



HRP-mediated polymerizations of acrylamide and sodium acrylate

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Previously, we reported the horseradish peroxidase (HRP) mediated free radical polymerization of methyl methacrylate.¹ The reaction medium was restricted to water/water-miscible co-solvent mixtures that solubilize both the monomer and enzyme. This paper describes HRP-mediated acrylamide polymerizations in aqueous medium with and without the addition of surfactants. Also, studies were conducted on polymerizations in concentrated emulsions using sorbitane monooleate as the surfactant. In aqueous medium, within 3 h, 98% yields of poly(acrylamide) were attained. By adding either anionic bis(2-ethylhexyl)sodium sulfosuccinate (AOT) or cationic cetyltrimethylammonium bromide (CTAB), the polymerization proceeded more rapidly so that within 60 and 75 min, respectively, 94% polymer yields were reached. When conducted in a concentrated emulsion formed with sodium monooleate, nearly quantitative yields resulted in 1.25 h. The weight average molecular weight (M_w) of the products ranged from 128 to 210 K and 106 to 154 K in aqueous medium and concentrated emulsions, respectively. In both of the above reaction systems, there was no apparent effect of the enzyme on the regulation of chain stereochemistry. The HRP-mediated polymerization of sodium acrylate conducted in aqueous medium gave poly(sodium acrylate) in yields up to 88% within 24 h with M_w ca. 119 K.

Introduction

Enzymes have proven to be powerful catalysts for the polymerization of a wide variety of monomers and macromonomers.^{2,3} They represent a family of 'environmentally friendly' natural catalysts that can function under mild reaction conditions. Horseradish peroxidase (HRP) is an oxido-reductase that acts on hydrogen peroxide and/or alkyl peroxide as an oxidant⁴ and on several reducing substrates such as phenol, hydroquinone, pyrogallol, catechol, aniline and *p*-amino-benzoate.⁵ The oxidative coupling of a variety of substrates such as phenols and aromatic amines catalyzed by HRP in the presence of hydrogen peroxide have been reported in aqueous,⁶ non-aqueous media,⁷⁻⁹ and at interfaces.¹⁰

The potential of using HRP and other oxidases to catalyze the free radical polymerization of vinyl monomers was first reported by Derango *et al.*¹¹ The polymers were formed in the presence of a large excess of oxidant (monomer:oxidant, 1.66:1.0 vol/vol, for *e.g.* 1.41:1.0 mol/mol for 2-hydroxyethyl methacrylate). Unfortunately, these workers gave only qualitative descriptors to describe whether a polymer was formed without further information on the polymer structure. Kobayashi and coworkers¹² reported the HRP-catalyzed polymerization of phenylethyl methacrylate. Similar to Derango *et al.*,¹¹ Kobayashi and coworkers¹² also published the formation of polymer using large quantities of oxidant (equimolar with respect to the monomer). More recently, the HRP-mediated free radical polymerization of acrylamide in water was reported.^{13,14} These polymerizations took place when β -diketones were used as initiators and the molar ratio of hydrogen peroxide to monomer was 1-66.^{13,14} That β -diketones will react under such conditions is related to their weakly bonded α -hydrogens. In fact, it had previously been shown that cyclic β -ketones, such as 5,5-dimethyl-1,3-cyclohexanedione, are substrates for chloroperoxidases which belong to the same subclass of enzymes as HRP (E.C. 1.11.a and 1.11.1.7, respectively).¹⁵ By analogy to

phenol, it was assumed that the enolic tautomeric form of 2,4-pentanedione is a key intermediate in the catalytic pathway.

This paper reports on the influence of reaction conditions and experimental variables to further extend our understanding of HRP-mediated free radical vinyl monomer polymerizations. Polymerizations of acrylamide and sodium acrylate were carried out at room temperature using HRP as an oxidoreductase, hydrogen peroxide as the oxidant and 2,4-pentanedione as the reductant. The influence of anionic (AOT) and cationic (CTAB) surfactants on HRP-mediated acrylamide polymerizations is reported. Behari *et al.*¹⁶⁻¹⁸ and others¹⁹ have also studied the effect of surfactant addition to free-radical polymerizations of acrylamide, but where chemical catalysts were used. Thus, comparisons were made between acrylamide polymerization kinetics when surfactants were added to these different polymerization systems. HRP-mediated polymerizations of acrylamide at room temperature were also studied in concentrated emulsions. A concentrated emulsion is a gel-like system whose internal phase ratio is greater than 0.74 (the volume fraction of the most compact arrangement of spheres of equal size).²⁰ Concentrated emulsions are of particular interest since, compared to inverse emulsion polymerizations, a much

Green Context

Utilising nature's catalysts is a very attractive option in a green chemistry context. Here we can read of the use of a horseradish peroxidase for the free radical polymerisation of acrylamide and sodium acrylate. Reactions can be carried out in water at ambient temperatures. They are shown to be a viable alternative to conventional free radical methods.

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smaller amount of organic solvent is needed to produce polymer latexes.

Results and discussion

In our studies of horseradish peroxidase mediated free radical polymerization of acrylamide/sodium acrylate polymerizations, all three reagents, the enzyme, the initiator and the oxidant need to be present for the polymerization to take place. In the absence of horseradish peroxidase, hydrogen peroxide or 2,4-pentanedione, no polymer formation was observed. Three different commercial peroxidases, horseradish peroxidase (HRP) type I, HRP type II, and soybean peroxidase (SBP) were evaluated to compare their catalytic activities for acrylamide polymerization. The reducing substrate 2,4-pentanedione and hydrogen peroxide were used in the ratio 1.5:1.0 mol/mol. The ratio of acrylamide to 2,4-pentanedione was fixed at 44:1 based on previous work by our laboratory on HRP II mediated methyl methacrylate polymerizations.¹ Comparative studies were conducted at room temperature (25 °C), in an aqueous medium, for 3 h (Table 1). The oxidases evaluated were normalized based on weight, *i.e.* the same quantities by weight of the oxidases were taken to determine their activity for acrylamide polymerization. HRP II and SBP appeared most promising. For example, the yields of poly(acrylamide) when using HRP II, HRP I and soybean peroxidase (SBP) were 98, 98 and 51%, respectively (entries 7, 8 and 9, Table 1). Furthermore, HRP II and SBP catalyzed polymerizations for 3 h gave poly(acrylamide) with M_w values of 209 and 282 K, and polydispersity index (PDI, M_w/M_n) values of 2.7 and 2.9, respectively (Table 1, entries 7 and 9). HRP I gave poly(acrylamide) having M_w and PDI values of 176K and 2.4, respectively (Table 1, entry 8). Based on the above results, their relative availability and cost, HRP II was selected for more detailed studies of its ability to mediate poly(acrylamide) polymerizations. A polymerization reaction carried out in the presence of denatured horseradish peroxidase (enzyme heated at 70 °C for 24 h) did not result in significant polymer formation.

The HRP II-mediated polymerization of acrylamide in water was monitored as a function of reaction time (Table 1, entries

1–7). There was an initial induction period of 60 min, where little polymerization occurred. The yields of poly(acrylamide) successively increased from 24 to 98% from 1.5 to 3 h. Measurements by GPC showed that the weight average molecular weight (M_w) of poly(acrylamide) products increased with reaction time (Table 1, entries 4–7) and ranged between approximately 128 and 209 K. Such an increase in molecular weight is not unusual for similar polymerizations with chemical catalysts systems.¹⁹ The tacticity for polyacrylamide (Table 1, entry 7) as determined by ¹³C NMR showed that the polymer is atactic.²¹ The methine carbon along the main chain of poly(acrylamide) gives rise to three distinct signals (triad sensitivity) which are further subdivided giving information at the pentad level. The low field and high field triplet peaks are assigned to rr (syndiotactic) and mm (isotactic) sequences, respectively. The central peak corresponds to heterotactic sequences (mr + rm). Thus, the repeat unit sequence distribution (Table 1, entry 7) by observation of triads using C_α (CH) resonances, gave syndio- (rr = 35%), hetero- (mr or rm = 51%) and isotactic (mm = 14%). Previously, we showed that the horseradish peroxidase mediated polymerization of methyl methacrylate (MMA) in different reaction conditions results in stereo-regular polymers with high syn-diad fractions.¹ However, in the present study, the poly(acrylamide) obtained was atactic.

Previously, HRP was used to polymerize *p*-alkylphenols at oil/water (reverse micelles)^{22–24} and air/water (Langmuir–Blodgett trough) interfaces.²⁵ Poly(*p*-alkylphenols) prepared in reverse micelles exhibited more uniform molecular weight distribution than those prepared in bulk organic solvents.^{23,24} Also, Behari *et al.*^{16–18} used surfactants to increase the kinetics of acrylamide free-radical polymerization with traditional catalyst systems. These findings encouraged us to study HRP-catalyzed acrylamide polymerizations in the presence of surfactants and in concentrated emulsions.

For aqueous HRP-mediated acrylamide polymerization, the induction period of 60 min without surfactant was reduced to 35 and 40 min when anionic bis(2-ethylhexyl)sodium sulfosuccinate (AOT) and cationic cetyltrimethylammonium bromide (CTAB) were used. Thus, both anionic and cationic additives enhanced the rate of the polymerization giving 94% isolated polymer yields in 60 and 75 min, respectively (see Fig. 1 and Table 1, entries 13 and 18). The M_w (PDI) values for poly(acrylamide)s formed by using anionic and cationic surfactants for 60 and 75 min reaction times were 141 000 (3.2) and 131 000 (3.2), respectively (see Table 1, entries 13 and 18). Behari *et al.*¹⁸ studied the effect of adding anionic (sodium oleate, sodium lauryl sulfate) and cationic (CTAB) surfactants above their CMC values to aqueous acrylamide polymerizations catalyzed by peroxydiphosphate/activator redox systems. Similar to the finding above, the addition of anionic surfactants enhanced the rate of acrylamide polymerization. However, in

Table 1 HRP II mediated free radical polymerization of acrylamide in aqueous medium, in presence of AOT surfactant, in the presence of CTAB surfactant and in sorbitane monooleate concentrated emulsion

Entry	System	Time/min	Isolated yield (%)	$10^{-3}M_w/g\ mol^{-1}$	PDI
1	Aqueous	30	—	—	—
2	Aqueous	45	—	—	—
3	Aqueous	60	2	N.D	N.D
4	Aqueous	90	24	128	2.7
5	Aqueous	120	72	132	2.9
6	Aqueous	150	83	178	2.9
7	Aqueous	180	98	209	2.7
8	Aqueous (HRP I)	180	98	176	2.4
9	Aqueous (SBP)	180	51	282	2.9
10	Aqueous + AOT	35	2	212	2.3
11	Aqueous + AOT	40	25	139	3.1
12	Aqueous + AOT	50	91	131	2.9
13	Aqueous + AOT	60	94	141	3.2
14	Aqueous + CTAB	40	2	282	2.9
15	Aqueous + CTAB	45	55	229	2.2
16	Aqueous + CTAB	50	81	192	2.3
17	Aqueous + CTAB	60	91	133	2.6
18	Aqueous + CTAB	75	94	134	3.2
19	Conc. emulsion	30	10	N.D	N.D
20	Conc. emulsion	45	85	154	5.2
21	Conc. emulsion	60	87	110	4.8
22	Conc. emulsion	75	99	107	4.2

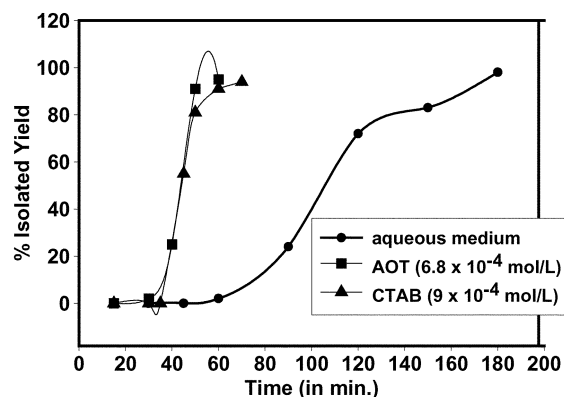


Fig. 1 Horseradish peroxidase mediated polymerization of acrylamide in aqueous medium with and without the addition of surfactants.

contrast to the present study, CTAB addition resulted in a decrease in the kinetics of acrylamide polymerization.¹⁹ Further work will be needed to explain this difference in response of the chemical and enzyme-based catalyst systems to CTAB.

The HRP-mediated polymerization of acrylamide, in a concentrated emulsion using toluene in the dispersed phase, and sorbitane monooleate as an oil-soluble liquid surfactant (hydrophile:lipophile balance of 4.3), was performed. When the acrylamide polymerization was conducted in an aqueous medium without surfactant addition, a 1 h induction period and a total reaction time of 3 h was required to reach nearly quantitative polymer formation. However, by using the emulsion technique (oil in water), the one-hour induction period was greatly reduced so that nearly quantitative polymer formation occurred within 1.25 h (Fig. 2, Table 1, entry 22). This is consistent with traditional free-radical polymerization systems when one moves from solution to emulsion polymerizations. For example, potassium peroxodisulfate catalyzed acrylamide polymerizations were found to occur more rapidly in a concentrated emulsion (dispersed in decane, internal phase ratio of 0.94) than in aqueous solution.²⁰ The product formed by this emulsion polymerization, after 1.25 h, had an M_w and PDI of 107 000 and 4.2, respectively (Table 1, entry 22). The repeat unit sequence distribution of this product was analyzed by ¹³C NMR. From observation of triads using C_α (CH) resonances, the sequence distribution was as follows: syndio- (rr = 34%), hetero- (mr and rm = 51%) and isotactic (mm = 15%). From these results, the randomness factor R was calculated ($R = 4[mm][rr]/[mr]^2$) and found to be 0.8. Since the value of R is near to 1.0, propagation closely follows Bernoulli statistics.²⁵ Comparison of the repeat unit sequence for polyacrylamides prepared in this study to those from peroxide initiated chemical polymerizations shows that they are similar. This suggests that HRP is not specifically associated with the propagating chain and the incoming monomer in such a way that might regulate chain stereoregularity.

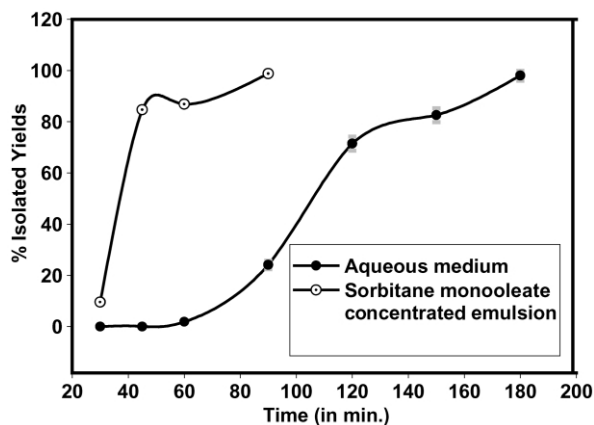


Fig. 2 Horseradish peroxidase mediated free radical polymerization of acrylamide in aqueous medium and in sorbitane monooleate concentrated emulsion (the error bars were generated by mean deviation of two replicates of reaction).

The molecular weight of the polyacrylamide obtained by using a concentrated emulsion was smaller than that synthesized in the aqueous medium (Table 1, entries 7 and 22). It may be that the transfer of hydrogen radical from the surfactant hydrophilic head-group to the propagating chain end results in higher rates of chain termination and, consequently, lower molecular weights than the reactions conducted in the aqueous medium.

HRP-mediated polymerization of sodium acrylate

The HRP-mediated polymerization of sodium acrylate at room temperature was studied in aqueous medium. The reducing

substrate 2,4-pentanedione and hydrogen peroxide were used in the ratio 1.5:1.0 mol/mol. The polymer yields increased from 38 to 88% from 2 to 24 h, respectively. Measurements by GPC showed that the weight average molecular weight (M_w) of poly(sodium acrylate)s formed ranged from 191 to 242 K (Table 2).

Table 2 HRP II mediated free radical polymerization of sodium acrylate in aqueous medium

Time/h	Isolated yield (%)	$10^{-3}M_w/g\ mol^{-1}$	PDI
2	38	242	5.0
4	44	212	5.1
6	49	200	5.3
8	79	203	4.9
16	85	208	5.0
24	88	191	5.2

Conclusions

Horseradish peroxidase type II (HRP II) was studied for the catalysis of acrylamide and sodium acrylate polymerizations at room temperature. HRP II functioned as an oxidant with hydrogen peroxide to transfer electrons under mild conditions to 2,4-pentanedione. We believe that 2,4-pentanedione then acts as the free radical initiator species for chain growth. When acrylamide was polymerized in aqueous solution, the addition of either AOT or CTAB was found to significantly reduce the lag-time for chain initiation and propagation reactions. With the addition of AOT or CTAB to acrylamide polymerizations in aqueous media, 94% isolated polymer yields were obtained in 60 and 75 min, respectively. Comparison of acrylamide polymerizations in aqueous media without surfactant versus in concentrated emulsions showed that the latter gave more rapid polymerizations but products with broader polydispersity. In addition, the above strategy for HRP-mediated free-radical polymerizations was found useful with sodium acrylate. Hence, after a 24 h polymerization in aqueous medium without surfactant, poly(acrylate) was prepared in 88% yield, M_w ca. 191 K, and PDI = 5.2. The chain stereochemistry of the polymers formed was found to be similar to that for free-radical polymerizations carried out without enzyme. This suggests that the propagating chain and the incoming monomer do not associate in such a way that would regulate chain stereoregularity. Currently, acrylamide polymerizations are performed with potassium peroxodisulfate at 40 °C or with azo compounds at 60 °C. Photoinitiators or redox polymerization systems that are used at around 40 °C must be stored at low temperatures under inert conditions due to their inherent instability. The chemical systems often involve heavy metals and/or toxic substances. Their storage requirements are costly and energy intensive. Since peroxidases are natural, safe, and stable in the absence of peroxides, they do not require stringent storage precautions. In addition, future work on enzyme-catalyzed free-radical polymerizations may lead to insights on how they can be more broadly used to control the stereochemistry during propagation reactions. Thus, considering current advantages and future opportunities, enzymatic free-radical polymerization have important environmental benefits.

Experimental

Materials

Horseradish peroxidase (Type II, activity 235 purpulloallin units/mg), Horseradish peroxidase (Type I, activity 100 purpul-

logallin units/mg), Soybean peroxidase (90 purpulloallin units/mg), hydrogen peroxide (30% (w/v)) were obtained from Sigma chemical company. 2,4-Pentanedione from Aldrich was distilled prior to use. Acrylamide, sodium acrylate, dioctyl sulfosuccinate sodium salt, cetyltrimethylammonium bromide, sorbitane monooleate, methanol obtained from Aldrich were used as received.

Instrumentation

Nuclear magnetic resonance. All polymer solutions were PFG filtered prior to analysis to remove interference that might have arisen due to the presence of small molecule impurities. The NMR data were recorded on Bruker AMX500 and DMX400 spectrometers. The ^{13}C NMR spectra were obtained using gated-decoupling and using an 8-s recycle delay between scans for 10K scans for quantitation of resonances. ^1H NMR spectra were recorded using a 4-second recycle delay between scans. The distribution of repeat unit sequences that differ in stereochemistry was analyzed for poly(acrylamide) by observing the NMR signals due to the methine carbon region in the ^{13}C NMR.²¹

Molecular weight measurements. The weight average molecular weights (M_w) of the polymer samples were determined by gel permeation chromatography (GPC). Studies by GPC were carried out using a Waters, Inc. Model 510 pump, two Shodex KB 80m and one Shodex KB 802.5 column, and a Waters 410 differential refractometer. The software used for molecular weight calculations was millennium chromatography manager version 2.15. Sodium dihydrogen phosphate, 20 mM, pH 7.0, was used as the eluent. Analyses were carried out at 35 °C, flow rate 1 mL min⁻¹ and with injection volumes of 10 μL . Polyethylene glycol standards with narrow polydispersity were used to generate a calibration curve.

Polymerization reactions

Polymerization of acrylamide in aqueous medium. Acrylamide (2.92 mmol in 4 mL water) in a dual inlet ampule was purged with nitrogen for 10 min. Into the above solution, HRP (8 mg in 0.2 mL water), hydrogen peroxide (0.046 mmol) and 2,4-pentanedione (0.068 mmol) were successively injected while stirring. The reactions were carried out at room temperature for a predetermined time period while maintaining both stirring and a nitrogen atmosphere. After the predetermined time, the reaction mixture was poured into an excess of methanol. The resulting precipitate was filtered off, washed with methanol and dried *in vacuo* (50 °C, 30 mmHg, 24 h). The enzyme was soluble in methanol and thus removed from the polymer.

Polymerization of acrylamide in aqueous medium in presence of surfactant. Acrylamide (2.92 mmol) was added to a solution of distilled water (3.75 mL) and CTAB (9.0×10^{-4} mol/L, 50 μL) or AOT (6.8×10^{-4} mol/L, 50 μL) in a dual inlet ampule under nitrogen atmosphere. An example of a typical reaction is the successive addition under a nitrogen atmosphere of 0.2 mL of HRP (40 mg mL⁻¹, 8 mg of enzyme), hydrogen peroxide (0.046 mmol) and 2,4-pentanedione (0.068 mmol). The reaction mixture was maintained under nitrogen with stirring at room temperature for a predetermined time period. Then, the reaction mixture was poured into a large excess of methanol. The precipitate obtained was separated by filtration,

washed with methanol and then with hot chloroform (to remove surfactant), and dried (*in vacuo*, 50 °C, 30 mmHg, 24 h).

Polymerization of acrylamide in concentrated emulsion. Toluene (42 μL) and sorbitane monooleate (17.2 μL) were degassed in a dual inlet ampule for 10 min. Acrylamide (2.92 mmol) dissolved in water (0.45 mL) was added to the surfactant solution with stirring. To the above was added HRP (8 mg in 0.3 mL water), hydrogen peroxide (0.046 mmol) and 2,4-pentanedione (0.068 mmol) while vigorously stirring. The polymerization was carried out under a nitrogen stream for different time periods. The polyacrylamide was isolated by precipitation in methanol and then dried in a vacuum oven (50 °C, 30 mmHg, 24 h).

Polymerization of sodium acrylate in aqueous medium. Sodium acrylate (4 mmol) was added to a solution of distilled water (1.5 mL) in a dual inlet ampule under nitrogen atmosphere. An example of a typical reaction is the successive addition under a nitrogen atmosphere of HRP (11.4 mg in 0.5 mL water), hydrogen peroxide (0.064 mmol) and 2,4-pentanedione (0.097 mmol). The reaction mixture was maintained under nitrogen with stirring at room temperature for a predetermined time period. Then, the reaction mixture was poured into a large excess of methanol. The precipitate obtained was separated by filtration, washed with methanol and dried (*in vacuo*, 50 °C, 30 mmHg, 24 h).

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