The Two Peptides Fragments of Goat Milk CSN1S2 Protein Blocked Insulin Receptor to Interact Ligand: In Silico Study

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Abstract. Insulin is a hormone that plays important role for tissue development and glucose homeostasis, it has significant implications for many chronic diseases, especially diabetes mellitus (DM). Alpha casein S2 (CSN1S2) isolated from Ethawah goat’s milk has eight peptides residue contain seven to twelve amino acid residues and has multiple functions, such as antimicrobial, immunomodulatory, and anti-oxidant. The purpose of this study is to observe the interaction between CSN1S2 protein peptides and Insulin Receptor. 3-D structure of Insulin Receptor is done by accessing RCSB PDB (ID: 4ZXB), protein was prepared using Discovery Studio BIOVIA 2019 and ligands were prepared using PyRx. Insulin receptor was docked to CSN1S2 protein peptides using HEX 8.0 software and visualized using Discovery Studio BIOVIA 2019.

The results of this study showed different binding patterns, most of the chemistry bond were resulted between protein and ligands interaction are hydrogen bonds and electrostatic, the binding energy of CSN1S2 fragment 41-47 and fragment 214-221 to insulin receptor are -186.9 kJ/mol and -172.7 kJ/mol respectively. The caprine CSN1S2 protein peptides have ability to be used as therapeutic agents, such as DM because they can bind to specific sites of the insulin receptor and may reduce the insulin resistance mechanism.

1. Introduction
Insulin is a hormone that plays important role for tissue development and glucose homeostasis, it secreted by β-pancreatic cells of Langerhans. The mechanism of insulin’s actions is mediated by insulin receptor (IR), a plasma membrane-resident glycoprotein and member of the receptor tyrosine kinase (RTK) family [1]. The structure of IR is a heterotetrametric formed of two extracellular α-subunits and two transmembrane β-subunits connected by disulfide bridges. The disulfide bonds in the α- and β-subunits involve C647 and C860 cysteines residue. In addition, there are α-disulfide bonds in residue C524 in the FnIII-1 domain and residue C682, C683 and C685 in the insert domain (ID). The intracellular portion of the α-subunit contains the kinase domain flanked by two regulatory regions, the juxta membranous region involved in the Insulin Receptor Substrate (IRS) 1-4, Shc, internalization receptor, and the C-terminal [2].

The role of insulin in physiological processes has significant implications for many chronic diseases, especially diabetes mellitus (DM) [3]. Mis-splicing of insulin receptor gene has been associated with insulin resistance, because there is a toxic effect of the CUG/CCUG expanded repeats, that leads to a higher expression of the INSR-A [4] or lacking exon 11 in α subunit of IR [5]. Previous studies were
finding out that the alpha casein S2 (CSN1S2) isolated from Ethawah goat’s milk has eight peptides residue contain seven to twelve amino acid residues [6]. It has multiple functions such as as antimicrobial, immunomodulatory, and anti-oxidant [7].

This study focuses on biological functions of CSN1S2 protein from goat milk to block IR to interact ligand and its effect on IR gene abnormalities.

2. Materials and Methods

2.1 Protein and Ligand Preparation

The 3-D structure protein structure of Insulin Receptor (PDB ID: 4ZXB) was downloaded in RCSB (www.rcsb.org) [8], then the water molecules and native ligands were removed by Discovery Studio BIOVIA 2019. The 3-D structure of ligands, CSN1S2 residue 41- NMAIHPR-47 and 214-TNAIPYVR-221 [9] energy was minimized using PyRx. The results are saved in PDB format.

2.2 Molecular Docking

Bioactive peptides of goat milk CSN1S2 residue 41-NMAIHPR-47 and 214-TNAIPYVR-221 as ligands and Insulin Receptor were docked using HEX 8.0.0. Docking results are saved in PDB format and HEX message which contains docking results information like total energy bond are saved in txt format.

2.3 Visualization

The interaction between ligands and receptor were visualized by Discovery Studio BIOVIA 2019. The results were analyzed to determine total energy binding, distance, and chemistry bond.

3. Result and Discussions

The result shows that the total energy from the interaction between the receptor and the ligand docking is -186.9 kJ/mol. There are 2 bonds formed between the caprine CSN1S2 amino acid residue fragment 41-NMAIHPR-47 and the Insulin Receptor. Both forms are Asn41 residual bonds with Glu329, the chemical bonds result are hydrogen and electrostatic respectively with salt bridge and attractive charge type (Figure 1).

Figure 1. Insulin receptor (blue) and bioactive peptide residue 41-NMAIHPR-47 fragment of caprine CSN1S2 protein (red).
Table 1. Interaction between insulin receptor and bioactive peptide residue 41-NMAIHPR-47 fragment of caprine CSN1S2 protein.

| Name                      | Interaction | Distance (Å) | Chemistry Bond     | Type                        | Energy Binding (kJ/mol) |
|---------------------------|-------------|--------------|--------------------|-----------------------------|-------------------------|
| IR – CSN1S2 41-NMAIHPR-47 | :ASN41:HT1 - E:GLU329:OE1 | 3.00         | Hydrogen Bond; Electrostatic | Salt Bridge; Attractive Charge | -186.9                 |
|                           | :ASN41:HT3 - E:GLU329:OE1 | 3.21         | Hydrogen Bond; Electrostatic | Salt Bridge; Attractive Charge |                         |

The result shows that the total energy from the interaction between the receptor and the ligand docking is -172.2 kJ/mol. There are 3 bonds formed between the amino acid residues of caprine CSN1S2 fragment 214-TNAIPYVR-221 and Insulin Receptor, there are Thr214 and Glu362, the chemical bonds are hydrogen and electrostatic with salt bridge and attractive charge type. In addition, there is a residual bond of Thr 214 with Glu 363, both chemical bonds are electrostatic with an attractive charge type. There is a residual Asn215 bond with Thr214, both chemical bonds are hydrogen with the conventional hydrogen bond type (Figure 2).

![Figure 2](image.png)  
Figure 2. Insulin Receptor (blue) and bioactive peptide residue 214-TNAIPYVR-224 fragment of caprine CSN1S2 protein (red).

Table 2. Interaction between insulin receptor and bioactive peptide residue 214-TNAIPYVR-224 fragment of caprine CSN1S2 protein.

| Name                      | Interaction | Distance (Å) | Chemistry Bond     | Type                        | Energy Binding (kJ/mol) |
|---------------------------|-------------|--------------|--------------------|-----------------------------|-------------------------|
| IR – CSN1S2 214-TNAIPYVR-221 | :THR214:HT1 - E:GLU362:OE1 | 2.25         | Hydrogen Bond; Electrostatic | Salt Bridge; Attractive Charge |                         |
|                           | :THR214:N - E:GLU363:OE2 | 3.34         | Electrostatic      | Attractive Charge           | -172.2                 |
|                           | :ASN215:HN - THR214:OG1 | 2.81         | Hydrogen Bond      | Conventional Hydrogen Bond  |                         |
Most of the chemistry bond were resulted between protein and ligands interaction are hydrogen bonds and electrostatic. The stability level of proteins is determined by the presence of hydrogen bonds. When the protein fold, 70% of the peptide groups and 65% of the polar side chains are buried inside the protein so they do not contact with the water [10], while the electrostatic bonds have major contribution to protein structure and the interaction of proteins with other biomolecules [11].

The two ligands, caprine CSN1S2 fragment 41-NMAIHPR-47 and fragment 214-TNAIPYVR-224 bind to the receptor (IR) with different binding patterns in the E domain, which is tyrosine kinase domain. The activity of tyrosine kinase determined by the phosphorylation state of the activation loop in the C lobe, and it contains three active sites of tyrosine autophosphorylation, Tyr1158, Tyr1162, Tyr1163 [1] and catalytic loop active site, Asp1132 [2].

Protein kinases have 12 subdomains with 300 amino acids, one of them shows the activation loop which has an important role in enzymatic activities starting with Asp, Phe, Gly and ending with Ala, Pro, Glu in subdomains 7 and 8. In in vivo and in vitro studies, mutation of asparagine (Asn 1050) in the activation loop influenced tyrosine autophosphorylation and kinase activation dependent ligand [12]. Based on statements above, the two ligands of caprine CSN1S2 interact in specific sites, but not on activation or catalytic loop. It assumed that receptor and ligand interaction may reducing the insulin resistance mechanism in low affinity because total energy binding results are not too high.

4. Conclusions
The two fragments of caprine CSN1S2 can bind to specific sites of the insulin receptor tyrosine kinase domain in low affinity, and it may reduce the insulin resistance mechanism in metabolic diseases, such as diabetes mellitus.

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