Molecularly Imprinted Polymers

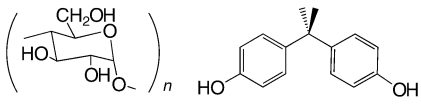
pH-Responsive Molecularly Imprinted Polymers

*Yasumasa Kanekiyo, Ryuichi Naganawa, and Hiroaki Tao**

Molecular imprinting is a powerful tool for the creation of molecular recognition materials having excellent molecular recognition ability.[1] In general, molecularly imprinted polymers (MIPs) are synthesized by the copolymerization of a functional monomer–template complex with a crosslinker. To create binding sites complementary to the shape of a template molecule, a high fraction of crosslinker is usually used, so that the binding sites are rigidly retained in the crosslinked polymer network. Accordingly, it is difficult to deform the binding sites for the sake of controlling binding characteristics. This is a significant drawback of MIP technique, and the development of a new type of MIP having flexible binding sites is desired.[2]

Recently, new types of MIPs that respond to stimuli are emerging. Alvarez-Lorenzo et al. have reported MIPs that change their rebinding abilities towards metal ions[3] and anionic molecules[4] in response to the temperature of the solution. In these systems, functional groups are dispersed in loosely crosslinked *N*-isopropylacrylamide (NIPA) polymer. Since NIPA polymer undergoes a reversible swelling–shrinking cycle in response to the external temperature, the rebinding ability is controllable by changing the solution temperature, which leads to a change in the structure of the binding sites.

Our group recently established a novel molecular imprinting strategy using amylose as a host matrix.[5] This method consists of helical inclusion-complex formation between



Amylose Bisphenol-A

amylose modified with acryloyl groups (acryloylamylose) and a template molecule in aqueous solution followed by radical copolymerization with a crosslinker (*N*,*N*′-methylenebisacrylamide). The resultant polymer retains a hydrophobic cavity that is complementary to the molecular shape of the template. It occurred to us that if acryloylamylose is copolymerized with a monomer having ionizable units such as carboxyl group, and the crosslinking density is appropriately tuned, the imprinted binding site should reversibly

[*] Dr. H. Tao, Dr. Y. Kanekiyo, Dr. R. Naganawa
National Institute of Advanced Industrial Science and Technology (AIST)
16-1 Onogawa, 305-8569 Tsukuba (Japan)
Fax: (+81) 29-861-8308
E-mail: hiro-tao@aist.go.jp

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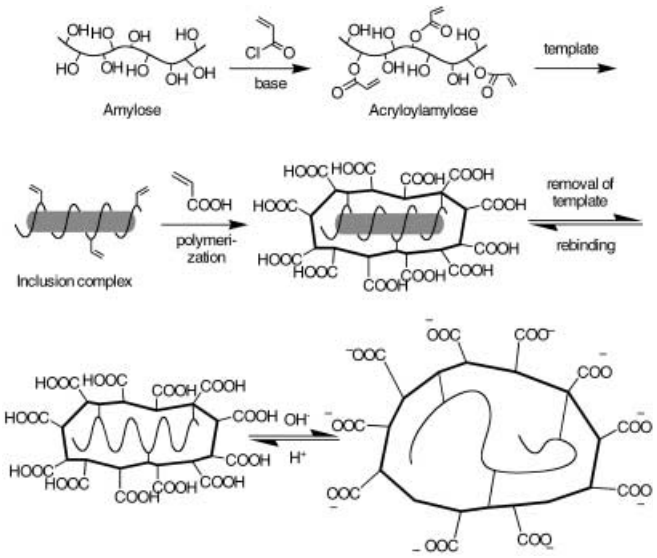

Scheme 1. Synthesis of amylose-based imprinted polymer and its pH-responsive structural change.

change its structure depending on the pH of the solution (Scheme 1).

Synthesis of acryloylamylose and the subsequent inclusion-complex formation with bisphenol-A as a template were conducted by the method already reported.[5] To control the crosslinking density of the imprinted polymers, the degree of substitution (DS)[6] of acryloylamylose was varied between 0.15 and 0.22. The higher DS results in the creation of more crosslinking points in the imprinted polymers. The stoichiometry of the complex between acryloylamylose and bisphenol-A was assessed by measuring the solubility difference of bisphenol-A in pure water and in aqueous acryloylamylose solution (Table 1). Acryloylamylose with DS = 0.22 shows a

Table 1: Complexation between Acryloylamylose and Bisphenol-A

DS[a]	solubility of bisphenol-A in water [mM]	in acryloylamylose solution[b] [mM]	[glucose unit]/ [complexed bisphenol-A]
0.15	1.12	6.38	27.5
0.22	1.12	5.84	30.7

[a] For the definition of DS, see Ref. [6]. [b] Concentration of acryloylamylose: 25 g L^{-1}.

10% lower complexation ability than the case of acryloylamylose with DS = 0.15. It is known that the intramolecular hydrogen bonding between hydroxyl groups plays an important role in stabilizing the helical conformation of amylose.[7] Hence, the modification of hydroxyl groups in amylose could disrupt the intramolecular hydrogen bonding and lower the complexation ability. In the present system, however, the difference in the complexation ability between the two acryloylamylose is relatively small.

After the formation of the inclusion complex, polymerization was carried out as follows: acrylic acid (10 mg) and 2,2′-azobis(2-amidinopropane) dihydrochloride (initiator, 1.6 mg) were added to an aqueous solution (2 mL) containing acryloylamylose (50 mg) and bisphenol-A (saturated). Bisphenol-A was excluded from the preparation of nonimprinted polymer. The stirring solution was irradiated by UV light (365 nm) at 25 °C for 3 h. The resultant polymer was copiously washed with acetone and methanol. Complete removal of bisphenol-A from the imprinted polymer was confirmed by HPLC analysis of the washing solvent. The reference polymer, which does not have ionizable units was also synthesized according to the same procedure mentioned above, except that acrylic acid was replaced with the same weight of acrylamide. These polymers were obtained as white powders, which were easily dispersible in aqueous solutions without any processing, such as grinding.

For the measurement of the rebinding ability, the imprinted polymer or nonimprinted polymer (5 mg) was dispersed in an aqueous solution of bisphenol-A (25 μM, 1 mL) and stirred at 25 °C for 3 h. After the reaction mixture was subject to centrifugation (90 000 rpm, 5 min) the amount of bound bisphenol-A was determined by HPLC analysis of the supernatant solution. Figure 1 shows the pH dependence

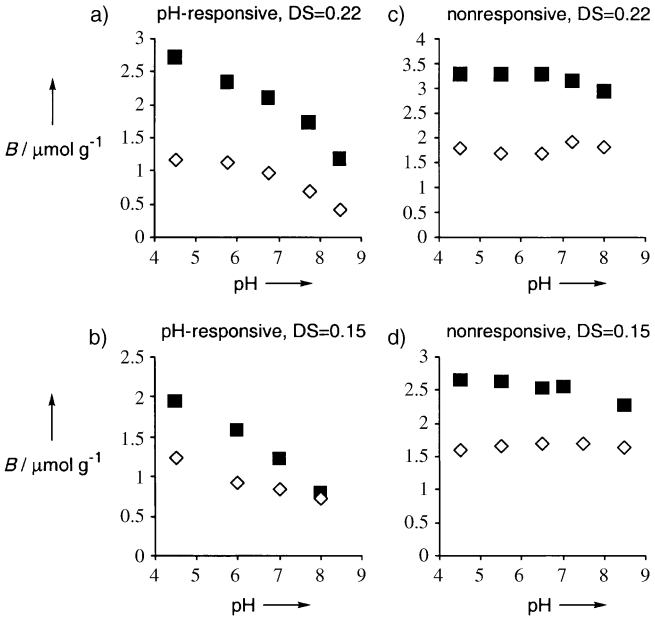

Figure 1. Rebinding abilities of imprinted polymers toward bisphenol-A at various values of pH: (■) imprinted, (◇) nonimprinted. pH-responsive polymers were copolymerized with acrylic acid, whereas nonresponsive polymers were copolymerized with acrylamide. *B* = binding ability.

of the rebinding abilities for the polymers. The polymers copolymerized with acrylic acid gradually decrease their rebinding abilities with increasing pH of the solution (Figure 1a and b). On the other hand, the polymers copolymerized with acrylamide show virtually no response toward pH variation (Figure 1c and d).[8] Deprotonation from the hydroxyl group in bisphenol-A is negligible in the measurement conditions as the pK_a value is 9.8.[9] Thus, we have verified that the introduction of carboxyl groups into the polymer matrix is essential for the creation of pH responsiveness.

The imprinted polymer synthesized from acryloylamylose with DS = 0.22 shows a higher rebinding ability than the case in which acryloylamylose with DS = 0.15 is used. For acryloylamylose with DS = 0.15, at higher values of pH the difference in the rebinding ability between the imprinted polymer and the nonimprinted polymer almost disappears, whereas for acryloylamylose with DS = 0.22 the difference still exists. These observations clearly support the view that the binding cavity created through the imprinting process is disrupted by a conformational change in amylose chain arising from the electrostatic repulsion between anionic carboxylate groups. It is understandable that the higher the crosslinking density, the better the rebinding ability because the imprinted binding site is rigidly retained. On the other hand, the recognition ability of the more rigid polymer is more difficult to erase.

We also examined the reversibility of the erasing and regeneration process for the molecular memory. While successively changing the acidity of an aqueous bisphenol-A solution, the rebinding abilities of the imprinted polymer that is copolymerized with acrylic acid were measured. The rebinding ability, as shown in Figure 2, reversibly decreases and increases by changing the conditions from acidic to basic and vice versa.[10]

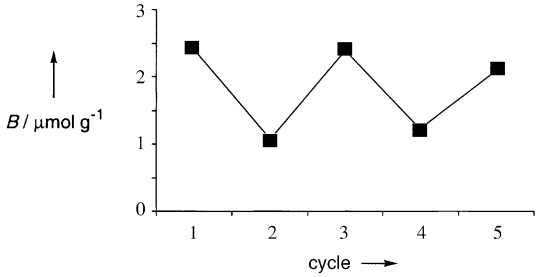

Figure 2. Reversible erasing and regeneration of molecular memory. The imprinted polymer copolymerized with acrylic acid was consecutively immersed in acidic (pH ≈ 4.5, odd cycles) and basic (pH ≈ 8.5, even cycles) solutions containing 25 μM of bisphenol-A. Acryloylamylose with DS = 0.21 was used for imprinting.

In conclusion, the work presented herein has demonstrated for the first time a novel strategy to create pH-responsive molecularly imprinted polymers using amylose as the host matrix. It is confirmed that changing acidity of the solution reversibly controls the rebinding ability toward bisphenol-A. We expect that the present system is expandable to the imprinting of other hydrophobic molecules. The system may have metal responsiveness arising from the complex formation with carboxylate groups, which is currently under investigation in our laboratory. Notably, the present system mainly consists of a naturally occurring product, which is abundantly obtained from common food products, such as wheat, potato, and rice. We thus believe that the present methodology is a promising way to develop environment-friendly separation materials, human-body-friendly drug delivery systems, etc. in the near future.

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Keywords: copolymerization · host–guest systems · inclusion compounds · molecular imprinting · nanostructures

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[10] The reversibility is gradually decreased with increasing acid–base cycles, which can be attributed to the hydrolysis of the ester bonds connecting amylose chain with crosslinking matrix.

