Deciphering Structures of Inclusion Complexes of Amylose with Natural Phenolic Amphiphiles

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ABSTRACT: Amylose inclusion complexes were prepared in aqueous solution with the amphiphilic moiety 3-pentadecylphenol via a direct mixing method. Attenuated total reflection Fourier transform infrared spectroscopy as well as differential scanning calorimetry confirmed the formation of amylose inclusion complexes. The morphology of the synthesized complexes is sensitive to temperature, and X-ray data revealed that the inclusion complexes exhibited distinct structures at different temperatures. Small-angle X-ray scattering data indicated ordered lamellar structures of the synthesized complexes at room temperature, and wide-angle X-ray scattering profiles showed the transformation of the crystalline structure as a function of the temperature. The results of this research will help to understand the relationship between the inclusion complex structures with temperature.

1. INTRODUCTION

Starch is composed of two distinct components: essentially linear or slightly branched amylose consisting of α-1,4-glycosidic bonds and highly branched amylopectin having α-1,4-glycosidic and α-1,6-glycosidic bonds. The amylose chains have a helical hydrophobic interior cavity, and it is known to form inclusion complexes via hydrophobic–hydrophobic interactions with hydrophobic guest moieties such as dyes, flavors, lactones, polymers, and lipids. The formation of the complex increases the physical and chemical stability of lipophilic guest molecules by shielding them against oxidation, evaporation, and decomposition. These kinds of inclusion complexes show their potential applications in food and pharmaceutical applications for nanoencapsulation, control release of the drugs, and flavor encapsulations.

Different ligands display a distinct interaction with amylose, and their interactions can significantly influence the structural properties of amylose inclusion complexes. The morphologies of the amylose inclusion complexes are known to be greatly affected by experimental conditions such as concentration of amylose and guest molecules, temperature, and pressure. Depending on the guest molecules, amylose inclusion complexes of 6, 7, or 8 glucosyl residues per helical turn are obtained for alcohols, acetone, lipids, and naphthol. Therefore, it is always interesting to explore distinct crystal structures of the resulting complexes of amylose with different complexing agents.

The impact of guest molecules and other experimental parameters on the morphological properties of synthesized inclusion complexes are most often explored by X-ray scattering techniques, and we have recently explored the structural characterization of amylose-polymer inclusion complexes by these techniques. In the present study, different concentrations of the pentadecylphenol (PDP) complexing agent is used to form amylose-PDP inclusion complexes. PDP is a naturally occurring amphiphilic phenolic surfactant obtained from cashew nut oils and has potential applications in medicine, resin additives, and fuel additives. Facilitating the formation of inclusion complexes with phenolic surfactants can extend the shelf life of food products via reducing enzymatic browning.

The objective of this work was to synthesize inclusion complexes of amylose with PDP and to further systematically elucidate the morphological characteristics via attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), wide-angle X-ray scattering (WAXS), and small-angle X-ray scattering (SAXS) characterization techniques. The effects of temperature and guest molecule concentration on the morphology of amylose-PDP inclusion complex were also investigated.

2. EXPERIMENTAL SECTION

2.1. Materials. Amylose with a molecular weight of ~180 kg/mol was received from Avebe. PDP was purchased from Sigma-Aldrich. PDP was recrystallized from petroleum ether and dried under vacuum at room temperature for 24 h.

2.2. Synthesis of Amylose-PDP Inclusion Complexes. Amylose-PDP complexes were prepared by suspending 500 mg of amylose in 15 mL of deionized water followed by mixing 5, 10, and 20% (w/w) PDP. The solution was rotated at room temperature for 2 h and transferred to a pressure vessel. The pressure vessel was heated to 160 °C for 1 h followed by cooling down to 80 °C. The solution was continuously rotated at 80 °C overnight. Thereafter, the solutions were cooled down to room temperature for 24 h. This process was repeated until the formation of inclusion complexes was confirmed by Fourier transform infrared spectroscopy and differential scanning calorimetry.

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The characteristic peaks of the substituted aromatic ring were observed in the FTIR spectra of PDP (a), amylose (b), and the inclusion complexes (c), (d), and (e) presented in Figure 1. The chemical compositions (20:1, 10:1, and 5:1 mass ratios) of amylose and the PDP guest molecule were used to form the amylose-PDP inclusion complex.31 The thermal properties of the amylose and inclusion complexes were characterized by differential scanning calorimetry (DSC) (Figure 2). The thermogram of amylose-PDP inclusion complexes showed an endotherm as compared to pristine amylose that arises due to dissociation of the inclusion complexes. A more detailed description of this behavior can be found elsewhere.32

Typically, the FTIR spectra of amylose-PDP inclusion complexes (Figure 1c,d) exhibited the vibrational peaks of amylose and PDP. However, due to the insertion of PDP into the amylose cavity, several characteristic absorption peaks of PDP between 1600 and 1000 almost vanished in the inclusion complex spectra. Moreover, the inclusion of the guest molecule splits the band of 990 cm$^{-1}$ into two separate peaks, and these peaks appeared at 993 and 1014 cm$^{-1}$ in the inclusion complex spectra.7 The intensities of these peaks decreased with increasing concentration of the PDP guest molecules.

The amylose characteristic peaks at 1640, 1080, and 855 cm$^{-1}$ were found to shift at 1645, 1076, and 860 cm$^{-1}$, respectively. The FTIR spectra of amylose-PDP inclusion complexes were characterized by differential scanning calorimetry (DSC) (Figure 2). The thermogram of amylose-PDP inclusion complexes showed an endotherm as compared to pristine amylose that arises due to dissociation of the inclusion complexes. A more detailed description of this behavior can be found elsewhere.7,11,32 By increasing the concentration of PDP, the endothermic enthalpy peak of the inclusion complexes sharpened due to the higher crystalline nature of PDP. Furthermore, the endothermic enthalpy increased with the concentration of PDP molecules, which proved to be better complexation between amylose and PDP at higher concentrations.

The effects of thermal treatment and concentration of PDP on inclusion complex structures were analyzed using synchrotron X-ray measurement. The SAXS patterns of the APD-5 and APD-20 inclusion complexes were recorded as a function of temperature. The SAXS patterns of the APD-5 and APD-20 inclusion complexes were recorded as a function of temperature. The SAXS patterns of the APD-5 and APD-20 inclusion complexes were recorded as a function of temperature. The SAXS patterns of the APD-5 and APD-20 inclusion complexes were recorded as a function of temperature.

Figure 1. ATR-FTIR spectra of (a) PDP, (b) amylose, (c) APD-5, (d) APD-10, and (e) APD-20. (i) Wavenumber range 750 to 3800 cm$^{-1}$. (ii) Magnified range of panel (i) between 750 and 1750 cm$^{-1}$.
temperature (25 to 160 °C and vice versa) as shown in Figures 3 and 4, respectively.

The scattering peaks at q of 0.7, 1.4, and 2.1 nm\(^{-1}\) suggested a lamellar structure of the amylose-PDP inclusion complexes at room temperature.\(^{33}\) Biais et al. reported that the lamellar structural organization of the amylose complex constituted by alternating crystalline and amorphous layers.\(^{34}\) The starting peak at 0.7 nm\(^{-1}\) is associated with the thickness of this lamellar morphology.\(^{35}\)

The van der Waals forces, mainly hydrophobic–hydrophobic interactions and H-bonds, play an important role in the inclusion complex formation. The strength of Van der Waals forces decreased with increasing temperature, and it had a significant influence on the inclusion complex morphology. It was observed that the inclusion complexes started to dissociate at 70 °C that is above the melting temperature of PDP (54 °C), and this is indicated by a reduction in the ordering of the lamellar structure. Furthermore, the SAXS patterns showed that the intensity of peaks decreased with increasing temperature and less well-defined peaks were obtained at the higher temperature. The cooling X-ray profile showed the reordering of the lamellar structure of the inclusion complexes. SAXS revealed the lamellar morphology and disruption of the ordered lamellar morphology of the amylose-PDP complexes with the temperature.

The concentrations of amylose and complexing agent have a significant impact on the morphology of the complexes. It was reported that the concentration of the complexing agents can induce more than one type of crystalline structure.\(^{36,37}\)

In the present study, the influence of the complexing agent concentration on the inclusion complexes was investigated, and SAXS diagrams of a higher amount of PDP (20 wt %) are recorded as a function of temperature. The heating and cooling cycles of the inclusion complex are presented in Figure 4a,b, respectively. As shown in Figure 4a, APD-20 showed two characteristic peaks at about 0.7 and 1.4 nm\(^{-1}\), which confirms a

![Figure 2](image_url)

**Figure 2.** Thermograms (heating scan) of amylose and inclusion complexes between amylose and three different concentrations of PDP (ADP-5, APD-10, and APD-20).

![Figure 3](image_url)

**Figure 3.** SAXS intensity profiles from APD-5 inclusion complex during (a) heating cycle (b) cooling cycle.
lamellar structure of the inclusion complex. However, higher PDP concentration reduced the intensity of the higher order peaks. These results indicated that increasing the amount of complexing agent might induce the random arrangements of well-defined domains of alternating layers of crystalline and amorphous regions.

The inclusion complexes were heated up to about 160 °C to redissolve the complexes followed by cooling back to room temperature to allow recrystallization of the complexes. The crystalline structure of the inclusion complexes during the heat treatment was investigated by WAXS. The representative WAXS patterns of the APD-5 and APD-20 complexes as a function of temperature are shown in Figure 5. As shown in Figure 5a, the diffraction patterns exhibit a typical crystalline structure displayed by different peaks at 10.7, 12.5, 12.8, 13.5, 14.0, 15.8, and 17.1 nm⁻¹ at 25 °C. After heat treatment, the intensities of the peaks changed and shifted to new positions at 11.5, 12.8, 14.5, and 17.4 nm⁻¹.

The transition to a different crystalline structure with temperature can be explained by temperature of crystallization and stabilizing the guest molecule in the hydrophobic cavity of the amylose molecules. The WAXS data demonstrated that the inclusion complex crystallizes into two polymorphs with the temperature. Similar transformations of the crystalline structure of amylose inclusion complexes during heat treatment have also been reported elsewhere. The complexes showed a stable crystal structures after the heat treatment, and it remains intact during cooling as well. Furthermore, a dominated sharp peak at 12.8 nm⁻¹ of the heat-treated sample suggested that the higher temperature induced the crystalline size of the complex.

The WAXS profile of APD-20 at room temperature (Figure 5c,d) displays more vivid peaks as compared to APD-5, and these peaks are located at 8.7, 10.7, 12.4, 12.8, 13.5, 14.0, 17.0 nm⁻¹. It is observed that the reorganization of less perfect crystalline structures occurred with the temperature via restructuring of amorphous and crystalline lamellae in order to provide more impeccable crystalline structures. The increasing number of peaks indicates that regularity of the inclusion complex increased with the amount of crystalline host molecules. Similar to APD-5, the peaks moved to new positions at 8.5, 9.2, 11.6, 12.9, and 17.5 nm⁻¹ after the heat treatment, which confirmed the transformations of the crystalline structure of the complex due to the heat treatment. Some variations in the diffracted intensity of the peak at 12.9 cm⁻¹ was observed with different concentrations of complexing agents due to different crystal sizes of the complex. Figure 6 displays SAXS and WAXS patterns at room temperature of APD-5, APD-10, and APD-20. The SAXS profile clearly shows the presence of lamellar patterns of the peaks.

4. CONCLUSIONS

The results of this study prove that PDP can be effectively inserted into the hydrophobic cavity of amylose in order to form amylose-PDP inclusion complexes. The formation of the inclusion complexes was confirmed by FTIR and DSC measurements. The DSC thermograms revealed an increased...
stability of the complex at higher concentrations of PDP. The crystalline structure and periodic organization of the complexes as a function of temperature were analyzed by SAXS and WAXS. Two different crystal structures of inclusion complexes were revealed by WAXS.

The complexation of PDP with amylose is a promising approach to improve the utilization of these kinds of complexes in food and drug industries, for instance, nanoencapsulation, controlled release of the drugs, and flavor encapsulations. In addition, the self-assembly of the inclusion complexes is expected to have potential applications in supramolecular chemistry for the fabrication of hierarchical morphologies, such as phase compatibilizers.

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