Specific Preparation of Artificial Xylan: A New Approach to Polysaccharide Synthesis by Using Cellulase as Catalyst

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Xylan, a xylose polymer having a $\beta(1\rightarrow4)$ glycosidic linkage in the main chain, is one of the most important components of hemicellulose in plant cell walls. Many studies on structure determinations, biosyntheses, and the chemical and physical properties of xylan have been performed in view of both fundamental science and practical applications.[1] Xylan is also an interesting macromolecule due to its supramolecular interaction with cellulose.[2] Naturally occurring xylan normally contains L-arabinose and 4-*O*-methylglucuronic acid as side chains, its hydroxy group often being acetylated, and occasionally contains the $\beta(1\rightarrow3)$ glycosidic linkage as minor unit. The synthesis of oligo- and polysaccharides consisting exclusively of a xylopyranose moiety connected through a $\beta(1\rightarrow4)$ glycosidic bond has, therefore, been a challenging problem in the synthetic field of polysaccharide chemistry.[3]

Recently, the use of an enzyme in glycosylation processes has become one of the most promising synthetic tools for construction of stereoregular oligo- or polysaccharides.[4] In the previous paper, we reported the first *in vitro* synthesis of cellulose, a stereoregular glucose polymer having a $\beta(1\rightarrow4)$ linkage, by utilizing β-cellobiosyl fluoride as a substrate monomer for cellulase, a hydrolytic enzyme of cellulose.[5] The stereochemistry of the glycosylating process between the anomeric center and the 4′-hydroxy group of each monomer was found to be under perfect control, leading to a selective formation of $\beta(1\rightarrow4)$ glycosidic bonds. The present communication deals with the first synthesis of artificial xylan, a linear xylose polymer composed exclusively of xylopyranose units, by a transglycosylation reaction catalyzed by cellulase with use of β-xylobiosyl fluoride **1** as a substrate monomer. It was postulated that a xylobiose (disaccharide) derivative would be a preferable substrate since it can be recognized by the catalytic site of the enzyme more strongly than a xylose (monosaccharide) derivative.[6] The β configuration of the anomeric fluorine atom of the substrate monomer was designed for the formation of a reactive intermediate, leading to a $\beta(1\rightarrow4)$ product (xylan) via the double-displacement mechanism at the active site of the enzyme.[5,7]

First, the enzyme specificity of the substrate monomer **1** for cellulase was tested. Figure 1 shows the hydrolytic behavior of **1** catalyzed by cellulase (*Trichoderma viride*) in deuterium oxide. The concentration of **1** was followed by means of ^{19}F NMR spectroscopy. When the solution was kept at 25 °C, a slight decrease of the substrate concentration was observed within the range of 0−60 min, indicating that a very slow hydrolysis of **1** (the carbon−fluorine bond cleavage to produce xylobiose) occurred even in the absence of cellulase. The addition of cellulase (shown by the arrow) caused a rapid hydrolysis within 40 min. The results clearly indicate that the monomer **1** can be recognized by cellulase and the carbon−fluorine bond is activated for hydrolysis.

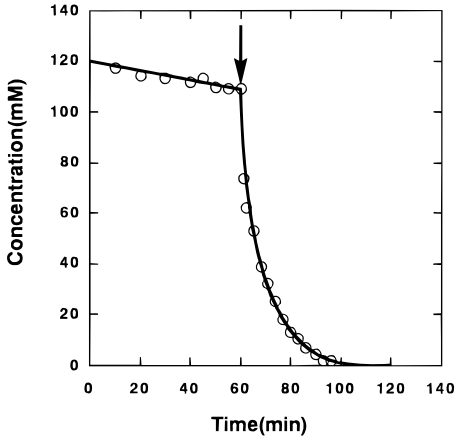
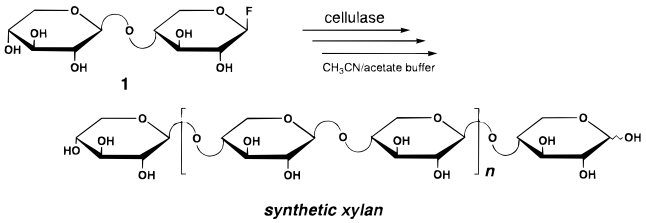


Figure 1. Cellulase-catalyzed hydrolysis (C−F bond cleavage) of β-xylobiosyl fluoride **1** in deuterium oxide. ↓: addition of cellulase.

Scheme 1



When the reaction was carried out under the polymerization conditions by adding acetonitrile as cosolvent, the substrate monomer **1** polymerized smoothly to afford the corresponding polycondensation products.

Table 1 summarizes the results of the solvent effect on the polymerization of **1** catalyzed by cellulase. The enzymatic polymerization reaction of **1** took place effectively when the reaction was carried out in an organic−water mixed solvent system. Of the organic solvents screened, acetonitrile was found to be the most effective for promotion of the polycondensation reaction. The ratio of acetonitrile to acetate buffer was also optimized, and the mixed solvent with the ratio of 5:1 gave the best result with respect to the yield of water-insoluble product (yield in parentheses in Table 1). In the absence of the cellulase catalyst in this solvent system, no condensation products were obtained. Acetone and methanol were also usable as cosolvents.

A typical procedure for the enzymatic polymerization is as follows: To a mixture of β-xylobiosyl fluoride[8] (1.0 g, 3.5 mmol) in a mixed solvent to acetonitrile (117.4 mL) and acetate buffer (pH = 5.0, 0.05 M) (7.8 mL) was added cellulase (50 mg, 75 units, 5.0 wt % for the substrate) from *Trichoderma viride* (activity with *O*-(carbomethoxy)cellulose: 1.5 units/mg solid, pH 4.5, 40 °C) in acetate buffer (15.6 mL), and the mixture was stirred at 25 °C. As the reaction proceeded, the initially homogeneous solution gradually became heterogeneous with a white precipitation of the product polysaccharide. After 2 h, the resulting suspension was heated at 90 °C for 10 min to inactivate the enzyme, and the precipitate (0.72 g) was centrifuged (4000 rpm, 10 min), decanted, and washed with distilled water (4 mL) two times to remove low molecular weight products (xylose, xylobiose, and xylooligomers of DP = 3−6), and dried in vacuo (P_2O_5) to give a white powdery water-insoluble product (0.37 g).

The cross-polarization/magic angle spinning (CP/MAS) solid-state ^{13}C NMR spectrum of the water-

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Table 1. Enzymatic Polymerization of 1 Catalyzed by Cellulase in Various Solvents[a]

entry	solvent[b]	yield (%)[c]
1	CH_3CN/buffer	72 (37)
2	$(CH_3)_2CO$/buffer	40 (22)
3	C_2H_5OH/buffer	28 (14)
4	CH_3OH/buffer	10 (4)
5	2-propanol/buffer	1 (∼0)

[a] Polymerized at ambient temperature (25 °C) for 2 h. [**1**] = 2.5×10^{-2} mol/L; cellulase, 5 wt % for **1**. [b] Organic solvent/0.05 M acetate buffer (pH = 5.0) = 5:1. [c] Isolated yield (weight percent of the product to **1**); solvent (organic solvent/acetate buffer = 5:1). In parentheses, the yield of the water-insoluble part is given.

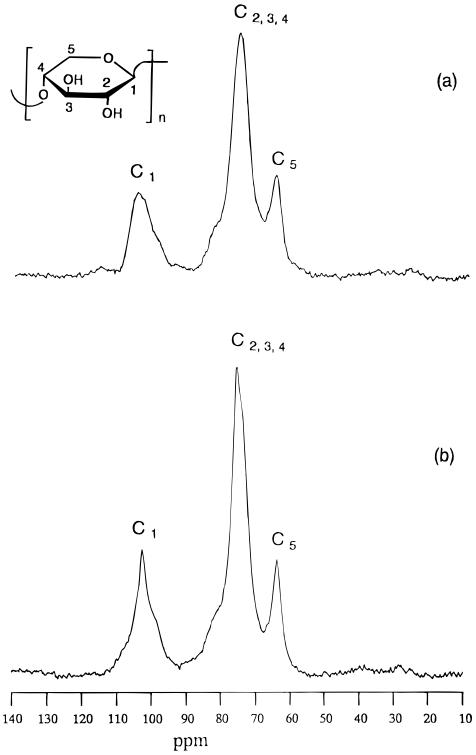

Figure 2. CP/MAS ^{13}C NMR spectrum of (a) artificial xylan and (b) natural xylan (from oat spelt).

insoluble product (Figure 2(a)) shows a signal at δ 103.5 ppm due to the C1 of the xylose unit. The signals ascribable to C2, C3, and C4 are observed in the region of δ 70−80 ppm. The signal at δ 64.1 ppm is assignable to C5. No signals from the methine carbon C3 (δ 85 ppm) adjacent to a $\beta(1\rightarrow3)$ glycosidic bond were observed in the products,[9] indicating that only products with $\beta(1\rightarrow4)$ linkages were formed. The CP/MAS ^{13}C NMR spectrum of a natural xylan (from oat spelt) is given as a reference (Figure 2(b). Each signal in the spectrum of the water-insoluble product (Figure 2a) corresponds to the signal in the spectrum of the natural xylan (Figure 2b). The shape of the signal in spectrum a is, however, slightly broader than that in spectrum b. This difference may be derived from the absence or existence of the minor branched unit (e.g., L-arabinose or 4-*O*-methylglucuronic acid) in the resulting polysaccharide and natural xylan, respectively. It is well accepted that even the existence of a small amount of a minor component causes a significant change for the crystallinity of xylan derivatives (the weight percent of the carbon atom due to the methoxy group of natural xylan is reported to be less than 2%).[10] The ^{13}C NMR spectrum of the water-soluble oligosaccharides in D_2O solution also indicated signals assignable to the corresponding carbon atom (C1−C5) of oligosaccharides having a $\beta(1\rightarrow4)$ linkage.

Treatment of the water-insoluble product with xylanase, a hydrolytic enzyme of xylan, in acetate buffer quantitatively afforded hydrolysis products of xylose and xylobiose. These results clearly indicate that the glycosidic bond formation occurred in a regio- and stereoselective manner between xylobiose units to afford a stereoregular polysaccharide having a $\beta(1\rightarrow4)$ linkage (*artificial xylan*).

In order to determine the molecular weight of the artificial xylan, it was converted to the corresponding carboxymethyl derivative using chloroacetic acid/NaOH. The gel permeation chromatographic (GPC) analysis of the carboxymethylated product revealed that the number-average molecular weight is at least 6.7×10^3 (water eluent containing 1 wt % lithium chloride, poly(ethylene glycol) standard), which corresponds to a degree of polymerization (DP) of 23.

The formation of the stereoregular polysaccharides can be explained by assuming the formation of a glycosyl−enzyme intermediate or a glycosyl oxocarbenium ion intermediate at an active site of cellulase with the elimination of fluoride anion. This reactive intermediate is then attacked by the 4′-hydroxy group of another molecule of monomer or oligomer which locates in a subsite of the enzyme, leading to the stereoselective formation of the $\beta(1\rightarrow4)$ linkage. Consequently, the stereochemistry of the product is retention of configuration via double inversion of the configuration concerning the C1 carbon atom of the β-xylobiosyl fluoride as observed for the cellulose synthesis.[5]

It is to be noted that different from natural xylan the artificial xylan prepared by the present enzymatic polymerization consists exclusively of D-xylopyranose residues with neither L-arabinose nor D-glucuronic acid as side chains. Further studies concerning the detailed reaction mechanism and inherent properties of the resulting artificial xylan are now in progress.

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