ON-OFF CONTROL OF DRUG PERMEATION THROUGH ANTIGEN-RESPONSIVE GELS

Takashi Miyata[1, 2], Noriko Asami[1], Tadashi Uragami[1]
[1] Faculty of Engineering and High Technology Research Center, Kansai University
Suita, Osaka 564-8680, Japan
[2] PRESTO, Japan Science and Technology Agency (JST)

Introduction

Stimuli-responsive gels that exhibit swelling/shrinking changes in response to environmental changes such as pH and temperature have many future opportunities as suitable materials for mimicking biomolecules and designing smart systems in the biochemical and biomedical fields [1]. For example, self-regulated drug delivery can be achieved by loading a drug within pH- and temperature-responsive gels. However, there are very few studies on molecule-responsive gels that undergo volume changes in response to a target molecule in spite of their potential applications in biomedical fields [2-6].

We have prepared novel antigen-responsive gels that have molecular recognition functions, based on the strategy that specific interactions such as antigen-antibody bindings can be used as stimuli-responsive cross-linking points [7, 8]. In addition, tumor-marker-responsive gels were synthesized by biomolecular imprinting in which lectin and antibody were utilized as ligands for a template tumor marker glycoprotein [9]. In this paper, a method for preparing antigen-responsive gels is reported that involves simple means of introducing stimuli-responsive cross-linking structures. This paper focuses on the controlled permeation of model drugs through the antigen-responsive gels. We discuss the effect of semi-interpenetrating polymer network (semi-IPN) in the gels on their antigen-responsive swelling behavior and controlled drug permeation.

Experiments

Preparation of antigen-responsive gels.

Two types of 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) were chemically modified using *N*-succinimidylacrylate (NSA) for the introduction of vinyl groups. The resultant vinyl-antibody was copolymerized with acrylamide (AAm) using redox initiators to synthesize the polymerized antibody. Antigen- antibody semi-IPN gels were prepared by the copolymerization of the vinyl-antigen, AAm and *N, N'*- methylenebisacrylamide in the presence of the polymerized antibody [7]. Antigen-antibody entrapment gels without a semi-IPN structure were also synthesized by using a native antibody in place of the polymerized antibody [8].

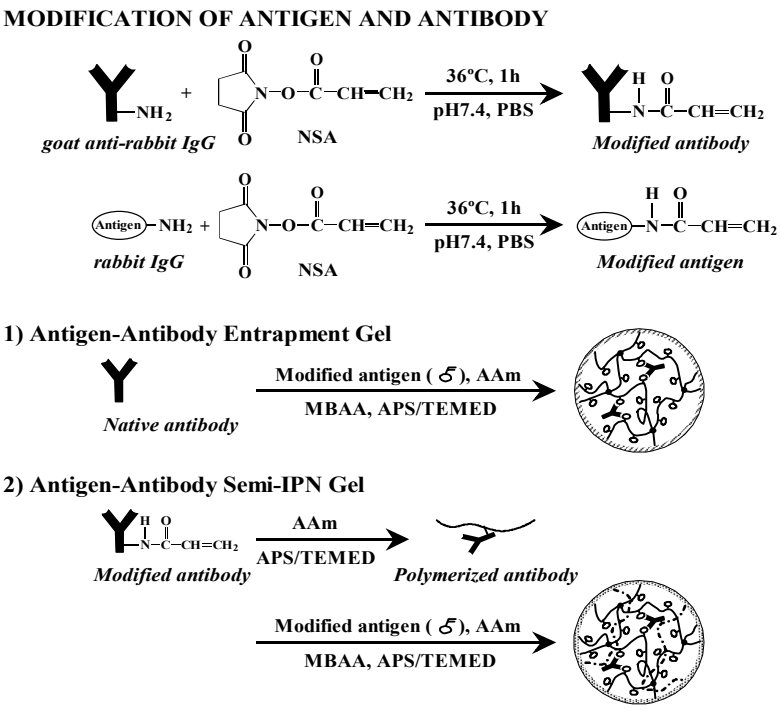

Figure 1. Preparation of antigen-antibody entrapment gel and antigen-antibody semi-IPN gel.

Swelling Measurements.

The 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 antigen at 25°C. The swelling ratio of the gels was determined from the ratio of their diameters.

Permeation experiments of drugs.

Permeation experiments were carried out at 25°C under magnetic stirring, using a diaphragm glass cell, in which the left-side chamber of the cell was filled with the phosphate buffer solution containing vitamin B_{12} and hemoglobin (1 mg/ml) as model drugs, and the antigen concentration in the phosphate buffer solution in the right-side chamber was stepwise changed between 0 and 4 mg/ml. The amounts of the solutes permeated through the gels were colorimetrically determined using a spectrophotometer.

Results and Discussion

Both antigen-antibody entrapment and semi-IPN gels swelled immediately in the presence of a free antigen. However, after the gels swollen in the presence of the antigen were immersed in a buffer solution without an antigen, the antigen-antibody semi-IPN gel shrank gradually but the entrapment gel did not. This suggests that the semi-IPN structure plays an important role in reversibly antigen-responsive swelling/shrinking behavior. Compressive modulus measurements 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. A native antibody in the antigen-antibody entrapment gel was released from the gel networks but that in the semi-IPN gel was not. This is due to that the polymerized antibody in the semi-IPN gel is trapped in a network containing grafted antigen. As a result, the antigen-antibody semi-IPN gel can shrink reversibly because the complexes between the polymerized antibody and grafted antigen are formed in a buffer solution without the free antigen.

The permeations of vitamin B_{12} (molecular weight = 1355) and hemoglobin (molecular weight = 64500) as model drugs through the antigen-antibody entrapment and semi-IPN gels were investigated using a diaphragm glass cell. The permeation rates of vitamin B_{12} through antigen-antibody entrapment and semi-IPN gels were much higher than those of hemoglobin. The antigen-antibody entrapment and semi-IPN gels could not control the permeation of vitamin B_{12} completely in response to stepwise

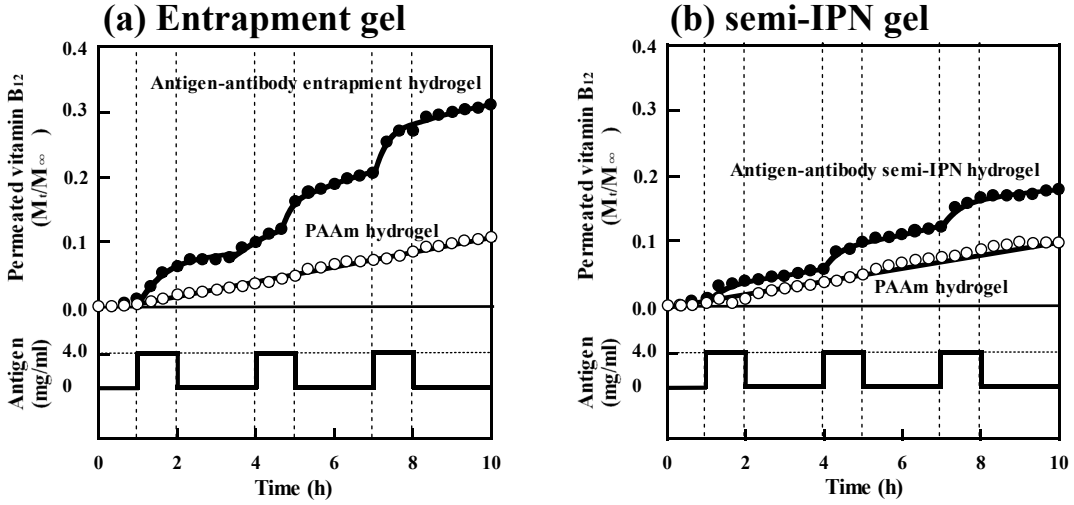

Figure 2. Permeation profiles of vitamin B_{12} from the PAAm (○) and antigen-antibody gel (●) having entrapment (a) and semi-IPN structure (b) in response to stepwise changes in the antigen concentration between 0 and 4mg/ml.

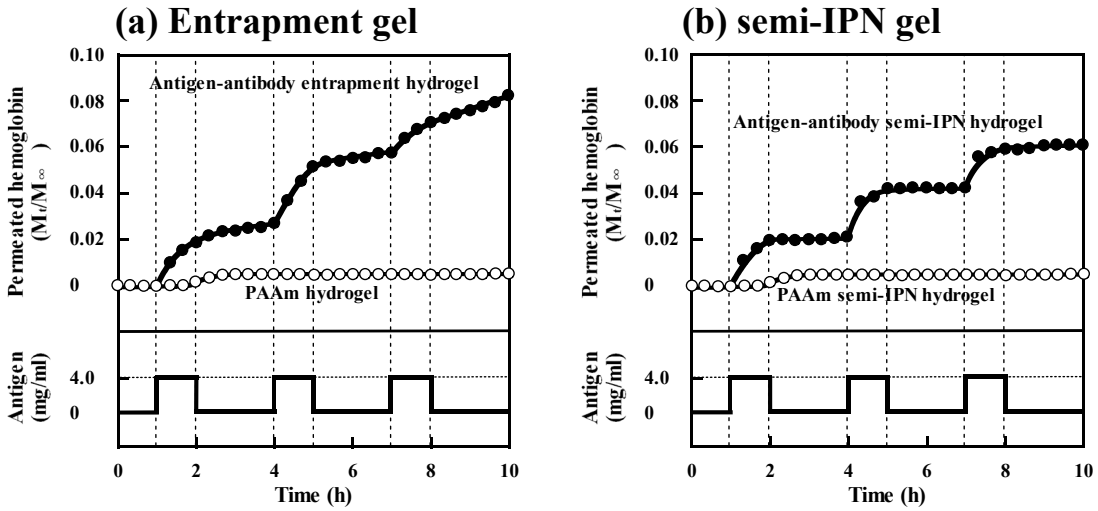

Figure 3. Permeation profiles of hemoglobin from the PAAm (○) and antigen-antibody semi-IPN gel (●) having entrapment (a) and semi-IPN structure (b) in response to stepwise changes in the antigen concentration between 0 and 4mg/ml.

changes in the antigen concentration (Figure 2). Similarly, hemoglobin permeation was not controlled sensitively through the antigen-antibody entrapment gel. However, hemoglobin was permeated through the antigen-antibody semi-IPN gel in the presence of an antigen but the hemoglobin permeation was completely depressed in its absence (Figure 3). Furthermore, the antigen-antibody semi-IPN gel enabled more sensitive ON-OFF control of the model drug permeation than the antigen-antibody entrapment gel. Thus, the pulsatile permeation of a model drug in response to the antigen concentration can be achieved by using the antigen-antibody semi-IPN gels (Figure 4).

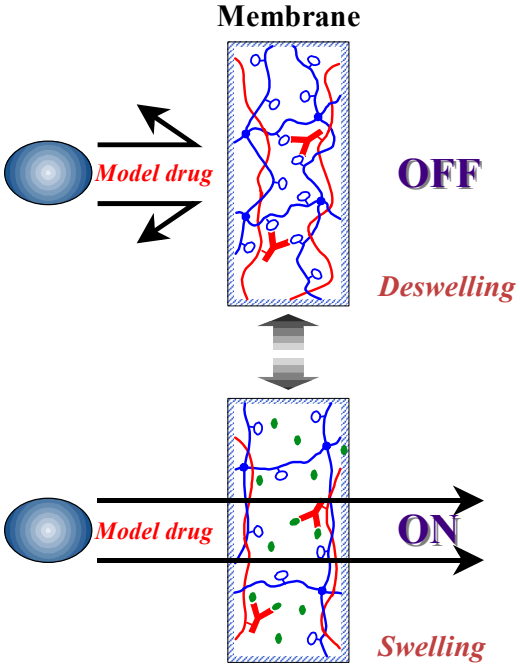

Figure 4. Proposed mechanism for drug permeation through an antigen-antibody semi-IPN gel in response to an antigen.

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.

References

1) T. Miyata, Supramolecular Design for Biological Applications (ed. Yui, N.) 2002, CRC Press, Boca Raton, pp. 191–225.
2) T. Miyata, T. Uragami, Polymeric Biomaterials (ed. S. Dumitriu) 2002 Marcel Dekker, New York, pp. 959–974.
3) T. Miyata, T. Uragami, K. Nakamae, *Adv. Drug Delivery Rev.*, **54**, 79 (2002).
4) T. Miyata, A. Jikihara, K. Nakamae, A. S. Hoffman, *Macromol. Chem. Phys.*, **197**, 1135 (1996).
5) T. Miyata, K. Nakamae, *Trend. Polym. Sci.*, **5**, 198 (1997).
6) T. Miyata, A. Jikihara, K. Nakamae, A. S. Hoffman, *J. Biomater. Sci., Polym. Ed.*, **15**, 1085 (2004).
7) T. Miyata, N. Asami, T. Uragami, *Nature*, **399**, 766 (1999).
8) T. Miyata, N. Asami, T. Uragami, *Macromolecules*, **32**, 2082 (1999).
9) T. Miyata, M. Jige, T. Nakaminami, T. Uragami, *Proc. Natl. Acad. Sci. USA*, **103**, 1190 (2006).