Journal of Molecular Catalysis B: Enzymatic 59 (2009) 177–184

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Enzymatic polymerization of phenols in room-temperature ionic liquids

Bilge Eker[a], Dmitri Zagorevski[b], Guangyu Zhu[a], Robert J. Linhardt[a,b,c], Jonathan S. Dordick[a,c,*]

[a] Department of Chemical and Biological Engineering, Rensselaer Nanotechnology Center, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA
[b] Department of Chemistry and Chemical Biology, Rensselaer Nanotechnology Center, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA
[c] Department of Biology, Rensselaer Nanotechnology Center, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

ARTICLE INFO

Article history:
Received 15 July 2008
Received in revised form 3 February 2009
Accepted 13 February 2009
Available online 6 March 2009

Keywords:
Enzymatic synthesis
Polyphenol
Solvent engineering
Room-temperature ionic liquids

ABSTRACT

Soybean peroxidase (SBP) was used to catalyze the polymerization of phenols in room-temperature ionic liquids (RTILs). Phenolic polymers with number average molecular weights ranging from 1200 to 4100 Da were obtained depending on the composition of the reaction medium and the nature of the phenol. Specifically, SBP was highly active in methylimidazolium-containing RTILs, including 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM(BF_4)), and 1-butyl-3-methylpyridinium tetrafluoroborate (BMPy(BF_4)) with the ionic liquid content as high as 90% (v/v); the balance being aqueous buffer. Gel permeation chromatography and MALDI-TOF analysis indicated that higher molecular weight polymers can be synthesized in the presence of higher RTIL concentrations, with selective control over polymer size achieved by varying the RTIL concentration. The resulting polyphenols exhibited high thermostability and possessed thermosetting properties.

1. Introduction

Phenol–formaldehyde resins have a long history of widespread use in surface coatings, adhesives, laminates, molding and friction materials, and abrasives [1], flame retardants [2], carbon membranes [3], glass fiber laminates [4], fiberboards [5], and protein-based wood adhesives [6]. While severe health and environmental concerns regarding the toxicity of formaldehyde has been noted for quite some time [7], recent legislation in many countries has limited conventional phenol–formaldehyde manufacturing and use [8]. As a result, alternative synthetic approaches have been sought, which do not involve formaldehyde yet provide simple, highly reproducible, and low-cost routes to phenolic polymers with thermosetting properties. One common alternative for formaldehyde-free phenolic polymerization involves free radical polymerization catalyzed by a chemical catalyst such as copper and copper complexes [9,10]. However, this approach is not effective for the polymerization of unsubstituted phenols [11], and still employs the use of toxic chemical catalysts leading to potential environmental problems [12].

An attractive alternative to chemical routes is the use of enzymes as catalysts. Nature provides a clear example of the power of enzymes in the preparation of phenolic polymers through the synthesis of lignin [13], the second most abundant polymer on earth [14]. Plant peroxidases catalyze the one-electron oxidation of phenolic monomers in the presence of H_2O_2, thereby generating free radicals that undergo radical transfer and coupling reactions to build the complex lignin macromolecules found throughout the plant kingdom [15,16]. The mild reaction conditions coupled with the highly reactive and stable peroxidase family of oxidative enzymes is also ideal for synthetic applications. In particular, soybean peroxidase (SBP) and horseradish peroxidase (HRP) have been used to synthesize phenolic polymers and copolymers from a wide range of phenols, including *p*-cresol, *p*-phenylphenol, various naphthols, and phenol itself, along with related anilines [16–18]. Both SBP and HRP yield similar polymeric products, which appear to be representative of the majority of plant peroxidases [19]. Unfortunately, in aqueous solutions, the poor solubility of phenolic monomers and the even lower solubility of the polymeric products result in low yields of oligomeric (predominantly dimers and trimers) that precipitate out of solution [20,21].

In addition to aqueous media, peroxidases are highly active in organic solvents and have been used to catalyze phenolic polymerizations in such milieu [17,22–25]. In some cases significant control over the polymer size and polydispersity has been achieved. For example, Dordick et al. observed changes in polyphenolic M_w from 1000 to over 26,000 Da, using 1,4-dioxane as the solvent at various

* Corresponding author at: Department of Chemical and Biological Engineering, Rensselaer Nanotechnology Center, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York 12180, USA. Tel.: +15182762899; fax: +15182762207.
E-mail address: dordick@rpi.edu (J.S. Dordick).

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doi:10.1016/j.molcatb.2009.02.018

water contents, with an optimal polymer size achieved in 85% (v/v) dioxane [17]. A functionally similar result was obtained by Pizzi et al. where polyphenolic M_w of the dimethylformamide (DMF)- and dimethylsulfoxide (DMSO)-soluble fractions were as high as 35,000 Da [26]. Water-miscible cosolvents have been used to support higher molecular weight polyphenolic synthesis. In addition to 1,4-dioxane–water mixtures, Oguchi et al. performed enzymatic oxidative polymerization of phenol in aqueous methanol solutions using HRP yielding number average molecular weights up to 5200 Da [18].

Despite the aforementioned examples, organic solvents suffer from several drawbacks, including poor solubility of highly polar compounds (including some phenolic monomers), and the relatively low solubility of the polyphenolic products. While highly polar aprotic solvents such as DMF and DMSO are able to solubilize high molecular weight fractions of polyphenols, primarily at very high solvent percentages [18,20,27], enzyme activity in these solvents is low [20,23,27]. RTILs, however, may provide the advantages of a nonaqueous environment that enables high solubility of phenols and their polymerized counterparts, while maintaining sufficient enzyme activity to allow efficient polymerization, all while providing "green" alternatives to volatile organic solvents.

In the present study, we have exploited RTILs as reaction media to support SBP-catalyzed polymerization of phenols. SBP-catalyzed oxidative polymerization reactions were performed in various aqueous RTIL solutions and different phenols were used as substrates for polymer synthesis. Higher molecular weight phenolic polymers were synthesized in solvent systems having higher RTIL contents, owing to the high dissolution capacity of RTILs. Finally, thermal analysis revealed that the resulting polyphenols were highly thermostable and had desirable thermosetting properties.

2. Materials and methods

2.1. Materials

Soybean hull peroxidase was purchased from Sigma–Aldrich (St. Louis, MO) as a solid powder (50 purpurogalin units/mg solid). All phenols, LiBr, and H_2O_2 (30%, w/w solution in water) were also obtained from Sigma–Aldrich and used without further purification. High purity RTILs (>98.5%) were obtained from Fluka (Milwaukee, WI) and polyethylene glycol (PEG) standards were obtained from Polymer Laboratories (Amherst, MA). All other chemicals employed were of the highest purity commercially available.

2.2. Enzymatic reactions

Phenolic polymerizations were carried out in a variety of RTIL/aqueous solutions on an orbital shaker (200 rpm) at 60 °C. Each reaction consisted of 20 mM of a phenolic substrate in the presence of 0.1 mg/ml SBP and 20 mM H_2O_2, the latter being added dropwise to the reaction mixture over a period of 2.5 h, and the reactions stirred for an additional 20 h. Several compositions of RTIL/aqueous solutions were prepared using BMIM(BF_4), and BMPY(BF_4) as the RTIL component ranging from 0 to 90% (v/v) in 10 mM phosphate buffer (pH 7). The pH values of all the aqueous RTILs were independent of the solvent composition and no change in pH was observed upon addition of RTIL (pH 7). Concentrations of phenols were determined by HPLC (Shimadzu LC-VP, Columbia, MD) with detection at 280 nm. Periodically, aliquots from the reaction mixtures were diluted fourfold with acetonitrile and analyzed on a reversed phase C18 column (4.6 mm × 150 mm, 5 μm, Alltech Alltima, Deerfield, IL). The eluent contained 0.1% (v/v) acetic acid in acetonitrile/water solution and was pumped with a linear gradient from 10 to 45% acetonitrile over 10 min and then isocratically at 45% acetonitrile for 20 min with a flow rate of 1 ml/min.

2.3. Gel permeation chromatography

Gel permeation chromatography was employed to determine polymer molecular weight. PLgel 3 μm MIXED-E column (molecular weight cutoff of 30 kDa) (Polymer Laboratories, Amherst, MA) was connected to the HPLC and 0.5 ml/min DMF was used as the mobile phase. PEG standards with peak molecular weights (M_p) of 21,030, 12,140, 8500, 4020, 1010, and 400 were used as molecular weight calibrants and the molecular weight distribution of the polymers was determined based on these PEG standards. LiBr (40 mM) was added to the DMF mobile phase to dissociate molecular aggregates of phenolic polymers during GPC analysis.

2.4. MALDI-TOF analysis

Mass spectra were acquired on an Ultraflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA) in linear and reflector modes. A "smart beam" Nd-YAG laser (λ = 355 nm) was set at ~70% maximum power. Changes in pulse ion extraction (PIE) time in the range of 20–300 ns did not significantly affect the oligomer ion distribution. PIE was set up for standard values of 20 ns in reflector and 100 ns in linear mode. MALDI-TOF analysis involved five different matrixes for sample preparation: 2,5 dihydroxybenzoic acid (DHB), dithranol, 2,4,6-trihydroxyacetophenone (THAP), 6-aza-2-thiothymine, and trans-2-indolacrylic acid. All matrixes produced satisfactory MALDI-TOF mass spectra in positive ion mode. The best negative ion mode mass spectra were obtained with trans-2-indolacrylic acid and THAP as matrixes.

DMF insoluble polymers were pretreated prior to MALDI-TOF analysis. The precipitates were centrifuged and the DMF was removed by washing with water and the solids were dried. The solids were then added to a mixture of DMF and methanol (4:1, v/v) or pure methanol to dissolve the solids. Molecular weights of the soluble fractions were determined based on MALDI-TOF analysis.

2.5. Thermal analysis

Thermal analysis was performed using a thermogravimetric analyzer (TGA) and a differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE). The flow rate of nitrogen was 50 ml/min, the sample size was 1–2 mg for both DSC and TGA, and the heating rate was 10 °C/min.

3. Results and discussion

While a growing literature exists on using enzymes in RTILs [28,29], very little work has been done with peroxidases in these solvents [30], and no reports exist on the polymerization of phenols catalyzed by peroxidases in RTILs. Therefore, we set out to assess the influence of RTILs on SBP catalysis. To that end, we used two different water miscible, commercially available RTILs as reaction solvents: 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM(BF_4)) and 1-butyl-4-methylpyridinium tetrafluoroborate (BMPy(BF_4)). Tetrafluoroborate was chosen as the anion due to its formation of stable RTILs with different cations, and its known capacity to support enzymatic catalysis [29,31]. SBP was used as the model peroxidase due its well-known operational stability, particularly in nonaqueous media [23,32]. A range of simple phenols were used as substrates for the synthesis of high molecular weight polymers, with *p*-cresol (4-methylphenol), employed as the initial model substrate in enzymatic polymerization reactions (Scheme 1).