Biochemically Controlled Thermal Phase Transition of Gels

Etsuo Kokufuta[†] and Toyoichi Tanaka*

Department of Physics and Center for Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received June 7, 1990; Revised Manuscript Received August 29, 1990

ABSTRACT: Recognition of biochemical reaction and subsequent conversion directly into mechanical work has long been a technological dream. Physical systems that respond specifically to a particular kind of molecule have similar capabilities to biological transducers, sensors, and actuators, in which molecular signals trigger biological activities. A gel is designed and synthesized that undergoes a discontinuous swelling–shrinking phase transition at a specific temperature when enzyme reaction takes place. The gel consists of a network of poly(*N*-isopropylacrylamide) in which liver esterase is immobilized. The enzyme hydrolyzes ethyl butyrate into ethanol and butyric acid. A change in substrate–product composition triggers the phase transition. A theoretical analysis of the phenomena is given.

Introduction

Recently, phase transitions and critical phenomena in polymer gels have attracted attention because of their scientific interest and technological importance.[1-3] Phase transitions accompanied by a reversible, discontinuous-volume change as large as several hundred times, in response to environmental changes, have been observed universally in gels made of synthetic and natural polymers.[1-10] Variables that may trigger the transition include temperature, solvent composition, pH, ionic composition, small electric field,[11,12] and light.[13,14] These parameters are all physical or chemical quantities and, therefore, are not specific. For example, in the solvent-induced phase transition of the acrylamide/sodium acrylate copolymer gels the solvent can be acetone, alcohol, or other nonpolar solvents. In the pH-driven phase transition of similar gels any types of buffers can be used. It will be of scientific interest and technological importance if a gel system is developed that undergoes the volume phase transition in specific response to only one kind of molecule.

The idea to change the volume of polymer gels in response to a specific type of molecule has been developed by several researchers in the field of controlled-release systems such as drug delivery systems.[15-17] Shinohara and Ishihara[18] used a hydrogel with entrapped glucose oxidase. The oxidation of glucose to gluconic acid changes the ionization of the gel and subsequently the degree of swelling. The pores of the gel were expanded, and the insulin previously incorporated within the gel was then released. The changes of the volume in the gels, however, were continuous and gradual as a function of glucose concentration. This letter reports the design and development of a gel that undergoes a discontinuous volume change in response to a biochemical reaction by sensing changes in substrate and product composition within the gel phase.

Design and Preparation of Gels

The strategy of the design of gels that respond to a specific kind of molecule is first to find a gel the undergoes phase transition within water at a moderate temperature. Then an enzyme that catalyzes a substrate will be entrapped within the gel. When the substrate molecule comes into the gel, it is catalyzed by the enzyme and converted into the products. A stationary state is established for the profile of substrate and product concentrations at the vicinity of the gel. The change in the solvent composition within the gel alters the phase equilibrium of the gel and induces the phase transition.

Rabbit liver esterase (EC 3.1.1.1) was immobilized in the gel by an entrapping method. An aqueous solution (100 mL) was prepared from purified *N*-isopropylacrylamide (main polymer constituent; Kodak; 7.8 g), *N*,*N*′-methylenebisacrylamide (cross-linker; Bio-Rad Laboratories; 0.133 g), and tetramethylethylenediamine (accelerator; Bio-Rad Laboratories; 240 μL). Esterase (Sigma; 3 mg) was dissolved in 1 mL of the solution. The cross-linking vs polymer molar ratio was 1:80. The solution was degassed under vacuum, mixed with 10 μL of aqueous ammonium persulfate solution (initiator; Mallinckrodt; 4 wt %), and transferred into glass capillaries with approximately 0.1-mm inner diameter. After gelation was completed, the gels were taken out of the capillaries and washed with water. As control samples, some of the gels were subjected to thermal treatment to inactivate the enzyme. Enzyme-free gels were also prepared as another control. All the samples were cut into a cylinder of 1-mm length, inserted into a micropipette (inner diameter 1 mm), and stored at 3 °C before use.

Observation of Phase Transition

Aqueous solutions of substrate and/or the products were introduced into the micropipette. The size and shape of the gel were monitored and analyzed at different temperatures by using an AVEC image processor (Hamamatsu Photonics, C1966). Ethanol and ethylbutyrate concentrations were determined by using gas chromatography (Hewlett Packard, 5880A). Production of ethanol was used to monitor the enzyme activity.

Figure 1 shows the effects of the substrate and product concentrations on the swelling curves of the enzyme-free gel. Reversible and discontinuous-volume phase transition was observed in the aqueous solutions of substrate (47.3 mM ethyl butyrate) and of products (ethanol and butyric acid, 47.3 mM each) and in water. Hysteresis was observed in all the swelling curves, which evidenced the first-order transition. The transition should therefore be characterized by two temperatures, T_c for collapsing and T_s for swelling. They decreased monotonically with a decrease of the substrate and an increase of the products.

Figure 2 shows the swelling curves of the enzyme-loaded gel in the saturated substrate solution, one with active

[†] Permanent address: Institute of Applied Biochemistry, University of Tsukuba, Tsukuba-shi, Ibaraki 305, Japan.

0024-9297/91/2224-1605$02.50/0

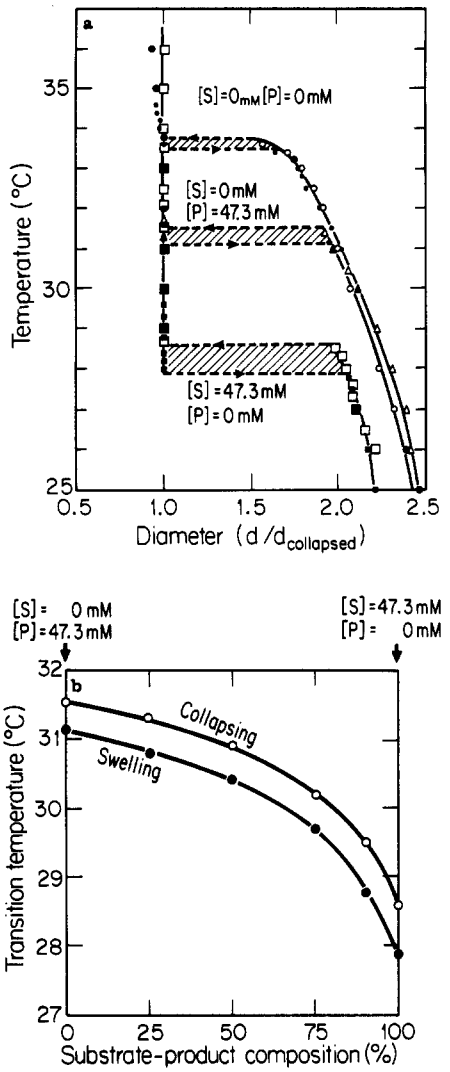

Figure 1. Effects of the substrate and products on the phase transition of enzyme-free *N*-isopropylacrylamide gel. (a) Temperature dependence of the gel diameter upon increasing temperature (open symbols) and decreasing temperature (solid symbols). The solvents are water (circles), a substrate solution containing 47.3 mM ethyl butyrate (squares), and a mixture of the products, ethanol and butyric acid (47.3 mM each, triangles). The diameter is normalized by the diameter in the completely collapsed state. (b) Transition temperatures were plotted as a function of the mixing ratio of the substrate, ethyl butyrate (47.3 mM), and the products, ethanol and butyric acid (47.3 mM each).

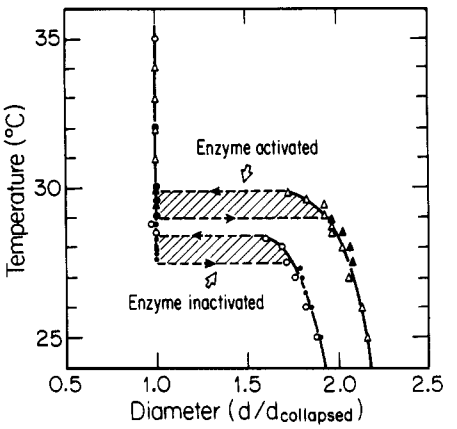

Figure 2. Diameter of liver esterase loaded *N*-isopropylacrylamide gel in a substrate solution as a function of temperature before and after inactivation of the immobilized enzyme. The diameter is normalized by the collapsed diameter. The substrate solution initially contained 47.3 mM of ethyl butyrate. When the enzyme has activity, the transition temperatures are T_c = 29.8 °C for the collapsing process and T_s = 29.1 °C for the swelling process, while they are lowered by inactivation to T_c = 28.4 °C and T_s = 27.6 °C. This indicates that the temperature range where a discontinuous-volume phase transition takes place in response to enzymatic reaction lies between 28.4 and 29.1 °C.

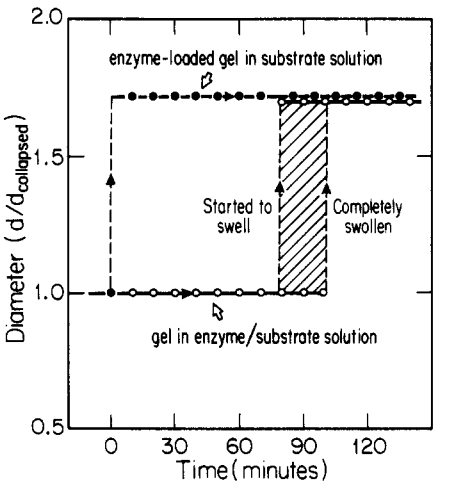

Figure 3. Time courses of the diameter in the swelling process of enzyme-free gel in the substrate solution containing 60 μg/mL esterase (open circles). The solid circles are for enzyme-loaded gel in the substrate solution. The enzyme-free gel was originally collapsed. Some portions of the gel started to swell at 80 min, but some portions did not swell until 100 min. This may be due to the inhomogeneities of the network structure of the gel. On the other hand, the enzyme-loaded gel is swollen from the beginning.

enzyme and the other with inactivated enzyme. With active enzyme, T_c = 29.8 °C and T_s = 29.1 °C. With inactivated enzyme, T_c = 28.4 °C and T_s = 27.6 °C. Discontinuous swelling and shrinking take place in response to enzyme reaction when the temperature was fixed between 28.4 and 29.1 °C.

The change in the phase-transition tempertaure was presumably caused by the change in the substrate and product composition induced by enzymatic reaction within the gel phase. An experimental support of this view is shown in Figure 3. When the enzyme-free gel was placed at 28.9 °C in an aqueous solution containing the substrate together with 60 μg/mL of esterase, the cylindrical gel, which was initially collapsed, swelled discontinuously 80–100 min later. Chromatographic analysis showed that at that time approximately 20% of the substrate was hydrolyzed. The phase transition apparently took place when the substrate/product ratio reached the value corresponding to T_s = 28.9 °C (Figure 1b). In the enzyme-loaded gel, a swollen state was reached from the beginning of the measurement. For the first several hours less than 1% of the entire substrate was hydrolyzed, while about 15% of the substrate was decomposed in 5 days. The former change is not enough to induce a significant change in the transition temperature (Figure 1b). Therefore, the phase transition of the enzyme-loaded gel was controlled by the concentrations of the substrate and the products within or at the very vicinity of the gel phase but not in the bulk solution (see Figure 4).

Theoretical Considerations

We now theoretically explain the effects of enzymatic reaction on the phase transition using the Flory–Huggins equation of state. The swelling curve is given by zero

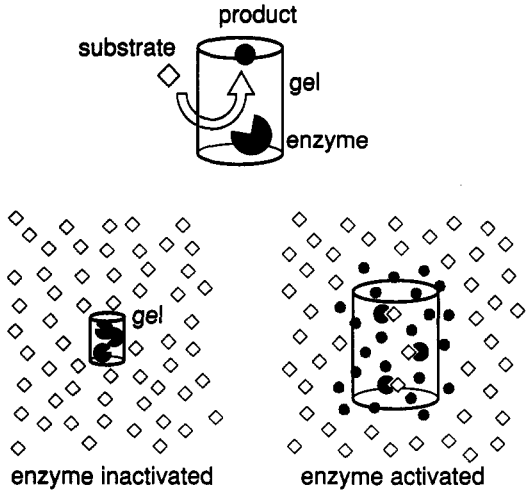

Figure 4. Schematic illustration of the phase transition of the gel induced by enzymatic reaction.

osmotic pressure for the gel[3,19]

$$\frac{1}{T} = \frac{\Delta S}{\Delta H} + \frac{k}{\Delta H}\left[\frac{v_1 \nu_e}{N\phi^2}\left\{(2f+1)\left(\frac{\phi}{\phi_0}\right) - 2\left(\frac{\phi}{\phi_0}\right)^{1/3}\right\} - \frac{2}{\phi} - \frac{2\ln(1-\phi)}{\phi^2}\right] \equiv g_{gel}(\phi) \quad (1)$$

where T is the absolute temperature, ΔS and ΔH are the differences in the entropy and enthalpy of polymer segment–segment and polymer segment–solvent contact, k is the Boltzmann constant, N is Avogadro's number, v_1 is the molar volume of water, ν_e is the total number of effective polymer chains, ϕ is the volume fraction of polymer network, and ϕ_0 is the volume fraction of dry network in the reference state at Θ temperature, and f is the number of counterions per chain. For N-isopropylacrylamide gels, ΔS and ΔH are negative, and the gels are swollen at low temperatures and shrunken at higher temperatures.

As the enzyme catalyzes the reaction, substrate is consumed and products are formed in the gel. A stationary condition is reached when diffusion of substrate and products along their gradients is balanced with their consumption and creation. In the first approximation these changes alter the contact free energy in proportion to the substrate concentration, σ_0, and to the enzyme concentration, $\epsilon = \epsilon_0\phi$, where ϵ_0 is the enzyme concentration at $\phi = 1$.

$$\Delta H = \Delta H_0 + \alpha_H \epsilon_0 \sigma_0 \phi \quad \text{and} \quad \Delta S = \Delta S_0 + \alpha_S \epsilon_0 \sigma_0 \phi \quad (2)$$

Here α's are constants. For simplicity, assume that α_H is zero. Then with enzyme reaction, the swelling curve is

$$\frac{1}{T} = g_{gel}(\phi) + \frac{\alpha_S \epsilon_0 \sigma_0 \phi}{\Delta H_0} \quad (3)$$

Since the transition temperature increases with enzyme reactions, $\alpha_S/\Delta H_0$ should be negative. The new term in eq 3 reduces Maxwell's loop, bringing the phase transition toward continuous transition. Indeed, the transition temperature increased by the enzyme reaction and the effect is larger with larger substrate concentration.

Conclusion

In this paper an enzyme reaction was chosen to change the environmental condition, thus altering the phase-transition threshold of gels. The concept introduced here, however, should be general, and various types of biochemical or chemical reactions, such as antibody–antigen or receptor–substrate interactions, could be used to induce the phase transition.

Acknowledgment. We thank Drs. A. Suzuki and S. Gorti for valuable discussions and Dr. S. Masamune for help in chemical analysis. Work was supported by the NSF, Grant DMR-8920401. The stay of E.K. in the U.S. was supported by the Ministry of Education, Science and Culture in Japan.

References and Notes

(1) Tanaka, T. *Phys. Rev. Lett.* **1978**, *40*, 820.
(2) Dusek, K.; Patterson, D. *J. Polym. Sci., Polym. Phys. Ed.* **1968**, *6*, 1209.
(3) Tanaka, T.; Fillmore, D. J.; Sun, S.-T.; Nishio, I.; Swislow, G.; Shah, A. *Phys. Rev. Lett.* **1980**, *45*, 1636.
(4) Ilavsky, M. *Macromolecules* **1982**, *15*, 782.
(5) Hrouz, J.; Ilavsky, M.; Ulbrich, K.; Kopecek, J. *Eur. Polym. J.* **1981**, *17*, 361.
(6) Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1984**, *81*, 6379.
(7) Erman, B.; Flory, P. J. *Macromolecules* **1986**, *19*, 2342.
(8) Hirotsu, S.; Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1987**, *87*, 1392.
(9) Otake, K., et al. *J. Chem. Phys.* **1990**, *91*, 1345.
(10) Amiya, T.; Tanaka, T. *Macromolecules* **1987**, *20*, 1162.
(11) Tanaka, T.; et al. *Science* **1982**, *218*, 467.
(12) Osada, Y. *Advances in Polymer Science*; Springer-Verlag: Berlin, 1987; Vol. 82, p 1.
(13) Suzuki, A.; Tanaka, T. *Nature* **1990**, *346*, 345.
(14) Mamada, A.; Tanaka, T.; Kungwatchakun, D.; Irie, M. *Macromolecules* **1990**, *23*, 1517.
(15) Hoffman, A. S. *J. Controlled Release* **1987**, *6*, 297.
(16) Siegel, R. A.; Firestone, B. A. *J. Controlled Release* **1990**, *11*, 181.
(17) Okano, T.; Bae, Y. H.; Jacobs, H.; Kim, S. W. *J. Controlled Release* **1990**, *11*, 255.
(18) Ishihara, K.; Kobayashi, M.; Shinohara, I. *Makromol. Chem., Rapid Commun.* **1983**, *4*, 327.
(19) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953.