Hybrid Hydrogels to Which Single-Stranded (ss) DNA Probe Is Incorporated Can Recognize Specific ssDNA

Yoshihiko Murakami[†] **and Mizuo Maeda***

Bioengineering Laboratory, RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako-Shi, Saitama 351-0198, Japan

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Hydrogels, which are three-dimensional cross-linked polymer networks that swell, but are insoluble in water, have various functional properties, such as the ability to absorb a significant amount of water and flexibility similar to a natural tissue. These properties have provided many potential applications, particularly in biotechnological and medical fields. In particular, stimuli-responsive hydrogels, which undergo volume transitions in response to physical or chemical changes in their environment, have been of great interest.[1] These intelligent materials may be suitable for designing devices for bioseparation,[2] biosensing,[3−5] actuation,[6,7] and controlled drug release.[8−10] To date, various hydrogels that are responsive to stimuli, such as changes in temperature,[11] pH,[12] ion concentration,[13] light,[14] and electric fields,[15] have been reported.

Among these stimuli-responsive hydrogels, noncovalently formed hybrid hydrogels containing synthetic polymers and biologically active molecules have shown interesting properties. The binding between an antigen and an antibody, for example, can introduce irreversible[3] or reversible[4] cross-links in the hydrogels. Since the competitive binding of a free antigen with an immobilized antibody results in a decrease in the number of cross-links, these hydrogels swell in the presence of a free antigen. On the other hand, hybrid hydrogels containing synthetic water-soluble polymers and recombinant protein modules are pH- and temperature-responsive, according to the properties of cross-linking protein modules in terms of structure, mechanical strength, and folding/unfolding processes.[16−19] These properties are useful in designing well-characterized pH- and temperature-responsive biomaterials.

We report here two novel hybrid hydrogels that can recognize single-stranded (ss) DNA. Our strategy was to incorporate ssDNA (as a probe to recognize a ssDNA sample) into polyacrylamide (polyAAm) hydrogels in directly grafting or semi-interpenetrating network (semi-IPN) manners. Three hydrogels, i.e., a hydrogel containing directly grafted ssDNA (Figure 1a-1), a hydrogel containing a ssDNA−polyAAm conjugate in a semi-IPN manner (Figure 1a-2), and a conventional polyAAm hydrolgel as a control (Figure 1b), were synthesized in this study (Figure 2).

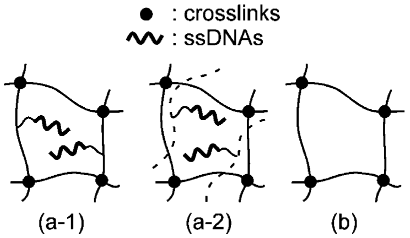

Figure 1. (a-1) Hydrogel containing directly grafted ssDNA, (a-2) hydrogel containing ssDNA−polyAAm conjugate in semi-IPN manner, and (b) conventional polyAAm hydrogel as control.

Methacryloyloxysuccinimide (MOSu) was synthesized from methacrylic acid and *N*-hydroxysuccinimide and purified. 5′-Aminoalkyl-modified ssDNA (5′-GCCACCAGC-3′, 0.65 μmol) was dissolved in 650 μL of 50 mM $Na_2CO_3/NaHCO_3$ buffer solution at pH 9.0, and then 150 μL of dimethyl sulfoxide containing MOSu (32.5 μmol) was added to the ssDNA solution. The mixture was incubated at 25 °C for 12 h. 5′-Methacryloyl-modified ssDNA was consequently obtained after purifying the mixture by reversed-phase high-performance liquid chromatography. The ssDNA−polyAAm conjugate was synthesized in 10 mM Tris-HCl buffer solution (pH 7.4) containing 5′-methacryloyl-modified ssDNA (0.6 mM), AAm (100 mM), ammonium persulfate (APS, 3 mM), and *N,N,N′,N′*-tetramethylethylenediamine (TEMED, 6 mM) at 25 °C for 18 h under a nitrogen atmosphere. The conjugate was then purified by gel filtration, dialysis, and lyophilization. The hydrogel containing directly grafted ssDNA and the hydrogel containing the ssDNA−polyAAm conjugate in a semi-IPN manner were synthesized by polymerizing AAm (1.40 M, i.e., 10% w/w of total reaction volume) in the presence of 5′-methacryloyl-modified ssDNA (0.07 mM) and the ssDNA−polyAAm conjugate (0.07 mM), respectively, using *N,N′*-methylenebis(acrylamide) (MBAAm, 3.24 mM, i.e., 0.5% w/w with respect to AAm) as a cross-linker, APS (4 mM), and TEMED (40 mM) in 10 mM Tris-HCl buffer solution (pH 7.4, 20 μL) in a Durham tube with an inner diameter of 3 mm at 25 °C for 24 h. The conventional polyAAm hydrogel was synthesized under the same conditions, except that 5′-methacryloyl-modified ssDNA and the ssDNA−polyAAm conjugate were not used. All the hydrogels were sliced with a length of 1 mm and immersed in water to remove residual chemicals and unreacted monomers.

The ssDNA responsiveness of these hydrogels was determined by the change in their swelling ratio induced by adding a ssDNA sample (the concentration of which was 10 equiv to that of the ssDNA probe in a hydrogel) to the 100 mM $MgCl_2$/10 mM Tris-HCl buffer solution (pH 7.4) in which each hydrogel was immersed. The ssDNA samples used were 5′-GCTGGTGGC-3′ (complementary to the ssDNA probe in the hydrogels), 5′-GCTGATGGC-3′ (one base mismatch to the ssDNA probe in the hydrogels), and 5′-GGCGTGTGC-3′ (uncomplementary to the ssDNA probe in the hydrogels). All experiments were performed in triplicate. The digital images of hydrogels using an optical microscope were quantitatively analyzed by graphic software. The d and d_0 were accurately determined by magnifying the images with four significant figures. The swelling ratio of the hydrogels, $(d/d_0)^3$, was determined from their diameter ratio, d/d_0, where d and d_0 were diameters of hydrogels with and without the ssDNA sample, respectively.

[†] Present address: Yokoyama Project, Kanagawa Academy of Science and Technology (KAST), KSP East 404, Sakado 3-2-1, Takatsu, Kawasaki, Kanagawa 213-0012, Japan.

* Corresponding author: e-mail mizuo@riken.go.jp; Fax +81-48-462-4658.

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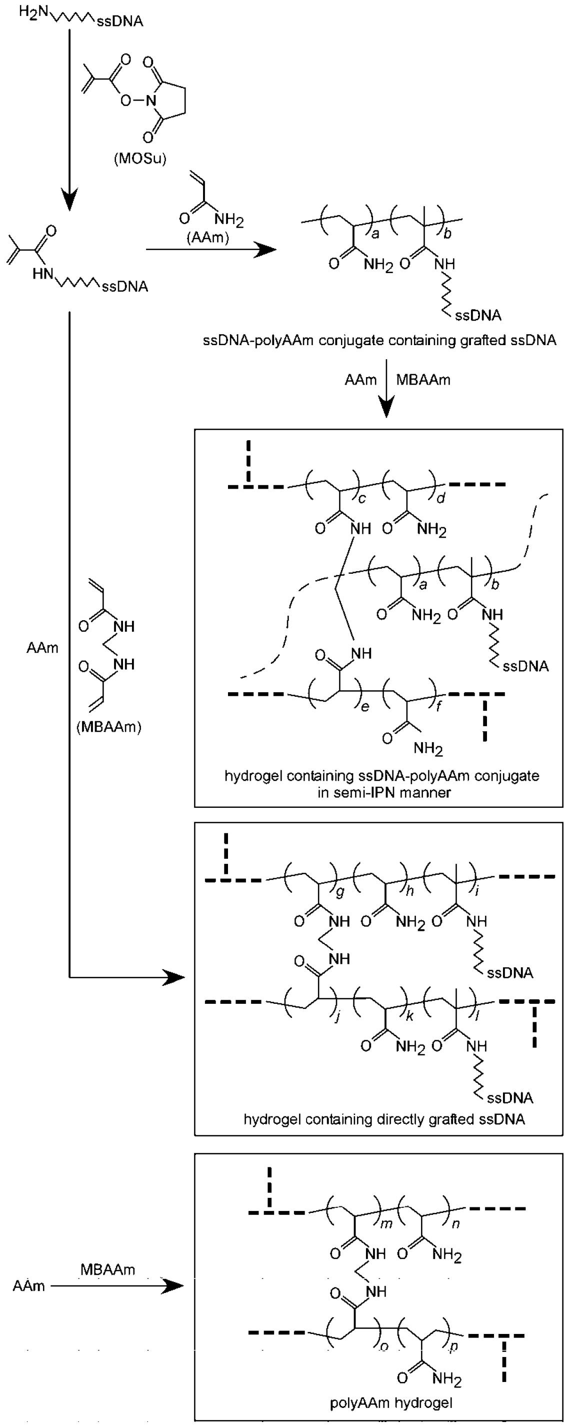

Figure 2. Strategy for the synthesis of hydrogels used in this study.

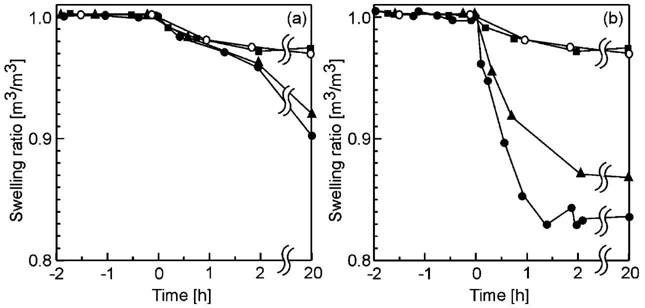

Figure 3. ssDNA responsivieness of hydrogels containing (a) directly grafted ssDNA and (b) ssDNA−polyAAm conjugate in semi-IPN manner. The ssDNA targets used were 5′-GCTGGTGGC-3′ (complementary to the ssDNA probe in hydrogels, closed circles), 5′-GCTGATGGC-3′ (one base mismatch to the ssDNA probe in hydrogels, closed triangles), and 5′-GGCGTGTGC-3′ (uncomplementary to the ssDNA probe in hydrogels, closed squares). The open circles show the case when ssDNA, 5′-GCTGGTGGC-3′, was added to a conventional polyAAm hydrogel.

The ssDNA samples were added to the buffer solution where the swelling of hydrogels attained equilibrium (Figure 3). The polyAAm hydrogel slightly shrunk when the ssDNA sample was added due to the change in osmotic pressure outside the hydrogel (open circles in Figure 3a,b). This phenomenon is presumably the same as that reported by Miyata et al. for the case in which a free antigen was added to a solution containing a polyAAm hydrogel.[3] On the other hand, since ssDNAs was not used as cross-linkers in our hydrogels, the hybridization between ssDNA sample and ssDNA probe might not induce the change in the structure of the hydrogels. We thus predicted that the ssDNA responsiveness of the hydrogel containing the directly grafted ssDNA and that containing the ssDNA−polyAAm conjugate in a semi-IPN manner were the same as that of the conventional polyAAm hydrogel. Interestingly, however, these two hydrogels showed the following DNA-sensitive properties: these hydrogels (1) shrunk in response to the ssDNA sample complementary to the ssDNA probe in the hydrogels, (2) recognized the one-base difference of the ssDNA sample, and (3) did not respond to the ssDNA sample uncomplementary to the ssDNA probe in the hydrogels. These results strongly suggest that the hydrogels to which the ssDNA probe was incorporated are responsive to complementary ssDNA samples; i.e., the hybridization between samples and probes greatly affects the change in the swelling ratio of hydrogels.

The results in Figure 3 also show that the hydrogel containing the ssDNA−polyAAm conjugate in a semi-IPN manner is more highly ssDNA-responsive than that containing directly grafted ssDNA. This indicates that the mobility freedom of ssDNA probe molecules is important for the responsiveness of hydrogels. The ssDNA probe was directly immobilized on the AAm main chain in the hydrogel containing the directly grafted ssDNA, while it was immobilized on the freely soluble polymer in the hydrogel containing the ssDNA−polyAAm conjugate in a semi-IPN manner, as shown in Figure 1. The high flexibility of the ssDNA probe molecule probably resulted in the high efficiency of DNA hybridization. The same phenomenon was also observed for the difference in DNA hybridization rate of ssDNA probe immobilized on DNA microarrays in comparison with that suspended in a solution (e.g., in one operation for DNA microarrays, a protocol calls for 15 h only for hybridization before washing, staining, and imaging,[20] while DNA hybridization sensing using a soluble ssDNA probe in capillary electrophoresis requires only 30 min[21]).

Thermodynamic study showed that the formation of DNA duplex is mostly accompanied by the uptake of structural water molecules, and so, the overall hydration of a duplex is mainly determined by its conformation and stability.[22,23] In addition, DNA duplex formation leads to the uptake of counterions into the hydrogels. These phenomena may lead to the “swelling” of the hydrogel in response to ssDNA samples. Hydrogels containing directly grafted ssDNA or the ssDNA−polyAAm conjugate in a semi-IPN manner, however, "shrunk" in response to the ssDNA samples. The rational explanation for this phenomenon is still unclear at present; however, one possible explanation is as follows. A recent report showed that a highly chemiluminescent reporter molecule, acridinium ester (AE), is readily hydrolyzed by water or hydroxide ion, when AE is tethered to ssDNA. The hydrolysis of the AE is, however, markedly inhibited, when ssDNA containing AE hybridized to a complementary ssDNA. Furthermore, mismatches near AE eliminate the ability of the double helix to strongly inhibit AE hydrolysis. These suggest that double-stranded (ds) DNA is dehydrated due to a low water activity within the minor groove of the helix compared to the case of ssDNA.[24] Thus, in our case, dsDNA (formed by the ssDNA probe in the hydrogels and the ssDNA sample) was dehydrated following the addition of the ssDNA target, and consequently water diffuses from the inside to the outside of the hydrogels. This phenomenon may induce the shrinking of hydrogels due to addition of the ssDNA sample. It was very interesting that the hydrogels shrunk even though there were many factors to lead to their swelling.

Our novel hydrogels shrunk in response to the ssDNA sample complementary to the ssDNA probe in the hydrogels. The change in the swelling ratio of hydrogels containing the ssDNA–polyAAm conjugate in a semi-IPN manner at equilibrium was approximately 17%. This is of the same order of magnitude as those previously reported for molecule-sensitive hydrogels.[3–5] On the other hand, a variety of DNA-sensing technologies have been reported including electrochemical DNA biosensors with a high sensitivity and responsiveness.[25,26] The limitation of our novel technology may be caused by a decrease in responsiveness upon the increase in the sample length because of a decrease in diffusion rate of ssDNA sample into hydrogels. The utilization of a porous hydrogel structure developed by freeze-drying to enhance the diffusion of the ssDNA sample[27] is one possible strategy to enhance the ssDNA responsiveness of the hydrogel. Although further work concerning the mechanism of the ssDNA responsiveness of the hydrogels proposed in the present study is required, the interesting results obtained indicate that the novel biomaterials can be used for DNA sensing, nucleotide-triggered drug delivery system, and other applications.

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