Insulin Drugs Development:

Influence of Mutations on Ligand-Receptor Interactions

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Insulin, a small protein hormone secreted by the pancreatic beta-cells, is principally responsible for controlling blood sugar levels in higher organisms. The inefficient production or improper utilization of insulin leads to diabetes mellitus. Introduction of mutations in an insulin molecule is one of the important approaches to drug development for treatment of diabetes mellitus. Generally, usage of mutations is aimed at activation of insulin and insulin receptor (IR) interaction. Such mutations can be considered as positive. Mutations that reduce the binding efficacy are negative. There are neutral mutations as well.

The aim of this work was to investigate influence of mutations on the ligand-receptor interactions of insulin and IR using different bioinformatics tools such as homology modeling, molecular dynamics, and docking. Firstly, all known primary sequences from UniProt database (www.uniprot.org) and sequences published in the literature for insulin superfamily peptides were aligned using the Muscle 3.7 program [1]. The obtained matrix shared amino acids in three functional groups: conservative acids (such as cysteines), variable acids, and acids responsible for ligand-receptor binding. The obtained matrix with mutation history of each site was used in further analysis. We also excluded some non-informative sites from consideration. The association between remained sites was evaluated using correlation coefficients computed under obtained mutation history.

After that, we selected the most perspective sites for introduction of mutations and constructed 3D structures of the mutant insulins using the MODELLER 9.9 software [2]. Further, we carried out molecular dynamics (MD) simulations for molecular relaxation, using the GROMACS 4.5.5 software package [3]. Obtained with MODELLER, molecules were immersed in the solvent using the tip4p water model. The solvated proteins were placed in the cubic boxes with periodic conditions, and the system energy was minimized to remove steric clashes. To compute the forces acting on each atom, the OPLS-AA force field was

applied [4]. Further we carried out docking of studied ligands into IR. Analysis of the conformational flexibility and ligand-receptor stability revealed two groups of mutation sites – positive (white circles) and negative (black and grey circles) for ligand-receptor binding.

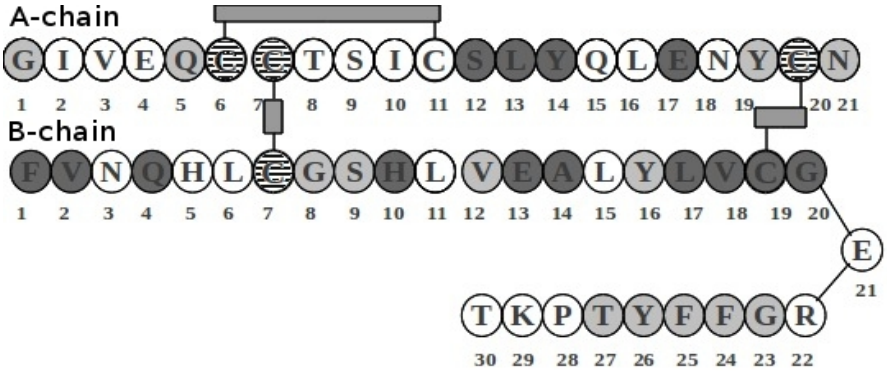

Figure. Insulin primary structure. White circles show sites for positive mutations, color circles – sites for negative mutations.

Furthermore, we showed that A8 is the most favourable site for the introduction of mutations. In addition, we revealed that His and Lys mutants have greater intrinsic alpha-helical propensity than Thr and exhibit enhanced affinities and stabilities. Based on these data we proposed that these peptides are the best candidates for developing new insulin drugs for treatment of diabetes mellitus.

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