Novel Synthetic Pathway to a Poly(phenylene oxide). Laccase-Catalyzed Oxidative Polymerization of Syringic Acid

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These has been much interest in polymerizations catalyzed by enzymes ("enzymatic polymerizations") as a new methodology of polymer syntheses.[1,2] Typical examples are the first in vitro synthesis of cellulose by cellulase-catalyzed polymerization of β-cellobiosyl fluoride via a nonbiosynthetic path[3] and the lipase-catalyzed ring-opening polymerization of macrolides, in which the macrolide showed unusually high reactivity in the catalysis.[4,5]

Poly(1,4-oxyphenylene) (poly(phenylene oxide), PPO) is widely used as high-performance engineering plastics, since the polymer shows high thermal stability and chemical resistance.[6] PPO is industrially prepared from 2,6-disubstituted phenol monomers using a copper/amine catalyst system.

Recently, enzymatic synthesis of polyaromatics has been developed.[7−12] Peroxidase-catalyzed polymerization of phenol and its various derivatives proceeded to give polymers with a complicated structure. The main structure was estimated to be of phenylene units[8] or of a mixture of phenylene and oxyphenylene units.[9,10] Bilirubin oxidase catalyzed the polymerization of 1,5-dihydroxynaphthalene to give the polymer, hardly soluble in common solvents.[11] The polymerization proceeded regioselectively to a phenylene polymer. *o*-Phenylenediamine was polymerized by peroxidase catalyst to give a soluble polymer having an iminophenylene unit, which is hard to synthesize by conventional oxidative polymerizations.[12] The present study deals with the first enzymatic synthesis of a PPO by oxidative polymerization of 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid) in an aqueous organic solvent. The present polymerization is a new type of the enzymatic polymerization involving eliminations of carbon dioxide and hydrogen from the monomer.

Laccase (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) has a role in lignin degradation in vivo.[13] In some cases, laccase preferentially catalyzed the polymerization of lignin-related substrates, leading to the formation of lignin-analogue polymers.[14] The laccase-catalyzed reaction of syringic acid in a buffer solution was reported,[15] in which the formation of oligo(phenylene oxide)s up to hexamer was observed but the detailed characterization of the oligomers was not well performed. In previous studies on the enzymatic oxidative polymerization of phenol derivatives using horseradish peroxidase catalyst, a mixture of a polar organic solvent and buffer was used as solvent to increase the solubility of the polymer formed during the reaction.[7−10] Here, the polymerization of syringic acid using laccase catalyst has been examined in an aqueous organic solvent.

The polymerization was carried out in acetone/acetate buffer (pH 5) (50:50 vol %) at room temperature under air for 24 h.[16] Laccase derived from *Pycnoporus coccineous* was used as catalyst. During the polymerization, powdery materials were formed. After 24 h, the monomer was consumed quantitatively. The yield of the methanol-insoluble part was 84%. The isolated polymer is soluble in common polar organic solvents, and its molecular weight was determined by gel permeation chromatography (GPC) to be 4.2×10^3. Interestingly, a polymer was not obtained from 2,6-dimethoxyphenol under similar reaction conditions.

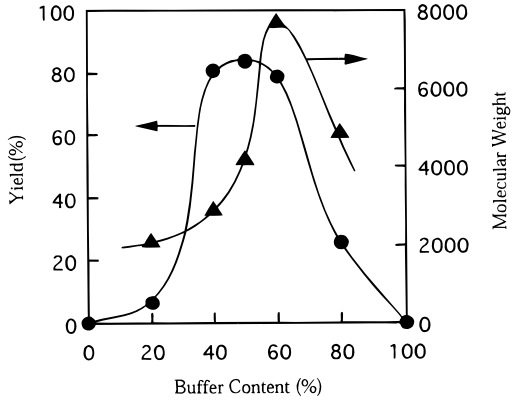

Figure 1. Effect of buffer content on the polymer yield and molecular weight. The polymerization was performed in a mixture of acetone and acetate buffer (pH 5) at room temperature for 24 h under air.

Scheme 1

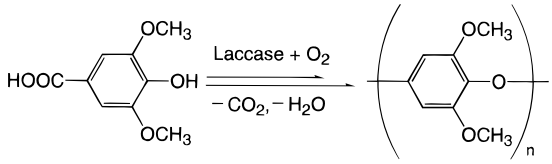

Table 1. Laccase-Catalyzed Oxidative Polymerization of Syringic Acid[a]

entry	solvent composition[b]	yield[c](%)	mol wt[d]
1	acetone (12.5)/buffer[e] (12.5)	84	4200
2	acetonitrile (12.5)/buffer[e] (12.5)	81	3800
3	1,4-dioxane (12.5)/buffer[e] (12.5)	86	2700
4	ethanol (12.5)/buffer[e] (12.5)	64	2800
5	methanol (12.5)/buffer[e] (12.5)	63	2400
6	methyl ethyl ketone (12.5)/buffer[e] (12.5)	62	3400
7	tetrahydrofuran (12.5)/buffer[e] (12.5)	84	3700
8	acetone (10)/buffer[e] (15)	80	7700
9	acetone (10)/chloroform (5)/buffer[e] (15)	82	11000
10	acetone (10)/chloroform (15)/buffer[e] (15)	83	18000

[a] Polymerization was performed at room temperature for 24 h under air. [b] In parentheses, solvent volume (mL). [c] Methanol-insoluble part. [d] Molecular weight of the peak-top determined by GPC using the chloroform eluent calibrated with polystyrene standards. [e] Acetate buffer (pH 5).

The polymer structure was confirmed by NMR and IR spectroscopies. There are five main peaks in the ^{13}C NMR spectrum of the polymer: four peaks at δ 92, 125, 153, and 156 due to the aromatics and a peak at δ 56 due to the methoxy carbon. The ^{1}H NMR spectrum shows two singlet peaks at δ 6.2 and 3.6, which are ascribed to protons of the phenyl and methoxy groups, respectively. In the IR spectrum, a characteristic peak at 1218 cm^{-1} ascribed to the C−O−C vibration of the phenylene oxide unit is observed. These results indicate the formation of poly(2,6-dimethoxy-1,4-oxyphenylene) by the enzymatic polymerization of syringic acid (Scheme 1).

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The effect of the buffer content in the mixed solvent (acetone/acetate buffer) on the polymer yield and the molecular weight is shown in Figure 1. The polymer was not obtained in cases of using either acetone or the buffer. The acetone content of 50% showed the highest yield. The highest molecular weight was obtained as 7.7×10^3 in the 40% content of acetone.

Organic solvents also affected the polymerization behavior (Table 1). The polymer was obtained in the equivolume mixture of various water-miscible organic solvents and the acetate buffer (entries 1−7). The use of acetone, acetonitrile, 1,4-dioxane, and tetrahydrofuran gave good results; the polymer yield was over 80%, and the molecular weight of the polymer was more than 3×10^3 (entries 1, 2, 3, and 7). The addition of 5 mL of chloroform, a very good solvent for the present polymer, to the mixed solvent of acetone and the acetate buffer (25 mL, 40:60 vol%) brought about the molecular weight increase of the product polymer to 1.1×10^4 in 82% yield (entry 9). When 15 mL of chloroform was added, the molecular weight further increased to 1.8×10^4 (entry 10). This powdery polymer sample possessed a glass transition temperature of 162 °C and no clear melting point below 300 °C measured by the differential scanning calorimeter.

In conclusion, PPO was synthesized by the enzymatic oxidative polymerization of syringic acid using laccase catalyst in the mixed solvent of a polar organic solvent and the acetate buffer. Oxidative polymerizations usually involve dehydrogenation of monomers, whereas eliminations of carbon dioxide as well as hydrogen from the monomer took place during the present polymerization. Further studies including the mechanism of the polymerization and the enzymatic synthesis of other PPO derivatives are now under way in our laboratory.

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(16) The following is a typical procedure for the polymerization (entry 1 in Table 1). Syringic acid (0.50 g, 2.5 mmol), a commercial reagent, was dissolved in a mixture of acetone (12.5 mL) and acetate buffer (12.5 mL). The polymerization was started by the addition of laccase solution (2.95 mg of protein). The reaction mixture was vigorously stirred under air at room temperature for 24 h. The solvent was evaporated under reduced pressure, and the residue was washed successively with water and methanol, followed by drying in vacuo to give 0.32 g of the polymer (yield 84%).

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