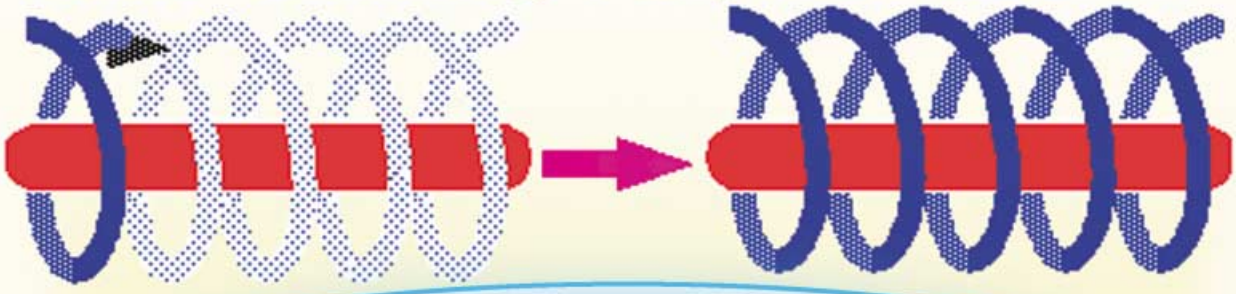
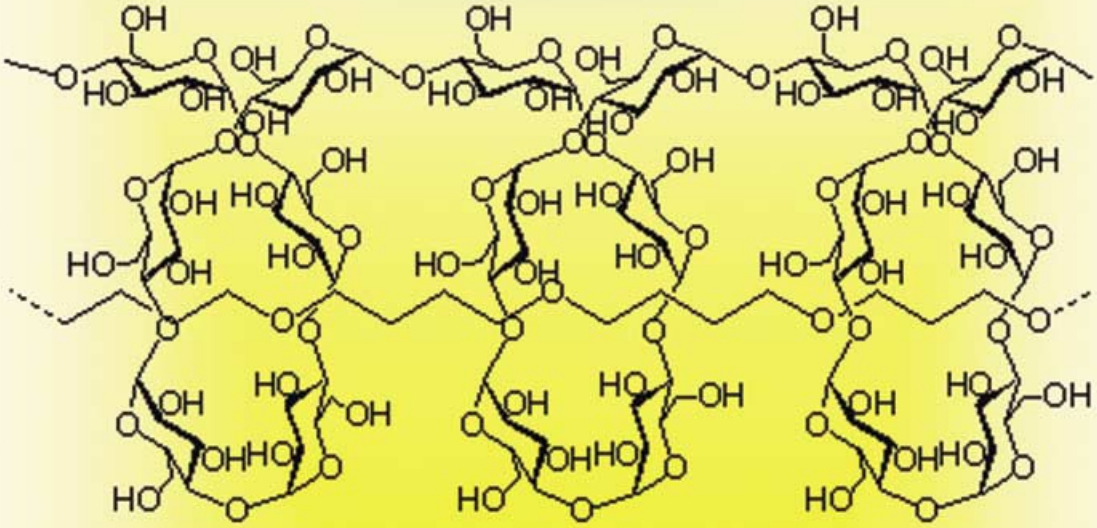
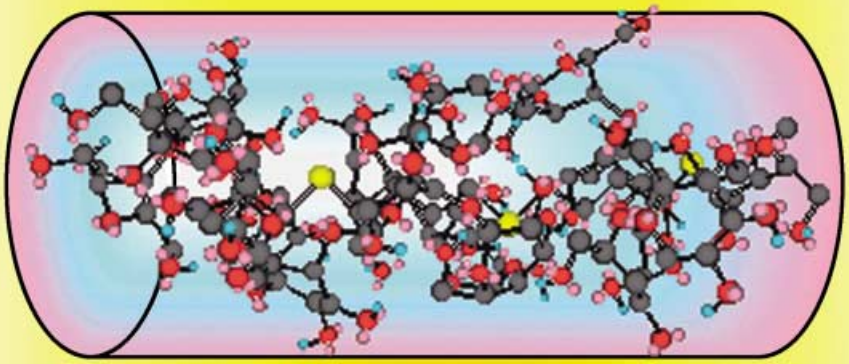
Vine-Twining Polymerization



Amylose twines around polyethers to form amylose-polyether inclusion complexes.





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  0947-6539/02/0815-3321 $ 20.00+.50/0

Vine-Twining Polymerization: Amylose Twines around Polyethers to Form Amylose – Polyether Inclusion Complexes

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Abstract: In this paper, we describe a new polymerization manner termed as "vine-twining polymerization" to produce amylose – polymer inclusion complexes. The polymerization was achieved by an enzymatic polymerization of α-D-glucose-1-phosphate monomer catalyzed by phosphorylase in the presence of polyTHF as a guest polymer. The structure of the product was determined by X-ray powder diffraction and ^{1}H NMR measurements to be the inclusion complex. The formation process of the inclusion complexes during the polymerization was also evaluated. Furthermore, the formation of the inclusion complexes by this polymerization method by using polyTHFs with various M_ns and end groups, as well as other polyethers as the guest polymers, was examined.

Keywords: carbohydrates · enzyme catalysis · helical structures · inclusion compounds

Introduction

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, for example, a double helix of DNA.[1] The research field on precision architecture of synthetic macromolecules with higher structural order, that is, "supramolecular chemistry", has recently been of importance from the viewpoint of corroboration of biological studies.[2, 3] Specially, the study on the supramolecular chemistry connected with polymerization chemistry is conceived as a significant research topic for the elucidation the biological mechanism of naturally occurring macromolecules.

Amylose, a natural polysaccharide, is a well-known host molecule that forms higher structurally ordered inclusion complexes with monomeric organic guest molecules.[4] However, the chemical formation of the complexes between amylose and polymeric guest molecules had been scarcely reported.[5, 6] The main difficulty for incorporating polymeric materials into the cavity of amylose is that the driving force for the binding is caused by only hydrophobic interactions. Amylose, therefore, does not have sufficient ability to include the long chains of the polymeric guests into its cavity.

Recently, we have found a new methodology for formation of amylose – polymer inclusion complexes by means of amylose-forming polymerization.[7] The first example of the method was achieved by an "*enzymatic polymerization*" of α-D-glucose-1-phosphate (Glc-1-P) catalyzed by phosphorylase in the presence of polyTHF as a guest polymer; this resulted in an amylose – polyTHF inclusion complex (Scheme 1).[8, 9] Polyesters, for example, a poly(ε-caprolactone), have also been used as the guest polymer in such methods, leading to the formation of an amylose – polyester inclusion complex.[10] When these guest polymers were just mixed with the synthetic amylose in the citrate buffer solution, the inclusion complexes were not obtained at all. This indicates that formation of the inclusion complexes is probably caused during the progress of the amylose-forming enzymatic polymerization in the above reaction system. The detailed formation process of the inclusion complexes during the polymerization reaction, however, has not been clear so far. Therefore, we have been carrying out studies on this type of the polymerization to reveal the formation process of the complexes. In this paper, we describe detailed studies on this type of polymerization system using polyTHFs as the guest polymers, with emphasis on understanding the relation between the formation process of the inclusion complex and the polymerization reaction. The

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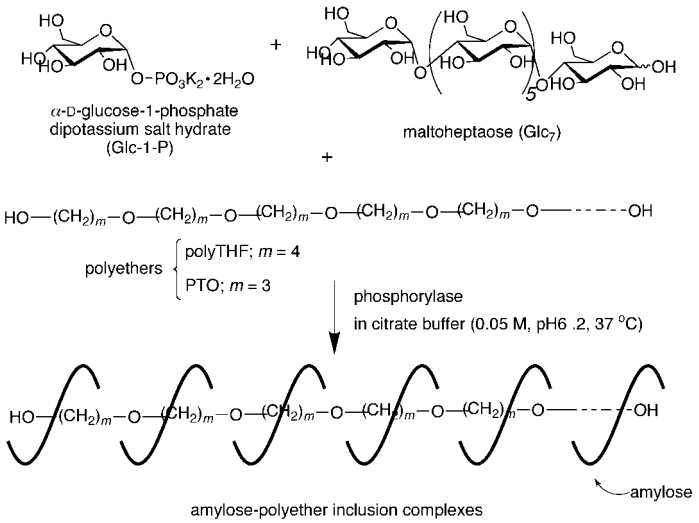

Scheme 1. Vine-twining polymerization.

image of this reaction system is similar to the way that vines of plants grow twining around a rod. Accordingly, we propose herein that the present polymerization manner for formation of polymer–polymer inclusion complexes is named as "*vine-twining polymerization*". Furthermore, we examined the formation of the inclusion complexes by this polymerization method using polyTHFs with various M_ns and end groups, as well as other polyethers as the guest polymers

Results and Discussion

Preparation of amylose–PolyTHF inclusion complexes: As previously reported,[7] 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 polyTHF with hydroxy end groups ($M_n = 4\,kg\,mol^{-1}$; units abbreviated to k in the following text: Scheme 1). The precipitated product was analyzed by X-ray powder diffraction (XRD) and ^1H NMR measurements. Figure 1a shows the XRD scan of the product in comparison with that of amylose and polyTHF (Figure 1b and 1c, respectively). The XRD scan

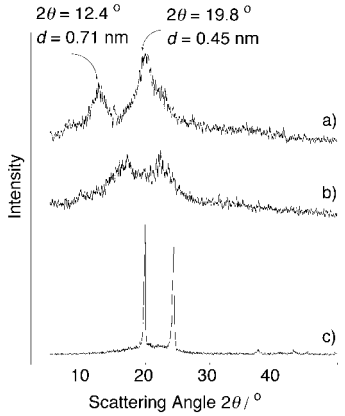

Figure 1. XRD scans of a) the product with polyTHF ($M_n = 4$ k) as the guest polymer, b) amylose, and c) polyTHF.

in Figure 1a exhibits two strong diffraction maxima at $2\theta = 12.4$ and 19.8°, corresponding to $d = 0.71$ and 0.45 nm, respectively. The XRD pattern of the product is completely different from that of amylose and polyTHF, and is similar to that of the inclusion complexes of amylose with monomeric compounds as shown in previous studies (Figure 1).[11, 12]

The ^1H NMR spectrum of the product in [D_6]DMSO shows the signal due not only to the amylose but also the polyTHF, in spite of washing with MeOH, which is a good solvent for polyTHF. Furthermore, the methylene peak H_β (C-CH_2CH_2-C) of polyTHF is broadened and shifted upfield ($\delta = 1.48$ ppm, Figure 2a) relative to that of an original polyTHF ($\delta = 1.50$ ppm, Figure 2b). A similar NMR pattern was also

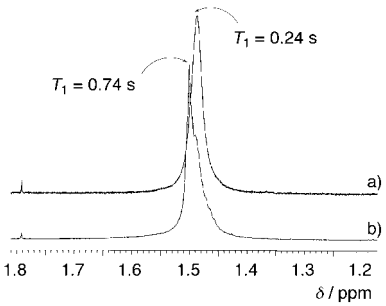

Figure 2. Expanded ^1H NMR spectra of C-CH_2CH_2-C signals of a) the inclusion complex and b) polyTHF in [D_6]DMSO.

observed in NaOD/D_2O solvent. The original polyTHF is insoluble in NaOD/D_2O, and no peak due to polyTHF appeared in the ^1H NMR spectrum of the suspension of polyTHF in NaOD/D_2O. The polyTHF in the product is probably solubilized in the alkaline solution by its inclusion in the cavity of amylose. Moreover, when polyTHF is added to the NMR sample of the product in [D_6]DMSO, two different signals due to methylene protons H_β are observed. These results suggest that the polyTHF of the product exists in a different environment from soluble polyTHF and interacts with the protons inside the cavity of the amylose. To confirm the structure of the product further, the spin-lattice relaxation time (T_1) measurement was carried out, because the T_1 measurements of inclusion complexes have often been used for the identification of their structures.[13, 14] The T_1 value of the methylene peak H_β of polyTHF in the product is 0.24 s, whereas that of the original polyTHF is 0.74 s. The shorter T_1 in the product confirms the restriction of the methylene movement due to included conditions. These XRD and NMR data can be taken to support the structure of the helical inclusion complex.

When the NMR sample of the product in [D_6]DMSO is kept at room temperature, the intensity of the methylene peak H_β of polyTHF gradually decreases and the solution becomes turbid. These observations indicate that polyTHF leaves the amylose cavity and precipitates owing to the lower solubility of the polyTHF in [D_6]DMSO. The degree of polymerization (DP) value of the precipitated polyTHF was calculated from the ^1H NMR data to be about 39 ($M_n = 2.8$ k), indicating that amylose prefers to include the lower molecular weight polyTHF present in the original polyTHF with an average

molecular weight of 4 k. The molecular weights of amylose in the inclusion complexes were 12 – 15 k as previously reported.[7]

Generally, one helical turn of amylose is composed of approximately six repeating glucose units when linear molecules of small cross-sectional area, for example, fatty acids, are included.[15–17] The repeat distance of the helix of amylose has been reported as 0.795 nm,[15–17] whereas the length of one unit of polyTHF is calculated as about 0.60 nm as shown in Figure 3.[18] Therefore, 4.5 repeating glucose units in amylose

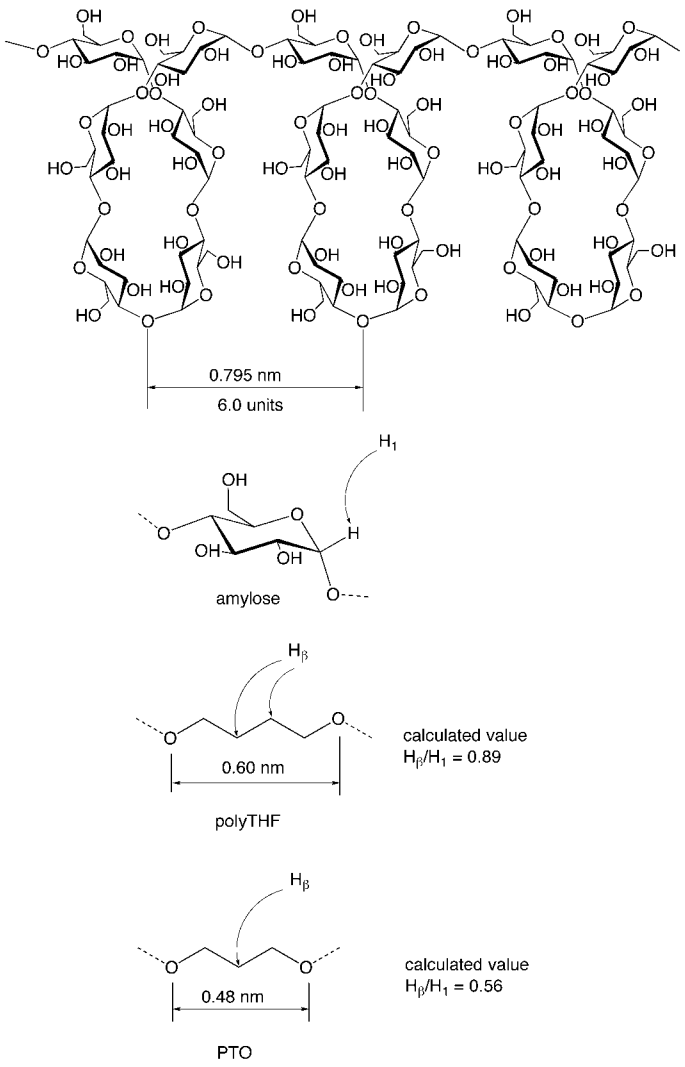

Figure 3. Illustration of repeat distance of amylose helix and unit lengths of polyTHF and PTO.

correspond to the length of one polyTHF unit. From the above calculations, the integrated ratio of the signal due to H_1 of amylose to the signal due to H_β of polyTHF (H_β/H_1) in the ^{1}H NMR spectrum should be 0.89. Actually, the integrated ratio of these two signals in the ^{1}H NMR spectrum of the product is 1.05, which is relatively close to the calculated value. This also supports the structure of the inclusion complex as shown in Scheme 1.

The preparation of the inclusion complexes by using polyTHFs with various M_ns (1, 2, 10, and 14 k) was also carried out. When the polyTHFs with M_ns = 1 and 2 k were used as the guest polymers, the XRD patterns of the products were the same as those of Figure 1a. Furthermore, the values of H_β/H_1 in the ^{1}H NMR spectra of the products are 0.90 (M_n = 1 k) and 0.91 (M_n = 2 k), which are close to the calculated value. These data indicate that inclusion complexes are also obtained when using polyTHF with M_ns = 1 and 2 k as the guest polymers. On the other hand, polyTHFs with higher M_ns than 4 k, such as 10 and 14 k, were not dispersed well in the citrate buffer of the polymerization solvent, and accordingly the inclusion complexes were not formed from these guest polyTHFs. To solve the problem, the polymerizations with these guest polymers were carried out in the following two-phase system. The polyTHF was dissolved in diethyl ether and the citrate buffer was added to the solution (1:5, v/v). Then the enzymatic polymerization of Glc-1-P took place with vigorous stirring to disperse the diethyl ether phase in the citrate buffer during the reaction. The XRD patterns of the products with polyTHFs with M_ns = 10 and 14 k obtained by the two-phase system indicated formation of the inclusion complexes. The structures of the inclusion complexes were also supported by the values of H_β/H_1 in the ^{1}H NMR spectra of the products, which were 0.80 (M_n = 10 k) and 0.83 (M_n = 14 k).

The surface morphology of the inclusion complex was investigated by atomic force microscopy (AFM). The AFM picture of the inclusion complex in Figure 4 shows striae separated by distances of 3.0 – 3.5 nm. The lateral dimensions of these striae are assumed to represent each inclusion complex. On the other hand, no such striation was observed in the AFM picture of pure synthetic amylose.

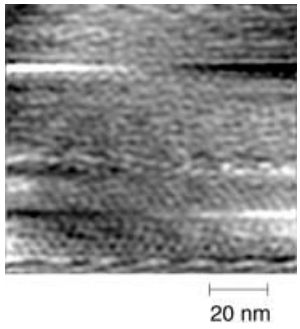

Figure 4. AFM picture of inclusion complex.

Speculation for formation process of inclusion complexes: The inclusion complex was not formed by mixing synthetic amyloses (DP = 75 – 90, M_n = 12.2 – 14.6 k) and polyTHF (M_n = 4 k) in the citrate buffer. This observation suggests that the inclusion complex forms during the enzymatic polymerization. To study the relation between formation of the inclusion complex and the enzymatic polymerization process, the following experiments were carried out. When polyTHF was added to the reaction solution immediately after the general enzymatic polymerization of Glc-1-P had started, an identical inclusion complex to that mentioned above was obtained, judging by the H_β/H_1 value in the NMR spectrum, which was approximately 1 (Figure 5a). However, the H_β/H_1 values decreased as the time delay between adding

  0947-6539/02/0815-3324 $ 20.00+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 15

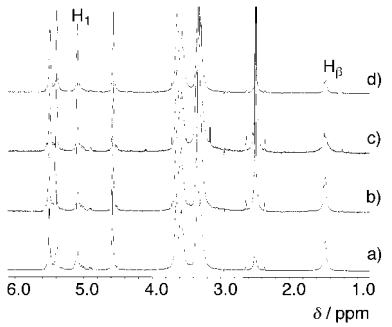

Figure 5. ^{1}H NMR spectra ([D$_6$]DMSO) of the products obtained by adding polyTHFs into the polymerization solution after a) 0 h, b) 1 h, c) 3 h, and d) 5 h after the enzymatic polymerization of Glc-1-P had started.

polyTHF into the solution and the start of the enzymatic polymerization was increased (Figure 5). The H_β/H_1 values of the products were 0.96, 0.78, and 0.33, respectively, when the times adding the polyTHFs were 1, 3, and 5 hours after the polymerization started. The amyloses obtained under these conditions were isolated and their DP values were determined by the ^{1}H NMR spectra to be 23 (1 h), 41 (3 h), and 45 (5 h). These observations reveal that the inclusion complexes were not formed after the polymerization produced amyloses with relative higher DPs. These results indicate that propagation of the enzymatic polymerization proceeds with the formation of the inclusion complex.

Effect of the end groups of polyTHFs: The effect of the end groups of the telechelic polyTHFs as the guest polymers was examined. The end groups employed were hydroxy (as mentioned above), methoxy, ethoxy, and benzyloxy groups. The products were characterized by means of the H_β/H_1 values in their ^{1}H NMR spectra. As shown in Table 1, the H_β/H_1 value of the product obtained with methoxy-terminated polyTHF is close to the calculated one, indicating formation of the inclusion complex from this guest polyTHF. The XRD scan of the product also exhibited the same pattern as that of the inclusion complex from hydroxy-terminated polyTHF. On the other hand, the H_β/H_1 value of the product from ethoxy-terminated polyTHF is much lower than the calculated one and its XRD scan did not show any clear-cut pattern. When the benzyloxy-terminated polyTHF was used, no inclusion complex was obtained. These results indicate that formation of the inclusion complex was affected by the bulkiness of the end groups of the guest polyTHFs.

Table 1. Effect of the end groups of polyTHFs.

end group	M_n[a] [kg mol^{-1}]	H_β/H_1[b]
hydroxy (OH)	4	1.05
methoxy (OCH_3)	3.2	0.89
ethoxy (OCH_2CH_3)	4.1	0.24
benzyloxy (OCH_2Ph)	3.1	0

[a] The M_n values were determined by the integrated ratios of peaks due to the end groups to the peaks due to the C-CH_2CH_2-C protons in the ^{1}H NMR spectra. [b] H_β/H_1 = the integrated ratios of the peaks due to H_1 of amyloses to the peaks due to C-CH_2CH_2-C protons of polyTHFs in the ^{1}H NMR spectra of the products.

Formation of inclusion complexes by using other polyethers as guest polymers: To investigate the effect of the alkyl chain lengths of the guest polyethers for formation of inclusion complexes, the enzymatic polymerization was carried out in the presence of polyethers with various alkyl chain lengths ($m = 2$, 3, and 4). The structures of the products were characterized by the XRD and ^{1}H NMR measurements. Figure 6 shows the XRD scans of the products from a) poly(tetramethylene oxide) (polyTHF, $M_n = 4$ k), b) poly(trimethylene oxide) (PTO, $M_n = 4$ k), and c) poly(ethylene oxide) (PEO, $M_n = 4$ k), and d) amylose. The product from PTO shows two strong diffraction maxima at $2\theta = 12.4$ and 19.8° (Figure 6b), which is the same pattern as that from polyTHF in Figure 6a. Furthermore, the ^{1}H NMR spectrum of the product in [D$_6$]DMSO shows the signals due to both amylose and PTO. The H_β/H_1 value in the NMR spectrum was 0.58, which is very close to the calculated value of 0.56 (Figure 3). These observations indicate that the inclusion complex was formed by using PTO as the guest polyether. On the other hand, the XRD pattern of the product obtained with PEO in Figure 6c is similar to that of amylose in Figure 6d. In addition, the peak due to PEO was not observed in the ^{1}H NMR spectrum of the product. No formation of the inclusion complex with PEO was detected from these analytical data. This is probably attributed to the hydrophilicity of PEO, which causes less hydrophobic interaction between PEO and the cavity of amylose. These results obtained above indicate that the hydrophobicities of the guest polymers are important factors for formation of the inclusion complexes by this type of the polymerization system.

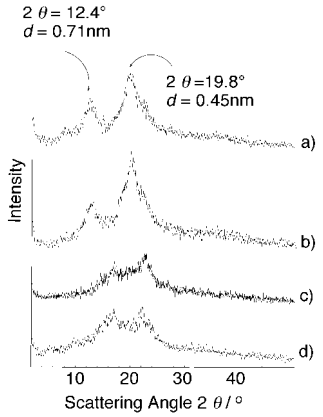

Figure 6. XRD scans of the products from a) polyTHF, b) PTO, c) PEO, and d) amylose.

Conclusion

The amylose–polyTHF inclusion complexes were obtained by means of the phosphorylase-catalyzed polymerization of Glc-1-P in the presence of polyTHFs. Another polyether, poly(trimethylene oxide), was also employed as the guest polymer for the polymerization system, giving rise to the corresponding inclusion complex. The experimental results in this paper suggest that the propagation of the polymerization

proceeds helically around the guest polyether chains, resulting in the inclusion complexes, similar to the way that vines of plants grow and twine around a rod. Therefore, we named this type of polymerization "vine-twining polymerization".

Experimental Section

Materials: Hydroxy-terminated polyTHFs with various M_ns were supplied from Hodogaya Chemical Co. and used as received. Methoxy-terminated polyTHF was prepared by ring-opening polymerization of THF initiated with methyl trifluoromethanesulfonate (MeOTf), followed by treatment with sodium methoxide, according to the usual manner for the ring-opening polymerization of THF. Ethoxy-terminated polyTHF was also prepared according to the same procedures by using EtOTf and sodium ethoxide. Benzyloxy-terminated polyTHF was prepared by ring-opening polymerization of THF according to the procedures described in the literature,[19] followed by termination with sodium benzyloxide. Phosphorylase (E.C.2.4.1.1), maltoheptaose, and PTO were prepared according to the literature.[20–22] Other reagents and solvents were used without further purification.

Typical procedures for preparation of inclusion complex: PolyTHF with hydroxy end groups ($M_n = 4$ k, 50.0 mg) was suspended in sodium citrate buffer (5.0 mL, 0.05 mol L^{-1}, pH = 6.20) with ultrasonic wave and heated to 37 °C. After addition of of maltoheptaose (Glc_7) primer (2.31 mg, 2 μmol), α-D-glucose 1-phosphate dipotassium salt hydrate (Glc-1-P; 186 mg, 500 μmol), and phosphorylase (6.40 mg; approximately 160 units; the unit definition is that one unit will form 1.0 μmol of Glc-1-P from glycogen and orthophosphate per min at pH 6.8 at 30 °C), the solution was stirred vigorously for 10 h at 37 °C. The precipitated product was collected by centrifugation, washed with methanol and water, and then lyophilized, to yield approximately 20 mg of the inclusion complex (yields ca. 21 % based on Glc-1-P and Glc_7, and ca. 4 % based on polyTHF); ^{1}H NMR (500 MHz, [D_6]DMSO) $\delta = 5.51$ (m, 1H; O-2 H of amylose), 5.41 (s, 1H; O-3 H of amylose), 5.10 (s, 1H; C-1 H of amylose), 4.59 (s, 1H; O-6 H of amylose), 3.65 – 3.58 (m, 4H; C-3 H, C-6 H, C-5 H of amylose), 3.40 – 3.28 (m; C-2 H, C-4 H of amylose, O-CH_2 of polyTHF, overlapping with HOD), 1.48 (s, 4H; C-CH_2CH_2-C of polyTHF).

Measurements: ^{1}H NMR spectra (500 MHz) were recorded on a Varian INOVA 500 spectrometer. Powder X-ray diffraction spectra were recorded on a Rigaku powder diffractometer unit, with $Cu_{K\alpha}$ (filtered) radiation ($\lambda =$ 0.154 nm) at 40 kV and 20 mA between 1.8 and 50° in two theta. AFM pictures were obtained on a SHIMADZU SPM-9500A in air in contact force mode.

Acknowledgements

We wish to thank Dr. H. Oikawa of Tohoku University and Dr. P. Qian of Tohoku National Industrial Research Institute for their support in AFM measurements. We acknowledge the gift of the polyTHF samples from Hodogaya Chemical Co., Kanagawa. We are also indebted to M. Karasu and A. Nakaya of our research group for technical assistance.

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Received: January 21, 2002 [F3813]

  0947-6539/02/0815-3326 $ 20.00+.50/0