Imprinting and Selective Binding of Di- and Tri-Peptides in Ultrathin TiO_2-Gel Films in Aqueous Solutions

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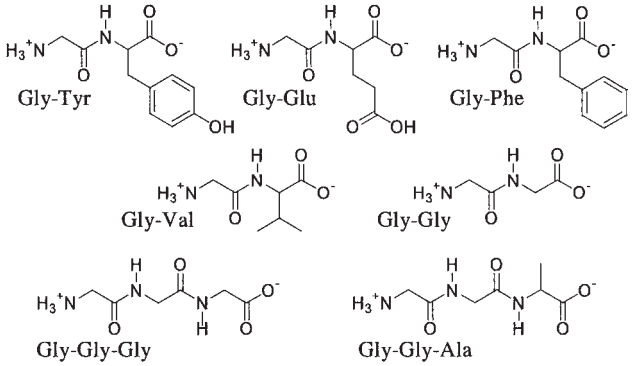
Di- and tri-peptides dissolved in water were alternately assembled with titanium *n*-butoxide to produce ultrathin nanocomposite films. Upon removal of the peptide templates, the titanium oxide film showed selective binding of peptides from dilute aqueous solution.

The host/guest chemistry has been extensively investigated during the past decades through advanced synthetic techniques and the supramolecular concept.[1] Systematic efforts were directed to design binding sites that are specifically applicable to structurally related, yet diversified guests such as sugar isomers and peptides.[2] Molecular imprinting is considered to be an effective method for this purpose, since it may produce precise cavities corresponding to the diversity of guest molecules.[3] We have reported that TiO_2-gel films prepared by the surface sol-gel process act as superior matrices for molecular imprinting.[4] Structural discrimination of guest molecules has been achieved for aromatic carboxyl acids and protected amino acids in polar organic media. However, it has not been clear whether the TiO_2-gel can discriminate subtle structural changes of biologically active compounds under physiological conditions (aqueous solution). We report herein the first case of imprinting and binding of short peptides in TiO_2-gel matrices in aqueous media.



In our previous studies, template molecules were introduced into TiO_2-gel films through complexation with titanium *n*-butoxide ($Ti(O^nBu)_4$) in organic solvents.[4] Since most of small peptides are insoluble in organic solvents, we prepared imprinted films by alternate adsorption of $Ti(O^nBu)_4$ in organic solvent and of peptides in water. A gold-coated QCM resonator (9 MHz) modified with mercaptoethanol was immersed in 100 mM $Ti(O^nBu)_4$ solution (in toluene/ethanol, 1/1) for 3 min, and washed with ethanol. The electrode was then dipped in pure water for 1 min to promote hydrolysis and condensation of chemisorbed alkoxides, and dried by flushing with N_2. It was then immersed in 10 mM aqueous glycyltyrosine (Gly-Tyr, pH 7) for 10 min, washed in pure water for 1 min, and dried. The electrode was left in air after each drying procedure until the frequency become steady within 1 Hz. These procedures were repeated 10 times at 30 °C, and then $Ti(O^nBu)_4$ was adsorbed to form the outermost layer.

Results of QCM experiments are summarized in Table 1. TiO_2-gel/Gly-Tyr composite film regularly grew at least up to 10 cycles with frequency shifts of 37 ± 21 Hz for $Ti(O^nBu)_4$ and 42 ± 30 Hz for Gly-Tyr. The total film thickness was 14 ± 3 nm, as estimated from QCM data.[5] Results for other peptides are also listed in Table 1. In all cases, the average frequency change for $Ti(O^nBu)_4$ adsorption was in a range of 23–39 Hz. On the other hand, the frequency change was small for glycylglutamic acid (Gly-Glu, 18 Hz) and glycylglycine (Gly-Gly, 27 Hz), but was larger for glycylglycylglycine (Gly-Gly-Gly, 56 Hz).

Table 1. Alternate adsorption of $Ti(O^nBu)_4$ and peptides

Metal alkoxide	Peptide	Freqency decrease/Hz		Desorbed peptide
		Metal alkoxide	Peptide	
$Ti(O^nBu)_4$	Gly-Tyr	37 ± 21	42 ± 30	53%[a]
	Gly-Glu	23 ± 10	18 ± 6	78%[a]
	Gly-Gly	28 ± 10	27 ± 17	62%[b]
	Gly-Gly-Gly	39 ± 16	56 ± 22	45%[b]

[a]Template peptides were removed by NaOH treatment. [b]Template peptides were removed by HCl treatment.

Template peptides were removed carefully in order to avoid damaging of the TiO_2-gel matrix. In the case of Gly-Tyr, QCM electrode was immersed in 10 mM NaOH solution for 10 min, rinsed in pure water for 3 min, briefly dipped in dilute HCl (pH 5), and then rinsed in pure water for 1 min. These procedures were repeated three times. Frequency increase after removal of Gly-Tyr template is 222 Hz, corresponding to 53% of the initially introduced Gly-Tyr (420 Hz). In the case of Gly-Glu, 78% of the introduced peptide was desorbed. Complete removal of the templates was not possible under mild conditions. This was confirmed by FT-IR measurement. The TiO_2-gel/Gly-Glu film gave an amide peak at 1630 cm^{-1} and peaks of titanium/carboxylic acid complex at 1445 cm^{-1} and 1550 cm^{-1}. Though the peak intensities were reduced after alkali treatment, 30-40% of the template still remained in the film. Gly-Gly and Gly-Gly-Gly templates could not be extracted by alkali treatment. Instead, immersion in dilute HCl (pH 5) for 1 min, followed by rinsing with deionized water for 3 min was effective. Their desorption ratios were 62% and 45%, respectively. In spite of incomplete removal of the templates, TiO_2-gel films showed reproducible binding for guest molecules. Thus, it is possible to evaluate imprinting effects without interference of the unremovable peptides.

Molecular recognition of the imprinted films was evaluated by *in-situ* QCM measurement using a one-sided folder (USI System, Fukuoka) in order to stabilize frequency measurement in

water. An imprinted film on the electrode was immersed in 20 mL of deionized water with mild stirring. After the frequency became constant, 20 μL of guest solutions (10 mM in water) was added, and the frequency change was monitored. Figure 1a shows QCM frequency changes during adsorption of several peptide molecules on a Gly-Tyr imprinted film. After adding Gly-Tyr, the frequency quickly dropped by about 12 Hz, and the change was essentially saturated within a few min at 17 Hz (0.066 nmol).[6] In contrast, the frequency decrease was not observed for glycylphenylalanine (Gly-Phe) guest. Glycylvaline (Gly-Val) gave gradual frequency decrement a few min after the injection. Addition of glycylglycylalanine (Gly-Gly-Ala) produced a frequency drift. Relative binding efficiency corrected by molecular weights of peptides is 57% for Gly-Val relative to rebinding of template Gly-Tyr. The lack of Gly-Phe binding suggests that the Gly-Tyr templated site contain a sub-site that strongly interacts with the phenol hydroxyl group.

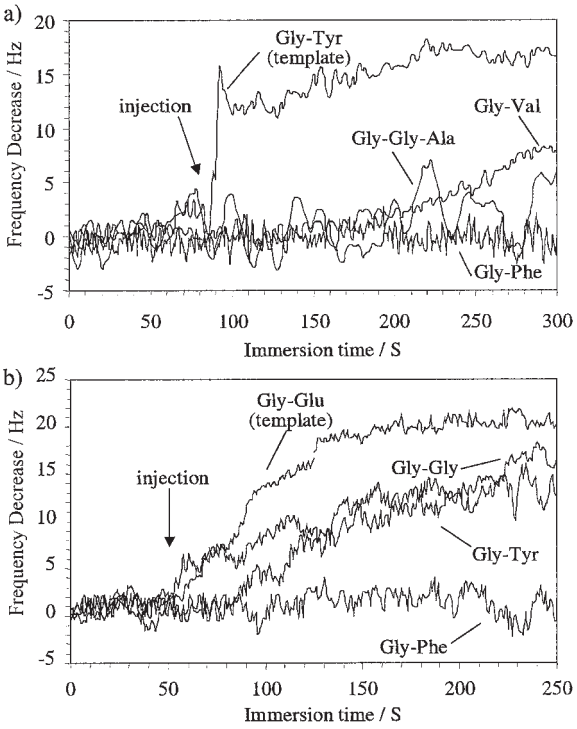


Figure 1. *In-situ* QCM frequency changes due to binding of peptides on Gly-Tyr imprinted (a) and Gly-Glu imprinted (b) TiO_2-gel films. Each guest molecule was injected at the points denoted by arrows. Concentration of the guest molecules is 10 μM, and pH is 7.0.

Selective recognition for a template molecule was also observed for a Gly-Glu imprinted film (Figure 1b). Rebinding of the template proceeded more slowly than the case of Gly-Tyr imprinted film and the binding saturation required more than 2 min. A possible explanation for this is retardation of the access of dissociated Gly-Glu at pH 7 to the negatively charged TiO_2-gel film. The binding efficiency of Gly-Tyr was 56% relative to rebinding of Gly-Glu template. Though frequency change was a little lower, small Gly-Gly molecule gave a binding efficiency close to that of the template. Adsorption of Gly-Phe was negligibly small.

As typically seen for Gly-Glu and Gly-Tyr imprinted films, peptide molecules with more than two hydroxyl groups exhibited high selectivities for the respective template. In contrast, TiO_2-gel films imprinted with peptides without functional side groups showed low selectivity. In fact, a Gly-Gly imprinted film gave frequency shifts of 2–3 Hz for both of the template and Gly-Tyr guest. Selectivity of a Gly-Gly-Gly imprinted film was still worse. Undoubtedly, the functional side groups of peptides improve recognition efficiency in addition to their shapes and sizes. An imprinted cavity containing a Gly-Glu template is illustrated schematically in Figure 2. TiO_2-gel can interact with the template molecule via metal coordination of carboxylate group, multiple hydrogen bonding, and electrostatic attraction. The flexibility and functional versatility of the TiO_2 network contribute to improved imprinting of multifunctional peptides.

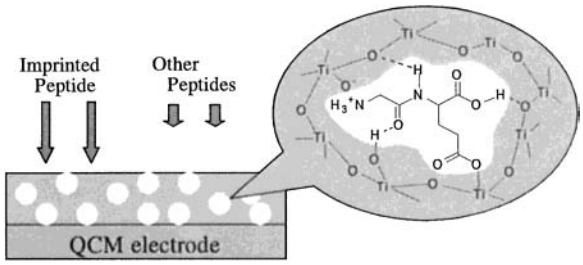


Figure 2. A schematic illustration of a Gly-Glu imprinted TiO_2-gel film.

It is noteworthy that imprinted TiO_2-gel films can detect 10 μM oligo-peptides in aqueous solution. Even though the QCM frequency is close to its precision limit, it is sufficient to confirm the selectivity. It is surprising that strong hydration of oligo-peptide molecules does not interfere with the selective binding. Low sensitivity of QCM measurement in water may limit its use under physiological conditions. Recently, Lahav *et al.* conducted detection of organic carboxylic acids by using a field-effect transistor (FET) device coated with a imprinted TiO_2-gel layer in a concentration range of 0.1 mM.[7] Such detection limit would be drastically improved, in the case of TiO_2-gel films imprinted with strongly binding oligo-peptides.

References and Notes

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5 Film thickness was calculated from the total QCM frequency shift (823 Hz) and the assumed density of $1,6 \pm 0.3$ g/cm^3 by using Sauerbery equation. The calculation procedure is given in following reference: I. Ichinose, H. Senzu, and T. Kunitake, *Chem. Mater.*, **9**, 1296 (1997).
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