Journal of Diabetes Science and Technology
Volume 6, Issue 6, November 2012

SYMPOSIUM

Fluorescence Resonance Energy Transfer Glucose Sensor from Site-Specific Dual Labeling of Glucose/Galactose Binding Protein Using Ligand Protection

Helen V. Hsieh, Ph.D., Douglas B. Sherman, Ph.D., Sandra A. Andaluz, M.S., Terry J. Amiss, Ph.D., and J. Bruce Pitner, Ph.D.

Abstract

Background:

Site-selective modification of proteins at two separate locations using two different reagents is highly desirable for biosensor applications employing fluorescence resonance energy transfer (FRET), but few strategies are available for such modification. To address this challenge, sequential selective modification of two cysteines in glucose/galactose binding protein (GGBP) was demonstrated using a technique we call "ligand protection."

Method:

In this technique, two cysteines were introduced in GGBP and one cysteine is rendered inaccessible by the presence of glucose, thus allowing sequential attachment of two different thiol-reactive reagents. The mutant E149C/A213C/L238S was first labeled at E149C in the presence of the ligand glucose. Following dialysis and removal of glucose, the protein was labeled with a second dye, either Texas Red (TR) C5 bromoacetamide or TR C2 maleimide, at the second site, A213C.

Results:

Changes in glucose-dependent fluorescence were observed that were consistent with FRET between the nitrobenzoxadiazole and TR fluorophores. Comparison of models and spectroscopic properties of the C2 and C5 TR FRET constructs suggests the greater rigidity of the C2 linker provides more efficient FRET.

Conclusions:

The ligand protection strategy provides a simple method for labeling GGBP with two different fluorophores to construct FRET-based glucose sensors with glucose affinity within the human physiological glucose range (1–30 mM). This general strategy may also have broad utility for other protein-labeling applications.

J Diabetes Sci Technol 2012;6(6):1286-1295

Author Affiliations: BD Technologies, Research Triangle Park, North Carolina

Abbreviations: (CCS) E149C/A213C/L238S, (DTT) dithiothreitol, (FRET) fluorescence resonance energy transfer, (GGBP) glucose/galactose binding protein, (IANBD) N,N′-dimethyl-N-(iodoacetyl)-N′-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine, (NBD) nitrobenzoxadiazole, (PBP) periplasmic binding protein, (PBS) phosphate buffered saline, (TR) Texas Red

Keywords: biosensor, fluorescence resonance energy transfer, glucose monitoring, protein engineering, reagentless sensor

Corresponding Author: Helen V. Hsieh, Ph.D., BD Technologies, 21 Davis Dr., Research Triangle Park, NC 27709; email address Helen_V_Hsieh@bd.com