Notes

Role of Temperature in Suppression of the Formation of Pummerer's Type Ketone in Enzymatic Polymerization of 4-Propylphenol: An in-Situ Variable Temperature ^{1}H NMR Study

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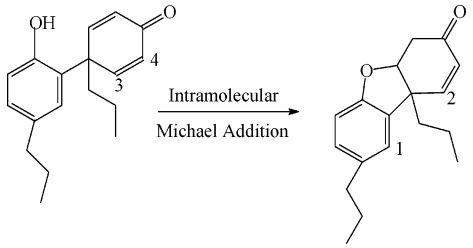
Introduction

Enzymatic polymerization has emerged as a promising alternative for the synthesis of functional polymers due to its inherit advantages such as the environmental compatibility, chemo- and stereoselectivity, and mild reaction conditions.[1] Horseradish peroxidase (HRP) catalyzed synthesis of poly(phenol)s and poly(aniline)s has been extensively explored owing to their potential applications in the areas of optoelectronics,[2−4] biosensors,[5] resins, and coatings.[1] Typically the reaction is carried out in an aqueous or a mixture of organic and aqueous media at room temperature, with H_2O_2 as an oxidant, and the product contains mixed repeated units of phenylene and oxyphenylene.[6]

In-situ ^{1}H NMR spectroscopy has been conformed to be an important technique for mechanistic studies involving the nature and position of coupling reactions occurring in the very early stages of enzymatic polymerization.[7] Moreover, the introduction of an internal standard provides a means for quantitative analysis of each of the reactive species.[8−11]

Our previous work on *p*-cresol has demonstrated that one major side product in the polymerization reaction is the formation of Pummerer's type ketone.[8] The Pummerer's type ketone is formed from the *ortho*−*para* coupled dimer by Michael addition as shown in Scheme 1.[9] Pummerer's type ketone has no enolic structure which is essential for initiation and subsequent propagation of the reaction, and therefore the polymerization terminates with the formation of the ketone. Although the ketone could be washed out from the polymer prepared, the yield of the polymer is significantly lowered due to the formation of this side product. Consequently, it is crucial to monitor and suppress the formation of Pummerer's type ketone for improving the yield of the polymeric product. An efficient way toward minimizing the formation of Pummerer's type ketone is to introduce bulky substituents on the *para* position of the monomer (relative to −OH).[8] However, this method obviates the pursuit of synthetic routes involving polymerization of many interesting unsubstituted monomers followed by postfunctionalization. It is known that temperature plays a key role in various free radical reactions. Hence, temperature may provide a handle on controlling the formation of undesired intermediates.

Scheme 1. Transformation of *Ortho*−*Para* Dimer to Pummerer's Type Ketone



This paper reports our initial finding on the role of reaction temperature on the formation of Pummerer's type ketone using variable temperature ^{1}H in-situ NMR techniques. In addition, the effect of reaction time on the formation of Pummerer's type ketone is also presented. The present study provides improved understanding of side reactions, the origin of color in the reaction products, and an effective way to minimize their concentrations.

Experimental Section

Materials. Horseradish peroxidase (HRP) (250 units/mg) was purchased from Sigma Chemical Co., St. Louis, MO. A stock solution of 10 mg/mL HRP in pH 7.0, 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 excess of H_2O_2, diluted H_2O_2 (5% in D_2O) was used. All deuterated solvents were purchased from Cambridge Isotope Laboratories Inc. and were used as received.

In-Situ ^{1}H NMR Measurement. In-situ spectra were recorded on a Bruker DRX-500 MHz NMR spectrometer with a 5 mm broadband probe equipped with variable temperature accessories. To a NMR tube 0.20 mL of monomer solution (7.5 mg/mL) in acetone-d_6, 0.05 mL of HRP in pH 7.0 phosphate buffer (prepared in D_2O), and 0.15 mL of pyrazine solution (2.0 mg/mL in D_2O) were added. The solution was shaken for 5 min prior to ^{1}H NMR measurement. Each ^{1}H NMR spectral data was recorded soon 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 time intervals of 5 min. A total of 15.0 μL of H_2O_2 was added. Two different methods were adopted for the variable temperature experiment. NMR spectra of sample solutions were recorded as a function of H_2O_2 addition at different temperatures, 273, 278, 283, and 298 K. (Because of the existence of inorganic salt, the freezing point of the solution is lower than 273 K.) In the latter case, a stepwise variable temperature

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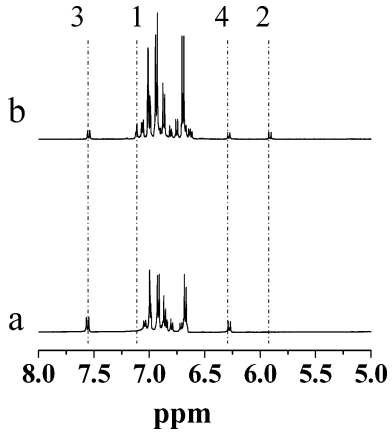

Figure 1. In-situ ^{1}H NMR spectra of 4-propylphenol acquired after addition of 6.0 μL of H_2O_2 at different reaction temperatures: (a) 273 K; (b) 298 K (aromatic region).

experiment was designed, wherein a stoichiometric amount of H_2O_2 was added into a solution containing the monomer and the enzyme at 273 K. The solution was then heated in a stepwise manner (the sample temperature was increased in increments of 5 K) until the sample reached a final temperature of 313 K. The sample was kept at a temperature of 313 K overnight. ^{1}H NMR was recorded at each step, and a total of 10 spectra were obtained. During all variable temperature experiments, the solution was equilibrated for 30 min at each desired temperature before data acquisition. To avoid the radio-frequency mismatch induced by the temperature variation, the probe was tuned and matched before each experiment.

The kinetics of formation of Pummerer's type ketone was investigated by recording the ^{1}H NMR spectrum at intervals of 1 min for 60 min, commencing immediately after the initiation of the reaction by the addition of 5.0 μL of H_2O_2 at 283 and 298 K.

Results and Discussion

The transformation from the *ortho*−*para* dimer to Pummerer's type ketone is shown in Scheme 1. The detailed assignments of resonances are based on the 2D NMR experiments, namely TOCSY (total correlation spectroscopy), HETCOR (heteronuclear correlation spectrum), and HMBC (heteronuclear multiple bond correlation), and will be reported elsewhere.[12] Figure 1 presents the aromatic region of the in-situ ^{1}H NMR spectra of 4-propylphenol after addition of 6.0 μL of H_2O_2 at 273 and 298 K. (For a better comparison, we only present the spectra acquired at 273 and 298 K.) For our purpose, we focused on the distinguishable resonances from Pummerer's type ketone and *ortho*−*para* coupled dimer. Because of severe overlap in the region of 6.5−7.0 ppm after the onset of polymerization, the clearly identifiable resonances such as the singlet at 7.06 ppm, the doublet at 6.00 ppm from protons "1" and "2" (as labeled in Scheme 1) in Pummerer's type ketone, and the doublets at 7.62 and 6.35 ppm originating from protons "3" and "4" in the *ortho*−*para* dimer are chosen as the sensors for following the progression of each of these species. A noteworthy aspect of the spectra obtained at different temperatures is that the resonances from Pummerer's type ketone are observed only at 298 K. Further, no Pummerer's type ketone was produced at 273 K. This result indicates that the transformation of the *ortho*−*para* dimer to Pummerer's type ketone is a temperature-sensitive process. It may also be concluded that a critical temperature for the transformation of the *ortho*−*para* dimer to Pummerer's type ketone is likely to exist in the temperature range of 273 and 298 K.

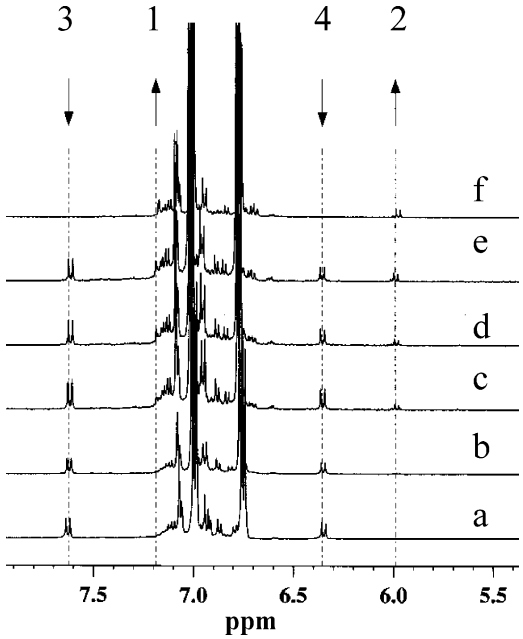

Figure 2. Determination of critical transition temperature to Pummerer's type ketone via stepwise variable temperature ^{1}H NMR experiment: (a) 273, (b) 293, (c) 303, (d) 308, (e) 313, and (f) 313 K overnight.

To find the critical transition temperature, the stepwise variable temperature experiment was performed. Figure 2 presents the in-situ variable temperature ^{1}H NMR spectra of poly(4-propylphenol) after the initiation of the reaction by addition of H_2O_2. At 273 K, no resonances from Pummerer's ketone were observed. Further the solution also remained colorless, and it is reasonable to conclude that Pummerer's type ketone is not formed at this temperature. The spectrum remains unchanged until the reaction mixture reached a temperature of 293 K. At 293 K, minor resonances from Pummerer's type ketone (on vertical expansion of the spectrum) at 7.06 and 6.00 ppm are observed. This indicates the formation of trace amounts of Pummerer's type ketone. It may be noted that the onset of formation of Pumemrer's type ketone occurs at this temperature (293 K). At temperatures lower than 293 K the formation of Pummerer's type ketone is negligible. When the temperature of the reaction mixture was increased beyond 293 K, an increase in the intensity of the peaks from Pummerer's type ketone accompanied by the simultaneous decrease in the intensity of peaks from the *ortho*−*para* dimer at 7.62 and 6.35 ppm was observed. This trend in the peak intensities continued until the complete disappearance of the peaks from the *ortho*−*para* dimer. The intensity of peaks corresponding to the Pummerer's type ketone reached its maximum after maintaining the reaction mixture at a temperature of 313 K overnight (12 h). This suggests that the dimer is completely and irreversibly transformed to the Pummerer's type ketone. It should be note that the critical transition temperature determined in this way may be higher than the real one owing to the inhibition of the enzyme activity via one-shot addition of H_2O_2.

The time dependence of the transformation was also investigated at different reaction temperatures after the initiation of the reaction (by one-shot addition of 5.0 μL of H_2O_2). For quantitative analysis, the peaks at 6.35

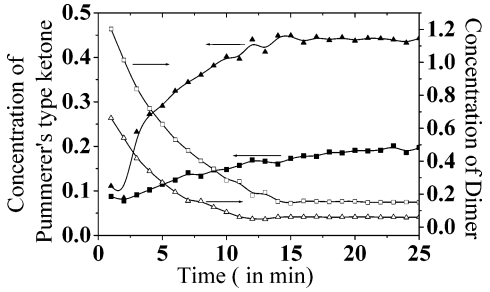

Figure 3. Integrated area of ^{1}H NMR resonances of Pummerer's type ketone at 298 (▲) and 283 K (■) and that of ortho−para dimer at 298 (△) and 283 K (□) as a function of reaction time.

and 6.00 ppm were selected as characteristic resonances for the *ortho−para* dimer and Pummerer's type ketone, respectively. Their integrated area was normalized with the area of an internal standard—pyrazine. At 273 and 278 K Pummerer's type ketone was not detected even after 75 min from the commencement of the reaction. The corresponding concentration variation of these two species at 283 and 298 K is presented in Figure 3. It can be seen that the concentration of *ortho−para* dimer decreases exponentially in the first 15 min, and during the same time frame the corresponding concentration of Pummerer's type ketone increases exponentially. After about 15 min the concentration of both the species stabilize. This simultaneous leveling off indicates that the *ortho−para* dimer is the only precursor for Pummerer's type ketone. It was also estimated that the concentration of Pummerer's type ketone formed at 298 K was approximately 2.25 times that formed at 283 K, implying the transition rate at 298 K is 2.25 times faster than that in 283 K.

It is evident that entropy has little contribution to this reaction. The calculation by HyperChem software using MM+ force field method shows that the internal energy for *ortho−para* dimer is 27.6 kcal/mol, whereas that for Pummerer's ketone is 26.9 kcal/mol. It is most likely that this 0.7 kcal/mol difference in enthalpy enables the transition to be thermodynamically favorable.

In summary, the ideal way to rule out the formation of Pummerer's type ketone is to prevent the *ortho−para* coupled dimer. In light of the transformation from *ortho−para* dimer to Pummerer's type ketone being extremely slow at low reaction temperature during experimental time scale, the control of reaction temperature would alone be sufficient in minimizing the formation of Pummerer's type ketone. The present study also suggests that the color of the reaction products is related to undesired side reactions.

Conclusion

The present work suggests that the temperature plays a key role in the transition of the *ortho−para* dimer to Pummerer's type ketone structure. Further, efficient inhibition of Pummerer's type ketone could be possible by carrying out the reaction at low reaction temperatures. The transition to Pummerer's type ketone is a thermodynamically favorable reaction and is extremely sensitive to the temperature. The minimization *ortho−para* coupling continues to be the ideal strategy for preventing the formation of Pummerer's type ketone.

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Supporting Information Available: The completely assigned ^{1}H NMR spectrum of purified Pummerer's type ketone. This material is available free of charge via the Internet at http://pubs.acs.org.

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