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A temperature-triggered fiber optic biosensor based on hydrogel-magnetic immobilized enzyme complex for sequential determination of cholesterol and glucose

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ABSTRACT

This work described a temperature-triggered fiber optic biosensor for sequential detection of cholesterol and glucose based on poly(*N*-isopropylacrylamide)-co-acrylamide)(P(NIPAAm-co-AAm))-magnetic immobilized glucose oxidase(GOD) complex (PMIGC) and magnetic immobilized cholesterol oxidase (COD). At 38 °C, the sensor will selectively detect cholesterol concentration with the detection range of 25–250 mg/dL since P(NIPAAm-co-AAm) is known to shrink above its lower critical solution temperature (LCST) of 36 °C, and will separate GOD from analytes so that PMIGC has no catalysis effect on glucose. When the temperature was switched to 25 °C (below LCST), PMIGC could catalyze the oxidation of glucose since GOD will be exposed to analytes due to the swell of P(NIPAAm-co-AAm). This sensor can be used for the detection of glucose with the detection range of 50–700 mg/dL. The optimal detection conditions for cholesterol were achieved with pH 7.0, 40 °C and 10 mg COD (in 75 mg carrier), and those for glucose were achieved with pH6.5, 35 °C and 12 mg GOD (in 90 mg carrier). The biosensor proposed is shown to have outstanding repeatability, selectivity and yield satisfactory detection results on practical samples.

1. Introduction

Glucose and cholesterol concentrations in human blood are clinically important in providing information for diagnostic procedures. It is well recognized that abnormal level of glucose in the blood is associated with diabetes mellitus, which is a group of metabolic disorders that accelerate long-term risks of complications such as heart disease, cardiovascular disease, kidney failure and blindness [1]. Cholesterol is an important biomarker for many diseases. The high levels of cholesterol in blood can cause the diseases including heart disease, hypertension, arteriosclerosis, coronary artery disease, cerebral thrombosis [2]. It has been reported that in mice models high glucose could lead to increased macrophage cholesterol accumulation, which is one of the main causes of atherosclerosis [3]. The results strongly suggested that high level of glucose in diabetes could accelerate the macrophage cholesterol accumulation and increase the risk of atherosclerosis. Therefore, the development of fast-responsive, reliable and low-cost method for sequential determination of glucose and cholesterol concentrations is of great importance to clinical medicine and human health.

Many methods have been developed for glucose or cholesterol assays, such as HPLC [4–7], electrode [8–11], electrochemical sensor [12–17], and optical sensing [18–21]. Q. L. Huang et al. developed a dual enzymatic-biosensor to perform simultaneous determination of glucose and cholesterol in serum and peritoneal macrophages of diabetic mice [22]. Fiber optic biosensors have many advantages such as high sensitivity, fast response, immunity from electrical interference [23], and can be promising tools to detect cholesterol and glucose concentrations.

Thermo-responsive hydrogels have tunable network structures [24] and the capability to respond to external temperature changes [25]. This characteristic makes them especially useful for the temperature controlled release of macromolecular drugs [26,27]. Poly(*N*-isopropylacrylamide) (PNIPAAm) is a well known temperature sensitive polymer and demonstrates a lower critical solution temperature (LCST) of ~32 °C in aqueous solution [28]. Poly(*N*-isopropylacrylamide-co-acrylamide)(P(NIPAAm-co-AAm)), formed by the introduction of segment acrylamide(AAm)

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to PNIPAAm, could slightly increase its LCST [29] and made the LCST close to human body temperature. If P(NIPAAm-co-AAm) is used to form complex with glucose oxidase (GOD), at the temperature higher than its LCST, the capsulated GOD will be isolated from the substrate due to the shrinkage of the hydrogen, which will not perform the enzymatic catalysis. On the other hand, at the temperature lower than its LCST, the hydrogel will swell and allow the enzyme to contact with substrate and cause the enzymatic catalysis reaction to occur. Therefore, the complex will have "on-off" switch effect for the GOD catalysis to glucose oxidation. Based on this idea, it will be possible to develop a dual-parameter biosensor by adding another immobilized enzyme (such as immobilized COD) and manipulating the temperature in a certain order so that glucose and cholesterol can be detected sequentially at different temperatures.

In our previous work, P(NIPAAm-co-AAm) was combined with the magnetic immobilized GOD to form P(NIPAAm-co-AAm)-magnetic immobilized GOD complex (PMIGC) and a temperature controlling fiber optic glucose sensor based on PMIGC was developed [30]. In this work, GOD and cholesterol oxidase (COD) were immobilized on Fe_3O_4@SiO_2(F)-@meso-SiO_2 nanoparticles, respectively. With optical oxygen sensing film [31] and lock-in technology [32], a fiber optic dual-parameters biosensor based on PMIGC and immobilized COD was fabricated to perform the sequential determination of cholesterol and glucose concentrations at different temperatures effectively. To the best of our knowledge, this temperature-triggered dual-parameters fiber optic biosensor based on PMIGC and immobilized COD has never been reported before.

2. Experimental

2.1. Materials and instrument

GOD (E.C. 1.1.3.4, 100 U mg^{-1}) was obtained from Aspergillus-niger. COD with a specific activity of more than 10U/mg was purchased from Aldrich-Sigma. Glucose, cholesterol and $Ru(bpy)_3Cl_2$ (99.0%) were obtained from Aldrich-Sigma. All reagents were with analytical grade and used without further purification. Double-distilled water was used throughout the experiments. The optical oxygen sensing membrane was prepared as previously described [31]. P(NIPAAm-co-AAm)-magnetic immobilized GOD complex (PMIGC) was prepared using our reported method [30]. The magnetic immobilized COD was prepared using our previous method [33].

A lock-in amplifier (SR830, Standford Research System, U. S. A.) was used for measuring the phase delay of sensor head.

2.2. Preparation of the sensor

The detecting system was similar to that in our previous work [30]. It consists of a lock-in amplifier, a LED with the excitation wavelength of 416 nm as the light source, a sensor head with an oxygen sensing membrane, a temperature controller, and a computer for data processing (see Fig. 1).

2.3. Measurements

For the determination of cholesterol and glucose concentrations at different temperatures, measurements were carried out using the setup shown in Fig. 1. The sensor head was placed into a tiny reaction cell containing PMIGC, immobilized COD, cholesterol and glucose buffer solution. An entire airtight reaction cell was used to eliminate the interference of oxygen from the open air. A temperature controller was introduced to control the temperature of the reaction cell. The temperature was firstly raised to 38 °C

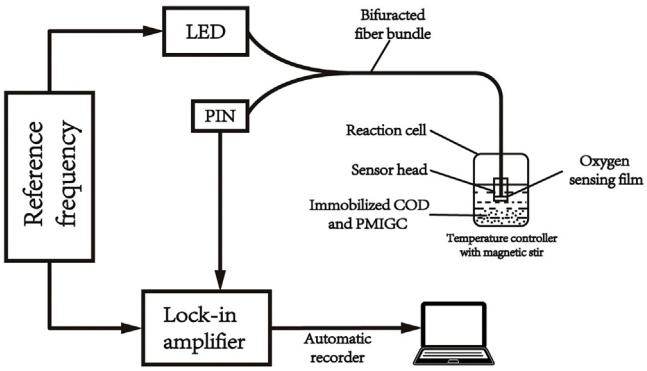

Fig. 1. Schematic diagram of the detecting system.

(above LCST) and the determination of cholesterol concentration was performed by recording the phase delay difference φ. Then the temperature was lowered to 25 °C (below LCST) and the determination of glucose concentration was carried out by recording φ. All the measurements were performed in triplicate. A simple washing of the sensor head, PMIGC and immobilized COD was needed before the following measurement. The magnetic carrier for immobilized COD and PMIGC would be beneficial to their separation from the solution.

3. Results and discussion

3.1. Principle of the sensor

This sensor is based on the fluorescence quenching and consumption of oxygen. The oxygen in the solution will be consumed by the oxidation of cholesterol and glucose catalyzed by COD and GOD, respectively. Using PMIGC and raising the temperature at above the LCST of P(NIPAAm-co-AAm), the cholesterol can be oxidized first with the catalysis of COD while PMIGC will not catalyze the oxidation of glucose. After the cholesterol oxidation is completed, the temperature is lowered below the LCST and the oxidation of glucose occur with the catalysis of PMIGC. Oxygen is consumed by both of the two oxidation processes. When the temperature is higher than LCST, the immobilized COD and optical oxygen sensing membrane act as the cholesterol sensor. When the temperature is lower than LCST, PMIGC and optical oxygen sensing membrane act as the glucose sensor. Therefore, with the same detecting system, the fiber optic cholesterol sensor and fiber optic glucose sensor can be fabricated. The oxygen concentration changes in these two oxidation processes are determined respectively by detecting the fluorescence of $Ru(bpy)_3Cl_2$ quenched by oxygen. Using a lock-in amplifier, the fluorescence quenching can be described as the phase delay difference φ of the sensor head [30]. By detecting φ in these two oxidation processes, the concentrations of glucose and cholesterol are determined.

3.2. Influence factors to the phase delay difference of the sensor

3.2.1. Influence factors to the phase delay difference of cholesterol sensor

In order to get sensitive detection of cholesterol, several influence factors including pH, temperature and GOD amount were studied. For the fiber optic cholesterol sensor, φ was defined as the difference between the phase delay of the sensor head with cholesterol concentration of 100 mg/dL and with no cholesterol in the solution.

The pH effect on the fiber optic cholesterol sensor was investigated over the range from pH 5.0 to 9.0. φ increases from pH 5.0 to 7.0 and reaches its maximal value at pH 7.0. When pH is higher