupy the hydrated exterior in the form of chain ends or loops. This type of "hairy micelle" structure is known to occur with block copolymers dispersed in a good solvent for one block and a nonsolvent for the other.[20] Salts of fatty acids also form spherical or rodlike micelles in which the hydrocarbon chains make up the interior of the micelle and the carboxylic acids face the aqueous exterior.[21] Solutions of EAA and fatty acids salts are optically inactive since the carboxylic acid groups are located in symmetric environments.

Vacuum CD studies of amylose have shown that the major optically active transition occurs at < 160 nm (Ref. 22) and is due to transitions of oxygen nonbonding electrons.[23] Interactions between transition dipoles of different oxygen atoms perturb their circular dichroism, and, hence, changes in dipole orientation and separation due to conformational changes will result in altered circular dichroism and optical rotation.[22,24] Rees[25] derived the semiempirical correlation, $[\phi]_{589} = -120(\sin\phi + \sin\psi) + 200$, where ϕ and ψ are the dihedral angles about the C(1)—O and O—C(4) glycosidic bonds of amylose. The Rees relation predicts $[\alpha]_{589} = [\theta]_{589}/1.62 = 152$, which corresponds to $[\alpha]_{365} = 435$ for amylose in the hydrated left-handed V-helical conformation (V_L, $\phi = -14, \psi = -8$).[26] This agrees well with values of $[\alpha]$ measured for amylose in aqueous butanol or DMSO,[27] compounds that are known to form V-type inclusion complexes with amylose.[1] Optical activities can also be influenced by intermolecular dipolar interactions between amylose molecules as well as by differential scattering of left- vs. right-handed circularly polarized light.[28]

Figure 1 presents optical rotation data ($[\alpha]_{365}$) as a function of the composition of EAA/AM mixtures in 10 mM NaOH, as well as similar data for solution mixtures of PA and BA with AM. $[\alpha]_{365}$ declined upon addition of EAA, reaching an asymptotic value of 240 at EAA/AM ratios > 0.5. Aging solutions for 1 day after mixing caused a further decrease in $[\alpha]_{365}$ for small values of EAA/AM, but no change in the asymptotic value. Little change in $[\alpha]_{365}$ was observed after 1 day of aging. Since the turbidity of these solutions increased with time (data not shown), it is likely that large EAA/AM aggregates require time to diffuse together for complete reaction. With an excess of small EAA micelles, however, the amylose can react quickly. $[\alpha]_{365}$ also declined upon addition of PA and BA, which are known to form V-type inclusion complexes with amylose,[6] reaching asymptotic values of 380 and 350, respectively, at fatty acid/AM ratios of 0.1. This ratio is very close to the value predicted (.095) if PA molecules in the *all-trans* conformation completely filled the interior of an amylose 6_1 helix.[13] Since a larger weight of EAA relative to PA or BA was required for complete complexation, it seems

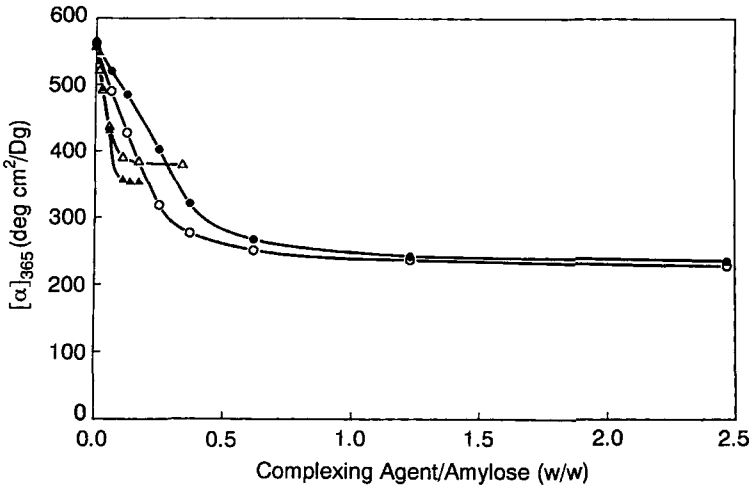

Figure 1 Specific rotation of solution mixtures of amylose in 10 m*M* NaOH with palmitic acid (△), behenic acid (▲), EAA [(●) 2 min after mixing], and EAA [(○) 1 day after mixing] as a function of weight complexing agent/weight amylose.

reasonable to assume that only the surface of the EAA micelle is accessible for reaction with amylose. Presumably, a dangling EAA chain end or loop on the micelle surface would be incorporated into the AM helix, forming a structure very similar to the FA/AM complex. Size-exclusion chromatography data for EAA (see below) showed no evidence for individual EAA molecules, so that the latter were probably not involved in complexes with AM. It should be noted, however, that the data do not exclude the possibility that the micellar structure of EAA is disrupted or altered upon complexing with AM.

Asymptotic values of $[\alpha]_{365}$ for EAA and PA/AM mixtures were much smaller than those predicted for the isolated *V*-type helix. Scattering does not appear to be the source of the discrepancy since Drude plots of optical rotation data for all solutions were linear and gave identical transition wavelengths, $L_k = 155 \pm 8$ nm. Intermolecular interactions between chromophores due to formation of small *V*-type amylose crystallites is a more probable cause. This might be expected to occur to a greater extent with hydrocarbon guest molecules that contain longer and less hydrated hydrocarbon chains. In particular, the surface of the EAA micelle would have hydrocarbon chains packed rather closely together, thus promoting local amylose helix crystallization. Similarly, Stipanovic and Stevens[23] found that vacuum CD bands for amylose films were less intense than those for amylose solutions, likely due to the hypochromic intermolecular dipolar interactions.

Although the EAA carboxylic acid group has no intrinsic optical activity, it may, upon complexation, show induced activity due to the asymmetric electronic environment of the interior of the amylose helix. By measuring the circular dichroism spectrum of the EAA/AM complex, the contribution of the EAA carboxyl group to the total optical activity can be assessed. Figure 2 shows ultraviolet circular dichroism (CD) spectra for solutions of EAA, EAA/AM (0.5/1), and AM in 3 m*M* sodium phosphate buffer, pH 7. The CD spectrum of AM is very similar to that reported by Lewis and Johnson[22] and began to show a negative band only below 200 nm. In the region of carboxylate pi–pi* transition (205–210 nm), EAA alone has zero ellipticity, whereas EAA complexed with amylose has $[\phi] = -110$ deg cm^2/dmol. The overall contribution of this transition to $[\alpha]_{365}$, calculated via the Drude equation (see Experimental), is less than 1 deg cm^2/Dg and, hence, is much smaller than the amylose contribution. The observation that EAA shows induced optical activity when complexed with AM implies that the acrylic acid moiety is restricted to a single or small number of environments rather than being in unrestrained motion.

Figure 3 shows the effect of pH on $[\alpha]_{365}$ for mixtures of EAA and amylose. Values of $[\alpha]_{365}$ are similar for 1–100 m*M* NaOH, pH 10–12.6, but were larger for water or 10 m*M* NaCl, pH 7. When solutions were mixed at pH 10, then adjusted to pH 7 with sodium phosphate buffer, values of $[\alpha]_{365}$ were identical to those obtained at pH 10. Therefore, it would appear that a portion of the EAA micelle is

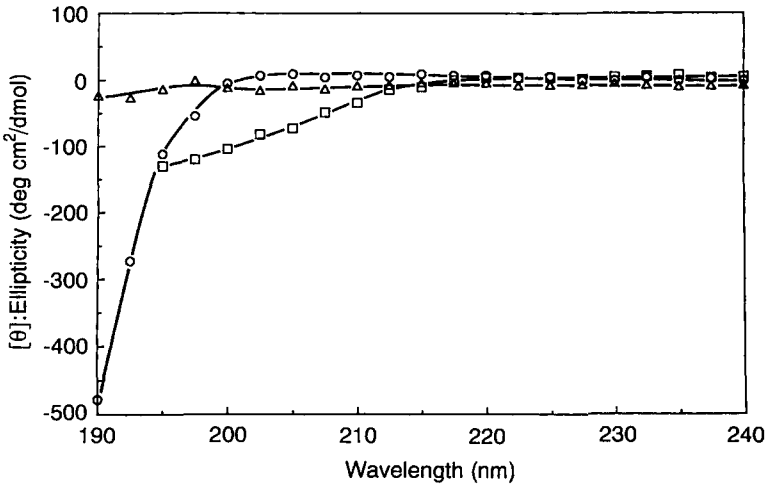

Figure 2 Circular dichroism spectra of amylose (○), EAA (△), and EAA/AM complex (□) in 3 m*M* sodium phosphate buffer, pH 7.

not available for reaction with amylose at pH 7. This may involve a phase change since solutions of carboxylic acids with long hydrocarbon chains such as PA became turbid below pH 9.

A similar aggregation was observed for EAA suspensions at pH 7, as shown by the Sepharose CL-2B size-exclusion chromatographs in Figure 4. EAA in 1 m*M* NaOH apparently forms polymeric micelles having a hydrodynamic diameter of 260 ± 40 Å as determined from polyacrylamide standards (Polysciences) of known hydrodynamic radii[29] and Porath's relationship between the partition coefficient and hydrodynamic radius.[30] After dialysis against water to pH 7, EAA eluted in the void volume, indicating that these micelles aggregated to form very large (> 1000 Å) particles.

Turbidities of the EAA/AM solution mixtures (Fig. 5) generally seemed to increase with increasing ionic strength of the solution. This is probably due to the screening out of electrostatic repulsion between the carboxylate anions of the EAA/AM complexes, thereby leading to destabilization. Turbidities reach a maximum at EAA/AM ratios of about 0.3–0.4, suggesting that the complexation is com-

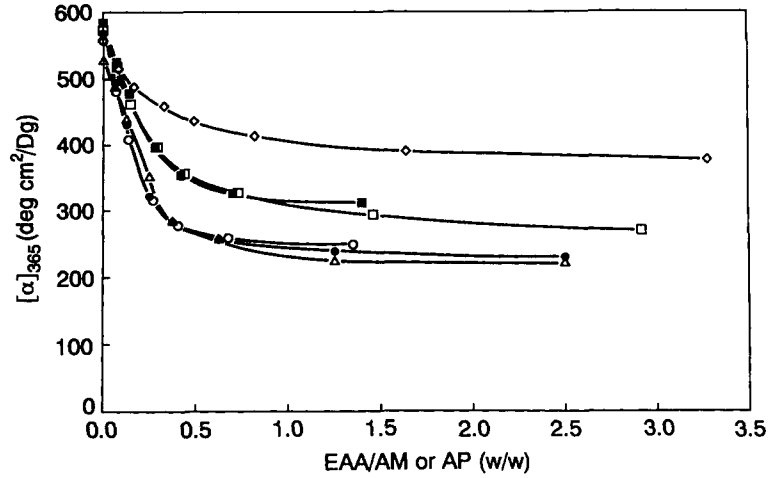

Figure 3 Composition dependence of specific rotation for EAA/AM complexes in 1 m*M* NaOH (○), 10 m*M* NaOH (●), 100 m*M* NaOH (△), water, pH 7 (□), 10 m*M* NaCl, pH 7 (■), and for EAA/AP complex in 10 m*M* NaOH (◇).

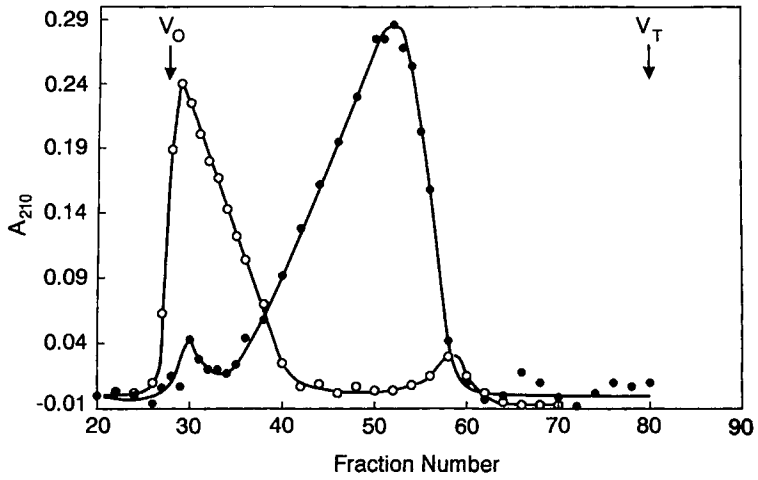

Figure 4 Sepharose CL-2B chromatograms of EAA in 1 m*M* NaOH, pH 10 (●), and EAA in water, pH 7 (○); 0.5–1.0 mL of 1% EAA solution was loaded onto a 50 × 1.5 cm column containing CL-2B. Fractions (1 mL) were collected at a rate of 10 mL/h, and EAA was monitored by the UV absorbance of the carboxyl group at 210 nm.

plete at this stoichiometry. For EAA/AM in 0.1 *M* NaOH, however, the turbidity reaches a maximum at an EAA/AM ratio of about 1.3, suggesting that the EAA/AM interaction may be weakened by pH values above 13. A value of 12.6 has been reported[31] for the pK_a of amylose, and, thus, the weaker interaction may be due to electrostatic repulsive forces between the glucose residues of amylose.

Optical rotation and turbidity data for solution mixtures of EAA with amylopectin in 10 m*M* NaOH are also shown in Figures 3 and 5, respectively. Much smaller changes in $[\alpha]_{365}$ and turbidity were seen for EAA/AP than for EAA/AM. This probably reflects a weaker interaction between AP and EAA, likely due to the shorter length of α-1,4-linked regions in AP and the α-1,6 branch points that may inhibit crystallization.

The effect of urea on the optical rotation of EAA/AM and EAA/AP was investigated because starch/EAA plastics that contained added urea had larger tensile elongations than did plastics without urea.[3] Plots of $[\alpha]_{365}$ vs. composition for solution mixtures

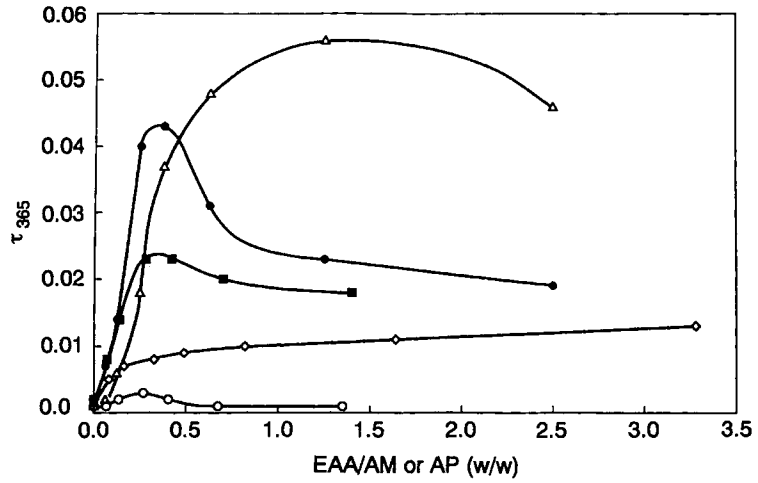

Figure 5 Composition dependence of turbidity of EAA/AM complexes in 1 m*M* NaOH (○), 10 m*M* NaOH (●), 100 m*M* NaOH (△), 10 m*M* NaCl, pH 7 (■), and for EAA/AP complex in 10 m*M* NaOH (◇).

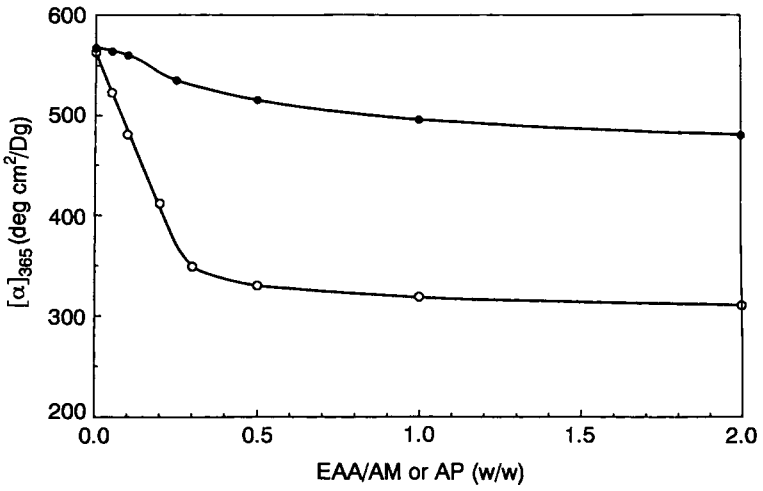

Figure 6 Composition dependence of specific rotation of EAA/AM (○) and EAA/AP (●) complexes in 8 *M* urea, 10 m*M* NaOH.

of EAA with AM and AP in 8 *M* urea, 10 m*M* NaOH are shown in Figure 6. $[\alpha]_{365}$ for AP declined from 565 deg cm^2/Dg when no EAA was present to 520 deg cm^2/Dg at EAA/AP = 0.5. This is much smaller than the decrease of about 125 deg cm^2/Dg observed for EAA/AP in 10 m*M* NaOH (see Fig. 3) and suggests that urea disrupts the EAA/AP complex to a large extent. $[\alpha]_{365}$ for EAA/AM complexes also decreased to a smaller extent when urea was present.

The temperature dependence of $[\alpha]_{365}$ for solution mixtures of EAA/AM 0.39/1 and EAA/AP 0.50/1 in 10 m*M* NaOH are shown in Figure 7. Little change in $[\alpha]_{365}$ for EAA/AM occurs, indicating that the EAA/AM complex is largely stable to temperatures greater than 90 degrees. This contrasts with the results of Bulpin et al.,[11] which demonstrate a melting point of 80 degrees for PA/AM complexes. Since the EAA micelles are much larger than PA, the entropy change upon complex disruption will be smaller for EAA/AM, and, consequently, higher temperatures will be required for melting. This argument requires a similar enthalpy change upon dissociation of both complexes. In support, Raphaelides and Karkalas[32] find little change in the enthalpy of dissociation of FA/AM complexes with changes in fatty acid chain length. For EAA/AP

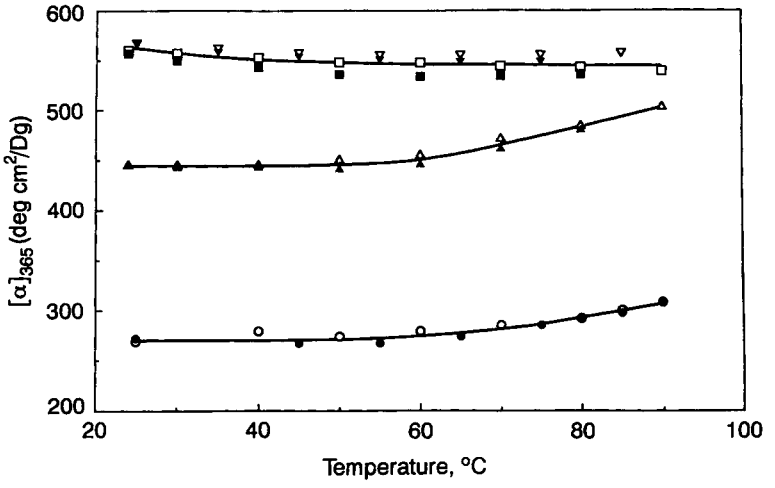

Figure 7 Temperature dependence of specific rotation for AP (□, ■), AM (▽, ▼), EAA/AM 0.39/1 (○, ●), and EAA/AP 0.5/1 (△, ▲) in 10 m*M* NaOH. Open symbols denote heating, whereas closed symbols denote cooling.

mixtures, the difference between values of $[\alpha]_{365}$ obtained for EAA/AP and AP at 90°C was approximately 30% of the difference at 25°C. Complexes of EAA/AP are therefore largely disrupted by temperatures above 90°C. This confirms our conclusions regarding the instability of EAA/AP relative to EAA/AM.

The temperature dependence of $[\alpha]_{365}$ for solution mixtures of EAA/AM 0.5/1 and EAA/AP 0.5/1 in 8 *M* urea, 10 m*M* NaOH are shown in Figure 8. On heating, $[\alpha]_{365}$ for AM increased abruptly at 70°C and continued to rise at 90°C. For AP, $[\alpha]_{365}$ also increased abruptly at 70°C, but reached a steady-state value of 570 at 80°C, corresponding to the specific rotation of free AP in solution. Comparison of these data with Figure 7 indicates that 8 *M* urea greatly lowers the dissociation temperature of the EAA/AM complex. Urea also caused a similar destabilization of EAA/AP complexes. Urea probably stabilizes the random coil form of AM and AP by disrupting intramolecular hydrogen bonding and by disrupting the ordered water structure that forms on exposure of the hydrophobic AM interior to bulk water. This latter mechanism probably also contributes to lowering the free energy of EAA in the unbound state in the presence of urea. It is interesting to note that dissociation of EAA/AP complexes in 10 m*M* NaOH began at 60°C, a lower initial temperature than the 70°C found for EAA/AP in 8 *M* urea. This may reflect the presence of a species of labile complex in 10 m*M* NaOH that is disrupted at room temperature in the presence of 8 *M* urea. Considerable hysteresis was exhibited on cooling of AM and AP solutions in 8 *M* urea, whereas none was observed when urea was absent. The hysteresis is likely due to supercooling that is necessary for nucleation of helical segments. The absence of hysteresis may reflect a greater resistance to disruption of certain helical segments when urea is absent. The reason why $[\alpha]_{365}$ did not return to the same values when solution mixtures of AM and AP with EAA were cooled to 30°C is presently unknown, but could involve an irreversible change in EAA micelle structure on heating in 8 *M* urea.

CONCLUSIONS

Optical rotation results show that a major conformational change occurs when amylose and amylopectin are mixed with EAA in aqueous suspension. This conformational change most likely results from the transition from a randomly coiled to the *V*-type helical conformation, with EAA occupying the interior of the polysaccharide helix. Confirming X-ray diffraction and solid-state NMR evidence are presented in the accompanying manuscript.[12] Only about $\frac{1}{3}$ by weight of EAA interacts with amylose and probably represents the hydrated exterior of the EAA micelle. Solutions at pH values > 10 are necessary to disperse aqueous EAA maximally and to allow optimal complexing with amylose. High concentrations of NaOH (> 0.1 *M*) weaken EAA/AM complexes, likely due to electrostatic repulsion be-

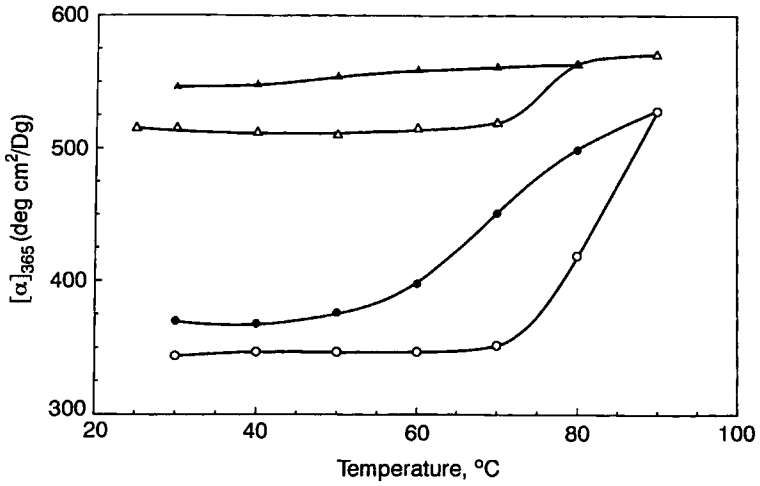

Figure 8 Temperature dependence of specific rotation for EAA/AP 0.5/1 (△, ▲) and EAA/AM 0.5/1 (○, ●) in 8 *M* urea, 10 m*M* NaOH. Open symbols denote heating, whereas closed symbols denote cooling.

tween ionized amylose hydroxyl and EAA carboxylate groups. Urea also weakens both EAA/AM and EAA/AP complexes, probably due to disruption of intramolecular hydrogen bonding and hydrophobic interactions with water. EAA/AP complexes are much less stable than are EAA/AM complexes, probably due to disruption of *V*-type crystallites by α-1,6 branches in AP.

These findings should help in understanding some of the physical and biodegradation properties that have been observed for starch/EAA films. For example, large increases in viscosity were found for starch pastes after addition of EAA.[5] AM and AP molecules are probably crosslinked by EAA micelles via *V*-type complex formation, leading to the viscosity increase. By varying the amount of complex formation through changes in temperature, AM/AP ratio, alkali concentration, or urea concentration, it should be possible to achieve the desired rheological properties for processing. Since the *V*-type complex readily crystallizes, increasing the amount of complex will likely give a stronger, more brittle film. The crystalline EAA/AM complex will also likely be resistant to amylase attack, as has been found for complexes of amylose with butanol[33] and fatty acid monoglycerides.[34]

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