Tunable Properties of Inclusion Complexes Between Amylose and Polytetrahydrofuran

Rachmawati Rachmawati, Albert J. J. Woortman, Katja Loos*

Amylose and polytetrahydrofuran (PTHF) are mixed in an aqueous solution to form inclusion complexes. DSC shows that immediate mixing results in complexes having lower melting temperatures compared with complexes prepared with longer mixing times. The washed complexes melt at higher temperatures compared with the corresponding unwashed complexes. XRD indicates that amylose–PTHF complexes diffract similar to amylose–fatty acids complexes (V$_{6I}$-amylose helices), with additional diffractions correlating with amylose–alcohol complexes (V$_{6II}$-amylose helices). This suggests that the structure of amylose–PTHF complexes is an intermediate or a mixture between V$_{6I}$ and V$_{6II}$-amylose. This shows that, besides residing inside the amylose helices, some PTHF chains are located in between the amylose helices.

1. Introduction

As a linear polysaccharide having α-(1 → 4)-glycosidic linkages, amylose is mainly present in nature as a component of starch. The presence of many hydroxyl groups in the amylose chain leads to several interesting characteristics and properties that are mainly due to intramolecular hydrogen bonds. As a single helix, amylose is known as V-amylose,[1] which is present as a left-handed helix that features a hydrophobic cavity inside and a hydrophilic surface outside.[2] These topographic properties facilitate amylose to act as a host molecule capable of including suitable guest molecules into its hydrophobic cavity to form inclusion complexes. A number of small molecules such as iodine, linear alcohols, and fatty acids are widely known to form good complexes with amylose. This offers opportunities for a variety of applications, as the complexes are able to self-assemble as a supramolecular assembly with higher ordered structures. Some possible applications are in the area of molecular imprinting,[3] as flavor encapsulation[4] and healthy diet[5] in food science, and as delivery system for fatty acids.[6] Larger molecules, such as polymers, were also reported to be able to form complexes with amylose.[7] For the same polymer, the length of the polymer chains play an important role in recognizing the host–guest interaction between the amylose and the polymer. Low molecular weight polymers are more favorable to be included by the amylose, compared to the ones with higher molecular weight.[8] Also, polymers with a hydrophilic backbone such as polyethyleneoxide, do not form inclusion complexes with amylose.[9] Even though the hydrophilicity of the polymer improves its solubility, certain levels of hydrophobicity should be maintained.

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* Supporting Information is available from the Wiley Online Library or from the author.
For amylose–polymer complexation, the methods which had been reported involved the use of a jet cooker or a pressure vessel [guest polymer: poly(ethylene-co-acrylic acid) (PEAA) and poly[ε-caprolactone] phosphate (PCL phosphate)], achemically modified amylose [guest polymer: polytetrahydrofuran (PTHF)], or via “vine-twinning polymerization” (guest polymers: polyesters, poly(ester-ether) (PEE), and polycarbonate). Those complexation methods open an interesting approach on utilizing amylose–polymer interactions. Furthermore, as it incorporates amylose as a biodegradable material, the resulting complexes can attract applications which require products with a certain life time.

PTHF is one among several hydrophobic polymers, which was reported to have good complexing ability with amylose. Besides being complexed with amylose via “vine-twinning polymerization” or via direct mixing with partially methylated amylose, PTHF can also be complexed with unmodified amylose. A facile method to prepare inclusion complexes between PTHF and unmodified amylose is possible by applying vibration and heating using water as a medium (direct mixing method). In this case, as amylose–PTHF inclusion complexes lead to molecules capable of self-organizing, the understanding of several factors which are involved in the complexation is important. This can bring added value in preparing polymer–polymer complexes with tailor-made properties. In this study, we report the use of the direct mixing method to investigate the speed of the complexation process along with the effects of different mixing times and mixing sequences on the resulting complexes. PTHF’s with different molecular weights were used as the guest polymers (Mn, 650–2900 g mol⁻¹) for complexation with amylose (Mn ≈ 200 kg mol⁻¹) to examine the effects of the degree of polymerization (DPn) of PTHF on the properties of the resulting amylose–PTHF complexes.

2. Experimental Section

2.1. Materials

Amylose with a molecular weight (Mv) of ~200 kg mol⁻¹ (amylose, from Avebe), hydroxyl terminated PTHF with molecular weights of 650, 1000, 2000, and 2900 g mol⁻¹, respectively (PTHF650, PTHF1000, PTHF2000, PTHF2900, from Aldrich), hydroxyl terminated PTHF (synthetic, Mn 2000 g mol⁻¹, sPTHF2000), ethanol (EtOH) (≥99.9%, from Emsure), and potassium carbonate (K₂CO₃) (≥99%, from Merck) were used as received.

2.2. Preparation of Amylose–PTHF Complexes

1 g of amylose was suspended in water (5% w/w) and 200 mg PTHF1000 (20% w/w based on amylose) were mixed and vibrated for 10 min in a ventilation oven at 85 °C (one-pot method, method OP). Afterwards, the suspension was while stirring heated to 160 °C in a pressure vessel (see Supporting Information Figure S1) and cooled down to a minimum temperature of 80 °C. The suspension was mixed under rotation in a ventilation oven at 85 °C for different times. For method IS (individual solubilization), 200 mg PTHF was suspended in water and vibrated for 10 min in a ventilation oven at 85 °C. At the same time, 1 g of amylose was suspended in water separately and heated to 160 °C in a pressure vessel and cooled down to 85 °C. The emulsified PTHF and the solubilized amylose were mixed together retaining an amylose concentration as 5% w/w in water. The mixtures were kept under rotation in a ventilation oven at 85 °C for different times. For PTHF2000/s2000/2900, the amylose inclusion complexes were prepared using method OP by utilizing 24 h vibration and 16 h mixing time.

2.3. Purification Methods

The amylose–PTHF1000 complexes that were prepared by method IS (0 h mixing time) and method OP (1 and 16 h mixing times at 85 °C) were diluted at 85 °C to 1% w/v (based on amylose concentration in water). The diluted complexes were centrifuged for 5 min at 2 000 rpm at room temperature. The supernatants were thrown away, and the precipitates were washed two times with hot water. The recovery of the washed products was around 30–45%, which was calculated gravimetrically based on the total weight of amylose and PTHF. The undiluted and unwashed products were freeze-dried and used as a reference. Water washing and subsequent freeze-drying was also applied for amylose–PTHF complexes (product recovery around 20% w/w). The water-washed products are herein stated as the W-washed products.

2.4. Differential Scanning Calorimetry (DSC)

The measurements were performed on a Perkin–Elmer Pyris 1 DSC that had been calibrated with indium. An empty pan was taken as a reference. The samples were weighed into DSC large volume cups (LVCs) as a suspension in water at a concentration of 10% w/w. The samples were equilibrated overnight before the measurement. The samples were heated and cooled under nitrogen in the range of 1–160 °C with a rate of 10 °C min⁻¹. Freeze dried samples were calculated as 97% dry matter.

2.5. X-Ray Diffraction (XRD)

The freeze-dried samples were put over saturated K₂CO₃ solution for seven days. The measurement was performed on a powder diffractometer (Bruker D8) using CuKα X-rays with a wavelength of 1.54 Å as the radiation source. The ranges of 2θ between 5° and 35° were obtained by scanning the samples with interval 0.05° at 8 s per step. The resulting data were smoothed using a fast Fourier transform (FFT) filter.

2.6. Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrophotometry (MALDI-ToF-MS)

MALDI-ToF-MS measurements were performed on a Biosystems Voyager-DE PRO spectrometer in a linear-delayed-positive mode.
The accelerating voltage was set to 20 kV with the grid voltage at 95%. Dithranol was used as the matrix, THF as the solvent and NaI as the cationization agent. PTHF (0.5 μL, 2 g L⁻¹) was mixed with 0.5 μL NaI (1 g L⁻¹) and 0.5 μL dithranol (20 g L⁻¹). Around 1 μL of the mixture was spotted on a MALDI plate and dried by air for 2 h before measurement.

3. Results and Discussion

3.1. Effects of the Mixing Method on Amylose–PTHF Complexes

To investigate the effect of mixing sequence between amylose and PTHF, two mixing methods were applied: either by mixing amylose and PTHF together in a one pot reaction (method OP) or emulsifying them separately followed by subsequent mixing (method IS). The mixing time was also investigated to see how fast the complexation took place. For both methods, water was used as the complexation medium. To enhance the complexation, heating to 160 °C was conducted to solubilize amylose, while the vibration at 85 °C in a ventilation oven was performed to emulsify PTHF. The complexation (mixing of amylose and PTHF) was conducted at 85 °C in a ventilation oven to avoid amylose retrogradation.

As shown in Figure 1, endothermic peaks were observed for both methods and for all mixing times (0, 1, 2, and 4 h). As the complexes were unwashed, the endothermic peak of PTHF was detected between 20 and 30 °C (see Supporting Information Figure S2). In the case of the 0 h-complexes, the complexation between amylose and PTHF occurred quickly: within 10 min for method OP and immediately for method IS. This shows that as long as both PTHF and amylose are well emulsified, the PTHF chains are easily included into the amylose helix. However, two different endotherm peaks (118 and 132 °C) were observed for the 0 h-complex prepared by method OP which suggests that two different crystal structures were formed. As comparison, the 0 h-complex that was prepared by method IS resulted in a single endotherm having a melting temperature (t_m) at 118 °C. Furthermore, longer mixing time after heating to 160 °C for both methods resulted in the similar type of complexes that melted at around 136 °C. This suggests that in the case of 0 h-complex prepared by method OP, small amount of complexes (t_m = 132 °C) were likely formed during the 10 min vibrated mixing at 85 °C, and most of the complexes (t_m = 118 °C) were formed after heating to 160 °C.

The higher t_m that was observed for 1, 2, and 4 h-complexes indicates that longer mixing time allows the complexes to organize in a more compact fashion compared to the corresponding 0 h-complexes. This organization resulted in a more crystalline structure that led to higher t_m. Albeit in the first run it is difficult to measure the melting enthalpy (∆H_m) exactly, the ∆H_m of 1, 2, and 4 h-complexes seem to increase slightly after longer reaction time. However, by comparing the two methods on the same mixing time, method OP resulted in complexes having a sharper endothermic profile than method IS. This difference is likely due to the fact that the one-pot-preparation of method OP allows both amylose and PTHF to interact during heating to 160 °C. In this case, there is the possibility that most of the complexes were already formed during the heating and the additional mixing time at 85 °C favored more crystal organization, while in method IS, heating to 160 °C was only applied to solubilize the amylose. During the first stage of immediate mixing (i.e., less than 5 s), some
complexes were formed ($t_m < 136 \, ^\circ\mathrm{C}$) and the rest of the complexes were formed during the mixing time at 85 $^\circ$C. Therefore, the overall endothermic peak of the resulting complexes that was prepared by method IS was observed to have a rather broad endothermic peak.

3.2. Combined Effects of Mixing Time and Water Washing on Amylose–PTHF1000 Complexes

The amylose–PTHF complexes settled as white precipitates, while the uncomplexed amylose remained as a clear solution above 70 $^\circ$C and the uncomplexed PTHF remained as a white emulsion. The distinctive behaviors of the three compounds make it possible for the amylose–PTHF complexes to be separated from the mixture. It is also expected that the purified complexes will have a higher enthalpy compared to the corresponding unwashed complexes. During the purification step, to remove uncomplexed soluble amylose, hot water was used to avoid retrogradation of the amylose.

Considering its high complexing ability with amylose, PTHF1000 was used as the guest molecule to study the effect of mixing time. As shown in Table 1 which is based on the first heating scan, the resulting $t_m$ of the 0 h-unwashed amylose–PTHF1000 complex (118 $^\circ$C) is lower than the $t_m$ of 1- and 16 h-unwashed complexes (around 136 $^\circ$C). The corresponding melting enthalpy ($\Delta H_m$) of the 0 h-unwashed complex is slightly higher (29 J·g$^{-1}$) than the 1- and 16 h-unwashed complexes which are 21 and 19 J·g$^{-1}$, respectively. This indicates that the crystallinity of the 0 h-complexes is probably lower but the complexes are formed in higher quantity than the ones that were prepared with a longer mixing time. The 0 h-unwashed complexes also recrystallized at some lower temperature. This was represented by a lower recrystallization temperature ($t_m$) at 93 $^\circ$C compared to the $t_m$s of the 1- and 16 h-complexes (95 and 97 $^\circ$C). The lower recrystallization enthalpies ($\Delta H_c$ between $-8$ and $-9$ J·g$^{-1}$) of the three complexes are likely due to the effect of the cooling rate of the DSC. Another possibility is due to the overlap of the exothermic peaks of the complexes with the recrystallization of the uncomplexed amylose. In addition, there are no significant differences on the second heating for the $t_m$s (133–134 $^\circ$C) and $\Delta H_m$s (11–12 J·g$^{-1}$) of the three unwashed complexes. The differences in both enthalpies and temperatures on the first heating/cooling for 1 and 16 h-complexes points out that the first stage of the amylose–PTHF complexation is represented by the resulted 0 h-unwashed complexes (low $t_m$). Given an additional heating and enough time, the low $t_m$ complexes likely develop into a more crystalline structure that shows similar thermal behavior with the 1- and 16 h-complexes.

In general, the $t_m$ and $\Delta H_m$ of the resulted complexes are as follow: unwashed < water-washed (Table 1). For the water-washed (W-washed) products, the $t_m$ of PTHF is still visible in the 0, 1, and 16 h-complexes. This $t_m$ of PTHF can either represent uncomplexed (free) or partly complexed PTHF. The presence of the endothermic peak of PTHF for the W-washed products due to the free PTHF is unlikely as the free PTHF does not precipitate and stays in solution, therefore the purification is expected to wash it away. However as the endothermic peak of PTHF is still visible, hot water was probably not the best solvent to purify the

| Table 1. DSC data of inclusion complexes between amylose and PTHF1000. |
|-----------------|-----------------|-----------------|-----------------|
| **Sample**      | **First heating scan** | **First cooling scan** | **Second heating scan** |
|                 | $\Delta H$ [J·g$^{-1}$] | Onset [°C] | Peak [°C] | $\Delta H$ [J·g$^{-1}$] | Onset [°C] | Peak [°C] | $\Delta H$ [J·g$^{-1}$] | Onset [°C] | Peak [°C] |
| Amylose         |                   |             |             |                   |             |             |                   |             |             |
| Inclusion complexes |                   |             |             |                   |             |             |                   |             |             |
| 0 h-unwashed    | 9.0              | 107.3       | 117.7       | 22.9              | 100.4       | 92.5        | -8.3             | 121.4       | 133.2       | 11.6            |
| 0 h-W-washed    | 22.3             | 110.8       | 122.2       | 29.6              | 99.5        | 92.9        | -14.1            | 123.4       | 133.9       | 23.0            |
| 1 h-unwashed    | 5.4              | 125.5       | 136.5       | 21.1              | 101.8       | 94.7        | -8.4             | 122.1       | 134.0       | 10.8            |
| 1 h-W-washed    | 10.8             | 125.4       | 135.2       | 34.7              | 105.3       | 99.2        | -18.4            | 126.5       | 133.5       | 28.5            |
| 16 h-unwashed   | 4.0              | 129.1       | 136.4       | 18.5              | 103.8       | 96.7        | -9.1             | 123.2       | 133.9       | 11.5            |
| 16 h-W-washed   | 11.2             | 125.7       | 136.0       | 31.2              | 104.1       | 97.7        | -20.2            | 124.9       | 134.2       | 29.8            |

The 0 h-complexes were prepared by method IS, while the 1- and 16 h-complexes were prepared by method OP. W denotes water.
complexes or the PTHF endothermic peak mostly came from the partly complexed PTHF.

By assuming that the PTHF peak is from partly complexed PTHF, the corresponding enthalpy is expected to be lower than the unwashed complex as most of the sample consists of amylose–PTHF complexes. However, in comparison to the unwashed complexes, the higher $\Delta H_m$ of the water-washed complexes are also accompanied by the higher $\Delta H_m$ of the PTHF. For example, $\Delta H_m$ of the amylose–PTHF 0 h-complex is 30 J g$^{-1}$ which is higher than the unwashed 0 h-complex ($\Delta H_m = 23$ J g$^{-1}$), but the $\Delta H_m$ of the PTHF (22 J g$^{-1}$) is also higher than the PTHF in the unwashed product ($\Delta H_m = 9$ J g$^{-1}$). As most of the uncomplexed (free) PTHF’s were washed away, there is also a possibility that the remaining PTHF that was detected by the DSC is from PTHF chains that reside in between the helices. The schematic representation of the organization of PTHF in amylose–PTHF complexes is shown in Figure 2.

To minimize the effect of the cooling speed on the complex recrystallization in the DSC, additional measurements were performed by applying an isothermic cooling for 1 h at 85 °C for the 1 h-complexes. The resulted $\Delta H_m$ values on the second heating are 11.5 J g$^{-1}$ for unwashed complexes and 26.5 J g$^{-1}$ for W-washed complexes. These values are similar to the ones shown in Table 1. This shows that 1 h isothermic at 85 °C was probably not sufficient to allow a complete recrystallization of the complexes.

3.3. XRD Measurements of Amylose–PTHF1000 Complexes

XRD was used to characterize the resulting structures of the amylose–PTHF1000 complexes (unwashed and W-washed), which were prepared by different mixing times (0, 1, and 16 h). In this case, it is important to note that XRD only characterizes the resulting crystal structures of the complexes. It cannot differentiate the presence of guest PTHF that resides inside and in between the helices because the two PTHF’s are considered as the building molecules of the unit cell of the complexes, together with amylose and water molecules. The XRD however can detect the uncomplexed (free) PTHF and uncomplexed amylose as they will form different crystal structures compared to the complexes therefore show distinctive diffraction patterns.

As shown in Figure 3, the 0 h-complexes show only one diffraction peak (20) at 20°, with a shoulder at 12–13° that becomes clearer for the water-washed (W-washed) products. This is in agreement with the DSC data (Table 1) that indicate that the low $t_m$ of the unwashed 0 h-complex is likely due to less crystallinity compared to the W-washed products. Furthermore, the high $t_m$ of the 1 and 16 h-complexes that show high crystallinity is also supported by the fact that the longer the complexation time, the sharper the diffraction peak at around 20°. However, while the DSC data show that there are endothermic peaks of PTHF for the
unwashed and water-washed complexes (0, 1, and 16 h), no diffraction peaks of PTHF (2θ: 19.9° and 24.4°) were detected by XRD. This illustrates that the peak by DSC related to PTHF is not from uncomplexed (free) PTHF. They rather represent the included PTHF’s that are arranged in between the amylose helices either partly or as a whole (see Figure 2).

The diffractions of amylose at around 17.1°–17.5° and 21.7°–22.9° are not clearly visible for the water-washed (W-washed) products. The diffraction peak at around 16.9° was observed as a shoulder-shaped peak which became sharper for 1- and 16 h-unwashed complexes. The DSC for the unwashed products showed that the retrogradation of amylose was visible for the unwashed products and negligible for the W-washed products. This shows that the purification by water washing eliminated the uncomplexed amylose successfully.

3.4. Structure Analysis of Amylose–PTHF1000 Complexes Based on XRD

The main diffraction peaks of the amylose–PTHF1000 complexes at 12.9°–13.1° and 19.6°–20.0° are associated with a d-spacing value of 0.68 and 0.45 nm for n = 1, respectively. These two 2θ values are slightly different than the ones that were reported for amylose–PTHF complexes by Kadokawa et al. and Kida et al. (12.4°–12.9° and 19.8°–20.1°)[9–11] and more similar to starch–decanal complexes which showed scattering angles at 7.5°, 13°, and 20°.[14] By using the indexes of the orthorhombic unit cell of amylose–fatty acids complexes reported by Zobel et al. (a = 13.6 Å, b = 23.7 Å, c = 8.1 Å),[15] the diffraction at 12.4°–12.9° and 19.8°–20.1° (d equals to 0.70 and 0.45 nm)[9–11] correspond to the reflections of plane 200 and 310, respectively (see Supporting Information, Table S1). The difference on the mentioned diffraction is likely due to the different packing of the PTHF in the resulted complexes as described before. The preparation of amylose–PTHF complexes via the so-called “vine-twinning polymerization” likely resulted in a complex in which the PTHF only reside inside the amylose helices. As for the arrangement where PTHF can be located inside and in between the amylose helices (see Figure 2), the main diffractions of the resulted complexes come thereby from different planes.

However, besides showing the two characteristic peaks (2θ) of the amylose inclusion complexes at 13.5° and 19.9°, the 1 h-complexes appear to have additional peaks (2θ) at around 17.0°/18.5°/20.6° that are more clearly visible for 16 h-complexes at 16.9°/18.4°/20.8°. The peaks are also visible on 0 h-complexes as shoulder-shaped peaks. As for the peaks at the range of 16.9°–17.1° and 20.9°–22.5° that correspond to d = 0.52 and 0.39–0.42 nm, respectively, they possibly result from uncomplexed amylose. As these peaks are small and rather sharp compared to the original amylose, they likely show that there are parts in one long chain of amylose which are guest free. Furthermore, for the 16 h-complexes that were unwashed and water washed, there are two peaks of 2θ between 20° and 23°. Although the calculated d = 0.40–0.42 nm is close to uncomplexed amylose, the d for the similar peak of the 16 h-water washed complexes at 20.3° is closer to the complex having a crystal structure similar to amylose–fatty acid complex (d = 0.44 nm, plane 310).[15] In addition, the presence of another peak at 18.4°–18.6° in the 1- and 16 h-complexes which associate with a d-spacing value of 0.48 nm also possibly associates to the diffraction from the complexes (plane 221).

As the diffractions of the complexes prepared by method OP resemble the amylose–fatty acid complex, the resulting
structure is therefore characterized as a $V_{6I}$-amylose. However, the DSC results for the W-washed products showed the endothermic peak of PTHF while no PTHF peak can be detected by XRD. It can be concluded that there is PTHF that resides in between the amylose helices. Furthermore, to accommodate guest PTHF’s in between the amylose helices, the resulting V-amylose is likely to adopt a certain structure that describes the most stable configuration. It is expected that the resulting crystal structure will be slightly different than the amylose–fatty acid. In this case, if PTHF is included inside the amylose helices as well as residing in between the amylose helices, a $V_{6I}$-amylose cannot accommodate the PTHF chains. This is due to the small dimensions of the unit cell of the $V_{6I}$-amylose, which provides no space for accommodating guest molecules in between the amylose helices.

Some diffractions such as the one at 22.1° can also be regarded as a diffraction from plane 531 of an amylose–$n$-butanol/$n$-pentanol complex ($V_{6I}$-amylose). Another peak at around 21° can also be a diffraction of a $V_{6I}$-amylose from the plane 450. The two peaks were reported for amylose–hexanoic acid which showed diffractions that fit with the packing of the $V_{6I}$-amylose similar to amylose–$n$-butanol/$n$-pentanol complex. In combination with the DSC data that indicates the presence of PTHF in between the amylose helices, the V-amylose in the 0-, 1-, and 16 h-amylose–PTHF1000 complexes possibly contains a mixture of $V_{6I}$ and $V_{6I}$-amylose configurations. However, there is also a diffraction at 21.9° which is unidentified. This means that there is also the possibility that the resulting crystal structure of amylose–PTHF1000 complexes described here have a different crystal dimension compared to the amylose–fatty acids complex or amylose–$n$-butanol/$n$-pentanol complexes.

### 3.5. Effects of Water Washing on Amylose–PTHF2000/\textit{s}PTHF2000 Complexes

Based on the above results and the thermal behavior, the crystallinity of the complexes is mainly affected by the mixing time, mixing sequence, and the purification method. The drying methods, which involve the use of freeze-drying seems to give no significant effects on the properties of the resulted complexes. In addition, methods OP and IS clearly give some advantages, such as an improved complexation and a tunable crystallinity of the resulting products in correlation with the mixing time. Therefore, it was also expected that the methods improve the amylose complexation with PTHF having higher molecular weights.

As shown in Table 2, the $\Delta H_m$ of the unwashed complexes is clearly visible (12–19 J·g$^{-1}$). This shows that longer vibration times increase the emulsification process of the PTHF, which resulted in an improved complexation. It is also important to note that the resulted complexes described in this section tend to crystallize at lower temperatures compared to the amylose–PTHF1000 complexes. This caused an overlap of the crystallization exotherm of the complex with the amylose retrogradation. Thus, the $\Delta H_s$ described for these complexes are considered for comparison purpose only.

In the case of amylose complexation with PTHF2000, two different hydroxyl terminated PTHFs were used: commercial (PTHF2000) and synthetic (sPTHF2000). On the first

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**Table 2.** DSC data of inclusion complexes between amylose and PTHF2000, sPTHF2000, and PTHF2900.

<table>
<thead>
<tr>
<th>Sample</th>
<th>First heating scan</th>
<th>First cooling scan</th>
<th>Second heating scan</th>
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<td></td>
<td>PTHF</td>
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<td>$\Delta H$</td>
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<td>Peak [°C]</td>
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</tr>
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<td>Unwashed</td>
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heating, both unwashed complexes melted at around 138 °C, but the amylose–sPTHF2000 complex showed a higher $\Delta H_m$ (19 J g$^{-1}$) than the amylose–PTHF2000 complex (12 J g$^{-1}$). This might be due to the high polydispersity of the guest PTHF2000 (see Supporting Information Figure S3). In this case, PTHF2000 chains with higher molecular weight ($\bar{M}_n$) than 2000 g mol$^{-1}$ were hardly complexed with amylose. Therefore, the resulting complexes were less as they were mostly constituted of amylose and PTHF2000 chains with lower $\bar{M}_n$. However, comparable $\Delta H_c$ (5 J g$^{-1}$) on the cooling and $\Delta H_m$ (11–12 J g$^{-1}$) on the second heating were obtained for both complexes. In addition, the $\Delta H_c$ of the amylose retrogradation for both complexes were also comparable (10 J g$^{-1}$). This suggests that the amount of uncomplexed amylose is the same for both complexes.

The higher $\Delta H_m$ for amylose–sPTHF2000 complexes indicates that guest polymers with narrower polydispersity arrange better in the resulted complex and thereby show a higher $\Delta H_m$. A high $\Delta H_m$ on the first heating was observed for the W-washed products of both amylose–PTHF2000 complexes (around 60 J g$^{-1}$). This $\Delta H_m$, which is almost twice the value of $\Delta H_m$ of W-washed amylose–PTHF1000 complexes (30–35 J g$^{-1}$, Table 1), correlates to the length of the PTHF2000 which is twice of PTHF1000. In this case, the longer chains of PTHF2000 occupy two times the spaces within the resulted structure of the complexes, thereby showing higher enthalpy compared to the amylose–PTHF1000 complexes. This shows that the amylose–PTHF complexation is greatly influenced by the preparation method. In this case, as the method described here improves the emulsification of amylose as well as PTHF and also allows a reasonable time of mixing (for complexation), the formation of amylose complexes with higher molecular weight of PTHF is therefore more feasible.

Additionally, the $\Delta H_m$ values of the free PTHF for the washed complexes of amylose–PTHF2000/sPTHF2000 were reduced (4–6 J g$^{-1}$) and the retrogradation of the amylose was negligible ($\Delta H_c$ around 2 J g$^{-1}$). This shows that the washed products of both amylose–PTHF2000 were more crystalline than the corresponding unwashed products. However, the W-washed amylose–sPTHF2000 complex started to melt at lower temperature ($t_m$ at 122 °C) compared to the amylose–PTHF2000 complex ($t_m$ at 129 °C). The broader endotherm of the former indicates that the resulted washed complex is less crystalline than the later. This broader peak is also observed on the second heating even though the $\Delta H_m$ of both complexes on the second heating were similar (around 23 J g$^{-1}$).

### 3.6. Effects of Water Washing on Amylose–PTHF2900 Complexes

As shown in Table 2, the unwashed product of amylose–PTHF2900 complex shows a higher $\Delta H_m$ (24 J g$^{-1}$) compared to the corresponding amylose–PTHF2000 complexes (12–19 J g$^{-1}$). This gives further evidence that longer vibration time leads to better emulsification of PTHF, thus enables longer PTHF chains to be complexed with the amylose. Nonetheless, the exotherm related to the retrogradation of amylose for the unwashed amylose–PTHF2900 complex is exceptionally high ($\Delta H_c$ around 14 J g$^{-1}$). This indicates that the high $\Delta H_c$ of the amylose–PTHF2900 complex likely correlate to the packing of the longer PTHF chains which resulted in a more crystalline structure. Furthermore, even though the $\Delta H_c$ of the amylose retrogradation is lower for the washed product (3 J g$^{-1}$), only a small increase of $\Delta H_m$ on the first heating, and a similar $\Delta H_m$ on the second heating of the unwashed product was observed. As a higher $\Delta H_m$ is expected for the washed product, some loosely bound PTHF2900 chains were washed away during the purification. This indicates that the amylose–PTHF2900 complexes are not as strong as the amylose complexes with lower molecular weight PTHF.

### 3.7. XRD Measurements of Amylose–PTHF2000/sPTHF2000/PTHF2900 Complexes

The diffractograms of the complexes of amylose–PTHF2000 and amylose–PTHF2900 complexes are depicted in Figure 4. PTHF2000 and PTHF2900 show two main diffraction peaks (2θ) at 19.9° and 24.4°. As expected, the peak at 24.4° from PTHF and the peak at 17.2° from amylose still appear for the unwashed complexes. However, the PTHF peak is also present for the W-washed amylose, which is more pronounced for amylose–PTHF2900 complexes. This shows that there are still some PTHF chains not included by the amylose.

In addition, the PTHF peak at 24.4° for the W-washed amylose complex with sPTHF2000 is sharper compared to the W-washed amylose–PTHF2000. This is in agreement with the DSC data (Table 2), in which the W-washed amylose–PTHF2000 showed a sharper endothermic peak with similar $\Delta H_m$ with the W-washed amylose–sPTHF2000. In this case, the free PTHF chains probably lead to a less crystalline structure of the complex. This suggests that the narrow polydispersity of sPTHF2000 likely conditioned all the available PTHF chains to access the amylose cavity. This resulted in a structure in which the number of partially included PTHF chains are larger than the wholly include PTHF chains. As in the case of a broader polydispersity of PTHF2000, the amylose probably favors selective PTHF chains with shorter chains, thus led to a sharper endothermic peak.

### 3.8. Structure Analysis of Amylose–PTHF2000/sPTHF2000/PTHF2900 Complexes Based on XRD

The main diffraction peaks (2θ) for the amylose–PTHF2000/sPTHF2000/PTHF2900 complexes appear at of 13.2° and...
20.0°, which correspond to the diffraction of an amylose–fatty acids complex from plane 111 and 310, compared to plane 200 and 310 from PTHF1000 complexes (see Supporting Information, Table S2). The difference is likely due to the arrangement of the longer chains of PTHF2000/sPTHF2000 and PTHF2900 that resulted in a modified crystal of the amylose–PTHF complex. In addition, even though the peak at around 13.2° of W-washed amylose–PTHF2900 complex only appears as a shoulder-shaped peak, there is a strong diffraction at 21.5°. This diffraction is also observed in a less intensity at 21.4° for the W-washed complexes of amylose–PTHF2000 and amylose–sPTHF2000. As mentioned before, this peak which correlates with a d-spacing of 0.41 nm was observed for 1 h-W-washed amylose–PTHF1000. This peak fits with the diffraction of plane 441 of an orthorhombic crystal of the amylose–n-butanol/n-pentanol complexes.\(^{16}\) This is an indication that there are some PTHF chains located in between the amylose helices in which the \(V_6\)-amylose expand to accommodate the guest molecules, known as \(V_6\)–amylose.\(^{16}\) Other diffraction peaks that indicate the presence of a \(V_6\)–amylose appear with low intensity at \(2\theta\) of 18.7°–19.0° (plane 530), 23.4°–23.6° (plane 550), 23.7°–23.8° (plane 222), 21.0°–21.3° (plane 450), 25.2°–25.5° (plane 402), 25.8°–26.5° (plane 422), 26.7° (plane 450), and 30.9° (plane 402).

There are also some diffraction peaks with a low intensity (\(2\theta\) at 6.9°, 7.6°, 8.3°, 8.9°, 18.2°, and 22.1°–22.4°) from the amylose–PTHF2000/sPTHF2000/PTHF2900 complexes that are still unidentified based on the calculation using either cell parameters of amylose–fatty acid,\(^{15}\) amylose–n-butanol/n-pentanol,\(^{16}\) or amylose–\(\alpha\)-naphthol complex.\(^{17}\) However, the main diffraction peaks at around 12.9°–13.2° and 19.6°–20.1° fit with the calculation of a unit cell of an amylose–fatty acids crystal, while the peak at 21.5° and some additional peaks correlate with the amylose–alcohol crystal. This indicates that the resulted \(V_6\)–amylose described here is probably the intermediate or the mixture between \(V_6\)–amylose and \(V_6\)–amylose. Considering the trend that longer complexation time and purification steps tend to result in complexes that show high \(f_m\) and more diffraction peaks, the methods described here can be used to tune the desirable level of crystallinity in the resulted amylose–PTHF complexes. Drying method by freeze-drying has no influences on the complexes.

4. Conclusion

Different methods for the complex formation, along with the variation on the mixing time and washing were investigated. It was observed that the inclusion complexes were already obtained after immediate mixing of soluble amylose and emulsified PTHF. Longer complexation time resulted in complexes with better crystallinity which indicates that the desirable properties of amylose–PTHF complexes can be tuned by varying complexation time. Although the definite crystal structure of amylose–PTHF inclusion complexes is not known yet, the results show that the included PTHF chains reside inside and in between the amylose helices. The XRD data revealed that the main diffractions of the resulted amylose–PTHF complexes correspond to the diffractions of an orthorhombic crystal of amylose–fatty acids complexes. In addition, some diffractions correlated to the cell parameters of the amylose–n-butanol/n-pentanol complexes. This indicates that the resulted structure of the amylose–PTHF complexes

![Figure 4. X-ray diffractograms of PTHF2000 (a), sPTHF2000 (b), PTHF2900 (c), and the unwashed (U) and water-washed (W) complexes between amylose and PTHF2000 (d), sPTHF2000 (e), and PTHF2900 (f).](image-url)
is a sixfold V-amylose helix in the form of a mixture or an intermediate of V$_{6I}$- and V$_{6II}$-amylose.

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