Structural Control in Enzymatic Oxidative Polymerization of Phenols with Varying the Solvent and Substituent Nature

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Control of the coupling selectivity (regioselectivity) in the horseradish peroxidase-catalyzed oxidative polymerization of 4-substituted phenols has been achieved by changing the hydrophobic parameters of the monomer substituent and organic solvent (π and log *P*, respectively), yielding soluble polyphenols with a different ratio of phenylene/oxyphenylene units.

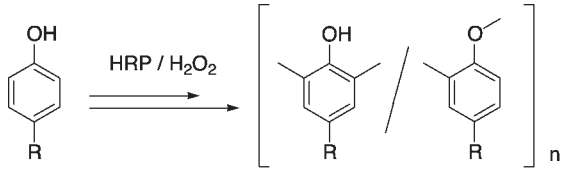
Enzyme catalysis is well known to be highly selective in substrates and reactions. In last decades, in vitro synthesis of polymers through enzymatic catalysis (''enzymatic polymerization'') has been extensively developed.[1] Enzyme catalysis has provided new synthetic strategy for useful polymers, most of which are otherwise very difficult to produce by conventional chemical catalysts. In vitro enzymatic syntheses of polymers via non-biosynthetic pathways, therefore, are recognized as a new area of precision polymer syntheses.

Catalytic oxidative polymerization of phenols is an environmentally benign production process of toxic formaldehyde-free phenolic polymers. Recently, their enzymatic syntheses have received much attention owing to extremely efficient catalysis of enzyme for their production under mild reaction conditions with facile procedures.[2]

In oxidative polymerization of phenol derivatives, the control of the coupling selectivity is often very difficult. Only some 2,6-disubstituted phenols are oxidatively polymerized to give poly(1,4-oxyphenylene) exclusively.[3] In case of phenol itself, on the other hand, conventional polymerization catalysts afford an insoluble product with non-controlled structure since phenol is a multifunctional monomer for oxidative polymerization.[3b] Polymerization of phenols with *o*-unsubstitution using horseradish peroxidase (HRP) gave the polymer consisting of a mixture of phenylene and oxyphenylene units.

In enzymatic reactions, solvent engineering has been extensively developed for tuning of solvent properties to achieve desired reactivity and selectivity. In aqueous organic media, HRP catalytic activity depended on both solvent and substrate hydrophobicities.[4] In hydrolase-catalyzed reactions in organic solvents, the enantioselectivity could be controlled by changing the solvent hydrophobicity and/or dipole moment to give optically active compounds.[5] Regioselectivity also depended on the solvent composition in hydrolase-catalyzed acylation of sugars and nucleosides.[6] On the other hand, little attention to coupling selectivity on enzymatic oxidative coupling of phenols has been received so far, probably owing to the free-radical coupling mechanism. We have reported that a soluble polymer was obtained using HRP catalyst in an aqueous methanol and the ratio of the C-C and C-O couplings changed with the solvent composition.[7]

In this paper, we have examined the coupling selectivity (regioselectivity) in the HRP-catalyzed polymerization of 4-substituted phenols in aqueous organic solvents (Eq. 1). The regioselectivity could be controlled by changing the hydrophobicity of the monomer and solvent, yielding the soluble polyphenols with a wide range of the unit ratio of phenylene/oxyphenylene (Ph/Ox). To our knowledge, this is the first clear-cut example on the coupling selectivity control in free-radical oxidative coupling of phenols.



(1)

The HRP-catalyzed oxidative polymerization was performed by using hydrogen peroxide (5%, equimolar ratio of phenol) as oxidizing agent in a variety of equivolume mixtures of water-miscible organic solvents and phosphate buffer (pH 7) at room temperature under air to give soluble polyphenols. The unit ratio was determined by titrating hydroxy group of the polymer.[7]

In this study, log *P*, where *P* is a partition coefficient for a given solvent between 1-octanol and water,[8] was used as parameter of solvent hydrophobicity. In case of 4-*t*-butylphenol (4-TBP) monomer, the polymer was obtained in 38–98% yields (Table 1). Number-average molecular weight (M_n) was estimated by size exclusion chromatography (SEC) as 390–1600 and the molecular weight value increased as a function of the hydrophobicity (log *P*). This may be due to the dependence of the polymer solubility upon the solvent hydrophobicity. The polymers in the range of unit ratio (Ph/Ox) from 85/15 to 47/53 were formed and the phenylene unit linearly decreased as the hydrophobicity increased (correlation coefficient = 0.994).

Table 1. Solvent Engineering for Structural Control of Polyphenol[a]

Organic solvent	log *P*	Yield /%	M_n[b]	M_w/M_n[b]	Ph/Ox[c]
Ethylene glycol	−1.36	38	390	2.0	85/15
Methanol	−0.77	83	570	1.9	72/28
1,4-Dioxane	−0.42	98	930	1.8	64/36
2-Propanol	0.05	87	1400	1.5	56/44
1-Propanol	0.25	64	1600	1.5	47/53

[a]Polymerization of 4-*t*-butylphenol (5.0 mmol) using HRP catalyst (440 units) in an equivolume mixture of an organic solvent and 0.1 M phosphate buffer (pH 7) (25 ml) for 3 h at room temperature under air. [b]Determined by SEC using DMF as eluent with polystyrene standards. [c]Determined by titration.

Besides 4-TBP, three 4-substituted phenols, 4-cyclohexylphenol, 4-isopropylphenol, and 4-ethoxyphenol (4-CHP, 4-IPP, and 4-EP, respectively), were enzymatically polymerized under the

similar reaction conditions. Figure 1 shows the relationships between log *P* of five organic solvents and the polymer structure. For all the monomers examined, a linear dependence was observed between log *P* and the unit ratio; the phenylene unit decreased as the hydrophobicity of the solvent increased. These data suggest that the polymer structure can be predicted by using log *P* of the organic solvent. The slope was relatively close in four cases. In the oxidative polymerization of the 4-substituted phenols catalyzed by soybean peroxidase or *Myceliophthora* laccase, the linear relationship between log *P* and the unit ratio was also observed (data not shown) and the slope was close to that using HRP catalyst.

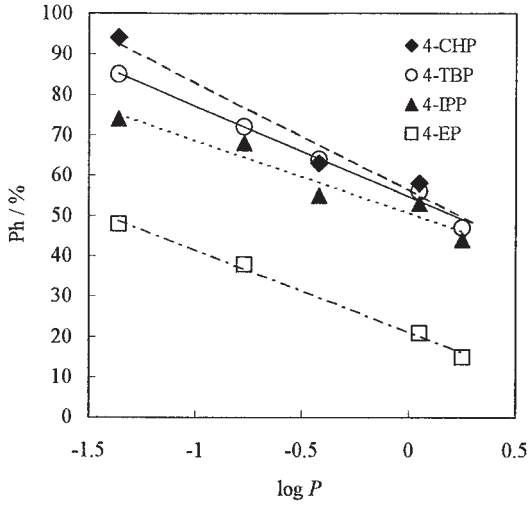


Figure 1. Relationships between log *P* of five solvents and polymer structure in HRP-catalyzed polymerization of 4-substituted phenols.

The polymer structure was also dependent on the monomer substituent. In order to evaluate the effect of the substituent, a hydrophobic substituent parameter, π, was used (Figure 2), which is derived from partition coefficient.[9] The first-order correlation was observed in ethylene glycol and methanol; however, other solvents did not show good linearity. In using other parameters such as electric and steric parameters, on the other hand, the correlation was not clear. These results indicate that the polymer structure strongly

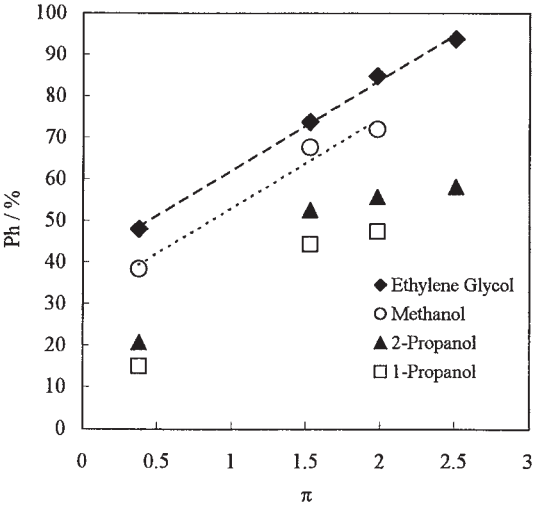


Figure 2. Relationships between π of four monomer substituents and polymar structure in HRP-catalyzed polymerization of phenols.

depended on the hydrophobic parameter of the substituent. The data of relationships between the polymer structure and solvent or substituent parameter will be useful to produce polyphenols with well-defined structure.

Furthermore, the production of the polyphenols with the wide range of the unit ratio was achieved. The polymer with the unit ratio of 94/6 was obtained from 4-CHP in 50% ethylene glycol, indicating the formation of poly(phenylene). The polymer mainly consisting of oxyphenylene unit (Ph/Ox = 4/96) was formed by the polymerization of 4-EP in 1,4-dioxane/phosphate buffer (80 : 20 vol%). These data indicate that the highly regioselective polymerization took place by selecting the appropriate selection of the monomer substituent and solvent composition, yielding poly(phenylene) or poly(oxyphenylene) selectively.

In conclusion, we have first achieved the control of regioselectivity in the free-radical oxidative polymerization of phenols by selection of the monomer substituent and solvent nature. The hydrophobic parameters of the monomer substituent and organic solvent (π and log *P*, respectively) strongly affected the polymer structure, and under appropriate conditions the polymerization proceeded regioselectively to produce a soluble poly(phenylene) or poly(oxyphenylene). The present study provides a clue to predict the polymer structure in the oxidative polymerization of phenols, which will contribute to the production of functional polymeric materials with controlled structure. Further investigations including mechanistic study on the regioselective polymerization using other oxidoreductases are under way in our laboratory.

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