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Making High Selective Molecule Imprinting Polymer (MIP) In Polar Solvent

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Abstract: Acrylamide was used as functional monomer to make high chiral selective molecule imprinting polymers against N^{α}-protected amino acid in polar solvent. The factor, which influence the efficiency of the polymer and the mechanism of chiral recognition were investigated.

Keywords: Molecule imprinting; chiral separation; stationary phase; amino acid.

Introduction

Molecule imprinting technology has made great progress in making chiral stationary phase with predetermined chiral selectivity against enantiomers such as amino acid and their derivatives; sugar and their derivatives; naproxen and methylbenzylamine[1-5]. Functional monomer and solvent commonly used in making MIP were methacrylic acid (MAA) and chloroform, but excess MAA with charge showed strong nonspecific interaction with print molecule, the hydrogen bonding ability of MAA with print molecule decreased in polar solvent such as acetonitrile. In order to improve the selectivity of MIP, "cocktail" polymerization using combined monomer MAA and 2-vinylpyridine (2-VP) was developed[6], but the strong ionic interaction between MAA and 2-VP would decrease the interaction between monomers and the print molecule, the efficiency of this system is not so high as expected. Now using acrylamide (AM) for hydrogen bonding leading to highly efficient polymer was reported[7], we believe that the high efficiency of this system is that AM shows strong "functional group complimentary" to the carboxy group of N^{α}-protected amino acid, so the interaction between the amide group on polymer and the print molecule are more specific. A series of MIPs using acrylamide against N^{α}-protected amino acids were synthesized, factors which control the selectivity of the polymer and the mechanism in chiral recognition were investigated.

Experiment

Materials

Ethylene glycol dimethacrylate (EGDMA) and trimethylolpropane trimethacrylate (TRIM) were purchased from Shanhu Chemical Plant (Shanghai, China); azo-bisisonitrile (AIBN) and acrylamide from Beijing Chemical Plant (Beijing, China); all amino acid

derivatives from Sigma (ST.Louis, MO); inhibitors in crosslinker and monomer were removed by active carbon; all solvents were of HPLC or analytical grade.

Preparation of MIPs

MIPs were prepared using photopolymerization, initiated by using 366nm UV lamp at 4 ℃ for 48 hr, the compositions of MIPs are shown in **Table 1**. The resulting polymers were ground, sieved and sedimented, particles less than 35 μm were collected and used as chiral stationary phase in HPLC.

High Performance Liquid Chromatography

LC-890A system from Beijing Xingda Technology Development Company consisted of two LC-05C pumps, a LC-830 UV-VIS detector (Soma optic LTD, Japan) was used to monitor the eluent, the system was controlled by a JS-3030 chromatographic operation station. MIP particles were slurried and packed using acetonitrile/water/acetic acid (50/45/5) into 4×250 mm columns at 30 MPa. Samples containing 40μg racemate in 20 μl volume were injected. The chromatographic results on MIP-CSPs are shown in **Table 2**.

Results and Discussions

Commonly the polymerization solvent was used as mobile phase in HPLC to simulate the chemical environment in which the print molecule and monomer can form stable complex. In pure polymerization solvent, highest selectivity and resolution were achieved, but enantiomers were eluted very slowly or even absorbed on the polymer, so modifiers such as acetic acid, water or methanol were required. When these modifiers were added into the mobile phase, chromatogram shapes were improved and analysis time reduced, but the selectivity and resolution decreased greatly, especially for acetic acid. For example, N-t-Boc-DL-Trp can be discriminated on polymer A with selectivity factor 2.37 and resolution factor 0.82 when 100% acetonitrile was used as the mobile phase; when 1% (v/v) water was added into the acetonitrile, selectivity and resolution decreased to 2.01 and 0.75 respectively. For polymer B, when acetic acid content in chloroform increased from 1% to 2.5%, the selectivity and resolution decreased from 2.83, 0.95 to 1.95 and 0.84 respectively. The modifiers can compete with enantiomers to form hydrogen bonds with the recognition sites, so the efficiency of the polymer decreased.

Polymer B shows high efficiency than polymer A, because the hydrogen bond between the print molecule and AM is more stable in less polar chloroform. But the hydrogen terms for the solubility parameters of acetonitrile and chloroform are 6.1 and 5.7 respectively[9], they have no distinct difference. AM can form strong hydrogen bond with print molecule too, and the solvent action of acetonitrile is higher than chloroform, most of amino acid derivatives are soluble in acetonitrile but not in chloroform, so we prefer to use acetonitrile in the printing process.

Table 1. Composition of MIPs

MIP	Imprinting molecule**	The ratio of imprinting molecule and functional monomer	Solvent
A	N-t-Boc-L-Trp	1:4	acetonitrile
B	N-t-Boc-L-Trp	1:4	chloroform
C	N-t-Boc-L-Trp	1:8	acetonitrile
D*	N-t-Boc-L-Trp	1:8	acetonitrile
E	N-Cbz-L-Phe	1:4	acetonitrile
F	N-Boc-L-Val	1:4	acetonitrile
G	N-t-Boc-L-Tyr	1:4	acetonitrile
H	N-Cbz-L-Trp	1:4	acetonitrile
I	N-Cbz-L-Tyr	1:4	acetonitrile
J	N-t-Boc-L-Ala	1:4	acetonitrile
K	N-Cbz-L-Ala	1:4	acetonitrile
L	N-Cbz-L-Ser	1:4	acetonitrile
M	N-t-Boc-L-Phe	1:4	acetonitrile

*Trimethylolpropane trimethacrylate (TRIM) was used as cross-linker
**Trp, Tyr, Phe, Ala, Ser, Val, Boc and Cbz are the abbreviation for tryptophan, tyrosine, phenylalanine, alanine, serine, valine, butoxy carbonyl and carbobenzyloxyl respectively.

Table 2. The chromatographic results of MIPs

MIP	Racemates	a	R*	Eluent
A	N-t-Boc-DL-Trp	2.37	0.82	acetonitrile
B	N-t-Boc-DL-Trp	2.83	0.84	chloroform/acetic acid, 99:1
C	N-t-Boc-DL-Trp	2.40	0.87	acetonitrile/acetic acid, 99:1
D	N-t-Boc-DL-Trp	2.05	0.84	acetonitrile/acetic acid, 99:1
E	N-Cbz-DL-Phe	1.85	0.90	acetonitrile/methanol, 99:1
F	N-Boc-DL-Val	1.65	0.24	acetonitrile/water, 99:1
G	N-t-Boc-DL-Tyr	2.54	1	acetonitrile/water, 99:1
H	N-Cbz-DL-Trp	2.92	1	acetonitrile/water, 99:1
I	N-Cbz-DL-Tyr	2.19	0.28	acetonitrile/water, 99:1
J	N-t-Boc-DL-Ala	1	**	acetonitrile/water, 99:1
K	N-Cbz-DL-Ala	1	**	acetonitrile/water, 99:1
L	N-Cbz-DL-Ser	1.26	0.1	acetonitrile/water, 99:1
M	N-t-Boc-DL-Phe	1.53	**	acetonitrile/water, 99:1

*Resolution factor R was calculated according to Ref. 8.
**The racemates were poorly or cannot be separated, R cannot be calculated.

The efficiency of polymer C is higher than that of polymer A, more functional monomers to print molecule leading to high selectivity. But polymer D crosslinked with TRIM shows similar efficiency to A.

To obtain stereospecific recognition, a “three point” interaction is necessary. If MIP was imprinted with N^{α}-protected amino acid using AM as functional monomer, the carboxyl and carbamide groups of print molecule could interact *via* hydrogen bond with the positioned amide group on the polymer, the third interaction should be the interaction between the side chain of amino acid with bonding sites. The side chains of Trp, Tyr and

Phe all contain a benzyl ring, the benzyl group can interact with amide group on the polymer *via* π-π interaction, the phenol group and pyrrole group of Tyr and Trp can offer hydrogen bonds with recognition sites, so polymers imprinted with these amino acid derivatives show high chiral selectivity. For Ala there is only a methyl group in the side chain which can not form hydrogen bond or π-π interaction with bonding sites, so no selectivity was observed on polymer J and polymer K. The side chain of Ser contains a hydroxymethyl group, the hydroxyl group can interact *via* hydrogen bond with bonding sites, so polymer L shows chiral selectivity to some degree. The moderate selectivity of polymer F may come from the N^{α}-protected group (butyloxy carbonyl group) of Val.

Conclusion

Acrylamide can be used in polar solvent to make high efficiency molecule imprinting polymer. The modifiers in the mobile phase influence the efficiency of the polymer greatly and the side chain of the amino acid derivatives is crucial for successful imprinting.

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