PREPARATION OF BIOMOLECULE-RESPONSIVE GELS BY BIOMOLECULAR IMPRINTING

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Introduction

Stimuli-responsive gels that undergo volume changes in response to environmental changes such as pH and temperature are smart material candidates for drug delivery systems and sensors in biomedical fields [1]. We have prepared biomolecule-responsive gels that can sense specific signal biomolecules such as glucose and protein, by using biomolecular complexes as reversible cross-linking points in gel networks [2-9]. Molecular imprinting is a promising method to create synthetic hosts having molecular recognition sites. In molecular imprinting, monomers having functional ligand groups for target (template) molecules are copolymerized with a large amount of cross-linkers while functional groups interact with template molecules to arrange ligands at suitable positions for recognizing target molecules. In this paper, we reveal that proteins and DNAs can be used as ligands in molecular imprinting and that using minute amounts of cross-linker enables resultant gels to undergo volume changes in response to a target biomolecule.

In this study, the biomolecule-responsive gels that exhibited volume changes in response to a tumor-specific marker glycoprotein and a target DNA were prepared by biomolecular imprinting using proteins and DNAs as ligands. We report dynamic biomolecule recognition of glycoprotein-imprinted gels having lectin and antibody molecules as ligands for a target tumor marker glycoprotein and DNA-imprinted gels having DNAs as ligands for a target DNA. This paper focuses on biomolecule-responsive behavior of the biomolecule-imprinted gels prepared with minute amounts of cross-linker.

Experimental Part

Preparation of tumor marker-imprinted gels

α-Fetoprotein (AFP) is a diagnostic tumor-specific marker glycoprotein widely used for the serum diagnosis of primary hepatoma. Tumor-marker-responsive gels can be strategically prepared by the biomolecular imprinting of lectins and antibodies as ligands for saccharide and peptide chains in target glycoproteins; association and dissociation behaviors of lectin-AFP-antibody complexes

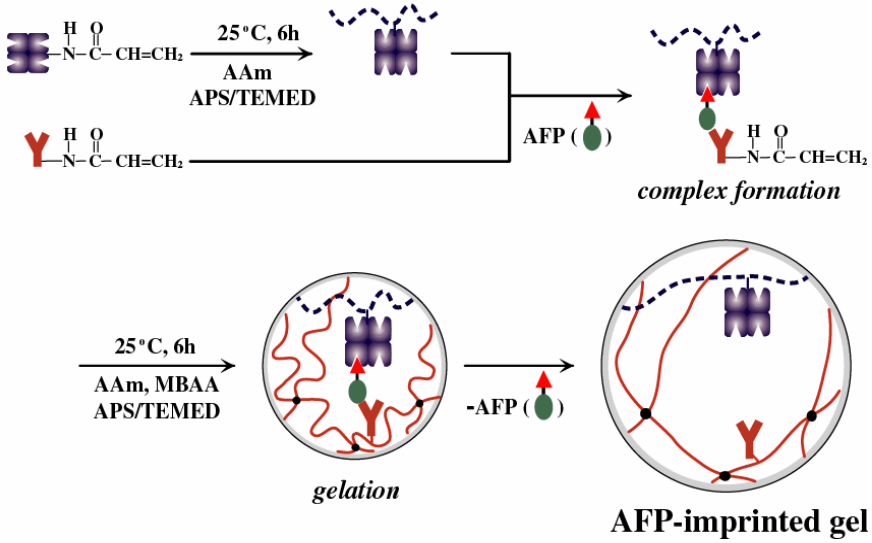

Figure 1. Synthesis of the tumor-marker-imprinted gels by using lectins and antibodies as ligands for template glycoprotein molecules.

in reversible cross-linking reactions cause AFP-responsive volume changes.

Bioconjugated gels containing lectins and antibodies to recognize saccharide and peptide chains in tumor specific marker glycoprotein (α-fetoprotein (AFP)) were prepared by biomolecular imprinting as follows (Figure 1): Lectin (concanavalin A; Con.A) and antibody (anti-AFP) were conjugated with *N*-succinimidylacrylate (NSA) to introduce polymerizable groups. After poly(acrylamide)(PAAm)-grafted lectins were synthesized by the copolymerization of acryloyl-lectin with AAm, acryloyl-antibodies were copolymerized with acrylamide (AAm) and *N, N'*-methylenebisacrylamide (MBAA) in the presence of template AFP and PAAm-grafted lectins to form lectin-AFP-antibody complexes. AFP-imprinted gels were prepared by removing AFP from resultant networks having lectin-AFP-antibody complexes. Nonimprinted gels were also prepared by copolymerizing acryloyl-antibodies with AAm and MBAA in the presence of PAAm-grafted lectin without template AFP.

Preparation of DNA-imprinted gels

5'-Aminoalkyl-modified DNA1 (3'-CACGCGCC-5') and DNA2 (3'-GGCGGACC-5'), which were complementary to a half sequence of a target DNA (3'-GGTCCGCCGGCGCGTG-5'), were chemically modified by coupling them with NSA in a Tris buffer solution to synthesize acryloyl-DNAs having a polymerizable group. A buffer solution containing the acryloyl-DNA1 and acryloyl- DNA2 was mixed with that of the target DNA as a template to form the DNA duplex (Figure 2). Then, DNA-imprinted gels were prepared by removing the template DNA from the network after the DNA duplexes having polymerizable groups were copolymerized with AAm and MBAA using redox initiators. Furthermore, nonimprinted gels were also prepared in a similar manner except without the addition of a template DNA.

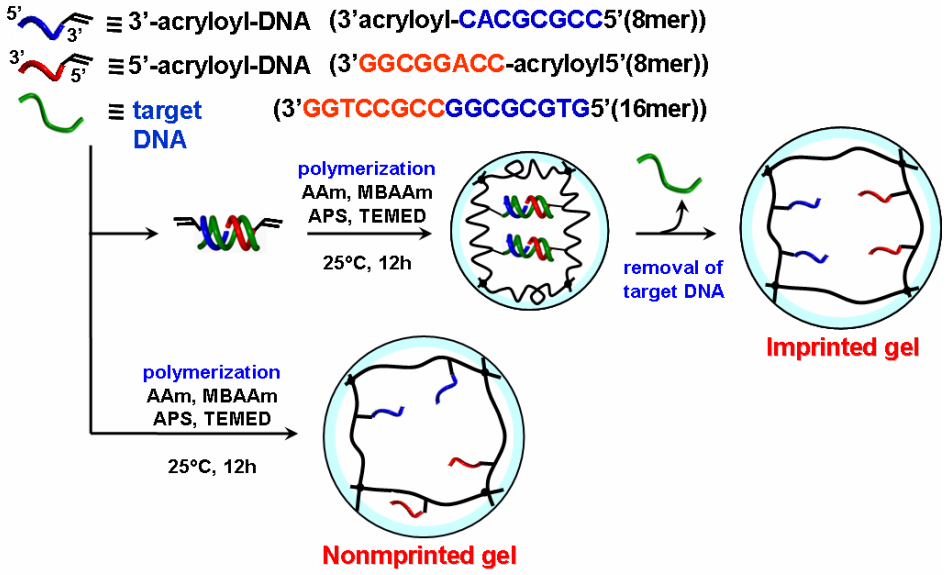

Figure 2. Preparation of DNA-imprinted and nonimprinted gels.

Swelling Measurements.

Bioconjugated gels were kept immersed in a buffer solution until equilibrium was reached at 25°C. After that, the gels were transferred and kept immersed in a buffer solution containing a desired amount of a target biomolecule at 25°C. The swelling ratio of the gels was determined from the ratio of their diameters by equation (1). The diameters of the gels swollen in a buffer solution (d_0) and a buffer solution containing a target biomolecule (d) were measured using an optical microscope.

$$Swelling\ ratio = \frac{V}{V_0} = \left(\frac{d}{d_0}\right)^3 \quad \cdots\cdots \quad (1)$$

Results and Discussion

Tumor Marker-Responsive gels.

AFP-responsive behaviors of AFP-imprinted, nonimprinted and PAAm gels were examined after swelling equilibria were attained in a phosphate buffer solution (pH 7.4). AFP-imprinted gels began to shrink as soon as they were immersed in phosphate buffer solutions containing AFP. Equilibrium swelling ratios of AFP-imprinted gels gradually decreased with increases in the AFP concentration of buffer solutions. In aqueous AFP solutions, however, PAAm gels did not exhibit volume changes and the nonimprinted gels experienced slight swelling. The compressive modulus measurements revealed that cross-linking densities of the AFP-imprinted gel gradually increased with increasing AFP concentrations in phosphate buffer solutions, but those of the nonimprinted and PAAm gels did not change at all. Lectins and antibodies in AFP-imprinted gels were distributed at optimal positions for the simultaneous recognition of AFP saccharide and peptide chains because gel networks were formed by biomolecular imprinting using AFP molecules as templates. Therefore, the presence of target AFP induced the formation of lectin-AFP-antibody complexes that could play a role at cross-linking points.

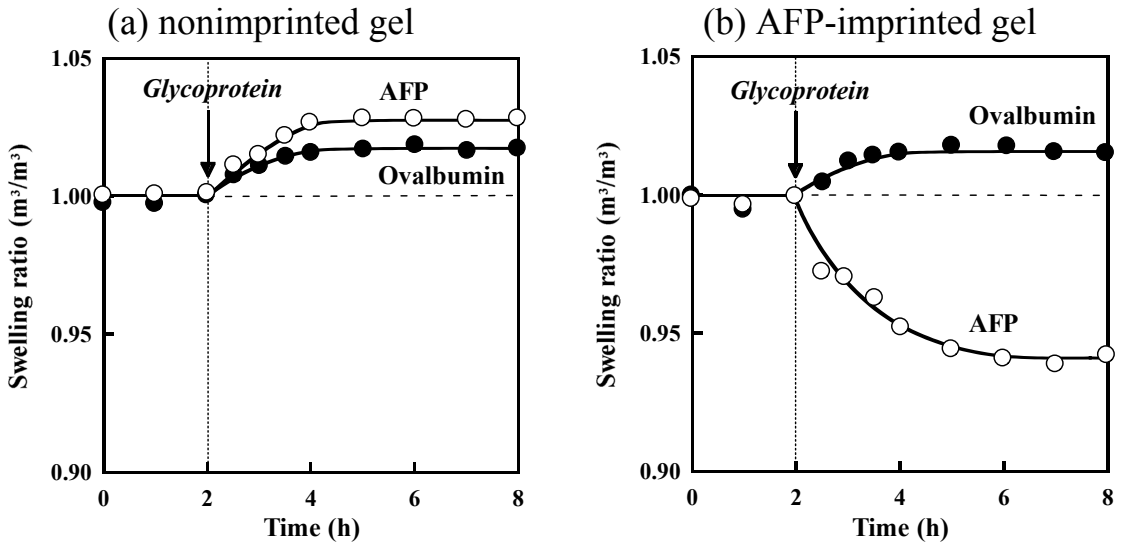

Figure 3. Swelling ratio changes of (a) nonimprinted and (b) AFP-imprinted gels following the addition of AFP (○)) and ovalbumin (●) after swelling had attained equilibrium in phosphate buffer solution at 25 ^{o}C.

To evaluate glycoprotein recognition function of AFP-imprinted gels, we examined swelling ratios of AFP-imprinted and nonimprinted gels in the presence of AFP or ovalbumin. Ovalbumin has a saccharide chain similar to AFP, but has a peptide chain different from AFP. Therefore, ovalbumin is recognized by the lectin ligand (Con.A), but not by the antibody ligand (anti-AFP) in AFP-imprinted and nonimprinted gels. The nonimprinted gels swelled slightly in the presence of AFP and ovalbumin (Figure 3 (a)). On the other hand, AFP-imprinted gels also swelled slightly in the presence of ovalbumin, but immediately shrank in the presence of AFP (Figure 3 (b)). These demonstrated that AFP-imprinted gels only shrank when both lectins and antibodies in the gels simultaneously recognized the saccharide and peptide chains of the target glycoprotein (Figure 4). Therefore, swelling or shrinking behaviors of AFP-imprinted gels in the presence of glycoproteins enable the accurate detection and recognition of glycoproteins with a double-lock function. Thus, tumor-marker-imprinted gels have a high potential in biomedical applications and may be used as sensor devices, since they can recognize a target glycoprotein and induce structural changes.

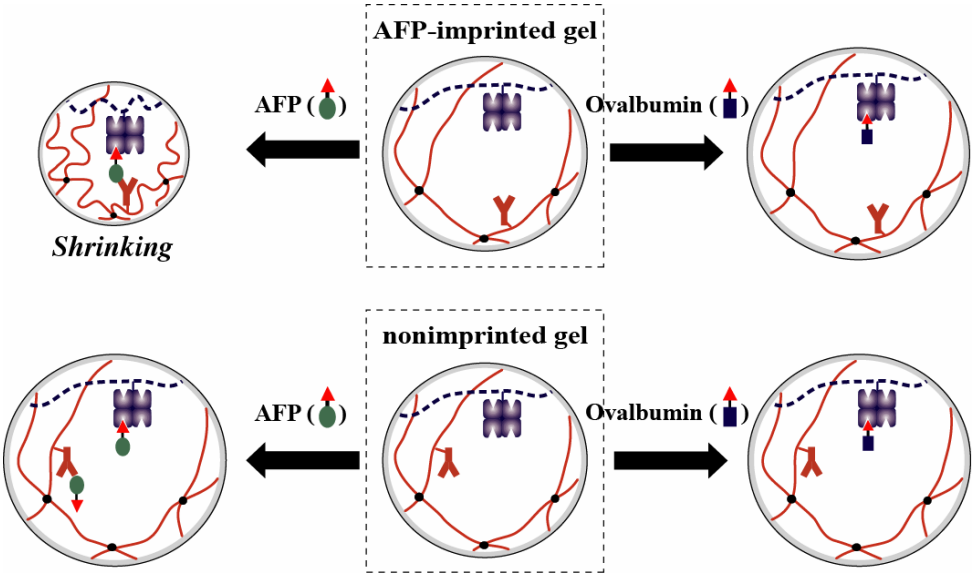

Figure 4. A schematic representation of glycoprotein-responsive behavior of tumor-marker-imprinted and nonimprinted gels.

DNA-responsive gels.

Sequence-specific DNA detection has been a topic of significant interest because of its application in the diagnosis of pathogenic and genetic diseases. Especially, an effective DNA-sensing system requires selectivity to sense single-nucleotide polymorphisms (SNPs), and sensitivity to detect a small number of copies of DNA. Many detection techniques have been developed which rely upon target hybridization with radioactive, fluorescent, and other types of labeled probes. Therefore, we tried to prepare DNA-responsive gels that can sense a sequence of a target DNA for developing smart devices for sequence-specific DNA detection.

Figure 5 shows swelling ratio changes of DNA-imprinted, nonimprinted and PAAm gels in a buffer solution containing a target DNA. DNA-imprinted and nonimprinted gels shrank immediately in the presence of target DNA, but PAAm gel did not. Furthermore, swelling ratio of the DNA-imprinted gel decreased more dramatically than that of nonimprinted gel. To reveal the mechanism responsible for the DNA-responsive behavior of the gels, cross-linking densities were determined by measuring compressive modulus. Cross-linking densities of the DNA-imprinted gel in the presence of a target DNA was higher that that in its absence. Furthermore, DNAs as ligands in the DNA-imprinted gels were distributed at optimal positions for the simultaneous recognition of target DNA because gel networks were formed by biomolecular imprinting using target DNA as template. These suggest that the DNA-responsive shrinking of the DNA-imprinted gels was caused by increased cross-linking density (Figure 6).

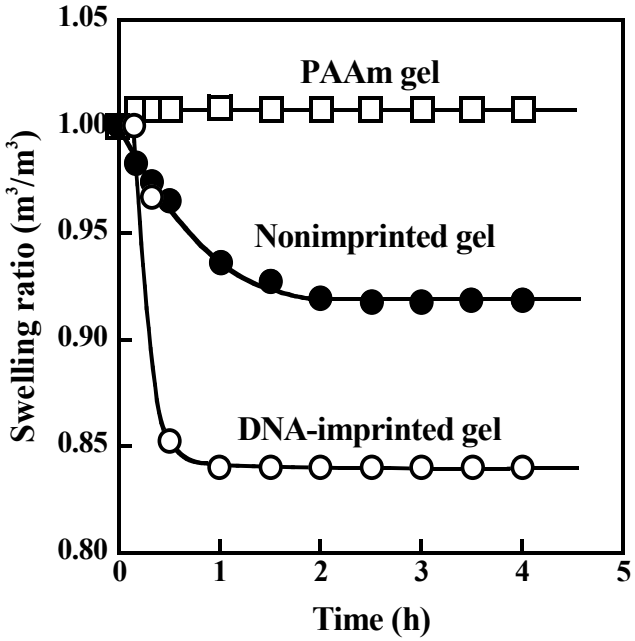

Figure 5. Time course of swelling ratio for DNA-imprinted gel (○), nonimprinted gel (●) and PAAm gel (□) in the presence of target DNA in 10mM Tris HCl-150mM NaCl buffer(pH 7.4) at 25°C.

We also investigated the swelling ratio of DNA-imprinted gels in a buffer solution containing DNAs having various sequences to evaluate their sequence recognition function. The swelling ratio of DNA-imprinted gels was strongly dependent upon the sequence of DNA in a buffer solution. Thus, as the DNA-imprinted gels can sense SNPs and induce their structural changes, they may prove useful in diagnosing genetic diseases that contain nucleotide mutations

As shrinking behavior of biomolecule-imprinted gels in response to target biomolecules enables the accurate detection and recognition of tumor marker glycoproteins and DNAs, they have many potential applications as smart devices in sensing systems and as molecular diagnostics.

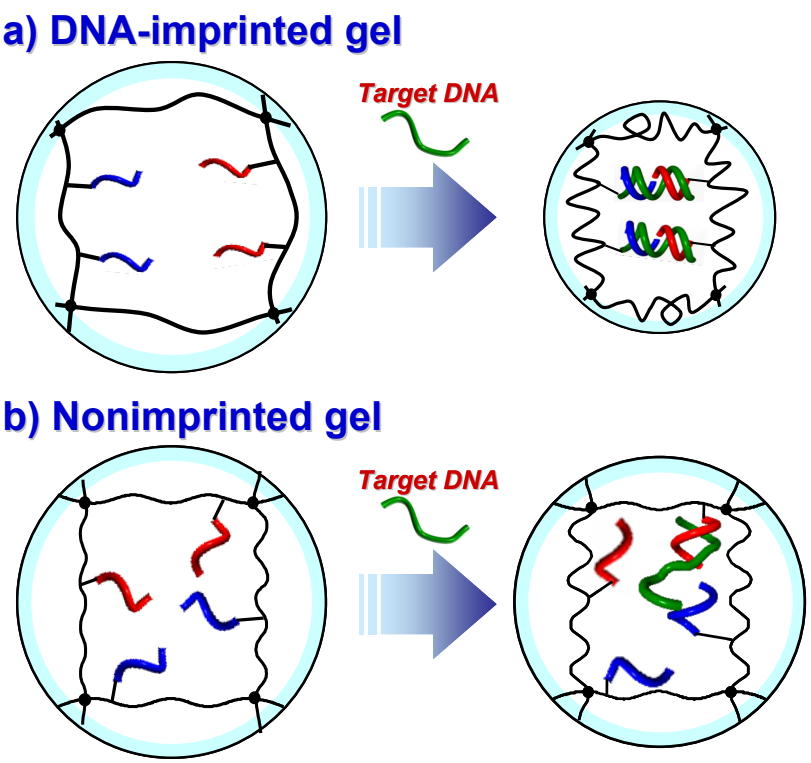

Figure 6. A schematic representation of DNA-responsive behavior of DNA-imprinted and nonimprinted gels

Acknowledgement. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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