A Molecularly Imprinted Polymer for the Reconstruction of a Molecular Recognition Region

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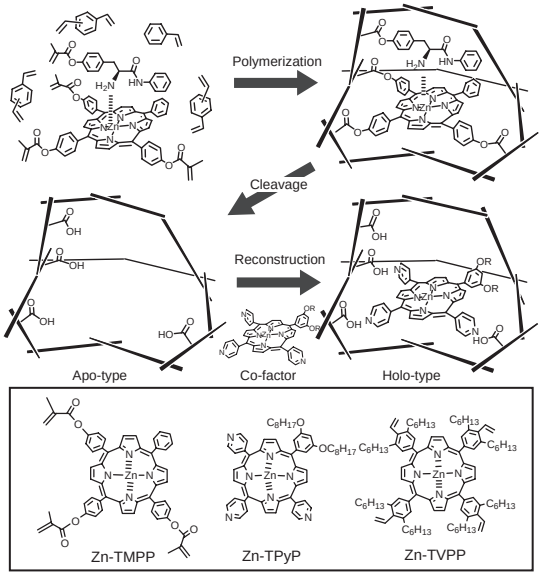
Cofactor-binding protein mimics were prepared by metalloporphyrin-based covalent molecular imprinting of amino acid derivatives, where an apo-type scaffold was obtained by removing the template and the porphyrin moieties from the resulting polymer after the imprinting process, and when the porphyrin derivative was incorporated as the cofactor to yield the holo-type binding sites, enantioselectivity was induced and the chirality of the target amino acids was recognized.

Cofactor-binding proteins such as hemoproteins and flavoproteins play an important role in biological functions and processes. Unique functional materials inspired by biological systems could be created by mimicking such conjugated proteins. For example, the hemoprotein myoglobin has a protoporphyrin IX iron complex as a prosthetic group, without which it cannot store dioxygen.[1] This intrinsic function of myoglobin has been engineered by replacing the prosthetic group,[2] in which apo-proteins were used as scaffolds for developing improved or new functions after reconstruction of a reaction center (holo-proteins) using specially designed molecules.

Synthetic polymers could be substituted for such natural apo-protein scaffolds if a desirable cofactor-binding field could be created in the polymer matrices to reconstruct holo-type sites for the development of useful functions. However, no conjugated protein-mimetic synthetic polymers have been reported so far, although a wide range of artificial receptors has been synthesized to date.[3] This paper describes a simple cofactor-binding protein mimic, namely, an apo-type synthetic polymer bearing reconstructable binding sites designed and synthesized by molecular imprinting, and capable of being converted into the corresponding holo-type after incorporation of the cofactor. Molecular imprinting, in which target molecules can be recognized according to their chemical and structural information, is a technique that has recently gained attention as a method for synthesizing artificial receptors.[4] Here, we designed apo-type protein mimics that could be converted to a holo-type binding site by binding a zinc(II) porphyrin derivative as a cofactor, for recognizing an enantiomer of tyrosine anilide (TyrAN) bearing a rigid structure with two interactive sites such as phenolic hydroxy and amino groups.

Reconstructable binding sites for TyrAN were prepared as illustrated in Scheme 1. The template (methacryloyl D-TyrAN), the removable porphyrin-based functional monomer, Zn–TMPP, and crosslinkers were copolymerized (A). Divinylbenzene and styrene were used as crosslinkers since the resulting polymer matrix can be resistant for the next cleavage conditions. Zn–TMPP and TyrAN moieties were removed from the polymer matrix by oxidative cleavage with H_2O_2, followed by hydrolysis with KOH to obtain the apo-form imprinted polymers (IP-D(apo)), in which four carboxylic acid residues were left in each cavity (B). From the absorbance decrease of the Soret band of Zn–TMPP residues in the treated polymer at 426 nm, measured by an integrating sphere-equipped spectrophotometer, about 65% of the porphyrin was removed.[7] The remained residues were not released by repeated treatments. When the rebinding porphyrin, Zn–TPyP, was assembled at the appropriate positions of the apo-binding sites (apo-type scaffold) where the Zn–TMPP residue existed before the cleavage step (C), enantioselectivity could be induced, likely because of the transformation of apo-type binding sites to holo-type binding sites.



Scheme 1. Preparation of the reconstructable binding site for TyrAN by molecular imprinting.

For the evaluation of binding property of the rebinding porphyrin to the apo-type scaffold, a binding isotherm was constructed for IP-D(apo).[7] Free base TPyP was used in this experiment, since Zn–TPyP was self-assembled by the metal center and the pyridyl groups at high concentrations. The binding saturated with increasing TPyP concentration, showing that a finite number of apo-type scaffolds existed in the IP-D(apo). The association constant was estimated to be $4.9 \times 10^4\ M^{-1}$ by Scatchard analysis, where a linear plot was obtained, indicating that the apo-type scaffolds produced were fairly homogeneous, as is the case with covalent molecularly imprinting systems.

When TyrAN was bound to the holo-type binding sites, a binding isotherm showing saturation behavior was obtained, with an estimated association constant of $1.37 \times 10^5\ M^{-1}$.[7] This figure was comparable to those of the previously reported zinc(II) porphyrin[5] and iron(III) porphyrin[6]-based imprinted

polymers. Because the association constant of tyrosine methyl ester to Zn–TPyP has been estimated to be $7 \times 10^3\ M^{-1}$,[7] the affinity appeared to be enhanced by the imprinting process. These results suggested that Zn–TPyP could be an effective rebinding porphyrin, leading to the reconstruction of holo-type imprinted binding sites for TyrAN.

As a reference for the holo-type binding sites made of the apo-type scaffold and Zn–TPyP, a D-TyrAN-imprinted polymer, IP-D(Zn–TVPP), was prepared by copolymerizing methacryloyl D-TyrAN with Zn–TVPP as an irremovable functional monomer and styrene/divinylbenzene as crosslinkers. After the radical polymerization, the TyrAN moiety was removed by hydrolysis to create chiral binding sites for D-TyrAN, leaving the covalently grafted Zn–TVPP moiety intact. Enantioselectivity was observed in batch binding tests using racemic TyrAN, and the separation factor, which was the ratio of the amount of bound template optical isomers to that of their corresponding antipodes, was 1.11 (Table 1). The observed transfer of chirality from the chiral template to the obtained polymers made of achiral monomers may have been due to the assembly of the porphyrin moiety and methacrylic acid residues complementary to the chiral template. In this case, the amino group of TyrAN could have coordinated to the zinc(II) porphyrin moiety, the phenolic hydroxy group of TyrAN could have interacted with the methacrylic acid residue by hydrogen bonding, and the rigid styrene/divinylbenzene polymer matrix could have acted as a barrier against rotation of the chiral center.

In order to probe the behavior of our designed conjugated-protein mimic system, induced enantioselectivity of the holo-type IP-D (IP-D(holo)) reconstructed with Zn–TPyP was examined by studying the binding behavior of racemic tyrosine anilide (DL-TyrAN). We also examined IP-D(apo) and IP-D(TPyP), a metal-free IP-D(holo) that was reconstructed using free base TPyP. The reconstructed binding sites in IP-D(holo) could have become cooperative binding sites consisting of both the Zn–TPyP moiety and methacrylic acid residues, if the binding site were reconstructed exactly as in IP-D(Zn–TVPP), i.e., if the reconstructed binding sites could recognize D-TyrAN enantioselectively as well as did IP-D(Zn–TVPP). As shown in Table 1, the separation factor of IP-D(holo) was roughly equal to that of IP-D(Zn–TVPP), suggesting that the reconstruction of chiral binding sites involving Zn–TPyP and methacrylic acid moieties was successfully achieved by rebinding of Zn–TPyP to IP-D(apo). In contrast, IP-D(TPyP) and IP-D(apo) showed almost no enantioselectivity. Since IP-D(TPyP) and IP-D(apo) had only methacrylic acid residues in the binding sites, these polymers may not have recognized the chirality of D- or L-TyrAN. The total amounts of D- and L-TyrAN bound were larger than the bound amounts of Zn–TPyP, meaning that nonspecific binding toward carboxylic acid residues unoccupied with Zn–TPyP in the polymer matrix could occur under the conditions employed.

Table 1. Enantioselectivity of the polymers tested

Polymers	Amount bound/μmol g^{-1}		Separation factor ($\alpha = B_D/B_L$)
	B_D (CV%)[a]	B_L (CV%)[a]	
IP-D(holo)	4.88 (0.50)	4.45 (0.35)	1.10
IP-D(apo)	4.66 (0.45)	4.55 (0.84)	1.02
IP-D(TPyP)	4.66 (2.71)	4.61 (2.50)	1.01
IP-D(Zn–TVPP)	3.71 (0.34)	3.35 (0.42)	1.11
IP-L(holo)	5.84 (2.35)	6.85 (2.48)	1.17
IP-L(apo)	6.39 (2.83)	6.64 (4.69)	1.04
IP-L(TPyP)	6.29 (2.73)	6.69 (2.38)	1.06
IP-L(Zn–TVPP)	3.55 (0.98)	4.10 (0.43)	1.15

[a] B_L and B_D are the amounts bound of D-TyrAN (initial concn: 12.5 μM) and L-TyrAN(initial concn: 13.5 μM), respectively. The bound amount of Zn–TPyP in IP-D(holo) was 5.1 μmol/g, and that of TPyP was 3.4 μmol/g. The bound amount of Zn–TPyP in IP-L(holo) was 5.9 μmol/g, and that of TPyP was 1.2 μmol/g. CV%: coefficient of variation (standard deviation ×100/mean)

To confirm the development of enantioselectivity during reconstruction, we also prepared L-TyrAN-templated IP-L(holo) and examined its enantioselectivity. As shown in Table 1, IP-L(holo) bound L-TyrAN more strongly than D-TyrAN ($\alpha = 1.17$). The degree of enantioselectivity was similar to that of IP-L(Zn–TVPP) ($\alpha = 1.15$), for which the reverse enantioselectivity of IP-D(Zn–TVPP) was observed, confirming template-directed formation of reconstructable binding sites.

The observed enantioselectivity would probably not have developed if Zn–TPyP had been randomly located in the apo-type scaffold, and, therefore, the reconstruction producing chiral recognition fields was achieved according to the design illustrated in Scheme 1. The intrinsic enantioselectivity of the present imprinting system was not high, which was shown in IP-D/L(Zn–TVPP). As a result, the observed separation factors of IP-D(holo) and IP-L(holo) were not large.

In conclusion, cofactor-binding protein mimics were prepared by metalloporphyrin-based covalent molecular imprinting. Reconstruction of the binding site was confirmed by the induction of enantioselectivity upon binding the cofactor. When a porphyrin derivative was incorporated with the apo-type scaffold as a cofactor to yield holo-type binding sites, enantioselectivity was induced, confirming that the binding site was precisely reconstructed by the binding of the cofactor. Cofactor binding imprinted polymer receptors, which can reconstruct or exchange their reaction centers, would allow us to develop new types of biomimetic and bioinspired materials, since damaged reaction centers can easily be replaced and even new functions introduced if the substitute were to have a similar structure with different functions.

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7 Supporting Information is available electronically on the CSJ-Journal Web Site, http://www.csj.jp/journals/chem-lett/index.html.

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