Co-polymerization of MTPC (methylene tri p-cresol) and m-cresol using CiP (Coprinus cinereus peroxidase) to improve the dissolution characteristics of the enzyme-catalyzed polymer

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ARTICLE INFO

ABSTRACT

MTPC (Methylene tri p-cresol) and m-cresol were copolymerized by Coprinus cinereus peroxidase in aqueous acetone. Although MTPC did not dissolve completely in the aqueous acetone, copolymerization was achieved owing to the radical transfer between solute and solid surface. Various polymerized products with different molecular weights and hydroxyl values were synthesized depending upon reaction compositions (ratio of MTPC to m-cresol and buffer to acetone). Poly(MTPC–m-cresol), a copolymer of MTPC and m-cresol, was mixed with a diazonaphthoquinone derivative to form a new type of photoresist, a thin film of which was formed on a silicon wafer and immersed in alkaline solution (tetramethylammonium hydroxide) to measure speed of dissolution. Poly(MTPC–m-cresol), with higher hydroxyl value (over 80%), showed remarkably improved dissolution characteristics (dark loss in alkaline solution decreased by almost half), which is prerequisite for sensitive photoresist polymer.

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1. Introduction

Photoresists are essential chemicals in semiconductor processing, LCD (liquid crystal display) processing, and many printing processes, with a rate of consumption that has steadily increased over the years [1]. Various polymers are currently being investigated as new photoresists because of the demand for high resolution and imagery, however, most conventional photoresists are still mainly composed of novolac resin and DNQ (diazonaphthoquinone) photoactive compounds. Most conventional novolac resins are synthesized by polymerizing various phenolics (o-cresols) with formaldehyde to produce resins with excellent film-forming properties, good adhesion, etch resistance, and high solubility in alkaline solution. However, the toxic properties of the formaldehyde used have led to considerable research efforts to find alternative routes to their synthesis.

Enzymatic polymerization of phenolics using peroxidase has been intensively studied [2,3,4] with several advantages over conventional polymerization having already been reported. Recently, we reported a successful enzymatic polymerization using the fungal peroxidase, CiP (Coprinus cinereus peroxidase), for the synthesis of polycardanol and poly(bisphenol A) [5,6]. Although the poly(bisphenol A) showed good resolution as a photoresist, its dissolution characteristics were not satisfactory because the poly(bisphenol A) had a lower hydroxyl value compared with that of conventional novolac resins. The ratio of the phenylene bond, not the oxyphenylene bond, must be increased to elevate the hydroxyl value of resulting polymer. Template polymerization using PEG can yield a polymer with a much higher hydroxyl value [7,8], however, separation of the pure polymer from the template–polymer complex is extremely difficult, thus polyphenol was not feasible as a photoresist polymer. In this paper copolymerization between MPTC (methylene tri p-cresol) and m-cresol was tried to increase the number of hydroxyl group per chain. Significant increase of OH-number per chain was achieved although there was a limit to elevate the hydroxyl value. The resulting copolymer (MTPC/m-cresol) showed much improved dissolution characteristics.

2. Experimental

2.1. Production and purification of the fungal peroxidase

C. cinereus IFO 8371 was used as the peroxidase-producing strain. The medium for the production of the peroxidase contained

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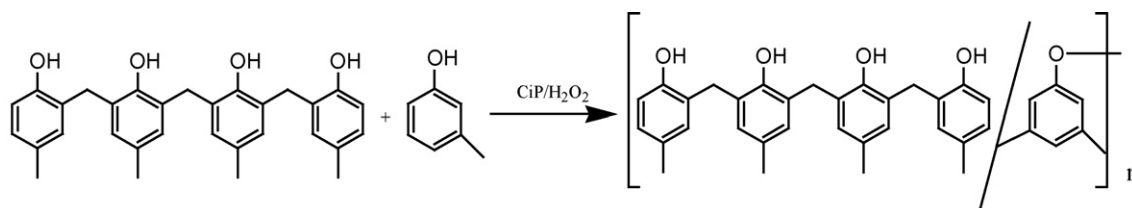
Fig. 1. Scheme of copolymerization of MTPC and *m*-cresol using CiP.

30 g/L glucose, 5 g/L peptone (Difco Lab., USA), and 3 g/L yeast extracts (Difco Lab., USA). The details of the production and purification of the fungal peroxidase have been previously reported [5]. Finally, the purified CiP was concentrated to a final concentration of 20,000 U/mL for the polymerization of bisphenol A.

2.2. Enzymatic copolymerization of MTPC and m-cresol

The reaction scheme for the copolymerization of MTPC (Waco Chemicals, Germany, CAS No 20837-68-7) and *m*-cresol (Aldrich, USA) is shown in Fig. 1. All the chemicals including MTPC and *m*-cresol were used as received without further purification. The typical enzymatic copolymerization of MTPC and *m*-cresol was carried out as follows: 3.24 g of MTPC and 2.16 g of *m*-cresol (20 mmol) were suspended in a mixture of 100 g acetone and 150 g phosphate buffer (100 mM, pH 7.0). 100,000 units of CiP were then added to the reaction mixture and stirred for 5 min. The reaction was initiated by adding 15% H_2O_2 solution (20 mmol) continuously at a rate of 4.0 mmol/h at 20 °C with gentle stirring for 5 h. After that, the reaction mixture was centrifuged at 5000 rpm and the supernatant decanted. The settled materials (white powdery) were washed three times with aqueous methanol (50:50, v/v) to remove any unreacted monomer and buffer. The washed material was dried *in vacuo* to give poly(MTPC–*m*-cresol).

2.3. Evaluation of peroxidase-catalyzed poly(MTPC–m-cresol) as a photoresist resin

The photoresist thin film was prepared on a silicon wafer by spin-coating from a 27 wt.% PGMEA (propylene glycol monomethyl ether) solution of the poly(MTPC–*m*-cresol) and a photosensitizer (PS-105, Koyo Chemicals, Japan) (80:20 wt.%). The resulting film was immersed in 2.38% TMAH (tetramethylammonium hydroxide) solution for 60 s. The differences in thickness before and after immersion were measured by employing Nanospec (NSR-19c MCS2, Nikon, Japan). The dissolution rate of the coating film (dark loss) was calculated from the differences in thickness.

2.4. Analytical methods

Peroxidase activity (U/mL) was measured as follows. Several microliters of the reaction solution were added to 2 mL of a 0.18 mM solution of ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) in 50 mM phosphate-citrate buffer (pH 5.0). One microliter of a 15% H_2O_2 solution was added to initiate the color generation reaction. One unit of peroxidase was defined as the amount of enzyme required to catalyze the conversion of 1 μmol of ABTS ($\varepsilon = 34,700\,\mathrm{cm^{-1}\,M^{-1}}$) per min at 25 °C. The molecular weights of the poly(MTPC–*m*-cresol) were determined by GPC (gel permeation chromatography). GPC analysis was carried out using a refractive index detector under the following conditions: PL4 mixed BB columns (TOSOH, Japan) and tetrahydrofuran as a solvent at 1.0 mL/min. The calibration curves for the GPC analysis were obtained using polystyrene standards. The hydroxyl

value was measured by titration methods using acetic anhydride [9].

3. Results and discussion

3.1. Peroxidase-catalyzed copolymerization of poly(MTPC–m-cresol)

In this study, peroxidase derived from *C. cinereus* and hydrogen peroxide were used as a catalyst and oxidizing agent, respectively. Polymerization of MTPC was not successful in any aqueous organic solvent mixture including methanol, ethanol, 2-propanol, acetone, and DMSO. Any significant increase of molecular weight in the MTPC reaction was not observed, implying that CiP was not able to generate a radical on the MTPC given its steric bulk interfering with its approach towards the reactive site of the peroxidase. Copolymerization of MTPC with *m*-cresol was attempted in an effort to obtain the polymeric product of MTPC since *m*-cresol is known to be polymerized easily through peroxidase-catalysis [10]. As shown in Table 1, the effects of the aqueous organic solvent mixture on the CiP-catalyzed copolymerization of MTPC–*m*-cresol were studied. In the hydrophilic aqueous solvent mixture (low log *P*, DMSO), the copolymerization did not take place at all. On the other hand, CiP yielded the polymerized product having a higher molecular weight in the relatively hydrophobic aqueous solvent mixture (high log *P*), as compared to that produced in the other solvents. Surprisingly, while MTPC was only suspended in aqueous acetone or aqueous 2-propanol, copolymerization of MTPC–*m*-cresol was accomplished successfully to yield polymeric product. This implies that the radical transfer between *m*-cresol radical and suspended but still particulate MTPC still takes place rapidly. Considering *m*-cresol was polymerized in aqueous DMSO mixture, radical transfer between MTPC and *m*-cresol was not thought sufficiently performed in aqueous DMSO mixture. Since radical transfer is known to depend on the radical lifetime, radical lifetime may be shorter in aqueous DMSO mixture rather than aqueous acetone or aqueous 2-propanol [11]. Fig. 2 shows the relationship between the log *P* of the organic solvent and the molecular weight of the poly(MTPC–*m*-cresol) whose polymerization was catalyzed by CiP in a mixture of organic solvent and phosphate buffer. An organic solvent that is too hydrophilic can penetrate and replace the water bound on the protein, possibly disturbing the authentic three dimensional protein structure [12]. A

Table 1
Effects of solvent on the peroxidase-catalyzed copolymerization of MTPC and *m*-cresol

Solvent	Yield (%)	Mn	Mw	Log *P*
DMSO	0	–	–	−0.69
Methanol	92.9	1536	2,633	−0.32
Ethanol	95.0	1902	3,794	0.056
Acetone	92.8	1866	7,530	0.23
2-Propanol	98.2	2446	10,293	0.42

MTPC (2.16 g) and *m*-cresol (3.24 g) were copolymerized by CiP in various aqueous organic solvents (buffer:solvent = 60:40, w/w).
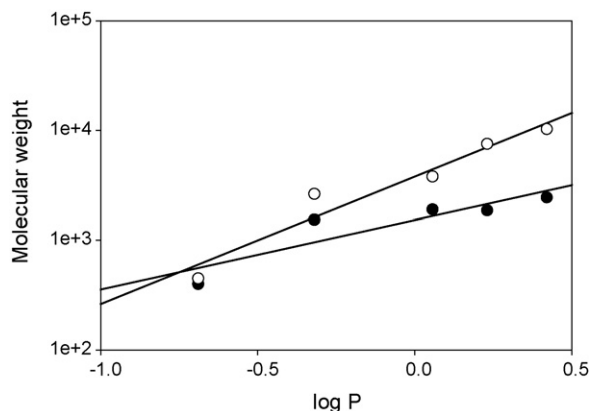
Fig. 2. The relationship between $\log P$ of the organic solvent and molecular weight of the CiP-catalyzed poly(MTPC–m-cresol) in a mixture of organic solvent and phosphate buffer (40:60 w/w). (●) Number-averaged molecular weight (Mn); (○) weight-averaged molecular weight (Mw).
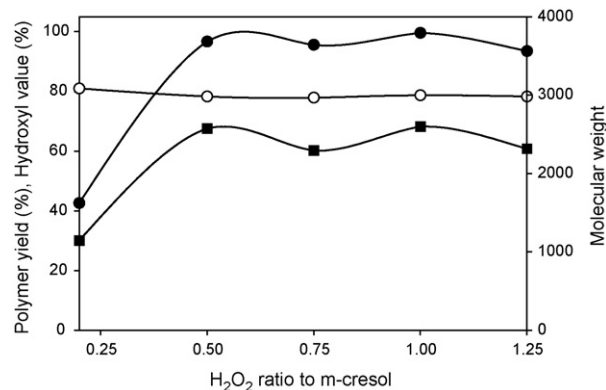

Fig. 3. The effects of hydrogen peroxide to m-cresol ratio on the copolymerization between MTPC and m-cresol. MTPC (4.68 g) and m-cresol (0.54 g) were copolymerized by CiP in aqueous acetone (buffer:solvent = 60:40, w/w). (●) polymer yield (%); (○) hydroxyl value (%); (■) weight average molecular weight of poly(MTPC–m-cresol).

similar relationship between the $\log P$ of the organic solvent and the molecular weight of poly(bisphenol A) could also be found [6]. Since the poly(MTPC–m-cresol) that polymerized in aqueous 2-propanol contained insoluble particulates, which can pose a serious problem for application as photoresist, subsequent experiments were performed in aqueous acetone.

3.2. Effects of MTPC to m-cresol ratio on the property of poly(MTPC–m-cresol)

While the homo-polymer of m-cresol showed 55% hydroxyl value, the copolymer of MTPC and m-cresol showed a much higher hydroxyl value. It is well known that oxyphenylene bonds can be formed through peroxidase-catalysis [6], still, novolac (phenol–formaldehyde resin) does not contain oxyphenylene linkages. Since MTPC contains no oxyphenylene linkages, the copolymer of MTPC and m-cresol was expected to have a small number of oxyphenylene linkages. An increase in the hydroxyl value (less the number of oxyphenylene linkage) was observed when the ratio of MTPC to m-cresol was increased, as shown in Table 2. Various copolymers having different hydroxyl values ranged from 55 to 82.8% can be obtained by incorporating MPTC. However, a larger MPTC ratio caused the reduction in the molecular weight of copolymer, ranging from 11,393 to 1708. This implies that generated radicals on m-cresol are easily transferred to MTPC and subsequently exhausted through radical–radical coupling, which may be a cause for the reduction in molecular weight of the copolymer. Although control of the hydroxyl value can be achieved by changing the composition of the aqueous organic solvent, the range of variation is known to be narrow (58–65%) [10]. On the contrary, copolymerization with MTPC can give more freedom in obtaining phenol polymers with a wide range of hydroxyl values.

3.3. Effects of hydrogen peroxide added on copolymerization of poly(MTPC–m-cresol)

Fig. 3 shows the effect of hydrogen peroxide on the copolymerization, including yield, hydroxyl value, and molecular weight. It is well known that 1 mol of hydrogen peroxide produces 2 mol of phenolic radicals by the catalysis of peroxidase. When hydrogen peroxide was supplied over 0.5 ratios to m-cresol, all the performance criteria such as yield, molecular weight and hydroxyl values varied only slightly. Under 0.5 ratios to m-cresol, the yield and molecular weight of the polymer was significantly reduced while the hydroxyl value remained constant, indicating that insufficient radical generation caused by a smaller volume of hydrogen peroxide resulted in the reduction of yield and molecular weight.

3.4. Effects of acetone to buffer ratio on copolymerization of poly(MTPC–m-cresol)

Table 3 summarizes the results of the copolymerization of MTPC and m-cresol in a mixture of acetone and buffer. In the case where the acetone concentration was less than 20%, MTPC and m-cresol were significantly insoluble in the medium. Even at this composition, the CiP catalyzed-polymerization yield was relatively high, as shown in Table 3. A lower concentration of acetone in the reaction medium can increase the stability of CiP, because less water bound on the CiP can be replaced by acetone molecules. Although the dissolution of MTPC and m-cresol is limited in a solvent mixture of a lower content of acetone, a higher yield of poly(MTPC–m-cresol) was nevertheless achieved. It was assumed that the polymerized product escapes from the reaction medium by precipitation and the vacant space could therefore be refilled with monomer.

Table 2
Effects of MTPC to m-cresol ratio on the peroxidase-catalyzed copolymerization of MTPC and m-cresol

MTPC:m-cresol (g:g)	Yield (%)	Mn	Mw	Polydispersity	Hydroxyl value (%)
4.86:0.54	99.6	1110	1,708	1.54	82.8
4.32:1.08	99.8	1219	4,485	3.68	81.5
3.24:2.16	98.5	1514	6,064	4.01	77.0
2.16:3.24	94.1	2099	6,677	3.18	75.8
0:5.4	94.6	3166	11,393	3.60	55.0

Various ratios of MTPC and m-cresol were copolymerized by CiP in aqueous acetone (buffer:solvent = 60:40, w/w).

Table 3
Effects of aqueous acetone on the peroxidase-catalyzed copolymerization of MTPC and m-cresol

Acetone:buffer	Yield (%)	Mn	Mw	Polydispersity	Hydroxyl value (%)
10:90	90.9	569	811	1.43	77.6
20:80	91.3	759	1,120	1.48	82.6
30:70	95.7	1,430	2,679	1.87	74.9
40:60	94.1	2,099	6,677	3.18	76.2
50:50	99.1	1,513	4,273	2.80	75.9

MTPC (2.16 g) and m-cresol (3.24 g) were copolymerized by CiP in various aqueous acetone mixtures.

Table 4
Effect of hydroxyl value and molecular weight of poly(MTPC–m-cresol) on the dissolution rate of polymer

Entry	Mn	Mw	Hydroxyl value (%)	Dark loss (A/s)
1	3166	11,393	55.0	969
2	1514	6,064	77.0	899
3	1110	1,708	82.8	866
4	1219	4,485	81.5	504

Changing the hydrophobicity of the solvent makes it possible to control regioselectivity [13]. The phenylene unit content of poly(bisphenol A) linearly decreased as 2-propanol concentration increased, varying in range from 65 to 58% [6]. A similar tendency was observed in the laccase-catalyzed polymerization of phenol in aqueous alcohol [14]. However, variation of buffer ratio to solvent did not result in the significant change of hydroxyl value in the copolymer of MTPC and m-cresol unlike the previous study [6]. Molecular weight of copolymer was decreased by increasing the buffer ratio as expected in previous report [6].

3.5. Improved dissolution characteristics of poly(MTPC–m-cresol)

Dark loss (dissolution rate at dark condition) is determined by measuring the loss of coating film when immersed in an alkaline solution (TMAH) at dark conditions. Photo-sensitizers (DNQ) contained in coating films are known to inhibit the dissolution of the coating polymer by forming a complex between the photosensitizer and phenolic polymers [15]. The Major interaction responsible between the photosensitizer and phenolic polymer is the hydrogen bond between the hydroxyl group of the phenolic polymer and the nitrogen atom of the photosensitizer. To reduce the dissolution rate of the coating polymer, a higher hydroxyl group content in coating polymer is required. As shown in Table 4, entry 1 polymer showed a higher dissolution rate although its molecular weight was higher than that of entries 2, 3, and 4. This was especially true for the copolymer (entry 4) having a higher molecular weight than entry 3 and with a similar hydroxyl value that showed a much improved performance for dark loss. The sharp contrast between dark loss and dissolution rate during UV illumination of coating polymer represents one the critical characteristics as a photoresist polymer. Poly(MTPC–m-cresol) can be used as better photoresist polymer since its dark loss can be decreased.

4. Conclusions

MTPC and m-cresol were copolymerized by C. cinereus peroxidase in aqueous acetone. Various polymerized products with different molecular weights and hydroxyl values were synthesized depending on the reaction conditions (ratio of MTPC to m-cresol, ratio of hydrogen peroxide to m-cresol, and the ratio of acetone to aqueous buffer). A new, positive type photoresist was developed by mixing poly(MTPC–m-cresol) and a photoactive compound such as DNQ. The hydroxyl value and molecular weight of the poly(MTPC–m-cresol) were the critical factors determining the dark loss of the formulated photoresist. The optimized poly(MTPC–m-cresol) was obtained by varying the hydroxyl value and molecular weight.

Acknowledgements

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