Artificial Biocatalyst Prepared by the Surface Molecular Imprinting Technique

Eiichi Toorisaka, Masahiro Yoshida, Kazuya Uezu, Masahiro Goto,* and Shintaro Furusaki
Department of Chemical Systems and Engineering, Graduate School of Engineering, Kyushu University, Hakozaki, Fukuoka 812-8581

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A novel artificial biocatalyst that catalyzes a hydrolysis reaction was prepared by a surface molecular imprinting technique. The properties of the artificial biocatalysts were investigated in the hydrolysis reaction of an amino acid derivative in a biphasic system. The enzyme-mimic biocatalyst showed a high activity and maintained high stability for a long time. We demonstrate that the surface molecular imprinting technique is effective for designing novel artificial enzyme materials.

To mimic highly organized functions observed in biological molecular recognition in biomolecules such as enzymes, antibodies and receptors is one of the most challenging themes. In an industrial application, however, instability of biomolecules *in vitro* is a inevitable problem to be solved. To date, various artificial bio-active materials have been studied to overcome the drawback in biomolecules.

Recently, the molecular imprinting technique[1,2] has attracted much attention to construct enzyme-mimicking polymers[3,4] which exhibit specific substrate recognitions on catalytic functions. The molecular imprinting technique is one of the promising approaches to design "tailor-made" catalytic sites. This technique has a large advantage to require neither a sophisticated molecular design nor cumbersome multi-step procedures in the preparation. However, it still has two fundamental drawbacks: difficulties to introduce water-soluble substrates as an imprinting molecule in the catalytic sites, and slow catalytic kinetics arising from the inner diffusion of substrates toward the catalytic sites formed deeply in the polymer matrix.

To conquer these problems, we have recently proposed a novel molecular imprinting technique for constructing recognition sites on the polymer surface called "surface molecular imprinting technique".[5] The imprinted polymer was prepared by polymerizing water-in-oil (W/O) emulsions containing a functional host molecule, an imprint molecule and a cross-linking agent. After polymerization the orientation of the functional host molecule can be fixed on the polymer surface. We have succeeded in preparing highly selective materials for an optical resolution of amino acids.[5]

In this study, we designed a novel functional host molecule possessing a high interfacial activity, considering that the host molecule should be located at water-oil interface to yield a high specific recognition. Furthermore we introduced an imidazole molecule as a functional group to catalyze the hydrolysis reaction of substrates. Using the newly synthesized functional host molecule, an enzyme-mimic polymer has been prepared by imprinting a substrate analogue (Nα-t-Boc-L-histidine) through the complex formation between a cobalt ion and the imidazole moiety. The catalytic properties of artificial biocatalysts are discussed on the hydrolysis reaction of an amino acid ester (N-t-Boc-L-alanine *p*-nitrophenyl ester) by comparing to several control experiments. This is the first report that an enzyme-mimic polymer prepared by the surface molecular imprinting technique exhibits a high catalytic activity.

The novel functional host molecule, oleyl imidazole (abbreviated with $1C_{18}IM$), was synthesized from oleic acid chloride and histamine dihydrochloride. The emulsion stabilyzer L-gultamic acid dioleylester ribitol (abbreviated with $2C_{18}\Delta^9GE$) was synthesized according to the previous work.[5] Divinylbenzene (abbreviated with DVB, Wako Pure Chemical Industries, Ltd.) was employed after treatment with silica gel to remove an inhibitor. Other reagents were of commercially available grade.

The artificial biocatalyst was prepared by the surface molecular imprinting technique utilizing W/O emulsions. A 20 cm^3 solution of DVB, in which $1C_{18}IM$ (150 mol/m^3) and $2C_{18}\Delta^9GE$ (30 mol/m^3) were dissolved, was mixed with 10 cm^3 toluene. A 15 cm^3 aqueous solution containing 100 mol/m^3 $Co(NO_3)_2$ (pH=7.0, buffered with 100 mol/m^3 acetic acid-sodium acetate) and 100 mol/m^3 Nα-t-Boc-L-histidine was added to the toluene solution, and the mixture was sonicated for 4 minutes to obtain W/O emulsions. After the addition of 0.18 g of powder initiator (2,2'-azobis(2,4'-dimethylvaleronitrile), Wako Pure Chemical Industries, Ltd.), the mixture was stirred at 55 °C for 3 h under a nitrogen atmosphere. The obtained polymer was dried *in vacuo* and ground into an appropriate size. The imprinted polymer was washed with 1000 mol/m^3 hydrochloric acid solution to remove cobalt ions and the imprint molecule Nα-t-Boc-L-histidine, and then filtered off. This procedure was repeated several times until the cobalt ions in the filtrate could not be detected. Finally, the N-t-Boc-L-histidine-imprinted polymer was dried *in vacuo*. The unimprinted polymer was similarly prepared as a reference polymer without cobalt ions and the imprint molecule Nα-t-Boc-L-histidine.

The hydrolysis reaction of the substrate was performed in a biphasic system of isooctane and a phosphate buffer solution. A 0.05 g of the polymers was placed in a sealed test tube (10 cm^3 volume), to which were added a 5 cm^3 isooctane solution containing 1.3×10^{-6} mol N-t-Boc-L-alanine *p*-nitrophenyl ester and a 5 cm^3 phosphate buffer (pH8.0) (Functional host molecule / substrate = 7.5 / 1 (mole ratio)). The mixture was shaken in a thermostated water bath at 35 °C. The hydrolysis activity of the

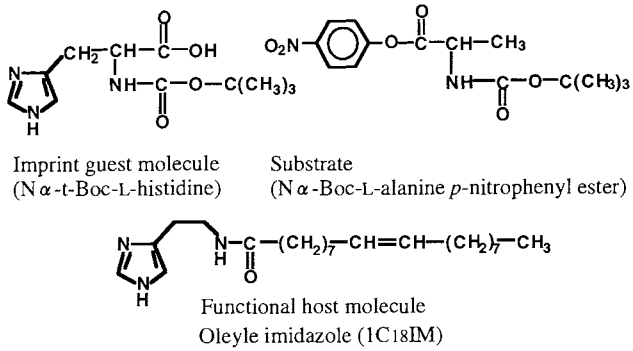

Figure 1. Molecular structures of the imprint guest molecule, the substrate for the hydrolysis reaction, and the functional host molecule.

imprinted polymer was determined by measuring the hydrolytic product (*p*-nitrophenol) produced in the phosphate buffer phase. The produced p-nitrophenol was detected at $\lambda = 400$ nm on a UV-vis spectrophotometer (JASCO). The degree of self-hydrolysis of the substrate was also measured without functional host molecules under the same conditions. Activity measurement was conducted at least three times and the date were plotted with the average values. The experimental errors were less than 5%.

After polymerization both the guest molecules and cobalt ions were removed from the polymer, where specific recognition sites were formed. The imprinted bulk polymer was ground into particles. The yield was ca. 90%. Figure 1 shows the molecular structures of the imprint guest molecule, the substrate for the hydrolysis reaction, and the functional host molecule. An oleyl chain was introduced to the imidazole derivative as the functional host molecule to enhance the interfacial activity. Nα-t-Boc-L-histidine was employed as the imprint guest molecule since the actual substrate N-t-Boc-L-alanine *p*-nitrophenyl ester is hydrolyzed by the functional host molecule during preparation of the enzyme-mimic polymer. By utilizing the substrate analogue as the imprinting molecule, the functional host molecules could be implanted on the surface of polymer so that they can fit a desirable formation around the substrate on hydrolysis reaction. In Figure 2 we illustrated the catalytic sites formed by three functional host molecules and an imprinting guest molecule through the complex formation with a cobalt ion.

Figure 3 exhibits the hydrolysis property of the enzyme-mimic polymer. The catalytic activity of the imprinted polymer is shown to be significant by comparing to the reactivity of the unimprinted polymer, the isooctane solution containing the same amount of the functional host molecules, and the self-hydrolysis of the substrate. The substrate N-t-Boc-L-alanine *p*-nitrophenyl ester was not self-hydrolyzed under the present experimental condition. The unimprinted polymer exhibited a little higher

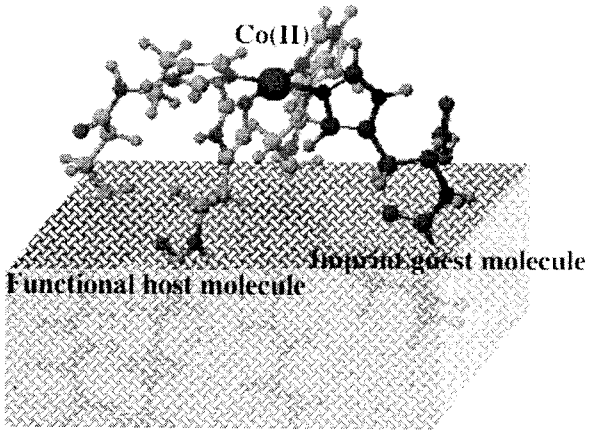

Figure 2. Formation of the catalytic site by the host molecule and the imprint guest molecule.

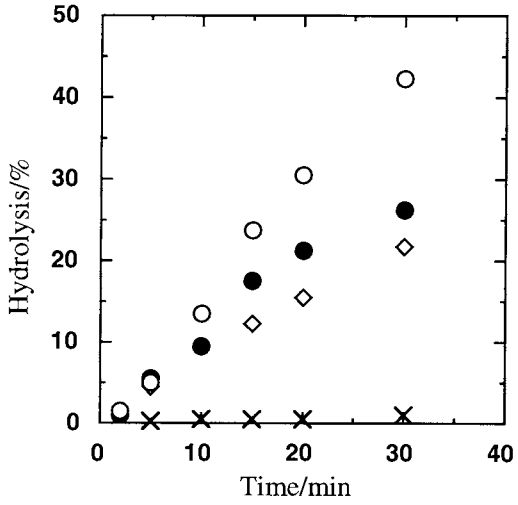

Figure 3. Hydrolysis reaction rate; (○) imprinted polymer, (●) unimprinted polymer, (◇) solution containing functional host molecule, (×) functional host molecule free (self-hydrolysis of substrate).

catalytic activity than that of the functional host solution. As the solubility of the functional host molecule in the reaction phase is not high enough, the unimprinted polymer possessing randomly dispersed host molecules show a better performance. Furthermore, the imprinted polymer exhibits much higher catalytic activity compared to that of the unimprinted polymer. These results suggest that complementary specific recognition sites were constructed by the imprinting guest molecule and by the functional host molecules which are specially positionized on the polymer surface.

In our conclusions, an enzyme-mimic polymer using a novel synthesized functional host molecule was prepared by the surface molecule imprinting technique. A substrate analogue was imprinted by utilizing the affinity between imidazole moieties in the functional host molecules and cobalt ions. The imprinted polymer exhibited a high catalytic activity for the hydrolysis reaction of an amino acid derivative. We are now expanding this novel surface-imprinting technique to create artificial polymers that mimic a variety of enzymes. It is our hope that the use of surface molecular imprinting technique for preparing artificial biocatalysts will find useful applications in the future.

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