Molecular Imprinting Micropolymerbeads Having Cooperative Effect of both Surfactant and Inosine Template

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ABSTRACT: Molecular imprinted polymer (MIP) microbeads were prepared by emulsion copolymerization of divinylbenzene (DVB) and methacrylic acid (MAA) in the presence of inosine (INO) template and laurylbenzenesulfonic acid (LBSA) as surfactant. The polymerization was carried out at 55°C under ultrasound exposure. The resulting copolymer microbeads, having 0.1–0.4 μm diameter, were observed to have a binding behavior of INO and surfactant to the polymer which was strongly dependent of the bulk pH; for example, at pH 3, 6, and 10 values of binding for INO to the imprinted copolymer were 3.7 μmol/g, 2.1 μmol/g, and 0 μmol/g, respectively. It was found that LBSA surfactant bound to the MIP microbeads similarly depending on pHs. At pH 3, 6, and 10, the LBSA values of binding were 23 μmol/g, 1.3 μmol/g, and 4.8 μmol/g. It was also noted that the surfactant binding was enhanced in the presence of the INO template. This demonstrated that a cooperative binding with the surfactant was cooperatively occurred in the presence of INO. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 565–571, 2010

Key words: molecular imprinting; inosine; cooperative effect; emulsion polymerization

INTRODUCTION

Many important reactions in biological systems involving biomolecules such as enzymes, antibodies and receptors occur by molecule recognition. Similar to these biosystems, artificial receptors made of synthetic polymers can be found in MIPs.[1-4] Since MIPs have provided selective recognition of targeted molecules, which their specific shape has been imprinted into synthesized polymer, the recognition sites in the polymer enable to bind these molecules. Therefore, such artificial receptors can be used in several applications such as separation, biosensor and drug delivery system.[5-7] Among these, MIP microbeads are expected to have many potential uses in these fields, for example, for packing MIP resins in a separation column. So far, there were many reports concerning the techniques for the preparation of MIP microbeads using precipitation polymerization, multistep swelling polymerization, dispersion polymerization and emulsion polymerization.[8-13] Among them, Sun et al., reported the separation of theophylline from green tea by a packing column with MIP microbeads, which he obtained by precipitation polymerization.[14] In the column separation of different kinds of green teas, teophylline imprinted poly(ethylene glycol dimethacrylate) microbeads were able to determine successfully the quantities of teophylline and caffeine in each samples. Koide et al. synthesized metal-imprinted microbeads in an oil-water micelle environment by using 10-(*p*-vinylphenyl)decanoic acid as a polymerizable surfactant and divinylbenzene (DVB) as a crosslinker.[15] The resulting imprinted microbeads were around 0.2–0.4 μm in diameter and the imprinting sites for metal ions were efficiently generated on the surface of the imprinted microbeads. On basis of these technical back ground, the present research focused on preparation of MIP microbeads as main subject of study. For the target molecule, inosine (INO) was used as a template (Fig. 1). Since INO consists of both base and sugar segments such as adenosine and adenine and the derivative is one of the raw materials of DNA and RNA, the template would be considered as a key chemical in the synthesis of amino acid. In addition, it is well known that INO is finally metabolized to purine and then uric acid, being the cause of gout disease.[16] Therefore, INO imprinted polymer microbeads are expected to be applied in the separation of such substances from liquids in the industrial food field. Also, INO imprinted polymer microbeads may be applied as potential drugs for treatment of gout disease in the medical field. Namely, experimental results obtained in INO imprinted polymer microbeads can apply for development to such field, since

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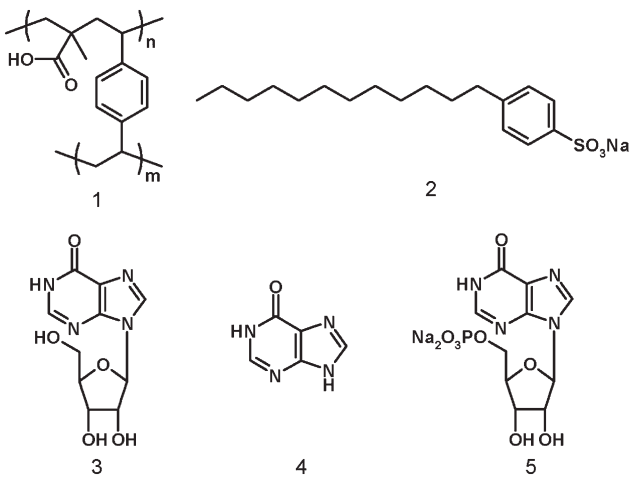


Figure 1 Chemical structures of 1 P(DVB-*co*-MAA), 2 laurylbenzenesulfonic acid (LBSA), 3 inosine, 4 hypoxanthine and 5 inosinic acid.

the MIPs selectively removes INO from mixture. Therefore, it might be expected that INO imprinted polymers would become either INO absorbents or metabolic inhibitor. Thus, it is of great interest to prepare and study INO imprinted polymers with microbead shape.

Through the present work relating with INO imprinted polymer microbeads, we found that a novel functionality of MIP microbeads which were prepared by using emulsion polymerization technique. That is, a cooperative effect between the target molecules and the surfactant was significantly effective for its recognition manner. As seen in Dai's report about hierarchically imprinted SiO_2 targeting Cu^{2+} ion with and without surfactant,[17] the binding behavior of Cu^{2+} in the presence of surfactant was higher than that in the absence of surfactant. Namely, hierarchically imprinted SiO_2 could cooperatively bind both Cu^{2+} and the surfactant. There was also reported as cofactor effect in MIP.[18] The cooperative binding of substrate and porphyrin cofactor was observed in enantioselectivity of MIPs. Thus, such cooperative effect became of much more interest for its possibilities in MIPs. On the other hands, there are still very few works that discuss about hierarchically MIP for targeting organic molecules so far. In addition, there has been no research on such cooperative effect of emulsion polymers. Therefore, the research results obtained in INO imprinted polymer microbeads by emulsion polymerization would strongly contribute to several fields. This kind of knowledge is useful, especially in construction of artificial enzyme by MIP.

To prepare MIP microbeads, methacrylic acid (MAA) as a functional monomer and DVB as a crosslinker were polymerized in the presence of both INO and laurylbenzenesulfonic acid (LBSA) as a surfactant under the ultrasound. After the preparation of INO imprinted polymers, binding experiments were done at different pH values for INO and other analogous substances such as hypoxanthine and inosinic acid (Fig. 1). Then, INO and LBSA binding was evaluated the same way in the presence of LBSA. Evidence of the cooperative effect between INO and LBSA was observed along the specific binding behaviors in the resulting MIP microbeads.

EXPERIMENTAL

Materials

MAA, DVB and laurylbenzene sulfonic acid (LBSA) were purchased from Nacalai Tesque, and inosin (INO) and other substrates of hypoxanthine and inosinic acid were from Tokyo Chemical Industry. MAA was purified by reduced pressure distillation before use. DVB was passed through a silica gel column to remove the inhibitor before polymerization. Other reagents and solvents were used without further purification.

Polymerization and purification

Imprinted beads with MAA and DVB segments, $P(MAA\text{-}co\text{-}DVB)_I$ (Fig. 1) were obtained in the presence of 2,2′-azobis[*N*-(2-carboxyethyl)-2-methylpropionamidine] *n*-hydrate as the initiator (Tokyo Chemical Industry) by emulsion polymerization. Herein, these monomers and LBSA surfactant were dispersed in water/chloroform medium. The procedure was as follows: firstly, MAA (15 mmol), DVB (15 mmol), LBSA (10 mmol), INO (15 mmol) and initiator (0.9 g) were added to distilled water (30 mL). The aqueous monomer solution and chloroform (270 mL) were set into round flask and bubbled with nitrogen gas for 1 h at room temperature. The polymerization was performed at 55°C for 3 h under ultrasound (Bronson) (45 kHz, 600 W). After the polymerization, chloroform was separated by evaporation and the resulting polymer was centrifuged at 3500 rpm for 15 min. Then, the solid polymer was redispersed into water and obtained by centrifugation for 15 min. his operation was repeated ten times. To extract LBSA from the resulting polymer, the samples were washed with ethanol and centrifuged. Finally, INO was extracted by washing in a $10^{-3}M$ HCl aqueous solution. Also, nonimprinting polymer [P(DVB-*co*-MAA)] was polymerized using the same procedure in the absence of INO.

Characterization of polymer microbeads

Morphology observations of the polymer microbeads were done by scanning electron microscopy (SEM)

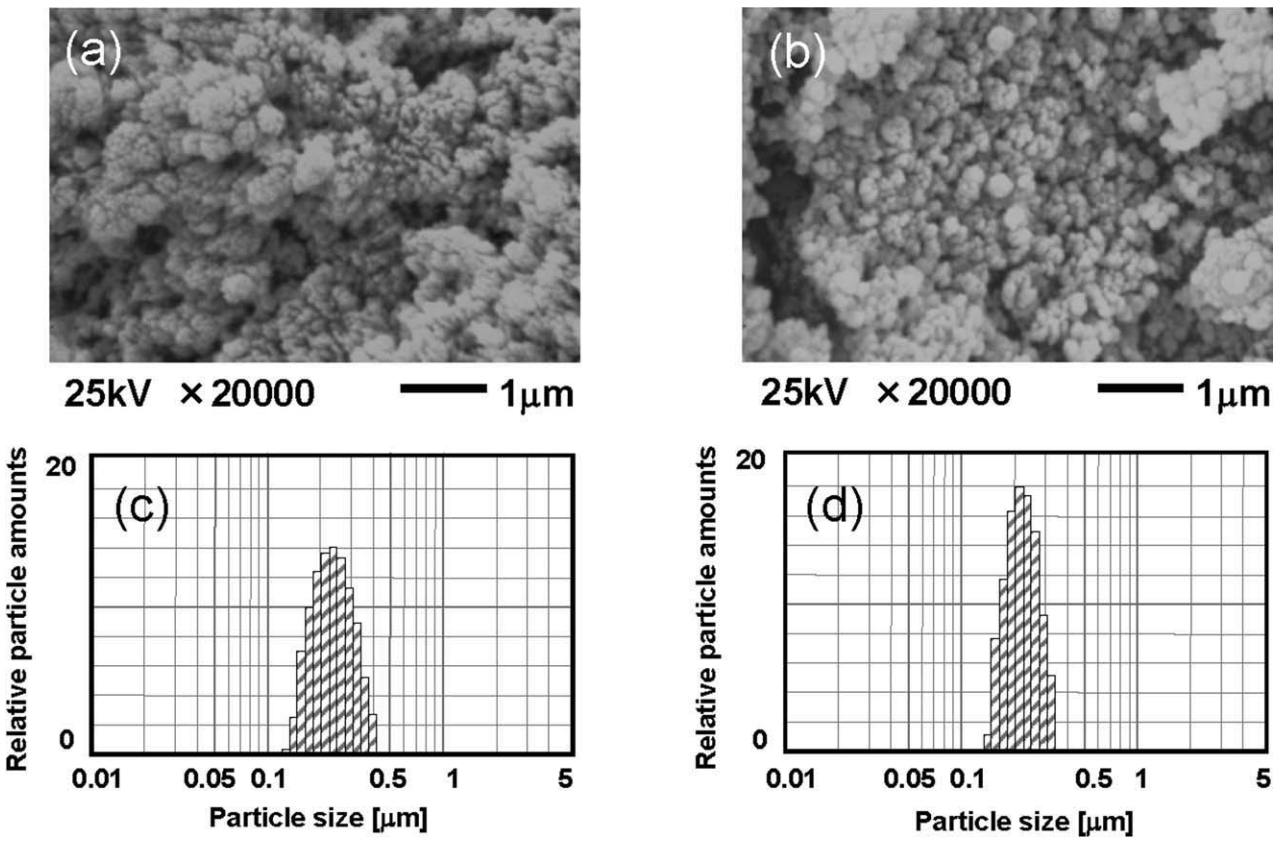


Figure 2 SEM images and particle size distributions of copolymer beads (a) and (c) P(DVB-*co*-MAA)$_I$ and (b) and (d) P(DVB-*co*-MAA) after extraction.

(JEOL JSM-5400). The samples were freeze-dried after centrifugation and then sputtered with gold under vacuum. Particle size distribution of the resultant polymer beads was measured by laser scattering diffraction method (LSD) (Shimadzu SALD-7000) in water. Then, BET surface areas of the resultant polymers were evaluated by micromeritics Flow Sorb 3 (Shimadzu) after freeze-drying. FTIR spectra of resultant polymer beads were observed using a FTIR spectrophotometer (Shimadzu IR-Prestige-21) with KBr tablet method. The characteristic COOH bond in the copolymer was used to estimate MAA contents (x_{MAA}) in the polymer beads.

Binding and recognition experiments of the imprinted polymers

Binding experiments of INO to the imprinted polymers were performed in aqueous substrate solution at various pH values. The substrate concentration in the aqueous solution was measured using a UV absorption spectrophotometer (JASCO V-570). The experiments were carried out in dialysis tube membrane (Spectrum Laboratories, Spectra/Por 1, molecular weight cut-off = 6–8kD) as follows: polymer powder (0.01 g) and water (2 mL) were put in a swelled dialysis tube membrane (20 × 50 mm^2). Then, the tube membrane was immersed in 20 μ*M*-INO aqueous solution (18 mL) and was shaken for 24 h at 30°C. The concentration difference before and after the equilibrium in outside of the dialysis tube membrane was evaluated by measuring the UV absorption of INO in the solution. Herein, the INO had the maximum absorption at 249 nm for the xanthine ring at pH 3 and 6 with the molar coefficients ε 12,300 M^{-1} cm^{-1}. However, the maximum absorption wavelength and molar coefficient of INO at pH 10 was shifted to 253 nm with 12,800 M^{-1} cm^{-1}. This was because of the chemical structure change of xanthine ring of INO at pH 10. Other inosine derivatives of hypoxanthine and inosinic acid were detected in the same manner with INO as mentioned earlier. In addition, the binding experiments of INO were performed similarly each with a 20 μ*M* aqueous solution. Therefore, the maximum absorption wavelengths and ε values of the other substrates at pH 3, 6, and 9 were as follows: hypoxanthine with 10,800 M^{-1} cm^{-1} at 250 nm (pH 3 and 6) and 10,700 M^{-1} cm^{-1} at 259 nm (pH 10), inosinic acid with 9100 M^{-1} cm^{-1} at 249 nm (pH 3, 6, and 10) and LBSA with 12,700 M^{-1} cm^{-1} at 225 nm (pH 3, 6, and 10).

To evaluate the equilibrium constant *K* for INO binding, Scatchard plots were used. For analyzing

the binding behavior of these substrates to the imprinted polymer, an aqueous solution of INO, hypoxanthine and inosinic acid was used in the range of 5–120 μ*M*. Scatchard analyzes were also performed in the presence of LBSA using an absorbance difference in INO and LBSA at 249 nm and 225 nm. Furthermore, the binding amounts of INO and LBSA in aqueous solution were also calculated.

RESULTS AND DISCUSSION

Characteristics of imprinted polymers microbeads

To evaluate morphology of resultant copolymers, samples were observed using SEM. Figure 2(a,b) show the SEM images of resultant imprinted polymer [P(DVB-*co*-MAA)$_I$] and nonimprinted polymer [P(DVB-*co*-MAA)]. The P(DVB-*co*-MAA) obtained (a) with and (b) without INO was in the range of 0.1–0.4 μm in diameter. To confirm the particle size distribution of the resultant beads, LSD was used. The data of LSD method for particle size distribution [Fig. 2(c,d)] confirmed the same results as the SEM pictures for both P(DVB-*co*-MAA)$_I$ and P(DVB-*co*-MAA). Therefore, the emulsion polymerization used under ultrasound condition caused the formation of the P(DVB-*co*-MAA) particles having submicrosize. We also measured the surface areas of P(DVB-*co*-MAA)$_I$ and P(DVB-*co*-MAA) by BET method, which were 242 m^2/g and 147 m^2/g, respectively. Also, to estimate MAA mole contents in the copolymers, MAA homopolymer and DVB were mixed at different weights and then the mixture was measured the IR spectra. From the obtained spectra, the MAA contents (x_{MAA}) in P(DVB-*co*-MAA)$_I$ and P(DVB-*co*-MAA) were estimated to be 10.3 mol % and 10.1 mol %, respectively.

Binding behavior of INO and LBSA into the imprinted polymers

It is known that one of the characteristics of imprinted polymers is the selective binding of template molecules. That means that imprint polymers become synthetic receptor materials having selective guest recognition.[1-4] In the present study, the binding amounts of INO and other substrates were measured. Since the P(DVB-*co*-MAA) contained COOH groups, which show acid–base equilibrium, the bulk pH values were adjusted at 3, 6, and 10 to control the dissociation condition of COOH groups. The binding amounts of INO and LBSA to the copolymers are listed in Table I. The data shows that the P(DVB-*co*-MAA)$_I$ is highly bound to INO at pH 3 and 6. The binding amount of INO being 3.7 μmol/g at pH 3 was somewhat higher than that of 2.1 μmol/g, when bulk pH was changed from pH 3 to 6. However, no binding was observed at pH 10. For P(DVB-*co*-MAA), the binding amounts decreased similarly, as the bulk pH changed from pH 3 to 10. This suggested that such copolymer containing both DVB and MAA segments showed a high binding ability to the INO at pH 3. It is apparent that the inosine substrate has a possibility to interact with the MAA segments at pH 3 by hydrogen bonds. It is better to consider that the bulk pH changed ability is due to hydrogen bonding between the COOH and INO, since the dissociation of the carboxylic acid groups of the MAA segments decreased in higher pH. In addition, INO shows different absorption peaks at pH 3 and 10 in the absorption spectra. This indicated that the template molecules behaved different due to the chemical structures at pH 3 and 10. Namely, the condition of pH 10 was quietly inconvenient for high binding of INO into the imprinted sites via hydrogen bonds.

To compare binding experiments for other substrates, the resultant values of the binding amounts of hypoxanthine and inosinic acid at pH 3 were 1.6 μmol/g and 0.5 μmol/g for P(DVB-*co*-MAA)$_I$, respectively. The binding amounts of the hypoxanthine were somewhat higher than the inosinic acid, indicating that the phosphoric acid segments of inosinic acid interfered in the binding of the imprinted sites by electrostatic repulsion at certain pH values. However, the values of hypoxanthine showed low binding relative to that of INO. This suggested that the molecule recognition occurred by hydrogen

TABLE I
Binding amounts of P(DVB-*co*-MAA)$_I$ and P(DVB-*co*-MAA) for Each substrates at Various pHs

Polymer	pH	Binding amounts [μmol/g]			
		INO	Hypoxanthine	Inosinic acid	LBSA
P(DVB-*co*-MAA)$_I$	3	3.7 (0.2)[a]	1.6 (0.3)	0.5 (0.1)	23 (1.8)
	6	2.1 (0.2)	1.3 (0.1)	0 (0)	1.3 (0.3)
	10	0 (0)	0 (0)	0 (0)	4.8 (1.0)
P(DVB-*co*-MAA)	3	1.0 (0.2)	0.7 (0.2)	0.6 (0.3)	23 (1.5)
	6	0.6 (0.2)	0.6 (0.1)	0 (0)	0.8 (0.3)
	10	0 (0)	0 (0)	0 (0)	5.3 (0.6)

[a] Standard deviation σ.

TABLE II
Equilibrium Constant K and Maximum Binding amounts B_{max} of P(DVB-*co*-MAA)$_I$ and P(DVB-*co*-MAA) for Each substrates at Various pHs

Polymer	Substrate	pH	$K_1/10^4$ (M^{-1})	B_{max1} (μmol/g)	$K_2/10^4$ (M^{-1})	B_{max2} (μmol/g)
P(DVB-*co*-MAA)$_I$	INO	3	7.2 (1.00)[a]	6.9	2.4 (0.99)	13
		6	5.9 (0.88)	4.0	2.0 (0.96)	8.0
	Hypoxanthine	3	5.4 (0.98)	3.2	1.6 (0.9)	7.0
		6	5.1 (0.92)	2.9	1.3 (0.88)	6.7
	Inosinic acid	3	–	–	3.4 (0.88)	1.2
P(DVB-*co*-MAA)	INO	3	–	–	1.6 (0.87)	5.6
		6	–	–	1.4 (0.88)	3.6
	Hypoxanthine	3	–	–	1.5 (0.8)	3.9
		6	–	–	1.4 (0.87)	3.7
	Inosinic acid	3	–	–	3.1 (0.85)	1.9

[a] Correlation coefficient of straight line.

bonding between MAA and hypoxanthine framework structure. Inosinic acid hardly bound to both copolymers, especially at pH 3. This was due to electrostatic repulsion between the phosphoric acid and the partially dissociated carboxylic acid of MAA in the polymer.

In addition to INO and other substrates, we observed binding amounts of LBSA to both imprinted and nonimprinted polymers. As listed in the Table I, it was noted that the binding of LBSA was strongly dependent on the pH value. For example, the value of LBSA was 23 μmol/g at pH 3, 1.3 μmol/g at pH 6 and 4.8 μmol/g for the imprinted P(DVB-*co*-MAA)$_I$. This implied that the dissociated MAA segments strongly contributed to the LBSA binding due to the electrostatic repulsion to the negatively charged LBSA. That is, the negatively charged MAA segments reduced the binding of LBSA at pH 10 by electrostatic repulsion. A comparison made between both imprinted and nonimprinted polymers for LBSA binding showed that the value of the LBSA binding, 23 μmol/g, was significantly higher than that of 3.7 μmol/g for INO at pH 3. Since the DVB and MAA monomers were emulsified with LBSA in water medium, these copolymers crosslinked by DVB also included LBSA. During the extraction process, the included LBSA was removed from the crosslinked polymer. As a result, the surfactant was imprinted in the crosslinked copolymer. So, the LBSA binding strongly suggested that the resultant copolymers contained the LBSA molecule volumetric space as shown by the imprinting effect. Even though there was no significant difference in the value of LBSA binding for P(DVB-*co*-MAA)$_I$ and P(DVB-*co*-MAA), the high value of LBSA binding was caused by the imprinting effect.

To furthermore obtain information, the binding kinetic parameter of the substrates was evaluated according to Scatchard analysis.[8,19] The Scatchard equation is as follows

$$B/[S] = (B_{max} - B)/K, \quad (1)$$

where B was binding amounts of various substrates to the polymer, B_{max} the apparent maximum number of binding sites, [S] substrate concentration and K equilibrium constant. In the present study, the adsorption isotherms of INO to the copolymers at various pH values were plotted. Then, Scatchard plots of [B] vs. [B]/[S] were converted from their adsorption isotherms. In the resultant plots, two straight line regions were obtained, except for those in the P(DVB-*co*-MAA) case. This meant that there were binding sites with specific and nonspecific recognition for INO in the P(DVB-*co*-MAA)$_I$ samples. From the left side straight line, specific binding equilibrium constant (K_1) and maximum

TABLE III
Binding amounts of P(DVB-*co*-MAA)$_I$ and P(DVB-*co*-MAA) for Each Substrates at Various pHs in the Presence of LBSA

				Binding amounts (μmol/g)			
Polymer	pH	INO	LBSA	Hypoxanthine	LBSA	Inosinic acid	LBSA
P(DVB-*co*-MAA)$_I$	3	3.8 (0.1)[a]	40 (2.8)	1.2 (0.3)	16 (1.8)	0.4 (0.1)	17 (0.6)
	6	0.5 (0.1)	0.4 (0.2)	–	–	–	–
	10	0.1 (0.1)	2.0 (0.4)	–	–	–	–
P(DVB-*co*-MAA)	3	1.1 (0.3)	16 (1.0)	0.5 (0.1)	17 (0.9)	0.3 (0.1)	17 (1.1)
	6	0.4 (0.1)	0.4 (0.1)	–	–	–	–
	10	0 (0)	2.5 (0.8)	–	–	–	–

[a] Standard deviation σ.

TABLE IV
Equilibrium Constant *K* and Maximum Binding amounts B_{max} of P(DVB-*co*-MAA)$_I$ and P(DVB-*co*-MAA) for Each substrates at Various pHs in the Presence of LBSA

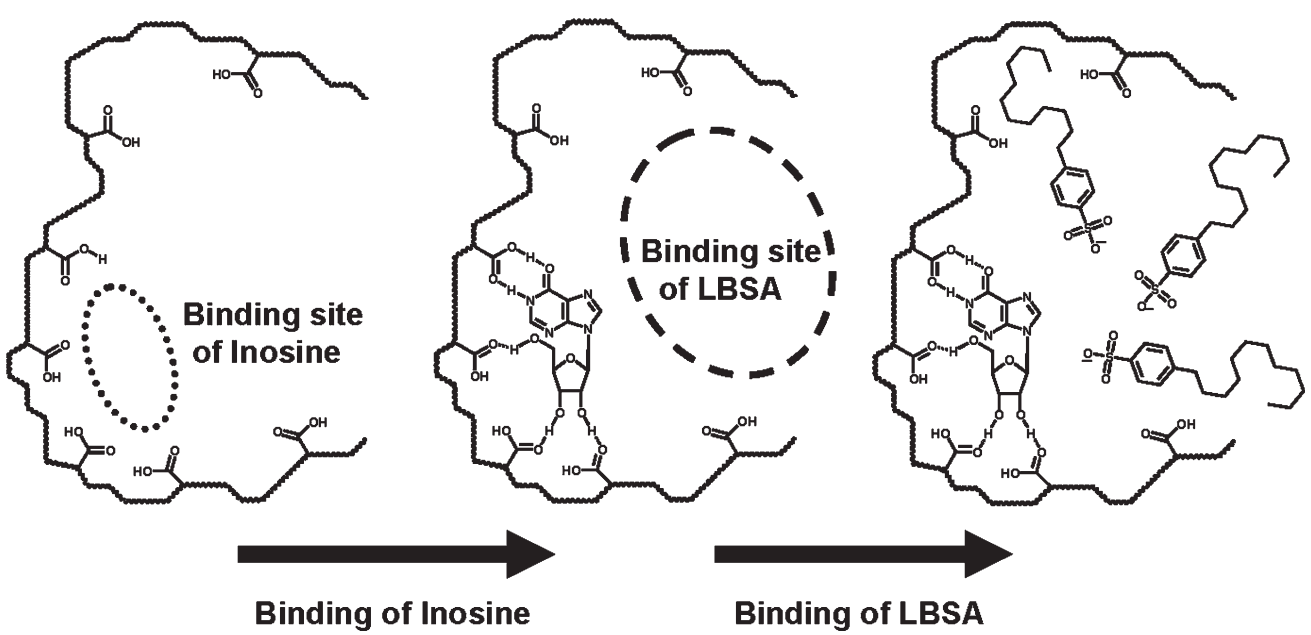
Polymer	Substrate	pH	$K_1/10^4$ (M^{-1})	B_{max1} (μmol/g)	$K_2/10^4$ (M^{-1})	B_{max2} (μmol/g)
P(DVB-*co*-MAA)$_I$	INO	3	7.2 (1.00)[a]	6.9	1.7 (0.99)	16
	Hypoxanthine	3	4.5 (0.84)	2.7	1.3 (0.94)	6.4
	Inosinic acid	3	–	–	3.0 (0.78)	1.1
P(DVB-*co*-MAA)	INO	3	–	–	2.1 (0.83)	4.2
	Hypoxanthine	3	–	–	1.7 (0.92)	2.4
	Inosinic acid	3	–	–	2.5 (0.65)	1.3

[a] Correlation coefficient of straight line.

binding amounts (B_{max1}) was estimated (Table II). The data showed that the P(DVB-*co*-MAA)$_I$ had high affinity to INO with $K_1 = 7.1 \times 10^4$ M^{-1} and $B_{max1} = 6.9$ μmol/g. This was due to the imprinting effect. Hypoxanthine was also bound to P(DVB-*co*-MAA)$_I$ with $K_1 = 5.4 \times 10^4$ M^{-1} and $B_{max1} = 3.2$ μmol/g. Also, the values of nonspecific binding equilibrium constant (K_2) and maximum binding amounts (B_{max2}) estimated from the right side region of the scatchard plots for INO-P(DVB-*co*-MAA)$_I$ were 2.4×10^4 M^{-1} and 13 μmol/g for the INO solution. This suggested that the INO binding to imprinting sites was mainly caused by the formation of hydrogen bonds between the hypoxanthine ring and the carboxylic group of MAA segments. However, our data suggested that the sugar segments of INO also played an important role in the INO binding.

Template supported cooperative binding of LBSA

Besides the experimental data, there is the possibility for the imprinted polymer to memorized both INO and LBSA. It was of interest to carry out binding experiments using a mixture of both INO and LBSA. The INO binding experiments were carried out in the presence of LBSA at pH 3, 6, and 10. Herein, 0.01 g of the copolymer was taken inside dialysis membrane tube with 2 mL water. After the both side of the tube was closed, the tube involved the copolymer was put in a 18 mL aqueous solution containing 20 μ*M*-INO and 20 μ*M*-LBSA. Then, the solution was equilibriumed for 24 h. The absorption spectra of the aqueous solution at the outside of the tube were measured. Table III shows the binding amounts of INO and LBSA at pH 3, 6, and 10 in the presence of LBSA. The resulting binding amount of INO was 3.8 μmol/g at pH 3. INO bound similarly as shown in Table I. However, it was noted that (DVB-*co*-MAA)$_I$ exhibited high binding to LBSA with 40 μmol/g. When the solution contained both INO and LBSA, the value of the binding amounts of LBSA for the mixed solution was two times higher compared to that of only LBSA in the solution. Because the binding amounts of LBSA to P(DVB-*co*-MAA)$_I$ at pH 3 increased in the presence of INO, it played important role in binding LBSA. The data in



Scheme 1 Binding mechanism of INO and LBSA to the MIPs.

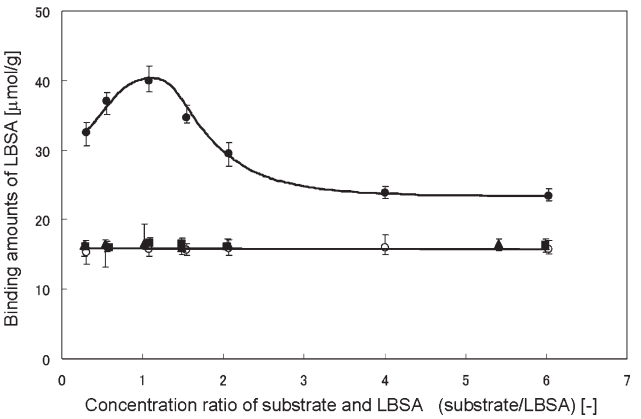


Figure 3 Binding amounts of LBSA to the copolymers in various mixture solutions of substrates and LBSA. (●) P(DVB-*co*-MAA)Ib in INO + LBSA solution, (○) P(DVB-*co*-MAA)Ib in INO + LBSA solution, (■) P(DVB-*co*-MAA)Ib in hypoxanthine + LBSA solution, (▲) P(DVB-*co*-MAA)Ib in inosinic acid + LBSA solution.

Table IV is shown to compare the effect of INO in the LBSA binding to the P(DVB-*co*-MAA)$_I$. The values of K_1 and B_{max1} for INO and hypoxanthine when the solution contained LBSA were 7.2×10^4 M^{-1}, B_{max1}= 6.7 μmol/g and 4.5×10^4 M^{-1}, B_{max1}= 2.7 μmol/g, respectively. The B_{max1} for hypoxanthine to P(DVB-*co*-MAA)$_I$ decreased as long as LBSA was present. These results suggested that the LBSA interfered in the binding of hypoxanthine to the P(DVB-*co*-MAA)$_I$. In contrast, the INO binding behaved almost similar to the cases of binding with and without LBSA. But, the binding of hypoxanthine was different from that of the INO system. This meant that the LBSA binding of INO and LBSA was cooperatively performed in the presence of INO. As illustrated in Scheme 1, INO supported the binding of the LBSA to the imprinted sites. To reveal the cooperative binding, the binding amounts of LBSA were measured using P(DVB-*co*-MAA)$_I$ and P(DVB-*co*-MAA) in a pH 3 solution having different concentrations of INO and LBSA. Figure 3 shows the binding amounts of LBSA measured at different ratio of substrate/LBSA. The ratio values of hypoxanthine/LBSA and inosinic acid/LBSA were similarly altered in the range of 0.25–6 in the binding solution for the P(DVB-*co*-MAA)$_I$. When the concentration ratio of INO and LBSA was about 1.2, the LBSA was absorbed in the copolymer with a binding amount of about 40 μmol/g. This value for the P(DVB-*co*-MAA)$_I$ was higher than that of about 16 μmol/g by changing the ratio of INO/LBSA in the P(DVB-*co*-MAA). However, other substrates had no effect on LBSA binding in the INO imprinted copolymer.

In conclusion, INO showed to have an important role in the LBSA binding of the P(DVB-*co*-MAA)$_I$. This was due to that the P(DVB-*co*-MAA)$_I$ imprinted with both INO and LBSA and acted with a cooperative binding in the imprinted copolymer.

CONCLUSION

By using emulsion polymerization of MAA and DVB in the presence of LBSA, INO imprinted polymers were obtained under ultrasound exposure conditions. The resultant copolymer beads, having 0.1–0.4 μm diameter, showed that the INO was able to bound significantly with a dependency of bulk pH. Namely, the imprinted polymer had higher binding amounts at pH 3 with a value of 3.7 μmol/g and less binding at pH 10 with a volue of 0 μmol/g. When LBSA was present in the INO solution, it was noted that the imprinted polymers exhibited a cooperative binding effect. The presence of INO enhanced the binding of LBSA to the imprinted polymer. That is, the LBSA emulsion polymerization also imprinted LBSA in the resulting crosslinked copolymer.

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