Abstract

According to the International Diabetes Federation (IDF), there were 371 million people in the age from 20 to 79 years worldwide affected by diabetes in 2012. This means diabetes has become a global epidemic disease and, therefore, the importace of insulin research still grows.

Insulin is a protein hormone that plays a key role in regulating blood glucose level which has a widespread impact on whole metabolism. Insulin acts through binding of its monomeric form to the insulin receptor. It is clear that insulin monomer has to undergo structural changes upon binding to the insulin receptor as the residues which are crucial for the interaction are burried within the native form. According to studies of highly active hormone analogs and the new information about the insulin-insulin receptor complex, there is a strong evidence that the C-terminal part of the B-chain is a dynamic element in insulin activation and receptor binding. Probably, there is also a great importance of the B-chain N-terminus and the transition between T and R conformations of insulin. However, the exact significance of the T and R states of insulin still remains unclear.

In this work, several new insulin analogs AibB3-insulin, AibB5-insulin, AibB8-insulin, *N*-MeAlaB8-insulin and D-ProB8-insulin were prepared for the purpose of studying significance of the T and R conformations of insulin and their relationship to the active form of the hormone when binding to its receptor. The α-aminoisobutyric acid, a non-proteinogenic aminoacid which is a strong inducer of helical structure in polypeptide chain, was incorporated into the B-chain N-terminus in positions B3, B5 and B8 to produce a conformation close to R-state of insulin. Furthermore, the amino acids *N*-MeAla and D-Pro were incorporated in the position B8 to produce molecules in the T-state of insulin. The newly synthetized insulin analogs were characterized by the RP-HPLC and mass spectrometry and the binding affinity was measured. We also obtained CD spectra of AibB3-insulin, AibB8-insulin, *N*-MeAlaB8-insulin and D-ProB8-insulin in the presence and absence of phenol to measure the ability of T→R transition of these analogs. Finally, using the 2D NMR experiments TOCSY and NOESY, we determined the three-dimensional structure of the AibB8LysB28ProB29-insulin, a new insulin analog synthetized and characterized previously.

Based on this work, we found that the insulin analog AibB8LysB28ProB29-insulin is not present in the R-state of insulin as we expected so that even the substitution of GlyB8 to Aib is not enough to produce the molecule in the R conformation. Therefore, it is probable

that the other insulin analogs with the Aib amino acid substitution are not in the R-state either. We proved that the glycine residue in the position B8 of the insulin molecule is crucial for its biological activity as the binding affinity of the B8-substitued analogs reduced to minimum. We also demonstrated that the main importance of the HisB5 residue consists in the structure determination during biosynthesis and folding of the insulin molecule due to low yields during the recombinant reaction of AibB5-insulin. We showed that the T→R transition is essential for the biological activity of the hormone whereas the binding affinity of the T-state analogs *N*-MeAlaB8-insulin and D-ProB8-insulin is negligible even though they were prepared with a great yield.

We conclude that the T-state is fundamental for the right folding of the insulin molecule and the ability of the T→R transition is crucial for full receptor binding.

Key words: insulin, insulin analog, insulin receptor, total chemical synthesis, binding afinity, T→R transition, CD spectroscopy, NMR spectroscopy