Combinatorial Molecular Imprinting for Formation of Atrazine Decomposing Polymers

Toshifumi Takeuchi,* Daigo Fukuma, Jun Matsui, and Takashi Mukawa
Laboratory of Synthetic Biochemistry, Faculty of Information Sciences, Hiroshima City University
3-4-1 Ozuka-higashi, Asaminami-ku, Hiroshima 731-3194

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Synthetic polymers capable of atrazine-selective decomposition were prepared by molecular imprinting. A combinatorial approach was employed using an automated polymer preparation/evaluation system for the selection of functional monomers to construct the atrazine decomposition sites. Combined use of methacrylic acid and 2-sulfoethyl methacrylate provided atrazine-decomposing polymers in which atrazine was converted to a non-toxic compound atraton and the imprinting effects enhanced the catalytic activity.

Atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine), a triazine herbicide, has drawn considerable concerns as an environmental issue since it has been widely used and caused serious pollution in soil and groundwater. For the remediation of environment, inactivation of atrazine would be a significant matter to be considered. Here, we report on the atrazine-decomposing polymers prepared by molecular imprinting, in which atrazine is bound to the imprinted polymers then converted to a biologically inactive compound, atraton (N^2-ethyl-N^4-isopropyl-6-methoxy-1,3,5-triazine-2,4-diamine).

The concept of molecular imprinting to yield functionalized polymers capable of molecular recognition, specific binding, and catalytic reaction is simple and straightforward. A target molecule to be recognized is added in the reaction mixture containing functional monomers, crosslinkers and solvents; followed by radical polymerization. Subsequently the target molecule is extracted from the resultant polymers to leave complementary binding and/or catalytic sites for the target molecule.[1]

For the development of atrazine-decomposing polymers, we employed a combinatorial approach in molecular imprinting. Combinatorial chemistry has been recognized as a powerful strategy to develop various lead compounds for pharmaceuticals. In the field of material sciences, combinatorial approach would be reasonably acceptable if automated systems for synthesis and high throughput screening were available. Recently, we have developed an automated system for molecularly imprinted polymer synthesis combined with a high throughput screening method, i.e., combinatorial molecular imprinting.[2] Compared to conventional "trial and error" approaches, combinatorial molecular imprinting enables us to speed up time-consuming steps such as designing imprinted polymers and optimizing preparation conditions.

For preparing the imprinted polymers bearing the atrazine-decomposing catalytic activity, atrazine was used as the template molecule with two species of functional monomers: one was contributed to construct atrazine-binding sites and the other was involved in the catalytic sites. Methacrylic acid (MAA) has already proven as a functional monomer to be effective for preparing atrazine imprinted polymers,[3] thus MAA was employed as the functional monomer for the binding site construction. Regarding the catalytic functional monomers, a basic monomer, 2-(dimethylamino)ethyl methacrylate (DMA) and four acidic monomers including monoacryloxy ethyl phosphate (AEP), itaconic acid (ITA), 2-sulfoethyl methacrylate (SEM), and 2-(trifluoromethyl)-acrylic acid (TFM) were employed to construct atrazine-decomposing polymer libraries, since atrazine is known to be decomposed under acidic and basic conditions.[4] In order to find a monomer suitable for catalytic site construction, combinatorial polymer libraries L-1(MAA+DMA), L-2(MAA+AEP), L-3(MAA+ITA), L-4 (MAA+SEM), and L-5(MAA+TFM) consisting of total 100 members (each 20 members × 5) were prepared using various ratios of MAA (0, 2, 4, 6, 8 mole ratio to atrazine added) and one of five catalytically active monomer candidates (2, 4, 6, 8 mole ratio). The experiments were conducted by the automated liquid handling system (Figure 1) and independent polymer preparations were carried out in triplicate.

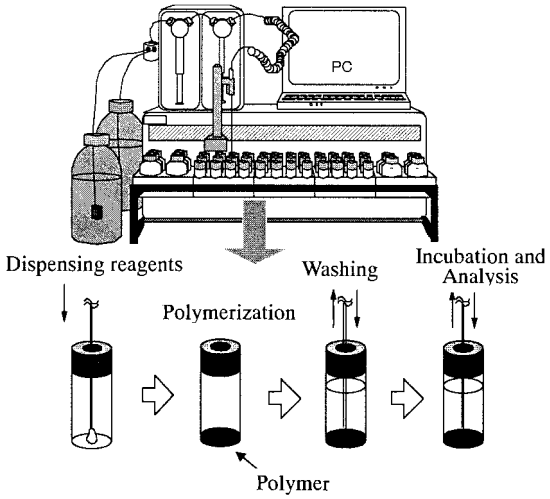

Figure 1. Schematic diagram of the automated system. Prepolymerization mixtures were prepared automatically in glass vials with caps (1.5 mL) by dispensing atrazine (1.95 μmol), ethylene glycol dimethacrylate (30.0 mg, 0.15 mmol), 2,2'-azobisisobutyronitrile (747 μg), functional monomers, and a solvent using a programmable liquid handler (Gilson 232XL) under nitrogen atmosphere. Chloroform was basically used as the solvent in the synthesis, but THF and DMF were used in the cases using ITA and AEP because of their solubility. The glass vials were placed under UV light for 12 h at 5 °C. Thinly coated polymers were washed by repeating dispensation and aspiration of methanol (3 times), methanol–acetic acid–water (10 times) and chloroform (3 times). Intervals taken between the dispensation and aspiration were 2 h.

Binding properties of polymers were investigated by automated batch binding tests, where polymers were incubated with atrazine in chloroform without the nucleophilic reagent methanol. As MAA contents were increased, the amount of atrazine bound showed a rising trend without significant dependence on the amount of the second monomer in all the libraries (data not shown). The second monomers exhibited different effects on the binding, that is

the amounts of atrazine bound increased with the amounts of SEM, AEP, ITA, and TFM (data not shown). However, in the case of DMA, the binding showed a decreasing trend. This could be due to competitive complexation of DMA and atrazine to MAA, resulting in the inhibition of atrazine-MAA complex formation that is essential for the generation of binding sites.

Binding selectivity for atrazine in a typical library member of atrazine:MAA:SEM = 1:6:2 (mole ratio) in L-4 was investigated by comparing the binding behaviors for atrazine and metribuzin (4-amino-6-*tert*-butyl-3-methylthio-1,2,4-triazin-5(4*H*)-one), a triazine herbicide. Selectivity factors calculated by dividing the bound amount of atrazine by that of metribuzin allowed a more careful comparison among the members (Figure 2). A trend can be seen that polymers prepared using more MAA and less SEM exhibited higher selectivity for atrazine. The results suggest that the strong interactions of highly acidic SEM with the basic compounds, atrazine and metribuzin, tend to increase non-specific binding. Taking it into consideration that SEM itself was not suitable for developing atrazine selectivity, MAA-rich polymers containing sufficient but minimum amount of SEM is preferable under the present conditions.

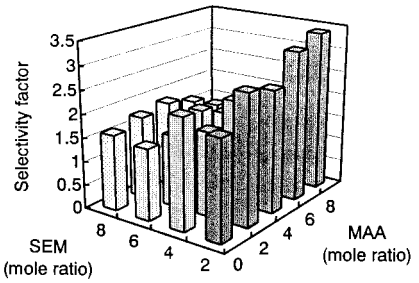

Figure 2. Selectivity of imprinted polymers in L-4 (atrazine: MAA:SEM=1:6:2, mole ratio). Selectivity is shown by selectivity factors calculated by dividing the bound amount of atrazine by that of metribuzin. More than unity means that the polymer has selectivity for atrazine. Binding tests were performed using atrazine or metribuzin dissolved in chloroform (500 μmol dm^{-3}, 1.50 mL). After 24 h incubation, supernatants (1.0 mL) were analyzed by HPLC.[5] Amount of atrazine or metribuzin bound was estimated by subtracting the free amount in the supernatants from the initial amount.

Immersing the thinly coated polymers on the bottom of vials with atrazine in chloroform containing 10% (v/v) methanol assessed activity for atrazine decomposition. After incubation for 24 h, the supernatants were analyzed by HPLC[5] to determine the degree of atrazine decomposition. Polymers prepared with SEM only exhibited the atrazine decomposition activity (Figure 3), while no other polymers showed such activity. Appearance of atraton suggested a possible mechanism of atrazine decomposition, in which methanol attacked the 6-position of triazine ring on atrazine activated by the SEM residues to allow a nucleophilic substitution. According to an estimated pK_a value of SEM (–1.43),[6] atrazine (pK_a: 1.7)[4] would be protonated when bound to the SEM-containing polymers, followed by the methanolysis. AEP is also acidic enough (estimated pK_a: 1.07)[6] to protonate atrazine, and exhibits a fairly strong binding property to atrazine; however, no catalytic activity was observed.[7] Moreover, the blank polymers prepared without atrazine yielded less atraton (data not shown), indicating that the imprinting enhanced the atrazine-decomposition activity.

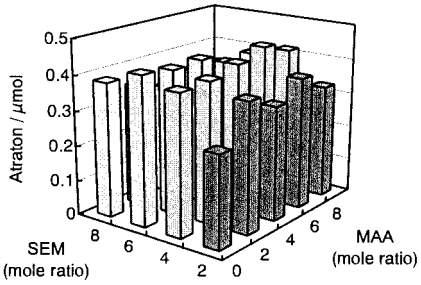

Figure 3. Atrazine decomposition in imprinted polymer libraries L-4 (atrazine:MAA:SEM=1:6:2, mole ratio). Catalytic tests were carried out as follows. Atrazine dissolved in chloroform containing 10% (v/v) methanol (500 μmol dm^{-3}, 1.50 mL) was dispensed into each polymer-coated vial. After 24 h incubation, supernatants (1.0 mL) were taken from the vials and amounts of atraton produced were analyzed by HPLC.[5]

Although further investigation should be addressed, the imprinted polymers capable of atrazine inactivation described here could provide a way to improve atrazine-polluted environments in conjunction with the use of imprinted polymer-based solid phase extraction previously reported.[8] Furthermore, the automated synthesis of small amount of diverse materials present here would be effective for establishing down-sizing and green chemistry-based material design/synthesis that should be considered for the reduction of pollutants to be used.

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References and Notes

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5 Column: Supelco LB-8-DB, 150 mm × 4.6 mm I.D, eluent: 33% (v/v) acetonitrile in 0.1 M ammonium acetate buffer (pH 6.0), 1 mL min^{-1}. The metabolites of atrazine was assingned by its UV–vis spectra to be atraton.

6 pK_a values of SEMA and AEP were estimated by Hammet and Taft Theory-based calculation with a software pKalc 3.2 (CompuDrug International Inc., U. S. A.).

7 The presence of methanol could lead to the decline of selective atrazine-uptake performance because such polar solvent could disturb the hydrogen bonding-based binding between atrazine and the polymers. Therefore, it is currently unclear whether AEP and TFM inherently possess no catalytic activity or the conditions employed are inappropriate for the monomers to develop the catalytic activity.

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