



# Designing peptide inhibitor of insulin receptor to induce diabetes mellitus type 2 in animal model *Mus musculus*



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## ABSTRACT

A designing peptide as agent for inducing diabetes mellitus type 2 (T2DM) in an animal model is challenging. The computational approach provides a sophisticated tool to design a functional peptide that may block the insulin receptor activity. The peptide that able to inhibit the binding between insulin and insulin receptor is a warrant for inducing T2DM. Therefore, we designed a potential peptide inhibitor of insulin receptor as an agent to generate T2DM animal model by bioinformatics approach. The peptide has been developed based on the structure of insulin receptor binding site of insulin and then modified it to obtain the best properties of half life, hydrophobicity, antigenicity, and stability binding into insulin receptor. The results showed that the modified peptide has characteristics 100 h half-life, high-affinity  $-95.1 \pm 20$ , and high stability 28.17 in complex with the insulin receptor. Moreover, the modified peptide has molecular weight 4420.8 g/Mol and has no antigenic regions. Based on the molecular dynamic simulation, the complex of modified peptide-insulin receptor is more stable than the commercial insulin receptor blocker. This study suggested that the modified peptide has the promising performance to block the insulin receptor activity that potentially induce diabetes mellitus type 2 in mice.

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## 1. Introduction

Diabetes mellitus type 2 (T2DM) patients increased year over year. International Diabetes Federation (2011) mention there were 366 million people suffer from T2DM and would increase to be 552 million people in 2030. This disease caused 4.6 million people death in 2011. Diabetes mellitus type 2 is a complex disease that attacks people ages 45–64 at developing country (Wild et al., 2004). One of the most common causes T2DM is insulin resistance that is due to inactivation insulin receptor function. The diminution of insulin receptor activity could be attributable to mutation or intercalation the binding between insulin and its receptor. The binding insulin into insulin receptor is necessary to activate tyrosine kinase of insulin receptor to drive signaling cascade to uptake glucose from the blood stream (Herrera and Rosen, 1986).

Many types of research explore the effective drugs to cure this disease by employ animal model. The most common the diabetic animal model was generated by induction of medicines, such as Streptozotocin in *Mus musculus*. However, the application of drugs in an animal would change systematically of physiology processes

that may not reflect of T2DM naturally in the model. Therefore, the development of T2DM animal model based on the insulin receptor (IR) blocker specifically is a warrant for exploring the potent of the anti-T2DM drug.

The insulin receptor blocker could be generated by modifying the structure of the IR-binding domain of insulin. The modification of the domain is possible to change its character that still able to bind into IR but without activating the function of the receptor. The binding between the modified IR-binding domain will block insulin bind with its receptor. Therefore, we have designed a peptide according to the structure of IR-binding domain of insulin to obtain an agent to block the insulin receptor activity. This study we have found the best peptide that computationally could be used to block insulin receptor activity that warrants for further study to develop the T2DM animal model.

## 2. Methods

### 2.1. Sample preparation

Sequence of insulin (P01326) and insulin receptor (P15208) of *Mus musculus* were retrieved from UniProt database (Wu et al., 2006). The sequence of peptide S961 used for reference was

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collected from website [www.phoenixpeptide.com](http://www.phoenixpeptide.com). The protein structure of insulin and insulin receptor were modeled by SWISSMODEL ExPasy (Biasini et al., 2014), and the structure of S961 was modeled by I-TASSER protein modeling server (Yang et al., 2015).

## 2.2. Inhibitor peptide design

The peptide was designed by modifying the IR-binding domain of insulin and compares to commercial insulin receptor inhibitor S961. The IR-binding domain was found in the sequence amino acid number 31, 32, 36, 46, 49, 50, 52, 70, 71, 74, 75, 77, 84, 87 and 89 of insulin *Mus musculus* sequence (P01326). Further, we took the part of the sequence of insulin from amino acid number 31 until 46, 49, 50 and 70 until 89, then mutated the amino acid randomly. The modified peptide then docked with the insulin receptor. The molecular docking was done to analyze the binding affinity and pattern of the modified peptide to the insulin receptor (IR). The docking process was done by HADDOCK (de Vries et al., 2010), and then were analyzed and visualized using Discovery Studio Visualizer (Dassault Systèmes Biova Inc., 2015).

## 2.3. Improving half-life of peptide inhibitor

The improving half-life of the peptide was done by substitute the N-terminal amino acid by another amino acid, then the half-life of the modified peptide was analyzed using ProtParam tool in ExPasy. The aimed of this modification was to increase half-life, the longer half-life is useful to produce the peptide recombinant and avoid the degradation by a protease in *E. coli*. The method used in this modification was altered on N-terminus peptide.

## 2.4. Improving binding affinity of peptide inhibitor

The improving binding affinity of the peptide was done by replacing amino acid in the coil region (residues no 14 until 28) with another amino acid that has more hydrophobicity based on index hydrophobicity in ProtScale tool ExPasy (Kyte and Doolittle, 1982). The lowest free energy of the modified peptide will be taken for further examination. The amino acid replacement and the energy stability was calculated by FoldX Yasara (Durme et al., 2011). Then we employed PSIPRED web server to analyze the secondary structure of the peptide.

## 2.5. Antigenicity analysis

Antigenicity analysis is crucial to examine the possibility of the peptide able to stimulate the immune system. The antigenic peptide will change physiology systemically and clearance of the second application of the peptide to the animal model. Therefore, the peptide should have minimum antigenic properties to avoid immune system reaction from the body. The antigenicity was improved by substitute the amino acid randomly, and then the antigenicity of the modified peptide was evaluated based on Kolaskar and Tongaonkar (1990) method using IEDB program. A peptide that had the lowest antigenicity was chosen as a candidate peptide inhibitor.

## 2.6. Protein stability analysis

The stability of complex between modified peptide and Insulin receptor were evaluated based on the molecular dynamic by using GROMACS (Groningen Machine for Chemical Simulations). The molecular dynamic is a technique to simulate the dynamic of complex molecules likes real condition in cells (Apol et al., 2010). This experiment, we used GROMACS version 4.5.5 for simulation of

the molecular dynamic, and GROMOS96 force field for calculating potential energy. The molecular dynamic was run until 8000 ps or 8 ns, and all parameters, including RMSD, RMSF, and potential energy were stored for every 0,2 ps.

## 3. Result and discussion

### 3.1. Peptide inhibitor design

The insulin receptor blocker was designed based on a modification of the IR-binding domain of insulin. The interaction of Insulin receptor in the insulin protein occurs in amino acid residues number 31, 32, 36, 46, 49, 50, 52, 70, 71, 74, 75, 77, 84, 87, 89. Therefore, we use the residues as a template to develop IR blocker by modifying the peptide. The peptide was mutated by substitute amino acids to adjust the peptide structure to form coil-helix-coil-helix-coil. The structure adjustment is important to maintain binding affinity of the peptide to IR without activating the function of the receptor. The simple scheme of designing peptide inhibitor showed in Fig. 1. The modification resulted in a peptide with structure coil-helix-coil-helix-coil, CGSHLVEALYLCV-GERGFTPGGPGAGDLQTLALEVAQQKR.

### 3.2. Potential peptide inhibitor

#### 3.2.1. Improving half-life in peptide inhibitor

The modified peptide has analyzed the half-life and stability using ProtParam ExPasy, and S961 peptide used as reference or control. The S961 peptide has 30 h half life, and modified peptide has 1,2 h. Hereafter we have substituted the N-terminus by amino acid A, M, F, V, I, C, L alternately for increasing the half-life. The alteration cysteine in the N-terminus with Valine, increasing half-life protein until 100 h. Based on in vivo research, N-terminal cysteine classified as an unstable peptide in the mammalian cell (reticulocyte), because there is a particular substrate in arginine t-RNA reticulocyte (Gonda et al., 1989). Arginilation has a significant role in activity regulation in vivo. If the site of arginilation is already recognized, it will act as inhibitor proteasome-dependent or independent degradation protein (Xu et al., 2009). As the result, Valine classified as a stable amino acid, nondegradable in the mammalian cell. It was proved by western blot result containing ubiquitin Valine did not show cleavage product (Gonda et al., 1989). This analysis we found the modified peptide with the amino residues as follows VGSHLVEALYLCVGERGFTPGGPGAGDLQTLALEVAQQKR has the longest half-life.

The modified peptide was docked with the insulin receptor to examine the binding affinity of the complex molecule. The result showed that binding energy of the modified peptide to the IR is still lower than S961, there are  $-50.0 \pm 5.5$  and  $-92.4 \pm 5.6$ , respectively. Further analysis using Discovery Studio Visualizer suggested that the binding pocket of this peptide is less hydrophobic. So, we modified the sequence in coil region that does not interact with insulin receptor to minimize structure alteration to maintain the peptide activity.

#### 3.2.2. Improving binding affinity peptide inhibitor

The improvement of binding affinity of the peptide into IR was made by substitute Glycine 21 with seven kinds of hydrophobic amino acid according to hydrophobicity index Kyte and Doolittle (1982). The changing of the amino acid (Glycine 21) might not affect the secondary structure. The result shows alteration Glycine 21 into Isoleucine increasing affinity score up to  $-73.5 \pm 4.6$ . Further, we also modified the Threonine 19 into nine kinds of amino acid (Ala, Met, Gly, Phe, Val, Ile, Cys, and Leu). These modifications to bring out a result that is changing Threonine 19 into Isoleucine increase affinity peptide into  $-76.1 \pm 5.9$ . Isoleucine

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