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HRP-catalyzed polymerization of sugar-based phenols in aqueous organic solvents

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Abstract

HRP-catalyzed polymerization of sugar-based phenols prepared by the reaction of sugar-lactones with tyramine was carried out in a buffer (pH 7)/organic solvent (1:1, v/v) mixture. When 1,4-dioxane, DMSO and alcohols such as methanol were used as organic solvents, the conversion rate was 10–20% and the molecular weight (M_n) of polyphenols was about 2500 in all solvents. The type of sugar moiety contained in phenol derivatives had little effect on the polymerization. The conversion rate and M_n increased in the presence of a lipid such as AOT (dioctyl sulfosuccinate). Maltose-based polyphenol showed the strongest interaction with concanavalin A.

Keywords: HRP-catalyzed polymerization; Sugar-based phenol; Sugar-lactone; Tyramine; Concanavalin A

1. Introduction

There have been many studies recently on enzymatic oxidative polymerization of phenols (Bruno et al., 2001; Kim, Uyama, & Kobayashi, 2003; Oguchi, Tawaki, Uyama, & Kobayashi, 2000). Compared with the conventional method for preparing phenol resins from novolacs and/or resols, this method has several advantages: no use of toxic formaldehyde, mild reaction conditions and facile procedures. It has also been shown that the products obtained using this method consist of a mixture of phenylene and oxyphenylene units, different from the composition of conventional phenol resins (Uyama, Kurioka, Kaneko, & Kobayashi, 1994). Polymerization of various kinds of phenol derivatives catalyzed by horseradish peroxidase (HRP) or other peroxidases has been studied. Many of them are phenols having alkyl or aryl groups (Akkara, Senecal, & Kaplan, 1991; Alva, Nayak, Kumar, & Tripathy, 1997; Dordick, Marletta, & Klibanov, 1987). Michon, Chenu, Kellershon, Desmadril and Gueguen (1997) studied HRP-catalyzed polymerization of tyrosine-containing peptides in an aqueous buffer (pH 8.7). The main products of this polymerization were dimers and trimers. Wang and Dordick (1998) studied the synthesis of thymidine-containing polyphenols using soybean peroxidase (SBP). A polymer having a molecular weight (M_n) of 21,700 and molecular weight distribution (M_w/M_n) of 1.2 was prepared in a solution of aqueous buffer (pH 7.0) containing 60% (v/v) CH_3CN. They also studied the polymerization of arbutin using HRP and SBP in aqueous buffer (Wang, Martin, Parida, Rethwisch, & Dordick, 1995). Water-soluble polymers with M_ns ranging from 1600 to 3200 (degree of polymerization up to 12) were synthesized, and the polymers were converted into poly(hydroquinone) by hydrolyzation. The interaction of sugar-containing polymers with dyes such as methyl orange or with lectins such as concanavalin A has been investigated as their functionality (Kobayashi, Sumitomo, & Ina, 1985; Uchida, Serizawa, & Akashi, 1999).

In this work, HRP-catalyzed polymerization of sugar-based phenols was carried out in aqueous organic solvents to synthesize functional polyphenols.

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2. Experimental

2.1. Materials

4-(2-Aminoethyl)phenol (tyramine), D(+)-glucono-1,5-lactone and HRP (activities: 100 units/mg) were purchased from Wako Pure Chemical Industries and used without further purification. The sugars used except for maltpentaose and organic solvents were commercial guaranteed ones. Maltpentaose was provided by Ensuiko–Seito Industry. Concanavalin A (Con A) from *Canavalia ensisiformis* was purchased from Sigma–Aldrich.

2.2. Methods

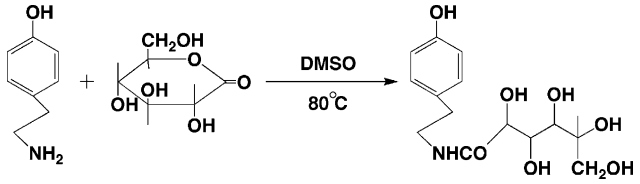
2.2.1. Oxidation of sugars

A typical run was as follows (Kobayashi et al., 1985). Maltose monohydrate (6 g, 16.5 mmol) was dissolved in water (25 ml), diluted with methanol (10 ml), and added to an iodine (8.5 g) solution in methanol (120 ml) at 40 °C. At this temperature, a 5% potassium hydroxide solution in methanol (200 ml) was added dropwise with magnetic stirring until the color of iodine disappeared. The solution was cooled externally in an ice-bath. The precipitate crystalline product was filtered and washed with cold methanol. The resulting potassium maltonate was then converted to free acid by passing the aqueous solution through a column of Amberlite IR-120B (H^+). The acidic elute was collected and concentrated in a rotary evaporator. Repeated evaporation of methanol and ethanol solution resulted in conversion of the maltonoic acid into maltonolactone containing a small amount of water. The yield was 5.7 g (95%).

Other sugars were oxidized in the same way, and the yields were the same as that of maltose.

2.2.2. Reaction of sugar-lactone with tyramine

A mixture of D(+)-glucono-1,5-lactone and tyramine of the same concentrations (16.8 mmol) was reacted in dimethyl sulfoxide (DMSO, 9 ml) at 80 °C for 24 h (Scheme 1). The product was precipitated in methanol and washed repeatedly with methanol. Other sugar-lactones were reacted in the same way. Yields and purities measured from nitrogen analysis were 81 and 71% for glucose, 42 and 85% for galactose, 27 and 71% for maltose, and 57 and 83% for maltpentaose, respectively.



Scheme 1. Synthesis of glucose-based phenol.

2.2.3. Enzymatic polymerization of sugar-based phenols

A typical run was as follows (Oguchi et al., 2000). Sugar-based phenol (0.6 g for monosaccharide or 0.9 g for disaccharide, 2 mmol) and HRP (20 mg) in a mixture of 15 ml of dioxane and 15 ml of phosphate buffer (pH 7) were placed in a 50 ml flask. Then 0.9 ml of 30% hydrogen peroxide (8 mmol) was added dropwise over a period of 2 h. The mixture was stirred at room temperature under air for 24 h. Then the reaction mixture was transferred to a MWCO 1000 membrane and dialyzed against deionized water for 3 days. The reaction products were recovered by lyophilizing the solution and were separated from HRP by extraction with methanol. Conversion rate of sugar-based phenols was calculated from the weights of the products. Then some of the products were acetylated to dissolve them in THF, and M_ns of the acetylated products were measured by GPC.

2.2.4. Interaction between maltose-based polymer and concanavalin A

Buffer solutions (0.1 M phosphate, pH 7.0) of Con A and maltose-based polymer were mixed and shaken at room temperature. At regular intervals, turbidity of the mixture was measured at 500 nm using a Hitachi U-2010 spectrophotometer (Kobayashi et al., 1985).

2.3. Measurements

NMR spectra were recorded on a JEOL EX-400 FT NMR spectrometer in deuterium oxide. GPC was carried out using a TOSOH GPC apparatus with an RI detector at 38 °C under the following conditions: two TSKgel (GMHHR-M) columns and THF eluent at a flow rate of 1.0 ml/min. The calibration curve was obtained using polystyrene standards. Thermal gravimetric analysis (TGA) was conducted using a SHIMADZU DTG-60 apparatus. The TGA was performed under nitrogen at a heating rate of 10 °C/min.

3. Results and discussion

3.1. Enzymatic polymerization of sugar-based phenols

HRP-catalyzed polymerization of glucose-based phenol was carried out in an equivolume mixture of organic solvents and pH 7 phosphate buffer (each 15 ml). This composition was selected for the solubility of sugar-based phenol. The polymerization proceeded homogeneously from the beginning to the end. Table 1 shows the conversion rates of glucose-based phenols and the M_ns of polymers prepared in various media. The conversion rates were below 20%, and the M_ns of polyphenols were 2000–2500 (degree of polymerization up to 8 with polydispersities of 1.2–1.3) in the aqueous organic solvents. 1,4-Dioxane was selected as an organic solvent for the following polymerization due to its good solubility for sugar-based phenols. Although the conversion rate and M_n were higher in buffer solution,

Table 1
HRP-catalyzed polymerization of glucose-based phenol in a solution of aqueous buffer containing 50% (v/v) organic solvent

Organic solvent	Conversion (%)	M_n	M_w/M_n
None[a]	25	4000	1.3
1,4-Dioxane	10	2500	1.3
DMSO	15	2000	1.3
Methanol	16	2500	1.2
Ethanol	13	2500	1.2
1-Propanol	19	2500	1.3
2-Propanol	7	–	–

Glucose-based phenol, 2 mmol.
[a] 0.4 mmol.

the degree of solubility of sugar-based phenols in water was relatively low.

A ^{13}C NMR spectrum (Fig. 1) of glucose-based polyphenol prepared in aqueous 1,4-dioxane showed signals for the sugar-alcohol component at 73–76 ppm and a signal for the amide carbonyl group at 177 ppm besides signals for the tyramine component at 155, 133, and 38–43 ppm. Chart 1 shows the proposed structure of glucose-based polyphenol prepared (Uyama et al., 1994).

Table 2 shows the effects of the kind of sugar on HRP-catalyzed polymerization of sugar-based phenols in 50% (v/v) aqueous 1,4-dioxane solutions. Although the conversion rate was about 10% with any sugar-based phenol, the M_ns of polyphenols increased with increase in the length of the sugar chain. However, the degree of polymerization was almost the same regardless of the kind of sugar. Tyramine was polymerized in aqueous ethanol solution because it is hardly soluble in aqueous 1,4-dioxane solution. In this case, the polymer prepared was insoluble in any of the solvents.

Template-assisted enzymatic polymerization of phenol in aqueous media has been reported to yield polyphenol/additive complexes having high molecular weights and high levels of electrical and optical activity (Bruno et al., 2001, 2002). The templates used include polystyrene sulfonate,

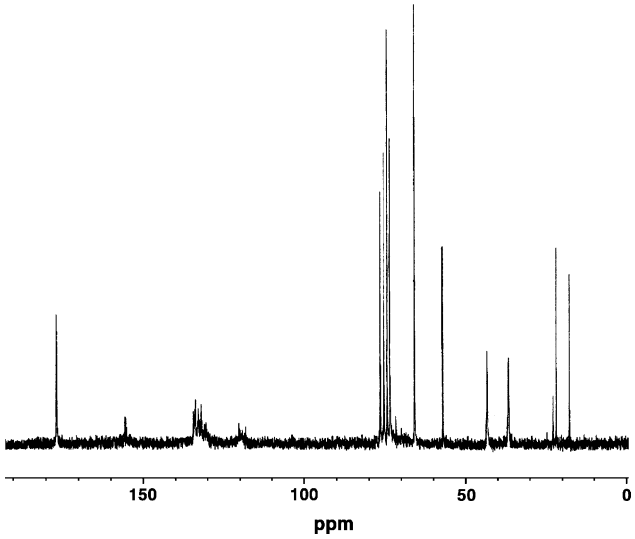


Fig. 1. ^{13}C NMR spectrum of glucose-based polyphenol measured in D_2O.

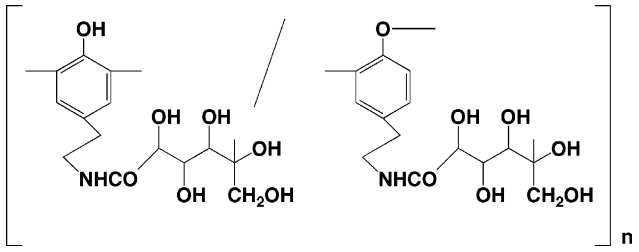


Chart 1. Proposed structure of glucose-based polyphenol.

dodecy benzene sulfonate and poly(ethylene glycol) (PEG). Improvement in the regioselectivity of polyphenol using PEG as a template has also been reported (Kim et al., 2003). We, therefore, examined the effects of additives such as PEG (M_n, 2000) and AOT (dioctyl sulfosuccinate) on the present polymerization. When equimolar additive with glucose-based phenol was added to the reaction mixture, the conversion rate and M_n of polyphenol were not affected by PEG, but were increased by AOT (conversion rate, 26%; M_n, 5400). AOT may act as a protector for the inactivation of HRP in aqueous 1,4-dioxane.

3.2. Properties of sugar-based polyphenol

The interaction of sugar-based polyphenol with Con A was investigated. Large aggregates appeared in a short time, resulting in an increase of the transmittance of the reaction solution with maltose-based polyphenol (Fig. 2,

Table 2
HRP-catalyzed polymerization of sugar-based phenol in 50% (v/v) aqueous 1,4-dioxane solution

Sugar	Conversion (%)	M_n (DP)	M_w/M_n
None[a]	24	Insoluble	–
Glucose	10	2500 (8)	1.3
Galactose	9	3000 (9)	1.2
Maltose	11	4000 (8)	1.2
Maltpentaose	12	5300 (6)	1.4

[a] In aqueous ethanol solution.

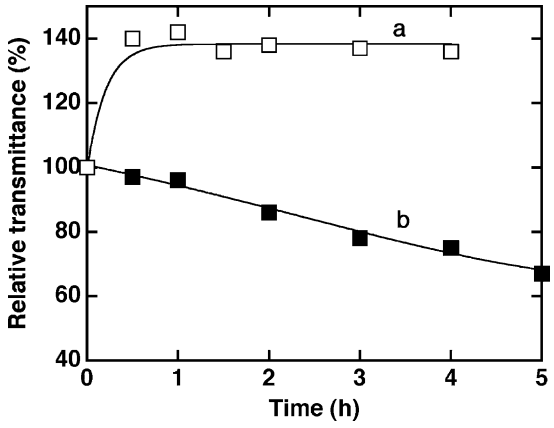


Fig. 2. Interactions of maltose (■) and maltose-based polyphenol (□) with Con A.

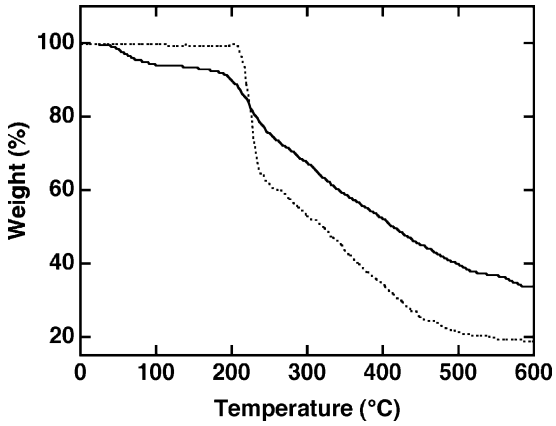


Fig. 3. TGA curves of glucose-based phenol (dotted line) and polyphenol (solid line).

curve **a**). On the other hand, the transmittance decreased with increase in reaction time with free maltose because of the formation of fine aggregates (Fig. 2, curve **b**). It is thought that maltose-based polyphenol bound easily to Con A due to the higher density of maltose molecules combined on a polymer chain different from free maltose (Uchida et al., 1999). This behavior was not observed with glucose-based polyphenol. This may be attributed to the structure of the sugar moiety, e.g. glucose has been changed to the corresponding sugar alcohol as shown in Chart 1.

Thermal properties of glucose-based polyphenol were determined by TGA, and a thermogram is shown in Fig. 3. The TGA analysis indicated that both glucose-based phenol (control) and its polymer began to degradate at about 200 °C. Though significant degradation occurred at this temperature with the control, that of polyphenol was moderate. The mass residue at 600 °C with the polymer was a little larger than that of the control. Further, the polymer did not have any melting point in the measured temperature range regardless of the control melted at about 200 °C, which were predetermined by differential thermal analysis. Thus, the thermal stability of glucose-based polyphenol was larger than that of its monomer.

4. Conclusions

Water-soluble polyphenols containing sugar moieties were synthesized by HRP-catalyzed polymerization of sugar-based phenols in aqueous organic solvents. However, the conversion rates of sugar-based phenols and the M_ns of the polyphenols prepared were low in all of the organic solvents used. They increased in the presence of an additive such as AOT. The interaction of sugar-based phenols with Con A was strongest with maltose-based polyphenol. Further investigations on ways to increase the conversion rates and M_ns of polyphenols and on applications of sugar-based phenols will be continued.

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