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PYRROLOQUINOLINE QUINONE GLUCOSE DEHYDROGENASE THERMISTOR FOR GLUCOSE DETECTION

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Abstract: An enzyme thermistor (ET) using pyrroloquinoline quinine glucose dehydrogenase (PQQGDH), an enzyme with high catalytic activity to glucose, was constructed to develop a thermal glucose sensor. The PQQGDH ET giving a wide linear relationship to glucose from 9μM to 100 mM. It is reproducible, stable, oxygen independent and inert to lactate and urea.
Keywords: pyrroloquinoline quinone glucose dehydrogenase, enzyme thermistor, glucose sensor

INTRODUCTION

Pyrroloquinoline quinine glucose dehydrogenase (PQQGDH) is an enzyme with high potential in glucose sensor devices, because of its high catalytic activity to glucose and insensitivity to oxygen [1-2]. Methods and devices for glucose sensing based on this enzyme published to date mostly included using an electrode [3-5] or an optical meter [6], sensors according to a thermal principle has not been seen reported. The aim to develop a PQQGDH based thermal glucose sensor is that thermal sensors are usually more inert or insensitive to electroactive or coloring impurities, and easy to operate.

EXPERIMENTAL

Measurements were performed in an aluminum thermostat as described by Danielsson [7]. The thermometric probe was designed as a polyacrylate tube which could fit to the immobilized enzyme column at one end. The rest of the probe contained gold tubing onto which a sensitive thermistor was attached. Two parallel fluid lines were installed in the thermostat column and one of them used as a reference system. The sample/buffer was pumped through the enzyme thermistor unit with a peristaltic pump at a selected flow rate normally set at a range of 0.3 ~ 0.8 ml/min; a 0.1 ml sample size loop was used to inject the sample. Measurement was performed after a stable baseline was reached. It was achieved by running the buffer at the measuring conditions. For an anaerobic detection, the buffer and the sample solution were thoroughly deoxygenated with N_2 before measurement.

RESULT AND DISCUSSION

Detect glucose in the absence and presence of electron acceptor

In the absence of phenanine methylsulfate (PMS), the enzyme thermistor only gave small thermal response which did not show any proportional relationship with the glucose concentration. In the presence of PMS, the thermal response was seen immediately increased and showed a linear relationship to a concentration of 100 mM which is still not the up limit of the linear response under the condition. The correlation coefficient of the straight line was 0.999.

Measurement at fixed PMS concentration showed that at low PMS concentration (10 mM), the detection limit of glucose is up to 25 mM, this was extended to 50 mM at 30 mM PMS and over 100 mM at 50 mM PMS (*Figure 1*). The detection linear range is seen increased as the electron acceptor's concentration increased. On the other hand, experiment carried at fixed glucose concentration showed that more PMS are required for higher concentration of glucose to reach a platform. The ratio was estimated 0.5:1 in concentration for PMS and glucose.

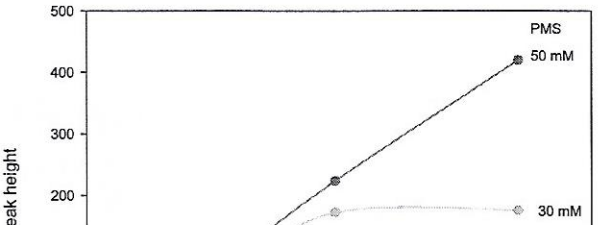

Figure 1. The dependence of thermal signal on glucose concentration recorded at fixed PMS concentrations.

The sensitivity and reproducibility of the PQQGDH ET

Sensitivity test showed that the ET could detect as low as 9 μM glucose. Correlation coefficient of the straight line for glucose concentration from 9 μM to 20 mM is 0.995. It is 0.999 when regress the data from 9 μM to 0.62 mM. it was found that the PQQGDH ET with one-off enzyme loading can measure glucose from 9 μM to 100 mM with linear response. The detection window can expand when the electronic recorded system is designed sufficiently sensitive.