Enantioselective Binding of Amino Acid Derivatives onto Imprinted TiO_2 Ultrathin Films[1]

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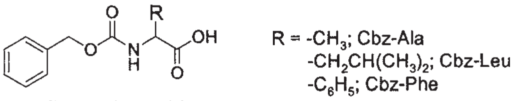
Ultrathin films of titanium oxide gel that are imprinted with carbobenzyloxy-L (and D)-amino acids (Cbz-Ala, Cbz-Leu, and Cbz-Phe) were prepared on gold-coated QCM electrodes. The imprinted films showed much better binding for template stereoisomers than the corresponding enantiomers. The chiral separation factor increased with increasing sizes of the amino acid side chain.

The biological organism is composed of macromolecules such as proteins, nucleic acids, and carbohydrates, in which chiral recognition plays intrinsic roles for their functioning. The corresponding chiral recognition in artificial systems has been achieved by introducing carbon chirality or molecular chirality on host molecules. Molecular imprinting has been examined as an alternate means to create chiral recognition sites, in order to facilitate chiral separations of amino acid derivatives, drugs, and sugar derivatives.[2] We have reported that ultrathin TiO_2-gel films as assembled by the surface sol-gel process are highly effective as matrices of molecular imprinting. Such imprinted films displayed satisfactory structural selectivities in the binding of aromatic carboxylic acids and protected amino acids.[3,4] An outstanding feature of these systems is the formation of multiple functional complementarity in the binding cavity by TiO_2-gel films alone. It is expected that such feature is similarly effective for creation of chiral binding sites. Thus, we examined enantioselective binding of chiral molecules onto imprinted TiO_2-gel films. Enantiomeric amino acid derivatives are selected as the first candidate.



Cbz-amino acid

We used L- and D-carbobenzyloxy (Cbz) amino acids (Cbz-Ala, Cbz-Leu, and Cbz-Phe) as template. The templates were mixed with $Ti(O\text{-}n\text{-}Bu)_4$ in toluene/ethanol (2/1, v/v) and stirred for more than 12 h to form complexes of the two components. TiO_2-gel template films were prepared essentially as reported by our previous paper[3b] (Figure 1). Gold-coated QCM electrodes (9 MHz) were used to monitor the film growth in the adsorption process, as constituted by cycles of chemisorption and activation (hydrolysis). Figure 1 shows the QCM frequency decrease in each adsorption cycle for the selected template solutions. The frequency uniformly decreased up to at least 10 cycles in either case. However, the average frequency change was rather different among the amino acid derivatives, as shown in Table 1: 66–82 Hz per cycle in the case of Cbz-Ala (L- and D-) and 46–53 Hz per cycle for Cbz-Phe (L- and D-).

The template molecules were removed from the gel films by dipping in 1% aqueous ammonia, as described before.[3b] The imprinted films showed the frequency increases corresponding to 19–26% of the adsorbed mass. The frequency change due to desorption and the desorption ratio for each imprinted film are included in Table 1. Rebinding experiments of L- and D-enantiomers were carried out for each imprinted film, as follows.[5]

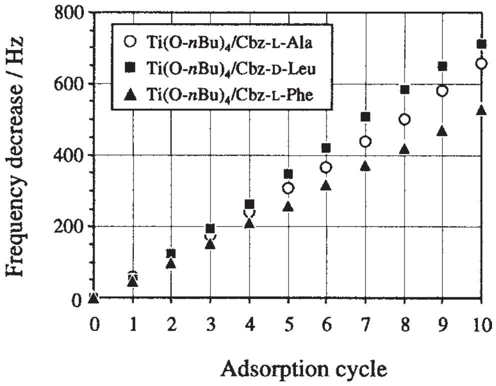


Figure 1. QCM frequency shifts ($-\Delta F$) during the assembly of TiO_2-gel/Cbz-amino acid template film. Stock solutions, (○): $Ti(O\text{-}n\text{Bu})_4$/100 mM, Cbz-L-Ala/12.5 mM, (■): $Ti(O\text{-}n\text{Bu})_4$/100 mM, Cbz-D-Leu/12.5 mM, and (▲): $Ti(O\text{-}n\text{Bu})_4$/100 mM, Cbz-L-Phe/5 mM in toluene/ethanol (2/1, v/v), were diluted by 20 times with toluene and used as dipping solution. The dipping process was carried out at 30 °C for Cbz-Ala and at 35 °C for Cbz-Leu and Cbz-Phe.

Imprinted films were immersed in 0.5 mM template (or, its enantiomer) solution in toluene for 10 min (sufficient for saturation of adsorption), washed in toluene, and dried by flushing with N_2 gas. The QCM electrode attached to the frequency counter was kept in a 10-mL sample bottle over silica gel to avoid the influence of humidity. These rebinding data include specific guest adsorption at the imprinted site in the interior of TiO_2-gel as well as non-specific adsorption onto the TiO_2-gel surface. We separately determined adsorption of guest molecules on the TiO_2-gel film *prior to* template removal. For example, the frequency change due to surface adsorption of Cbz-L-Ala on its imprinted film was 22 Hz, and it was subtracted from the frequency change for total guest binding ($\Delta F_{\text{total}} = 65$ Hz). Thus, the net frequency change for the specific guest binding is estimated as 43 Hz, as shown in Table 1.

Generally, the template molecules gave much larger frequency changes than their enantiomers. The enantiomeric difference in the frequency changes, $\Delta\Delta F_{\text{enantio}} = \Delta F_{\text{t}}$ (template) $-\Delta F_{\text{e}}$ (its enantiomer), becomes larger with increasing sizes of the side chain: Cbz-Ala < Cbz-Leu < Cbz-Phe. The selectivity of the imprinted films is directly represented by the frequency changes for the D- and L-forms. Thus, chiral separation factor (as enantioselectivity) was defined as a ratio of frequency changes for template and its enantiomer ($\Delta F_{\text{t}}/\Delta F_{\text{e}}$, Hz/Hz). In the case of Cbz-Ala, the $\Delta\Delta F_{\text{enantio}}$ values were less than 5 Hz,

Table 1. Adsorbed and desorbed masses during imprinting process of Cbz-amino acids and chiral separation factor

Template		Adsorption		Frequency change due to desorption /desorption ratio	Frequency change due to specific guest binding[a] ($\Delta F_{\mathrm{total}}$)		Chiral separation factor[c], $\Delta F_{\mathrm{t}}/\Delta F_{\mathrm{e}}$
		immersion time/temp.	frequency shift per cycle (total frequency shift)		L-form	D-form	
Cbz-Ala	L	3 min/30 °C	66 ± 8 Hz (659 Hz)	153 Hz/23%	$\underline{43}$[b] Hz (65 Hz)	38 Hz (60 Hz)	1.13
	D		82 ± 10 Hz (818 Hz)	146 Hz/18%	41 Hz (67 Hz)	$\underline{44}$ Hz (70 Hz)	1.08
Cbz-Leu	L	3 min/35 °C	94 ± 17 Hz (939 Hz)	243 Hz/26%	$\underline{38}$ Hz (74 Hz)[d]	22 Hz (58 Hz)[e]	1.73
	D		71 ± 10 Hz (711 Hz)	206 Hz/26%	24 Hz (62 Hz)	$\underline{41}$ Hz (79 Hz)	1.71
Cbz-Phe	L	3 min/35 °C	53 ± 4 Hz (530 Hz)	110 Hz/21%	$\underline{40}$ Hz (69 Hz)	20 Hz (49 Hz)	2.00
	D		46 ± 7 Hz (464 Hz)	87 Hz/19%	32 Hz (44 Hz)	$\underline{54}$ Hz (66 Hz)	1.68

[a] Frequency change due to surface adsorption is subtracted from the frequency change due to total guest binding ($\Delta F_{\mathrm{total}}$). [b] Frequency shift for template was underlined. [c] Defined as the ratio of frequency changes due to binding of template and its enantioamer (ΔF_{t} (template)/ΔF_{e} (enantiomer), Hz/Hz). [d] 57 Hz in CH_3CN. [e] 48 Hz in CH_3CN.

and the chiral separation factor was about 1.1 in either of the L- and D-imprinted films. In contrast, the template molecule was bound much better than its enantiomer in the Cbz-Leu or Cbz-Phe imprinted film. The $\Delta\Delta F_{\mathrm{enantio}}$ values for these optical isomers were 16–22 Hz, and the chiral separation factor amounted to 1.7–2.0. These results are summarized in Table 1.

Chiral molecular imprinting has been often conducted by using amino acid derivatives as reference compounds. Particularly, L-phenylalanine anilide (L-Phe-NHPh) has been used frequently as template. For example, Shea et al. reported a separation factor of 4–8 for resolution of racemic Phe-NHPh using methacrylic acid (MAA) as functional monomer.[6] Exceptionally high separation factors of 17.8, 14.2, and 5.21 for DD-, DL-, LD-isomers respectively were found in the case of a polymer imprinted against *N*-Ac-L-Phe-L-Trp-OMe.[7] However, organic polymers imprinted with N-protected amino acids give much smaller chiral separation factors: 1.84 for racemate resolution of Cbz-Phe in MAA-ethylene glycol dimethacrylate (EDMA) copolymer system and 2.36 in acrylamide-EDMA copolymer system in acetonitrile.[8] These latter values are very close to what we found for Cbz-Phe. It is clear that the TiO_2-gel imprinted film is capable of chiral recognition of amino acid derivatives, comparable to those of functionalized cross-linked polymers. As seen from Table 1, the chiral separation factor is enhanced from 1.1 for Cbz-Ala to 1.7–2.0 for Cbz-Phe with increasing sizes of the amino acid side chain. This trend is similar to what we found for binding of a series of Cbz-protected L-amino acids.[3b] It is noteworthy that the imprinted cavity is characterized with geometrical, functional and steric structures complementary to those of individual template molecules.

We conclude from our results that TiO_2-gel films provide a versatile medium for imprinting of complex molecules such as amino acid derivatives. In comparison with cross-linked polymers, the amorphous network of metal oxides can, by itself, produce imprinting sites of varied functionalities: metal coordination, proton donor, proton acceptor, polar region, and apolar domain, as illustrated in Figure 2. Spatial disposition of such individual domains is built in the cavity, making it enantioselective. Such structural flexibility and functional diversity are greatest advantages of the TiO_2-gel for molecular imprinting. The surface sol-gel process should be useful in analytical and separation technologies.

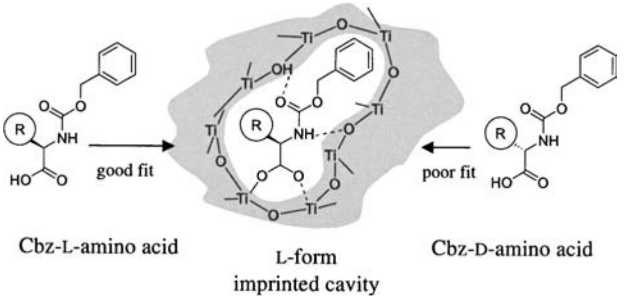


Figure 2. A schematic presentation of relative binding of enantiomers in a Cbz-L-amino acid imprinted TiO_2-gel film.

References and Notes

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1 This paper is dedicated to Professor Teruaki Mukaiyama (Tokyo University of Science) on the occasion of his 75th birthday.

2 a) G. Wulff, *Angew. Chem.*, *Int. Ed. Engl.*, **34**, 1812 (1995). b) O. Ramstörm and R. J. Ansell, *Chirality*, **10**, 195 (1998).

3 a) S.-W. Lee, I. Ichinose, and T. Kunitake, *Langmuir*, **14**, 2857 (1998). b) S.-W. Lee, I. Ichinose, and T. Kunitake, *Chem. Lett.*, **1998**, 1193. c) I. Ichinose, T. Kikuchi, S.-W. Lee, and T. Kunitake, *Chem. Lett.*, **2002**, 104.

4 M. Lahav, A. B. Kharitonov, O. Katz, T. Kunitake, and I. Willner, *Anal. Chem.*, **73**, 720 (2001).

5 The binding to the Cbz-Ala imprinted films was evaluated by *in situ* QCM measurement. The QCM electrode was placed in toluene (1 mL) with stirring until the frequency became stabilized, and then 30 μL of 10 mM guest solution in toluene was added and frequency was measured.

6 B. Sellergren and K. J. Shea, *J. Chromatogr.*, **635**, 31 (1993).

7 O. Ramstörm, I. A. Nichollos, and K. Mosbach, *Tetrahedron*: *Asymmetry*, **5**, 649 (1994).

8 a) M. Kemp and K. Mosbach, *J. Chromatogr., A*, **691**, 3 (1995). b) M. Kemp and K. Mosbach, *J. Chromatogr., A*, **691**, 317 (1995). c) C. Yu and K. Mosbach, *J. Org. Chem.*, **62**, 4057 (1997).