

Comparing the Performance of the Optical Glucose Assay Based on Glucose Binding Protein with High-Performance Anion-Exchange Chromatography with Pulsed Electrochemical Detection: Efforts to Design a Low-Cost Point-of-Care Glucose Sensor

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Abstract

Background:

The glucose binding protein (GBP) is one of many soluble binding proteins found in the periplasmic space of gram-negative bacteria. These proteins are responsible for chemotactic responses and active transport of chemical species across the membrane. Upon ligand binding, binding proteins undergo a large conformational change, which is the basis for converting these proteins into optical biosensors.

Methods:

The GBP biosensor was prepared by attaching a polarity-sensitive fluorescent probe to a single cysteine mutation at a site on the protein that is allosterically responsive to glucose binding. The fluorescence response of the resulting sensor was validated against high-performance anion-exchange chromatography (HPAEC) with pulsed electrochemical detection. Finally, a simple fluorescence reader was built using a lifetime-assisted ratiometric technique.

Results:

The GBP assay has a linear range of quantification of 0.100–2.00 μM and a sensitivity of 0.164 μM^{-1} under the specified experimental conditions. The comparison between GBP and HPAEC readings for nine blind samples indicates that there is no statistical difference between the analytical results of the two methods at the 95% confidence level. Although the methods of fluorescence detection are based on different principles, the response of the homemade device to glucose concentrations was comparable to the response of the larger and more expensive tabletop fluorescence spectrophotometer.

Conclusions:

A glucose binding protein labeled with a polarity-sensitive probe can be used for measuring micromolar amounts of glucose. Using a lifetime-assisted ratiometric technique, a low-cost GBP-based micromolar glucose monitor could be built.

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Abbreviations: (GBP) glucose binding protein, (HPAEC) high-performance anion-exchange chromatography, (LED) light-emitting diode, (LOD) limit of detection, (LOQ) limit of quantification, (PBPs) periplasmic binding proteins, (PED) pulsed electrochemical detection, (PMT) photomultiplier tube, (Ru(bpp)) Bis(2,2'-bipyridine)-(5-isothiocyanato-phenanthroline) ruthenium II(hexafluorophosphate)

Keywords: binding protein, biosensor, diabetes, glucose monitor

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