Preparation of Inclusion Complexes Composed of Amylose and Strongly Hydrophobic Polyesters in Parallel Enzymatic Polymerization System

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ABSTRACT: We describe the preparation of inclusion complexes composed of amylose and strongly hydrophobic polyesters in a parallel enzymatic polymerization system. This was achieved by carrying out phosphorylase-catalyzed enzymatic polymerization of α-D-glucose 1-phosphate from maltoheptaose, giving rise to amylose as a host, and lipase-catalyzed enzymatic polymerization of dicarboxylic acids and diols, leading to the guest polyesters, simultaneously. When the numbers of methylene units in a dicarboxylic acid and a diol as the monomers for the guest polyester were 8, the corresponding amylose−polyester inclusion complex was obtained. On the other hand, use of the monomers having methylene units of 10 and 12 hardly gave the corresponding amylose−polyester inclusion complexes.

Introduction

Amylose, a natural linear polysaccharide with helical conformation linked through (1→4)-α-glycosidic linkages, is one component of starch, which has been studied for many years in the material research fields because of its low cost, biodegradability, and renewability. It is a well-known host molecule that readily forms inclusion complexes with slender guest molecules having relatively lower molecular weight by hydrophobic interaction between guest molecules and the cavity of amylose.[1] However, little has been reported regarding the formation of inclusion complexes composed of amylose and polymeric compounds.[2] The principal difficulty for incorporating polymeric materials into the cavity of amylose is that the driving force for the binding is only due to weak hydrophobic interactions. Amylose, therefore, does not have the sufficient ability to include the long chains of polymeric guests into its cavity.

By means of the enzymatic method for direct construction of amylose,[3] we have developed the new methodology for the preparation of inclusion complexes composed of amylose and synthetic polymers,[4] which was achieved by the enzymatic polymerization forming amylose in the presence of guest polymers. The representation of this reaction system is similar to the way that vines of plants grow twining around a rod. Accordingly, we have proposed that this polymerization method for the preparation of amylose−polymer inclusion complexes is named "vine-twining polymerization".

In this polymerization system, the hydrophobicity of the guest polymers has been a very important factor because the driving force for the formation of the inclusion complexes is probably hydrophobic interaction. For example, when this polymerization was carried out using the guest polyethers having appropriate hydrophobicities such as poly(trimethylene oxide) and poly(tetramethylene oxide), these hydrophobic polyethers were included in the cavity of amylose to form the inclusion complexes.[4c] However, in addition to no formation of the inclusion complex from hydrophilic polymer such as poly(ethylene oxide), the preparation of the inclusion complexes has not been achieved from the polymers with strong hydrophobicity, for example poly(hexamethylene oxide), attributed to their aggregation in the aqueous buffer of the solvent for the enzymatic polymerization.

To obtain the inclusion complex from a strongly hydrophobic guest polymer, we have been investigating a parallel enzymatic polymerization system as a new polymerization method. In the previous review, we briefly reported a possibility for the formation of the inclusion complex by means of this system[4e] because we had confirmed that the inclusion complex was partially existed in the products produced by carrying out amylose-forming enzymatic polymerization described above and lipase-catalyzed enzymatic polycondensation of sebacic acid and 1,8-octanediol,[5] simultaneously; the latter polymerization gave the strongly hydrophobic polyester. However, the detailed characterizations have not yet been established, and thus we have been carrying out the continuous study on this parallel enzymatic polymerization system.

In this paper, we would like to report the comprehensive results in the above-mentioned parallel enzymatic polymerization system to obtain the inclusion complexes composed of amylose and the strongly hydrophobic polyesters. As the monomers for the guest polyesters, we employed the dicarboxylic acids and the diols having methylene units of 8, 10, and 12, hereafter denoted as Diacids-8, -10, -12 and Diols-8, -10, -12, respectively.

Experimental Section

Materials. Phosphorylase (ca. 300 units/mL) was supplied from Ezaki Glico Co. Ltd., Osaka, Japan.[6] Lipase from Pseudomonas cepacia (ca. 50 units/mg) was purchased from Fluka Chemie GmbH. Maltoheptaose (G_7) was prepared by selective cleavage of one glycosidic bond of β-cyclodextrin under acidic conditions.[7] Other reagents and solvents were used without further purification.

Preparation of Amylose−Polyester Inclusion Complexes in Parallel Enzymatic Polymerization System. A typical experimental procedure for the preparation of amylose−polyester inclusion complex using Diacid-8 and Diol-8 was as follows. Diacid-8 (40.5 mg, 0.2 mmol), Diol-8 (29.2 mg, 0.2 mmol), G_7 (2.3 mg, 2 μmol), and G-1-P (sodium salt form, 152.1 mg, 0.5 mmol) were

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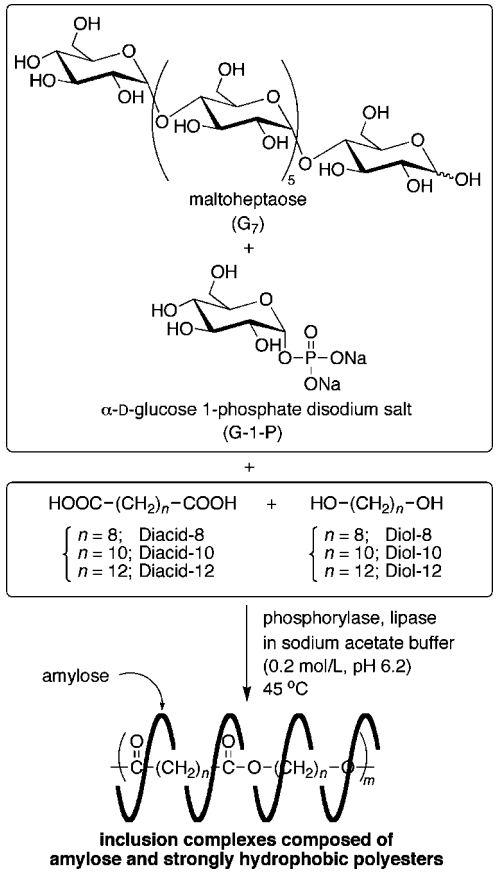
Scheme 1. Preparation of Inclusion Complexes Composed of Amylose and Strongly Hydrophobic Polyesters in the Parallel Enzymatic Polymerization System



mixed in sodium acetate buffer (2.0 mL, 0.2 mol/L, pH = 6.2). After adjustment of the pH value to 6.2 using acetic acid aqueous solution (0.2 mol/L) was carried out, lipase (150 units) and phosphorylase (24 units) were added to this mixture, and then the obtained suspension was stirred vigorously for 24 h at 45 °C. The insoluble fractions were separated by filtration, and the product was isolated from the materials by washing with water, acetone, and chloroform and then dried under reduced pressure at room temperature to yield ca. 12.9 mg of the inclusion complex. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.13−1.26 (br, −C−(CH_2)$_4$−C− due to the polyester, Diacid-8, and Diol-8), δ 1.34−1.61 (br, −$CH_2$$CH_2$C(=O)− and −$CH_2$$CH_2$OC(=O)− due to the polyester,

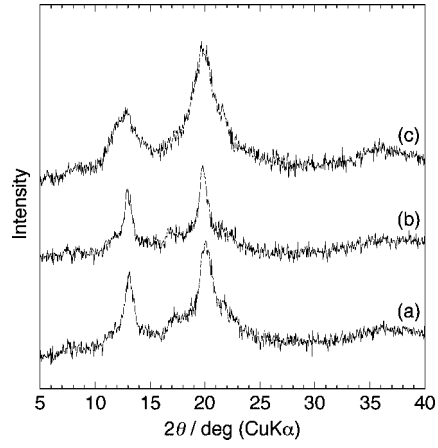


Figure 1. XRD patterns of the products obtained by the parallel enzymatic polymerization using (a) Diacid-8 and Diol-8, (b) Diacid-10 and Diol-10, and (c) Diacid-12 and Diol-12.

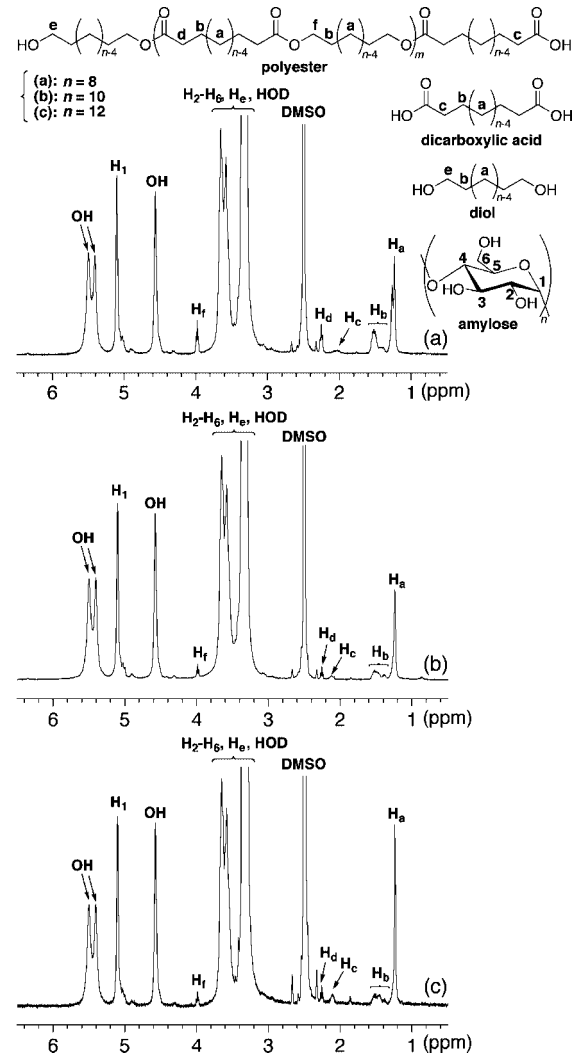


Figure 2. ^{1}H NMR spectra of the products obtained by the parallel enzymatic polymerization using (a) Diacid-8 and Diol-8, (b) Diacid-10 and Diol-10, and (c) Diacid-12 and Diol-12.

−$CH_2$$CH_2$C(=O)OH due to the polyester and Diacid-8, −$CH_2$$CH_2$OH due to the polyester and Diol-8), δ 1.90−2.14 (br, −CH_2−C(=O)OH due to the polyester and Diacid-8), δ 2.17−2.30 (br, −CH_2−C(=O)O− due to the polyester), δ 3.08−3.91 (m, **H_2**−**H_6** due to amylose, −CH_2OH due to the polyester and Diol-8, overlapping with HOD), δ 3.91−4.04 (br, −CH_2OC(=O)− due to the polyester), δ 4.56, 5.41, 5.50 (OH), δ 5.10 (br, **H_1** due to amylose).

Extraction of Guest Compounds from Inclusion Complexes. A typical experimental procedure for extraction of the guest compounds from the inclusion complex obtained using Diacid-8 and Diol-8 was as follows. The inclusion complex (50.0 mg) was dissolved in DMSO-d_6 (2 mL). After the solution was stirred for 3 h at room temperature, chloroform (10 mL) was added to the solution. The resulting precipitate was isolated by filtration, washed with chloroform, and then dried under reduced pressure at room temperature to yield fraction 1 (48.7 mg). Subsequently, the filtrate was evaporated and dried under reduced pressure at 60 °C to obtain fraction 2. Fraction 2 was washed with water (ca. 30 mL), and the resulting filtrate was evaporated and dried under reduced pressure to obtain fraction 3 (1.3 mg). On the other hand, the residue was washed with 0.1 mol/L aqueous ammonia (ca. 30 mL) and dried under reduced pressure to yield fraction 5 (2.6 mg). The filtrate was evaporated and dried under reduced pressure to give fraction 4 (2.4 mg).

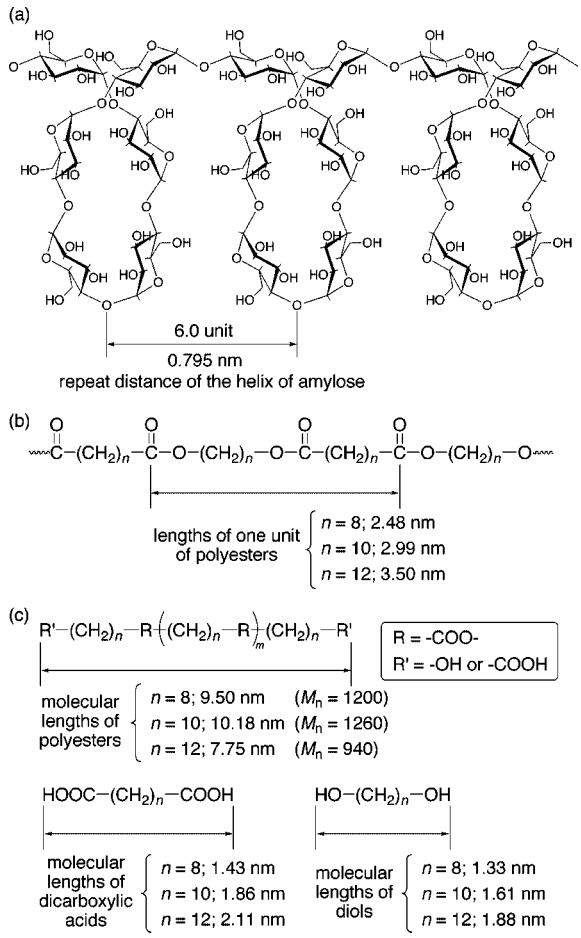


Figure 3. Illustrations of (a) repeat distance of amylose helix, (b) lengths of one unit of polyesters, and (c) molecular lengths of polyesters, dicarboxylic acids, and diols.

Table 1. Effect of the Number of Methylene Units in Dicarboxylic Acids and Diols on Formation of Inclusion Complexes in the Parallel Enzymatic Polymerization

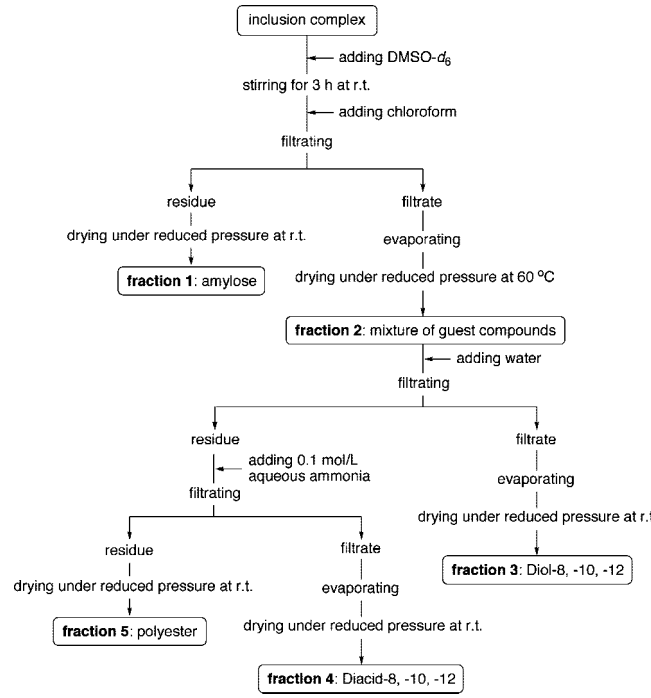
run	no. of methylene units in dicarboxylic acid and diol	length of one unit of polyester (nm)[a]	$\mathbf{H_f}/\mathbf{H_1}$[b] actual: theoretical	$\mathbf{H_a}/\mathbf{H_1}$[b] actual: theoretical
1	8	2.48	0.15:0.21	0.82:0.86
2	10	2.99	0.05:0.18	0.57:1.06
3	12	3.50	0.05:0.15	0.87:1.21

[a] The values were calculated with MM2 in the CS Chem 3D program package. [b] The actual values were estimated by ^{1}H NMR measurements.

Amylose-Forming Polymerization in the Presence of Dicarboxylic Acids. A typical experimental procedure for amylose-forming polymerization in the presence of Diacid-8 was as follows. Diacid-8 (40.5 mg, 0.2 mmol), G_7 (2.3 mg, 2 μmol), and G-1-P (152.1 mg, 0.5 mmol) were mixed in sodium acetate buffer (2.0 mL, 0.2 mol/L, pH = 6.2). After adjustment of the pH value to 6.2 using acetic acid aqueous solution (0.2 mol/L) was carried out, phosphorylase (24 units) was added to this mixture, and then the resulting suspension was stirred vigorously for 24 h at 45 °C. The following procedures were same as those for the parallel enzymatic polymerization described above. Yield of the product was ca. 2.6 mg. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.19−1.25 (br, −C−(CH_2)$_4$−C− due to Diacid-8), δ 1.41−1.52 (br, −CH_2CH$_2$C(=O)OH due to Diacid-8), δ 2.13−2.21 (br, −CH_2−C(=O)OH due to Diacid-8), δ 3.01−3.92 (m, $\mathbf{H_2}$−$\mathbf{H_6}$ due to amylose, overlapping with HOD), δ 4.58, 5.41, 5.51 (OH), δ 5.11 (br, $\mathbf{H_1}$ due to amylose).

Amylose-Forming Polymerization in the Presence of the Polyester. The polyester was prepared by polycondensation of Diacid-8 (80.9 mg = 0.4 mmol) and Diol-8 (58.5 mg = 0.4 mmol) catalyzed by lipase (300 units) in water (1.0 mL) for 24 h at 45 °C.[5] The isolated polyester (12.2 mg, M_n = 3010, M_w/M_n = 1.29) was suspended in sodium acetate buffer (2.0 mL, 0.2 mol/L, pH = 6.2) using an ultrasonic wave. After the addition of G_7 (2.3 mg = 2 μmol) and G-1-P (152.1 mg = 0.5 mmol) to the suspension, adjustment of the pH value to 6.2 using acetic acid aqueous solution (0.2 mol/L) was carried out. Phosphorylase (13.5 units) was added to this suspension, and then the suspension was stirred vigorously for 24 h at 45 °C. The following procedures were same as those for the parallel enzymatic polymerization described above. Yield of the product was ca. 21.5 mg. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.10−3.91 (m, $\mathbf{H_2}$−$\mathbf{H_6}$ due to amylose, overlapping with HOD), δ 4.57, 5.40, 5.50 (OH), δ 5.10 (br, $\mathbf{H_1}$ due to amylose).

Scheme 2. Extract Operation of the Guest Compounds from the Inclusion Complex



Polyester-Forming Polymerization in the Presence of Amylose. Amylose was prepared by the enzymatic polymerization of G-1-P (152.1 mg, 0.5 mmol) from G_7 (2.3 mg, 2 μmol) catalyzed by phosphorylase (13.5 units) in sodium acetate buffer (2.0 mL, 0.2 mol/L, pH = 6.2) for 6 h at 45 °C. Amylose (15.0 mg), Diacid-8 (40.5 mg, 0.2 mmol), and Diol-8 (29.2 mg, 0.2 mmol) were mixed in sodium acetate buffer (2 mL, 0.2 mol/L, pH = 6.2). After adjustment of the pH value to 6.2 using acetic acid aqueous solution (0.2 mol/L) was carried out, lipase (150 units) was added to this suspension, and then the suspension was stirred vigorously for 24 h at 45 °C. The following procedures were same as those for the parallel enzymatic polymerization described above. Yield of the product was ca. 17.1 mg. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15−1.31 (br, −C−(CH_2)$_4$−C− due to Diacid-8 and Diol-8), δ 1.42−1.59 (br, −CH_2CH$_2$C(=O)OH and −CH_2CH$_2$OH due to Diacid-8 and Diol-8), δ 2.10−2.20 (br, −CH_2−C(=O)OH due to Diacid-8), δ 3.10−3.92 (m, $\mathbf{H_2}$−$\mathbf{H_6}$ due to amylose, −CH_2OH due to Diol-8, overlapping with HOD), δ 4.58, 5.41, 5.50 (OH), δ 5.10 (br, $\mathbf{H_1}$ due to amylose).

Measurements. The ^{1}H NMR spectra were recorded using a JEOL ECX400 spectrometer (JEOL Ltd.). The X-ray diffraction (XRD) measurements were performed at a scanning speed of 2θ = 2°/min using a Geigerflex RAD-IIB diffractometer (Rigaku Co.) with Ni-filtered Cu Kα radiation (=0.154 18 nm). The gel permeation chromatography (GPC) analyses were performed by using a HITACHI pump L-2130 and a HITACHI RI detector L-2490 under the following conditions: Shodex GPC KF-804 L

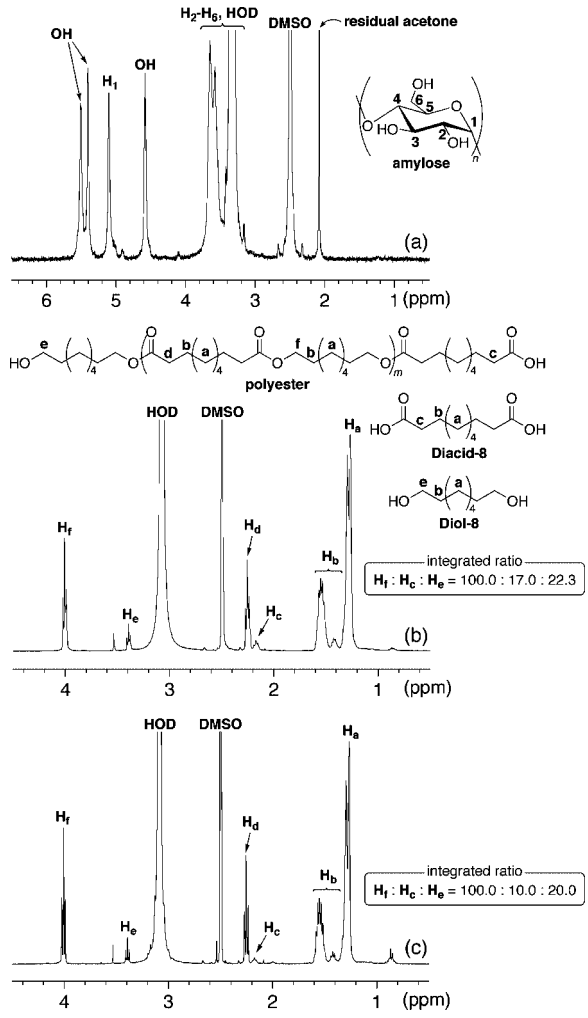


Figure 4. ^{1}H NMR spectra of (a) fraction 1, (b) fraction 2, and (c) fraction 5 obtained by operation in Scheme 2 using the inclusion complex from Diacid-8 and Diol-8.

Table 2. Effect of the Number of Methylene Units in Dicarboxylic Acids on Formation of Inclusion Complexes Composed of Amylose and Dicarboxylic Acids

run	no. of methylene units	$\mathbf{H_a}$ of dicarboxylic acid/$\mathbf{H_1}$[a] actual:theoretical
1	8	0.13:0.74
2	10	0.40:0.86
3	12	0.66:1.01

[a] The actual values were estimated by ^{1}H NMR measurements.

Table 3. Molecular Weights of the Guest Polyesters Included in the Cavity of Amylose

		M_n		
run	no. of methylene units in dicarboxylic acid and diol	estimated by ^{1}H NMR	estimated by GPC	M_w/M_n[a]
1	8	1200	1950	1.46
2	10	1260	1260	1.20
3	12	940	1350	1.13

[a] Estimated by GPC measurements.

and KF-803 L columns with chloroform as the eluent at a flow rate of 1.0 mL/min at 40 °C.

Results and Discussion

The preparation of the inclusion complexes was performed in two enzymatic polymerizations, which were the phosphorylase-catalyzed polymerization of G-1-P from G_7, giving rise to amylose, and the lipase-catalyzed polycondensation of Diacids-8, -10, -12 and Diols-8, -10, -12, leading to the polyesters (Scheme 1). We employed lipase from Pseudomonas cepacia in this study because it was reported that yield of the polyester in the polycondensation catalyzed by lipase from this origin was higher than that of the polyesters obtained by lipases from other origins.[5] The insoluble fractions were isolated, and the products were separated from the materials by washing subsequently with water, acetone, and chloroform. The products were characterized by the following XRD and ^{1}H NMR measurements.

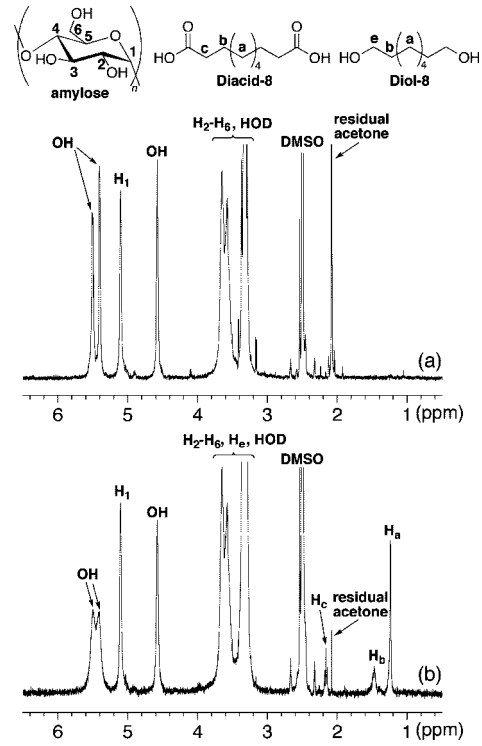


Figure 5. ^{1}H NMR spectra of the products obtained by (a) amylose-forming polymerization in the presence of polyester and (b) polyester-forming polymerization in the presence of amylose.

The XRD patterns of all products show two diffraction peaks at 2θ = ca. 13° and 20° (Figure 1), which are similar to those of the inclusion complexes of amylose with monomeric compounds[8] and with polymers.[4] This indicates that the products are inclusion complexes composed of amylose and the guest compounds. At first, we describe the detailed characterizations of the inclusion complex obtained using Diacid-8 and Diol-8 as follows.

The ^{1}H NMR spectrum in DMSO-d_6 of the product shows the signals not only due to amylose but also due to the guest polyester (Figure 2a), in spite of washing with acetone and chloroform as the good solvents for the polyester and its monomers. The NMR result is also taken to support the formation of the inclusion complex.

Generally, one helical turn of amylose is composed of ca. 6 repeating glucose units when linear molecules of small cross-sectional area such as fatty acids are included (Figure 3a).[9] The repeat distance of the helix of amylose was reported as ca. 0.795 nm (Figure 3a).[9] On the other hand, the length of one unit of the polyester having methylene units of 8 was presently calculated to be ca. 2.48 nm (Figure 3b).[10] On the basis of the values described above, the theoretical integrated ratios of the signal $\mathbf{H_1}$ due to H-1 of amylose to the signal $\mathbf{H_f}$ due to the $-CH_2OC(=O)-$ ($\mathbf{H_f/H_1}$) and to the signal $\mathbf{H_a}$ due to the $-C-(CH_2)_4-C-$ ($\mathbf{H_a/H_1}$) in the ^{1}H NMR spectrum of the inclusion complex was calculated to be 0.21 and 0.86, respectively

(run 1 in Table 1). The actual integrated ratio of $\mathbf{H_f/H_1}$ in the ^{1}H NMR spectrum of the product was 0.15, which was smaller than the theoretical value. On the other hand, the actual integrated ratio of $\mathbf{H_a/H_1}$ was 0.82, which was in good agreement with the theoretical value. The signal $\mathbf{H_f}$ is attributed to the methylene protons in only the polyester ($-CH_2OC(=O)-$), whereas the signal $\mathbf{H_a}$ is ascribed to the methylene protons in both the polyester and its monomers. On the basis of these results, we assumed that amylose included not only the polyester but also its monomers, i.e., Diacid-8 and Diol-8.

Therefore, to investigate the ratio of the polyester, Diacid-8, and Diol-8 which existed in the cavity of amylose, these guest compounds were extracted from the inclusion complex according to the operation in Scheme 2. After stirring the inclusion complex in DMSO-d_6 for 3 h, chloroform was added to the solution, and the precipitated product was isolated by filtration to give fraction 1, which was confirmed by ^{1}H NMR measurement to be amylose alone (Figure 4a). The ^{1}H NMR spectrum in DMSO-d_6 of fraction 2 obtained by evaporation and dryness of the filtrate shows the signals due to methylene protons of $-CH_2OC(=O)-$ at around 4.0 ppm, $-CH_2-C(=O)O-$ at around 2.2−2.3 ppm, and aliphatic chains at around 1.2−1.3 ppm (Figure 4b). These data indicated that the fraction 2 was a mixture of the polyester, Diacid-8, and Diol-8. Subsequently, fraction 2 was washed with water to obtain fraction 3 (filtrate). In addition, the residue was washed with 0.1 mol/L aqueous ammonia to separate fraction 4 (filtrate) and fraction 5 (residue). The fractions 3, 4, and 5 were Diol-8, Diacid-8, and the polyester, respectively, which were confirmed by ^{1}H NMR measurements.

The average molecular weight and the molecular length of the included polyester were estimated by the ^{1}H NMR spectrum of fraction 5 to be 1200 and 9.50 nm, respectively (Figures 3c and 4c),[11] and the molecular lengths of Diacid-8 and Diol-8 were calculated to be 1.43 and 1.33 nm, respectively (Figure 3c).[10] On the basis of the ^{1}H NMR spectra of fractions 2 and 5, the ratio of methylene protons of $-CH_2OC(=O)-$ in the polyester, methylene protons of $-CH_2-C(=O)OH$ in Diacid-8, to methylene protons of $-CH_2OH$ in Diol-8 was calculated to be 100:7.0:2.3 (Figure 4).[12] Taking into consideration of all the above calculations, the ratio of volumes occupied by the polyester, Diacid-8, Diol-8, and nonincluded space in the cavity of amylose was calculated to be 92.2:3.2:1.0:3.6.[13] This result indicates that amylose included relatively a large amount of the polyester in the present parallel enzymatic polymerization system.

On the other hand, the followings are the detailed characterizations of the inclusion complexes obtained using Diacids-10, -12 and Diols-10, -12, which were performed by the same manners as those for the product from Diacid-8 and Diol-8 as described above. In the ^{1}H NMR spectra of the products, the actual integrated ratios of $\mathbf{H_f/H_1}$ were fairly smaller than the theoretical ratios of them (Figure 2b,c, runs 2 and 3 in Table 1), indicating that the polyesters having methylene units of 10 and 12 hardly included in the cavity of amylose. To investigate the detailed ratios of the guest compounds existed in the cavity of amyloses, they were extracted from the inclusion complexes according to the operation in Scheme 2, and the ratios of volumes occupied by the polyesters, Diacids-10, -12, Diols-10, -12, and nonincluded spaces in the cavity of amyloses were estimated. Consequently, these ratios were calculated to be 45.6:8.0:0.1:46.2 and 54.2:26.1:2.0:17.7 for the inclusion complexes obtained using the monomers having methylene units of 10 and 12, respectively. These results indicate that relatively larger amounts of the Diacids-10, -12 were included in the cavity of amylose compared with the case of the inclusion complex obtained using Diacid-8 and Diol-8. Because the hydrophobicities of Diacids-10 and -12 are stronger than those of Diacid-8, they would be readily included in the cavity of amylose. Actually, when we investigated amylose-forming polymerization in the presence of Diacid-8, Diacid-10, or Diacid-12, individually, the dicarboxylic acids having larger numbers of methylene units were easily included in the cavity of amylose, confirmed by ^{1}H NMR measurements (Table 2). These results indicate that those Diacids-10 and -12 were predominantly included in the cavity of amylose in the parallel enzymatic polymerization system to disturb the inclusion of the polyesters. In addition, the polyesters obtained using Diacids-10, -12 and Diols-10, -12 would be aggregated in the aqueous buffer more than those obtained using Diacid-8 and Diol-8 due to stronger hydrophobicity and accordingly separated from the cavity of amylose. Because of these results and considerations, the polyesters would be hardly included in the cavity of amylose in the case of using Diacids-10, -12 and Diols-10, -12.

The molecular weights (M_ns) of the polyesters included in the cavity of amylose evaluated by means of GPC and ^{1}H NMR measurements were in the ranges of 1260−1950 (M_w/M_n = 1.13−1.46) and 940−1260, respectively (Table 3). On the other hand, the M_ns of the polyesters that were not included in the cavity of amylose in the parallel enzymatic polymerization system using the guest monomers having methylene units of 8, 10, and 12 were estimated to be 2280, 1540, and 1010, respectively, by means of GPC measurements.[14] These values were almost same as those of the polyesters included in the cavity of amylose.

To demonstrate that the inclusion complex composed of amylose and the strongly hydrophobic polyester could be prepared by only the present parallel enzymatic polymerization system, we performed following two experiments. First, amylose-forming polymerization was performed in the presence of the polyester having methylene units of 8. In the ^{1}H NMR spectrum of the product, the signals due to only amylose were observed (Figure 5a), indicating that this polymerization method did not afford the corresponding inclusion complex. On the other hand, polyester-forming polycondensation was carried out in the presence of amylose. Consequently, although the monomers for the polyester were included in the cavity of amylose, the polyester was not included in it, confirmed by ^{1}H NMR measurement (Figure 5b). These results indicate that the inclusion complex composed of amylose and the strongly hydrophobic polyester was prepared by only the present parallel enzymatic polymerization system.

Conclusions

In this paper, we described that the preparation of the inclusion complexes composed of amylose and the strongly hydrophobic polyesters in the parallel enzymatic polymerization system, in which amylose-forming polymerization and guest polyester-forming polymerization took place simultaneously. When the number of methylene units of dicarboxylic acid and diol as the monomers for the guest polyester was 8, the corresponding amylose−polyester inclusion complex was obtained. On the other hand, use of the monomers having methylene units of 10 and 12 hardly gave the corresponding amylose−polyester inclusion complexes.

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(10) The calculation of this value was performed with MM2 in the CS Chem 3D program package.

(11) The number of ester groups in one polyester molecule was calculated to be 6.66 from the integrated ratio of the methylene peak $\mathbf{H_f}$ due to $-CH_2OC(=O)-$ to the methylene peaks $\mathbf{H_c}$ and $\mathbf{H_e}$ due to $-CH_2C(=O)OH$ and $-CH_2OH$ end groups in the ^{1}H NMR spectrum. The average degree of polymerization (DP) of the polyester was calculated to be 3.83 from the following equation: DP = (number of ester group + 1)/2. On the basis of the DP value (3.83), molecular weight (312.4), and length (2.48 nm) of one unit of the polyester, the average molecular weight and molecular length of the polyester were estimated to be 1200 and 9.50 nm, respectively.

(12) The integrated ratio of methylene peaks $\mathbf{H_f}$, $\mathbf{H_c}$, to $\mathbf{H_e}$ in fraction 2 was estimated to be 100.0:17.0:22.3, whereas that in fraction 5 was estimated to be 100.0:10.0:20.0 by means of ^{1}H NMR measurements (Figure 4b,c). The signals $\mathbf{H_c}$ and $\mathbf{H_e}$ in fraction 2 are ascribed to methylene protons of $-CH_2C(=O)OH$ and $-CH_2OH$ groups due to the polyester end groups, Diacid-8, and Diol-8, respectively. On the other hand, these signals in fraction 5 are attributed to methylene protons of $-CH_2C(=O)OH$ and $-CH_2OH$ groups due to only the polyester end groups, respectively. Therefore, the ratio of methylene protons due to $-CH_2OC(=O)-$ in the polyester, methylene protons due to $-CH_2C(=O)OH$ in Diacid-8, to methylene protons due to $-CH_2OH$ in Diol-8 can be estimated by subtracting the integrated values of the signals $\mathbf{H_c}$ and $\mathbf{H_e}$ in fraction 5 from those in fraction 2.

(13) The ratio of methylene protons due to $-CH_2OC(=O)-$ in the polyester, methylene protons due to $-CH_2C(=O)OH$ in Diacid-8, to methylene protons due to $-CH_2OH$ in Diol-8 were exactly calculated to be 100.00:6.96:2.28 (Figure 4). On the other hand, the numbers of the methylene protons in each one molecule were 13.32, 4.00, and 4.00, respectively. Therefore, the molar ratio of the polyester, Diacid-8, to Diol-8 was calculated to be 7.51:1.74:0.57. The numbers of the methylene protons due to $-C-(CH_2)_4-C-$ in each one molecule of the polyester, Diacid-8, and Diol-8 were 61.28, 8.00, and 8.00, respectively, because the number of the methylene protons in the polyester is estimated by multiplication of the DP value (3.83) with the number of the methylene protons in one unit of the polyester (16). Taking into consideration of the molar ratio and the numbers of the methylene protons in each one molecule, the ratio of the methylene protons $\mathbf{H_a}$ due to the polyester, Diacid-8, to Diol-8 were calculated to be 460.21:13.92:4.56. Assuming the integrated value of the signal $\mathbf{H_1}$ in amylose is 100, that of the signal $\mathbf{H_a}$ in the guest compounds is 82 (run 1 in Table 1). Here, the integrated value of the signal $\mathbf{H_a}$ can divide into 78.83 for the polyester, 2.39 for Diacid-8, and 0.78 for Diol-8, on the basis of the ratio of the methylene protons $\mathbf{H_a}$ due to their guest compounds. The numbers of glucose units in amylose corresponding to the lengths of the polyester (9.50 nm), Diacid-8 (1.43 nm), and Diol-8 (1.33 nm) were calculated to be 71.70, 10.79, and 10.04, respectively, on the basis of the number of glucose unit in one helical turn of amylose (6 units) and the repeat distance of the helix of amylose (0.795 nm). These values lead to $\mathbf{H_1/H_a}$ values of 1.170 (= 71.70/61.28), 1.349 (= 10.79/8.00), and 1.255 (= 10.04/8.00), respectively. Therefore, the numbers of glucose units in amylose, i.e., the integrated values of the signal $\mathbf{H_1}$, corresponding to the integrated values of the signal $\mathbf{H_a}$ due to the polyester, Diacid-8, and Diol-8 described above were calculated to be 92.2, 3.2, and 1.0, respectively. Therefore, the ratio of volumes occupied by the polyester, Diacid-8, Diol-8, to nonincluded space in the cavity of amylose was estimated to be 92.2:3.2:1.0:3.6.

(14) The polyesters that were not included in the cavity of amylose were isolated by the following procedures. After the insoluble fraction obtained by the parallel enzymatic polymerization was separated by filtration, the residue was washed with water, acetone, and chloroform. The soluble fractions in acetone and chloroform were collected and then evaporated and dried under reduced pressure at room temperature to obtain the nonincluded polyester.

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