

Development of Nanoprobes as Glucose Biosensors

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ABSTRACT

In 2005, the American Diabetes Association (ADA) reported that 20.8 million people have diabetes in the USA. The objective is to focus on the development of glucose sensitive nanoprobes that could be inserted into erythrocytes, red blood cells (RBC). Glucose oxidase (GOx), dextran, and the fluorescent dye pairs, Texas Red (TR), and Alexa Fluor 647 (AF 647), were utilized to develop the nanoprobes. TR, the donor fluorophores, was labeled to dextran while AF 647, the acceptor fluorophores, was labeled to GOx. The fluorescent probes are based on a competitive binding technique and uses the chemical transduction method of fluorescence resonance energy transfer (FRET) to detect the presence of glucose. Dextran binds to GOx, but in the presence of glucose, the donor-dextran gets displaced from the acceptor-GOx, resulting in a decrease in acceptor fluorescence with a corresponding increase in donor fluorescence.

Keywords: biosensor, glucose, diabetes, nanosensor.

INTRODUCTION

The monitoring of glucose is imperative among all diabetics. Diabetes is a widely spread disease in the world. According to the ADA by the year 2020, there will be 250 million people affected by diabetes. Diabetics monitor their glucose levels several times a day, followed by appropriate actions to maintain normal glycemia. Monitoring glucose level will help diabetics to prevent adverse affects of diabetes. A noninvasive glucose sensor would help improve the lives of many people by reducing the pain associated with the testing. The glucose sensors commercially available are electrochemical sensors that still require extraction of blood samples.

Recently, increase focus has been placed upon the development of sensors and biosensor devices for the long-term monitoring and managing of health conditions. In particular, glucose sensors have been heavily investigated [1]. The main objective in these investigations has been to develop an ideal sensor with ultra-sensitivity, high selectivity, reliability, limited biofouling, and low cost.

Many scientists are investigating glucose sensors based on fluorescence spectroscopy. It has the advantages of high sensitivity and superior specificity provided by molecular recognition [2].

There are many fluorescent probes that monitor glucose accurately. For example, a series of experiments were performed using FRET and competitive binding technique. McShane et al. [3] utilized Concanavalin A and dextran which were labeled with acceptor and donor dyes respectively. In the presence of glucose, the labeled dextran was displaced from the Concanavalin A resulting in a change in fluorescence. The sensors are being placed in microspheres, which have yet to be investigated for biocompatibility and to ensure that the toxic Concanavalin A does not leach out of the microspheres.

Glucose concentration can be estimated from FRET efficiency, and the ratiometric nature of the FRET analysis method allows compensation for variations in instrumental parameters, assay component concentrations, and measurement configuration [4].

MATERIAL

Glucose Oxidase 2 mg/ml, pH 7.0 (Sigma – G2133 – lot 034K1462)
Sodium Bicarbonate 84.01 MW, 0.1 M, pH 8.3 (Sigma - S-6297 - lot 50K0241)
Alexa Fluor 647 1300MW, 10 mg/ml (Molecular Probes – A20173 – lot 100E5-1)
DMSO 78.13 MW (MU Recycling – D-5879 – lot 100F-0269)
ddH₂O
PBS 0.01M, pH 7.4 (Sigma - P-3813 - lot 075K8206)
Beta-D (+) glucose 180.2 MW, 100 mg/ml (Sigma, G5250, Batch # 014K1265)
Texas Red-dextran 5mg/ml (molecular weight 70,000 Da, 4.5 mol TR/mol of dextran)

INSTRUMENTATION

A UV-Vis absorbance spectrometer (Beckman DU 520) was used to collect absorbance spectra and perform catalytic activity tests. The slit size (4 nm) and scanning speed (595 nm/min) were held constant throughout all the experiments. A scanning fluorescence Spectrometer (FluoroMax-3 Jobin Yvon, Hobira) was used to collect fluorescence emission spectra by exciting the sample at 595 nm. The slit size and integration time were 4 nm and 0.5 s, respectively.

METHODS

Glucose Oxidase (GOx), dextran, and the fluorescent dye pairs, Texas Red (TR), and Alexa Fluor 647 (AF 647), were utilized to develop the nanoprobe. TR, the donor fluorophores, was labeled to dextran while AF 647, the acceptor fluorophores, was labeled to GOx. The fluorescent probes are based on a competitive binding technique and uses the chemical transduction method of FRET to detect the presence of glucose. Dextran binds to GOx and results in an energy transfer from the donor to the acceptor fluorophores. But in the presence of glucose, the donor-dextran gets displaced from the acceptor-GOx, resulting in a decrease in acceptor fluorescence with a corresponding increase in donor fluorescence. The response of the nanoprobe to glucose was tested in FluoroMax-3 spectrofluorometer. 40 mg AF 647-GOx was added to 20 mg TR-dextran in a 1 ml microcuvett of PBS. Initially, baseline fluorescence was obtained. Then various concentration of glucose was added and the change in fluorescence was recorded.

RESULTS AND DISCUSSION

The results showed a decrease in the acceptor fluorescence and an increase in the donor fluorescence as glucose was added to the solution. The glucose displaced the dextran as it binds to the GOx. Figure 1 shows a decrease in acceptor against a normalized donor peak.

The nanoprobe displayed a sensitivity of at least 0.5 mM below 5 mM and a sensitivity of at least 3 mM above 5 mM, which meets our initial requirements for an implantable glucose sensor.

It is interesting to note when the data is plotted donor/acceptor fluorescence vs. glucose concentration, the data follows the Michaelis-Menten equation as expected for competition between the donor-dextran and glucose as in figure 2. The nanoprobe displayed a sensitivity of at least 0.5 mM below 5 mM and a sensitivity of at least 3 mM above 5 mM, which meets our initial expectations.

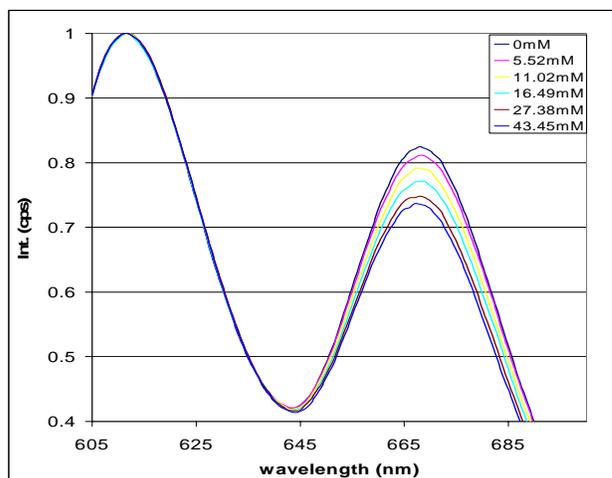


Figure 1: Normalized donor peaks of TR-Dextran showing a change in the acceptor fluorescence in the presence of glucose.

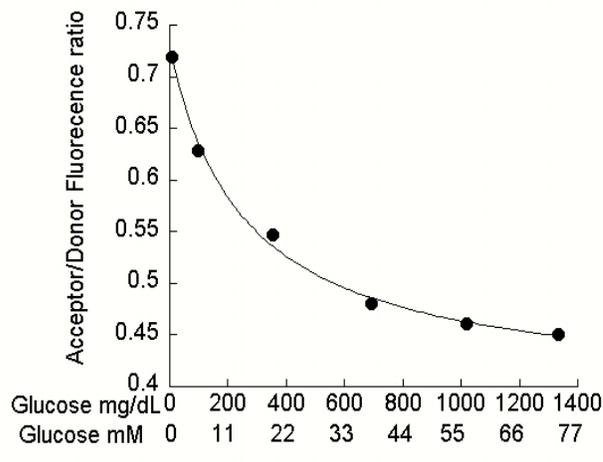


Figure 2 displays the nanoprobe response (change in acceptor response) upon glucose addition. As the glucose concentration increases, the acceptor fluorescence decreases.

CONCLUSION

The nanoprobe biosensor responded well to different glucose concentrations. This results well be used to encapsulate in the red blood cells.

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