Molecular Imprinting of Protected Amino Acids in Ultrathin Multilayers of TiO_2 Gel

Seung-Woo Lee, Izumi Ichinose, and Toyoki Kunitake*
Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka 812-8581

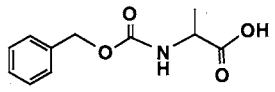
(Received August 5, 1998; CL-980601)

A complex of carbobenzyloxy-L-alanine and titanium *n*-butoxide was adsorbed to hydroxylated surfaces repeatedly to produce ultrathin multilayers, which, upon removal of the amino acid template, showed selective binding of carbobenzyloxy-L-amino acids.

Molecular imprinting of amino acid derivatives have been examined by several groups for the purpose of developing specific receptors for immunoassay mimics, biosensors and chromatographic separation.[1] In such studies, hydrogen bonding interactions with guest peptide units were enhanced by using acrylamide, methacrylic acid, or an amino acid derivative as gel components along with cross-linker monomers, and by burying peptides in the matrix.[2-5] A technique called surface imprinting has been developed particularly for large template molecules of biological origin.[6]

We recently established a new procedure to prepare ultrathin layers of metal alkoxide gels by sequential chemisorption and activation.[7] This surface sol-gel process can be used to design individual metal oxide layers with molecular precision, and has since been extended to formation of composite layers of inorganic and organic components.[8] It was also possible to conduct molecular imprinting of azobenzene carboxylic acid by using TiO_2 gel layers as matrix.[9] The imprinted gel films displayed structural selectivity among aromatic carboxylic acids in rebinding experiments.

We examined in this study molecular imprinting of amino acid derivatives that attracts much interests from the practical viewpoint. The imprinting procedure is schematically shown in Figure 1a. The electrode surface of quartz crystal microbalance(QCM)[10] is first hydroxylated and then subjected to adsorption of titanium alkoxide complexes. Subsequent washing, drying and hydrolysis complete one adsorption cycle.



Cbz-L-Ala

In the current procedure, a mixture of 100 mM Ti(O-*n*Bu)$_4$ and 25 mM carbobenzyloxy-L-alanine(Cbz-L-Ala) was stirred in 2:1(vol/vol) toluene-ethanol at room temperature for more than 12 h, and then added with 275 mM water. This stock solution(0.1 ml) was diluted with 2 ml of toluene and used as dipping solution. A gold-coated QCM electrode (9 MHz) modified with mercaptoethanol was immersed in a dipping solution for 3 min at room temperature, washed in toluene for 1 min to remove the physisorbed complex. The resonator was then immersed in water-saturated toluene for 3 min, and dried by flushing with N_2 gas.

Figure 1b displays QCM frequency decreases due to adsorbed mass in each cycle. Uniform adsorption was observed up to 15 cycles with frequency shifts of 140 to 160 Hz per cycle. Three sets of independent experiments gave close mass increases: 2100 to

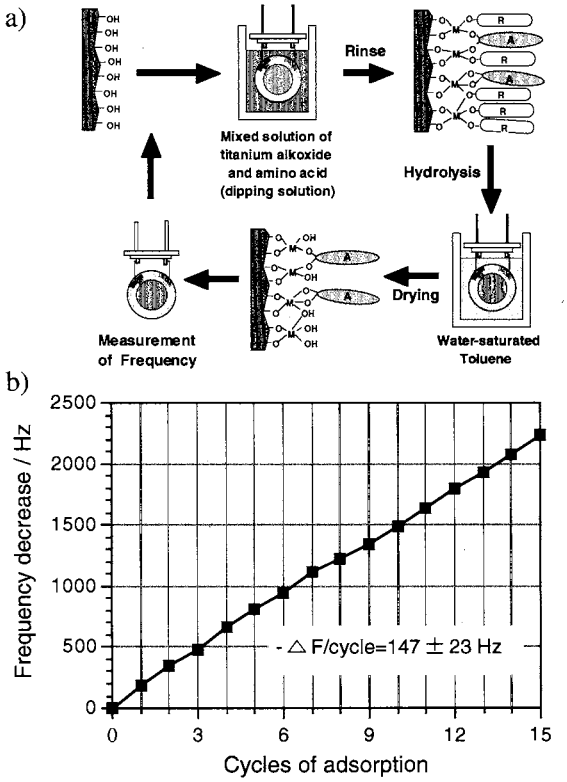


Figure 1. Molecular imprinting process by the surface sol-gel process (a), and QCM frequency shifts in the adsorption of the Ti(O-*n*Bu)$_4$ / Cbz-L-Ala complex (b). A and R denote the template molecule and unhydrolyzed butoxide group of Ti(O-*n*Bu)$_4$. Stock solution was diluted with 2 ml toluene. Stock solution: Ti(O-*n*Bu)$_4$; 100 mM, Cbz-L-Ala; 25 mM, H_2O; 275 mM in toluene / ethanol = 2:1. Adsorption procedure: [1] adsorption; 1 min at r.t(20-23 °C), [2] washing; 1 min in toluene, [3] hydrolysis; 3 min in water-saturated toluene.

2300 Hz for 15 cycles. The incorporation of the template as Ti-complex was confirmed by an FT-IR spectrum(Reflection-Absorption Mode, Nicolet Magna-IR 860 spectrometer) of the adsorbed film which indicated the lack of the $\nu_{C=O}$ peak of the template itself at 1726 cm^{-1} and the presence of the $\nu_{C=O}$ peak of the Ti-carboxylate complex at 1426 and 1561 cm^{-1}.[11]

The template molecule, Cbz-L-Ala, was removed from the gel film by dipping in 1 wt% aqueous ammonia for 20 min and washing in deionized water and in dilute hydrochloric acid(pH 4), followed by washing in water and in methanol and drying over silica gel. The frequency increase upon template removal was 459 Hz(the average of three experiments), corresponding to 21 ± 2% of the adsorbed mass. Characteristic peaks of the template was essentially absent in FT-IR spectra of this film at 1000 to 2000 cm^{-1}.

In situ QCM measurements were carried out for rebinding experiments of template and other molecules, as described before.[9] The imprinted film was kept in 2 ml of acetonitrile with stirring

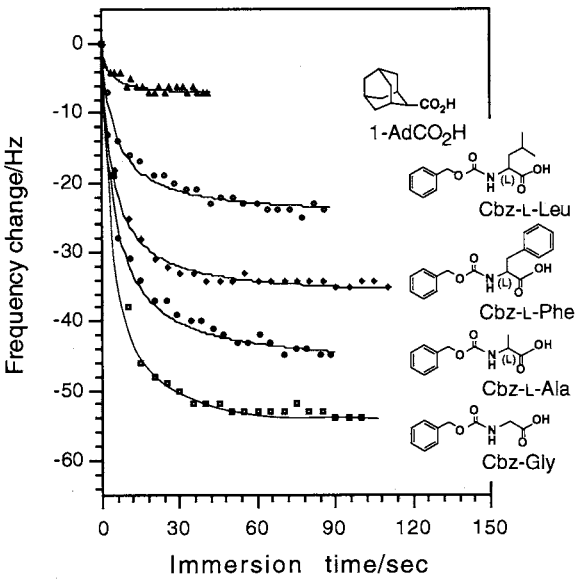


Figure 2. In situ QCM frequency decreases due to binding of a series of guest molecules.

until the frequency became stabilized, and then 10 μ l of 50 mM guest solution in acetonitrile was added and frequency was measured. Since guest molecules were completely removed after each in situ experiment, and a single film could be used for repeated binding experiments. Figure 2 gives selected examples of the guest binding experiment. The binding is saturated in 30 to 60 s. Among the peptide derivatives, Cbz-Gly showed the largest binding and the extent of binding became suppressed with increasing sizes of the side chain, although Cbz-L-Leu and Cbz-L-Phe are reversed. It is noteworthy that the original template, Cbz-L-Ala, is less efficiently bound than Cbz-Gly. However, the binding efficiency is not determined solely by the size of guest molecules. Adamantane-1-carboxylic acid, 1-$AdCO_2H$, gave much smaller binding than all the amino acid derivatives, in spite of its smaller molecular weight. Table 1 summarizes the results of guest binding at saturation. It is clear that all the protected amino acids are bound better than the other, more conventional carboxylic acids. These binding data strongly suggest that the imprinting has produced a specific receptor site in the TiO_2 gel film. As illustrated in Figure 3, this receptor cavity is probably composed of carboxylate site, hydrogen bonding site and hydrophobic site(s). The carboxylate-binding site is common to all the guest molecules, as discussed in our previous paper.[9] In contrast, the hydrogen bonding site may be created only by using amino acid derivatives as template. The hydrophobic site is required for accommodation of hydrophobic moieties of guest molecules.

Table 1. Rebinding efficiency for Cbz-L-Ala imprinted TiO_2 film

guest MW	saturation -△F of binding Hz/pmol	relative binding efficiency mol/mol
benzoic acid (122.12)	5/ 37[a]	0.21
octanoic acid (144.21)	7/ 44	0.25
cinnamic acid (148.16)	0/ 0	0
1-$AdCO_2H$ (180.25)	7/ 35	0.20
Cbz-Gly (209.20))	53/230	1.32
Cbz-L-Ala (223.23)	43/170	**1.00**
Cbz-L-Pro (249.27))	33/120	0.69
Cbz-L-Leu (265.31)	24/ 81	0.47
Cbz-L-Phe (299.33)	34/100	0.59

[a] A frequency decrease of 1 Hz corresponds to mass increase of 0.9 ng.

The desorbed mass in the template removal process was 21% of the originally adsorbed mass. This corresponds to a 10 : 1 molar ratio of the titanium oxide moiety and guest molecule, if titanium alkoxide is completely hydrolyzed, and a 6 :1 molar ratio, if half

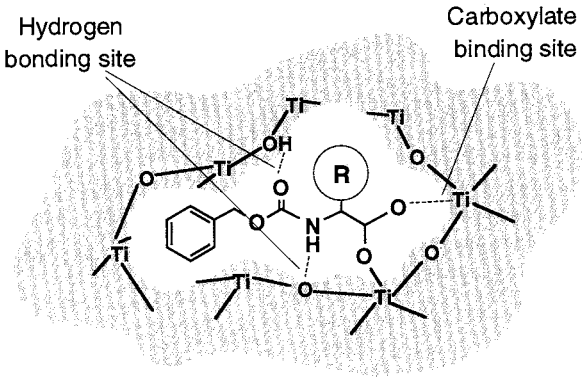


Figure 3. A schematic illustration of an imprinted film. R denotes the side chain of Cbz-amino acid derivatives.

of the butoxy group is left unreacted. Anyway, it is probable that receptor cavities are surrounded by single layers of the titanium oxide network.

In conclusion, it is established that the surface sol-gel process is quite useful for imprinting of molecules that are made of varied functional units like amino acid derivatives.

References and Notes

1 O. Ramstörm and R. J. Ansell, *Chirality*, **10**, 195 (1998).
2 C. Yu and K. Mosbach, *J. Org. Chem.*, **62**, 4507 (1997).
3 J.-M. Lin, T. Nakagama, K. Uchiyama, and T. Hobo, *Biomedical Chromatography*, **11**, 298 (1997).
4 K. Yano, T. Nakagiri, T. Takeuchi, J. Matsui, K. Ikebukuro, and I. Karube, *Anal. Chim. Acta*, **357**, 91 (1997).
5 M. Yoshikawa, J. Izumi, and T. Kitao, *Polymer Journal*, **29**(3), 205 (1997).
6 M. Kempe, M. Glad, and K. Mosbach, *J. Mol. Recogn.*, **8**,35 (1995).
7 I. Ichinose, H. Senzu, and T. Kunitake, *Chem. Lett.*, **1996**, 831; I. Ichinose, H. Senzu, and T. Kunitake, *Chem. Mat.*, **9**, 1296 (1997).
8 I. Ichinose, T. Kawakami, and T. Kunitake, *Adv. Mater.*, **10**, 535 (1998).
9 S.-W. Lee, I. Ichinose, and T. Kunitake, *Langmuir*, **14**, 2857 (1998).
10 Y. Lvov, K. Ariga, I. Ichinose, and T. Kunitake, *J. Am. Chem. Soc.*, **117**, 6117 (1995); Y. Ebara and Y. Okahata, *J. Am. Chem. Soc.*, **116**, 11209 (1994).
11 C. J. Brinker and G. W. Scherer, " Sol-Gel Science, The Physics and Chemistry of Sol-Gel Processing," Academic Press, San Diego (1990); C. Roger and M. J. Hampden-Smith, *J. Mater. Chem.*, **2**(10), 1111 (1992).