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Formation of Amylose–Poly(tetrahydrofuran) Inclusion Complexes in Ionic Liquid Media

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In this study, we found that amylose–poly(tetrahydrofuran) (PTHF) inclusion complexes were formed when a mixture of amylose with PTHF was stirred in 1-butyl-3-methylimidazolium chloride of an ionic liquid at high temperature under reduced pressure, followed by cooling and precipitation by addition of methanol.

Amylose, which is a polysaccharide with helical conformation linked through (1 → 4)-α-glycosidic linkages, is a well-known host compound that forms inclusion complexes with various low-molecular-weight compounds mainly by hydrophobic interaction between the guest molecules and the cavity of amylose.[1] On the other hand, only a few studies regarding the formation of inclusion complexes of amylose with polymeric compounds were reported in the 1990s.[2] Amylose in solid state constructs stable crystalline structure associated with the formation of double helixes.[3] In addition, a single-stranded amylose in aqueous solution also gradually forms a double helix, giving water-insoluble product. Due to the stable double helix of amylose, it may be difficult to incorporate polymeric compounds into the cavity of amylose by only hydrophobic interaction between amylose and the guest polymers.

Recently, some preparation methods for amylose–polymer inclusion complexes have been reported. For example, we have developed a new method named "vine-twining polymerization" for the preparation of inclusion complexes composed of amylose and synthetic polymers, which is achieved by phosphorylase-catalyzed polymerization to form a single-stranded amylose[4] in the presence of guest polymers.[5] The key point of this method is that an enzymatically produced amylose-chain forms inclusion complexes with guest polymers before construction of stable double helix. On the other hand, Akashi et al. have also found that the inclusion complexes were formed using partially methylated amyloses as effective hosts.[6] The double helix may slightly loosen, because the intra- and intermolecular hydrogen bonds of the amylose derivatives are weakened compared with those of the original amylose. Therefore, the guest polymers are allowed to incorporate into the cavities of the amylose derivatives.

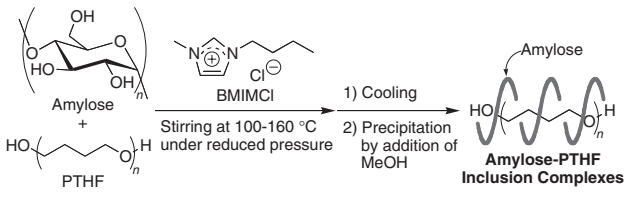
If some solvents are found to loosen the double helix of amylose, the development of new and facile preparation methods for amylose–polymer inclusion complexes would be expected. Because of low solubility, however, disentanglement of the helix does not commonly take place in usual solvents. For this purpose, we have noted ionic liquids (ILs), which have been found to be used as good solvents for polysaccharides.[7] Such solubility of polysaccharides in ILs has inspired us to develop a new method for formation of amylose–polymer inclusion complexes, because we assume that the double helix of amylose loosens by dissolution in ILs.

In this study, therefore, we investigated formation of amylose–polymer inclusion complexes in an IL media. 1-Butyl-3-methylimidazolium chloride (BMIMCl) is known as a good solvent for starch,[8] and it was actually confirmed by visual observation that amylose was dissolved in BMIMCl at higher temperature. On the other hand, poly(tetrahydrofuran) (PTHF) has been used as a guest polymer in the aforementioned previous studies to prepare the inclusion complexes.[5a,5c,5k] Therefore, BMIMCl and PTHF were employed as a media and a guest polymer, respectively.

Prior to the following attempt for formation of inclusion complexes, BMIMCl (500 mg) was preheated at 140 °C under reduced pressure (ca. 5 mmHg) to remove most water. Then, PTHF (number-average molecular weight (M_n): 5600, 50 mg) and amylose (M_n = 38000, 20 mg) were added to the anhydrous BMIMCl. After the mixture was vigorously stirred at the prescribed temperatures and times under reduced pressure (ca. 5 mmHg), the mixture was cooled to room temperature and methanol was added to the mixture to obtain a precipitate (Scheme 1). The isolated precipitate was washed with methanol, chloroform, and water to remove BMIMCl, PTHF, and water-soluble oligosaccharides obtained by hydrolysis of amylose, and then dried under reduced pressure at room temperature to yield the product.[13]

The characterization of the resulting product was performed by means of X-ray diffraction (XRD) and ^{1}H NMR measurements. The XRD pattern of the product obtained at 120 °C for 60 min (Run 4 in Table 1) shows two diffraction peaks at 2θ = ca. 13 and 20° (Figure 1), which is similar to that of the inclusion complexes of amylose with monomeric compounds[9] and the polymers shown in our previous papers.[5] In addition, the ^{1}H NMR spectrum in DMSO-d_6 of the product shows the signals not only due to amylose but also due to PTHF, in spite of washing with chloroform, which is a good solvent for PTHF (Figure 2). The integrated ratio of the signal $\mathbf{H_1}$ of amylose to the signal $\boldsymbol{\beta}$ of PTHF ($\boldsymbol{\beta}/\mathbf{H_1}$) was calculated to be 0.84. This value is almost the same as the theoretical $\boldsymbol{\beta}/\mathbf{H_1}$ value (=0.89) estimated by taking into consideration of the helix repeat distance of amylose and the length of one unit of PTHF, as previously reported.[5a] The results of XRD and ^{1}H NMR measurements indicate that the product is inclusion complex composed of amylose and PTHF.

When the operation was carried out at lower temperature (100 °C) than that described above, the $\boldsymbol{\beta}/\mathbf{H_1}$ value in the



Scheme 1. Formation of amylose–PTHF inclusion complexes in BMIMCl.

Table 1. Effect of temperature and time on formation of amylose–PTHF inclusion complexes in BMIMCl[a]

Run	Temp. /°C	Time /min	Yield /mg	β/H_1[b]	M_n of amylose in the product[b]
1	100	2	15.2	0.12	26000
2	100	60	13.3	0.40	19000
3	120	2	15.3	0.24	30000
4	120	60	12.2	0.84	17000
5	140	2	15.4	0.33	32000
6	140	60	6.0	0.82	17000
7	160	2	6.5	0.88	15000
8	160	60	0	—	—

[a]Reaction conditions: BMIMCl: 500 mg, pressure: ca. 5 mmHg, amylose ($M_n = 38000$): 20 mg, PTHF ($M_n = 5600$): 50 mg. [b]Estimated by ^{1}H NMR measurements.

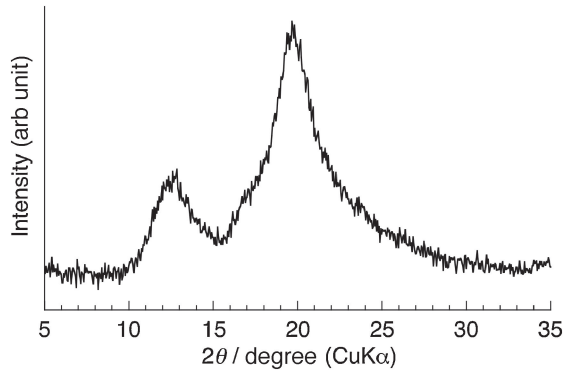


Figure 1. XRD pattern of the product obtained under the conditions at 120 °C for 60 min (Run 4 in Table 1).

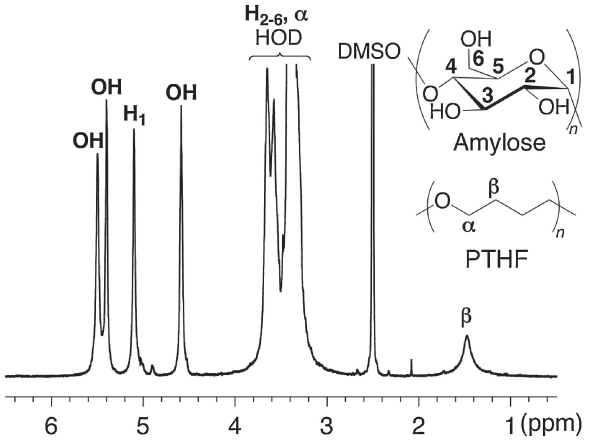


Figure 2. ^{1}H NMR spectrum in DMSO-d_6 of the product obtained under the conditions at 120 °C for 60 min (Run 4 in Table 1).

^{1}H NMR spectrum of the product decreased (Run 2 in Table 1). This is probably because the double helix of amylose did not loosen by heating in BMIMCl at temperatures below 100 °C. On the other hand, although the operation at higher temperature (140 °C) led to the formation of inclusion complex ($\boldsymbol{\beta/H_1} = 0.82$), the yield of the product was decreased (Run 6 in Table 1). In addition, the product was not obtained by the operation at much higher temperature (160 °C) (Run 8 in Table 1). This is because the amylose main-chain was hydrolyzed in the slight presence of moisture remaining in BMIMCl at such high temperatures, giving water-soluble oligomeric amyloses that do not have ability to form inclusion complex with PTHF. The ^{1}H NMR spectrum of the water-soluble fractions revealed the existence of oligomeric amylose.

On the other hand, when the operation was performed for shorter time (2 min) than that described above, the $\boldsymbol{\beta/H_1}$ values were decreased (Runs 1, 3, and 5 in Table 1) with the exception of the run at 160 °C (Run 7 in Table 1). However, the yield of the product obtained at high temperature was low.

The M_ns of amyloses in the products were evaluated by the integrated ratio of the signal $\mathbf{H_1}$ of amylose chain (δ 5.1) to the signal $\mathbf{H_1}$ (α and β) of the reducing terminus (δ 4.9 (α) and 4.3 (β)) in the ^{1}H NMR spectra (DMSO-d_6 containing a small amount of D_2O). In all cases, the M_ns of amyloses in the products were lower than that of the employed amylose, and particularly longer stirring time led to lower M_ns, which is reasonably explained by occurrence of hydrolysis of amylose main-chains (Table 1).

As described above, we found that the amylose–PTHF inclusion complexes were formed by stirring a mixture of amylose with PTHF in BMIMCl at high temperature. This may be explicable by the following process. First, the double helix of amylose probably loosened in BMIMCl at high temperature because the hydrogen bonds between hydroxy groups of amylose would be weakened under such conditions. Then, the helical structure was regenerated from single-stranded amylose in BMIMCl during cooling and precipitation. Because PTHF was not dissolved in BMIMCl as confirmed by visual observation, this would prefer to be inside of the amylose helix as a hydrophobic space rather than in BMIMCl. Therefore, we assume that the formation of the inclusion complex occurred during the regeneration of helical structure of amylose in the cooling process. To confirm disentanglement of the double helix of amylose in BMIMCl at high temperature, we investigated the thermal behavior of amylose in BMIMCl by differential scanning calorimetry (DSC) measurements.

The DSC thermogram of amylose in BMIMCl (30 wt %) at the heating rate of 10 °C min^{-1} shows a broad endothermic peak in the range between ca. 110 and 165 °C (Figure 3). A similar phase transition associated with gelatinization has been observed for amylose-rich starch (pea starch) in water at high temperatures (95–121 °C).[10] Therefore, we suggest that conformational transition of amylose probably occurred from double helix to random coil in BMIMCl at such temperatures. Because an onset of phase transition of amylose in BMIMCl is appeared at 111 °C, we believe that operation at higher temperature than 111 °C is required for the formation of amylose–PTHF inclusion complexes.

As a comparative experiment, a mixture of amylose with PTHF was stirred in dehydrated DMSO as a good solvent for these polymers instead of BMIMCl in an argon atmosphere under atmospheric pressure at 120 °C for 60 min.[11] In the ^{1}H NMR spectrum in DMSO-d_6 of the product, the $\boldsymbol{\beta/H_1}$ value was calculated to be 0.30, which was much lower than that of the product obtained using BMIMCl at 120 °C for 60 min (Run 4 in Table 1). In addition, the XRD pattern of the product showed the

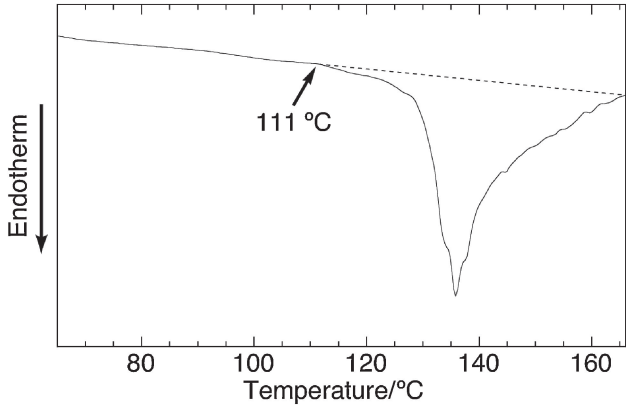


Figure 3. DSC thermogram of amylose in BMIMCl (30 wt %) at the heating rate of 10 °C min^{-1}.

diffraction peaks due to both the amyloses which formed and did not form the inclusion complex. This indicates that use of DMSO as a media did not efficiently result in the formation of inclusion complexes composed of amylose and PTHF. Because it is well known that DMSO is included in the cavity of amylose,[12] amylose–DMSO inclusion complexes were preferentially formed in the above experiment, causing difficulty in inclusion of PTHF in the cavity. The above suggests that use of BMIMCl as the media is of great advantage to form amylose–PTHF inclusion complexes.

In summary, we reported a facile method for formation of amylose–PTHF inclusion complexes, which was achieved by stirring a mixture of amylose with PTHF in BMIMCl at high temperature under reduced pressure, followed by cooling and precipitation by addition of methanol.

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