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Selective control of sensitivity to imidazole derivatives of interference-based biosensors by use of a phase transition gel

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Sensitivity to histidine of an interference-based biosensor coated with a phase transition gel is controlled by swelling and shrinking the gel, while that to imidazole is not changed significantly.

Interference-based biosensors are used for the detection and determination of inhibitors for biocatalysts including enzymes.[1] Inhibitors for enzymes are generally toxic and interference-based biosensors are more sensitive to a large number of toxic inhibitors. However, such sensors are essentially unselective to a specific inhibitor. For example, a sensor based on cytochrome oxidase is sensitive to cyanide, azide and H_2S[2] while a biosensor based on heme peptide (HP) is also sensitive to some inhibitors including imidazole and histidine.[3,4]

If one wishes to determine two inhibitors at the same time, two independent pieces of information have to be acquired. In response to this, an electrochemical/piezoelectric dual response biosensor was developed by modifying an electrode of a quartz crystal resonator with HP.[5] In this case, a decrease in the catalytic activity of HP (for H_2O_2 reduction) and an increase in the mass of the bound inhibitor are measured on the basis of the catalytic current and the resonance frequency, respectively. This dual transduction system is, however, not simple since both electrochemical and piezoelectric equipment must be employed. An alternative way to obtain two independent kinds of information is the use of a sensor array with some different biocatalysts. However, a two-channel electrochemical set-up and two different, but similar biocatalysts are necessary.

In the present work, a single interference-based biosensor is coated with a gel that exhibits a volume phase transition.[6] Permeability of the gel to substances can be controlled by changing an environmental factor, such as temperature. As the gel is shrunk, the permeability to some substances may be lowered, due chiefly to the reduced pore size of the polymer network. In this case, the suppression of the permeability to a large molecule should be more significant than that to a small molecule. On the basis of this effect, we intended to selectively control the sensitivity of the interference-based biosensor to inhibitors (Fig. 1). As a result, we are able to acquire two independent pieces of information, one from the sensor in the swollen state and the other from the sensor in the shrunken state.

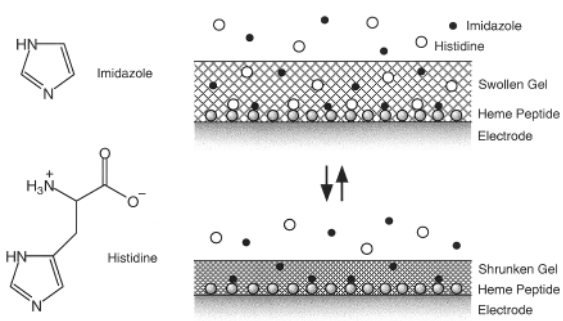

Fig. 1 Schematic depiction for the selective control of sensitivity to imidazole and histidine by shrinking and swelling the gel coated on a heme peptide-modified electrode.

This technique should also be important for the control of the apparent activity and selectivity of biocatalysts. Phase transition gels have been used to control the apparent activity of an enzyme immobilized in or on the gel.[7–14] However, to the best of our knowledge, the selective control of the apparent activity (equivalent to the sensitivity of a sensor) has not, as yet, been reported.

An indium tin oxide (ITO)-coated glass plate (area *ca.* 0.25 cm^2) was treated with a 10% toluene solution of (3-aminopropyl)triethoxysilane to introduce the amino group onto the electrode surface. The electrode was then treated with pH 7.4 phosphate buffer (0.0667 M) containing 1 g L^{-1} heme undecapeptide (HP, Sigma) and 20 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide so as to immobilize HP covalently to the electrode surface *via* amide bonding. The HP electrode thus obtained was further coated with a phase transition gel to obtain a gel–HP electrode. A 10 μL aliquot of a freshly prepared aqueous solution containing 700 mM *N*-isopropylacrylamide (NIPA), 7 mM *N*,*N*′-methylenebisacrylamide as a cross-linking agent, 0.087 mM $(NH_4)_2S_2O_8$ as a polymerization initiator, and 39 mM *N*,*N*,*N*′,*N*′-tetramethylethylenediamine as a reaction accelerator was applied to the electrode surface and was left for 5 h to obtain a gel. The gel–HP electrode thus obtained was then rinsed thoroughly with water (< 30 °C). The gel exhibited a volume phase transition at *ca.* 33 °C. It is known that NIPA-based gels are swollen at lower temperatures and shrunken at higher temperatures.[7,8,11,14] In the swollen state, the thickness of the present gel was *ca.* 1 mm while in the shrunken state, the gel was *ca.* an order of magnitude thinner.

Electrochemical measurements were performed at 25 °C (swollen gel) or 45 °C (shrunken gel) with a potentiostat LC-4C (BAS). Reference and counter electrodes were Ag/AgCl/NaCl(sat.) and a platinum wire, respectively while the electrolyte solution was 0.0133 M phosphate buffer, pH 7.4. The working electrode was polarized at +150 mV *vs.* Ag/AgCl, and a hydrogen peroxide solution was added to the electrolyte (final concentration, 10 μM), followed by the addition of an inhibitor (imidazole or histidine).

First an HP electrode not coated with the gel, was investigated. In response to the addition of hydrogen peroxide, a cathodic current was observed (1.6 nA at 25 °C and 3.9 nA at 45 °C). This is based on the electron transfer from the ITO electrode to compounds **I** and **II** of HP (oxidized forms) [eqns. (1)–(3)]

$$\text{Ferric HP} + H_2O_2 \rightarrow \text{Compound } \mathbf{I} + H_2O \quad (1)$$

$$\text{Compound } \mathbf{I} + H^+ + e^- \rightarrow \text{Compound } \mathbf{II} \quad (2)$$

$$\text{Compound } \mathbf{II} + H^+ + e^- \rightarrow \text{Ferric HP} + H_2O \quad (3)$$

Addition of imidazole or histidine suppressed the cathodic current since imidazole and histidine coordinate to the vacant coordination site of HP at which hydrogen peroxide reacts. These results have already been reported and exploited for the determination of imidazole derivatives.[3–5]

In the present paper, the response R is defined by eqn. (4)

$$R = 1 - (i/i_0) \quad (4)$$

where i_0 is the cathodic current observed after the addition of H_2O_2 (10 μM) and i is the current measured after addition of an

inhibitor (imidazole or histidine) (background current subtracted from both of the currents). In other words, the response *R* is the degree of interference. Even though the measurements were carried out at both 25 and 45 °C, relationships between the response to the inhibitor and the inhibitor concentration (Fig. 2) were similar for the HP electrode without the gel.

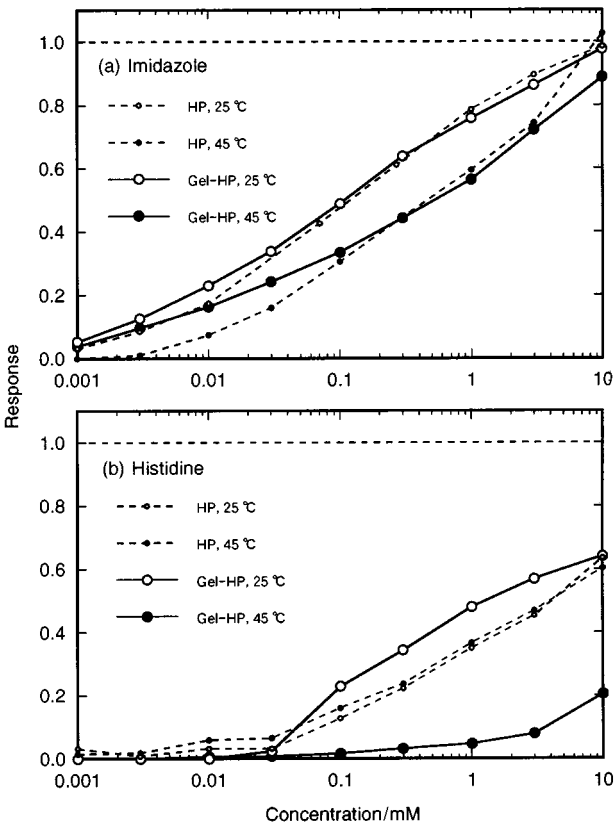

Fig. 2 Responses of the HP and gel–HP electrodes to imidazole (a) and histidine (b) at 25 °C (swollen gel) and 45 °C (shrunken gel) as a function of their concentrations.

The HP electrode coated with the phase transition gel, the gel–HP electrode, was then examined. At 25 °C, when the gel is swollen, the responses to both imidazole and histidine were almost the same as those obtained from the HP electrode without gel [Fig. 2(a) and (b)]. At 45 °C, where the gel is shrunk, while the responses of the gel–HP electrode to imidazole were similar to those of the HP electrode [Fig. 2(a)], the gel–HP electrode exhibited significantly smaller responses to histidine at 45 °C than did the HP electrode [Fig. 2(b)]. This temperature-dependent behavior was reversible for the gel–HP electrode. Additionally, the presence of both analytes (imidazole or histidine) did not interfere in the determination of each. At 45 °C, addition of histidine (final concentration, 1 mM) to 3 mM imidazole led only to a slight increase in the response, as expected from Fig. 2.

Thus, the sensitivity of the gel–HP electrode to histidine was lowered by shrinking the gel without significant changes of the sensitivity of imidazole. In other words, two independent responses can be acquired in the swollen and shrunken states. The concentration of histidine in the shrunken gel is lower than that in the solution by more than one order of magnitude. There are two possible reasons for this selective control of the sensitivity: (i) histidine (M = 155) is larger than imidazole (M = 68) so that the former permeates less effectively into the fine polymer network of the shrunken gel; and (ii) histidine, which has one positive and one negative charge at pH 7.4, should be more hydrophilic than imidazole, and hence would permeate less efficiently into the shrunken gel, which is relatively hydrophobic. In future work, we will examine the possibility of the determination of two interferants in one solution on the basis of the two responses obtained at swollen and shrunken states. Additionally, not only the size and hydrophilic/hydrophobic effects, but also electrostatic and some other effects will be exploited for the selective control of sensitivity of biosensors.

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