Macromolecules

Volume 36, Number 6 March 25, 2003

Communications to the Editor

Self-Beating Motion of Gels and Modulation of Oscillation Rhythm Synchronized with Organic Acid

Ryo Yoshida,*,† Keigo Takei,† and Tomohiko Yamaguchi‡

Department of Materials Engineering, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan, and National Institute of Advanced Industrial Science and Technology, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan

Received December 12, 2002
Revised Manuscript Received February 6, 2003

The TCA cycle (Krebs cycle) is a well-known key metabolic process that takes place in living organisms. In this study, we have prepared the biomimetic polymer gel which undergoes such a metabolic process with cyclic changes. The speed of cyclic change becomes faster with an increase in concentration of organic acid. In conventional stimuli-responsive gels, either swelling or deswelling is driven by on−off switching of external stimuli such as temperature,[1−3] pH,[4−6] electric field,[7,8] specific chemicals,[9−11] etc. In contrast, self-beating motion of gels and the modulation of self-beating rhythm were first achieved here. Although there is a lot of work to realize on−off control of monotonic swelling or deswelling, there is no report to realize on−off control of self-beating motion by switching external stimuli repeatedly. The periodic self-beating motion of the gel is produced by dissipating chemical energy of the oscillating reaction, i.e., the Belousov−Zhabotinsky (BZ) reaction.[12−15] The BZ reaction was originally designed from the analogy of TCA cycle. The reactants consist of organic acid such as citric acid or malonic acid, oxidizing agent, and metal catalyst. The roles of oxidizing agent and metal catalyst correspond to those of respiration and enzyme in living system, respectively. The overall process is the oxidation of an organic acid by an oxidizing agent in the presence of metal catalyst under an acidic condition. In the course of the reaction, a cyclic reaction network of intermediates is spontaneously created, as in the TCA cycle. As a result, the catalyst ion periodically changes between reduced and oxidized states under homogeneously stirred conditions.

Our gel involves a built-in system of energy conversion from chemical oscillation to mechanical oscillation. The catalyst of the BZ reaction, ruthenium(II) tris(2,2′-bipyridine) ($Ru(bpy)_3^{2+}$), is covalently bonded to the polymer chain of *N*-isopropylacrylamide (NIPAAm). When the gel is immersed in the aqueous solution containing the substrate of the BZ reaction except for the catalyst, the substrates penetrates into the polymer network, and the BZ reaction occurs in the gel. The polymer has lower critical solution temperature (LCST) because of thermosensitive constituent NIPAAm. The LCST of the polymer in the oxidized Ru(III) state becomes higher than that in the reduced Ru(II) state due to the charge increase of the catalyst.[16,17] At constant temperature, therefore, redox changes of the catalyst lead to hydrophilic changes of the polymer chains. Consequently, periodic redox changes induced by the BZ reaction produce al swelling−deswelling changes of the gel.

NIPAAm (0.156 g), $Ru(bpy)_3^{2+}$ monomer (8.2 mg) into which a vinyl group was introduced, *N*,*N*′-methylenebis-(acrylamide) (2.8 mg) as a cross-linker, and 2,2′-azobis-(isobutyronitrile) (6.6 mg) as an initiator were dissolved in 1 mL of O_2-free methanol. This monomer solution was injected into a glass capillary with 620 μm i.d., and the both ends were sealed. The gelation was carried out at 60 °C for 20 h. To remove unreacted monomers, the gel was immersed in methanol for a week with replacing methanol once a day. The gel was carefully hydrated through dipping it in a graded series of water−methanol mixtures, for 1 day each in 25, 50, 75, and 100% (v/v) of water.

† The University of Tokyo.
‡ National Institute of Advanced Industrial Science and Technology.
* To whom correspondence should be addressed: phone and fax +81-3-5841-7112; e-mail yoshida@bmw.t.u-tokyo.ac.jp.

10.1021/ma0259618 CCC: $25.00
Published on Web 02/22/2003

The gel was cut into a rod shape with 1 mm length and immersed into 90 μL of an aqueous solution containing malonic acid (MA), sodium bromate ($NaBrO_3$), and nitric acid (HNO_3) in the microchamber, which was maintained at 20 °C. The gel was fixed in the chamber by using a glass needle attached to micromanipulator (Leica, MP-6). The swelling−deswelling oscillations of the gel accompanying the redox oscillations were observed under a microscope (Olympus BX-50) equipped with a black−white CCD camera and a video recorder. We used monochromatic light (390 nm) passed through a blue filter on the halogen light source. Color changes due to the periodic oxidation and reduction of the $Ru(bpy)_3$ moieties within the gel were measured by means of transmitted light. The transmittance was then converted to 8-bit gray scale changes by using the image processing software (NIH image 1.61).

After the gel is soaked in the solution, the ruthenium catalyst autonomously exhibits periodic changes between the oxidized (Ru(III)) and the reduced (Ru(II)) state. Since the gel size is smaller enough than the wavelength of chemical wave, the redox changes can be regarded to occur homogeneously without pattern formation.[18] Accompanying the redox oscillations, mechanical oscillations of the gel occur; the gel swells during the oxidized state and deswells during the reduced state. Typically, the self-beating continues for 2 or 3 h under this experimental condition. The length of time will depend on the volume of outer solution.

Figure 1 demonstrates the oscillating behavior of the gel under different concentrations of MA when the other substrate concentrations were fixed. Figure 1A shows the spatiotemporal diagram constructed by image-processing procedure, expressing both the redox changes inside the gel and the motion of the gel edge. The gel edge fluctuates periodically due to swelling−deswelling changes. Figure 1B shows the time course of redox oscillation at a fixed position in the gel which was expressed as 8-bit gray scale changes. As the [MA] increases, both the period and the amplitude of redox changes decrease.

As an inherent behavior of the BZ reaction, the abrupt transition from steady state (nonoscillating state) to oscillating state occurs with a change in controlling parameter such as chemical composition, etc. This change is termed "bifurcation". In the previous study, we demonstrated bifurcation structure for the solution system which consists of $MA/NaBrO_3/HNO_3/Ru(bpy)_3$-$Cl_2$. Such a behavior is also observed for our oscillating gel, although there is no direct relationship between solution and gel systems due to mechanical effects as described later. Figure 2 shows the period and amplitude of the swelling−deswelling oscillation as a function of [MA]. At lower concentration, oscillation does not take place (steady state). When the concentration exceeds a certain value, a stable and periodic oscillation takes place (oscillating state). At higher concentration, however, the oscillation stops and the gel becomes the steady state again. In the oscillating state, both the period and the amplitude decrease with the increase in [MA]. It is common knowledge that the period of the BZ reaction becomes shorter as the concentration of substrates increases. The reason why the mechanical amplitude also decreases with decreasing period can be considered as follows. First, when the redox oscillation takes place with very high frequency, mechanical change of the gel cannot follow the chemical oscillation because

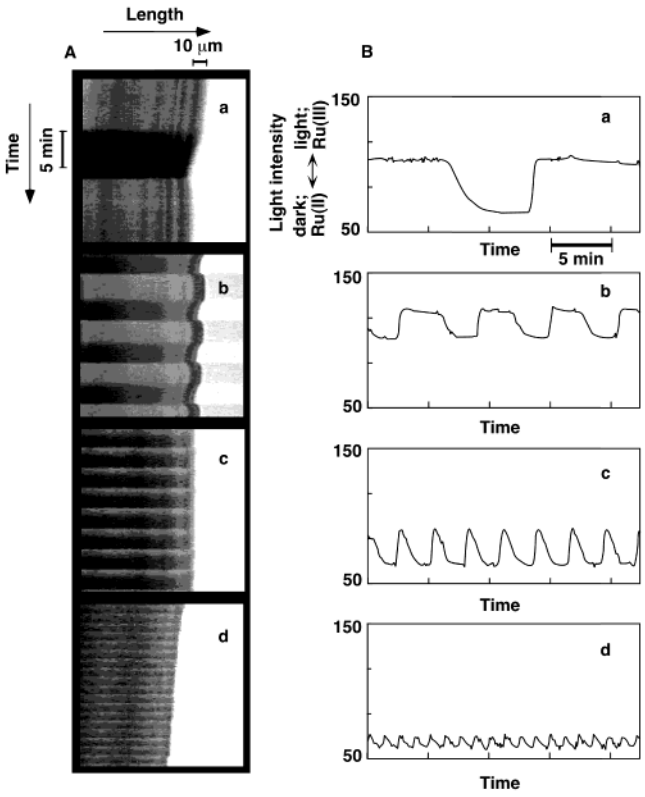

Figure 1. (A) Spatiotemporal pattern of gel oscillation constructed from digitized sequential images. The cylindrical poly(NIPAAm-*co*-$Ru(bpy)_3$) gel was immersed in 90 μL of the aqueous solution containing malonic acid ((a) 15, (b) 50, (c) 75, and (d) 150 mM), sodium bromate (84 mM), and nitric acid (0.3 M) at 20 °C. The magnified image around the gel edge was recorded on a VTR through a CCD camera. The video images were transferred through a digital time base corrector to a computer equipped with an image-acquisition board. The redox reaction was thus recorded as 8-bit gray scale changes. One-pixel line along the moving direction of the gel edge in the image was stored every 3 s. 440 frames (i.e., 22 min) of the stored line images were sequentially lined up as a function of time by the computer. (B) Time course of redox changes in the gel. The redox state is presented by transmitted light intensity, which is expressed as an 8-bit (256) gray scale value (dark, reduced state; light, oxidized state).

the swelling−deswelling response of the gel to the redox changes is rate-determining for the mechanical oscillation. Second, the degree of redox changes also becomes smaller with shorter period, as shown in Figure 1.

From these results, it is expected that the rhythmical motion of the gel can be controlled by changing [MA] during the oscillation. If the [MA] is changed within the concentration region of oscillating state, the beating rhythm (period and amplitude) of the gel would be varied. On the other hand, if the [MA] is switched between the concentration regions of steady state and oscillating state, on−off control of the beating would be possible. Whether there is a hysteresis or not when the condition is varied continuously and repeatedly in series would also be an important subject. Figure 3 shows the oscillating behavior of the gel when the stepwise change in [MA] was repeated between lower concentration (10 mM) in steady state and higher concentration (25 mM) in the oscillating state. At [MA] = 10 mM, the redox oscillation does not occur, and consequently the gel exhibited no swelling−deswellng changes. Then the concentration was quickly increased to 25 mM. Immediately after increasing concentration, the gel started self-beating. The beating stopped again as soon as the

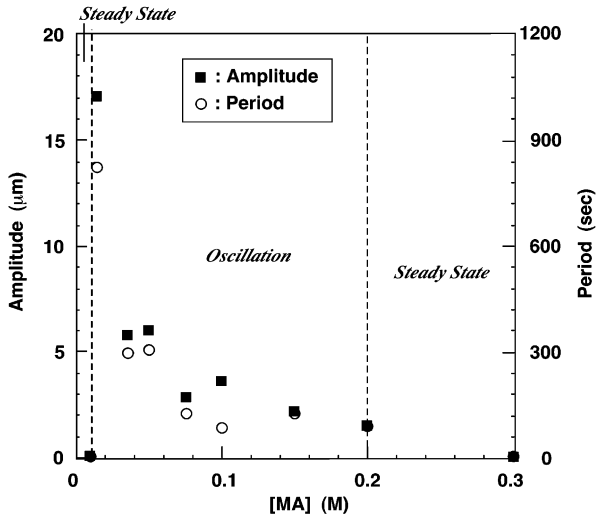

Figure 2. Period and amplitude of the swelling−deswelling oscillation for the gel as a function of the concentration of MA at 20 °C. The concentrations of other substrates are fixed at $[NaBrO_3] = 84$ mM and $[HNO_3] = 0.3$ M.

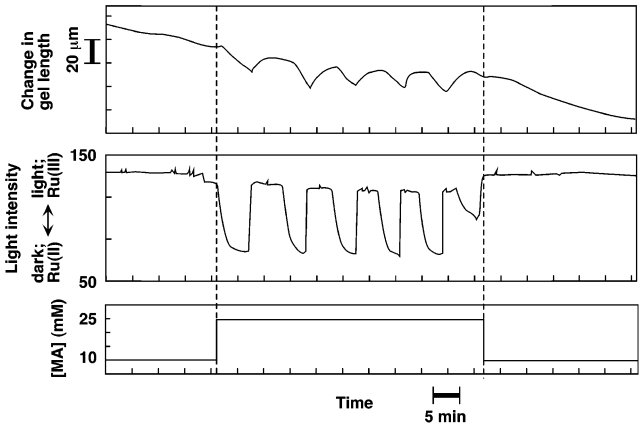

Figure 3. Change in oscillating behavior of the gel in response to the stepwise concentration changes of MA between 10 and 25 mM (others: $[NaBrO_3] = 84$ mM, $[HNO_3] = 0.3$ M, 20 °C). The swelling−deswelling profiles were pictured as a track of the moving gel edge in the spatiotemporal diagram obtained.

concentration was decreased back to the initial value. It was clarified that beating occurred or stopped without hysteresis. In these ways, reversible on−off regulation of self-beating triggered by MA was successfully achieved. In this case, the self-beating occurs at higher concentration and stops at lower concentration. If the stepwise concentration changes are supplied across the upper limit of oscillating region, an inverse response would be possible; that is, oscillation occurs at lower concentration and stops at higher concentration. Since there are some organic acids that can be the substrate for the BZ reaction (e.g., citric acid), the same regulation of beating is possible by using those organic acids instead of MA. And also, as the gel has thermosensitivity due to the NIPAAm component, the beating rhythm can be also controlled by temperature.

As demonstrated above, the gel exhibits an autonomous swelling−deswelling cycle by metabolizing substrates under constant conditions. The cyclic rhythm varies depending on the external concentration of organic acid. Such self-beating behavior will create a lot of new fields for application of gels. As an application to micromachine, we are attempting to prepare the gel actuator exhibiting autonomous ciliary motion by the microfabrication technique using X-ray lithography.[19] A microprojection structure array was formed on the surface of gel strip. It was observed that the array moved in series with the propagation of chemical wave like living ciliary motion. The actuator may serve as a microconveyer to transport micro- or nanoparticles on the surface. Further, we may expect to develop a new type of intelligent self-beating micropump for controlled drug release, which enables the design of a microchip to release drug periodically without power supply from the exterior. For the purpose of such a biomedical application in the future, in this study, MA was selected as a controlling substance because organic acid exists in a body. For these purposes, basic analyses for microfabrication of the gel and for the oscillating behavior in microenvironment are under investigation.

Acknowledgment. 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 (No. 11167206).

References and Notes

(1) Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1984**, *81*, 6379−6380.
(2) Okano, T., Ed. *Biorelated Polymers and Gels: Controlled Release and Applications in Biomedical Engineering*; Academic Press: Boston, 1998.
(3) Yoshida, R.; Uchida, K.; Kaneko, Y.; Sakai, K.; Kikuchi, A.; Sakurai, Y.; Okano, T. *Nature (London)* **1995**, *374*, 240−242.
(4) Siegel, R. A.; Falamarzian, M.; Firestone, B. A.; Moxley, B. C. *J. Controlled Release* **1998**, *8*, 179−182.
(5) Dong, L. C.; Hoffman, A. S. *J. Controlled Release* **1991**, *15*, 141−152.
(6) Beebe, D. J.; Moore, J. S.; Bauer, J. M.; Yu, Q.; Liu, R. H.; Devadoss, C.; Jo, B. H. *Nature (London)* **2000**, *404*, 588−590.
(7) Tanaka, T.; Nishio, I.; Sun, S.-T.; Ueno-Nishio, S. *Science* **1982**, *218*, 467−469.
(8) Osada, Y.; Okuzaki, H.; Hori, H. *Nature (London)* **1992**, *355*, 242−244.
(9) Kataoka, K.; Miyazaki, H.; Bunya, M.; Okano, T.; Sakurai, Y. *J. Am. Chem. Soc.* **1998**, *120*, 12694−12695.
(10) Miyata, T.; Asami, N.; Uragami, T. *Nature (London)* **1999**, *399*, 766−769.
(11) Oya, T.; Enoki, T.; Grosberg, A. Y.; Masamune, S.; Sakiyama, T.; Takeoka, Y.; Tanaka, K.; Wang, G.; Yilmaz, Y.; Feld, M. S.; Dasari, R.; Tanaka, T. *Science* **1999**, *286*, 1543−1545.
(12) Zaikin, A. N.; Zhabotinsky, A. M. *Nature (London)* **1970**, *225*, 535−537.
(13) Field, R. J., Burger, M., Eds. *Oscillations and Traveling Waves in Chemical Systems*; John Wiley & Sons: New York, 1985.
(14) Kapral, R., Showalter, K., Eds. *Chemical Waves and Patterns*; Kluwer Academic Publishers: Dordrecht, 1995.
(15) Epstein, I. R.; Pojman, J. A. *An Introduction to Nonlinear Chemical Dynamics: Oscillations, Waves, Patterns, and Chaos*; Oxford University Press: New York, 1998.
(16) Yoshida, R.; Takahashi, T.; Yamaguchi, T.; Ichijo, H. *J. Am. Chem. Soc.* **1996**, *118*, 5134−5135.
(17) Yoshida, R.; Sakai, T.; Ito, S.; Yamaguchi, T. *J. Am. Chem. Soc.* **2002**, *124*, 8095−8098.
(18) Yoshida, R.; Tanaka, M.; Onodera, S.; Yamaguchi, T.; Kokufuta, E. *J. Phys. Chem. A* **2000**, *104*, 7549−7555.
(19) Tabata, O.; Hirasawa, H.; Aoki, S.; Yoshida, R.; Kokufuta, E. *Sensor Actuators A* **2002**, *95*, 234−238.

MA0259618