Novel Glucose Biosensor Based on the Microcantilever

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ABSTRACT

We report a novel technique for micromechanical detection of biologically relevant glucose by immobilization of glucose oxidase (GOx) onto a microcantilever surface. Microfabricated cantilevers have recently attracted considerable interest in the development of a wide range of novel physical, chemical, and biological sensors. This paper describes the combination of this novel technology with enzyme specificity to construct a highly selective glucose biosensor. The enzyme-functionalized microcantilever undergoes bending due to a change in surface stress induced by the reaction between glucose and the GOx immobilized on the cantilever surface. The common interferents for glucose detection in other detection schemes have been tested and have shown no effect on the measurement of blood glucose level by this technique.

INTRODUCTION

Recent advances in designing and fabricating microcantilever beams capable of detecting extremely small forces, mechanical stresses, and mass additions offer the promising prospects of physical, chemical, and biological sensing with unprecedented sensitivity and dynamic range [1-3]. Microcantilevers, the simplest micro-electro-mechanical-system (MEMS) components, are easily micromachined and mass-produced. Molecular adsorption, when confined to one surface of a cantilever, results in differential surface stress that leads to cantilever bending. The transduction mechanism of a microcantilever sensor is based on the changes in the deflection and resonance frequency induced by environmental factors in the medium in which the cantilever is maintained. The surface stress can be calculated from the magnitude of cantilever bending by using Stoney's formula [4]:

$$z = \frac{3(1-\gamma)L^2}{t^2 E} d\sigma \ , \qquad (1)$$

where z is the cantilever deflection, $d\sigma$ is the differential surface stress, γ is the Poisson's ratio, E is the Young's modulus for the substrate and L and t are the length and thickness of the cantilever, respectively.

In recent years, the unique ability of biomolecules to recognize other molecules has been investigated in the development of microcantilever-based biosensors. Microcantilever-based biosensors can offer many advantages over other biosensor designs; for example, the microcantilevers can easily be fabricated into multiple-element arrays, and the sensor does not require the use of external probes or labeling. General applications of this label-free detection

method have been shown for DNA hybridization, the detection of single-base mismatches [5,6], and nanomechanical motion induced by antibody-antigen interaction [7, 8].

Diabetes mellitus is a disease in which cells fail to take up glucose due to either a lack of insulin (Type I) or an insensitivity to insulin (Type II). The associated elevation of blood glucose levels for prolonged periods of time has been linked to a number of problems, including retinopathy, nephropathy, neuropathy, and heart disease. The diagnosis and management of diabetes require daily monitoring of blood glucose levels. Tight control of the glucose level in the blood is a very important way to delay the onset and dramatically slow the progression of complications from diabetes [9, 10].

Here we report the study of a novel glucose biosensor based on the microcantilever. Glucose deflection was achieved by immobilizing a layer of glucose oxidase (Gox) on the surface of a microcantilever and then detecting the mechanical bending induced by the enzyme reaction that took place on the cantilever surface in the presence of glucose. A major advantage of such a direct transduction is the high selectivity due to the high specificity of GOx.

EXPERIMENTAL SECTION

GOx, bovine serum albumin (BSA), and glutaraldehyde (GA) were obtained from Sigma Chemical Company (USA). β-D-glucose, ascorbic acid, 3-hydroxytyramine hydrochloride, uric acid, 4-acetamidophenol, catechol, D-fructose, and D-mannose were used as received from Aldrich (USA). Other chemicals employed were all of analytical grade. High-purity deionized water was prepared with a Milli-Q water system (≥ 18 MΩ).

Cantilever deflection measurements were carried out by measuring optical beam deflection with a commercially available atomic force microscope head (Digital Instruments, Santa Barbara, CA).

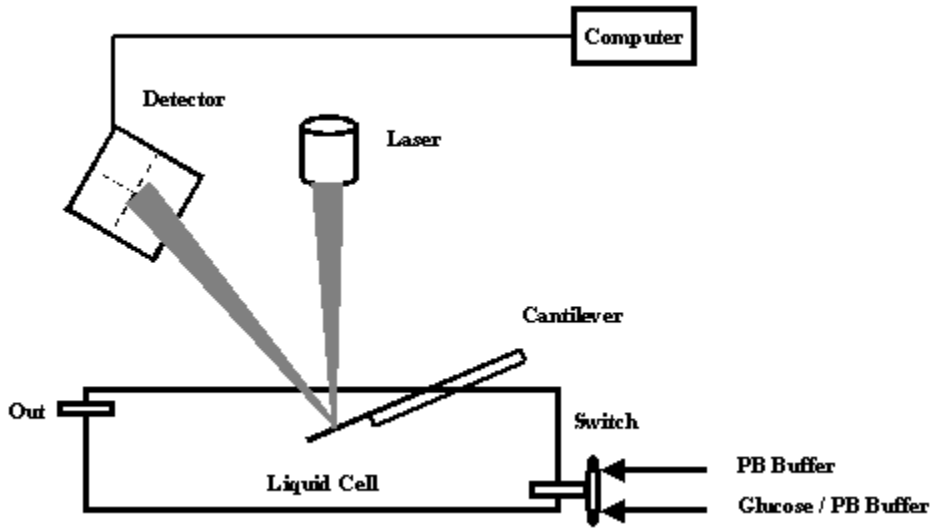

Figure 1. A schematic diagram of the experimental setup. Fluid flow was controlled by a syringe pump connected to an in-line injection valve and 1-mL sample loop.

The experiments were performed in a flow-through glass cell in which the microcantilever was immersed in distilled water. The liquid flow was controlled by a syringe pump (IITC, Inc., Woodland Hill, CA) equipped with a low-pressure liquid chromatography injector valve and injection loop. A schematic of the experimental arrangement is shown in Fig. 1. The bending of the cantilever was measured by monitoring the position of a laser beam reflected off the cantilever onto a position-sensitive detector. For all of the experiments, standard rectangular silicon tipless cantilevers from MikroMasch (USA) were used. The dimensions of the cantilever are 350 μm long, 35 μm wide, and 1 μm thick. Its force constant is 0.03 N/m. On one side of the microcantilever, a 2.5-nm adhesion layer of chromium and then a 25-nm layer of Au were deposited by using an electron-beam evaporator (Thermionics, Port Townsend, WA). Before film deposition, the microcantilevers were carefully cleaned for 10 min in acetone and for 10 min in absolute ethanol and were then immersed in piranha solution (three parts 30% hydrogen peroxide, seven parts concentrated sulfuric acid) for 30 s. They were then rinsed with water and ethanol and were dried in an oven at 80°C for more than 1 h. Then they were ready for the GOx deposition. The cleaned cantilevers were usually stored in vacuum desiccators.

RESULTS AND DISCUSSION

Functionalization of GOx onto the surface of the microcantilever

GOx was immobilized onto the cantilever surface by being cross-linked with GA in the presence of BSA. The enzyme stock solution was prepared by dissolving 20 mg of GOx, 5 mg of BSA, and 40 μL of 50% GA in sequence in 1 mL of PB solution (pH = 7). A microsyringe was used to withdraw 5 μL of the enzyme solution and to dispense it onto the cantilever surface. The cantilever was stored in a refrigerator and dried at 4^{o}C overnight. The cantilever was rinsed with PB solution thoroughly befo it was used for the measurements. The influence of the GOx concentration in the enzyme solution on the deflection response of the cantilever has been tested, and the result showed that higher concentrations of GOx in the enzyme solution cause larger deflections of the cantilever. In all of our subsequent measurements, a 20-mg/mL solution of GOx in the enzyme solution was used.

Deflection response of glucose on the microcantilever

The enzyme-functionalized microcantilever was used to study its deflection response to various concentrations of glucose. The cantilever was first equilibrated with PB solution in the flow cell system until a stable baseline was obtained. Then one concentration of β-D-glucose in same PB solution was injected into the flow cell by syringe pump at a flow rate of 2 mL/h, and the deflection *vs* time profile was recorded. Fig. 2a shows the 5-mM glucose deflection profile for the enzyme-functionalized microcantilever. A clear change in the deflection of the cantilever was observed as the glucose sample solution passed over it. The cantilever continuously bent toward the Au side while glucose solution flowed through the cell. When the glucose sample solution was replaced by the PB washing solution, the deflection of the cantilever arrived at a plateau. A control experiment was carried out on a blank microcantilever. When a 5-mM glucose in PB solution passed over the cantilever, it bent slightly toward the silicon side (Fig. 2b).

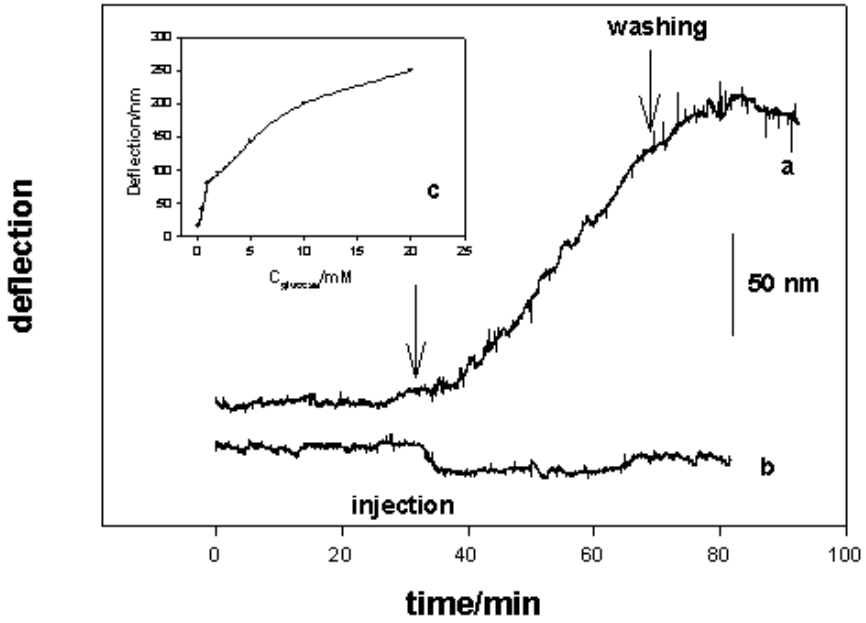

Figure 2. Response of microcantilevers to glucose. (a) Response of a GOx-functionalized microcantilever to a 5-mM concentration of glucose in PB. (b) Response of blank microcantilever to a 5-mM concentration glucose. (c) The calibration curve. Flow rate: 2 mL/h; sample loop: 1 mL.

The cantilever maintained its deflection position when the glucose solution fully filled and stayed in the flow cell. When the washing process began, the cantilever returned to its original position. It displayed totally different response properties from those of the enzyme-functionalized microcantilever. The slight downward deflection was thought to be due to a change in the refractive index because the buffer solution contains a 5-mM concentration of glucose. The GOx-functionalized cantilever deflections were tested by recording profiles of the deflection as a function of time with the injection of different concentrations of glucose in a PB solution at a flow rate of 2 mL/h. The deflection increased with glucose concentration within the range studied. The calibration plot is shown in Fig. 2c.

Specificity and Selectivity

The specificity of the GOx-functionalized microcantilever was tested by injection of other sugars, such as D-fructose and D-mannose. The cantilever did not show any response to those sugars but displayed a highly specific response to glucose.

To demonstrate the possibility of using this sensor use to monitor blood glucose, some co-existing species present in biological fluids were also tested for selectivity. Such species as ascorbic acid, 4-acetaminophen, catechol, and 3-hydroxytyramine appear to cause a slight deflection downward because they alter the refractive index. None of these species interfered the measurement of glucose, suggesting GOx-functionalized microcantilevers could be used as highly selective glucose sensors.

CONCLUSION

Our study has shown that a novel glucose biosensor has been developed based on microcantilever technology. This sensor was found to be highly selective and specific in its response to glucose over a range of concentrations. This work demonstrated that the combination of microcantilever sensor technology with an enzyme-specific reaction has produced a novel glucose sensor.

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