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ORIGINAL ARTICLE

Horseradish peroxidase-catalyzed polymerization of *ortho*-imino-phenol: Synthesis, characterization, thermal stability and electrochemical properties

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Abstract Enzymatic polymerization of phenols has been investigated extensively over the last decades. However, involving imine functional group in the side chain of an oligophenol and its effect on polymerization is poorly understood. Therefore, the influence of the imine functionality in the side chain of oligophenol for enzymatic polymerization is explored in this work. *Ortho*-imine substituted phenol, (*E*)-2-((*p*-tolylimino)methyl)phenol (PTIMP), was enzymatically polymerized using horseradish peroxidase (HRP) enzyme in aqueous organic solvents and hydrogen peroxide (H_2O_2) as an oxidant. Different parameters (solvent system, pH and reaction temperature) on polymerization were investigated. EtOH/pH 6.0 buffer (50:50 vol.%) at 25 °C in 24 h under air was found to be the optimum polymerization condition with 65% of yield and M_n = 6100 g/mol (DP ≈ 29, PDI = 1.09). Polymerization of PTIMP in the presence of HRP enzyme catalyst leads to the formation of an oligophenol containing phenylene and oxyphenylene repeat units. The resulting oligophenol is soluble in most of the organic solvents. Characterization of oligo(PTIMP) was achieved by NMR, UV–Vis, CV, FT-IR spectroscopy and thermogravimetric analysis.

1. Introduction

Enzymes have gained increasing attention due to their unique properties including high stability, selectivity, catalytic activity, low solubility in water and low toxicity. Enzymes have been used in a wide range of fields including biochemistry, chemistry, pharmaceutical applications, medicine and industry (food, textile, and etc.) [1,2]. Enzymes are usually derived from renewable resources and known as green catalysts. For exam-

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ple, Horseradish peroxidase (HRP) enzyme is obtained from horseradish plant [3].

In the last few decades, enzymatic polymerizations of phenolic compounds have been extensively investigated by many polymer research groups since they require milder reaction conditions (physiological pH, ambient temperature and pressure) and facile methods [4,5]. Oxidative polymerizations of a variety of phenols catalyzed by peroxidase enzymes produced various polyphenols with excellent toughness and high thermal stabilities [6]. Dordick et al. reported the first study on the polymerization of phenolic compounds using HRP enzyme in aqueous organic solvents [7]. HRP is a widely used enzyme catalyst in enzymatic reactions and has received considerable attention due to its catalytic properties under a variety of conditions. HRP enzyme activated by hydrogen peroxide (H_2O_2) is known to oxidize a wide range of aromatic compounds including phenols, anilines, phenolic acids, amines, indoles, and sulfonates to form free radicals, which then undergo polymerization via Carbon–Carbon (C–C) and Carbon–Oxygen (C–O) couplings [8,9]. Polymerization occurs via couplings of phenolic monomers through *ortho*- and *para*-positions given in the formation of phenylene and oxyphenylene repeat units.

Phenol–formaldehyde resins are extensively used in industrial applications as surface coatings, laminates, adhesives, and friction materials due to their excellent toughness and thermal-resistant properties. In the literature, phenol–formaldehyde resins are also reported as good antioxidants for plastics and rubber. But, formaldehyde is known to be a toxic compound, and the health hazards of formaldehyde have become a public concern. Therefore, it is necessary to discover environmentally benign and green synthesis methods for the production of phenol–formaldehyde resins [4,10,11]. Enzymatic oxidative polymerization is considered as a benign method compared to the conventional oxidative polymerizations. Polymerization of phenol derivatives using peroxidase enzymes provided an alternative opportunity for the production of polyphenols (phenolic resins) with no need to use toxic formaldehyde comonomer in the process [12]. A variety of phenolic compounds have been subjected to the enzymatic polymerization using HRP enzyme [13]. Phenol derivatives having different functionalities can be chemoselectively polymerized using this method without involving side groups during polymerization [14,15]. Conducting polymers can also be efficiently produced by enzymatic polymerization [16,17]. Moreover, HRP is used to remove phenolic compounds (especially, chlorophenols labeled as "priority pollutants" by the Environmental Protection Agencies) from wastewater [18].

Polyazomethines have gained widespread interest due to their useful properties such as high thermal stability, electrical and magnetic properties, mechanical properties, semiconducting and crystalline properties, and etc [19]. We previously described enzyme-catalyzed polymerization of a phenolic compound containing *para*-imine functionality at RT under air, affording an oligophenol with high thermal stability and good electroactivity [20]. We have pursued the study of *ortho*-imine functionality in the side chain of oligophenols as a means of tailoring the physical properties and to develop new types of materials. For this purpose; a new type of imine functionalized phenol derivative, PTIMP, was synthesized with a condensation reaction of 2-hydroxybenzaldehyde and *p*-toluidine. Obtained PTIMP monomer was then enzymatically polymerized in the presence of HRP enzyme and H_2O_2 oxidizer under different reaction conditions. A wide pH range from 5.0 to 9.0 and reaction temperatures from 20 to 55 °C were examined. The optimum polymerization was achieved in EtOH/pH 6.0 phosphate buffer (50:50 vol.%) at 25 °C resulting in the highest molecular weight (M_n = 6100 g/mol, DP * 29, PDI 1.09) and yield (65%). A new type of useful and high performance phenolic oligomer, which is not easily obtained by conventional polymerization methods, has been prepared by enzymatic polymerization of *ortho*-imine functionalized phenol employing HRP enzyme catalyst. Enzymatic polymerization of PTIMP leads to the formation of an oligomer containing phenylene and oxyphenylene repeat units as reported in previous studies. Oligo(PTIMP) was characterized using ^{1}H and ^{13}C NMR, FT-IR, CV, UV–Vis, TGA and GPC analyses.

2. Experimental

2.1. Materials and reagents

2-Hydroxybenzaldehyde (salicylaldehyde, Merck, Catalog #800640), *p*-toluidine (Merck, Catalog #808315), phosphate buffers (pH = 5.0, 6.0, 7.0, 8.0 and 9.0), hydrogen peroxide (H_2O_2, Sigma–Aldrich, Catalog #18304), *N,N′*-dimethylformamide (*N,N′*-DMF, Merck, Catalog #110983), dimethylsulfoxide (DMSO, Merck, Catalog #802912), chloroform ($CHCl_3$, Merck, Catalog #102431), acetone (Merck, Catalog #100013), methanol (MeOH, Merck, Catalog #106007), ethanol (EtOH, Sigma–Aldrich, Catalog #34923), dichloromethane (CH_2Cl_2, Sigma–Aldrich, Catalog #24233) and 1,4-dioxane (Merck, Catalog #103115) were used without further purification. Horseradish peroxidase (HRP) enzyme was purchased from AppliChem (practical grade II, Catalog #A3800) and used as received.

2.2. Synthesis of (E)-2-((p-tolylimino)methyl)phenol (PTIMP)

(*E*)-2-((*p*-tolylimino)methyl)phenol (PTIMP) was synthesized according to the synthetic routes reported in the literature [21]. In a 50 mL flask, 10.0 mmol 2-hydroxybenzaldehyde and 10.0 mmol *p*-toluidine were dissolved in 20 mL of ethanol and a piece of molecular sieve was added to the flask. The solution was stirred and refluxed at 80 °C for 4 h. The product, (*E*)-2-((*p*-tolylimino)methyl)phenol (PTIMP), was obtained by removal of ethanol, and followed by flash column chromatography (SiO_2 column, elution with 9:1 hexane/acetone) (Fig. 1).

FT-IR (KBr) of PTIMP: 3027 cm^{-1} (aromatic, =CH), 2916 cm^{-1} (azomethine, —N=CH—), 1605 cm^{-1} (—CH=N—), 1504 cm^{-1} (—C=C—), 1283 cm^{-1} (=C—O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.2 (s, H1), 8.9 (s, H6), 7.6 (d, H5), 7.3 (t, H3), 7.2 (m, H4, H7, H7′, H8 and H8′), 6.9 (d, H2), 2.33 (s, H9). ^{13}C NMR (100 MHz, DMSO-d_6) δ:

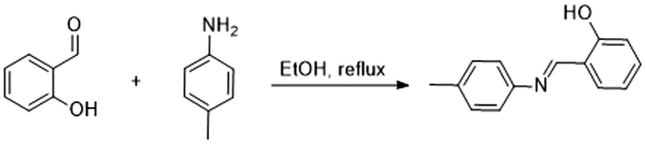

Figure 1 Preparation of the monomer.