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Communications

Enzyme-Initiated Miniemulsion Polymerization

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Enzyme-catalyzed polymerization in vitro has gained considerable attention in the last two decades as an efficient tool in the polymerization of various monomers, such as saccharides, esters, phenols, and aromatic anilines; however, the polymerization of vinyl monomers using enzymes has been more limited, perhaps due to the hydrophobicity of most common vinyl monomers. Enzyme-initiated miniemulsion polymerization is demonstrated herein as a way to polymerize hydrophobic vinyl monomers such as styrene. By application of enzyme-initiated radical polymerization in miniemulsion, stable poly(styrene) latexes are prepared with a particle size near 50 nm. A very small amount of enzyme and surfactant is required to facilitate the miniemulsion polymerization, whereas a relatively high polymerization rate and conversion are achieved.

Introduction

Enzyme-catalyzed polymerization in vitro has been a research focus for the past two decades for the preparation of various polymers.[1,2] The high selectivity, mild reaction conditions, and environmental compatibility inherent in enzymatic reactions have made this approach a very attractive alternative in the synthesis of complex, stereoselective, and bioactive compounds, which are often difficult to obtain by conventional chemical routes. To date, polymers such as poly(saccharides), poly(esters), poly(phenols), and poly(anilines) have been the synthesized by enzyme-catalyzed polymerizations.[3−14] The enzymatic polymerization of hydrophobic vinyl monomers such as styrene, however, has scarcely been reported.[15,16]

Oxidoreductases, especially horseradish peroxidase (HRP), are known to have the ability to catalyze the oxidation of phenols, anilines and their derivatives.[4,10−14] The potential of HRP and other oxidases to catalyze the free radical polymerization of vinyl monomers was first reported by Derango et al.[17] The polymers were formed in the presence of a large excess of hydrogen peroxide. It was claimed that the oxo-iron(IV) π-radical cation generated by HRP and H_2O_2 may contribute to the polymerization. Poly(acrylamide), poly(methyl methacrylate), and poly(styrene) were synthesized by HRP and other oxidases with β-diketones as initiators.[15,16,18−22] It was found that no polymer was produced if a low ratio of H_2O_2:monomer was used in the absence of β-diketones. Thus, another mechanism was proposed where β-diketone radicals generated by HRP-catalyzed oxidation of the β-diketone with H_2O_2 may initiate the polymerizations.[15]

One reason why enzymatic polymerizations of vinyl monomers have not been thoroughly investigated may be that the majority of common vinyl monomers are barely soluble in water, which is the traditional reaction medium for the application of biocatalysts. Indeed, hydrophilic vinyl monomers were utilized in most previous studies using HRP as an initiator.[17−20,22] Cosolvents have also been utilized to allow polymerization of hydrophobic monomers.[15] However, the yields of polymer were relatively low under these conditions, i.e., 21.2% in THF-H_2O and only 3.8% in methanol-H_2O cosolvent after 24 h.[15]

Here we demonstrate a simple way to realize the enzyme-initiated polymerization of hydrophobic vinyl monomers in an aqueous system via the application of miniemulsion polymerization. A miniemulsion is an aqueous dispersion of relatively stable oil droplets with a size range of 50−500 nm in which polymerizations are performed. In the ideal case, there is little exchange of material between droplets and the droplets act as nanoreactors.[23] Miniemulsion polymerizations are robust to

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variations in chemistry, with traditional radical polymerizations,[24,25] controlled radical polymerizations such as ATRP,[26] RAFT,[27,28] NMP,[29] and coordination-insertion polymerizations[30−33] being carried out effectively in them. Additionally, miniemulsion polymerizations are considered to be "green" processes because they use water in place of organic solvents. Moreover, miniemulsion polymerizations produce polymers of uniform composition and final latexes with excellent shear stability exceeding those of conventional emulsion polymerizations. The methodology demonstrated here may also allow for the enzyme-initiated polymerization of various hydrophobic vinyl monomers other than styrene, with possible advantages over conventional polymerizations such as use of milder reaction conditions that might be especially suitable when using thermosensitive monomers or for chemoselective polymerizations.[34]

Experimental Details

Materials. Styrene (J.T. Baker) was purified by removing the inhibitor *tert*-butylactechol by passing the monomer through a column packed with inhibitor remover (Aldrich), followed by distillation under vacuum. Hexadecane (Aldrich, 99%) and sodium dodecyl sulfate (SDS; J.T. Baker. 99.8%) were used as received. Deionized water was generated with a U.S. Filter Systems Deionizer and was used without further purification. Horseradish peroxidase (HRP; TCI America), hydrogen peroxide (Aldrich, 30% w/w), and 2, 4-pentanedione (ACAC; Alfa Aesar, 99%) were used as received.

Polymerization. The miniemulsion was prepared by adding a solution of degassed styrene and hexadecane to a solution of sodium dodecyl sulfate in deionized water. The mixture was stirred under nitrogen in an ice bath for 10 min and then sonicated with a Fischer model 30 sonic dismembrator operated at 70% power output for approximately 10 min, while being stirred under nitrogen and cooled in an ice bath. HRP was dissolved in a small amount of deionized water and purged with nitrogen for 10 min. A portion of the HRP solution was injected in the styrene miniemulsion and stirred for 5 min, followed by adding ACAC and H_2O_2 simultaneously. The miniemulsion was stirred under nitrogen, and samples were drawn at intervals. The samples were poured into a large excess of cold methanol. The polymer was precipitated and filtered, washed with cold methanol, and dried under vacuum at 30 °C.

Characterization. The dried polymer was dissolved in chloroform (J.T. Baker, HPLC) and filtered through a 0.2 μm syringe filter. GPC analyses were carried out using three columns (American Polymer Standards styrene-divinylbenzene 100, 1000, and 10^5 Å) at 30 °C. The columns were connected to a Viscotek GPCMax pump and autoinjector and a Waters 410 refractive index detector and calibrated with narrow polystyrene standards (Polymer Laboratories). Chloroform was used as the eluent at a flow rate of 1.0 mL/min, and the injection volume was 100 μL.

Latex particle sizes were analyzed using quasielastic light scattering (QELS, Protein Solutions DynaPro). The conversion of styrene monomer conversion was determined gravimetrically.

Results and Discussion

Kinetic Study of HRP-Catalyzed Miniemulsion Polymerization. Assuming the polymerization follows the mechanism outlined in the Introduction,[15] it is believed that H_2O_2 oxidizes HRP and the oxidized metal center is reduced by ACAC, yielding ACAC-derived radicals in the aqueous phase. These radicals presumably can do two things to initiate the polymerization, either (i) enter the monomer droplets and start the polymerization directly in the droplet or (ii) oligomerize the small fraction of styrene that is present in the aqueous phase, with these active styryl chains entering the droplets as they get more hydrophobic with increasing chain length.

Table 1. Recipe for HRP-Catalyzed Miniemulsion Polymerization

miniemulsion			
H_2O	2.88 g	HRP	2.4 mg
Styrene	0.689 g	H_2O_2 (30 wt %)	7 μL
SDS	0.0155 g	ACAC	9 μL
Hexadecane	0.0162 g		

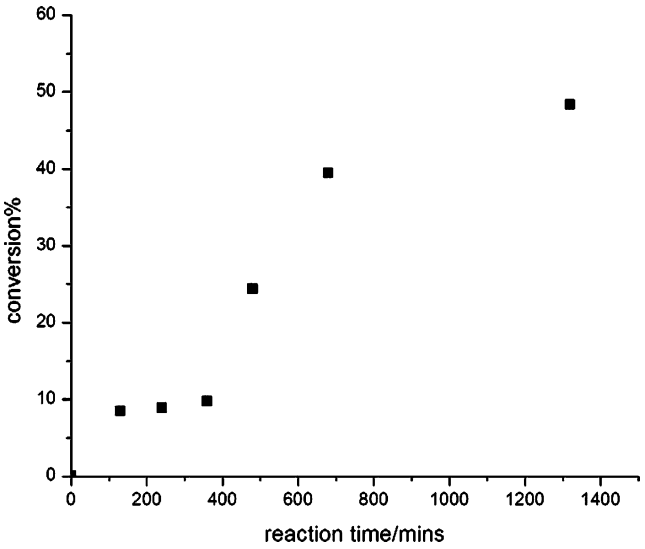

Figure 1. Relationship between of conversion and reaction time of the HRP mediated miniemulsion using the recipe in Table 1.

The main recipe used for HRP-initiated miniemulsion polymerization is shown in Table 1. It is interesting to note that the conversion of styrene proceeded slowly until 360 min, as shown in Figure 1. After 360 min, the conversion of styrene increased significantly, followed by a gradual decrease in polymerization rate after around 800 min. Because this kinetic behavior was quite unusual, the main experiment was repeated three times, and in all cases, a period of slow conversion was observed.

There are many possible causes of these observations. The consumption of ACAC and H_2O_2 with time, which decreases the radical initiation rate, could cause the decreased polymerization rate observed at long times. A loss of enzyme activity or degradation of HRP with increasing reaction time could also contribute to this phenomenon. Concerning the slow conversion at short reaction times, one potential cause is an imbalanced ratio of ACAC:H_2O_2 in the aqueous phase. Although the molar ratio of ACAC:H_2O_2 was chosen to be close to an optimal ratio reported previously for another polymerization system (set at 1.3:1, (Table 1)),[35] the actual ratio of ACAC:H_2O_2 in the aqueous phase, where the enzyme is presumed to reside, could be much lower due to the preferential partitioning of ACAC into the monomer droplets. Additionally, it is possible that a different initiation mechanism operates under these conditions. Derango et al. suggested a different mechanism could operate under some conditions and found that, with a low ratio of vinyl monomer:H_2O_2, polymerization could take place even without ACAC (vide supra).[17,34] In the present work, the concentration ratio of styrene to H_2O_2 in the aqueous phase may be quite low. Assuming that all of the H_2O_2 was dissolved in the aqueous phase that was saturated with styrene, the molar ratio of styrene to H_2O_2 was around 0.04 at the start of the polymerization.[36] These conditions may favor the alternate mechanism of Derango et al. Furthermore, the drastic changes in polymerization rate

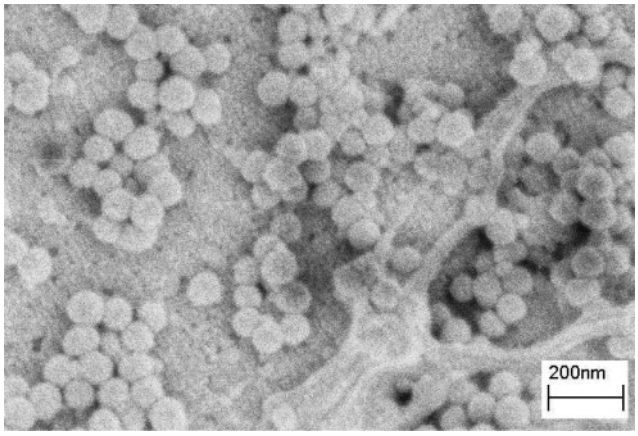

Figure 2. SEM photograph of poly(styrene) latex nanospheres obtained from HRP-mediated miniemulsion with the recipe in Table 1 after 24 h.

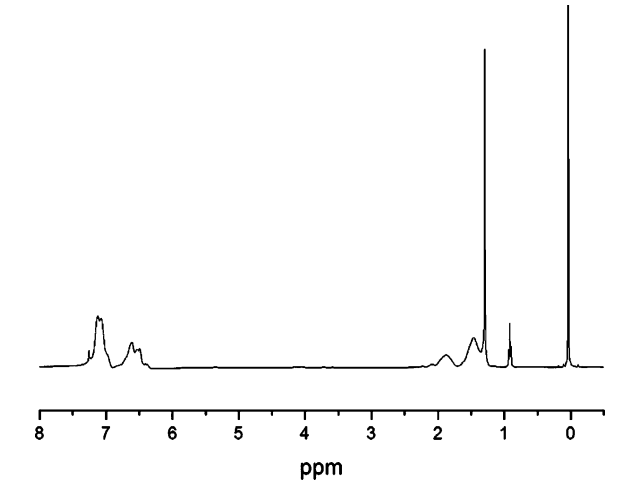

Figure 3. ^{1}H NMR spectrum of HRP catalyzed poly(styrene) with the recipe in Table 1 after 24 h using $CDCl_3$ as solvent.

at certain times throughout the process may be indicative of the system operating via different mechanisms at different times.

Although a much smaller amount of HRP and H_2O_2 were used than in the previous report,[15] the conversion after 24 h, around 48%, was still much higher than previously reported using cosolvent solutions. Moreover, a stable poly(styrene) latex with a narrowly distributed particle size distribution around 50 nm was achieved, as shown in Figure 2. The ^{1}H NMR spectrum of the poly(styrene) is shown in Figure 3.

There was a discontinuity in the number average molecular weight (M_n) of poly(styrene) in this HRP-initiated miniemulsion polymerization, similar to the trend observed in conversion. The M_n was almost constant at the beginning of the polymerization and then sharply increased afterward, as shown in Figure 4. It is worth pointing out the relatively high molecular weight of the poly(styrene), as high as 406k, compared to the previous enzyme-mediated work using a cosolvent, which gave molecular weights of ~30k.[15] A low concentration of ACAC in the aqueous phase may lead to a low total radical concentration, accounting for the high molecular weight of the poly(styrene). The radical segregation effect in miniemulsion also favors high molecular weights by inhibiting termination of propagating radicals.

Effects of Reaction Temperature. It is well-known that temperature has significant effects on the reactivity of enzymes. Therefore, the conversion of styrene at different temperatures was measured after about 24 h. As shown in (Table 2),

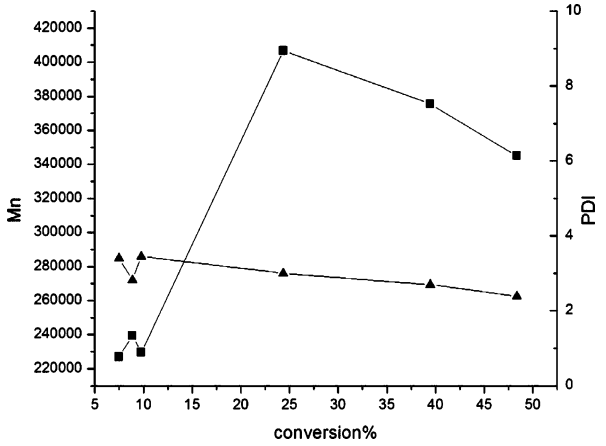

Figure 4. Relationship between M_n, PDI, and conversion using the recipe in Table 1

Table 2. Effects of Reaction Temperature on the HRP-Catalyzed Miniemulsion Polymerization

exp[a]	temperature/ K	reaction time/ mins	conversion%	average particle size/ nm
1	279	1355	50.2	50.63
2	296	1320	48.3	54.09
3	305	1375	9.8	47.42

[a] The recipe was shown in Table 1.

Table 3. Effects of the Amount of Enzyme on the HRP-Catalyzed Miniemulsion Polymerization

exp	HRP/ mg	ACAC/ μL	H_2O_2/ μL	conversion%	average particle size/ nm
4	2.4	9	7	48.3	54.09
5	5.0	9	7	98.3	62.76
6	9.0	9	7	100.0	65.16

temperature has a dramatic influence on the miniemulsion polymerization, and the conversion was lower at high temperatures. At 305 K, only 9.8% conversion was achieved after 24 h, whereas a much higher conversion could be achieved at a lower temperature of 279−296 K. The result is reasonable given that a previous study showed that HRP has its highest activity at 278 K and gradually loses its activity at higher temperatures.[37]

Amount of Enzyme. The effect of the amount of enzyme used in the miniemulsion polymerization was investigated. The conversion in three experiments with increasing HRP concentration was measured after 24 h. With a doubling of HRP in experiment 4, the conversion in experiment 5 was greatly improved from 48.3% to almost a full conversion (Table 3).

Effect of H_2O_2/ACAC Ratio. Previous studies have indicated the ratio of ACAC:H_2O_2 has a major influence on HRP-mediated polymerizations.[15,16,18,19,22] At low concentrations, hydrogen peroxide functions as an electron acceptor and initiator, whereas at higher concentrations, it can inhibit enzyme activity. Therefore, a study was carried out for 24 h at 296 K to determine how the concentrations of hydrogen peroxide and 2,4-pentanedione affected the outcome of styrene miniemulsion polymerizations. Four control experiments were carried out first. In these control experiments, one component in the reaction (HRP, ACAC, or H_2O_2) was removed from the recipe to see the impact of each individual component on the polymerization. No poly(styrene) was formed in these control experiments except

Table 4. Effects of H_2O_2 and ACAC on the HRP-Catalyzed Miniemulsion Polymerization

exp	HRP/ mg	ACAC/ μL	H_2O_2/ μL	conversion%
control 1	2.4	0	0	0
control 2	2.4	0	7	trace
control 3	2.4	9	0	0
control 4	0	9	7	0
7	2.4	40	7	32.7
8	2.4	20	7	51.2
9	2.4	9	7	48.3
10	2.4	9	15	52.6
11	2.4	9	40	33.4

for a trace of polymer in the case where ACAC was not used (Table 4). These results agreed with the results reported before.[15]

As shown in (Table 4), with an increase of ACAC concentration from 9 to 20 μL, the conversion only slightly increased from 48.3% to 51.2%. Further increasing the concentration of ACAC, however, lead to a slight decrease in the conversion. A similar phenomenon was observed by altering the concentration of H_2O_2. Increasing the amount of from 7 to 15 μL contributed an increase in conversion from 48.3% to 52.6%, while further increasing H_2O_2 resulted in a decrease in the final yield. Thus it is reasonable to assume that there should be an optimal ratio of ACAC:H_2O_2 in the HRP catalyzed miniemulsion polymerization, although such conditions were not identified here.

Conclusion

Enzymatic miniemulsion polymerization was demonstrated in this work to be a way to use enzymes to polymerize hydrophobic vinyl monomers. Stable poly(styrene) latexes were synthesized by HRP initiated miniemulsion polymerization. A very small amount of HRP, H_2O_2, and ACAC was required to facilitate the miniemulsion polymerization while a relatively high conversion was achieved.

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References and Notes

(1) Kobayashi, S.; Uyama, H.; Kimura, S. *Chem. Rev.* **2001**, *101*, 3793−3818.
(2) Gross, R. A.; Kumar, A.; Kalra, B. *Chem. Rev.* **2001**, *101*, 2097−2124.
(3) Kobayashi, S.; Shoda, S.-i.; Kashiwa, K. In *Enzymatic polymerization. The first in vitro synthesis of cellulose via non-biosynthetic path catalysed by cellulase*; Atlanta, GA, 1991; ACS: Washington, DC, 1991; pp 417−418.
(4) Dordick, J. S.; Marletta, M. A.; Klibanov, A. M. *Biotechnol. Bioeng.* **1987**, *30*, 31−36.
(5) Kobayashi, S.; Shimada, J.; Kashiwa, K.; Shoda, S. *Macromolecules* **1992**, *25*, 3237−3241.
(6) MacDonald, R. T.; Pulapura, S. K.; Svirkin, Y. Y.; Gross, R. A.; Kaplan, D. L.; Akkara, J.; Swift, G.; Wolk, S. *Macromolecules* **1995**, *28*, 73−78.
(7) Henderson, L. A.; Svirkin, Y. Y.; Gross, R. A.; Kaplan, D. L.; Swift, G. *Macromolecules* **1996**, *29*, 7759−7766.
(8) Knani, D.; Gutman, A. L.; Kohn, D. H. *J. Polym. Sci., Part A: Polym. Chem.* **1993**, *31*, 1221−1232.
(9) Knani, D.; Kohn, D. H. *J. Polym. Sci., Part A: Polym. Chem.* **1993**, *31*, 2887−2897.
(10) Uyama, H.; Kurioka, H.; Kaneko, I.; Kobayashi, S. *Chem. Lett.* **1994**, *3*, 423−426.
(11) Akkara, J. A.; Senecal, K. J.; Kaplan, D. L. *J. Polym. Sci., Part A: Polym. Chem.* **1991**, *29*, 1561−1574.
(12) Akkara, J. A.; Salapu, P.; Kaplan, D. L. In *Polyaniline synthesized by enzyme-catalyzed reactions in organic solvents*; San Francisco, CA, 1992; ACS: Washington, DC, 1992; pp 374−375.
(13) Liu, W.; Kumar, J.; Tripathy, S.; Senecal, K. J.; Samuelson, L. *J. Am. Chem. Soc.* **1999**, *121*, 71−78.
(14) Samuelson, L. A.; Anagnostopoulos, A.; Alva, K. S.; Kumar, J.; Tripathy, S. K. *Macromolecules* **1998**, *31*, 4376−4378.
(15) Singh, A.; Ma, D.; Kaplan, D. L. *Biomacromolecules* **2000**, *1*, 592−596.
(16) Shan, J.; Kitamura, Y.; Yoshizawa, H. *Colloid Polym. Sci.* **2005**, *284*, 108−111.
(17) Derango, R. A.; Chiang, L. C.; Dowbenko, R.; Lasch, J. G. *Biotechnol. Technol.* **1992**, *6*, 523−526.
(18) Durand, A.; Lalot, T.; Brigodiot, M.; Marechal, E. *Polymer* **2001**, *42*, 5515−5521.
(19) Kalra, B.; Gross, R. A. In *HRP-mediated polymerization of acrylamide and sodium acrylate*; Washington, DC, 2000; American Chemical Society: Washington, DC, 2000; pp 1828−1829.
(20) Lalot, T.; Brigodiot, M.; Marechal, E. *Polym. Int.* **1999**, *48*, 288−292.
(21) Tsujimoto, T.; Uyama, H.; Kobayashi, S. *Macromol. Biosci.* **2001**, *1*, 228−232.
(22) Durand, A.; Lalot, T.; Brigodiot, M.; Marechal, E. *Polymer* **2000**, *41*, 8183−8192.
(23) Chou, Y. J.; El-Aasser, M. S.; Vanderhoff, J. W. *J. Dispersion Sci. Technol.* **1980**, *1*, 129−150.
(24) Ugelstad, J.; El-Aasser, M. S.; Vanderhoff, J. W. *J. Polym. Sci., Part C: Polym. Lett.* **1973**, *11*, 503−513.
(25) Schork, F.; Luo, Y.; Smulders, W.; Russum, J.; Butte, A.; Fontenot, K. *Polym. Part.* **2005**, *175*, 129−255.
(26) Matyaszewski, K.; Qiu, J.; Tsarevsky, N. V.; Charleux, B. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 4724−4734.
(27) Russum, J. P.; Barbre, N. D.; Jones, C. W.; Schork, F. J. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 2188−2193.
(28) de Brouwer, H.; Tsavalas, J. G.; Schork, F. J.; Monteiro, M. J. *Macromolecules* **2000**, *33*, 9239−9246.
(29) Prodpran, T.; Dimonie, V. L.; Sudol, E. D.; El-Aasser, M. S. *Macromol. Symp.* **2000**, *155*, 1−14.
(30) Held, A.; Kolb, L.; Zuideveld, M. A.; Thomann, R.; Mecking, S.; Schmid, M.; Pietruschka, R.; Lindner, E.; Khanfar, M.; Sunjuk, M. *Macromolecules* **2002**, *35*, 3342−3347.
(31) Claverie, J. P.; Viala, S.; Maurel, V.; Novat, C. *Macromolecules* **2001**, *34*, 382−388.
(32) Quemener, D.; Heroguez, V.; Gnanou, Y. *Macromolecules* **2005**, *38*, 7977−7982.
(33) Tomov, A.; Broyer, J. P.; Spitz, R. *Macromol. Symp.* **2000**, *150*, 53−58.
(34) Uyama, H.; Lohavisavapanich, C.; Ikeda, R.; Kobayashi, S. *Macromolecules* **1998**, *31*, 554−556.
(35) Kalra, B.; Gross, R. A. *Biomacromolecules* **2000**, *1*, 501−505.
(36) Andrews, L. J.; Keefer, R. M. *J. Am. Chem. Soc.* **1950**, *72*, 5034−5037.
(37) Nicell, J. A.; Bewtra, J. K.; Bewas, N.; St. Pierre, C. C.; Taylor, K. E. *Can. J. Civil Eng.* **1993**, *20*, 725−735.

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