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LETTERS

Autonomous Chemomechanical Oscillations in a Hydrogel/Enzyme System Driven by Glucose

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An autonomous chemomechanical oscillator, driven by membrane-controlled enzymatic conversion of a physiological substance, glucose, to hydrogen ion, has been constructed. The oscillator consists of a pH-sensitive, hydrophobic polyelectrolyte hydrogel membrane based on poly(*N*-isopropylacrylamide-*co*-methacrylic acid), and the enzyme glucose oxidase. The system is configured as a transport cell, with the membrane separating two compartments. A solution containing glucose at constant concentration flows through one compartment (Cell I). Glucose permeates the membrane into the other compartment (Cell II), containing glucose oxidase, which converts glucose to hydrogen ion. Hydrogen ions in turn regulate membrane charge, swelling, and glucose permeability, establishing a negative feedback loop. The membrane's response to hydrogen ion exhibits hysteresis, and under proper conditions a feedback instability is created, leading to oscillations in membrane swelling and permeability, and in pH measured in Cell II. The range over which pH oscillates is shifted in the alkaline direction by reducing methacrylic acid content. Period of oscillations increases with time, and ultimately oscillations cease. Both of these phenomena appear to be due to the buildup of gluconate ion in Cell II, which buffers and slows down pH variations.

1. Introduction

Investigations of synthetic chemical oscillators,[1−4] such as the Belousov−Zhabotinsky (BZ) reaction, have yielded numerous insights into periodic behaviors exhibited by biological systems,[5−8] including cAMP pacemakers in slime molds,[9] intracellular glycolytic[10] and calcium[11,12] oscillations, spontaneous beating of the sinoatrial node,[13] the embryonic cell cycle,[14] circadian rhythms,[15] and ultradian hormone secretion.[16,17] Similarly, autonomous oscillating synthetic membrane systems,[18] including the Teorell membrane oscillator[19−22] and lipid-,[23,24] polyelectrolyte-,[25] and polyamino acid-based systems,[26,27] have been touted as analogues of biomembranes whose electrical potentials or permeabilities exhibit temporal fluctuations. Systems involving both membranes and enzymes have also been studied as oscillating biomimetics.[28−32]

Polyelectrolyte hydrogels swell and deswell in response to changes in their chemical environment.[33−36] Hydrogels are mechanically similar to soft biological materials, and periodic, chemomechanical swelling pulsations of hydrogels might be harnessed to rhythmically gate the transport and delivery of biologically active substances, or to exert periodic forces on their environments, again mimicking rhythmic physiological processes.

Yoshida et al.[37] demonstrated periodic swelling changes in hydrogels bathed in media exhibiting chemical oscillations. In one system, a polyacid hydrogel was suspended in a continuous

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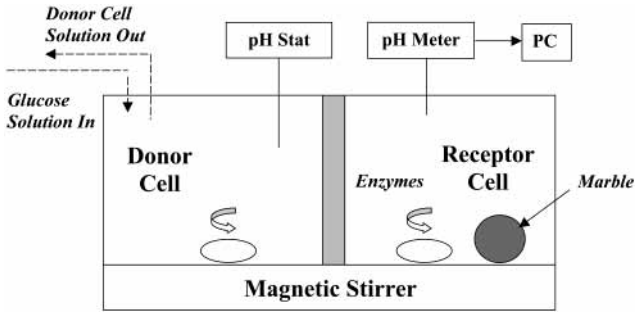

Figure 1. Schematic of glucose-driven chemomechanical oscillator.

stirred tank reactor (CSTR) in which a peroxide-sulfite-hexaferrocyanate pH-oscillator reaction was running.[37] In a second system,[38,39] a hydrogel with covalently attached BZ-catalytic redox groups was immersed in a medium containing all other BZ reactants. In both systems, oscillations in concentrations of ionizing species led to oscillations in fixed charge density, and hence swelling, of the gel. While these systems are interesting as biomimetic models (and exhibit other interesting behaviors such as wave propagation[40]), their ability to function in biological environments is limited, since they must be continuously supplied with toxic redox reactants.

We have constructed a chemomechanical oscillator whose rhythmic pulsations are driven by a constant level of a physiological substance, glucose. This oscillator is based on the bistable swelling/collapse behavior of poly(*N*-isopropylacrylamide-*co*-methacrylic acid) [p(NIPA-*co*-MAA)] hydrogel membranes.[33,34] Such membranes exhibit sharp, hysteretic changes in swelling and permeability to glucose with change in pH, resulting from changes in fixed charge density in the membrane.[41,42] By coupling these transitions to rapid enzymatic conversion of glucose to hydrogen ion in a chamber, a chemomechanical feedback instability is established, resulting in sustained oscillations.

2. Materials and Methods

The glucose-driven chemomechanical oscillator, illustrated in Figure 1, is a side-by-side transport cell (Crown Glass), consisting of two 80 mL cells containing 75 mL saline solutions [50 mM NaCl, with 0.01 w% 2-bromo-2-nitro-1,3-propanediol (antimicrobial; Aldrich)]. The cells are separated by a p(NIPA-*co*-MAA) hydrogel membrane (NIPA, Kodak; MAA, Polysciences), lightly cross-linked with tetraethylene glycol dimethacrylate (EGDMA, Polysciences). Saline containing a constant concentration of glucose flows through Cell I at 1.37 mL/min, and pH in Cell I is clamped at 7.0 using a pH-stat. Cell II contains 5000 units glucose oxidase (Sigma: 234 I.U./mg) and 27000 units catalase (Sigma: 10 700 I.U./mg), along with a 12.5 gm piece of marble. Both cells are well stirred (600 rpm) and water jacketed at 37 °C.

Hydrogel membranes are synthesized by mixing various mole ratios of NIPA and MAA with 0.5 mol % EGDMA and dissolving 500 mg of this mixture in 500 mg water and 500 mg methanol. Solution polymerization is initiated with 5 mg ammomium persulfate (Polysciences) and 20 μL tetramethylenediamine (Aldrich), and carried out overnight at 10 °C between glass plates separated by a 250 μm spacer. After washing in methanol and water, membranes are conditioned in pH 4.5 saline solution at 37 °C, and then mounted into the aperture (radius 1 cm) between Cell I and Cell II.

3. Results

To demonstrate oscillations, we follow pH in Cell II over time. Figure 2 displays a pH record obtained with 50 mM glucose solution pumped through Cell I, and with a membrane synthesized with 10 mol % MAA. Although glucose concentration in Cell I is fixed, pH fluctuates in a nearly periodic manner in Cell II. The pH "troughs" are initially relatively deep, but converge to a nearly constant value after a few periods, while the pH "peaks" converge more rapidly to their final value. The period of oscillation increases with time, and oscillations ultimately cease after numerous periods.

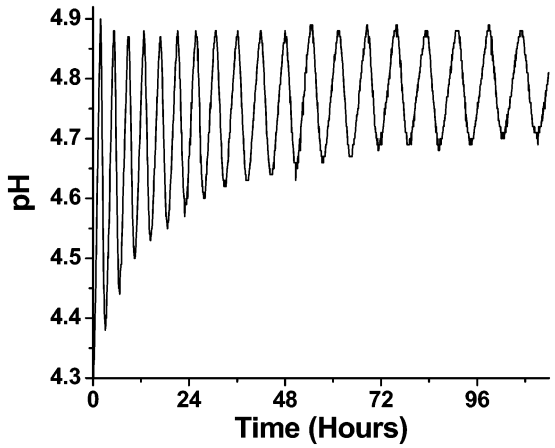

Figure 2. pH oscillations observed for a membrane synthesized with 90 mol % *N*-isopropyl acrylamide (NIPA) and 10 mol % methacrylic acid (MAA). Glucose feed into Cell I is 50 mM.

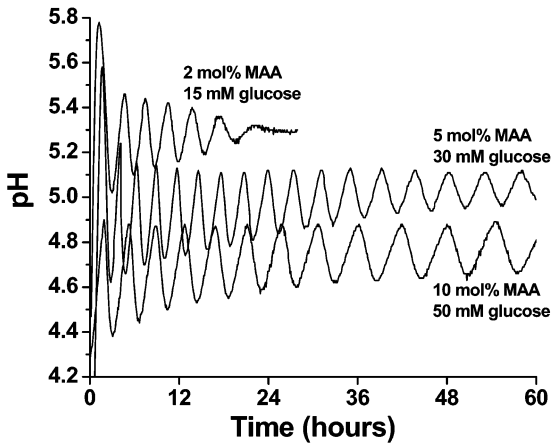

Figure 3. Effect of MAA content on pH oscillations. As MAA content decreases, the range of oscillations shifts in the alkaline direction, and smaller concentrations of glucose fed into Cell I are required to elicit oscillations. The early cutoff of oscillations for 2 mol % MAA may be due to nonoptimal selection of feed glucose concentration. No oscillations are observed when MAA content is below 2 mol %.

The pH oscillations in Cell II are accompanied by coherent alternations in the membrane's stiffness, which can be observed since the membrane exhibits small vibrations in response to hydrodynamic agitation derived from stirring.

The pH range over which oscillations occur is shifted in the alkaline direction by reducing MAA content in the membrane, as shown in Figure 3. Glucose feed concentrations used to elicit oscillations are reduced with decreasing MAA content. Below 2 mol % MAA, the membrane does not swell at 37 °C and 50 mM NaCl, and no oscillations are observed.

4. Discussion

We propose the following mechanism for chemomechanical oscillations. Starting with the membrane in its charged, swollen state, glucose rapidly crosses the membrane from Cell I into

Cell II, and is converted to gluconic acid by the net reaction[43]

$$\text{Glucose} + O_2 \xrightarrow[\text{Catalase}]{\text{Glucose Oxidase}} \text{Gluconic Acid} + \frac{1}{2}O_2$$

Gluconic acid readily dissociates into gluconate$^-$ and H^+ ($pK_a = 3.6$), lowering pH in Cell II. Hydrogen ions then diffuse from Cell II into the hydrogel membrane, where they bind and neutralize carboxylate (MAA) groups in the bordering layer, which subsequently collapses to a dense, taut skin. (Because Cell I is held at pH 7.0, regions of the membrane close to Cell I remain swollen.) Following skin formation, transmembrane flux of glucose to the enzymes, and hence production of H^+ in Cell II, are substantially attenuated. During this phase the pH in Cell II rises, reducing the supply of H^+ to the membrane. Eventually the membrane-bound hydrogen ions in the skin layer are released and diffuse into Cell I, where they are carried away in the drain line. The skin layer then disappears, the hydrogel membrane reverts to its high-permeability state, and the system is poised to repeat the sequence of events.

Oscillations are observed over a bounded range of glucose concentration in Cell I. Below a minimal concentration of glucose, production of H^+ in Cell II is insufficient to form a collapsed skin. When glucose concentration is too high, residual flux of glucose through the skin layer engenders enough H^+ formation to maintain the skin indefinitely.

We previously demonstrated[41,42] that p(NIPA-*co*-MAA) membranes, when exposed to constant pH 7.0 in Cell I and to solutions of varying acidic pH in Cell II, exhibit first-order swelling/collapse transitions in which membrane permeability to glucose is attenuated more than ten-fold upon collapse, but is restored upon reswelling. Collapse and reswelling, which occur in a layer adjacent to Cell II, display pH-hysteresis, i.e., the collapse pH is below the reswelling pH. The proposed mechanism for chemomechanical oscillations can therefore be interpreted in terms of principles utilized in the analysis and design of a variety of chemical oscillators.[2−4,44,45] Many such oscillators feature a component or reaction mechanism exhibiting bistability, plus a "feedback" species, whose level crossings cause the bistable element to flip back and forth between states or branches of behavior. In the present system the pH-hysteretic membrane serves as the bistable element, and hydrogen ion, whose production rate is controlled by glucose flux through the membrane, is the feedback species.

The first-order phase transition in the p(NIPA-*co*-MAA) membrane, with bistability inside the hysteresis band, results from competition between hydrophobic forces associated with the NIPA units (and protonated MAA units at lower pH values), which favor membrane collapse, and electrostatic/osmotic forces that are present when MAA units are ionized at higher pH values and which favor membrane swelling.[33−36] For example, the Flory−Rehner−Donnan equation of state for certain hydrophobic pH-sensitive hydrogels predicts a region of bistability in the swelling versus pH characteristic.[46,47] Without committing to this particular model, we argue that within the band of bistability, the hydrophobic and electrostatic/osmotic interactions establish two attractors, one collapsed and one swollen, respectively, separated by a free energy barrier. This barrier disappears outside the band. Thus a swollen membrane layer bordering Cell II remains swollen until the lower pH-limit of the hysteresis band is crossed, while a collapsed skin persists until the upper pH-limit of the band is surpassed.

The system will oscillate only if circumstances permit pH in Cell II to cross both ends of the hydrogel's hysteresis band. For example, the limited range of glucose concentrations in Cell I supporting oscillations may now be ascribed to trapping of the hydrogel layer bordering Cell II in either the swollen or the collapsed state when glucose concentration is below or above that range, respectively.

Initial undershoots seen in the pH records in Figures 2 and 3 are believed to be due to sluggishness of membrane response to changes in pH in Cell II. These undershoots eventually disappear, and the period of oscillations in pH increases with time. We attribute amplitude transients and drift in period of the pH waveform to steady accumulation of gluconate$^-$ in Cell II, which acts as a pH buffer. The buffer effect reduces the slew rate of pH, allowing the membrane's swelling to follow pH more closely. The ultimate cessation of pH oscillations is presumed to be due to accumulation of sufficient gluconate$^-$ in Cell II to prevent pH from dropping below the lower edge of the membrane's pH-hysteresis band, trapping the membrane in its swollen, high permeability state.

The purpose of added marble is to accelerate pH swings in the system. In previous experiments with marble absent,[42,48] many hours or even days were required to traverse the hysteresis band of the membrane, and at most a pair of oscillations was seen before pH in Cell II converged to a stationary value. In those studies, gluconate$^-$ may have accumulated before any more oscillations could occur. Marble ($CaCO_3$) provides a shunt pathway for removal of H^+ through the heterogeneous net reaction $2H^+ + CaCO_3 \rightarrow Ca^{2+} + H_2O + CO_2\uparrow$.[49,50] By including this shunt and increasing the feed glucose concentration, pH swings in Cell II are both accelerated and kept in the range of the hysteresis band, enabling oscillations.

The alkaline shift in pH-oscillations, and the lower glucose concentrations in Cell I required to elicit oscillations with decreasing MAA content in the membrane, both shown in Figure 3, are explained in terms of the competition between hydrophobic and electrostatic/osmotic forces governing swelling and collapse. At constant ionic strength and temperature, the relative strengths of these forces in a hydrogel are determined primarily by the fixed charge density of the hydrogel membrane, which is the product of the mole fraction of MAA in the polymer and the fraction of MAA groups that are ionized, the latter increasing with pH. Assuming that the limits of the pH-hysteresis band can be identified with particular fixed charge densities that are, to first order, unaffected by membrane composition, it follows that reducing MAA content in the membrane will result in an alkaline shift in the hysteresis band, and hence the range of pH-oscillations. To target pH in Cell II into an increasingly alkaline range, concentration of glucose flowing into Cell I must be reduced, as this leads to decreased flux of glucose into Cell II and decreased enzymatic production of H^+.

5. Conclusions

We have demonstrated the capability of a polyelectrolyte hydrogel/enzyme system to undergo autonomous oscillations, which are driven by nonlinear chemomechanical feedback between swelling and collapse of the gel, and enzyme-catalyzed conversion of glucose to hydrogen ion. This system, in contrast to previously studied oscillating gel systems, is driven by a physiological substrate and shows promise for biomedical applications. On the other hand, the present implementation requires marble which, though not toxic, will ultimately be depleted. We believe that the accelerating role played by marble can be replaced by increasing the ratio of the membrane surface to the volume of Cell II.

We have also shown that the pH range over which oscillations are seen can be manipulated by changing the doping of MAA.

However, this method only permits relatively small alkaline shifts. To function in a physiologically buffered environment, the range of operation must be shifted close to physiological pH, and MAA must be replaced with ionizable groups having a pK_a that brings about such a shift.

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