Notes

Synthesis of Poly(amino acid)−Polyphenol Hybrids by Oxidative Cross-Coupling

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Received June 3, 2004
Revised Manuscript Received August 9, 2004

Introduction

Recently, there has been much interest in a variety of naturally occurring polymers derived from renewable resources for material applications.[1] Among them, amino acid-based polymers including polypeptides have been significantly developed owing to their specific properties such as biodegradability, biocompatibility, etc.[2] Poly(aspartic acid), synthesized by thermal or acid-catalyzed polymerization of aspartic acid, followed by alkaline hydrolysis, is known to be a water-soluble biodegradable polymer and industrially used as chelator.[3] Poly(succinimide) (PSI), a starting polymer of poly(aspartic acid) synthesis, is highly versatile for design and synthesis of functional polymers;[4] the succinimide group of PSI can react with various nucleophiles to produce poly(amino acid)s with tailor-made structure.

For the past decades, enzymatic synthesis of polyphenols has been extensively investigated.[5] Peroxidase induces the oxidative polymerization of phenol derivatives under mild reaction conditions to produce a new class of polyphenols in good yields, which are expected as an alternative of conventional phenol−formaldehyde resins since the enzymatic process does not involve the use of toxic formaldehyde. We have developed a new catalyst for the oxidative coupling; iron−*N*,*N*′-ethylenebis(salicylideneamine) (Fe−salen), a model complex of heme-containing enzymes,[6] showed high catalytic activity for oxidative polymerization of various phenols.[7,8] Fe−salen is useful as catalyst for the oxidative coupling of phenols in organic solvents, in which enzymatic activities normally decrease greatly.

Very recently, we have expanded scope of the oxidative polymerization of phenols by the enzyme or enzyme model catalyst to production of high molecular weight soluble polymers from phenol-containing precursor polymers as a starting substrate; we have proposed a new concept of "polymerization of polyfunctional macromolecules". Generally, gelation takes place in the reaction of the polyfunctional macromolecules. For example, it was reported that the oxidative coupling of phenol-containing polymers by laccase or Fe−salen catalyst produced the insoluble cross-linked gels.[9] On the other hand, we have found that soluble high molecular weight polymers were exclusively formed by the precise design of the phenol-containing precursor polymer under the selected reaction conditions.[10,11] High molecular weight poly(amino acid)s were obtained by the oxidative coupling of poly(glutamine) or poly(asparagine) having a phenol moiety in the side chain using Fe−salen as catalyst.[10] Furthermore, Fe−salen catalyzed the oxidative coupling of enzymatically synthesized polyphenols to produce ultrahigh molecular weight polymers ($M_w > 10^6$).[11]

For development of high-performance polymers from renewable resources, hybrid polymeric materials have been extensively studied since they are expected to show unexpected hybrid properties derived from unique combinations of different polymers. Very recently, we have expanded the oxidative coupling of phenol-containing precursor polymers to synthesis of cellulose−polyphenol hybrids.[12] They were obtained by the oxidative coupling of a phenol-containing cellulose derivative and an enzymatically synthesized polyphenol. During the reaction, the cross-coupling between them preferentially took place; however, a small amount of the homocoupled product of the polyphenol was formed as byproduct. Thus, the purification procedure was required to isolate the desired cellulose−polyphenol hybrid.

In this study, we have synthesized poly(amino acid)−polyphenol hybrids by the Fe−salen-catalyzed oxidative cross-coupling of the poly(amino acid)s having a phenol moiety in the side chain and the enzymatically synthesized polyphenols, polyfunctional macromolecules (Scheme 1). By selecting the reaction conditions, the coupling reaction between different types of phenolic polymers was investigated in detail. By the control of the intermolecular oxidative coupling, the soluble hybrids were exclusively formed, preventing the production of the homocoupled polymers and insoluble gels.

Results and Discussion

Previously, we reported that poly(α/*β*-*N*-(2-(4-hydroxyphenyl)ethyl)asparagine, a phenol-containing poly(asparagine) derivative (**1**; $M_n = 3.7 \times 10^4$, $M_w/M_n = 2.3$), was subjected to the oxidative coupling by the Fe−salen catalyst, yielding a soluble high molecular weight poly(amino acid) quantitatively.[10] In this study, we selected **1** as backbone of the hybrid and examined the oxidative grafting of poly(*m*-cresol) (**2**; $M_n = 2.5 \times 10^3$, $M_w/M_n = 2.5$) on **1**. Poly(*m*-cresol) which was synthesized by peroxidase-catalyzed oxidative polymerization[13] was reported to be also oxidatively polymerized by the Fe−salen catalyst to produce a ultrahigh molecular weight polymer.[11] The oxidative coupling of **1** and **2** was performed using Fe−salen and hydrogen peroxide as catalyst and oxidizing agent, respectively, in the presence of a small amount of pyridine in *N*,*N*-dimethylformamide (DMF) at room temperature under air. In the

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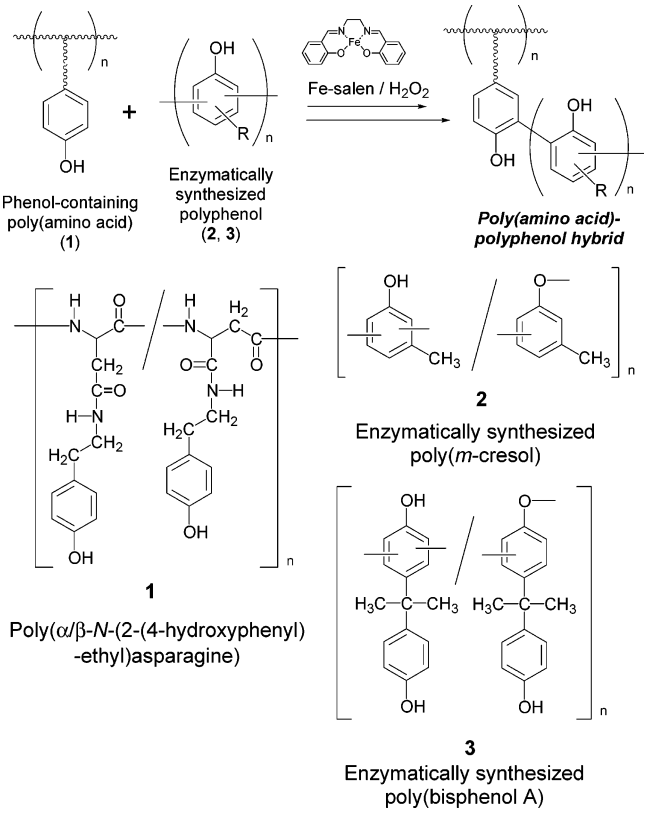
10.1021/ma048897y CCC: $27.50
Published on Web 09/25/2004

Scheme 1



course of the coupling, the molecular weight change of the reaction mixture was monitored by size exclusion chromatography (SEC) without further purification.

Figure 1 shows SEC traces of the coupling of **1** and **2** with different amount of hydrogen peroxide. In the SEC traces of the reaction mixture with an RI detector (Figure 1A), the peak due to **2** became much smaller only by the addition of a small amount of hydrogen peroxide. When the molar ratio of hydrogen peroxide to phenolic group of precursor polymers (**1** and **2**) was beyond 25%, this peak completely disappeared. The peak intensity increased as a function of hydrogen peroxide amount, whereas the elution volume scarcely changed. No peaks were additionally observed between these peaks during the coupling reaction. The number-average molecular weight and its index of the final product were 6.4×10^4 and 1.4, respectively. These data suggest that the grafting of **2** on **1** preferentially took place without the homocoupling of **1** and **2** to form the poly(amino acid)−polyphenol hybrid quantitatively.

In the SEC traces measured with a UV detector at 340 nm (Figure 1B), where only the polymeric phenol had the absorbance but **1** did not, the peak due to **2** rapidly decreased with the addition of hydrogen peroxide, and only one unimodal peak appeared newly at the lower elution volume during the coupling, the gradual shift of the peak of **2** to the lower elution volume was not observed. These data strongly support the selective formation of poly(amino acid)-*graft*-polyphenol (Figure 2A).

When the oxidative cross-coupling was carried out at the higher concentrations of the substrates and catalyst, the SEC peak due to the resulting hybrid polymer shifted at the much lower elution volume with the addition of hydrogen peroxide (Figure 1C). These results indicate that at the higher substrate and catalyst

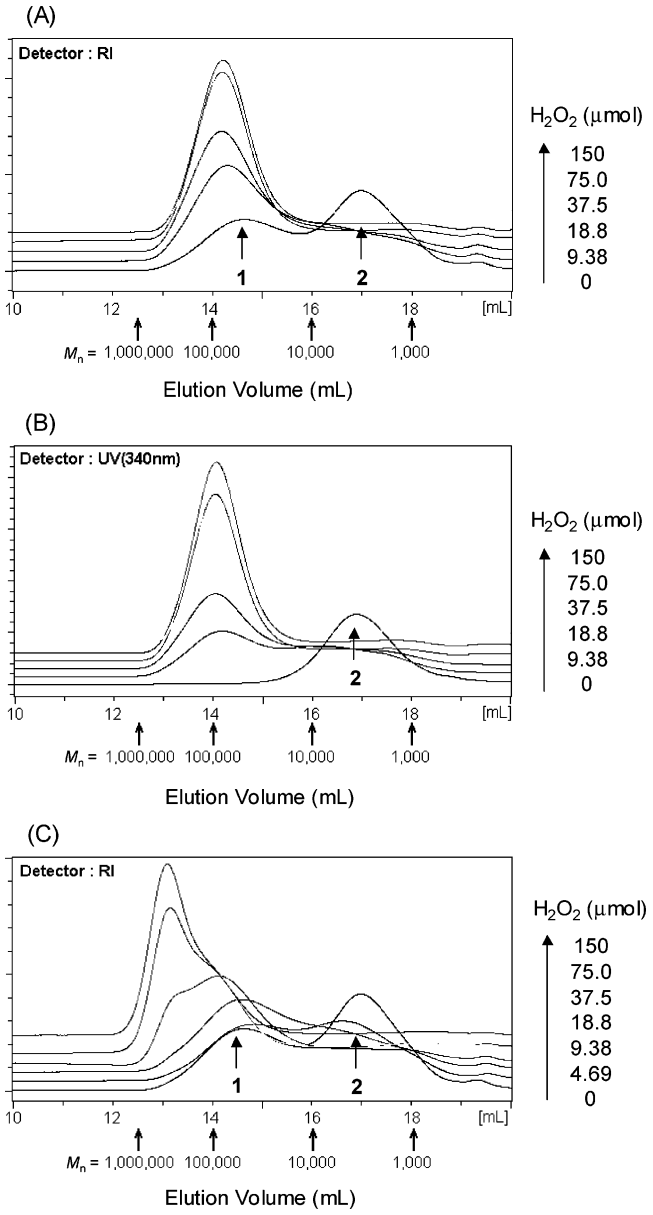


Figure 1. SEC traces of products by oxidative cross-coupling of **1** and **2** at low substrate and catalyst concentrations with (A) RI detector, (B) UV detector (340 nm), and (C) the coupling at higher substrate and catalyst concentrations with RI detector. The oxidative coupling of **1** (50 μmol of phenol unit) and **2** (100 μmol of phenol unit) was carried out by using Fe−salen catalyst ((A) and (B): 3.75 μmol; (C): 2.5 μmol) in DMF ((A) and (B): 5.0 mL; (C): 1.5 mL) at room temperature for 24 h under air.

concentrations not only the oxidative grafting of **1** on **2** but also the coupling most probably between the resulting hybrids take place to produce the polymers with $M_n = 2.0 \times 10^5$ and $M_w/M_n = 1.8$ (Figure 2B). The insoluble gels were immediately formed by the addition of hydrogen peroxide at the further higher concentrations, where the reaction of **1** and **2** takes place indiscriminately to form a network polymer by the intermolecular coupling. These results show clearly that the concentration of the substrate and catalyst is crucial for the production of the soluble hybrid polymers.

To confirm the polymer structure, we carried out the alkaline hydrolysis of peptide linkage of the *graft*-(Figure 2A) and *hybrid*-polymers (Figure 2B) and analyzed the hydrolysates by SEC and NMR. With use of the UV detector, the number-average molecular weight

Table 1. SEC−VISC−RALLS Analysis of Poly(amino acid)−Polyphenol Hybrids

sample	mol wt M_w	intrinsic viscosity IV_w (dL/g)	radius $R_{g,w}$[a] (nm)	radius $R_{h,w}$[b] (nm)	Mark−Houwink−Sakurada values[c] a	Mark−Houwink−Sakurada values[c] log K
1	7.85×10^4	0.239	8.19	6.28	0.958	−5.30
hybrid (**1** and **2**)[d]	1.43×10^6	0.110	17.0	13.1	0.449	−3.71
hybrid (**1** and **3**)[e]	1.67×10^6	0.113	18.0	13.9	0.507	−4.08

[a] Radius of gyration. [b] Hydrodynamic radius. [c] Mark−Houwink−Sakurada equation; $[\eta] = KM^a$. [d] Oxidative coupling product of **1** and **2**. [e] Oxidative coupling product of **1** and **3**.

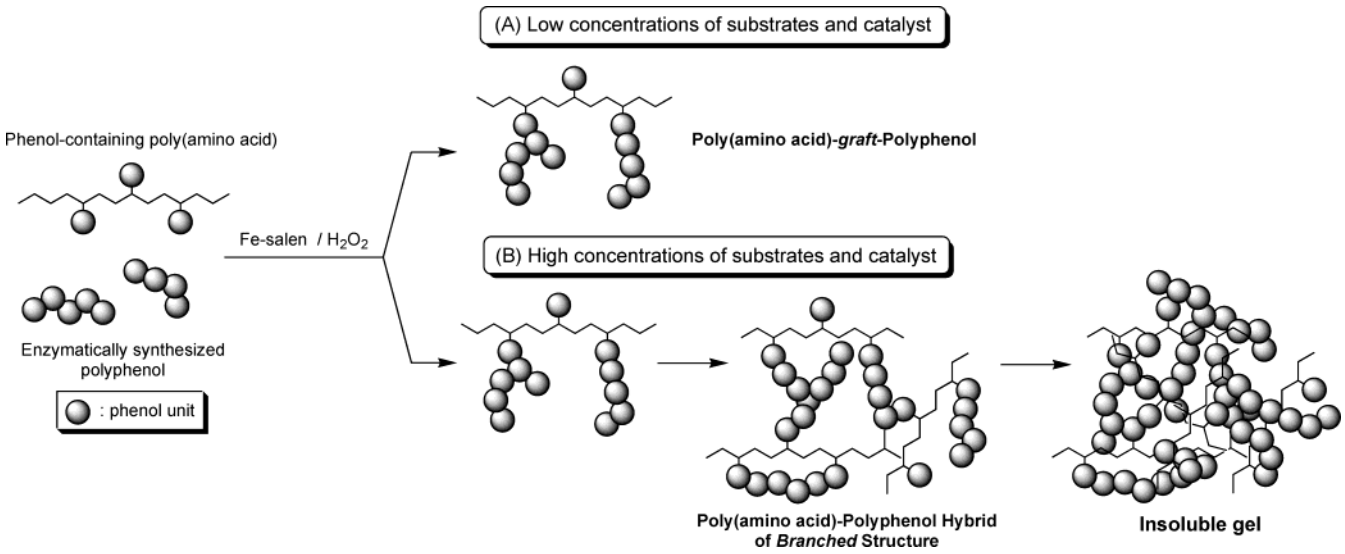


Figure 2. Schematic representation of hybrid polymer formation via the oxidative cross-coupling.

and its index of the hydrolysate of the *graft*-polymer were 3.0×10^3 and 3.9, respectively, and those of the hydrolysate of the *hybrid*-polymer were 7.0×10^3 and 6.1. The tyramine group was observed in the ^{1}H NMR spectra of both hydrolysates (data not shown). From these results, it was confirmed that during the reaction of **1** and **2** the oxidative cross-coupling of the phenol moieties containing in both precursor polymers proceeds to give the poly(amino acid)−polyphenol hybrid. Furthermore, the small difference of the molecular weight between **2** and the hydrolysate of the *graft*-polymer supports that the grafting of **2** on **1** mainly takes place and the intermolecular coupling of the resulting *graft*-polymer is negligible. On the other hand, the much larger molecular weight of the hydrolysate of the *hybrid*-polymer strongly suggests the occurrence between the formed hybrid polymers and/or the phenolic polymers.

We synthesized poly(bisphenol A) (**3**; $M_n = 3.8 \times 10^3$, $M_w/M_n = 1.8$) by the peroxidase catalyst and examined the cross-coupling with **1**. Under the selected reaction conditions, the oxidative grafting of **3** on **1** preferentially took place; the molecular weight of the products slightly increased in comparison with that of **1** by the SEC analysis (Figure 3A). The large increase of the molecular weight was observed at the higher concentrations of the substrates and catalyst (Figure 3B). At the further higher concentrations, however, the formation of insoluble gels immediately took place, as observed with the case of **1** and **2** above.

We measured the absolute molecular weight of the hybrid polymers obtained at the high concentration of the substrates and catalyst by a combination of SEC with an on-line viscometer (VISC) and a right angle laser light scattering (RALLS) detector (SEC−VISC−RALLS analysis) (Table 1). For both hybrid polymers, the molecular weight was very high ($M_w > 10^6$). The parameters obtained by Mark−Houwink−Sakurada

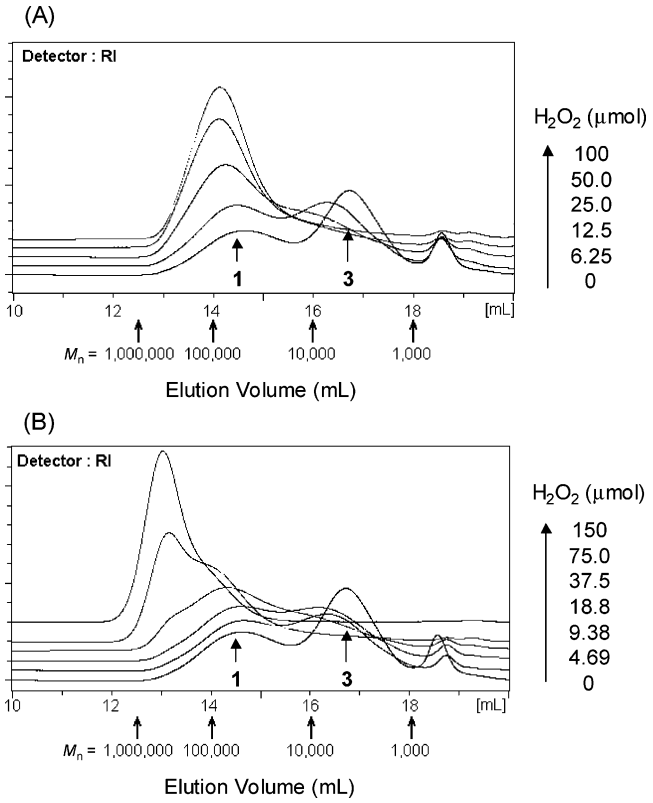


Figure 3. SEC traces of products by oxidative cross-coupling of **1** and **3** with RI detector at (A) low and (B) high substrate and catalyst concentrations. The oxidative coupling of **1** (50 μmol of phenol unit) and **3** (100 μmol of phenol unit) was carried out by using Fe−salen catalyst ((A): 3.75 μmol; (B): 2.5 μmol) in DMF ((A): 5.0 mL; (B): 1.5 mL) at room temperature for 24 h under air.

equation ($[\eta] = KM^a$) are often used for the molecular conformation of polymers in solution. The a value of

these hybrid polymers was around 0.5, which is lower than that of the rigid starting substrate, poly(amino acid) **1** ($a > 0.9$), and a linear polymer ($a = 0.6-0.8$). These results suggest that the present hybrid polymers of poly(amino acid)s and polyphenols are of branched structure (Figure 2B).

Conclusion

In this study, we achieved the precise control of oxidative cross-coupling of phenol-containing precursor polymers for the first time to produce a new class of poly(amino acid) hybrids. In the coupling of phenol-containing poly(amino acid) (**1**) and enzymatically synthesized polyphenols (**2** and **3**) using the Fe−salen catalyst, the grafting of the polyphenols on **1** proceeded under appropriate reaction conditions, leading to exclusive formation of poly(amino acid)-*graft*-polyphenol. At a higher concentration of the substrates and catalyst, the molecular weight further increased to give a high molecular weight hybrid polymer of branched structure. The present study provides a new strategy for production of useful and functional polymeric materials from a variety of combination of starting polymers, including bio-based polymers such as proteins and lignins.

Experimental Section

Materials. Fe−salen and horseradish peroxidase (100 units per mg) were purchased from Tokyo Kasei Inc. and Wako Pure Chemical Industries, Inc., respectively, and used without further purification. PSI was kindly donated by Prof. Toyoji Kakuchi (Hokkaido University, Japan). Poly(α/β-*N*-(2-(4-hydroxyphenyl)ethyl)asparagine) (**1**),[10] enzymatically synthesized poly(*m*-cresol) (**2**),[13] and poly(bisphenol A) (**3**)[14] were synthesized according to the literature. Other reagents and solvents were commercially available and were used as received.

Oxidative Coupling of 1 and 2. A typical run was as follows. A mixture of **1** (11.7 mg, 50 μmol of phenol unit), **2** (10.8 mg, 100 μmol of phenol unit), and Fe−salen (1.2 mg, 3.75 μmol) was dissolved in DMF (5.0 mL) containing 0.4 vol % pyridine. The coupling started by the addition of a quarter equivalent of hydrogen peroxide (30%) with respect to all amount of the phenol units of **1** and **2** under air. The same amount of hydrogen peroxide was added three more times every 15 min. After 24 h, the reaction mixture was subjected to the SEC analysis. The oxidative cross-coupling of **1** and **3** was performed under similar reaction conditions.

Measurements. SEC analysis was carried out by using a Tosoh GPC-8020 apparatus equipped with refractive index (RI) and UV detectors under the following conditions: TSKgel α-3000 and α-M columns and DMF containing 0.10 M LiCl eluent at a flow rate of 1.0 mL/min at 60 °C. The calibration curves were obtained using polystyrene as standard. SEC−VISC−RALLS analysis was performed by using a Tosoh GPC-8020 apparatus equipped with refractive index (RI) and UV detectors on-line combined with a TriSEC dual detector model 270 apparatus (Viscotek Co.) under the same conditions of SEC analysis. NMR spectra were recorded on a Bruker DPX400 spectrometer.

Acknowledgment. This work was partly supported by Program for Promotion of Basic Research Activities for Innovative Bioscience and by the 21st century COE program, COE for a United Approach to New Materials Science.

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MA048897Y