The Postgenomic Research and Technology Programme in South Western Sweden

Postdoc Programme

Proteomic studies of protein phosphorylation and expression in insulin resistance and diabetes

Svante Resjö

Home department: Department of Cell and Molecular Biology, Lund University
External lab: Department of Electrical Measurements, Lund Institute of Technology
Doctors degree: 2002-02-22, Department of Cell and Molecular Biology, Lund University

Project description:

Type 2 diabetes is a disease that is becoming increasingly more common. It is characterised by insulin resistance and/or decreased insulin secretion. The effects of insulin are mediated through protein phosphorylations and the glucose induced insulin secretion from the pancreatic beta cells can also be affected by phosphorylation of the proteins involved in that process. Thus, knowledge of the phosphorylations occurring in these cells during insulin stimulation/secretion is important for the understanding of the defects in these processes associated with type 2 diabetes.

Traditionally, cellular protein phosphorylation has been investigated by studying single proteins. Recently, there has been rapid method development in two important areas, namely two-dimensional gel electrophoresis and mass spectrometry. The combination of these techniques has greatly simplified the study of global cellular phosphorylation patterns. However, the sensitivity of mass spectrometrical analysis of phosphopeptides is limited due to the negative charge of the phosphate group.

The goal of the first part of my postdoc period is to develop more sensitive methods for the mass spectrometrical detection of phosphopeptides. The phosphate group of phosphoserine and phosphothreonine can be substituted with groups that improve ionisation by beta elimination and Michael addition. I will evaluate the suitability of different reagents for this reaction. In addition to this I will also combine ion exchange and immobilised metal affinity chromatography to enrich phosphopeptides from tryptic digests.

During the second part of my postdoc period I will use these methods to study protein phosphorylations during insulin signalling and insulin secretion. A number of insulinotropic substances (such as GLP-1, GIP, VIP, PACAP and glucagon) have been shown to increase cAMP levels. cAMP exerts many of its effects by activating PKA and I intend to use proteomics to study global protein phosphorylation patterns in beta cells stimulated with these substances.

Phosphodiesterase 3B (PDE3B) is expressed in tissues with an important role in energy metabolism. Inhibition of PDE3B in beta cells causes a potentiation of the glucose induced insulin secretion. In adipocytes, PDE3B is an important part of the antilipolytic signal transduction pathway of insulin. In order to gain insight into the role of PDE3B I will use specific inhibitors of PDE3B as well as transgenic animal models to study how protein phosphorylation and expression is affected by PDE inhibition.