

Research on Potato Glucose Biosensor Based on Click Reaction and its Application for Chemical Sensors

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Abstract: Biological sensing, qualitative analysis, quantitative DNA and protein are one of the important research issues in biosensor theory. With the improvement of people's living standards and increase of the elderly Population, the incidence of diabetes is rising and becomes the third dangerous disease which is only less than cardiovascular disease and cancer. To this end, based on the click reaction, a facile and efficient method for the preparation of functional glucose oxidase has been developed. It proposes a new method for biological sensing, qualitative analysis, quantitative DNA and protein. This dissertation focuses on how to choose the most appropriate and sensitive analyte specific recognition element. The experimental results show that the potato glucose biosensor is effective and feasible, and has better expansibility. *Copyright © 2013 IFSA.*

Keywords: Chemical sensors, Potato glucose, Biosensor, Click reaction, Electrochemical analysis.

1. Introduction

Using of animal and plant tissue as a biological biosensor catalytic materials, their life is often longer than the pure enzyme preparation, and this is because the body has many natural biological process indispensable for the enzyme reaction and other bimolecular coenzyme auxiliary and synergies role of the enzyme reaction efficiency and longer life expectancy. We know that in organisms, many of recognition between the biomolecule and the identified relationships, such as antigen-antibody recognition, the enzyme-matrix identified aptamer-protein recognition, the biosensor is the use of

biologically active substances and molecular recognition properties between the target molecule [1], the entire event is generated biometric concentration conformation change is converted into visible, can output various signals (electrical, optical signals), depending on the signal strength to be detected visually the amount of analyte [2].

Biosensors make up by the signal recognition component and a signal converter constituted. Where combined with the combination and function of matter called identification element, the biologically active molecules or chemical probes constituted with them directly and to identify test substances. Conversion part is called the converter [3], it is to identify the element component and being produced

by chemical or physical changes into optical signal, such as fluorescence spectroscopy, electrochemical workstation. Biosensor is different from physical and chemical sensor is characterized by containing biological active substances (nucleic acid, enzyme, protein etc.) receptors [4]. Biosensor is the nature of the processes of the biometric various changes in element detection and quantification is easy to convert a signal, which can be life substances and chemicals detection and monitoring, with a sample to be measured highly specific and selective detection system. In short, the biosensor is an analytical device, it is the biological elements (events can lead to biometrics) and physical elements (can be converted recognition event) skillfully combined and used for biological analysis.

British researchers put forward the concept of the enzyme electrode in 1962 [5]; they tried to put the high sensitivity and high specificity of enzymatic reaction electrode combination. In 1967, the first enzyme glucose sensor emerged. Continuously developed subsequent microbial immune biosensors. In the 21st century, life science era [6], DNA research is becoming increasingly important, the use of bio-sensors to detect nucleic acid naturally become a research hotspot. DNA Biosensors is to the presence of DNA (or DNA concentration) through the transition to the measurement converter, visible, such a recording of optical signals [7]. That has been developed for DNA electrochemical biosensor, DNA optical sensor, DNA piezoelectric sensors. In recent years, with the aptamer study [8], the emergence of new aptamer-based biosensor using aptamer as recognition element to detect the presence of the target protein, and the photoelectric conversion signal.

Click reaction is 2001 Nobel Prize winner Professor Sharpless Scripps Research Institute: Cu (I) catalyzed azide formed with terminal acetylene carbon Asia diamine, the mild reaction conditions, selectivity, biological compatibility [9].

In this paper, click reaction the function of glucose oxidase (GOx) is fixed to gold nanoparticles (AuNPS) surface, and apply it to the detection of glucose. Cu (I) catalyzed azide formed with terminal acetylene carbon Asia diamine, the mild reaction conditions, selectivity.

2. Biosensor Design

2.1. Composition of Biosensor

Signal amplification, signal transmission, the signal conversion system, transducer, biological recognition element, data processing, data display system is an important part of the biosensor. Currently there are widespread uses of fiber-optic-based biosensors [10], electrochemical biosensors. Biosensor computer is for data analysis processing and data display. Fiber-type silicon biosensor applications rays, photomultiplier tubes, photodiodes, etc. for transducer photomultiplier maximum energy

conversion efficiency; electrochemical biosensor bit applications sensitive field effect transistor and a glass electrode as a transducer [11].

Nucleic acids, receptors, antibodies, bacteria, cells and enzymes are commonly used in biological recognition element sensitive material produced. Nowadays, used in environmental monitoring biosensor are the most microbial cells and enzymes. The biometric components using immobilized enzymes which with high sensitivity, specificity and strong short response time; however difficult to extract the pure enzyme, the enzyme is relatively short shelf life, easy inactivation [12]. It is only in the physicochemical proper conditions or based on certain soluble cofactor basis, the enzyme will produce effect. On the basis of microbial sensor made of some microbial cells, did not see a strictly limited physicochemical conditions, has a longer shelf life, service life, the cost is not high, but the regeneration rate is slow, long response time, in certain situations with poor selectivity and specificity.

2.2. The Basic Principles of Biosensors

Usually, functional biosensor using biological recognition element component to immobilized enzyme technology as a foundation immobilized cells. Identification of the measured object, perception, and based on the law to be identifiable signal conversion by the converter to produce analyte-specific responses, information, or other biological sensing element for sound, electricity, light, etc. and easy detection signal conversion, indirect access to relevant information analyte. Usually, the enzyme, enzyme components, cell, cell membrane, cell, organism, antibody, nucleic acid, organization, receptor, organic molecules are biologically sensitive element. Converter are the main types of conductivity, light, electric, acoustic intensity measurement type, current, impedance, measurement of heat type, "molecular" electronic type and mechanical type etc. The schematic diagram of a biosensor device as show in Fig. 1.

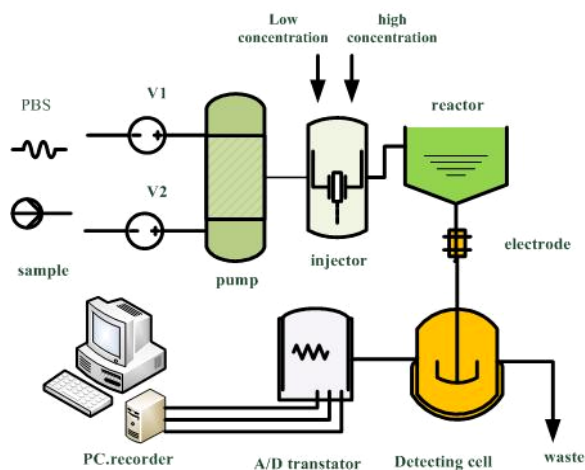


Fig. 1. Schematic diagram of a biosensor device.

3. Simulation Testing and Experimentation

3.1. Reagents and Instruments

All electrochemical experiments were performed on CHI660C electrochemical workstation (CH instruments) on, and the three electrode cell system of traditional. Diameter of 2 mm gold disk electrode as working electrode, a reference electrode was a saturated KCl Gan pro electrode (SCE), the counter electrode is carbon rod. In this paper all potentials with the SCE. Electrochemical quartz crystal microbalance (QCM, USA) studied using HP4395A impedance analyzer (USA HP). Gold-plated AT-cut 9 MHz piezoelectric quartz crystal (PQC, crystal diameter 12.5 mm, electrode diameter 6.0 mm, JA5B type) were purchased from Beijing Chen Jing Electronics Co., experimental single-touch solution, and touch on the gold electrode surface working electrode. Transmission electron microscopy (TEM) images collected from Hitachi 800 transmission electron microscope. UV-vis spectra collected from the UV-2450 spectrophotometer (Shimadzu, Japan). Gold chloride acid (HAuCl₄) and Azusa trisodium citrate were purchased from Shanghai Chemical Reagent Company. Aniline and Tiberius were purchased from Shantou West Chen plants and Sinopharm Chemical Co., Ltd. (Shanghai), distilled before the experiment after purification. Chitosan (CS, 90wt.% Deacetylation) and p-benzoquinone (BQ) were purchased from Sinopharm Chemical Co., Ltd. (Shanghai). Ferrocenecarboxylate (FcMA) were purchased from Suzhou Time-chem Technology Co., Ltd. (Suzhou). All other reagents were of analytical grade or higher purity. 0.60 wt.% CS solution consisted of 0.2 M pH 3.34 acetate buffer configurations. Glucose solution before use in the preparation of one day was well-balanced effect of glucose mutarotation. Human serum samples are taken from Hunan Normal University Hospital. All solutions are using Milli-Q ultrapure water now with the current. Experiments were performed at room temperature (23 ± 1 °C) temperature.

3.2. Preparation of the Sensor

The bare glassy carbon electrode respectively 1.0, 0.3 and 0.05 μm diameter Al₂O₃ in deerskin polishing paste to make a mirror, followed by acetone, the volume ratio of 1:1 HNO₃, NaOH and distilled water for sound processing. Before the modification, the glassy carbon electrode was placed 0.6 mol/L of H₂SO₄ scan to get a stable cyclic voltammogram so far. Pipette 5 μL AzNPs/G0x dropped on the glassy carbon electrode surface. Finally, take 2.0 μL 5% Nafion solution was dropped on the electrode surface, dry naturally, that is namely detection sensor. The schematic diagram of DNA mutation detection as show in Fig. 2.

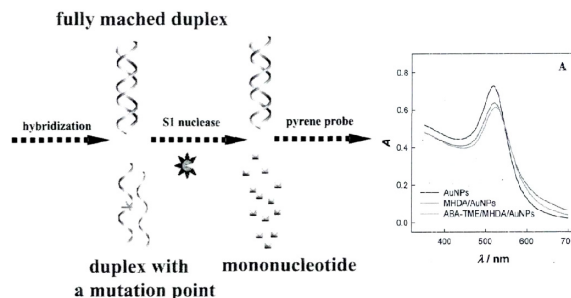


Fig. 2. Schematic diagram of DNA mutation detection.

4. Results and analysis

4.1. Characterization of Gold Nanoparticles

Structure of citrate stabilized gold nanoparticles by transmission electron microscopy and UV visible spectrophotometers were used to characterize the. A Fig. 3 gold nanoparticle dispersed evenly, average particle diameter is 12-23 nm. UV-visible absorption spectroscopy is used to confirm the formation of gold nanoparticles. Gold particles have a characteristic absorption at 520 nm peak, so you can prove successful synthesis of gold nanoparticles.

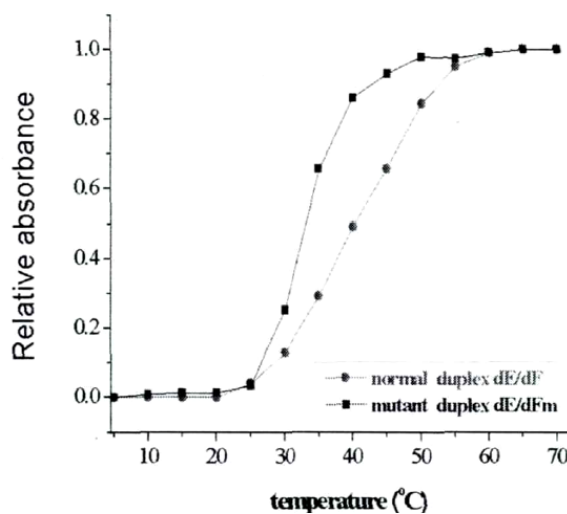


Fig. 3. The normal 10-mer duplex DNA (dE/dF).

Through the DNA melting curve, calculate the melting temperature of two DNA samples (T_m). As shown in Fig. 4, for the normal DNA samples, the measured melting temperatures are 40 °C and 50 °C. For the mutant DNA samples, the measured melting temperatures are 45 °C and 55 °C. Thus, the normal melting temperature of DNA (T_m) is higher than the melting temperature of mutant DNA (T_m). This is because, two single stranded DNA normal after natural annealing process can form double stranded DNA is quite stable, not easy chain, T_m is relatively high. One single stranded DNA point mutation, after annealing treatment is not easy to form a stable

double stranded DNA, easy solution chain; T_m is lower than that of normal DNA.

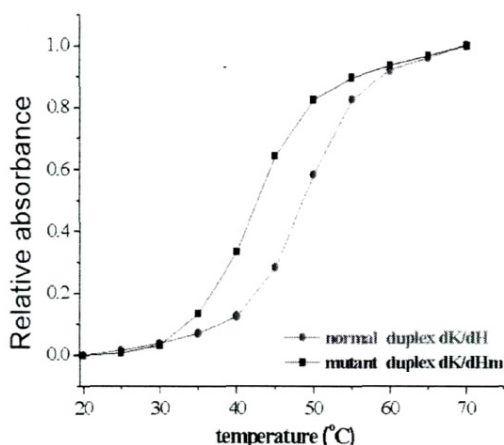


Fig. 4. The normal 15-mer duplex DNA (dK/dH).

4.2. Electrochemical Biosensor

AC impedance spectroscopy is a powerful tool for study of electrode surface properties; it can be more in-depth study of electrode resistance, which confirmed that the gold nanoparticles functionalized with glucose oxidase by linking reactions were well combined with the experiment.

We also evaluated the reproducibility of electrode, long-term storage stability and selectivity. The relative standard deviation of test the same electrode for 5 consecutive times (RSD) of 4.3 %, reflects the very good repeatability. Stored at 5°C the month after next, relative to the initial response, CS/HRP-PTBA/Au_{plate}/Au electrode response only dropped 9.54 %, while CS/HRP/PTBA/Au_{plate}/Au decreased by 13.3 %, showed that the enzyme in the synthesis of polymer compared to chemical synthesis polymer has more excellent ability to maintain enzyme activity. The electrode stability of this enhancement can be attributed to the synthesis of the enzyme under mild conditions, the destructive enzymes minimal. Such as Fig. 5, glucose, adding UA and AA no significant response, indicates that the sensor has good anti jamming capability.

4.3. Electrochemical Detection of Glucose

Under the optimized experimental conditions, Fig. 6 is a GCE/AzNPs/GOx sensor in the working potential, 0.6 V of different concentration of glucose current response and the working curve is shown in Fig. 6. In PBS, the continuous addition of glucose solution of different concentration response relationship, "current and glucose concentration from Fig. 6 can be obtained, the linear range for the detection of glucose the sensor is 5 μ M-1.82 mM, the linear equation was $I(\mu A) = 0.3453 + 0.023C(\mu M)$,

the sensitivity is 280 $\mu A / mM/cm^2$, and the minimum detection limit of the method was 33 0.5 μM .

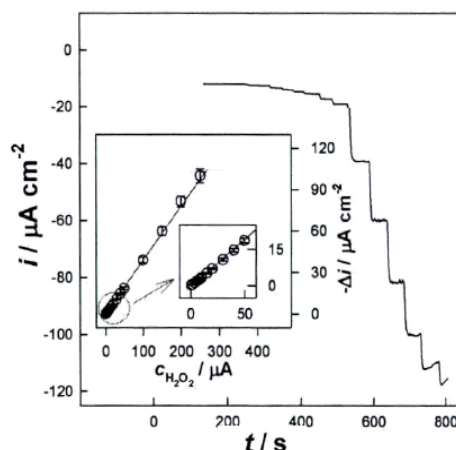


Fig. 5. CS/HRP-PTh/AUplate/Au.

Table 1. Determination of glucose in serum samples.

Samples	Added(10^{-4} M)	Found(10^{-5} M)	R.S.D(%)
A	0.2	1.32	1.4
	3.0	1.90	1.75
	5.0	6.78	2.3
B	0.0	2.11	0.78
	3.0	3.34	1.8
	5.0	5.67	1.2

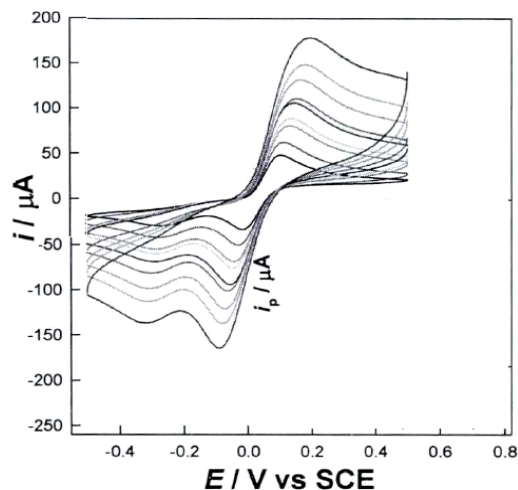


Fig. 6. Plots of the anodic (i_{pa}) and cathodic (i_{pc}) peak currents versus the square roots of scan rates.

5. Conclusions

In this paper, explore new methods for biological sensing, qualitative analysis, quantitative DNA and protein. Biosensor is the use of biological active substances (nucleic acid, protein, enzyme, cell and molecular recognition between functions), the concentration, the target substance detected

conformational changes and other changes of biological processes microscopic into physical chemical signals visible, measurable (such as fluorescence signal, signal), so as to achieve the target molecular purposes.

Biosensor design, focusing on how to choose the most appropriate and sensitive analyte specific recognition element, which directly determines the quality and sensitivity of the sensor selectivity level of antigen-antibody, enzyme-matrix and the aptamer-protein are very good candidate combinations. There are many different types of biosensors, such as the enzyme sensor, a glucose sensor, and immune sensor. Bio sensing method as a biotechnology a superior means of monitoring and detection methods, with good application value and development space. This paper studies based on fluorescence probe and nucleic acid aptamer biosensor and applied to the analysis of nucleic acids and proteins and detection.

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