Bioluminescent Assays for Studying Insulin Biology
Donna Leippe, Michael P. Valley, Gediminas Vidugiris, Natasha Karassina, James J. Cali and Jolanta Vidugiriene
Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711

1. Introduction

The interplay between glucose, lipid, protein and amino acid metabolism is complex and must be finely regulated in response to changing environments. Hormones such as insulin and glucagon have a central role in communicating environmental conditions and coordinating metabolic pathways at the cellular, organ and whole organism level.

To facilitate studies of insulin, glucagon and lipid function we have developed new bioluminescent homogeneous, in-solution immunoassays based on a luciferase complementation system. The system consists of two subunits that have weak binding affinity and must be brought into proximity to restore a functional luciferase and generate a light signal. The subunits were attached to two anti-insulin antibodies or two anti-glucagon antibodies to develop homogeneous immunoassays with ranges of 10 pM to 8 nM for insulin and 0.5 pM to 1 nM for glucagon. The immunoassays have been used to measure GSIS in model systems including cell lines and human islet microtissues. The assay requires small volumes of sample and can be performed in 384-well plates to enable the rapid measurement of large numbers of samples, such as may occur during perfusion or screening experiments.

We have also used bioluminescent metabolite assays to study insulin action on specific cells and tissues. These metabolite assays are based on an enzyme-coupled, bioluminescent NADH detection technology that was developed for monitoring key metabolic pathways, such as glycolysis, glucose-uptake, lipolysis and lipogenesis. Hepatic glucose overexpression was measured in a liver microtissue model system using a glucose assay to measure glucose production, which could be inhibited by insulin treatment. Effects of insulin on adipocytes were investigated using differentiated 3T3-L1 MBX adipocytes. In this cell system insulin was observed to increase glucose uptake and suppress lipolysis using glucose uptake and glycerol assays.

2. Lumit™ Immunoassays

• Lumit™ Immunoassays are bioluminescent immunoassays.
• They utilize the NanoBiT™ technology, a structural complementation system based on a small and very bright NADH luciferase.
  - Large BT: 18Da domain with no luciferase activity (gLBiT)
  - Small BT: a peptide (11 aa) which has low affinity for gLBiT (sLBiT)
• The BTs must be in proximity to generate an active luciferase
• The BTs can be conjugated to proteins, including antibodies

When labeled antibodies bind to their analyte, the BTs are brought into proximity and form an active luciferase

Lumit™ Immunoassays are in-solution, no-wash immunoassays

3. Lumit™ Insulin and Glucagon Immunoassays

4. Insulin Secretion Measurements

Measurement of Glucose-Stimulated Insulin Secretion in Cell Models

5. Compatibility with Miniaturization and Semi-Automated Dispensing

The immunoassays are amenable to the miniaturization and automation needed for rapid measurements of large numbers of samples.

6. Bioluminescent Metabolite Assays

• The Metabolite Assays are based on a bioluminescent NAD(P)H detection technology and coupled enzyme reactions
• They can be used for the rapid detection of multiple metabolites
• Sample types include cell culture media, cell lysates and tissue homogenates

7. Insulin Action on Lipid Metabolism

• Cellular glucose and lipid metabolism can be altered in response to insulin
• These changes can be detected using metabolite assays in several cellular model systems including adipocytes, myotubes and hepatocytes

3T3-L1 MBX Fibroblasts → Adipocytes

Insulin Inhibition of Lipolysis Measured by Glycerol Secretion

Adipocytes were treated for 50 min with combinations of insulin (25 nM) and glucagon (150 nM). Lipolysis was assayed by measuring glycerol released into the medium. Isoproterenol-stimulated lipolysis was inhibited 2-fold by insulin.

8. Insulin Action on Glucose Metabolism

Insulin Stimulation of Glucose Uptake in Adipocytes

Inhibition of Glucose Uptake

Treatment of adipocytes with insulin increased glucose uptake in a dose-dependent manner. This is due to the insulin-induced translocation of GLUT4 to the plasma membrane. The bioluminescent assay measures the uptake of the commonly used glucose analog 2DG.

Inhibition of Glucagon Secretion Measured by Glucose Secretion

In a liver cell model, islets of Langerhans were incubated with 10 nM glucagon, the immunoassay was used to measure the glucose secreted into the medium.

9. Summary

Bioluminescent Assay can facilitate the study of insulin and glucagon secretion and action

• Bioluminescent immunoassays were developed to measure insulin and glucagon with picomolar sensitivity and large linear range
• The Insulin immunoassay was used to measure secreted insulin in functional GSIS tests of cells and islet microtissues
• The assays are compatible with low volumes and semi-automated dispensing to accelerate the measurement of large numbers of samples
• Bioluminescent metabolite assays have been developed for several key metabolites that can serve as markers for important cellular metabolic pathways
• The assays were used to study the action of insulin in model systems to measure cellular responses such as increases in glucose uptake, decreases in glucose production and decreases in lipid catabolism

References:

www.promega.com
Corresponding author: donna.leippe@promega.com