Uniform-Sized Molecularly Imprinted Polymer Material for Propranolol. Recognition of Propranolol and Its Metabolites

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The molecularly imprinted polymers have considerable molecular recognition ability, and can be used as separation media and antibody mimics.¹ Usually, non-aqueous bulk polymerization techniques² are utilized to obtain molecularly imprinted polymers, which are followed by smashing, sieving and classifying of the block polymer to utilize for separation media or antibody mimics.³ Though suspension polymerization methods, which require an aqueous suspension medium, can produce spherical polymer beads, water is thought to weaken the interaction between the template and functional monomers.⁴ Recently, we prepared uniform-sized molecularly imprinted polymer materials for isomers of diaminonaphthalene⁵, a chiral amide derived from (*S*)- α -methylbenzylamine⁶ and (*S*)-naproxen⁷, where a typical multi-step swelling and polymerization method⁸ with water as the suspension medium was used. However, the prepared stationary phases showed an equivalent molecular recognition ability to those prepared by non-aqueous bulk polymerization methods.^{9,10}

Previously, molecularly imprinted polymers for propranolol and β -adrenergic antagonists (β -blockers)¹¹⁻¹⁷ were prepared through a bulk polymerization method, and used for chiral stationary phases in HPLC¹¹, antibody mimics in radioligand binding assay^{12,13}, solid phase extraction¹⁴ and chiral selectors in capillary electrochromatography.¹⁵⁻¹⁷ On these molecularly imprinted polymer materials, evaluation was performed only for the imprinted molecule. In this study, we prepared uniform-sized molecularly imprinted polymer materials for racemic propranolol and (*S*)-propranolol by a multi-step swelling and thermal polymerization method and evaluated selectivity of the obtained molecularly imprinted polymer materials for propranolol and its metabolites by using aqueous-rich mobile phases.

Experimental

Materials

Ethylene dimethacrylate (EDMA) and methacrylic acid were purchased from Tokyo Chemical Industry (Tokyo, Japan) and Wako Pure Chemical Industry (Osaka, Japan), respectively. Both monomers were purified by general distillation techniques *in vacuo* to remove the polymerization inhibitor. 2,2'-Azobis(2,4-dimethylvaleronitrile) were purchased from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industry (Tokyo, Japan), respectively, and used without further purification. (*S*)-(-)-Propranolol hydrochloride and racemic propranolol hydrochloride were purchased from Tokyo Chemical Industry (Tokyo, Japan). 4-Hydroxypropranolol hydrochloride was obtained from Sumitomo Chemical Co. (Osaka, Japan). *N*-Desisopropylpropranolol hydrochloride was purchased from the ICI Pharmaceuticals Co. (Macclesfield, UK). 5-Hydroxy- and 7-hydroxypropranolols were synthesized as hydrochlorides according to the method of Oatis *et al.*¹⁸ The structures of propranolol and its metabolites, 4-hydroxy-, 5-hydroxy- and 7-hydroxy- and *N*-desisopropylpropranolols, are illustrated in Fig. 1. Other reagents and solvents were used without further purification.

Water purified with a Nanopure II unit (Barnstead, Boston, MA, USA) was used for the preparation of the eluent and the sample solution.

Multi-step swelling and polymerization method

Uniformly sized, polystyrene seed particles utilized as the shape template were prepared by an emulsifier-free emulsion polymerization and purified by a previously reported method.¹⁹ The size of the seed particle was *ca.* 1 μm in diameter. Preparation of uniformly sized, macroporous, molecularly imprinted polymer beads as well as non-imprinted polymer beads by a

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Fig. 1 Structures of propranolol and its metabolites used in this study.

multi-step swelling and polymerization method was carried out as follows. A water dispersion of the uniformly sized, polystyrene seed particles (0.497 g ml^{-1}), 0.17 ml , was admixed with a micro-emulsion prepared from 0.48 ml of dibutyl phthalate as activating solvent²⁰, 0.02 g of sodium dodecyl sulfate and 5 ml of distilled water by sonication. This first-step swelling was carried out at room temperature for 15 h with stirring at 125 rpm until micro oil droplets were completely disappeared. To the swollen particles, a micro-emulsion prepared from 0.375 g of 2,2'-azobis(2,4-dimethylvaleronitrile), 5 ml of toluene, 12.5 ml of water and 10 ml of 4.8% polyvinyl alcohol solution was added. This second-step swelling was carried out at room temperature for 2 h with stirring at 125 rpm . To the dispersion of swollen particles, a dispersion of 5 ml of EDMA, 7.0 mmol (*ca.* 0.60 g) of methacrylic acid, 0.02 g of sodium dodecyl sulfate, 12.5 ml of water and 10 ml of 4.8% polyvinyl alcohol solution was added. This third-step swelling was carried out at room temperature for 2 h with stirring at 125 rpm . When the template molecule was added, 2.0 mmol (0.59 g) of (*S*)-propranolol hydrochloride or racemic propranolol hydrochloride was admixed with the monomers utilized to prepare the dispersion for the third-step swelling. After the third-step swelling was completed, the polymerization procedure was started at 50°C under argon atmosphere with slow stirring for 24 h . The dispersion of polymerized beads was poured into 250 ml of water to remove the suspension stabilizer (polyvinyl alcohol), and the supernatant was discarded after sedimentation of the beads. The polymer beads were redispersed into methanol, and the supernatant was again discarded after sedimentation. This procedure was repeated three times in methanol and twice in tetrahydrofuran (THF), then the polymer beads were filtered on a membrane filter and washed with THF and acetone followed by drying at room temperature.

The prepared beads were packed into a stainless-steel column ($4.6 \text{ mm i.d.} \times 100 \text{ mm}$) by a slurry technique using methanol as the slurry medium to evaluate their chromatographic characteristics.

Chromatography

The HPLC system used was composed of an LC-9A pump, an SPD-6A spectrophotometer, a Rheodyne 7125 injector with a $20\text{-}\mu\text{l}$ loop, a C-R6A integrator (all from Shimadzu, Kyoto, Japan). The flow-rate was maintained at 1.0 ml min^{-1} . Fluorometric detection was performed with excitation and emission wavelengths at 285 and 340 nm for propranolol and at 310 and 380 nm for its metabolites. All separations were carried out at 25°C using a water bath (Thermo Minder Lt-100, Taitec Co., Saitama, Japan). The eluents are prepared by using phosphoric acid, sodium dihydrogenphosphate, disodium hydrogenphosphate and acetonitrile. The eluent used was specified in the legends of tables and figures.

Results and Discussion

The molecularly imprinted polymer materials were prepared by a multi-step swelling and thermal polymerization technique with water as a suspension medium. EDMA and methacrylic acid were used as cross-linker and host monomers, respectively, and the obtained molecularly imprinted polymer was evaluated by using an aqueous-rich mobile phase. Table 1 shows the capacity factors of racemic propranolol and its metabolites, 4-hydroxy-, 5-hydroxy- and 7-hydroxy- and *N*-desisopropylpropranolols, on the uniform-sized molecularly imprinted polymer material for racemic propranolol and the non-imprinted one, where selectivity is represented by the ratio of the capacity factors of the racemate on molecularly imprinted and non-imprinted poly-

Table 1 Capacity factors of propranolol and its metabolites on the uniform-sized molecularly imprinted polymer material for racemic propranolol and the non-imprinted one^a

	Molecularly imprinted polymer for racemic propranolol k'^b	Non-imprinted polymer k'	Selec- tivity ^c
Propranolol	31.8	9.25	3.44
4-Hydroxypropranolol	11.1	4.79	2.32
5-Hydroxypropranolol	12.4	5.01	2.47
7-Hydroxypropranolol	11.3	5.51	2.06
<i>N</i> -Desisopropylpropranolol	10.2	7.31	1.39

a. HPLC conditions: mobile phase, 20 mM phosphate buffer (pH 5.1)/CH₃CN=50/50(v/v); flow-rate, 1.0 ml min⁻¹; loaded amount, 50 ng.

b. k' is a capacity factor.

c. Selectivity is represented by $k'(\text{imprinted})/k'(\text{non-imprinted})$.

mer materials, $k'(\text{imprinted})/k'(\text{non-imprinted})$. Since hydrophobic and electrostatic interactions played an important role in the retentivity of propranolol and its metabolites⁷, the metabolites of propranolol were less retained on the obtained molecularly imprinted polymer than propranolol. The obtained polymer materials showed high selectivity for propranolol and moderate selectivity for its metabolites. Although the highest selectivity was obtained with 5-hydroxypropranolol

among the hydroxypropranolols tested, there were not so large differences in the selectivity. However, the selectivity for *N*-desisopropylpropranolol was lower than those for hydroxypropranolols. This result suggests that the interaction of the *N*-isopropyl group with the molecularly imprinted polymer plays an important role in recognition of propranolol and its metabolites. On the other hand, the molecularly imprinted material showed little selectivity for other basic compounds such as chlorpheniramine and tolperisone, acidic compounds such as ibuprofen and aspirin, and neutral compounds such as benzene and benzophenone.

Table 2 shows the capacity factor, enantioseparation factor and resolution of propranolol and its metabolites on the uniform-sized molecularly imprinted polymer material for (*S*)-propranolol and the non-imprinted one, where selectivity is represented by the ratio of the capacity factors of (*S*)-enantiomer on the molecularly imprinted and non-imprinted polymer materials, $k'_S(\text{imprinted})/k'_S(\text{non-imprinted})$. As shown in Table 2, enantioseparation of *N*-desisopropylpropranolol was not attained, but enantioseparations of propranolol and hydroxypropranolols were attained. This might be due to that the *N*-isopropyl group could play an important role in chiral recognition of propranolol and its metabolites, as described above.

Table 3 shows the capacity of the molecular imprinted polymer for (*S*)-propranolol and the non-imprinted

Table 2 Capacity factor, enantioseparation factor, resolution and selectivity of propranolol and its metabolites on the uniform-sized molecularly imprinted polymer material for (*S*)-propranolol and the non-imprinted one^a

	Molecularly imprinted polymer for (<i>S</i>)-propranolol			Non-imprinted polymer	Selectivity ^c
	$k'_S{}^b$	α^c	R_s^d	k'	
Propranolol	75.6	2.97	0.77	9.25	8.18
4-Hydroxypropranolol	17.3	2.42	0.53	4.79	3.60
5-Hydroxypropranolol	21.4	2.29	0.56	5.01	4.27
7-Hydroxypropranolol	19.3	1.81	0.48	5.51	3.50
<i>N</i> -Desisopropylpropranolol	14.0	1.00	—	7.31	1.92

a. HPLC conditions as in Table 1. b. k'_S is the capacity factor of (*S*)-enantiomer. c. α is an enantioseparation factor. d. R_s is resolution.

e. Selectivity is represented by $k'(\text{imprinted})/k'(\text{non-imprinted})$.

Table 3 Capacity of the uniform-sized molecularly imprinted polymer material for (*S*)-propranolol and the non-imprinted one^a

Loaded amount of propranolol/ng	Molecularly imprinted polymer for (<i>S</i>)-propranolol			Non-imprinted polymer	Selectivity ^c
	$k'_S{}^b$	k'_R	α	k'	
50	25.5	75.6	2.97	9.25	8.18
100	23.7	66.7	2.82	9.18	7.26
200	22.5	58.4	2.60	9.17	6.37
400	20.8	46.0	2.21	9.12	5.05
1000	18.4	38.0	2.06	9.02	4.22

a. HPLC conditions as in Table 1. b. k'_R is the capacity factor of (*R*)-enantiomer. c. Selectivity is represented by $k'_S(\text{imprinted})/k'_S(\text{non-imprinted})$.

one. With an increase in the loaded amount of racemic propranolol hydrochloride from 50 ng to 1000 ng, the capacity factor of (*S*)-propranolol was drastically decreased, while a slight decrease in that of (*R*)-propranolol was observed. On the other hand, a decrease in the capacity factor of propranolol on the non-imprinted polymer material was negligible. This means that the limited number of chiral recognition sites is present on the molecularly imprinted polymers. However, it is thought that the capacity of the obtained molecularly imprinted polymer is sufficient enough for the purpose of trapping of propranolol and its metabolites in biological fluids. Because the plasma levels of propranolol and its metabolites are low after administration of propranolol.²¹

In conclusion, the results obtained above indicate that the molecularly imprinted polymers prepared show high selectivity for propranolol and moderate selectivity for propranolol metabolites. This means that the obtained molecularly imprinted polymer material could be used for selective trapping of propranolol and its metabolites in biological fluids. Further study is in progress in our laboratory.

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