Multienzymic Synthesis of Poly(hydroquinone) for Use as a Redox Polymer

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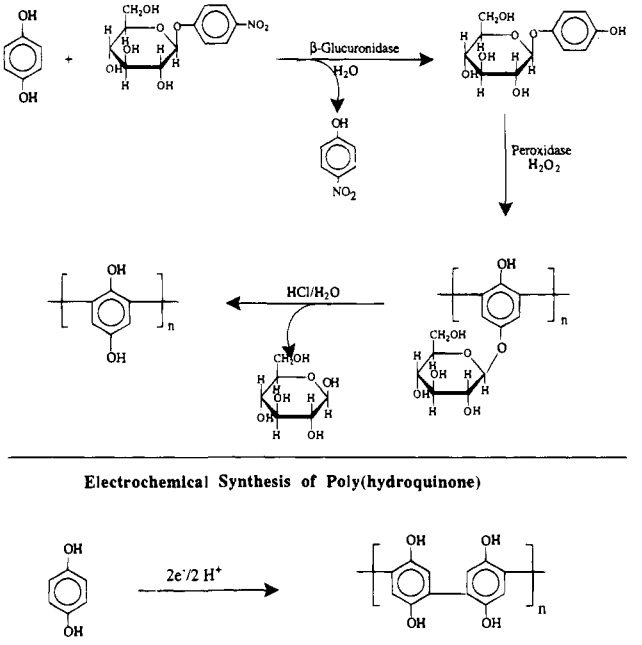
Redox-active polymers are used in a wide range of applications including electrical conductors, batteries, electrode coatings for sensors, catalysts for electrochemical reactions, antioxidants, and corrosion or degradation reaction inhibitors.[1] Poly(quinone)s are a particularly interesting class of redox polymers as they can undergo direct two-electron oxidation and reduction, and they have high charge densities.[2] Poly(quinone)s are typically prepared via chemical[2] or electrochemical methodologies,[3] and invariably involve complex processes that are difficult to scale and produce large amounts of byproducts. An alternative synthetic strategy is to use enzymes to catalyze poly(quinone) formation from available hydroquinone precursors. Peroxidases are highly effective phenol oxidizing catalysts.[4] Unfortunately, the direct peroxidase-catalyzed oxidation of hydroquinones leads to minimal polymerization as the hydroquinones are oxidized directly to the benzoquinones.[5] In the present work, we have developed a general synthetic methodology that incorporates a multienzymic approach to overcome this limitation. Specifically, we utilize an enzyme to catalyze the monoglycosylation of hydroquinone in aqueous solution. The free hydroquinone phenolic moiety is then a substrate of peroxidase for the formation of the poly(hydroquinone glycoside) derivative, which is subsequently converted to poly(hydroquinone) by chemical removal of the sugar protecting group. The entire process (depicted in Scheme 1) can be performed in aqueous solution due to the hydrophilic nature of the sugar blocking group.

Numerous enzymes are capable of attaching sugars to nucleophilic compounds; however, the reaction must be regiospecific and block only one of hydroquinone's two hydroxyl groups. To that end, a number of glycosidases were screened for their ability to catalyze the synthesis of hydroquinone glycoside in aqueous buffer.[6] Most glycosidases gave low yields of hydroquinone glycoside; however, β-glucuronidase (from bovine liver) catalyzed the efficient transfer of β-D-glucose to hydroquinone in aqueous solutions to give glucose-β-D-hydroquinone (arbutin).[7] Hydrolysis of the sugar donor was minimal, and the primary product was arbutin.[8] β-Glucuronidase proved to be a highly versatile glycosyl transferring enzyme. In addition to hydroquinone, a variety of other nucleophiles could be used as glucosyl acceptors including other sugars and secondary alcohols, and enantioselectivity was observed (Table 1). Indeed, the (*R*)-(+)-2-butanol reacts 7-fold faster than the (*S*)-(−)-isomer.

Scheme 1. Chemoenzymatic Methodology for Synthesizing Poly(hydroquinone)



Polymerization of arbutin was catalyzed separately by peroxidases from horseradish (HRP) and soybean hulls (SBP) in aqueous buffer over a wide range of pH values and resulted in water-soluble polymers with M_n's ranging from 1600 to 3200 (degree of polymerization up to 12 with polydispersities of 1.1−1.2).[9] Both peroxidases gave optimal molecular weight poly(arbutin)s at pH's between 6.0 and 7.5 with isolated yields of

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(6) The following enzymes (and their respective glycoside substrates) were studied: α- and β-glucosidases (*o*-nitrophenyl α- and β-D-glucosides); α- and β-galactosidases (*o*-nitrophenyl α- and β-D-galactosides); β-glucuronidase from bovine liver and *Escherichia coli* (*o*-nitrophenyl β-D-glucoside and cellobiose).

(7) Arbutin is also a natural product found in the leaves of various berry plants (Klein, G. *Handbüch der Pflanzenanalyse*; J. Springer: Vienna, Austria, 1932; Vol. 3, Part 2). Arbutin has been prepared by plant cell culture using suspension cultures of *Rauwolfia serpentina* (Lutterbach, R.; Stöckigt, J. *Helv. Chim. Acta*, **1992**, *75*, 2009). Interestingly, the diglycoside is a major byproduct of the plant cell culture.

(8) Arbutin was purified from the enzymatic reaction mixture by evaporating the water under vacuum and applying the residue to flash silica chromatography (72:6:4 ethyl acetate/MeOH/H_2O). The isolated yield of arbutin was 62% (1.65 g). Arbutin formation was confirmed by ^{1}H- and ^{13}C-NMR: ^{1}H-NMR (D_2O) δ (ppm) 3.55 (4 H, m, br, H-6′,5′,4′), 3.77 (1 H, m, H-3′), 3.94 (1 H, d, H-2′), 4.97 (1 H, d, H-1′), 6.87 (2 H, d, H-2,6), 7.05 (2 H, d, H-3,5); ^{13}C-NMR (DMSO-d_6) δ (ppm) 61.04 (C-6′), 70.03 (C-4′), 73.55 (C-2′), 76.84 (C-5′), 77.08 (C-3′), 101.9 (C-1′), 115.8 (C-2,6), 117.9 (C-3,5), 150.6 (C-1), 152.4 (C-4). This represents a 1.4 ppm downfield shift in the C-1′ carbon vs glucose, thereby indicating regioselective formation of arbutin. Arbutin is also available from Sigma, and this was the material used for subsequent peroxidase catalysis.

(9) The peroxidase-catalyzed reactions were performed with 0.1 M arbutin, 0.2 M H_2O_2, and 0.12 mg/mL peroxidase (horseradish or soybean). The arbutin (20 mL) and H_2O_2 (10 mL) were pumped separately over a period of 3 h into a 20 mL solution of peroxidase. The solution was stirred (100 rpm at 25 °C) for an additional 12 h after all of the arbutin and H_2O_2 were added. The reaction mixture turned dark brown during the polymerization, yet remained completely water soluble. The reaction was terminated by lyophilizing the solution. Isolated yields were based on the weight of polymer following dialysis with a 1000 MWCO membrane against deionized water. Values of M_w and M_n were also based on dialyzed retentate material and were determined using HPLC-GPC with Ultrahydrogel columns (2000, 500, and 250 Å columns in series). GPC calibration was performed with PEG standards of M_w = 1450, 5000, and 9000.

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Table 1. β-Glucuronidase Activity and Specificity in Aqueous Buffer[a]

acceptor	enzyme concn (mg/mL)	init reactn rate (mmol (mg of enz)$^{-1}$ h^{-1})	yield (8 h, %)	isolated yield (% (g))
1-butanol[b]	3	17.3	>95	91.2 (2.02)
hydroxyethyl acrylate[c]	3	16.8	>95	90.6 (2.52)
hydroxyethyl methacrylate[c]	3	16.4	93	88.7 (2.59)
(*R*)-2-butanol	5	13.6	>95	91.5 (2.03)
(*S*)-2-butanol	15	1.94	42	38.5 (0.85)
hydroquinone[d]	15	1.71	65	62.1 (1.65)
o-nitrophenyl glucoside[e]	6	0.94	57	52.0 (2.41)

[a] Unless otherwise indicated, the concentrations of *o*-nitrophenyl β-D-glucoside and acceptor were 0.1 and 0.6 M, respectively. The reactions were performed in 50 mM Na acetate buffer, pH 5.0. Yields (to the glycosylated products) and initial rates were determined by gas chromatography following silylation of the free hydroxyl groups and were specific toward the formation of the glycosidic bond. The enzyme's specific activity was 560 units/mg. [b] (*o*-Nitrophenyl β-D-galactoside was nearly as effective as *o*-nitrophenyl β-D-glucoside (yield of 75% after 8 h for the former). [c] The products of these reactions underwent autopolymerization during purification to the sugar-based poly(meth)acrylate which was insoluble in water, yet highly swellable. [d] The hydroquinone concentration was 0.3 M. [e] The transfer product was identified as a disaccharide that coelutes on GC (following silylation) with *o*-nitrophenyl lactoside.

ca. 70% following dialysis to recover the polymer. ^{13}C-NMR and elemental analysis confirmed that the poly(arbutin) retained its sugar moieties throughout the polymerization reaction.[10] Deglycosylation of the poly(arbutin) was performed at 60 °C in 5 M HCl for 24 h to give quantitative yields of a dark brown powder. ^{1}H-NMR and ^{13}C-NMR of the deglycosylated product were performed in DMSO-d_6 and showed no evidence of sugar moieties. Loss of the hydroquinone hydroxyl protons in the ^{1}H-NMR spectrum via deuterium exchange with D_2O in DMSO is qualitatively indicative of a poly(hydroquinone) structure rather than that of a poly(benzoquinone).[11] The poly(hydroquinone) is soluble (>2%, w/w) in THF, DMSO, DMF, acetone, and methanol. This is different from poly(hydroquinone) prepared electrochemically, in which the polymer (dp ≈ 6) is insoluble in these solvents,[3d] and may be due to the ortho–ortho coupling of the arbutin during peroxidase catalysis. The difference in structures between the enzymic and electrochemical synthetic routes is depicted in Scheme 1.

Poly(hydroquinone) has distinct redox properties and can undergo two-electron oxidation per hydroquinone unit to the poly(benzoquinone). Electrochemical properties of poly(hydroquinone) were examined in both aqueous and organic solutions following coating of the polymer on a glassy carbon electrode.[12] Cyclic voltammetry in 0.1 M H_2SO_4 showed clear oxidation and reduction peaks at 480 and 280 mV, respectively (Figure 1), which were nearly identical to those of hydroquinone itself under acidic conditions. A negative shift in redox potentials occurred as the pH increased (Figure 1); a plot of the midpoint redox potential as a function of pH is linear with a slope of −58.2 mV/pH unit (Figure 1, inset). This is in agreement with $2e^-/2H^+$ transfer and provides quantitative confirmation of the formation of poly(hydroquinone) as opposed to partial oxidation to the poly(benzoquinone) during removal of the sugar moieties from poly(arbutin). A cyclic voltammogram taken in CH_3CN containing 0.1 M benzenesulfonic acid gives clear oxidation and reduction peaks at 820 and 550 mV, respectively. In both aqueous and organic solutions, no changes in the redox potential occurred after 500 cycles. Thus, the poly(hydroquinone)–poly(benzoquinone) couple is stable and reversible.

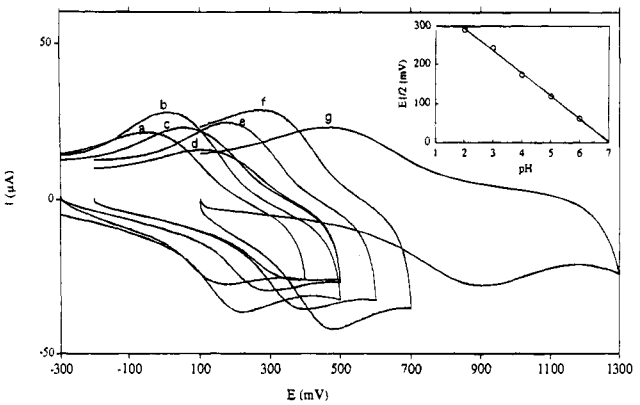

Figure 1. Cyclic voltamograms of poly(hydroquinone) in different solutions: (a) pH 6; (b) pH 5; (c) pH 4; (d) pH 3; (e) pH 2; (f) 0.1 M H_2SO_4; (g) 0.1 M benzenesulfonic acid in CH_3CN. For pH's 2–6, 0.1 M $LiClO_4$ was added. Inset: Correlation between midpoint potential and solution pH.

In conclusion, poly(hydroquinone) with interesting electrochemical properties has been prepared via a combined bienzymic/chemical strategy. The high fidelity of enzymatic catalysis provides the means to prepare unique monomers for subsequent peroxidase catalysis and chemical deblocking. This strategy, in addition to being applicable to hydroquinone, is expected to be applicable to biphenols, oligophenols, and anthraquinones to produce a wide range of redox-active polymers.

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Supporting Information Available: Representative ^{1}H- and ^{13}C-NMR spectra (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(10) Poly(arbutin): ^{13}C-NMR (DMSO-d_6) δ (ppm) 60.7 (C-6′), 69.7 (C-4′), 73.4 (C-2′), 76.6 (C-3′,5′), 101 (br, C-1′), 117 (C-2,3,5,6), 154 (br, C-1,4). Elemental anal. Calcd: C, 53.33; H, 5.19; O, 41.48. Found: C, 53.38; H, 5.12; O, 41.50.

(11) Poly(hydroquinone): ^{13}C-NMR (DMSO-d_6) δ (ppm) 118 (C-2,3,5,6), 154 (br, C-1,4). Importantly, no evidence of benzoquinone exists (lack of peaks at 137 and 187 ppm). Therefore, the poly(hydroquinone) appears to be in the fully reduced state upon deglycosylation of poly(arbutin). ^{1}H-NMR (DMSO-d_6): δ (ppm) 6.76 (br, H-3,5), 9.35 (br, OH). Deuterium exchange shows loss of peak at 9.35 ppm. Elemental anal. Calcd: C, 66.67; H, 3.70; O, 29.63. Found: C, 66.54; H, 3.83; O, 29.63.

(12) Poly(hydroquinone) was dissolved in DMSO at a concentration of 2% (w/w). Ten microliters of this solution was spread onto an aluminum-polished glassy carbon electrode with a cross-sectional area of 0.45 cm^2. The solvent was evaporated in air, resulting in formation of a thin film on the electrode.