Preparation of Biomolecule-Responsive Bioconjugate Gels Using Biomolecular Interactions as Reversible Cross-Linkings

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1. Introduction

Stimuli-responsive gels that exhibit swelling changes in response to environmental changes such as pH and temperature have many future opportunities as suitable materials for designing smart systems in the biochemical and biomedical fields. However, there are very few reports on the stimuli-responsive gels that can respond to a specific biomolecule in spite of many potential applications. 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 cross-linking points. Based on this strategy, we have prepared novel antigen-responsive gels by using antigen-antibody bindings as stimuli-responsive cross-linking points. This paper describes two types of biomolecule-responsive gels that undergo swelling changes in response to specific biomolecules, which were prepared by using biomolecular interactions such as antigen-antibody bindings and saccharide-lectin bindings.

2. Experimental

Antigen-responsive gels were prepared by the copolymerization of a modified antigen having a vinyl group, acrylamide (AAm) and N,N'-methylenebisacrylamide (MBAA) in the presence of PAAm-grafted antibody to form an antigen-antibody binding (Fig. 1). Glycoprotein-responsive gels were prepared by biomolecular

Fig. 1. Synthesis of the bioconjugate gel having antigen-antibody bindings as cross-linking points.

Fig. 2. Synthesis of the imprinted gel having lectin and antibody as ligands for a print glycoprotein (AFP).
imprinting using lectin and antibody as ligands for a print glycoprotein (α-fetoprotein (AFP)) (Fig. 2). In the biomolecular imprinting, a modified antibody having a vinyl group was copolymerized with AAm and MBAA in the presence of AFP and PAAm-grafted lectin to form a lectin-glycoprotein-antibody complex and then the AFP was removed from the resultant network.

3. Results and Discussion

The gels having antigen-antibody bindings at cross-linking points were swollen in the presence of rabbit IgG, but were not in the presence of other proteins. In addition, the antigen-antibody gels having a semi-IPN structure showed reversible swelling changes in response to stepwise changes in the rabbit IgG concentration. The measurements of their cross-linking density revealed that their reversibly antigen-responsive behavior was due to dissociation and association of the antigen-antibody bindings in the presence and absence of a free antigen, respectively (Fig. 3). Furthermore, the antigen-responsive gels controlled drug permeation in response to the antigen concentration.

On the other hand, the imprinted gels having both lectin and antibody were shrunken in the buffer solution containing glycoprotein like α-fetoprotein (AFP), but a nonimprinted gel was not. The glycoprotein-responsive shrinking of the imprinted gels was caused by the formation of lectin-glycoprotein-antibody complex that acted as a cross-linking point (Fig. 4). Thus, biomolecule-responsive gels can be prepared by using biomolecular interactions as stimuli-responsive cross-linking points.

References:
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- Introduction
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- Biomolecule-Crosslinked Gels
  - Antigen-Responsive Gels
- Biomolecule-Imprinted Gels
  - Glycoprotein-Responsive Gels
- Conclusions
Intelligent Materials

- Biomolecule separator
  - Adsorption
  - Desorption
  - pH
  - Temperature
  - Electric field

- Controlled release
  - pH
  - Temperature
  - Electric field

- Stimuli-responsive absorbent
  - pH
  - Temperature
  - Electric field

- Membrane separation
  - pH
  - Temperature
  - Electric field
  - OFF
  - ON

- Artificial muscle
  - pH
  - Temperature
  - Electric field
Volume Change of Gels

\[ \Pi = \Pi_{el} + \Pi_M + \Pi_{ion} = \nu kT \left[ \frac{\phi}{2\phi_0} - \left( \frac{\phi}{\phi_0} \right)^{1/3} \right] - \frac{\Delta F}{\nu} \phi^2 - \frac{kT}{\nu} \left[ \ln(1-\phi) + \phi \right] + f \cdot v \cdot kT \left( \frac{\phi}{\phi_0} \right) \]

Crosslinking Interaction Charge

Biomolecular interaction (Our strategy)
Two Types of Biomolecule-Responsive Gels

Biomolecular interaction

Target

Mickey

Minnie

Pluto

Triangle
Preparation of Biomolecule-Responsive Bioconjugate Gels Using Biomolecular Interactions as Reversible Cross-Linking

- Introduction - Our Strategy -

- Biomolecule-Crosslinked Gels
  - Antigen-Responsive Gels

- Biomolecule-Imprinted Gels
  - Glycoprotein-Responsive Gels

- Conclusions
(1) Biomolecule-Crosslinked Gels

Antibody + Antigen → Antigen-antibody binding

Our Strategy

A) Chemical Modification of Antigen and Antibody

\[
\text{Antigen} + \text{Rabbit IgG} \rightarrow \text{Modified antigen}
\]

\[
\text{Antigen} + \text{Goat anti-rabbit IgG} \rightarrow \text{Modified antibody}
\]

B) Preparation of Gels Having Antigen-Antibody Bindings

a) Antigen-antibody semi-IPN hydrogel

\[
\text{Modified antibody} + \text{AAm} \xrightarrow{\text{APS/TEMED}} \text{Polymerized antibody}
\]

b) Antigen-antibody entrapment hydrogel

\[
\text{Native antibody} + \text{Modified antigen} \xrightarrow{\text{MBAA, APS/TEMED}} \text{Antigen-Antibody Entrapment Hydrogel}
\]

\[
\text{Modified antigen} + \text{AAm} \xrightarrow{\text{MBAA, APS/TEMED}} \text{Antigen-Antibody Semi-IPN Hydrogel}
\]
Antigen-Responsive Swelling

Fig. Effect of the antigen concentration in the phosphate buffer solution on the equilibrium swelling ratio of the PAAm semi-IPN hydrogel (○) and antigen-antibody semi-IPN hydrogel (●).
Fig. Swelling ratio changes of the antigen-antibody semi IPN hydrogel 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.
Reversibly Antigen-Responsive Swelling

(a) Entrapment hydrogel
(b) Semi-IPN hydrogel

Fig. Swelling ratio changes of the PAAm hydrogel (■), antigen-antibody hydrogel (□) in response to stepwise changes in the antigen concentration between 0 and 4mg antigen.
Mechanism for Antigen-Responsive Behavior

(a) Antigen-antibody entrapment hydrogel

(b) Antigen-antibody semi-IPN hydrogel
Antigen-Responsive Permeation

Fig. Swelling ratio changes of the PAAm semi-IPN hydrogel (○) and antigen-antibody semi-IPN hydrogel (▲), and permeation profile of a model drug from their membranes in response to stepwise changes in the antigen concentration between 0 and 4 mg/ml at 25°C.
Antigen-Responsive Permeation

(i) Antigen

Drug

Antigen Sensor

Drug Delivery

(ii) No antigen

Drug

Antigen Sensor

Drug Delivery

OFF

Shrinking

ON

Swelling
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Glycoprotein-Responsive Gels

Cancer

Liver

Diagnostic tumor-specific marker

α-Fetoprotein (AFP)

Saccharide

Peptide

Biomolecular Imprinting

Modified protein

Interaction

Polymerization

Removal of AFP

AFP-imprinted gel
Fig. Swelling ratio changes of AFP-imprinted gel (□) nonimprinted gel (○) and PAAm gel (●) in PBS containing AFP (40 µg/ml).
Mechanism of Glycoprotein-Responsive Behavior

AFP imprinted gel

Nonimprinted gel
Biomolecule-Responsive Bioconjugate Gels

(1) Biomolecule-crosslinked gels

(2) Biomolecule-imprinted gels
1. The hydrogels having antigen-antibody bindings at cross-linking points underwent reversible swelling changes in response to a specific antigen.

2. The measurements of their cross-linking density demonstrated that their reversibly antigen-responsive behavior was due to dissociation and association of the antigen-antibody bindings in the presence and absence of a free antigen, respectively.

3. The antigen-responsive hydrogels controlled drug permeation in response to the antigen concentration.

**CONCLUSIONS**

**(1) Biomolecule-crosslinked gels**

1. The hydrogels having antigen-antibody bindings at cross-linking points underwent reversible swelling changes in response to a specific antigen.

2. The measurements of their cross-linking density demonstrated that their reversibly antigen-responsive behavior was due to dissociation and association of the antigen-antibody bindings in the presence and absence of a free antigen, respectively.

3. The antigen-responsive hydrogels controlled drug permeation in response to the antigen concentration.

**(2) Biomolecule-imprinted gels**

1. The AFP-imprinted hydrogels having both lectin and antibody shrank in the buffer solution containing AFP, but a nonimprinted hydrogel did not.

2. The glycoprotein-responsive shrinking of the AFP-imprinted hydrogels was caused by the formation of lectin-glycoprotein-antibody complex that acted as a cross-linking point.

We conclude that biomolecule-responsive hydrogels can be prepared using biomolecular interactions as stimuli-responsive cross-linking points.