Abstract

The regulation of metabolic and mitogenic cellular processes is a complex system, depending on the precise function of several signalling cascades. One example is the insulin pathway, which is mediated by a group of three sequentially and structurally highly similar hormones (insulin, insulin-like growth factor (IGF) -I and -II) and their homologous tyrosine kinase receptors (IR-A, IR-B, IGF-1R). Such a high degree of homology leads to crosstalk in receptor communication, with each of the ligands triggering different biological responses. The design of insulin analogues activating primarily metabolic effects or IGF antagonists suppressing an unfavourable mitogenic response is one of the main goals in this research field, which can be facilitated by identification of the regions responsible for receptor binding.

The work included in this thesis is focused on the effects on receptor interactions following the introduction of selected elements from the IGF-I primary sequence into the IGF-II molecule. In particular, these include a point mutation of Ser^{29} to Asn, an insertion of Gly-Ser after Arg^{34}, an insertion of Pro-Gln after Ser^{39}, or combination of both insertions. Although the IGF-II modifications described here negatively affected binding affinity towards IR-A, they did not enhance the IGF-1R binding. The optimisation of the recombinant production of IGF-II and its analogues in the heterologous *E. coli* based expression system was followed by a detailed biophysical characterisation of IGF-II, which lead to clarification of the mechanism behind the signal broadening in NMR spectra. The NMR diffusion and relaxation experiments revealed that the line broadening observed for IGF-II NMR signals can be attributed to the dynamic conformational heterogeneity of the molecule rather than aggregation, as frequently quoted in the literature.

(In Czech)