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Peroxidase-Catalyzed Polymerization of 1-Hydroxypyrene

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ABSTRACT

Peroxidase-catalyzed polymerization of a hydroxy substituted polynuclear aromatic compound, 1-hydroxypyrene, is reported. The fluorescence spectrum of the synthesized polymer shows a dramatic red shift compared to that of the monomer. The enzymatic polymerization of 1-hydroxypyrene demonstrates the capability of peroxidase in catalyzing the oxidation of polynuclear aromatic molecules, and offers new possibilities for the design and synthesis of polymers with interesting electrical and optical properties in an environmentally friendly way.

Key Words: Horseradish peroxidase; Enzymatic polymerization; 1-Hydroxypyrene.

INTRODUCTION

Peroxidase-catalyzed polymerization of phenols and anilines has been extensively studied due to its potential in the synthesis of electrically active polymers in an environmentally benign way.^[1] In the presence of H₂O₂, peroxidase, such as horseradish

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peroxidase (HRP), can oxidize phenols and anilines to generate corresponding radicals. These radicals may couple together through radical coupling and transfer to form dimers, trimers, tetramers, oligomers, and polymers.^[2] In a suitable solvent system, polymers with modest molecular weight may be realized.^[1b] Typical monomers used for this peroxidase-catalyzed polymerization are phenol, aniline, and their substituted derivatives.^[2b] The use of phenol monomers with different chromophores substituted at the para position has resulted in the synthesis of macromolecular dyes, such as polyazophenols, with interesting optical properties.^[3] So far, very few hydroxy group substituted polynuclear aromatic hydrocarbons have been investigated using this peroxidase-catalyzed polymerization.

In this paper, we report the peroxidase-catalyzed oxidation of a polynuclear aromatic hydrocarbon substrate, 1-hydroxypyrene, to form the products shown in Sch. 1. 1-Hydroxypyrene is a planar molecule with dimensions much larger than that of typical phenol substrates and thus serves as an interesting challenge to test this peroxidase-catalyzed polymerization. In addition, pyrene derivatives are highly fluorescent molecules with long lifetimes and sensitive solvatochromic shifts.^[4] It has been demonstrated that pyrene derivatives are useful fluorescent probes^[5] due to the efficient emission in comparison to other polyaromatic fluorophores. Therefore, one of the major objectives of this work is to produce a new type of pyrene-based macromolecular fluorescent dye using peroxidase-catalyzed polymerization of 1-hydroxypyrene.

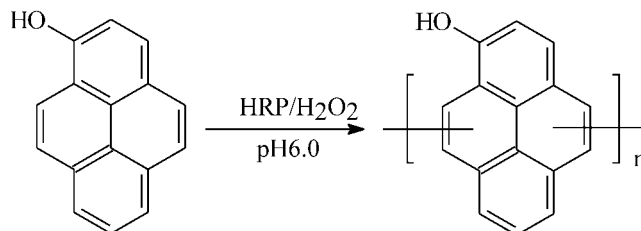
EXPERIMENTAL

Materials

Horseshoe peroxidase (EC 1.11.1.7) (200 unit/mg) was purchased from Sigma with RZ > 2.2. A stock solution of 10 mg/mL in pH 6.0, 0.1 M phosphate buffer was prepared. The monomer, 1-hydroxypyrene, was obtained from Aldrich and used as received. All other chemicals and solvents used were commercially available, of analytical grade or better and used as received.

Enzymatic Synthesis of Poly(1-Hydroxypyrene)

The synthesis of poly(1-hydroxypyrene) was performed in a mixture of solvent and buffer. Hundred milligram of 1-hydroxypyrene was added into a 50 mL mixture of 50%



Scheme 1.



ethanol and 50% aqueous buffer. After the monomer was totally dissolved, 0.5 mL HRP stock solution was added. H_2O_2 (0.2 M) equal molar to monomer was pumped into the solution gradually. After equal molar H_2O_2 was added, the reaction was left stirring for at least 3 h. The synthesized products were isolated by centrifugation, purified by washing with the mixture of ethanol and water (30% ethanol and 70% water). The obtained precipitates were dried in a hood at room temperature, and then vacuum dried in an oven. Other solvents system such as the mixture of 50% acetone and 50% buffer may also be used as solvent in the reaction. The synthesized products show similar properties as obtained in the ethanol/buffer mixture.

UV-VIS and Fluorescence Spectroscopy

UV-VIS and fluorescence spectroscopy were used to monitor the process of enzymatic polymerization of 1-hydroxypyrene. A stock solution was prepared by dissolving 5.9 mg 1-hydroxypyrene in 2.7 mL ethanol (equal to 10 mM). H_2O_2 (50% w/v) was diluted to a concentration of 5 mM into a mixture of 50% ethanol/buffer. To a 3 mL quartz cell which contained 3 mL of 50% ethanol/buffer mixture, 10 μL 1-hydroxypyrene stock solution and 15 mL HRP stock solution (10 mg/mL) were added. A UV-VIS spectrum was recorded on a Perkin-Elmer Lambda 9 spectrophotometer before the addition of H_2O_2 . After each addition of 2 μL of the diluted H_2O_2 solution, a spectrum was recorded.

The same conditions were used in the measurement of the fluorescence spectra. Fluorescence spectra were recorded prior to the addition of H_2O_2 and after each incremental addition of H_2O_2 to the reaction.

RESULTS AND DISCUSSION

1-Hydroxypyrene was enzymatically oxidized in a mixture of 50% ethanol and 50% buffer (0.01 M phosphate) at pH 6.0 at room temperature as shown in Sch. 1. The process of the reaction was monitored in situ by UV-VIS and fluorescence spectroscopy. The monomer, 1-hydroxypyrene, shows three major absorption peaks in the mixture of ethanol/water at ~ 240 , ~ 276 , and ~ 345 nm, respectively [Fig. 1(a)]. H_2O_2 was added to initiate and facilitate the reaction. The major absorption peaks from the monomer decreased gradually with the addition of H_2O_2 , and a new absorption peak at ~ 450 nm emerged. This peak gradually increased with an absorption tail extending to longer wavelength, indicating that oxidized products have a longer conjugation length. The fluorescence spectrum of the monomer, 1-hydroxypyrene, gives three emission peaks at ~ 374 , ~ 394 , and ~ 416 nm, respectively [Fig. 1(b)]. As the reaction progressed, the fluorescence was monitored in situ. The fluorescence intensity of the monomer decreased gradually with the addition of H_2O_2 into the reaction media. After an equal molar amount of H_2O_2 (relative to monomer) was added to the solution, the fluorescence of the monomer was completely quenched, indicating the total conversion of the monomer.

Similar reaction conditions as described above were used in the bulk synthesis of the poly(1-hydroxypyrene). 1-Hydroxypyrene is a very active substrate in the HRP-catalyzed



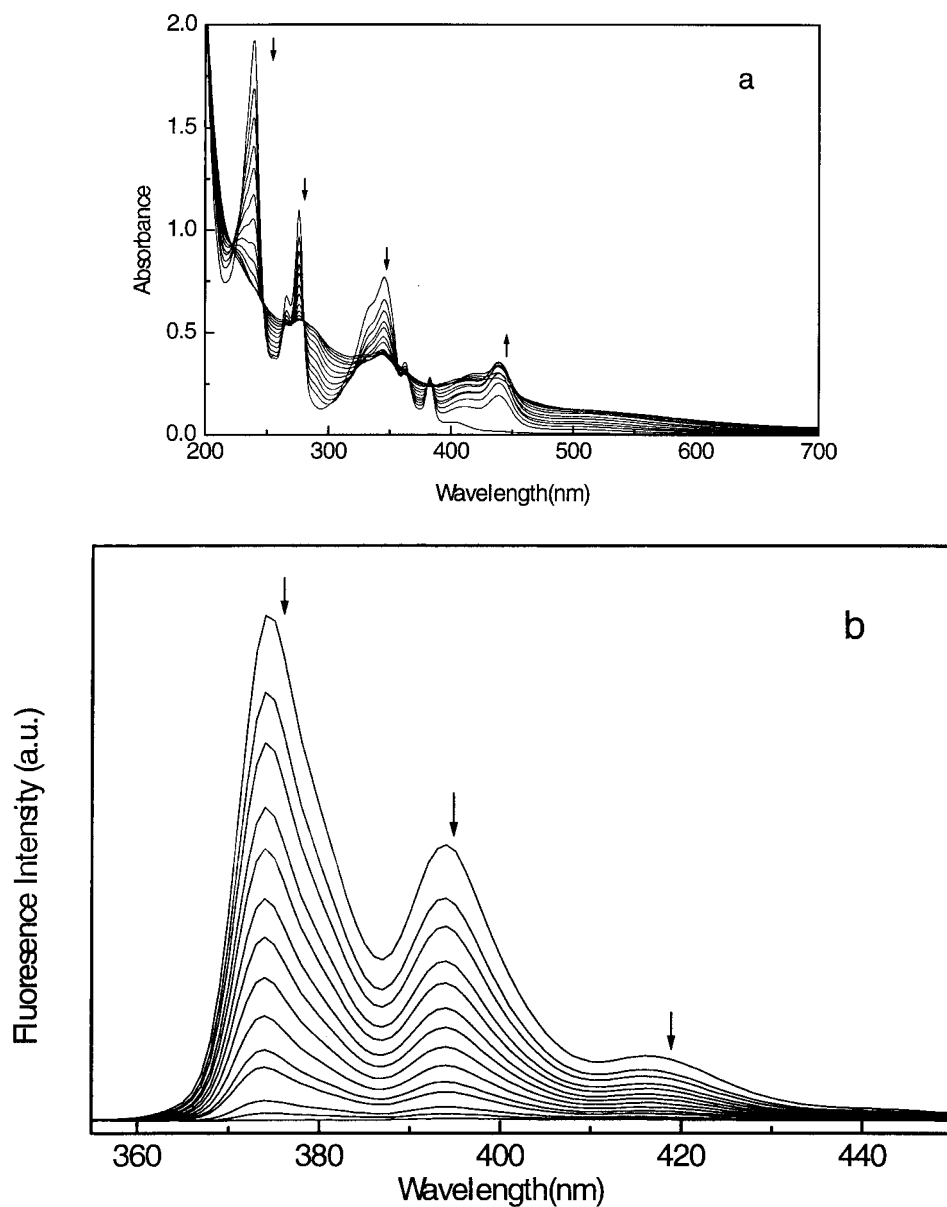


Figure 1. UV-VIS (a) and fluorescence spectra (b) for HRP-catalyzed oxidation of 1-hydroxypyrene. The reaction conditions are the same for recording the spectra (a) and (b). The exciting wavelength for the recording spectra (b) is 355 nm.



oxidation reaction. With the progress of the reaction, the products formed precipitated as a powdery material. The synthesized products were isolated by centrifugation, purified by washing with a mixture of ethanol and water (30% ethanol and 70% water). The obtained precipitate was dried in a fume hood at room temperature and then dried in a vacuum oven. A brown-yellow powder was obtained after the precipitates were dried. The isolated yield of the reaction was $\sim 80\%$. The powder was slightly soluble in dioxane and DMF.

The enzymatically synthesized poly(1-hydroxypyrene) does not show any fluorescent emission in the ethanol/water mixture during the reaction as was discussed previously. However, the synthesized products do show strong fluorescence in anhydrous solvents, such as ethanol, dioxane, and DMF etc. The absence of the fluorescence emission from the synthesized product during the reaction is due to the presence of water in reaction system.^a The fluorescence spectra of the monomer and polymer of 1-hydroxypyrene in dioxane are shown in Fig. 2. The monomer of 1-hydroxypyrene shows a strong fluorescence peak at ~ 394 nm in anhydrous dioxane with a shoulder at ~ 416 nm. However, the peak of the polymer fluorescence spectrum shifts significantly to longer wavelength with a peak at ~ 482 nm and a shoulder peak at ~ 506 nm. A red shift of ~ 70 nm for the major peak of the polymer was observed compared to that of the monomer. The photographs of the quartz cells containing the monomer and polymer solutions on excitation by a 360 nm UV lamp are shown in the inset in Fig. 2. Typically, the solution of the monomer in dioxane shows purple blue light emission, while the solution of the synthesized product in dioxane gives blue green light emission. The pyrene derivatives readily aggregate together at high concentration to form excimer, which usually causes a red shift of the emission. In this case, the significant red shift of the emission of the synthesized products is not due to the formation of the excimer, since low concentrations of monomer and polymer have been used in these measurements. The observed dramatic red shift of emission of the polymer compared to that of the monomer is attributed to the red shift of the absorption.

It has been previously reported that the structures of enzymatically synthesized polyphenols are complicated due to the presence of both C—C (two carbons on different aromatic rings coupled together, usually at the ortho position) and C—O—C (the oxygen from the hydroxy group on one aromatic ring coupled with the carbon on another aromatic ring) couplings in the reaction.^[1a,3] The main chain of the synthesized polyphenols is usually a mixture of phenylene and oxyphenylene units. The structure of the synthesized product, in the present work, was characterized by ¹H NMR and FTIR spectroscopy (data not shown). The primary results show a similar structural feature as that observed with enzymatically synthesized polyphenols in that both C—C and C—O—C coupling appear to be involved in the reaction. As one can see from the molecular structure of 1-hydroxypyrene, several radical resonance structures may be formed. Thus, more positions on the pyrene ring are available for coupling compared to that of the phenol. The structure of the enzymatically synthesized oligomers and polymers of 1-hydroxypyrene are expected to be more complicated in structure compared to

^aThe reasons for the fluorescence quenching of the synthesized products due to the presence of water are still under investigation.



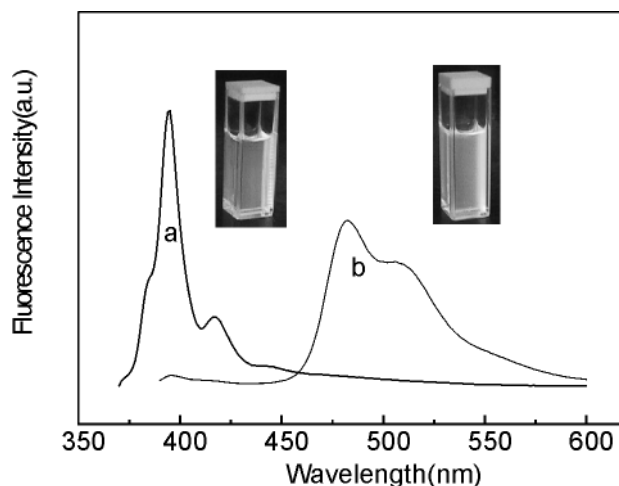


Figure 2. The fluorescence spectra of the monomer (a) and synthesized products (b) in pure dioxane. The exciting wavelength for measuring these spectra is 355 nm. The insets show the photos of the quartz cells that contain monomer and polymer solution pumped by a 360 nm UV lamp.

polyphenols. The details on the coupling positions and the final structure of the synthesized products are still under investigation.

CONCLUSION

In conclusion, we have shown that 1-hydroxypyrene, a polynuclear aromatic compound, can be oxidatively polymerized by an environmentally friendly peroxidase-catalyzed reaction. The feasibility of this enzymatic oxidation of 1-hydroxypyrene demonstrates the ability of peroxidase in catalyzing the oxidation of polynuclear aromatic molecules. The product of this reaction shows a dramatic red shift in the fluorescence spectra compared to that of the monomer, resulting in a new type of pyrene-based macromolecular fluorescent dye. Such types of polynuclear aromatic hydrocarbons offer exciting new possibilities for electronic and photonic materials. The detailed mechanism for this peroxidase-catalyzed oxidation of polynuclear aromatics is still under investigation and will be the subject of future publications.

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