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Molecular Recognition in a Supramolecular Hydrogel to Afford a Semi-Wet Sensor Chip

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Many artificial receptors and chemosensors have been developed for recent years, and their functions were intensely evaluated in homogeneous solution.[1] When these are attached to solid supports such as a polymer resin or an electrode, one frequently suffers from the fact that the excellent original function is greatly suppressed by such immobilization.[2] In two-dimensional immobilization of the receptors using monolayer films, on the other hand, very weak signals in response to guest-binding require special equipment in many cases.[3] These problems prevent a lot of potentially useful chemosensors from practical applications.[4]

Recently, we developed a semi-wet peptide/protein array using a supramolecular hydrogel.[5a] An intermediate property (so-called "semi-wet") of the hydrogel between aqueous solution and dry solid can entrap enzymes noncovalently and provide a suitable reaction medium to native enzymes. This finding stimulates us to envisage that artificial receptors can be immobilized in a gel matrix without loss of the molecular recognition capability displayed in solution. We describe herein that the molecular recognition features (the binding selectivity and affinity) of artificial chemosensors are practically retained even in the hydrogel. Furthermore, the miniaturization of the functionalized hydrogel produces a new molecular recognition chip useful for convenient and high-throughput assay to plural analytes.

For a gel matrix, we employed a supramolecular hydrogel consisting of a glycosylated amino acid type of hydrogelator **1**.[5b,c] Figure 1a shows an AFM image of the hydrogel of **1** spread on mica substrate. Many fibers with nanometer width are entangled to form spaces of micrometer-size that may be potentially used as a suitable cavity for molecular recognition. In addition, the present hydrogel is so transparent that spectroscopic analysis of the events inside the gel matrix can be readily carried out (Figure 1b).

We previously demonstrated in the case of another hydrogelator similar to **1** that the self-assembled fiber is stabilized through well-developed intermolecular hydrogen bonding networks and van der Waals packing of the hydrophobic rings.[5d] So as not to disturb the gel structure, we initially assumed that the key interaction in the artificial receptors should be orthogonal against interactions operated in the hydrogel fibers. Thus, an artificial receptor **2** was used which binds phosphate derivatives via coordination chemistry in aqueous solution.[6a,b] Fluorescence titration of **2** with phosphate in the hydrogel shows the increase in the fluorescence intensity (Figure 2a) in the manner similar to that in aqueous system. The titration curve (Figure 2b) displays the typical saturation that gave us an apparent binding constant of 1.1×10^5 M^{-1}, the value of which is only 3-fold smaller than that in aqueous solution. The titration experiments for various anions were conducted, and the results are summarized in Figure 2b. Apparently, the fluorescence enhancement occurs for phenyl phosphate (p-Ph: 6.2×10^4 M^{-1}) and phosphotyrosine (pY: 2.0×10^5 M^{-1}), and the affinity is scarcely different from that in aqueous solution. On the other hand, sulfate anion did not cause any considerable fluorescence change, indicating that the anion selectivity of **2** was in good agreement with that obtained in aqueous solution. For rapid and efficient sensing, the hydrogel was miniaturized and arranged into an array on a glass support (Scheme 1). The heated solution (10 μL) containing **1** and **2** was spotted on

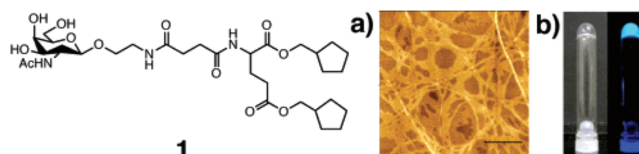


Figure 1. (a) An AFM image of a supramolecular hydrogel of **1** on mica. Scale bar: 1 μm. (b) Photograph of a supramolecular hydrogel of **1** containing **2** in the presence of phenyl phosphate (p-Ph) under natural light (left) or UV light irradiation (λ_{ex} = 365 nm) (right).

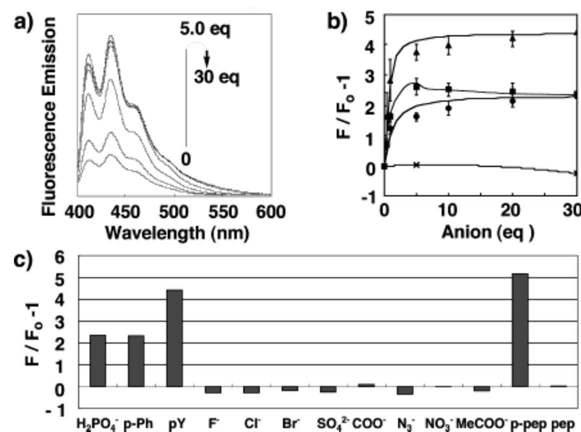
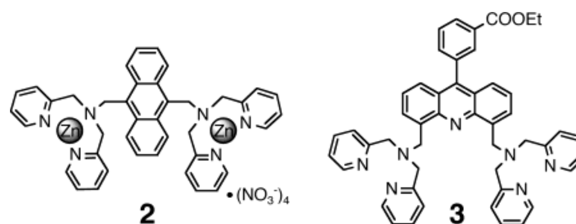


Figure 2. (a) Fluorescence spectral change (MCPD) of **2** (20 μM) upon the addition of phosphate embedded in the hydrogel **1**: $[H_2PO_4^-] = 0-600$ μM, λ_{ex} = 380 nm. (b) Fluorescence titration plots of **2** (λ_{em} = 435 nm) with various anions: p-Ph (●), pY (▲), phosphate (■), or sulfate (×). (c) The emission intensity change of a hydrogel array containing **2** by addition of various anions. Conditions: [**2**] = 40 μM, [anion] = 200 μM.



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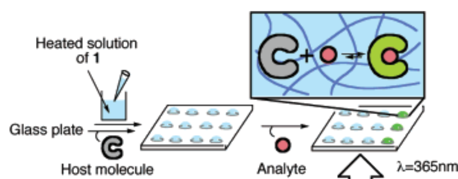
Scheme 1. Preparation Scheme of a Semi-Wet Sensor Array



a glass plate prior to gelation and stood for 1 h to yield a hydrogel array. Figure 2c shows a histogram of the fluorescence intensity change of the array by addition of various anions. Clearly, the fluorescence was intensified only in spots where phosphate and its derivatives (p-Ph, pY, and phosphorylated peptide (p-pep)) were injected. It is also noteworthy that, in this down-sized hydrogel chip, less than 1 μL is needed and the analytes diffuse rapidly in the gel matrix so that the spectral change reaches equilibrium within 15 min. Thus, we conclude that the hydrogel provides a suitable microenvironment for the immobilization of the artificial receptor along with retaining its recognition function.

High-throughput analysis for metal cations is also carried out when we use a semi-wet molecular recognition chip involving a fluorescent Zn^{2+} receptor **3**.[6c] Figure 3a shows a photo of the hydrogel chip after corresponding cations were put into each spot. Strong blue emission was observed only in two spots to which Zn(II) or Cd(II) solution is injected, whereas a very weak emission is observed in others containing Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cu^{2+}, Fe^{3+}, Co^{2+}, or Ni^{2+}. Because the present method does not require any chemical modification of chemosensors for immobilization, a wide variety of commercially available probes can be fixed by simple mixing with the hydrogel **1**. For instance, a Ca (II) probe (CG-2)[7] and a pH probe (SNARF-1)[8] are embedded in a supramolecular hydrogel array. For CG-2, strong green emission was observed in spots containing Ca^{2+}, Zn^{2+}, Cd^{2+}, Mg^{2+}, Co^{2+}, or Ni^{2+} (see Figure 3b) but any signal increase was not induced by other cations (Na^{+}, K^{+}, Cu^{2+}, or Fe^{3+}). In the case of a pH probe chip, the hydrogel color changes from orange to red as the pH shifts from 6.0 to 9.0 (in Figure 3c) due to a seesaw type of change in the two emission peaks at 585 and 644 nm (see Supporting Information). The behaviors observed in the receptor **3**, CG-2, and SNARF-1-embedded hydrogel are almost same as those in aqueous solution.

Based on the present supramolecular immobilization, an integrated molecular recognition chip may be readily prepared by use of many chemosensors bearing various selectivities for high-throughput assay.[9] As a proof-of principle experiment, the above-mentioned four probes were integrated to one glass plate (**2**, **3**, CG-2, and SNARF-1 from the top to bottom) and four distinct analytes, that is p-pep, Zn^{2+}, Ca^{2+}, and pH, were simultaneously analyzed from the mixed solution. Parts d and e of Figure 3 show the analyzed results for six kinds of mixtures including p-pep, Zn^{2+}, Ca^{2+}, and OH^{-} (i.e., pH) in different concentrations. When the mixed solution containing p-pep, Zn^{2+}, and Ca^{2+} at basic pH was put on the gel spots of the lane 2, four probes emit the corresponding fluorescence in response to the correct guest without being disturbed by other analytes, compared to lane 1. In lines 3−5, smear spots clearly correspond to the missing component (Ca^{2+}, Zn^{2+}, or p-pep respectively in lanes 3, 4, and 5) in the analyzed mixture. In the lane 6, the mixture containing Ca^{2+}, Zn^{2+}, and p-pep at neutral pH shows strong emission at the receptor **2**, **3**, and CG-2 and weak, orange emission of SNARF-1. These indicate that the integrated supramolecular sensor chip may be potentially applied to mixed-sample analysis without tedious isolation processes.

In conclusion, good coincidence of the binding selectivity and signal changes in the semi-wet hydrogel system with those in

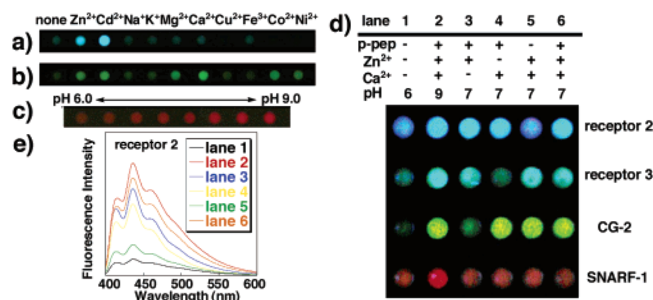


Figure 3. Photographs of sensing patterns of a semi-wet chemosensor chip containing (a) **3** (80 μM), (b) CG-2 (50 μM) in the presence of various metal cations, or (c) SNARF-1 (100 μM) at various pHs. (d) A photograph of an integrated molecular recognition hydrogel chip for mixed solution assay: **2** (20 μM), **3** (80 μM), CG-2 (50 μM including [EDTA] = 1 mM), or SNARF-1 (10 μM) from the top to bottom. Six mixed solutions including [p-pep] = 0 (−) or 30 μM (+), [$Zn(NO_3)_2$] = 0 (−) or 120 μM (+), [$Ca(NO_3)_2$] = 0 (−) or 1 mM (+), pH 7.0 or 9.0. These spectral changes are shown in Supporting Information.

solution system was demonstrated although apparent binding constants are slightly depressed. The present semi-wet supramolecular sensor chip may be expected to possess several advantages over the conventional systems, that is: (i) no covalent attachment of artificial receptors to solid matrix is required in contrast to the polymer-based system, (ii) molecular recognition function can be retained under the semi-wet conditions, (iii) miniaturization and array-types of arrangement of the molecular recognition hydrogel are feasible for high-throughput sensing in many analytes, (iv) since the microspaces constructed by the gel network are accumulated in a 3D manner, the signal/noise ratio is greatly improved relative to the 2D systems so that the change can be easily distinguished with either the naked eye or a simple digital camera.

Acknowledgment. This paper is dedicated to the occasion of the 60th birthday of Prof. Seiji Shinkai.

Supporting Information Available: Fluorescence spectra of chemosensors in the chip and experimental protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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