Doctoral dissertation

Insulin – metallacarborane conjugates: a new strategy to enhance the biological efficacy of peptide drugs

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Summary

A major global healthcare challenge, diabetes mellitus affects 0.5 billion people and ranks among the top eight causes of death. To date, many hypoglycaemic drugs have been developed, but for a significant number of patients, insulin remains the only drug to achieve acceptable blood glucose-lowering activity. Unfortunately, due to its unfavourable pharmacokinetics, use of insulin poses many problems, therefore insulin analogues have been developed. They are structurally similar to the regular insulin but thanks to minor structural changes, their pharmacokinetic profile is altered. Research on insulin analogues has led to the development of fast-acting and long-acting insulins which significantly improve glucose control and simplify dosing schemes. The development of long-acting insulins is especially crucial because they are often used in the treatment of type 2 diabetes mellitus which is the most prevalent type of diabetes. Out of 4 marketed long acting insulins, 3 of them largely rely on the introduction of chemical modifications to the insulin, resulting highly albumin affine insulin analogues. This strategy can be further enhanced by the modifications with new, strong albumin binding molecules such as metallacarboranes.

Metallacarboranes are abiotic, fully synthetic coordination compounds composed out of metal cation which is coordinated by two carborane ligands. This class of compounds emerged in the recent decade as an interesting platform for the development of new biologically active molecules due to the mix of unique physicochemical properties that include: self-assembly and strong affinity to the serum albumin. Both of these properties are important in the design of new long-acting insulin analogues. Therefore, the goal of this thesis was to synthesise first insulin-metallacarborane (INS-MC) conjugates and study their biological and physicochemical properties.

As a proof-of-concept, this multidisciplinary project unfolds across several stages to explore the role of metallacarborane conjugation in protein modification, using insulin as the reference model. The project began with an extensive and thorough literature review, focusing on the non-covalent interactions of metallacarboranes with other (bio)molecules. The findings were compiled into a review article, serving as a predictive guide for the properties of metallacarborane conjugates, among others. The goal of the next phase was focused on targeted the development of conjugation-compatible metallacarboranes. This part of research focused on the assessment of available synthetic routes as well as determination of the structure-activity relationship based on the antiproliferative assays. Generated data helped to identify biocompatible structural motives that were used to obtain conjugation-ready metallacarboranes. With proper metallacarboranes substrates, a method to selectively lysine B chain of insulin was developed. That included synthesis of protected insulin, followed by conjugation reaction,

deprotection step and final purification. Obtained INS-MC were fully characterised with liquid chromatography, mass spectrometry and circular dichroism. In the next step, INS-MC conjugates were tested for their albumin binding with surface plasmon resonance (SPR) and fluorescence quenching of albumin. Biological activity of the conjugates was tested with cell-based assays where glucose uptake and antiproliferative activities were assessed.

Conducted studies allowed to rationally design and synthesise metallacarborane building blocks for INS-MC conjugations. Developed methods resulted in 3 different, high purity INS-MC conjugates. Modifications had profound impact on albumin binding capabilities of insulin – INS-MC had 10-fold greater affinity towards albumin than commercially available insulin detemir and degludec which are known for their albumin binding. Biological tests indicated that INS-MC retained signalling ability of the native insulin. Synthesised INS-MC conjugates are promising candidates for next-generation basal insulins. Based on initial activity screening, a group of highly promising metallacarborane-based antimicrobial leads was identified. Overall, conducted studies indicate that metallacarboranes are versatile building blocks that can be used in various areas of biomedical research.