

**P029** Analysis of insulin-containing vesicle proteome.

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While extensive genetic and biochemical analysis has identified a number of proteins that regulate insulin vesicles, vesicle proteins and their complexes remain poorly characterised and several aspects of vesicle cycle regulation are not well understood. In this study a proteomic analysis of the insulin-containing vesicle was undertaken to obtain better understanding of vesicle regulation. Two complementary 2-dimensional gel electrophoresis methods were used for vesicle proteome analysis: (a) isoelectric focusing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (IEF/SDS-PAGE) and (b) benzyl hexadecyl ammonium chloride/SDS-PAGE (16 BAC/SDS-PAGE). The IEF/SDS-PAGE method provided superior resolution of soluble proteins while 16-BAC/SDS-PAGE improves separation and resolution of integral membrane proteins. So far 24 proteins were identified including the heat shock cognate protein (Hsc70) - involved in uncoating of clathrin-coated vesicles -, vacuolar ATP synthase (v-ATPase) subunits, glucose-regulated protein 54, 78 and 94. Several proteins not directly attached to vesicles, but involved in vesicle cycle regulation copurified with vesicles for example actin and tubulin. Both actin and tubulin form core complex with synapsin proteins, synaptotagmin and SNAP-25 during vesicle docking thus explaining their presence on insulin vesicles.