

# BIOMOLECULE-RESPONSIVE BEHAVIOR OF SMART GELS HAVING BIOMOLECULAR COMPLEXES AS REVERSIBLE CROSS-LINKS

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## Introduction

Stimuli-responsive gels undergo abrupt changes in volume in response to environmental changes such as pH and temperature. Stimuli-responsive gels have attracted considerable attention as smart materials in the biochemical and biomedical fields, since they can sense environmental changes and induce structural changes by themselves. The fascinating properties of such stimuli-responsive gels suggest that they have many future opportunities as suitable materials for the design of smart biomaterials and self-regulated drug delivery systems [1]. Furthermore, several researchers focused on biologically stimuli-responsive gels that can respond to glucose for constructing self-regulated insulin delivery systems [2-6]. Recently, biomolecule-responsive gels that exhibit swelling/shrinking changes in response to specific signal biomolecules like tumor-specific markers have become increasingly important as smart devices for drug delivery systems and molecular diagnostics.

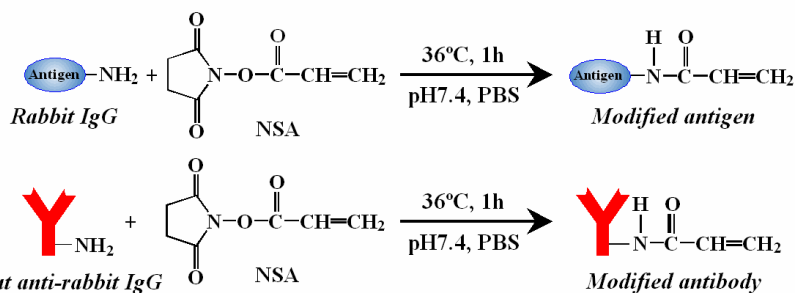
Stimuli-responsive behavior of most gels reported previously was mainly based on changes in affinity of polymer chains for solvents or by changes in their charged groups. However, the cross-linking structure of a gel is also an important factor to determine its swelling behavior. This led us to a strategy that novel biomolecule-responsive gels can be prepared by using stimuli-responsive complexes as reversible cross-linking points. Based on this strategy, we have prepared two types of biomolecule-responsive gels (biomolecule-cross-linked gel and biomolecule-imprinted gel) by using biomolecular complexes as reversible cross-linking points [7-9]. This paper describes biomolecule-responsive behavior of biomolecule-cross-linked gels that were prepared using biomolecular complexes such as antigen-antibody complexes and DNA duplexes.

## Experiments

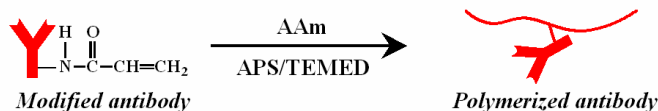
### Preparation of antigen-antibody cross-linked gels

Bioconjugated gels having antigen-antibody bindings as cross-linking points were prepared as follows (Figure 1): An antigen (Rabbit IgG) and an antibody (goat anti-rabbit IgG)

### 1) Introduction of vinyl groups into antigen and antibody



### 2) Copolymerization of vinyl-antigen and acrylamide



### 3) Preparation of an antigen-antibody semi-IPN gel

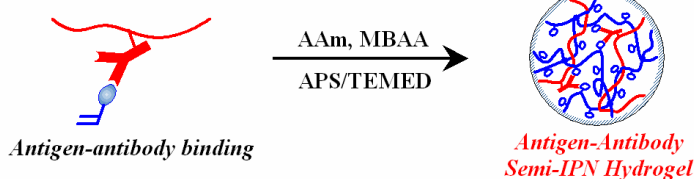


Figure 1. Preparation of antigen-responsive gels.

were modified using *N*-succinimidylacrylate (NSA) for the introduction of acryloyl groups. The resultant acryloyl-antibody was copolymerized with acrylamide (AAm) using redox initiators to synthesize the polymerized antibody. Antigen-antibody gels having a semi-interpenetrating polymer network (semi-IPN) structure were prepared by the copolymerization of the acryloyl-antigen, AAm and *N,N'*-methylenebisacrylamide (MBAA) in the presence of the polymerized antibody.

### Preparation of DNA-cross-linked gels

5'-Aminoalkyl-modified ssDNA (5'-GCGCTGGCC-3') and 5'-aminoalkyl-modified ssDNA (5'-GGCCTGCGC-3') having its one base mismatch sequence were chemically modified by coupling them with NSA in a Tris buffer solution to synthesize ssDNAs having a acryloyl group (Figure 2). A buffer solution of the acryloyl-ssDNA was mixed with that of the acryloyl-ssDNA having one base mismatch sequence to form the DNA duplex having acryloyl groups. Then, bioconjugated gels having DNA duplexes at cross-linking points (DNA-cross-linked gels) were prepared by the copolymerization of DNA duplex having acryloyl groups, acrylamide (AAm) and MBAA using redox initiators.

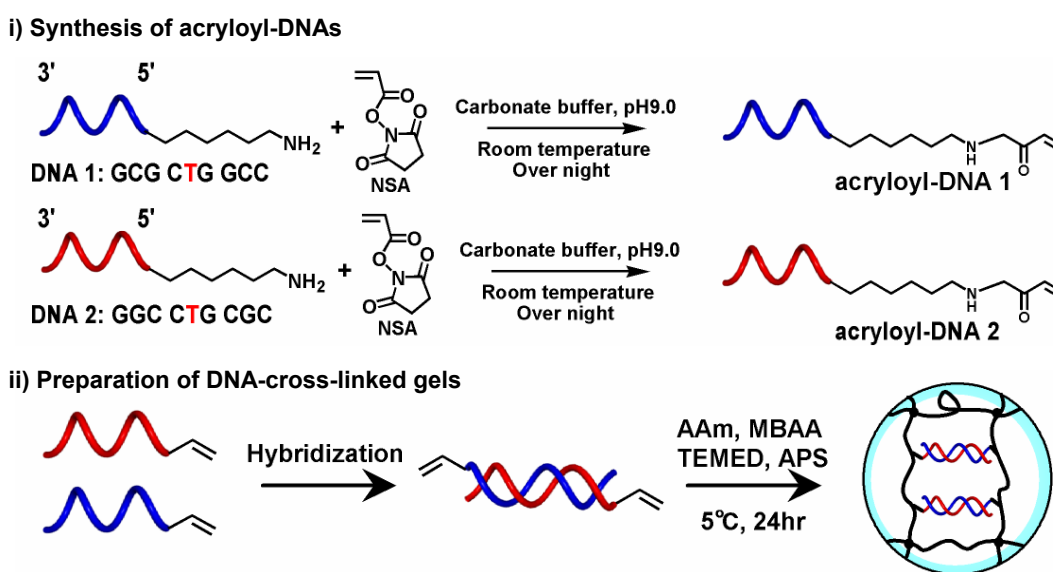


Figure 2. Preparation of DNA-cross-linked gels.

### 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.

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

## Results and Discussion

### Antigen-Responsive gels.

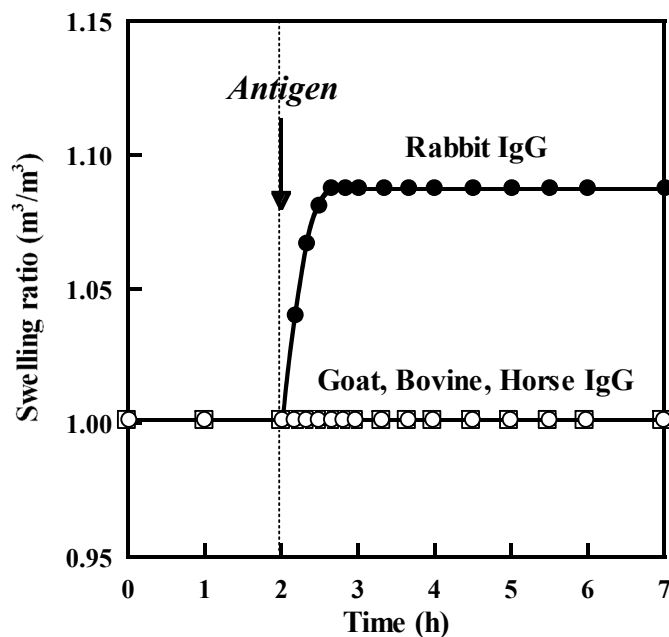
Swelling ratio of the antigen-antibody semi-IPN gels was measured in a phosphate buffer solution containing various proteins. The swelling ratio of the antigen-antibody semi-IPN gels did not change by the addition of goat, bovine and horse IgG, whereas addition of rabbit IgG led to a drastic increase

(Figure 3). This result means that the gel can recognize a specific antigen and change its structure chemo-mechanically.

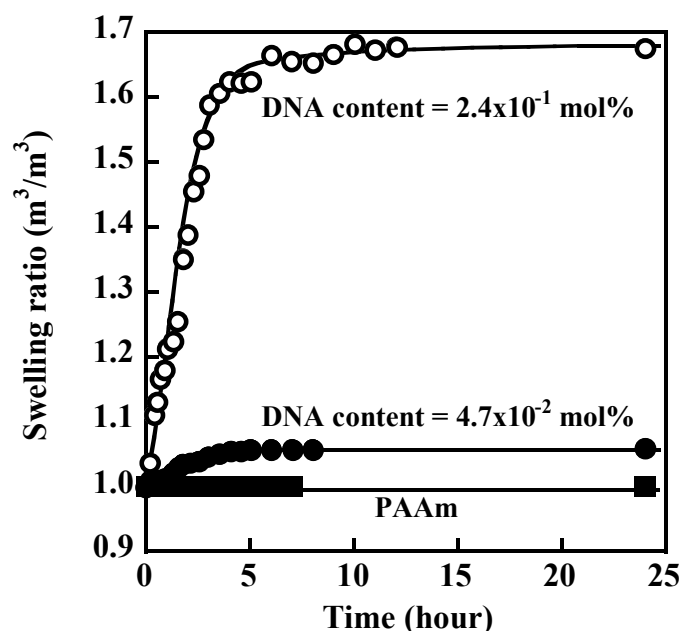
We also investigated swelling/shrinking behavior of the antigen-antibody semi-IPN gels when they were immersed in a buffer solution with and without rabbit IgG. The antigen-antibody gel with a semi-IPN structure swelled immediately in the presence of rabbit IgG, and shrank gradually in its absence. The antigen-antibody semi-IPN gel exhibited a reversible change in swelling behavior in response to stepwise changes in the antigen concentration, but the gel without a semi-IPN structure did not. This suggests that the semi-IPN structure plays an important role in reversibly antigen-responsive swelling/shrinking behavior. Some investigations revealed that the antigen-responsive behavior of the gels results from changes in their cross-linking density due to the association and dissociation of the antigen-antibody binding in response to the free antigen. In the antigen-antibody semi-IPN gel, the polymerized antibody is trapped in a network containing grafted antigen, and so the gel can shrink reversibly because the cross-linking between the polymerized antibody and grafted antigen reforms.

#### DNA-Responsive gels.

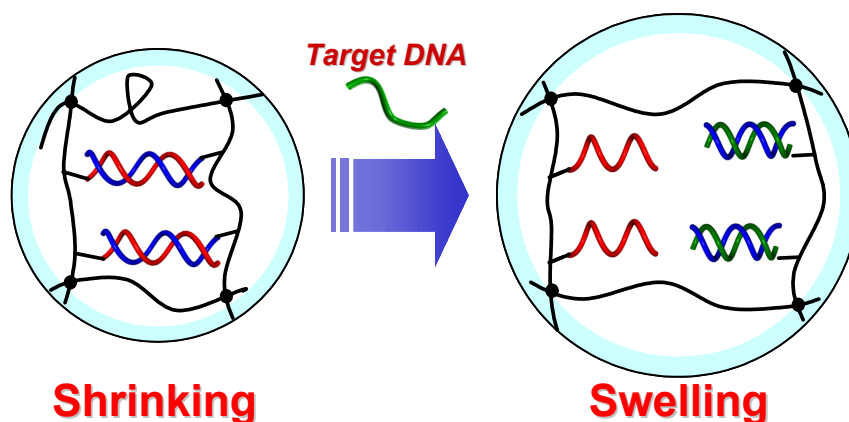
Swelling ratios of the DNA-cross-linked and PAAm gels were examined in a Tris buffer solution containing target ssDNA (5'-GGCCAGCGC-3'). The DNA-cross-linked gel swelled abruptly following the addition of a target ssDNA, but PAAm gel did not change at all (Figure 4). This indicates that the DNA-cross-linked gel is target ssDNA-responsive, and has a ssDNA sensing function. We performed compressive modulus measurements to examine the changes in the cross-linking density of the DNA-cross-linked gel following the addition of target ssDNA. The cross-linking density of the DNA-cross-linked gel in a buffer solution containing target ssDNA was lower than that in a solution without ssDNA. Consequently, investigations into the



**Figure 3.** Swelling ratio changes of the antigen-antibody semi IPN gel following the addition of rabbit IgG (●), goat IgG (○), bovine IgG (□) and horse IgG (■) after its swelling had attained equilibrium in phosphate buffer solution. The concentration of the antigen in the phosphate buffer solution was 4 mg/ml.



**Figure 4.** Swelling ratio changes of mismatch DNA-cross-linked gels with a ligand DNA content of  $4.7 \times 10^{-2}$  (●) and  $2.4 \times 10^{-1}$  (○) in a Tris-NaCl buffer solution (pH 7.4) containing target DNA at 25°C.



**Figure 5. Target DNA-responsive swelling behavior of DNA-cross-linked gels.**

cross-linking density demonstrated that the DNA-responsive swelling of the DNA-cross-linked gel resulted from a decrease in the cross-linking density of the gel due to the dissociation of the DNA duplex in the presence of target ssDNA (Figure 5).

These results mean that the DNA-cross-linked gel can recognize a sequence of ssDNA and then change its structure chemo-mechanically. These fascinating properties of the DNA-cross-linked gels suggest that they have many future applications as suitable materials for designing intelligent systems.

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