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ENZYMATIC SYNTHESIS OF POLY(HYDROXYSTILBENE)S. A NEW CLASS OF LUMINESCENT DYE

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Dedicated to the memory of Professor Sukant K. Tripathy.

Key Words: Luminescence; Enzymatic synthesis; Horseradish perioxidase; Hydroxystilene; Macrodye

INTRODUCTION

In 1987, Tang and VanSlyke [l] first demonstrated fabrication of light emitting diodes (LED) using organic luminescent dyes. Since then, the organic LEDs have been extensively investigated. The potential of making large area displays from easily processable polymer has driven much of recent research into the development of polymeric light-emitting diodes [2]. Holmes *et al*. first demonstrated the possibility of using poly(phenyl vinylene) (PPV) for light emitting diodes [3]. The emission range of conjugated organic materials can be readily tuned by modifying the structure of the chromophore. This should allow light emission to be engineered over the entire spectral range. The goal of synthesizing efficient polymeric blue emitters is still an active area of research. A number of

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different approaches have been investigated towards the synthesis of blue wavelength emitting polymers. These include using different substituents to change the extent of conjugation, copolymerizing with different materials to control the conjugation length, using side chain chromophores and blending blue light emitting chromophores with polymers [4].

In our research group, we have been employing enzyme catalyzed polymerization for the synthesis of conjugated polymers such as polyanilines and polyphenols. Enzymatic polymerization provides an attractive alternate approach to chemical synthesis. Enzymatic reactions usually proceed under much milder reaction conditions and are environmental friendly.

In vitro synthesis of cellulose, ring-opening polymerization of polyester by lipase, and polyaromatics by horseradish peroxidase have been extensively studied [5]. Peroxidases are also advantageous due to their wide working range and facile reaction conditions [6], Dordick *et al*. first demonstrated that horseradish peroxidase (HRP) can catalyze polymerization of phenolic monomers in a mixed organic solvent system [7]. Since then, several modified enzymatic reactions such as reversed micelles [8], Langmuir monolayers [9] and template assisted synthesis [10], have been investigated. Peroxidases are also gaining special interest due to the possibility of forming direct aromatic rings to ring coupled products. Polymers with conjugated backbones have potential applications as electro-optic and electronic materials [11]. Optoelectronic materials like polyaniline, water-soluble conjugated polyaniline complex [10] photodynamic azobenzene [12] and fluorescent naphthol-based polymers were demonstrated [13].

In this paper, we describe the synthesis and characterization of polymers from stilbene based phenols by enzyme catalyzed polymerization reactions and some interesting properties that were observed. Stilbene derivatives are one of the most important fluorescent chromophores. This class of compounds has been extensively developed for fluorescent whitening agents for paper and fiber products. They are also used in detergents. Our group recently reported the synthesis of polyazophenols [14] by the oxidative coupling of azophenol using HRP. In addition to the expected ortho coupling, some meta coupling was also observed. As a result, a macromolecule with essentially 100% dye content and an unusual articulated structure was formed. These macrodye molecules are glassy and good optical quality thin films can be easily fabricated. We realized that the same design motif could be extended to photoluminescent phenolic dye molecules.

EXPERIMENTAL

Materials

4-Hydroxystilbene (4HS) and 4-nitro-4’hydroxystilbene (4NOHS) were purchased from Acros Chemical Co. and used as received. Hydrogen peroxide, 1,4-dioxane, and sodium acetate were purchased from Aldrich Chemical Co. Horseradish peroxidase Type II was obtained from Sigma Co.

Measurements

UV-Vis spectra were recorded on a Perkin-Elmer Lambda 9 spectrometer. Fluorescence spectra were measured on a SLM 8100 emission spectrometer. Fourier transform infrared (FT-IR) spectra were obtained on a Perkin-Elmer 1760x spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX500 spectrometer.

Polymerization

The general procedure for the enzymatic polymerization reaction involved mixing a solution of the monomer in 1,4-dioxane/sodium acetate buffer (pH = 6) solution and HRP solution. In the case of 4-hydroxystilbene, the ratio of dioxane to the buffer solution was 4 to 1 and in the case of 4-nitro-4'-hydroxystilbene, it was 1 to 1. The final concentration of horseradish peroxidase in the reaction mixture was 0.1 mg/mL. Hydrogen peroxide was then added to initiate the reaction. The amount of hydrogen peroxide is one equivalent with respect to the monomer concentration. The reaction solution was stirred overnight to make sure the reaction was complete. The precipitate was collected by centrifugation and washed repeatedly with acetone/water (3:7) solution to eliminate any monomer residue and enzyme. The product was dried in a vacuum oven for 12 hours before characterization.

RESULTS AND DISCUSSION

The UV-Vis absorption spectra of poly(4HS) and its monomer are shown in Figure 1.

Poly(4HS) exhibited a broad structured band from 275 to 355 nm, with maxima at 300 and 324 nm. These absorption features are similar to the monomer and only slightly red-shifted and are attributed to the stilbene A band region corresponding to the n-π^* (^1B-^1A) transition [15]. The similarity of the band structures between the polymer and monomer indicates that the polymer retains the stilbene structural skeleton. The absorption shift to longer wavelength upon polymerization may be attributed to the increase in conjugation length due to coupling between aromatic rings. NOHS has an absorption maximum at 370 nm which is about 30 nm red-shifted from 4-hydroxystilbene due to intramolecular charge transfer due to the nitro group. The HRP polymerized poly(NOHS) showed two distinct bands in the UV-Vis spectrum at 370 nm and at 270 nm. The 370 nm peak is due to the NOHS monomer. The peak at 270 nm may have resulted from the isolated nitrobenzene group that has an absorption maximum at 268.5 nm [15]. This implies that in some cases saturation of the stilbene double bond happens after a radical was induced by HRP. The conjugation length was thus broken and a certain amount of non-conjugated nitrobenzene was formed. This conjecture was further

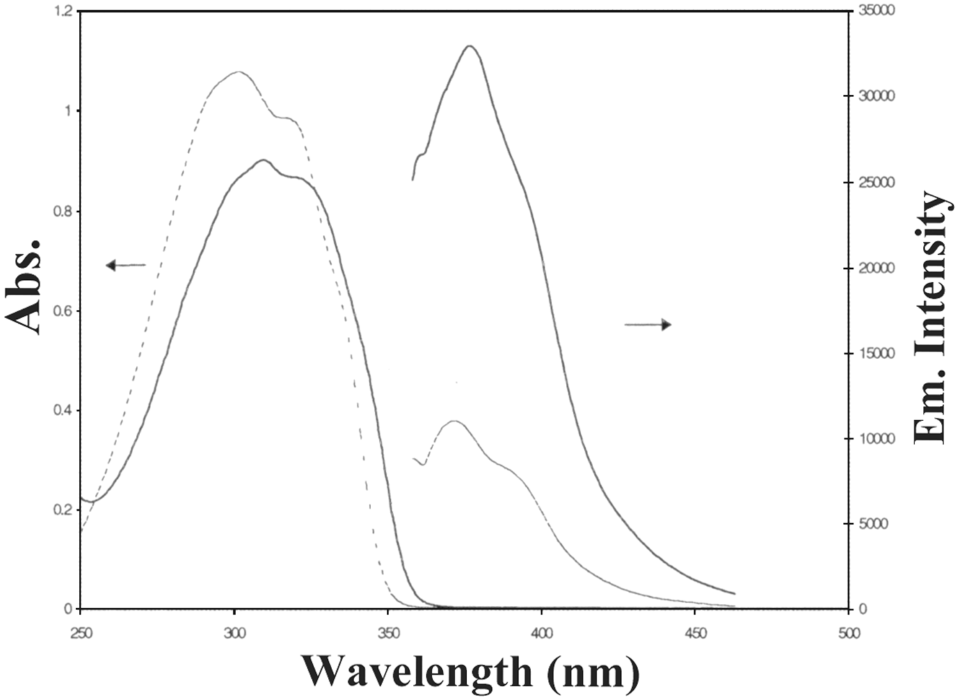

Figure 1. Absorption and emission spectra of 4-hydroxy stilbene (dashed line) and poly(4HS) (solid line) in 1,4-dioxane.

verified by the characterization of the emission properties of the polymer as described below.

In the fluorescence study, poly(4HS) showed less structure and has a slightly red-shifted emission peak than the monomer. The shift in the wavelength may be due to the conjugation length change after polymerization. Normally polymerization of fluorescent chromophores results in decreased fluorescence due to increased local concentration quenching effects [16]. But to our surprise, the emission intensity of poly(4HS) is much stronger than the monomer. It has been reported that HRP synthesized polyaromatics will produce randomly branched molecules [17]. The random ortho and meta linkage of the chromophore results in an articulated structure, though the UV-Vis absorption spectrum of poly(4HS) implies that the chromophore structure is retained in the polymer. The irregularity of polymer conformation prevents close packing of the chromophores and reduces the loss of emission in the solid state. Figure 2 shows the absorption and emission spectra of spin coated poly(4HS) film.

Poly(NOHS) in 1,4-dioxane solution exhibited an emission maximum at 490 nm when excited at 390 nm. Its emission feature is similar to the monomer emission spectrum with comparable emission intensity (Figure 3).

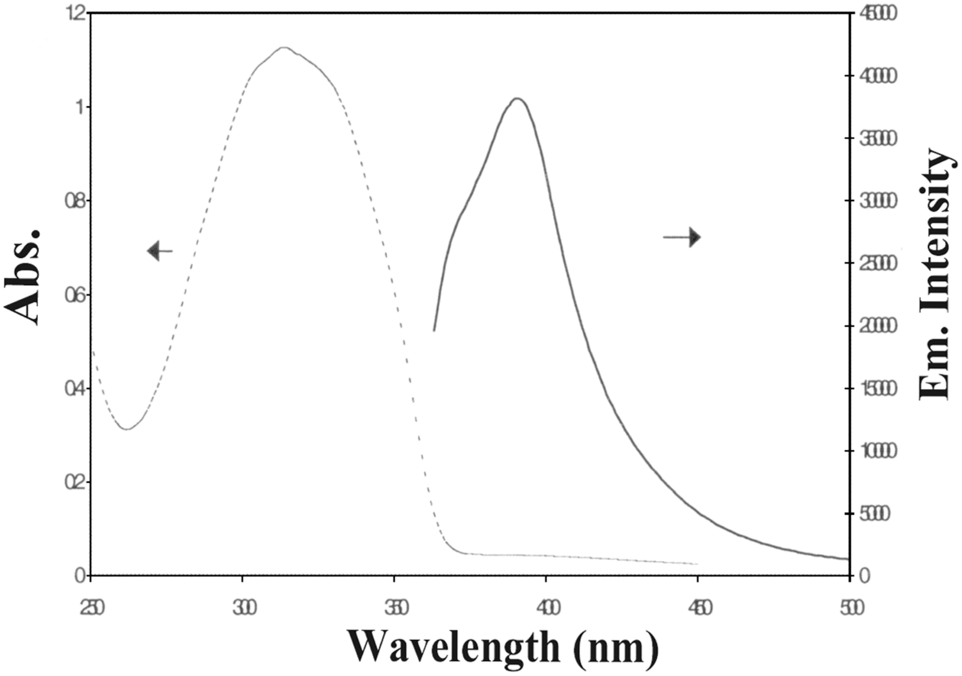

Figure 2. Absorption and emission spectra of poly(4HS) film spin-coated on a quartz slide.

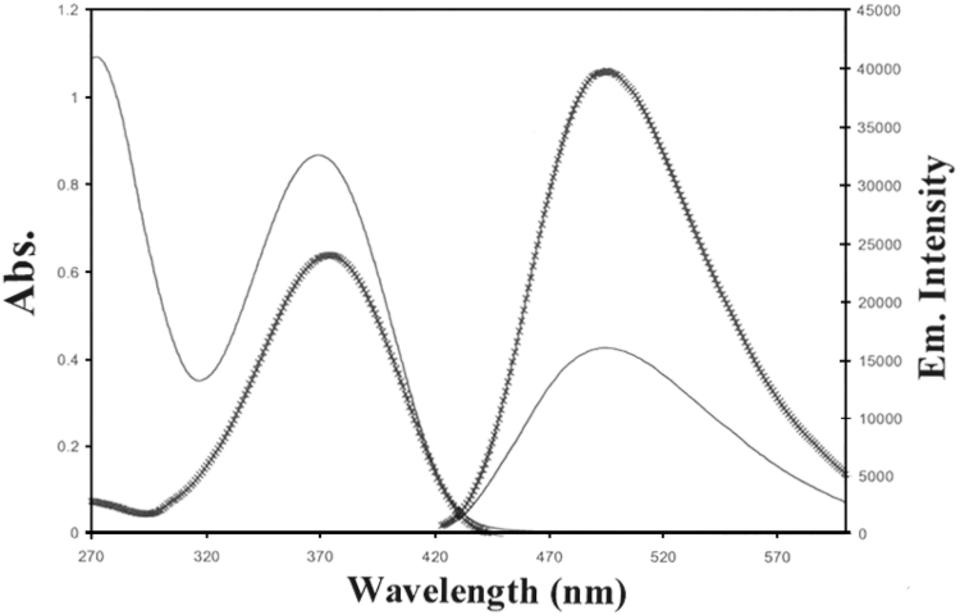

Figure 3. Absorption and emission spectra of 4-nitro-4'-hydroxy stilbene (dashed line) and poly(NOHS) (solid line) in 1,4-dioxane.

The DMF solution of poly(NOHS) formed a yellowish optical quality film by spin coating. The absorption spectrum of the film showed two bands, one at 270 nm and the other at 370 nm. These features are similar to the absorption spectrum obtained from the poly(NOHS) in 1,4-dioxane solution. The fluorescence spectrum has an emission maximum at 520 nm, about 30 nm red-shifted from the solution spectrum. Although poly(4HS) maintained the same emission intensity in both solution and film, the emission intensity of poly(NOHS) obtained from the spin-coated films is fairly low. This may be due to the higher conversion of the alkene double bond to a saturated bond on the stilbene moiety.

The FTIR spectra obtained from poly(4HS) and its monomer are shown in Figure 4.

In the polymer spectra, it shows a broad peak from 3500 to 3300 cm^{-1}, and a small peak at 3045 cm^{-1}. These represent the OH intermolecular stretching and aromatic C-H stretching, respectively. These are almost identical to those observed in the case of the monomer, indicating that the polymer has a significant phenolic character. A second observation is the appearance of a new peak at 1600 cm^{-1}. This can be assigned as the C=O vibration of a quinoid or ketonic group. This structure formation may result from the free radical resonance to the 2 position of the phenol ring and subsequent 2-6’ coupling. Quinoid functionality has been observed in both electrochemical and enzymatic polymerizations. A similar FTIR spectral response was observed in the case of poly(NOHS).

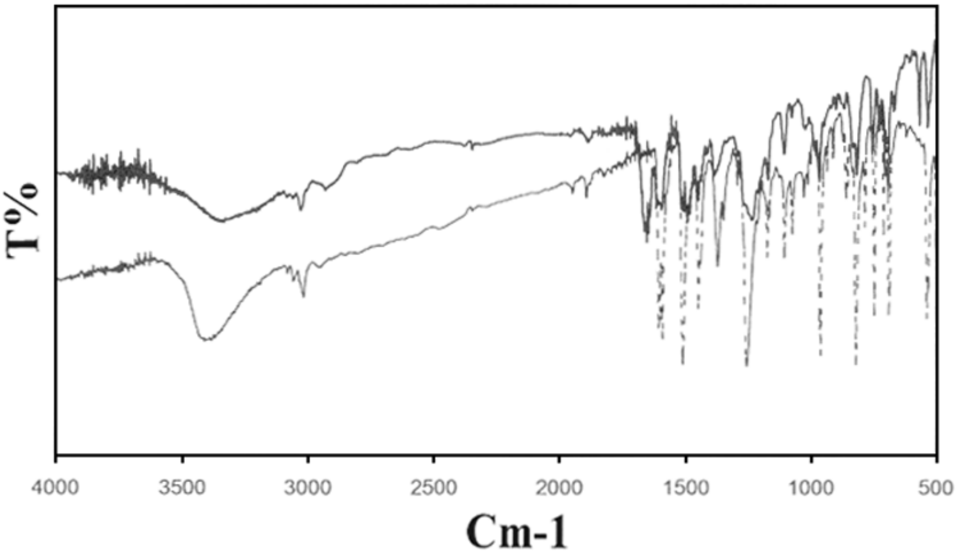

Figure 4. FTIR spectra of monomer (dashed line) and polymer (solid line) of 4-hydroxystilbene in KBr.

In the proton NMR spectra, both poly(4HS) and poly(NOHS) showed indistinguishable broad peaks at 6 to 8 ppm in the aromatic region that are characteristics of different and closely packed hydrogens as polymer formed. Two distinctive small peaks further upfield (4.5 and 5.4 ppm) were also observed. These are not due to the aromatic hydrogens. These may be tentatively assigned to the hydrogens on a dihydrofuran moiety, which may result from an 8-5 coupling between two monomers. Tomioka *et al*. [18] reported that chemically synthesized 2,3-diphenyl-2, 3-dihydrofuran has a similar pattern in the same region. This conjecture is also supported by peroxidase oxidation of E-coniferyl alcohol (4-hydroxy-3-metboxycinnamyl alcohol). Under peroxidase catalyzed polymerization, this naturally occurring lignin precursor turns to dehydrodiconiferyl alcohol as one of the major products [19]. This is a result of radical formation on the vinylic carbon followed by dimerization. This may support the observation of poly(NOHS) UV-Vis absorption spectrum. The observation of a peak at 270 nm can be attributed to the unconjugated nitrobenzene structure attached to the dihydrofuran group. The GPC results show that the poly(NOHS) has a molecular weight around 7000 compared to a polystyrene standard in a DMF column, while the molecular weight of poly(4HS) is only about 3000. Both polymers can be spin coated from organic solvents into continuous good optical quality thin films. Preliminary attempts of fabricating a light emitting device from poly(4HS) were made. The device was fabricated by spin-coating a film of poly(4HS) on an indium tin oxide (ITO) anode and evaporating an aluminum cathode over the polymer film. Because poly(4HS) emits in the UV range, some tris(8-quinolinolato) aluminum (Alq3) was deposited on the top of ITO in order to observe the light emission. As anticipated, yellow light from Alq3 was seen when a voltage was applied. This demonstrated that the poly(4HS) could be electrically pumped emitting UV light which induces fluorescence from Alq3.

In summary, a new class of HRP catalyzed stilbene based luminescent polymers were synthesized. Poly(4-hydroxystilbene) prepared enzymatically exhibits fluorescence in the same wavelength range as the 4-hydroxystilbene moiety. The fluorescence intensity increased about 3 times compared to the monomer upon polymerization. The polymer can be processed into good optical quality films, and no apparent fluorescence quenching was observed in the films. Poly(4-nitro-4’-hydroxystilbene) has a maximum emission wavelength in the visible range (490 nm) showing a comparable emission intensity as the monomer. Unfortunately, solid state quenching was observed in the poly(NOHS) film. We are encouraged by the lack of quenching in the case of the poly(4HS) film. The species obtained from the HRP catalyzed oxidation of 4-hydroxystilbene are coupled at various positions of the aromatic ring. The macrodye thus synthesized also showed promising electroluminescence behavior. The effect of different substitution groups to the HRP catalyzed polymer structure and how they may influence the light emission properties are under investigation. HRP polymerization is a promising route to develop other glassy dye materials whose emission wavelength can be tuned across the visible wavelength region.

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