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Formation of an Amylose−Polyester Inclusion Complex by Means of Phosphorylase-Catalyzed Enzymatic Polymerization of α-D-Glucose 1-Phosphate Monomer in the Presence of Poly(ε-caprolactone)

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The biological macromolecules such as proteins, nucleic acids, and polysaccharides accommodate the important in vivo functions associated with “living”. The important biological functions of these macromolecules appear to be controlled by not only their first-order structures but also those of higher-order structures, e.g., a double helix of DNA.[1] The research field on precision architecture of synthetic macromolecules with higher structural order, i.e., “supramolecular chemistry”, therefore, has recently been of importance from the viewpoint of contact with study on biological works.[2] Specially, the study on the supramolecular chemistry connected with polymerization chemistry is conceived as a significant research topic to elucidate the biological mechanism of naturally occurring macromolecules.

Amylose, a natural helical polysaccharide, is a well-known molecule forming higher structural ordered complexes with monomeric organic guest molecules.[3] However, the chemical formation of the complexes between amylose and polymeric guest molecules had been scarcely reported.[4] Recently, we have found a new methodology for formation of an amylose−polymer inclusion complex by means of amylose-forming polymerization.[5] Such a new method was achieved by an enzymatic polymerization of α-D-glucose 1-phosphate monomer (Glc-1-P) catalyzed by phosphorylase in the presence of polyTHF as a guest polymer.[6]

This communication reports formation of an amylose−polyester inclusion complex by means of this polymerization technique using telechelic poly(ε-caprolactone) (PCL) with hydroxy end groups as a new guest polymer (Scheme 1).[7] In addition to the preparation of the inclusion complex, we also describe the unique property of the included PCL under alkaline conditions.

The phosphorylase-catalyzed enzymatic polymerization of Glc-1-P from maltoheptaose (Glc$_7$) as a primer was carried out in the presence of a telechelic PCL (DP = 9.0, M_n = ca. 1000)[8] with hydroxy end groups in citrate buffer.[9,10] The precipitated product was characterized by means of the ^{1}H NMR and X-ray diffraction (XRD) measurements. The ^{1}H NMR spectrum of the product shows the signals due not only to the amylose but also to the PCL. The XRD scan of the same product indicates two strong diffraction maxima at $2\theta = 12.4°$ and 19.8°, corresponding to $d = 0.71$ and 0.45 nm, respectively (Figure 1b). The XRD pattern is completely different from that of amylose (Figure 1a) and is similar to that of the inclusion complex of amylose with polyTHF.[5] These analytical data mentioned above can be taken to support the structure of the helical inclusion complex.

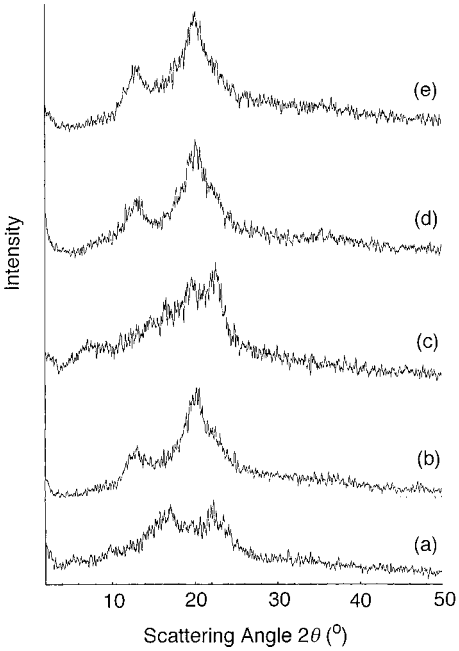

Figure 1. X-ray powder diffraction scans of amylose (a), the product from PCL (M_n = ca. 1000) in citrate buffer (b), the product from PCL (M_n = ca. 2000) in citrate buffer (c), the product from PCL (M_n = ca. 2000) in a mixed solvent of citrate buffer and acetone (d), and the product from PCL (M_n = ca. 2000) in a mixed solvent of citrate buffer and ethanol (e).

The enzymatic polymerization of Glc-1-P proceeded in the presence or absence of the PCL similarly. In the presence of the PCL, however, the product was precipitated from citrate buffer solution earlier than that in the absence of the PCL. This is probably due to the inclusion of the hydrophobic PCL in the cavity of amylose. The DP value of amylose in the inclusion complex, therefore, was smaller than that obtained by the general enzymatic polymerization of Glc-1-P; ca. 56 and 90, respectively.[11] The DP value of the included PCL was also estimated by the ^{1}H NMR spectrum to be ca. 8.5, which is close to that of the original PCL (DP = 9.0). The data indicate that amylose equally included every fraction of the PCL.

Generally, one helical turn of amylose is composed of ca. 6 repeating glucose units when linear molecules of small cross reactional area, e.g., fatty acids, are included.[12] The repeat distance of the helix of amylose has been reported as 0.795 nm,[12] whereas the length of one unit of PCL is presently calculated as ca. 0.84 nm,[13] as shown in Figure 2. From the above calculations, the integrated ratio of the signal due to H$_1$ of amylose to the signal due to H_γ $(-CH_2CH_2C\mathit{H}_2CH_2CH_2(C{=}O)O-)$ is assessed to be 0.31. Actually, the integrated ratio of these two signals in the ^{1}H NMR spectrum of the product was 0.28, which is in good agreement with the calculated value. This also supports the structure of the inclusion complex as shown in Scheme 1.

When a PCL with higher molecular weight (M_n = ca. 2000) was used as the guest polymer for the experiment same to above, the PCL was not dispersed well in the

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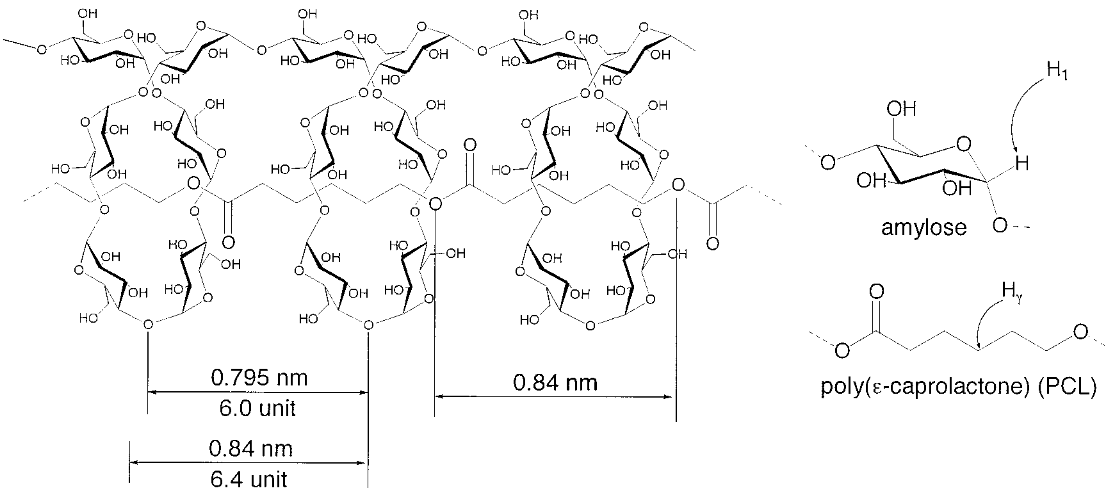
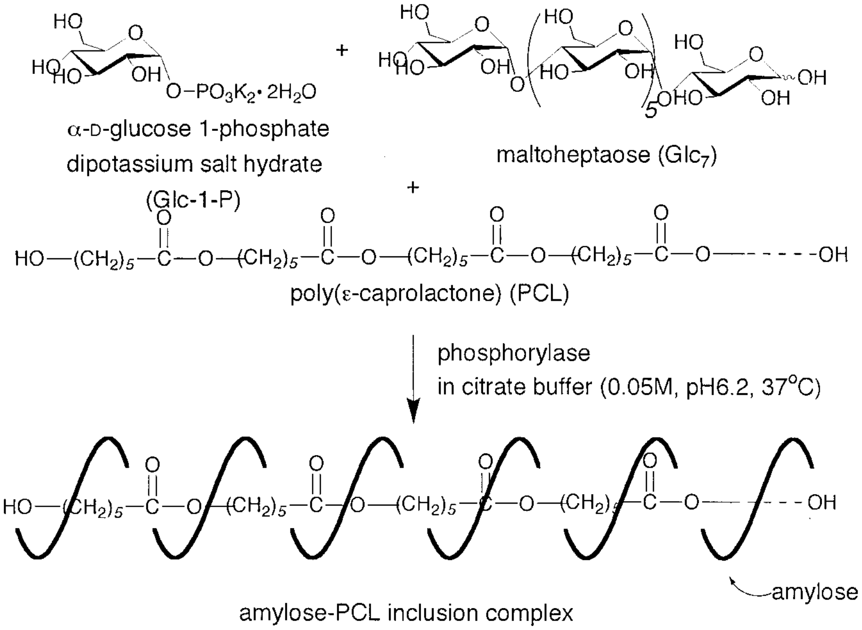


Figure 2. Illustration of the repeat distance of amylose helix and the length of one PCL unit.

Scheme 1



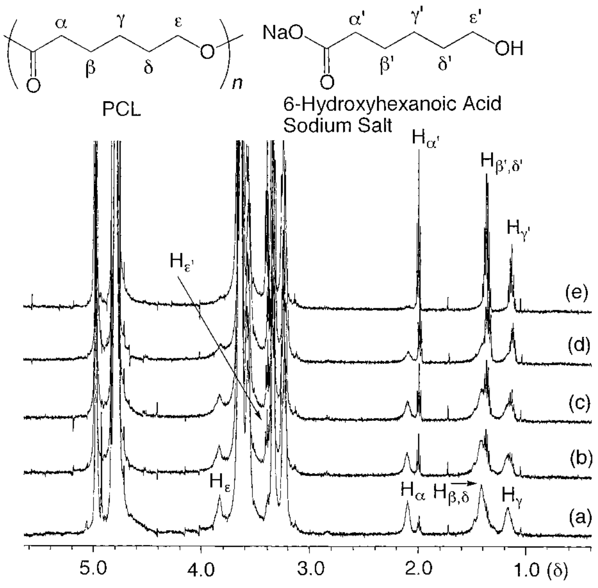


Figure 3. ^{1}H NMR spectra of the inclusion complex after 3 min (a), 30 min (b), 1 h (c), 5 h (d), and 20 h (e) in 1.0 mol/L NaOD−D$_2$O.

citrate buffer, and accordingly, the inclusion complex was not formed. For example, the XRD scan of the product does not show any clear-cut pattern (Figure 1c). To be dispersed in the reaction solvent, the polymerization was carried out in a mixed solvent of citrate buffer and acetone (5:1, v/v). The obtained product was characterized by the ^{1}H NMR and XRD (Figure 1d) measurements to be the inclusion complex. Similarly, the mixed solvent system of citrate buffer and ethanol also gave the inclusion complex as characterized by the XRD scan in Figure 1e.

The stability of the included PCL under alkaline conditions was examined by means of ^{1}H NMR analyses in 1 mol/L NaOD−D$_2$O solvent. Pure PCL was insoluble in that solvent and immediately hydrolyzed to give 6-hydroxyhexanoic acid sodium salt. On the other hand, the included PCL in the amylose helix was soluble in the solvent (Figure 3a) and gradually hydrolyzed over several hours as shown in Figure 3b−e. This observation indicates that the PCL was stabilized from the alkaline, due to its inclusion in the cavity of amylose.

In conclusion, the amylose−polyester inclusion complex was formed by means of the enzymatic polymerization of Glc-1-P in the presence of PCL. The unique property of the included PCL in the alkaline solution was examined, in which the PCL was soluble and stabilized in that solution by its inclusion in the cavity of amylose. Detailed studies on how the complex forms during the polymerization process as well as the variation of polyesters as the guest polymers are now in progress.

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References and Notes

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(6) Amylose can be prepared by an in vitro approach from Glc-1-P catalyzed by phosphorylase enzyme (Cori, G. T.; Cori, C. F. *J. Biol. Chem.* **1940**, *135*, 733). The enzymatic polymerization initiated from a primer of maltoheptaose (Glc$_7$) proceeds through the following reversible reaction, where a glucose unit is transferred from Glc-1-P to the nonreducing 4-OH terminus of a (1→4)-α-glucan chain, resulting in inorganic phosphate (P): $((\alpha,1\rightarrow4)\text{-Glc})_n + \text{Glc-1-P} \rightleftarrows ((\alpha,1\rightarrow4)\text{-Glc})_{n+1} + \text{P}$.

(7) The inclusion complex was not formed by mixing amylose (DP = 75−90, M_n = 12 200−14 600), prepared by the general enzymatic polymerization of Glc-1-P, and the PCL (M_n = ca. 1000) in citrate buffer as previous reported, because the PCL diol was quite hydrophobic and incompatible with amylose.[4b] Therefore, to increase the hydrophilicity of the PCL diol sufficiently to permit entry into the cavity of amylose, the chemical modification of the hydroxy end groups to phosphate groups was carried out. Furthermore, complexes were not obtained by mixing oligomeric amylose with lower DPs, such as 7.0 and 17.0, and the PCL in citrate buffer as well.

(8) The PCL used was prepared by ring-opening polymerization of ε-caprolactone initiated with 1,4-butanediol. Therefore, the DP value was calculated by the integrated ratio of the peak due to $-CH_2CH_2OH$ of the polymer ends to the peak due to $CH_2C{=}O$ of the polymer units by the ^{1}H NMR spectrum.

(9) As a typical experimental procedure, PCL (DP = 9.0, 0.05 g) was suspended in 5.0 mL of sodium citrate buffer (0.05 mol/L, pH = 6.20) with ultrasonic waves and kept at 37 °C. After addition of maltoheptaose (0.0023 g, 2 μmol), α-D-glucose 1-phosphate dipotassium salt hydrate (0.186 g, 0.5 mmol), and phosphorylase (0.0064 g, ca. 160 units), the solution is stirred vigorously for 10 h at 37 °C. Then, the reaction mixture was placed overnight in the refrigerator to precipitate the product. It was collected by centrifugalization, washed with acetone and water, and lypophilized to give 0.0211 g of an inclusion complex (yields 10.2% based on Glc-1-P and 3.8% based on PCL). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.29 (m, 2H, H_γ), 1.53 (m, 4H, H_β and H_δ), 2.27 (t, 2H, H_α), 3.28−3.40 (m, 2H, H-2, 4, overlapping with HOD), 3.58−3.65 (m, 4H, H-3, 5, 6), 3.98 (t, 2H, H_ϵ), 4.59, 5.41, 5.51 (3s, 3H, OH), 5.41 (s, 1H, H-1).

(10) Phosphorylase (E.C.2.4.1.1) was prepared from potato according to the following literature: Kamogawa, A.; Fukui, T.; Nikuni, Z. *J. Biochem.* **1968**, *63*, 361. The activity was measured as ca. 5 units/mg. One unit forms 1.0 μmol of Glc-1-P from glycogen and orthophosphate per minute at pH 6.8 at 30 °C.

(11) The DP values were calculated by the integrated ratio of the peak due to H_1 protons of amylose chain to the peaks due to H_1 (α and β) protons of the reducing terminus by the ^{1}H NMR spectra.

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

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