Video Article

Synthesis of Plant Phenol-derived Polymeric Dyes for Direct or Mordant-based Hair Dyeing

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Abstract

Effective hair dyeing through *in situ* incubation of keratin hair with the products of fungal laccase-catalyzed polymerization of plant phenols has been previously demonstrated. However, the dyeing process takes a long time to complete compared to commercial hair-dyeing products. To overcome this bottleneck, pre-synthesized polymeric products of the oxidative reaction of *Trametes versicolor* laccase on catechin and catechol, either with or without mordant agents (*e.g.,* $FeSO_4$), were here employed to achieve permanent keratin hair dyeing in various colors and shades. The laccase action in acidic sodium acetate buffer led to a deep black coloration after coupling reactions between the plant phenols. The colored dye products were then desalted and concentrated with ultrafiltration. The dyes, with or without mordant agents, caused a significant increase in ΔE values (*i.e.,* color difference value) in gray human hair within 2.5 hours. In addition, different keratin colors and shades were induced depending upon the mordanting and pH changes. The dyed hair also exhibited a strong resistance to detergent treatments, indicating that our methods can give rise to permanent hair dyeing. Overall, our work has provided novel insight into developing eco-friendly hair-dyeing methods as alternatives to commercial toxic diamine-based dyes.

Video Link

The video component of this article can be found at http://www.jove.com/video/54772/

Introduction

Laccases are oxidases that are active towards phenolic and polyphenolic compounds. They have been identified in various living organisms, including plants, fungi, insects, and bacteria. Their enzymatic actions contribute to several morphogenetic phenomena[1]. The enzymes catalyze single-electron oxidation of the substrates, resulting in the formation of radicals that are further coupled to small organics and to solid surfaces. Such coupling processes lead to syntheses of oligomers and polymers and to surface functionalizations[2, 3]. When laccase substrates are from natural sources, such as plant phenolics, the enzymatic reactions are of great interest with respect to green chemistry. Here, both reactants and catalysts are from natural sources. In addition, the resulting products are similar to the natural products, since the overall reactions mimic the *in vivo* syntheses of natural phenolic polymers-including plant lignin, poly(flavonoid), and humus-wherein small plant phenolic compounds are highly cross-linked by oxidase-induced radical coupling[4].

Products derived from laccase-catalyzed coupling reactions of plant-derived phenols can be used to dye gray hair through *in situ* incubation and can be developed as alternatives to commercially available dyes[1]. Such alternatives are important, since commercial hair-dyeing agents are based on *p*-phenylenediamine (PPD), PPD-related diamine compounds, and hydrogen peroxide, which have been shown to be toxic, carcinogenic, and allergenic to humans[5, 6]. In the laccase-catalyzed coupling reactions, the laccases and plant phenols functionally replace hydrogen peroxide and *p*-phenylenediamine, respectively[7]. However, the dyeing speed of the laccase-based systems is much slower than that of the commercial one. In general, the PPD-based dyeing agents require less than one hour to achieve effective color change in keratin hair, while the laccase-based reactions require an overnight incubation[7]. The slow dyeing kinetics could be explained by two possible phenomena. First, the use of a low-pH buffer (*e.g.,* pH 5) to maximize laccase activity has been observed to decrease the degree of swelling in the keratin matrices, thus inhibiting deep penetration of dyes into the matrices. Indeed, agents allowing the dyeing reactions to proceed in high-pH conditions have been shown to be integral to commercial hair-dyeing products[8]. Second, the number of possible chromophore molecules exhibiting strong adsorption to keratinous surfaces during the polymerization reaction has been shown to be proportional to the incubation time (*i.e.,* the extent of polymerization). For example, the transformation of dopamine to polydopamine was shown to induce a strong adhesion to many surfaces that was concomitant with the formation of a black color[9].

In the current work, pre-synthesized polymeric products obtained from *T. versicolor* laccase-catalyzed oxidation of catechin and catechol were used to treat keratin hair for dyeing. We hypothesized that the adsorption ability of the polymers would be much stronger than that of the monomeric plant phenols and that they would initially form low-molecular-weight oligomers. Results demonstrated that, when using the pre-synthesized polymers, the enzymatic oxidation power was no longer necessary. This indicates that the pH can be controlled and that metal ions can be used in hair-dyeing treatments, regardless of enzyme activity. This protocol provides a simple and fast method to dye keratin hair in various shades of color while using eco-friendly and renewable plant-derived phenolics (**Figure 1**).

Protocol

1. Preparation of Plant Phenol-derived Polymeric Dyes

1. Dissolve catechol (0.1 g) and (+)-catechin hydrate (0.1 g) in 32 ml of 100 mM sodium acetate buffer (pH 5.0) and 8 ml of absolute ethanol.
2. Add 10 mg of *T. versicolor* laccase to the catechol- and catechin-containing buffer. Mix vigorously and pour the solution into a square Petri dish. Incubate the dish at room temperature in a shaking incubator (25 rpm) for 24 hr. Dramatic color change of the solution from transparent to dark black can be observed with the naked eye after laccase-induced coupling reactions.
3. Centrifuge the solution for 10 min at 20,000 x g in order to spin down insoluble polymeric particles. Use the deep black supernatant for further desalting.
4. Desalt the reaction solution with a 5 kDa ultrafiltration disc. After concentrating the reaction volume to 20 ml through ultrafiltration, exchange the reaction buffer by adding 300 ml of distilled water. Finally, using filtration, concentrate the volume of the solution to 25 ml.

2. Dyeing Solutions for Gray Keratin Hair

1. Prepare the following six polymeric solutions: polymeric dyes, polymeric dyes/$FeSO_4$, polymeric dyes/$FeSO_4$ in pH 3 water, polymeric dyes/$FeSO_4$ in pH 11 water, polymeric dyes/$FeSO_4$ with acetic acid, and polymeric dyes/$FeSO_4$ with ammonia.
    1. For the polymeric dye, mix 5 ml of distilled water with 1 ml of the desalted polymeric dyes (1.4).
    2. For the mordant solutions, add 0.33 g of $FeSO_4$ to the mixture from step 2.1.1. Vigorously vortex to dissolve the $FeSO_4$ completely.
    3. For the pH 3 or 11 water solution, adjust the pH of 5 ml distilled water using 1 N HCl or 1 N NaOH. Then add 1 ml of the desalted polymeric dyes (1.4) and 0.33 g of $FeSO_4$.
    4. For acetic acid- or ammonia-treated solution, mix 1.0 ml of glacial acetic acid or 1.0 ml of ammonia water with 5 ml of distilled water. Then add 1 ml of the desalted polymeric dyes (1.4) and 0.33 g of $FeSO_4$.

2. For the plant monomers, mix catechol (0.1 g) and (+)-catechin hydrate (0.1 g) in 6 ml of distilled water, with or without 0.33 g of $FeSO_4$, to soak the hair in.
3. As soon as the dyeing solutions from step 2.1 and 2.2 are prepared, completely soak 5-cm long gray human hair tresses (0.2 g) in the solutions. Incubate the hair tresses at 32 °C in a shaking incubator (160 rpm) for 2.5 hr.
4. Afterwards, take out the hair tresses and rinse them with running water. Use an electronic hair drier to remove the moisture. Color change caused by the polymeric dyes can be visualized with the naked eye.
    1. To obtain the color parameters (*i.e., L**, *a**, and *b**), employ a conventional colorimeter according to the manufacturer's protocol. Crumple the hair tresses into a ball, thus allowing them to be measured with the lens of a colorimeter. Repeat this process on a different region of the hair with the colorimeter lens.
    2. Measure the color parameters of each dyed tress seven times. Calculate averages and standard deviations of the parameters. Calculate ΔE using the formula: $[(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$.

3. Color Durability Tests

1. Dissolve 200 mg of sodium dodecyl sulfate (SDS) in 40 ml of distilled water. Soak the dyed hair completely in the SDS-containing water for 5 min at room temperature. Take out the hair tresses and then rinse them with enough running water to remove the detergents. Use an electronic hair drier to remove the moisture.
2. To obtain the color parameters (*i.e., L**, *a**, and *b**), employ a conventional colorimeter according to the manufacturer's protocol. Repeat the soaking described in step 3.1 once more with the same dyed hair and then measure the parameters again.
3. Dissolve 800 mg of SDS in 40 ml of distilled water. As described in step 3.1, repeat the soaking twice more with the same dyed hair. Overall, treat each dyed hair tress with the SDS solution a total of four times.

Representative Results

First, the dyeing ability of polymeric dyes was compared to that of plant-derived monomers (*i.e.,* catechin and catechol). The polymeric dyes induced a significant change in the color of gray keratin hair (**Figure 2A** and **Figure 3**), while the innate gray color of the hair remained very stable with plant monomers (**data not shown**). The effects of mordanting agents on the dyeing abilities of polymeric products were then evaluated. As seen in **Figure 2A**, the addition of Fe ions led to no increase in the ΔE value of gray keratin hair. However, the hair color was significantly changed with the use of Fe ions (**Figure 3**). To check whether mordanting agents can induce coloration with plant phenolic monomers, Fe ions were also added to the monomers without oxidative polymerization. As soon as the Fe ions were dissolved with the monomers in an acidic sodium acetate reaction buffer, a deep black color appeared immediately (**data not shown**). Moreover, complexes of Fe and plant monomeric phenols proved to be effective at dyeing gray human hair, resulting in an efficient transformation of the gray color to deep black (**Figure 3**). The ΔE value resulting from the Fe-plant phenol dye was higher than that of the polymeric dyes (**Figure 2A**).

To evaluate whether a change in pH affects the dyeing efficiency of keratin hair with Fe ion-polymeric dye complexes, low pH (from pH 3-adjusted and acetic acid-containing waters) and high pH (from pH 11-adjusted and ammonia-containing waters) were tested. As seen in **Figure 2A**, such pH changes led to little change in the ΔE value, contrary to the effect of polymeric dyes. However, the visible colors from dyed hair did change markedly depending on which pH-adjusting agent was used (**Figure 3**). Interestingly, the hair color resulting from the use of HCl differed from that resulting from acetic acid, despite their apparently similar roles in pH control. Different hair colors also resulted from NaOH and ammonia. All three color parameters (*i.e., L** ranging from 0 (black) to 100 (white), *a** ranging from -100 (green) to 100 (red), and *b** ranging from -100 (blue) to 100 (yellow)) of our dyed hair were then compared, as shown in **Figure 2B**. Although the ΔE values differed only slightly for the different dyeing conditions, the color parameters were relatively disparate (**Figure 2B**), consistent with the diverse visible colors of the hair tresses (**Figure 3**).

Finally, the detergent resistance of the dyed hair was checked. As seen in **Figure 4**, all dyed hair generally maintained their ΔE values against repeated SDS treatments, indicating that the polymeric dyes with or without mordanting resulted in permanent dyeing. When the plant monomers without polymerization were used, the ΔE values were inversely proportional to the number of SDS treatments applied (**Figure 4**). In contrast, the dyeing was more stable when polymeric dyes only or with $FeSO_4$ were used.

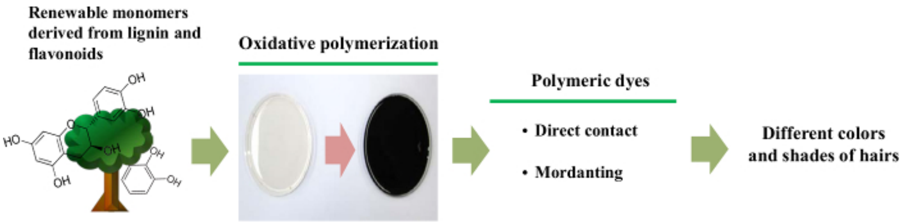

Figure 1. Scheme for plant phenol-based polymeric dye synthesis and its application in keratin hair dyeing. A simple and fast method to dye keratin hair in various shades of color while using eco-friendly and renewable plant-derived phenolics is shown. Please click here to view a larger version of this figure.

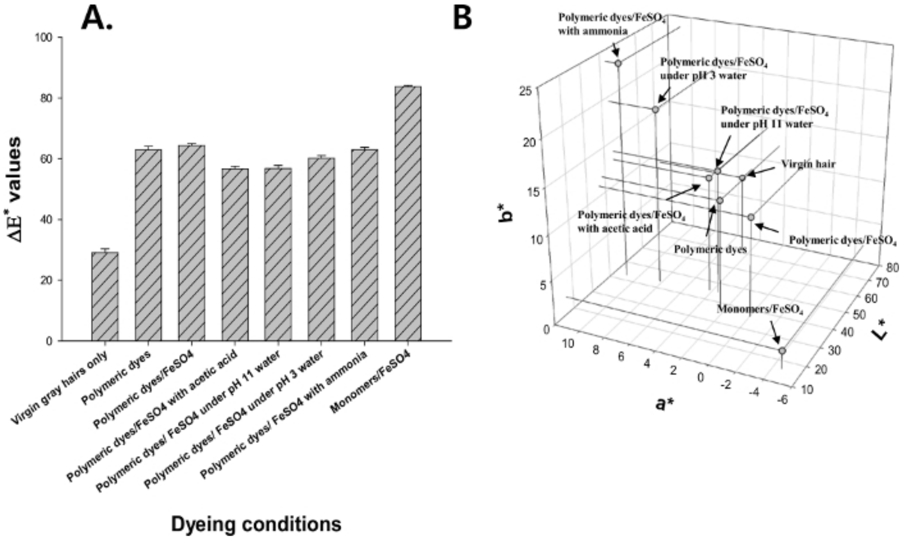

Figure 2. Color evaluation of dyed hair. (**A**) ΔE values (±SD, n=7) and (**B**) distribution of the three color parameters (*L**, *a**, and *b**) under different reaction conditions. Please click here to view a larger version of this figure.

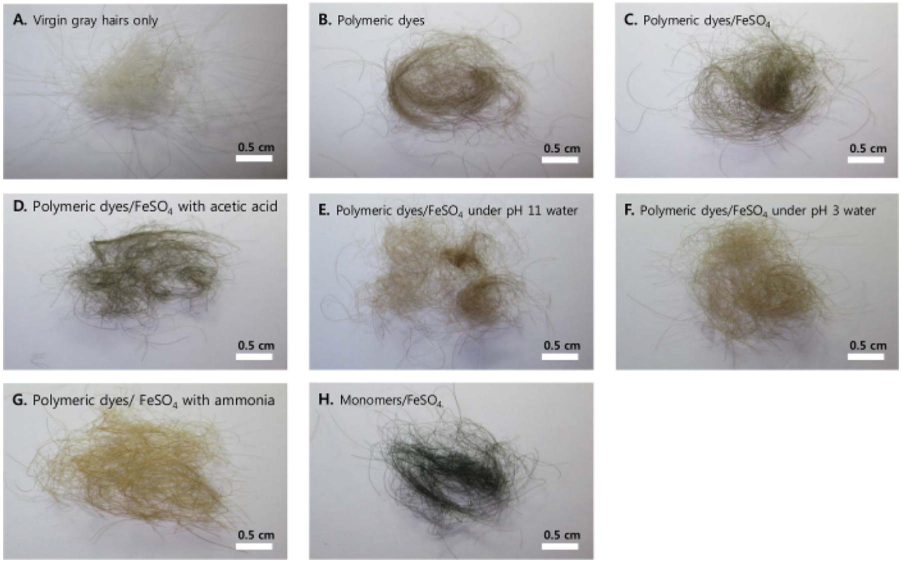

Figure 3. Photographical images of hair dyes under different reaction conditions. (**A**) Virgin gray human hairs only; (**B**) Polymeric dyes; (**C**) Polymeric dyes/$FeSO_4$; (**D**) Polymeric dyes/$FeSO_4$ with acetic acid; (**E**) Polymeric dyes/$FeSO_4$ in pH 11 water; (**F**) Polymeric dyes/$FeSO_4$ in pH 3 water; (**G**) Polymeric dyes/$FeSO_4$ with ammonia; (**H**) Monomers/$FeSO_4$. Scale bar is 0.5 cm. Please click here to view a larger version of this figure.

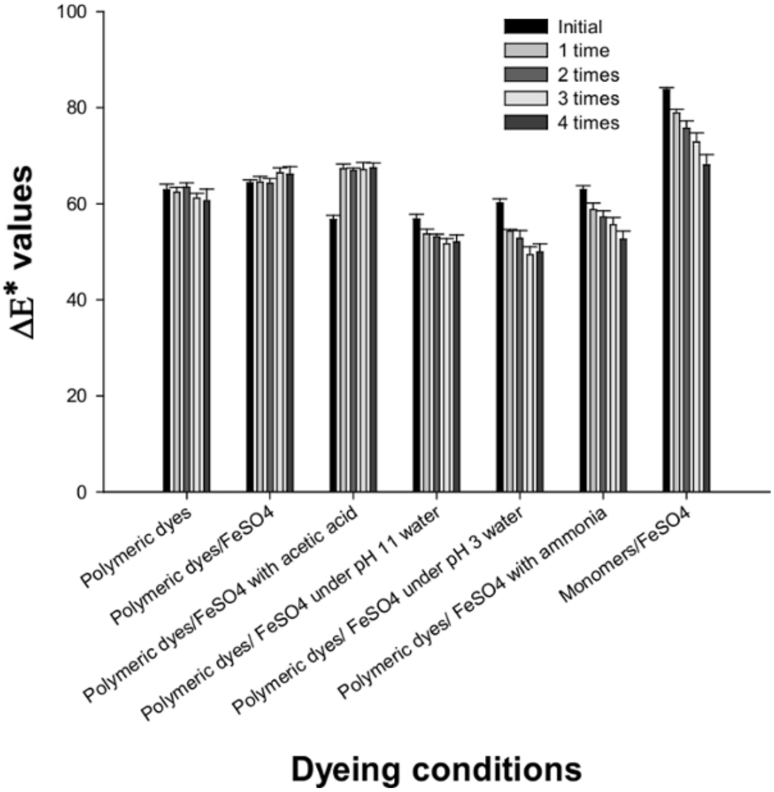

Figure 4. Color durability of dyed hair to repeated detergent treatments. The greater stabilizing effects of direct dyeing (Polymeric dyes) and mordanting with polymeric products (Polymeric dyes/$FeSO_4$) rather than mordanting with monomers (Monomers/$FeSO_4$) were shown (±SD, n=7). Please click here to view a larger version of this figure.

Discussion

Interestingly, our method reduced the time it took to dye keratin hair with oxidant-induced polymerizations of natural phenolics. It also induced diverse colors in the hair through simple manipulations of the polymeric dyes, such as changing the pH and applying mordant.

In situ incubation of keratin hair with laccase-catalyzed oxidation of plant phenols requires overly long incubation times to attain effective dyeing[7]. Such slow dyeing kinetics may be due to the poor binding abilities of plant phenols and to the initial oligomers having very low molecular weights, thus delaying the starting time of actual hair dyeing until polymers with relatively high molecular weights are formed. The fast hair dyeing and color durability observed when directly using the polymeric products (**Figures 2A** and **4**) strongly support the above hypothesis. The increased binding ability and presence of a chromophore in the polymers may have contributed to the fast speed of permanent hair dyeing. Although other researchers have shown that some monomeric phenolics are able to bind well to certain surfaces, the binding durability in the presence of external stimuli is unclear, as the authors evaluated the binding phenomena under very mild conditions[10, 11]. In addition, a chromophore was not present in the monomeric compounds tested. Therefore, the most critical step in this protocol is to use the products obtained after 24 hr incubated reactions, because a large number of high-molecular-weight, colorful phenolic polymers exhibiting strong binding properties are thus sufficiently formed.

Several studies have used metal ion-phenol complexes to dye fabrics[12, 13]. There are two possible mechanisms for these dyeing phenomena: the metal ion-phenol complexes act as chromophores, or the metal ions bidirectionally coordinate the phenols and the surfaces to be dyed, leading to strong attachment of the complexes. The variability in hair color depending upon the presence of Fe ions (**Figures 2B** and **4**) is consistent with both of the above mechanisms, because coordination of the metal ions with the polymeric products and monomers can result in a change and generation of color, respectively. In addition, the detailed coordination bonding structures may have been modulated by pH changes, as observed in drug delivery systems[14]. It is thus not unreasonable to suggest that mordanting concomitant with pH changes would result in diverse color changes through structural transformations of the chromophore. In general, mordanting is believed to enhance the dyeing ability of natural plant extracts[13]. However, the similarity of the ΔE values of the polymeric dyes with and without Fe ions (**Figure 2A**) indicated that the coordination of the Fe with the dyes mainly changed the chromophore, resulting in dyed hair of different colors, as seen in **Figure 3**. High pH would lead to high ΔE values of dyed hair, because high pH agents such as ammonia water are known to cause keratin fibers to become swollen, thus increasing dye diffusion rates[8]. Such diffused dyes are then captured when the hair matrices retighten. However, ΔE values of dyed hair change little with high pH or ammonia, indicating that the ability of polymeric dyes to bind onto the hair surface better describes the hair dyeing in this protocol than does keratin swelling.

Color durability is very important in hair dyeing, and the dyeing methods should produce dyes that are durable in the presence of external stimuli. In particular, daily shampooing is the most significant stimulus that fades dyed hair[8]. As seen in **Figure 4**, our dyeing methods were very resistant to repeated SDS treatments. The greater stabilizing effects of direct dyeing (Polymeric dyes) and mordanting with polymeric products (Polymeric dyes/$FeSO_4$) rather than mordanting with monomers (Monomers/$FeSO_4$), as shown in **Figure 4,** may have been due to the greater molecular weights of polymeric dyes. The efficiency with which the metal ions bridged the polymeric dye and keratin surface apparently depended upon the average molecular size of the dye. Hair is composed of various substances, including melanin, amino acids, proteins, and lipids[15]. Further studies should be conducted to confirm whether this protocol is effective on a variety of human hair types. In addition, even though our materials are derived from natural sources, a strict toxicity test to human cells is required in order to fully guarantee non-toxicity.

Overall, we here provided a well-defined method to induce permanent keratin hair dyeing, using eco-friendly materials within a commercially acceptable short period of time. Based on our data revealing the properties of the direct binding of polymeric dyes to keratin hair, further methods allowing our polymeric dyes to act as active components in commercialized PPD-based hair-dyeing products will be developed.

Disclosures

The authors declare that they have no competing financial interests.

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