Abstract

Insulin acts as a key hormone in the blood glucose levels maintaining mechanisms. Outside this metabolic function it also has a growth hormone functionality. The interaction of insulin with the two existing insulin receptor isoforms - IR-A and IR-B, which are variously represented in the human body is determining insulin. IR-A, supposed to be mainly responsible for the mitogenic function of insulin, is located in the brain or lymphatic cancer and fetal tissue, whereas IR-B, performing metabolic function is located in adipose and muscle tissue. Present aim is to design such insulin analogs that would preferentially bind to IR-B, and could thus more efficiently carry out physiological metabolic function of insulin necessary for patients with diabetes. Based on the recently solved 3D structure of insulin bound to IR, it was found that the C-terminus of the B-chain of insulin must undergo conformational change bending it in about 90°, for efficient binding to IR.

The aim of this thesis was the preparation and characterization of two insulin analogs with bridging C-terminus of the B-chain in positions B26-B29 and B27-B29 using disulfide bridge. This could fix a bended structure of the B chain end and could help to increase the affinity of IR and specificity for IR-B. The preparation was carried out using solid phase synthesis, cyclization and subsequent enzymatic semi-synthesis using trypsin. Both analogs were prepared successfully, but in low yields. These yields, however, were sufficient for subsequent binding tests. Binding assays for the IR-A and IR-B were performed to determine by the binding affinity of both analogs to human insulin receptor isoforms, and their dissociation constants determined. From the values obtained, we can conclude that in both cases there was an increased specificity for IR-B compared to human insulin. However, the affinity of the individual isoforms was significantly reduced. This thesis yielded two successfully prepared insulin analogs with fourth disulfide bridge. The data obtained can be used to prepare further similar analogs, which having desirable properties could find applications in pharmaceutical use. (In Czech)

Key words: insulin, analog, insulin receptor, disulfide bridge, solid-phase synthesis, enzymatic semisynthesis