Transactions of the Materials Research Society of Japan 24 [3] 453-456 (1999)

Molecularly Imprinted Resin for Recognition of Amino Acids Using Self-Assembly at o/w Interface

Shigeo Nakamura, Hiroki Takeuchi, Hidehiko Nakamura, Mizuo Maeda and Makoto Takagi

Department of Chemical Systems and Engineering, Graduate School of Engineering, Kyushu University
Hakozaki, Higashi-ku, Fukuoka, 812-8581, Japan
Fax: 81-92-642-3603, e-mail: takagtcm@mbox.nc.kyushu-u.ac.jp

Molecular imprinting technique gives a method of synthesizing host polymers for binding target molecules. The concept of molecular imprinting is as follows: A target (guest) molecule is incorporated into polymerizable compounds (host monomers), and the complex is then polymerized to give a resin. Upon washing out the target molecules, the cavities remaining within the polymer matrix keep the memory about the shape and the chemical properties of the target molecule. We are developing a new technique for forming template on the surface of resin. By this surface imprinting method, we prepared a resin that recognizes the species of amino acids. L-Phe-imprinted resin was synthesized by polymerization of the o/w emulsion from potassium oleate and divinylbenzene in water in the presence of L-Phe. This resin had a higher adsorptive activity of L-Phe than non-imprinted resin. On the other hand, L-Phe imprinted resin can absorb L-Val less effectively than non-imprinted resin. It was suggested that the specific binding site for L-Phe was formed on the surface of L-Phe imprinted resin. Our method should be available for preparing materials that have specific affinities to various kinds of compounds.

Key words: molecular imprinting, surface template polymerization, self-assembly, molecular recognition, amino acids

1. INTRODUCTION

Fields relating to design and synthesis of artificial host molecules have been developed in recent years. Molecular recognition plays an important role for the development. Molecular imprinting is got much attention as a method for preparing artificial host polymers[1-3], in contrast with supramolecular chemistry using precision organic synthesis. Molecular imprinting was proposed about 20 years ago and this is a method of obtaining a imprinted host by polymerizing vinyl monomers assembled around the guest molecule.

The concept of molecular imprinting is as follows. 1) Forming a complex of guest molecules and functional monomers that can interact with the guest. 2) Polymerizing the complex with cross-linker and immobilizing the environment around the guest. 3) After washing out the guest molecules, it remains holes that memorize the shape and the chemical property of the guest. Molecular imprinting is a method that can easily get functional materials that possess an ability of molecular recognition in large amount and low cost.

However the conventional methods (Fig. 1a) of molecular imprinting have some week points. The rate of host-guest complexation is slow because the recognition sites form inside of the polymer. It is difficult of the application to biochemical compounds that are mostly water-soluble because the polymerization is carried out in homogenous organic solvent system.

We have developed the novel method of molecular imprinting named "surface imprinting"[4] (Fig. 1b). This method is polymerizing the emulsion of guest molecules, cross-linker and functional surfactant that can complex with guest. The problems above should be solved by our surface imprinting.

We have already applied surface imprinting to prepare metal ion selective resins[5-7]. This result

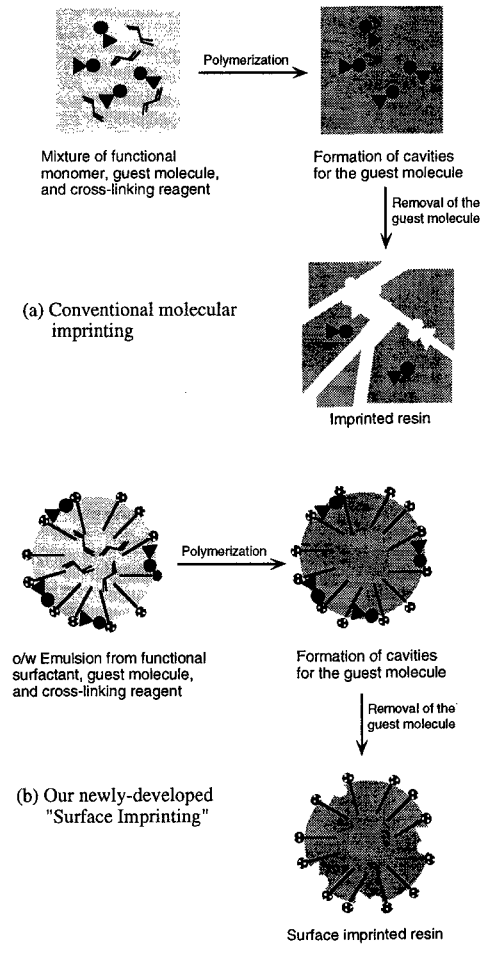


Fig. 1. Schematic Illustrations of the Conventional Molecular Imprinting and Our Surface Imprinting.

suggested that this novel method should be useful for not only inorganic ions but also water-soluble organic compounds. Amino acids are very important biochemical compounds, and Mosbach et al. have obtained the resin that recognize amino acid by conventional method of molecular imprinting[8-11]. In this study, we applied our new surface imprinting to amino acid derivatives as target molecules.

2. EXPERIMENTAL

2.1 Materials

Potassium oleate was purchased from Tokyo Chemical Industry Co., Ltd. Divinylbenzene was a generous gift from Nippon Steel Chemical Co., Ltd. and was used after treatment with silica gel to remove an inhibitor. 2,2'-Azobis(4-methoxy-2,4-dimethylvaleronitrile) (V-70) was purchased from Wako Pure Chemical Industries, Ltd. L-Phenylalanine hexyl ester was synthesized from L-Phe and 1-hexyl alcohol by use of p-toluenesulfonic acid in toluene. L-Phenylalanine methyl ester (L-PheOMe) and L-valine methyl ester (L-ValOMe) were purchased from Nacalai Tesque, Inc. 4-[4-(Dimethylamino)phenylazo]phenylisothiocyanate (DABITC) was purchased from Dojindo Laboratories. Reversed phase high performance liquid chromatography analysis was performed by Hitachi HPLC system of L-6200 intelligent pump and L-4200 UV-Vis detector.

2.2 Preparation of Resin

L-Phe-imprinted resin was synthesized as follows. The mixture of 1.65 g of potassium oleate as a functional monomer, 10 ml of divinylbenzene as a cross-linker and V-70 as a radical initiator was added into 80 ml of water. It was treated by a probe-type sonicator for 5 cycles of 20 sec sonication and 1 min interval at 0 °C and o/w emulsion was formed. A half-gram of L-phenylalanine hexyl ester as a template in 20 ml of water was added to the emulsion then the complexation occurred on the surface of micelle. Polymerization was carried out with stirring at 25 °C for 8 hr under nitrogen atmosphere. Diluted hydrochloric acid was added until pH 3 to aggregate the resin. The collected resin by filtration was washed 3 times with 200 ml of HCl (pH 3) containing 10% of dioxane to remove the template from the surface of the resin. Non-imprinted resin was synthesized similarly without addition of template.

2.3 Adsorption of Amino Acid Derivatives

One gram of imprinted or non-imprinted resin was dispersed in 45 ml of 5 mM phosphate buffer (pH 6.8) containing 0.1 M KCl and 5 ml of dioxane. Fixed amount of 46 mM L-PheOMe or L-ValOMe was dropped into the dispersion. Then pH was adjusted to 7.0 by adding the small amount of 0.1 M KOH. After 1 hr stirring 1 ml of the dispersion was withdrawn as a sample. The solution of amino acid derivative was added again and these operations were repeated. Sample solutions were treated by membrane filter (0.20 μm) to remove the resin. For HPLC analysis, 100 μl of filtered sample, 100 μl of *N,N*-dimethyl-*N*-allylamine solution (30 mM in water : 1-propanol = 1 : 1) and 400 μl of DABITC solution (5 mM in acetone) was mixed and incubated for 12 hr at 50 °C. Then the reaction mixtures were evaporated to dryness and were dissolved in 1 ml of ethanol. The ethanol solution was applied to HPLC analysis and the conditions were as follows. Eluent A was phosphate buffer (0.1 M, pH 6.6) : methanol : butanol = 76.5 : 23.5 : 0.5 and eluent B was methanol. Gradient was from eluent A : eluent B = 30 : 70 to 15 : 85 for 10 min and from 15 : 85 to 0 : 100 for 40 min. Flow rate was 0.5 ml / min, the injected amount of sample was 10 μl and detected wavelength was 418 nm.

3. RESULTS AND DISCUSSION

We have used some types of L-Phenylalanine alkyl esters as template molecules, and checked which gives the best result (data not shown). When the ester alkyl chain was too short the template would distribute in water phase, and when it was too long it would be difficult to wash out the template because it would be deeply buried inside of the polymer particles. From the preliminary investigation, L-Phenylalanine hexyl ester was chosen as a template molecule.

The amount of L-PheOMe absorbed on the surface of the resin was calculated from the amount in the filtrate. Figure 2 shows the absorption isotherms of L-PheOMe to L-Phe-imprinted or non-imprinted resin. L-Phe-imprinted resin absorbed L-PheOMe about twice as much as non-imprinted resin. This imprinted effect suggests that there exist specific binding sites for L-Phe on the surface of the imprinted resin.

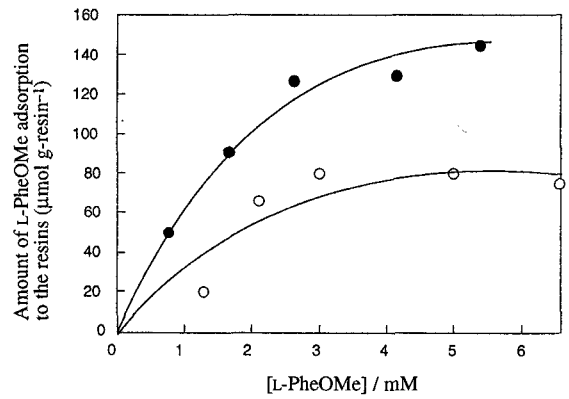


Fig. 2. Adsorption isotherm of L-PheOMe to the resins. ●, L-Phe-imprinted resin; ○, non-imprinted resin.

L-ValOMe was chosen as a control of L-PheOMe in order to know whether the imprinted effect was specific to the amino acid residue. Figure 3 shows the absorption isotherms of L-ValOMe to L-Phe-imprinted or non-imprinted resin. In contrast to the case of L-PheOMe, the amount of absorption to non-imprinted resin was almost double that of L-Phe-imprinted resin. This result shows that the absorption to L-Phe-imprinted resin is selective to L-Phe.

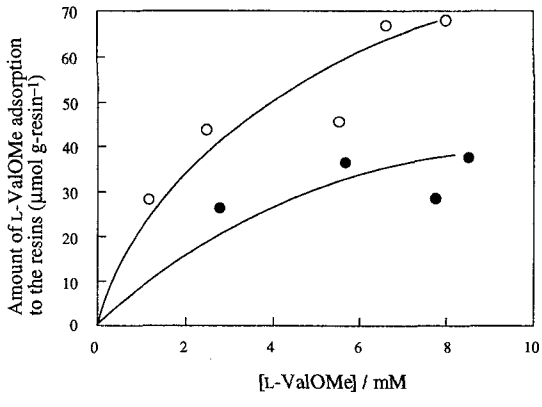


Fig. 3. Adsorption isotherm of L-ValOMe to the resins. ●, L-Phe-imprinted resin; ○, non-imprinted resin.

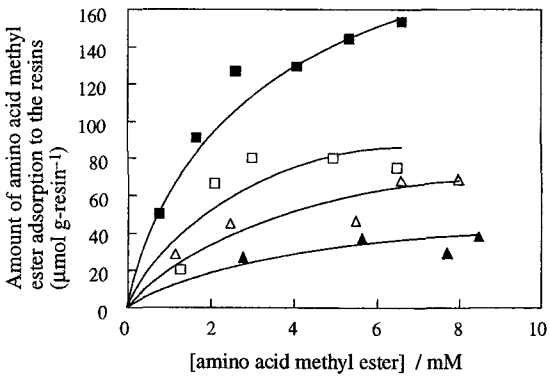
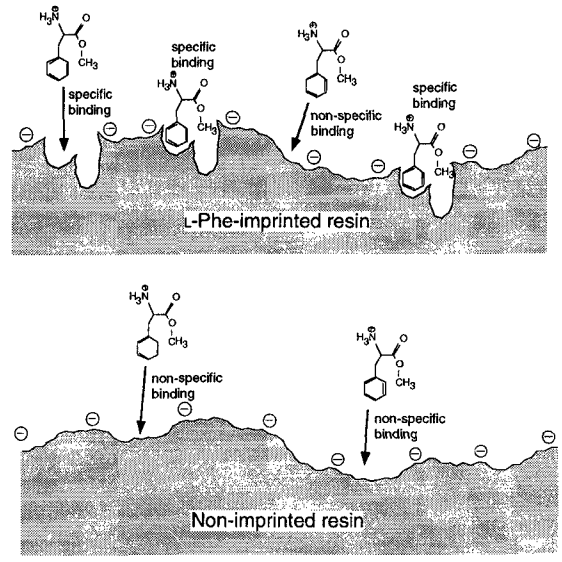
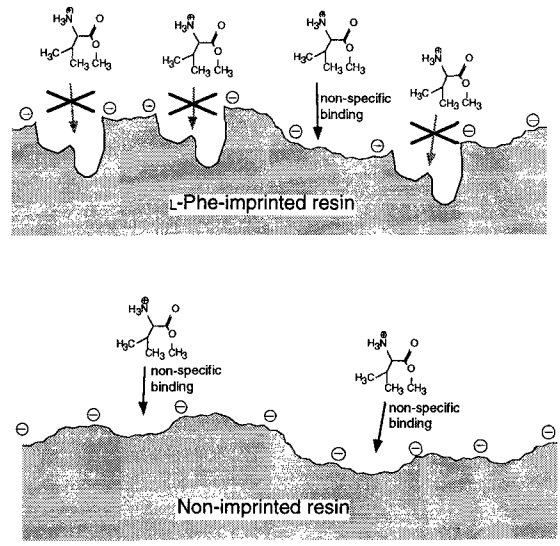


Fig. 4. Adsorption isotherm of L-PheOMe and L-ValOMe to the resins. ■, L-PheOMe to L-Phe-imprinted resin; □, L-PheOMe to non-imprinted resin: ▲, L-ValOMe to L-Phe-imprinted resin; △, L-ValOMe to non-imprinted resin:

Figure 4 is a combination of Fig. 2 and 3 on the same scale. Comparing the adsorption amount of L-PheOMe and L-ValOMe, non-imprinted resin can bind both of them at almost the same amount. On the other hand, L-Phe-imprinted resin effectively bound L-PheOMe but inhibited the binding of L-ValOMe. These phenomena can be explained as follows (Fig. 5). Non-imprinted resin has an electrostatic interaction with amino acid derivatives because there are carboxyl groups of oleate on the surface. This is the origin of the non-specific binding of amino acid derivatives (Fig. 5a,b, lower). In the case of L-Phe-imprinted resin, there are both non-specific and specific binding sites that has memorized the shape and the character of L-Phe on the surface. So the number of non-specific binding site on the surface of L-Phe-imprinted resin is smaller than that of non-imprinted resin. Absorption of L-PheOMe to L-Phe-imprinted resin was observed as the sum of specific and non-specific binding (Fig. 5a, upper). Imprinted effect would appear when the number of specific binding site was big and the site was so effective. In the case of L-ValOMe, the binding to L-Phe-imprinted resin was inhibited because L-ValOMe does not fit to the specific binding site of L-Phe. Absorption of L-ValOMe to L-Phe-imprinted resin was observed only the remained non-specific binding (Fig. 5b, upper). Thus L-Phe-imprinted resin absorbed L-ValOMe less effectively than non-imprinted resin.



(a) Specific and non-specific binding of L-PheOMe to L-Phe-imprinted resin (upper) and non-specific binding to non-imprinted resin (lower)



(b) Non-specific binding of L-ValOMe to L-Phe-imprinted resin (upper) and non-specific binding to non-imprinted resin (lower)

Fig. 5. Proposed Binding Modes of L-PheOMe and L-ValOMe to the resins.

Chiral recognition by this type of resin could not be observed (data not shown). It is necessary to improve the condition of polymerization and change the system

for evaluation of the resin performance. However we can get the resin that recognizes selectively the templated amino acid by our new surface imprinting technique. It can be applied to the other water-soluble compounds and it should be a useful method to supply novel materials for molecular recognition.

4. REFERNCES

1. K. Mosbach, *Trends Biochem. Sci.*, **19**, 9-14 (1994).
2. G. Wulff, *Angew. Chem. Int. Ed. Engl.*, **34**, 1812-32 (1995).
3. H. S. Andersson and I. A. Nicholls, *Recent Res. Devel. in Pure & Applied Chem.*, **1**, 133-57 (1997).
4. M. Maeda, *Bunseki*, **6**, 429-33 (1993).
5. H. Kido, T. Miyajima, K. Tsukagoshi, M. Maeda and M. Takagi, *Anal. Sci.*, **8**, 749-53 (1992).
6. K. Tsukagoshi, K. Y. Yu, M. Maeda and M. Takagi, *Bull. Chem. Soc. Jpn.*, **66**, 114-20 (1993).
7. M. Murata, S. Hijiya, M. Maeda and M. Takagi, *Bull. Chem. Soc. Jpn.*, **69**, 637-42 (1996).
8. M. Kempe and K. Mosbach, *J. Chromatogr. A*, **694**, 3-13 (1995).
9. O. Ramström, I. A. Nicholls and K. Mosbach, *Tetrahedron Asymmetry*, **5**, 649-56 (1994).
10. B, Sellergren and K. J. Shea, *J. Chromatogr. A*, **690**, 29-39 (1995).
11. I. A. Nicholls, O. Ramström and K. Mosbach, *J. Chromatogr. A*, **691**, 349-53 (1995).

(Received December 11, 1998; accepted January 29, 1999)