MS09-1-1 Insulin plasma concentration determination – details of a well-established sandwich assay #MS09-1-1

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Abstract
Insulin is a hormone produced in the pancreatic beta cells from which it is released after food intake, when the blood glucose level rises. Insulin signals cells to absorb glucose from the blood for energy usage or storage for future use. Insulin thereby helps to keep the blood glucose level in balance. A certain amount of circulating insulin is needed, even in phases without food intake, because the liver keeps producing glucose and releasing it into the bloodstream to supply cells with glucose all the time. A fasting insulin level should never be zero, which it might be in a person with untreated Type 1 diabetes. However, an excessive insulin level is just as problematic as a sign of insulin resistance, prediabetes or Type 2 diabetes. Thus, it is important from a diagnostic point of view to know the concentration of insulin in blood.
The monoclonal antibodies (mAbs) HUI-018 and OXI-005 were developed and found to work in a sandwich immunoassay for human insulin (HI) by Novo Nordisk in the 1980's and have since been used extensively. Initially, the approximate epitopes of different insulins for HUI-018 and OXI-005 were identified by indirect methods. In this study, the insulin epitopes for OXI-005 and HUI-018 were determined using X-ray crystallography in order to achieve a more thorough understanding. The crystal structure of the HUI-018 Fab in complex with HI was determined and OXI-005 Fab crystal structures were determined in complex with HI and porcine insulin (PI) as well as on its own. Based on the Fab complex crystal structures with the insulins a molecular model for simultaneous binding of the Fabs to PI was built and this model was validated by small angle X-ray scattering. The epitopes for the mAbs on insulin were found well separated from each other as expected from luminescent oxygen channelling immunoassay results for different insulins. The affinities of the OXI-005 and HUI-018 Fabs for HI, PI and DesB30 HI were determined using surface plasmon resonance. The KDs were found to be in the range of 1-4 nM for the HUI-018 Fab, while more different for the OXI-005 Fab (50 nM for HI, 20 nM for PI and 400 nM for DesB30 HI) supporting the importance of residue B30 for binding to OXI-005.
Finally, the design of other insulin and insulin analogue specific concentration determination assays is discussed on the background of a number of experimental structures of other insulin recognising Fabs in complex with their targets.

References
Johansson E., Wu X., Yu B., Yang Z., Cao Z., Wiberg C., Jeppesen C.B., Poulsen F. (2021) Insulin binding to the analytical antibody sandwich pair OXI-005 and HUI-018: Epitope mapping and binding properties. Protein Sci 30(2), 485-496.

Insulin bound to the Fabs of HIU-018 and OXI-005: