ChemComm

Synthesis of an amylose–polymer inclusion complex by enzymatic polymerization of glucose 1-phosphate catalyzed by phosphorylase enzyme in the presence of polyTHF: a new method for synthesis of polymer–polymer inclusion complexes

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Received (in Cambridge, UK) 10th October 2000, Accepted 23rd January 2001
First published as an Advance Article on the web 14th February 2001

The enzymatic polymerization of α-D-glucose 1-phosphate (Glc-1-P) with phosphorylase in the presence of polytetrahydrofuran (polyTHF) leads to an amylose–polyTHF (polymer–polymer) inclusion complex; the present reaction system provides a new method for the preparation of polymer–polymer inclusion complexes.

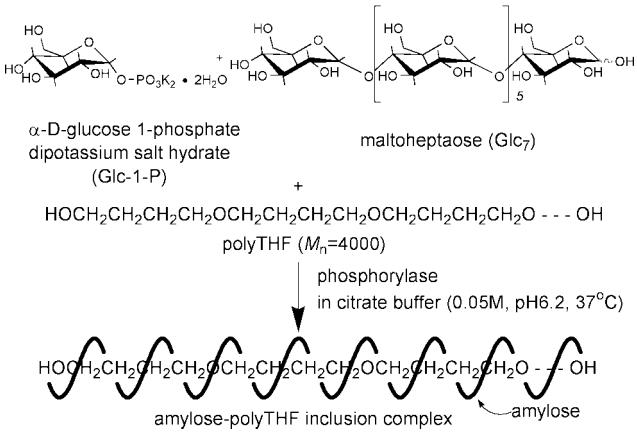
Recent studies on molecular recognition and self-assembly in the field of supramolecular chemistry have received considerable attention because of possible applications in numerous scientific fields, such as materials science and chemical sensing.[1] As it concerns those research fields, host–guest chemistry, which is often compared to the relation between an enzyme and a substrate, has been of importance for chemists, from the viewpoint not only of pure chemistry but also in connection with biological work.[2] Representative host molecules have cyclic structures like crown ethers[3] and cyclodextrins.[4] Amylose, a natural linear polysaccharide linked through (1→4)-α-glycosidic linkages, is also a well-known host molecule forming helical inclusion complexes with monomeric organic compounds by hydrophobic interaction between guest molecules and the cavity of amylose.[5] Although amylose has been reported to form inclusion complexes with a few polymeric guest molecules, *i.e.* polymer–polymer inclusion complexes,[6] the scope and limitation for formation of polymer–polymer inclusion complexes using not only amylose but also the other host molecules has not been well surveyed. The polymeric hosts may not have sufficient ability to include the long chains of the polymeric guests directly into those cavities.

Amylose has been prepared by an *in vitro* approach from α-D-glucose 1-phosphate (Glc-1-P) monomer catalyzed by phosphorylase enzyme.[7] 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 non-reducing 4-OH terminus of a (1→4)-α-glucan chain, resulting in inorganic phosphate (P).

$$((\alpha, 1 \rightarrow 4)\text{-Glc})_n + \text{Glc-1-P} \rightleftharpoons ((\alpha, 1 \rightarrow 4)\text{-Glc})_{n+1} + \text{P}$$

This polymerization forming amylose has inspired us to develop a new method for preparation of polymer–polymer inclusion complexes, because we have assumed that the polymerization proceeds with the formation of an inclusion complex when the enzymatic polymerization is carried out in the presence of a hydrophobic synthetic guest polymer. Here, we report this new method for preparation of the amylose–polymer inclusion complex by enzymatic polymerization of Glc-1-P monomer catalyzed by phosphorylase enzyme in the presence of polyTHF as a hydrophobic polymer (Scheme 1).†

When the enzymatic polymerization of Glc-1-P from Glc_7 as a primer catalyzed by the phosphorylase (E.C.2.4.1.1)[8] in citrate buffer was carried out in the presence of polyTHF (M_n = 4000), the inclusion complex was obtained (Scheme 1), and its structure was characterized by X-ray powder diffraction and ^{1}H NMR measurements.



Scheme 1

The X-ray powder diffraction scan of the product indicates two strong diffraction maxima at 2θ = 12.4 and 19.8°, corresponding to d = 7.1 and 4.5 Å, respectively. The X-ray 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.[9] These data indicate that the product has a conformation similar to that of the helical inclusion complexes obtained from amylose and monomeric guests.

The ^{1}H NMR spectrum in DMSO-d_6 of the product in Fig. 1 shows the signals due not only to the amylose but also the polyTHF, in spite of the washing with MeOH, which is a good solvent of polyTHF. Furthermore, the methylene peak $\mathbf{H_a}$ of polyTHF is broadened and shifts to upfield (δ 1.48) compared to that of the original polyTHF (δ 1.50). This is because each methylene group of polyTHF is basically immobile and interacts with the protons inside the cavity of the amylose. When polyTHF was added to the NMR sample of the product in DMSO-d_6, two different signals due to methylene protons $\mathbf{H_a}$ of polyTHF were observed. This result suggests that the polyTHF of the product exists in a different environment. These NMR data can be taken to support the structure of the helical inclusion complex, which was also confirmed based on the spin-lattice relaxation time (T_1) measurements in the ^{1}H NMR analysis.‡ The T_1 value of the methylene peak $\mathbf{H_a}$ of polyTHF in the product was 0.24 s, whereas that of the original polyTHF was 0.74 s. The shorter T_1 in the product confirms the restriction of the methylene movement due to included conditions.

When the NMR sample was kept at rt, the intensity of the methylene peak $\mathbf{H_a}$ of polyTHF gradually decreased and the solution became turbid. These observations indicate that polyTHF was coming out of the amylose cavity and precipitating owing to the relative lower solubility of the polyTHF in DMSO-d_6. The degree of polymerization (DP) value of the precipitated polyTHF was calculated from ^{1}H NMR analyses to be *ca.* 39 (M_n = 2800), indicating that amylose preferred to

DOI: 10.1039/b008180i

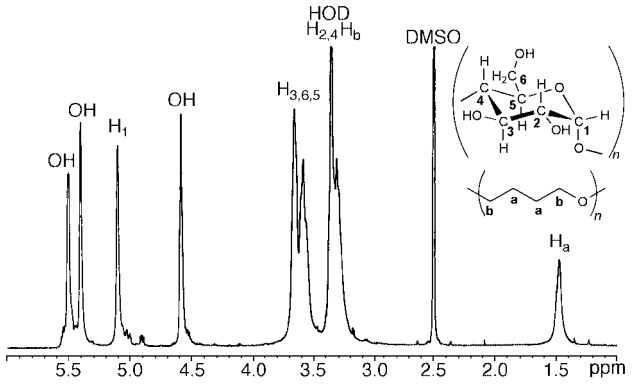


Fig. 1 ^{1}H NMR spectrum of the product in DMSO-d_6. Chemical shifts were referenced to DMSO (δ 2.50 ppm).

include the relative lower molecular weight polyTHF present in the original polyTHF, with an average molecular weight of 4000 (by ^{1}H NMR); the molecular weight was also estimated by gel permeation chromatographic (GPC) measurement as 3200.

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.[10] The repeat distance of the helix of amylose has been reported as 7.95 Å,[10] whereas the length of one unit of polyTHF is presently calculated as *ca*. 6.0 Å,§ as shown in Fig. 2. Therefore, 4.5 repeating glucose units in amylose correspond to the length of one polyTHF unit (Fig. 2). From the above calculations, the integrated ratio of the signal due to **H_1** of amylose to the signal due to **H_a** of polyTHF (H_a/H_1) in the ^{1}H NMR spectrum is assessed to be 0.89. Actually, the integrated ratio of these two signals in the ^{1}H NMR spectrum of the product was *ca*. 1, relatively close to the calculated value. This also supports the structure of the inclusion complex as shown in Scheme 1.

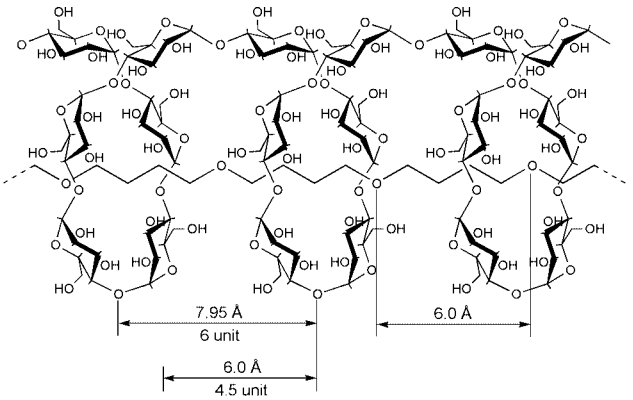


Fig. 2 Illustration of repeat distance of amylose helix and length of one polyTHF unit.

The molecular weight of the amylose in the inclusion complex was evaluated by means of GPC measurement after the acetylation of the inclusion complex and the precipitation of the products into i-PrOH.[11] The GPC value of the precipitated triacetyl amylose was 22800 (M_w/M_n = 1.29), which corresponds to M_n = 12800 of the original amylose. This value is in good agreement with the molecular weight values determined by ^{1}H NMR spectra (M_n = 12200–14600, DP = 75–90).¶ These values correspond to 99–119 Å of molecular lengths in helical form,‖ whereas the chain length of the included polyTHF (M_n = 2800) is calculated as *ca*. 230 Å.§ Therefore, one polyTHF molecule is probably included by two amylose molecules.

The inclusion complex was not formed by mixing amylose (DP = 75–90, M_n = 12200–14600) and polyTHF (M_n = 4000) in the same solvent as described above (sodium citrate buffer, 0.05 mol L^{-1}, pH = 6.20) at 37 °C. This observation suggests that the inclusion complex forms during the enzymatic polymerization. Such a formation behavior for the inclusion complex was further supported by the following experiments. 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_a/H_1 value in the NMR spectrum, which was *ca*. 1. However, the H_a/H_1 values decreased as the time delay between adding polyTHF into the solution and the start of the enzymatic polymerization was increased (after 1 h; H_a/H_1 = 0.96, after 3 h; H_a/H_1 = 0.78, after 5 h; H_a/H_1 = 0.33). These observations reveal that the inclusion complex was not formed after the polymerization produced amyloses with relative higher molecular weights. These results indicate that polymerization proceeds with the formation of the inclusion complex.

In conclusion, we have synthesized an amylose–polyTHF (polymer–polymer) inclusion complex by the enzymatic polymerization of Glc-1-P with phosphorylase in the presence of polyTHF. The present reaction system provides a new method for the preparation of polymer–polymer inclusion complexes. Detailed studies on how the complex forms during the polymerization process are now in progress.

We acknowledge the gift of the polyTHF sample from Hodogaya Chemical Co., Ltd., Kanagawa. We are also indebted to Ms M. Karasu and Messrs K. Fujita and S. Nagase of our research group for performing X-ray measurement and technical assistance.

Notes and references

† Synthesis of the amylose–polyTHF inclusion complex: typically, polyTHF (M_n = 4000, 50.0 mg) was suspended in 5 mL of sodium citrate buffer (0.05 mol L^{-1}, pH = 6.20) with ultrasonication and heated to 37 °C. After addition of 2.31 mg (2 μmol) of maltoheptaose (Glc_7) primer, 186 mg (500 μmol) of α-D-glucose 1-phosphate dipotassium salt hydrate (Glc-1-P), and 6.40 mg (~160 units) of phosphorylase, the solution was stirred vigorously for 10 h at 37 °C. The precipitated product was collected by centrifugation, washed with MeOH and water, and then lyophilized, to yield *ca*. 20 mg of the inclusion complex (yields *ca*. 21% based on Glc-1-P and Glc_7, and *ca*. 4% based on polyTHF).

‡ The measurements of T_1 values of inclusion complexes have often been used for the identification of their structures. The T_1 values of the inclusion complexes are shorter than those of the corresponding individual molecules.[3]

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

¶ The DP values were calculated by the integrated ratio of the peak of **H_1** of amylose chain (δ 5.1) to the peaks of **H_1** (α and β) of the reducing terminus (δ 4.9 (α) and 4.3 (β)) by the ^{1}H NMR spectra in DMSO-d_6–D_2O. The DPs were changeable depending on reaction conditions, especially the activity of enzyme.

‖ The reported DP 75 and 90 corresponds to 75/6 = 12.5 and 90/6 = 15 helical turns, and hence 12.5 × 7.95 Å = 99 Å and 15 × 7.95 Å = 119 Å represent the length of helical amylose and not that of the extended molecule.

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