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Effect of Temperature on the Enzymatic Polymerization of 4-Propylphenol: An In Situ ^{1}H-NMR Study[#]

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ABSTRACT

The effect of temperature on the concentration of the major reactive species as a function of H_2O_2 addition in the enzymatic polymerization of 4-propylphenol was investigated using in situ ^{1}H-NMR spectroscopy. We have studied the trend of monomer depletion and the formation of predominant dimers with an incremental addition of H_2O_2 at different temperatures. The trends for depletion of monomer are very similar while the trends for the dimers display dramatic differences at the investigated temperatures. The dynamic equilibrium stage of dimers was observed only in the case of low reaction temperature. The comparison of the in situ spectra recorded at different reaction temperatures suggests that less undesired side reactions occurred at low temperature.

Key Words: Enzymatic polymerization; Variable temperature; In situ ^{1}H-NMR spectroscopy.

[#]Dedicated to the memory of Professor Sukant K. Tripathy.

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INTRODUCTION

Over the past few decades, enzymatic polymerization has attracted the attention of researchers due to its one-step reaction procedure and environmental compatibility. Horseradish peroxidase (HRP) catalyzed synthesis of polyphenols has been especially investigated extensively because of wide potential applications in the areas of photonics,[1–3] bio-sensors,[4] and phenolic resins and coatings.[5] Typically this reaction is carried out in an aqueous-organic or aqueous solution at room temperature, with H_2O_2 as an oxidant.[6] The polymerization reaction involves continuous initiation and coupling steps during which the two kinds of radicals, the initially produced phenoxy radicals and the subsequently formed phenyl radicals, co-exist and propagate simultaneously. Therefore, normally the prepared poly(phenol)s contain mixed repeating units of phenylene and oxyphenylene.[7] To prepare polymers with well-defined structure, many strategies have been developed such as the polymerization of monomer with unique propagation center,[8,9] the manipulation of solvent system to enhance the propagation of one kind of radicals.[10] However, to date the effect of temperature on such processes has not been investigated.

It is well known that temperature plays an important role in various free radical reactions. The reactivities of the free radicals are sensitive to temperature; consequently, it is expected that temperature can be utilized to minimize undesired products and tailor the propagation of the radicals towards formation of desired polymers.

High resolution NMR is a powerful analytical tool in investigating the mechanism of reactions by monitoring the state of the intermediates and analyzing the products. Our previous work using an in situ ^{1}H-NMR technique has demonstrated its importance in studying the coupling mechanism of the enzymatic polymerization during the very early stages of the reaction for different phenolic monomers.[11]

In this paper, we apply in situ ^{1}H-NMR, combined with a variable temperature (VT) technique to investigate the effect of temperature on the enzymatic polymerization of 4-propylphenol. We focus mainly on (a) monitoring the consumption of monomer and the formation of major dimers as a function of H_2O_2 addition at the very early stage of reaction; and (b) evaluating the relative amount of side reactions at different reaction temperatures.

EXPERIMENTAL

Materials

Horseradish peroxidase (250 units/mg) was purchased from Sigma Chemical Co., St. Louis, MO. A stock solution of 10 mg/mL HRP at pH 7.0, in 0.1 M phosphate buffer solution was prepared in D_2O. H_2O_2 (50% water solution), 4-propylphenol, and pyrazine (used as reference standard for quantitative analysis) were obtained from Aldrich Chemical Co., Inc., Milwaukee, WI. To avoid the inhibition of HRP due to an excess of H_2O_2, dilute H_2O_2 (5% in D_2O) was used. All deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. and were used as received.

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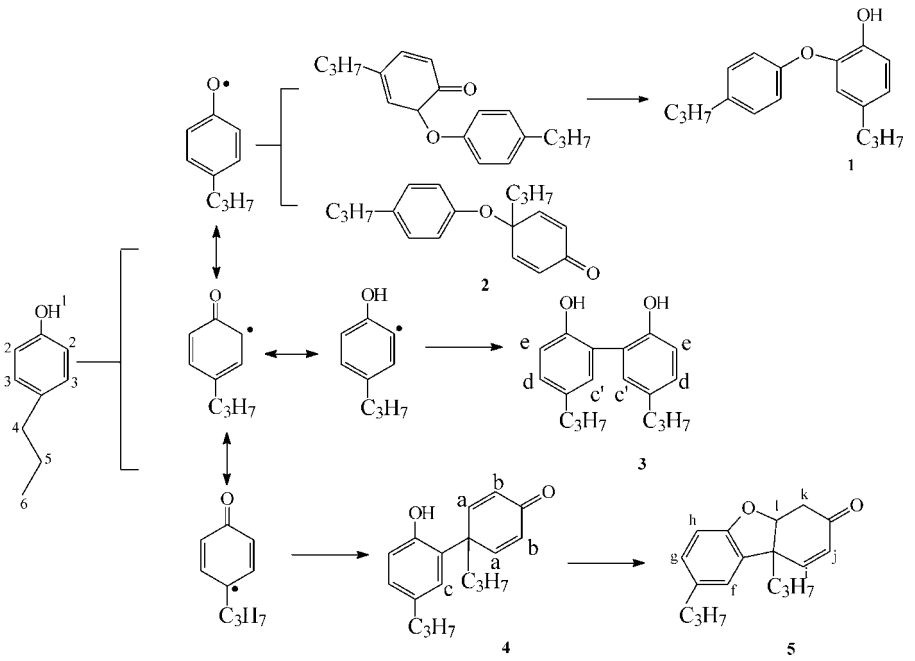
In Situ ^{1}H-NMR Measurements

In situ ^{1}H-NMR spectra were recorded on a Bruker DRX-500 MHz NMR spectrometer with a 5 mm broadband probe equipped with variable temperature accessories. The in situ polymerization was carried out in the 5 mm NMR tube. To a NMR tube, 0.20 mL of 7.5 mg/mL of monomer solution in acetone-d_6, 0.05 mL of HRP in phosphate buffer and 0.15 mL of 2.0 mg/mL pyrazine solution in D_2O were added. The solution was shaken for 5 min and equilibrated at the desired temperature for half an hour prior to ^{1}H-NMR measurement. Each ^{1}H-NMR spectrum was soon recorded (approximately 1 min) after the addition of 3.0 μL of H_2O_2 to the NMR tube. Incremental addition of H_2O_2 was carried out at every 5 min, and a total of 15.0 μL of H_2O_2 was added. Typical optimized parameters for acquiring ^{1}H-NMR spectrum with reasonable signal-to-noise ratio are: a 10 kHz spectral width, a 13.1 μs (90° pulse) pulse width, a 32 K time domain data points, a 1.6 s acquisition time, a 1.0 s relaxation delay, 16 transients and with water suppression by a presaturation technique. The free induction decay (FID) data were processed with 0.3 Hz line broadening prior to Fourier transformation. The spectrum was internally referenced by assigning the chemical shift of —CH in pyrazine at 8.70 ppm with respect to tetramethylsilane (TMS).

RESULTS AND DISCUSSION

The possible coupling reactions for 4-propylphenol that may occur at the early stage of the polymerization are illustrated in Sch. 1. These include C—O—C coupled products



Scheme 1. Possible coupling products for 4-propylphenol at early stage.

1 and **2**, the ortho–ortho C—C coupled product **3**, ortho–para coupled product **4**, and the Pummerer's type ketone **5** via intramolecular Michael addition from dimer **4**.

Figures 1 and 2 are stacked plots of selected in situ ^{1}H-NMR spectral data of enzymatic polymerization of 4-propylphenol in the aromatic and aliphatic regions, respectively. The spectra were recorded at 293 K with an incremental addition of H_2O_2. The labeling of each proton is presented in Sch. 1. Before the addition of H_2O_2, the resonance signals are from monomer and pyrazine. Since pyrazine is a symmetrical molecule, only one singlet at 8.7 ppm is observed. Two doublets at 6.7 and 6.9 ppm in Fig. 1 are assigned to protons 2 and 3 in 4-propylphenol. The alkyl protons 4, 5, and 6 in 4-propylphenol appear at 2.4, 1.5, and 0.8 ppm, respectively as shown in Fig. 2. After the addition of 3.0 μL of H_2O_2, new peaks appear immediately in the region of 7.6–6.0 ppm suggesting the onset of polymerization. With the addition of H_2O_2, the intensity of monomer peak decreases and new peaks emerge due to the formation of various oligomeric species. After 12.0 μL of H_2O_2, the new peaks become distributed widely indicating the formation of various dimers and other oligomers. The complex nature of the spectral pattern as a result of overlapping of resonances arising from several reaction products with varying concentrations, has necessitated the use of 2D correlation experiments like COSY (COrrelated SpectroscopY), TOCSY (TOtal Correlation SpectroscopY) (spectrum not shown) and model compounds with NMR spectral simulation software for the proper assignments of the predominant resonances. With this approach, the peaks at 7.62, 6.35, and 7.08 ppm are assigned to the protons a, b, and c in the ortho-para C—C coupled dimer **4**. The overlapped peak at 7.08 ppm and the doublets at 7.01 and 7.12 ppm are due to the protons c, d, and e in the ortho-ortho C—C coupled dimer **3**. The change in the local chemical environment of the aliphatic side group is also reflected in the formation

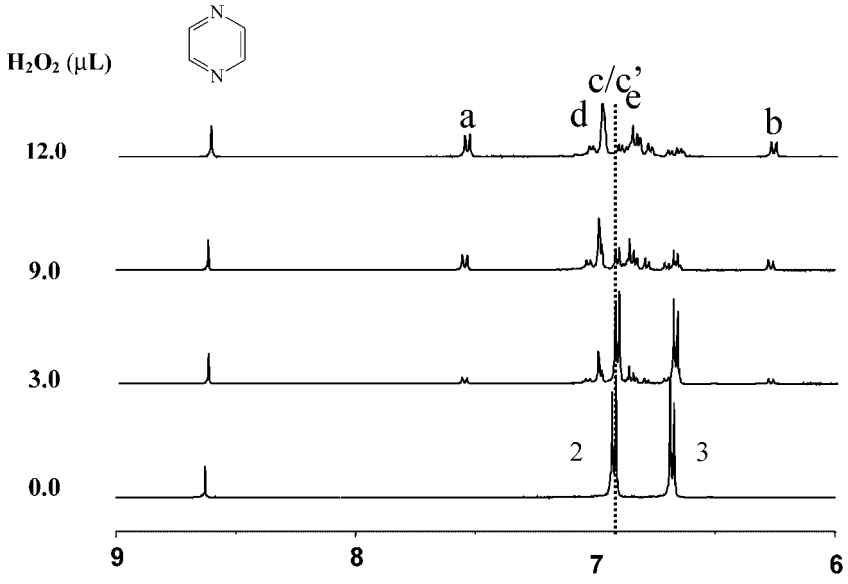


Figure 1. Selected in situ ^{1}H-NMR spectral data of enzymatic polymerization of 4-propylphenol (aromatic region) at 293 K.

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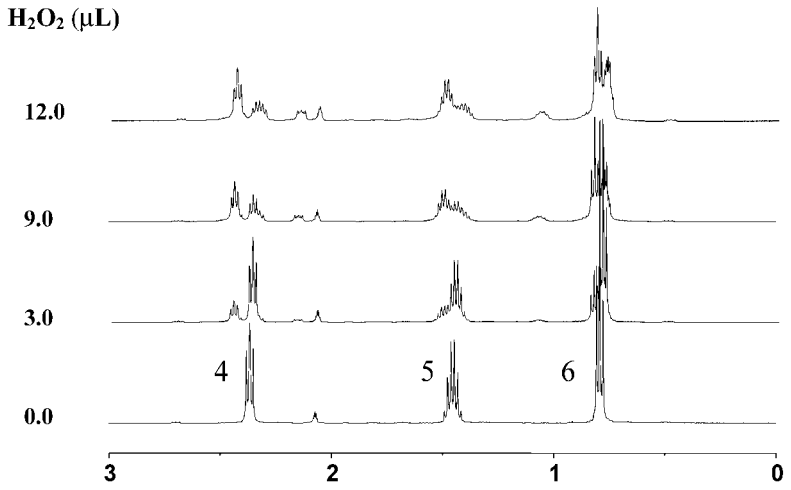

Figure 2. Selected in situ ^{1}H-NMR spectral data of enzymatic polymerization of 4-propylphenol (aliphatic region) at 293 K.

of new peaks as well. To investigate the temperature effect on the reactive species, similar measurements were also performed at temperatures 273, 283, and 288 K.

Pyrazine is chemically inert and does not interfere in this reaction. It is used as an internal standard in this experiment for quantitative analysis by assigning its resonance area to be unity. The concentration of each reactive species could be obtained by normalizing their integrated area relative to that of pyrazine. In the present work, the monomer resonance at 6.90 ppm and the peak for the predominant dimers at 7.08 ppm are selected as the representative resonances. Figures 3 and 4 show the concentration of monomer and dimer formed as a result of enzymatic polymerization as a function of H_2O_2 addition at various temperatures ranging from 273 to 293 K.

Figure 3 shows clearly that the decrease in the concentration of monomer follows the same trend at all tested temperatures. The relative monomer concentration initially reduced at a faster rate before the addition of 9.0 μL of H_2O_2. Subsequently, the rate of decrease of monomer concentration slowed down. Such behavior agrees well with our previous observation on *p*-cresol[12] and *p*-sulfonated phenol.[13]

Until now, two kinds of trends for the formation of dimers have been observed during the enzymatic polymerization of phenols.[11] In the case of *p*-sulfonated phenol at ambient temperature, the growth profile for dimers as a function of H_2O_2 can be divided into three stages.[13] Stage 1 consists of the proliferation of the dimers where the concentration of dimers increases rapidly. In stage 2, a dynamic equilibrium between the dimers formation and their consumption is attained. This results in the dimers concentration remaining almost constant even though the monomer is being consumed continuously during this stage. In the last stage of the dimers profile, the concentration of dimers decreases gradually due to their participation in the polymerization. On the other hand, in the case of polymerization of *p*-cresol at ambient temperature, however, no stage 2, i.e., a dynamic equilibrium for dimer, was observed.[12]

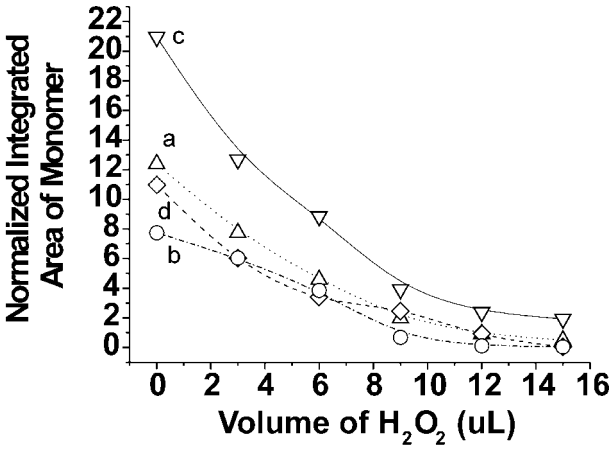

Figure 3. Integrated area of monomer as a function of H_2O_2 at tested temperatures.

More interestingly, in the present work, two distinct profiles for the dimer concentration were observed by simply varying the reaction temperature. In Fig. 4, all three stages (shown in Fig. 4) are observed for dimers at temperature 273 and 283 K. However, the profile of these stages changed significantly as the reaction temperature was increased. At reaction temperatures 288 and 293 K, only the stages for proliferation and reduction of dimers were observed (Stages 2 and 3 in Fig. 4). These results suggest that the formation and consumption of dimers can be manipulated simply by altering the reaction temperature.

There are some additional weak resonances (2.5–3.0, 1.8–2.25 ppm, 0.9–1.25 ppm) observed in the aliphatic region of the spectrum (Fig. 5). These are believed to be from the

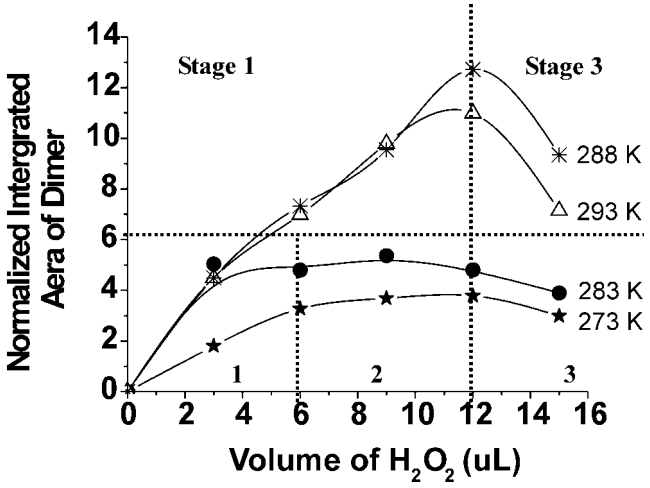

Figure 4. Integrated area of dimers as a function of H_2O_2 at tested temperatures.

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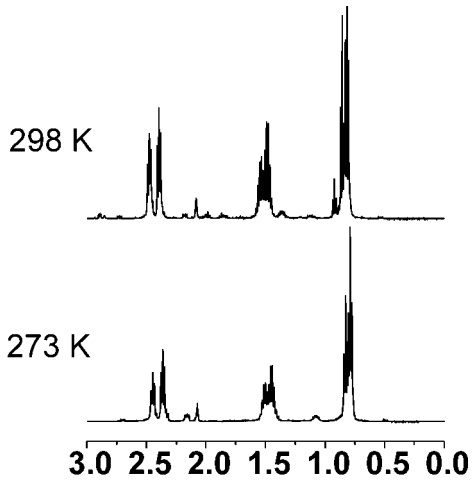

Figure 5. In situ spectra acquired after addition of 6.0 μL of H_2O_2 at 298 and 273 K (aliphatic region).

side reactions. The assignments of these peaks are still under investigation. However, recently we were able to isolate one of the major side products by thin layer chromatographic (TLC) technique.[14] The detailed structural analysis of this major side product by one- and two-dimensional NMR techniques suggests that it is a Pummerer's type ketone (Structure **5** in Sch. 1), which is formed through an intramolecular Michael addition as shown in Sch. 1.[14] The relative intensities of these peaks change as a function of temperature (Fig. 5). For example, these weak resonances have become pronounced at an elevated temperature (298 K). These data suggest that side reactions are minimized at low reaction temperatures.

CONCLUSION

For the first time, the effect of temperature on the enzymatic polymerization of 4-propylphenol has been investigated using an in situ ^{1}H-NMR technique. The in situ ^{1}H-NMR experiments indicate that the trend for monomer concentration as a function of H_2O_2 addition is identical at all investigated temperatures, whereas the trends for dimers as a function of H_2O_2 addition is strongly temperature dependent. The difference between concentration profiles of the dimers as a function of H_2O_2 addition at high reaction temperature compared to a lower temperature is the disappearance of dynamic equilibrium stage in the former. Our data established that the formation of side products is sensitive to the reaction temperature.

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