Virtual Screening and Prediction of Binding of Caprine CSN1S2 Protein Tryptic Peptides to Glucokinase

Fatchiyah Fatchiyah[1,2*+], Atika Hanoum Rahasta[2+], James R. Ketudat Cairns[1,3]

[1]Research Center of Smart Molecule of Natural Genetics Resources, Brawijaya University, Indonesia

[2]Biology Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Indonesia

[3]School of Chemistry, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

Correspondence author: Fatchiyah F, Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University. Indonesia. Tel: +62-341-575-841.E-mail: fatchiya@ub.ac.id ;

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ABSTRACT

Introduction: Glucokinase (hexokinase D) is an enzyme that phosphorylates glucose in hepatocytes totrap it in the cell and prime it for conversion to other compounds, yet this enzyme has low affinity to bind with glucose. In Diabetes Mellituspatients, the blood glucose level is poorly controled. **Material and Methods:** This study explored the possibility to induce glucokinase activity with bioactive peptides derived from the goat milk protein CSN1S2 by in- silico docking approach. Two bioactive tryptic peptides, CSN1S2 residues 41-47 and 214-221, were successfully docked to glucokinase and found to bind to the activation site. **Results:** Amino acid residues Asn41, Ala43, His45 and Arg221 from these peptides provided the major contribution to docking to glucokinase. Asn41 made more interactions with glucokinase than the other residues in the peptide, including hydrogen bonds and salt-bridge These bioactive peptides appear to help glucokinase to bind glucose, since the number of hydrogen bonds between the protein and the glucose was higher and their distances shorter in the complex with the peptides without disturbing the glucose position for phosphorylation. **Conclusion:** Thus, the activation effect of the CSN1S2 derived bioactive peptides for glucokinase binding affinity of glucose is indicated by this study.

Keywords: CSN1S2, Diabetes, Goat Milk, Glucokinase, In-Silico.

1. INTRODUCTION

Diabetes Mellitus (DM) is an alarming degenerative disease. Symptoms such as inability to uptake glucose and store it cause high levels of glucose in the blood (1). The number of patients is constantly increasing. Potentially, in 2030, Indonesia alone will have 21.3 million people with this disease (2, 3).

The inability to convert glucose in Diabetes Mellitus type 2(DMT2) is because of the damage to insulin production in the pancrease or mutations in or down regulation of the insulin receptor. Many studies focus on reverting this dysfunction, either by induction by chemicals or nutrition (4).

Other studies are focusing on finding alternative enzyme besides insulin which will help to convert glucose into metabolic intermediates, so later the body will be capable to store it. One of the enzymes involved in blood glucose regulation is glucokinase (GCK, hexokinase D, hexokinase IV) (5). This enzyme plays a key role in glucose uptake and metabolism by converting it to glucose-6-phosphate, which serves as the beginning step of glycolysis, glycogen synthesis, the pentose phosphate pathway and various other metabolic uses of glucose. In the homeostatic state, GCK will only convert a small amount of glucose at normal blood glucose concentration (5 mM). Because GCK has low affinity for glucose with $K_{0.5}$ of 7 mmol/L (6), many studies are searching how to activate it. One way to increase the enzymatic activity of GCK is by inducing this enzyme with an activator (7, 8). An activator might be a small molecule that can help increase the glucokinase catalytic rate. This small molecule could bind to the enzyme to induce its activity (9). Since GCK has two distinct structures between the active and non-active one (7), this activator could help the enzyme move toward the active conformation and to bind glucose more rapidly.

In this study, we would like to test a possible nutritional effect on GCK enzymatic activity. A common nutritient used for treatment for Diabetes Mellitus in Indonesian is CSN1S2 (4). Eight bioactive peptides have been identi-

fied in the CSN1S2 protein (10). In this study, the binding of CSN1S2 peptides was simulated to investigate whether and how CSN1S2 may work as an activator. By in-silico and 3D protein molecular docking, the possibility of binding and activation of GCK was approachedon a molecular level.

2. METHODS

Protein and Bioactive Peptides Preparation

The glucokinase structure used for this study is PDB ID 1v4s (http://www.rcsb.org/pdb/explore/explore.do?structureId=1v4s) [6]. Bioactive peptides from CSN1S2 were previously separated and identified by MALDI-TOF (10). All these bioactive peptides have different lengths and positions in the CSN1S2 sequence. Therefore before docking, of GCK and bioactive peptides were prepared using PyMol for arranging their original position and cut off ligands or any activator from the GCK structure. Further, water molecules also added by using MEGA 6.0. After the preparation was done, the structures were used in the docking phase.

Docking Molecules

Docking was based on methods from a previous study (Mohan et al., 2015) with some modification on not using active site clustering docking. Docking is done for all CSN1S2 bioactive peptides to GCK, all bioactive peptides to glucose and GCK to glucose. The software used for molecular docking was Cluspro 2.0. All docking models were saved and bonds between molecules were also observed. All docking used blind docking and default parameter. This means docking was not done specificly at the activator site, but by random initial placement of the peptides. Docking was done to glucokinase X-ray crystal structure modelsin both the active and inactive conformations (7). Each of these structures was docked with each of the 8 CSN1S2 bioactive peptides.

Analyzing structure

Analysis was done by visualization in PyMol and Ligplot+. Ligplot+ was used to illustrate the binding in 2D to see hydrogen bonds and the residues which potentially play major roles in determining the binding, while PyMol was used to illustrate bonds in 3D.

3. RESULTS

For the first screening of the 8 CSN1S2-derived bioactive peptides, docking was used to measure its capability of binding with glucose, glucokinase and the glucose-glucokinase complex. Table 1 lists all parts of the bioactive peptide chain that successfully bound to glucose, glucokinase and complexes of the two. From this table, it can be seen from the peptide 41-NMAIHPR-47 amino acid residue numbers 41, 43 and 45 always bound to glucose, glucokinase and the glucose-glucokinase complex. From peptide derived from GCK 214-TNAIPYVR-221, amino acid number 221 was the only residuethat was always bound to the three complexes.

Based on Figure 1(A and B), data were collected in Table 1 to compare the binding sites of CSN1S2 bioactive peptides. Three of eight bioactive peptides derived from CSN1S2 displayed significant binding to GCK according to docking results. After analysis in Ligplot+, only two bioactive peptides bound to the GCK complexes's previously described allosteric site (Kamata et al., 2004). These bioactive peptides bound to GCK by different parts of their chains. As seen in Figure 1(A-B), the bioactive peptides 41-NMAIHPR-47 and 214-TNAIPYVR-221 appeared to bind to GCK. 41-NMAIHPR-47 bound to GCK via five different residues in the GCK chain, Arg63, Glu96, Tyr215, Tyr214 and Met235. In these interactions, GCK acted as the recipient while the peptides acted as donors in hydrogen bonds. Points where 41-NMAIHPR-47 was successfully bound are on GCK's allosteric site (Kamata et al. ,2004). The other bioactive peptide which could bind to GCK's allosteric site is 214-TNAIPYVR-221. TheGCK (as a recipient) interacting residues that successfully bonded with 214-TNAIPYVR-221(as a donor) are Arg63, Asp158, Ala456, Tyr215, Thr65, Tyr214, Glu96 and His218. The binding site of 214-TNAIPYVR-221 has more residues compared to 41-NMAIHPR-47, in 214-TNAIPYVR-221 there was 8 GCK residues that they can bind. When successfully bound to glucose, GCK will change its shape. This shape change brings two parts of GCK close together and binds glucose in between. The same interaction also occured on this study when the glucokinase complex bound to GCK-41-NMAIHPR-47 and GCK-214-TNAIPYVR-221. This interaction can be seen in Figure 1(C,D and E).

Bioactive peptide	Glucose	Glucokinase	Glucose-GCK
41-NMAIHPR-47	Asn41	Asn41	Asn41
	Ala43	Ala43	His45
	His45	His45	Arg47
		Arg47	
182-KISQYYQK-189	Ile183	Lys182	-
	Ser184	Ile183	-
	Tyr186	Ser184	-
		Gln188	-
214-TNAIPYVR-221	Tyr219	Thr214	Thr214
	Arg221	Asn215	Asn215
		Ala216	Ala216
		Ile217	Ile217
		Pro218	Val220
		Val220	Arg221
		Arg221	
		Tyr222	
		Leu223	

Table 1. Peptide chains from CSN1S2's bioactive peptide that binding with Glucose, Glucokinase, and both of them in complexes.

Activator Site(7)	Activator site 41	Activator site 214
Arg63	Arg63	Arg63
Ser64	Glu96	Thr65
Gln98	Tyr214	Glu96
Ile159	Tyr215	Asp158
Ile211	Met235	Tyr214
Tyr214		Tyr215
Tyr215		His218
		Ala456

Table 2. Comparasion of the originally described activator site (6) and residues of glucokinase to bind with CSN1S2 bioactive peptides

Compared to the interaction of bioactive peptides with the glucokinase in its inactive form (as shown in Table 3–supplementary data), interactions in its inactive form appear to be different from the interaction with its active form. This interaction showed a lot more different residues on different part of glucokinase. Such as Glu51, Asp205, Glu256, Arg186, Asn204, Glu51, Pro59, Leu243, Leu58, Met202, His50, Val182 and Ala201 that are successfully bound by 41-NMAIHPR-47. The214-TNAIPYVR-221peptide also interacted with the inactive glucokinase in outside of its allosteric site, including residues Glu196, Arg186, Arg447, Ala201, Val182, Val199, Glu196, Val203, and Ile189. In Figure 1(F), a mechanism is predicted where bioactive peptides can attach to glucokinase inactive or active conformation atdifferent positions on glucokinase.

Figure 1 (C-D-E) also compares the glucose binding site from GCK before docking with the CSN1S2 bioactive peptides and after peptide docking. Originally, glucose can bind at 5 different residues of GCK with only 6 hydrogen bonds spread on theglucose molecule. Those residues are Thr168, Glu256, Ala259, Gln287 and two hydrogen bonds on residue Gly258. After docking, glucose can bind with 7 different residues of GCK with 10 hydrogen bonds, with each glucose chain has at least 2 residues bound to it. Those residues are Lys169, Cys230, Gln287, Glu290 and two hydrogen bonds on residue Asp205, Glu256 and Asn204. The average distance of these hydrogen bonds is 2.93, while that in the original bound structure is 3.10. This apparent strengthening of binding was also observed in glucose binding with GCK-214-TNAIPYVR-221, in which glucose interacted with 6 different GCK residues and made 9 hydrogen bonds with an average distance of 2.904. Those residues are Cys230, Gln287, Glu290 and two hydrogen bonds on Asn204, Asp205, Glu256. The orientation of the glucose from all the complexes have a free sixth carbon. This sixth carbon will later get phosphorylated to change glucose into glucose-6-phosphate.

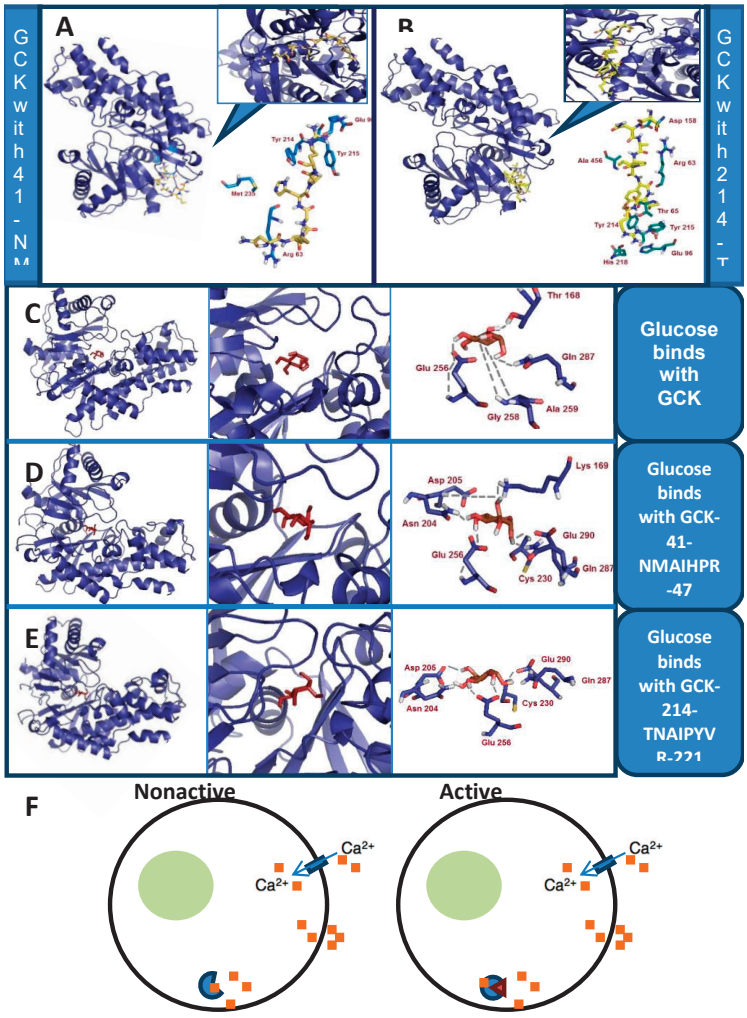

Figure 1. All interactions between glucokinase, bioactive peptides and glucose and an introduced mechanism for peptide binding. (A-B) Three-dimensional structure of docking results comparing binding of 41-NMAIHPR-47 with GCK and 214-TNAIPYVR-221 with GCK. (C,D and E) Three-Dimensionstructure and hydrogen bonds made from glucokinase and glucose bound compared in three different structures. The structure in C shows the original bonding between glucokinase and glucose in the crystal structure. The other two structures are glucokinase complexed with 41-NMAIHPR-47 interactions with glucose (D), and glucokinase complexed with 214-TNAIPYVR-221 interactions with glucose (E).(F) Mechanism prediction of Glucokinase and bioactive peptide interactions. (Orange blocks are peptides; Blue circle is glucokinase; Red triangle is glucose; Blue block is calcium pump.)

4. DISCUSSION

Based on studies by Farrell, et al., as referred in (10), one active compound that has been proven to have a significant amount of effect on health (such as antihypertensive, anti-allergy, anti inflammation, immunomodulator, and act as anti-oxidative) is CSN1S2 from Ethawah goat milk. In this study, another possible activity of this compound was investigated. Kamata et al. (7) identified a GCK allosteric site in their description of the human GCK structure. This allosteric site can be activated by activator and proven to promote activity of GCK itself (11).The residues that contribute to the allosteric site are Arg63, Ser64, Gln98, Ile159, Ile211, Tyr214 and Tyr215. Correspondingly, Arg63, Tyr214 and Tyr215 are the binding sites for 41-NMAIHPR-47 and 214-TNAIPYVR-221. The binding is evidenced by several hydrogen bonds in these two complexes, in which the bioactive peptide played the role of the donor of hydrogens to GCK. A hydrogen bond is stronger than a van der Walls interaction. Thus, these bonds provided for a tight peptide-protein interactions. Other than taking role as donor of hydrogen on this interaction,Asp41 and Arg47 (from 41-NMAIHPR-47) interacted to GCK by salt bridge interactions via peptide's amino terminus. Salt bridges function in stabilizing proteins (12). Not only a tight bound was made, but also a new complex of stable protein were made. Another bond also made by Arginine (amino acid number 221 for 214-TNAIPYVR-221) which tightly bound to four different residues of GCK, with average distance of only 2.0 Å. McDonald and Williams (13) reported that a hydrogen bond can make a tight interaction between two compounds. A good interaction will have not only one hydrogen bond to the compound, but interactions from all binding site residues linked together. So, the binding

Bioactive Peptide	Name	From Chemistry	To	To Chemistry	Distance	Category	Types Of
41-NMAIHPR-47	:ASN41:HT1 - A:GLU248:OE1	:ASN41:HT1	H-Donor;Positive	A:GLU248:OE1	2,83977	Hydrogen Bond;Electrostatic	Salt Bridge;Attractive Charge
	:ARG47:HH11 - A:GLU96:OE1	:ARG47:HH11	H-Donor;Positive	A:GLU96:OE1	1,75439	Hydrogen Bond;Electrostatic	Salt Bridge;Attractive Charge
	:ARG47:NH2 - A:GLU96:OE2	:ARG47:NH2	Positive	A:GLU96:OE2	5,58805	Electrostatic	Attractive Charge
	:ASN41:HD21 - A:GLU248:O	:ASN41:HD21	H-Donor	A:GLU248:O	2,86483	Hydrogen Bond	Conventional Hydrogen Bond
	:ASN41:HD22 - A:GLU248:O	:ASN41:HD22	H-Donor	A:GLU248:O	2,8939	Hydrogen Bond	Conventional Hydrogen Bond
	:HIS45:HN - A:ARG63:O	:HIS45:HN	H-Donor	A:ARG63:O	2,25581	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG47:HN - A:GLU67:OE1	:ARG47:HN	H-Donor	A:GLU67:OE1	2,61287	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG47:HH11 - A:TYR215:O	:ARG47:HH11	H-Donor	A:TYR215:O	2,85603	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG47:HH21 - A:TYR215:O	:ARG47:HH21	H-Donor	A:TYR215:O	2,02011	Hydrogen Bond	Conventional Hydrogen Bond
182-KISQYYQK-189	:LYS182:HZ1 - A:GLU272:OE1	:LYS182:HZ1	H-Donor;Positive	A:GLU272:OE1	1,71168	Hydrogen Bond;Electrostatic	Salt Bridge;Attractive Charge
	:LYS182:HZ2 - A:GLU272:OE2	:LYS182:HZ2	H-Donor;Positive	A:GLU272:OE2	1,74253	Hydrogen Bond;Electrostatic	Salt Bridge;Attractive Charge
	:LYS189:HZ1 - A:ASP311:OD1	:LYS189:HZ1	H-Donor;Positive	A:ASP311:OD1	1,84262	Hydrogen Bond;Electrostatic	Salt Bridge;Attractive Charge
	A:ARG303:HH12 - :GLN188:O	A:ARG303:HH12	H-Donor	:GLN188:O	1,95407	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG303:HH12 - :LYS189:O	A:ARG303:HH12	H-Donor	:LYS189:O	2,5128	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG303:HH21 - :GLN188:O	A:ARG303:HH21	H-Donor	:GLN188:O	2,63691	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG303:HH21 - :LYS189:O	A:ARG303:HH21	H-Donor	:LYS189:O	2,08907	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG327:HH12 - :TYR187:O	A:ARG327:HH12	H-Donor	:TYR187:O	2,83524	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG327:HH12 - :GLN188:OE1	A:ARG327:HH12	H-Donor	:GLN188:OE1	1,69326	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG327:HH21 - :TYR187:O	A:ARG327:HH21	H-Donor	:TYR187:O	2,03003	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG327:HH21 - :GLN188:OE1	A:ARG327:HH21	H-Donor	:GLN188:OE1	2,91012	Hydrogen Bond	Conventional Hydrogen Bond
214-TNAIPYVR-221	:THR214:N - A:ASP158:OD2	:THR214:N	Positive	A:ASP158:OD2	4,59096	Electrostatic	Attractive Charge
	:THR214:HG1 - A:ASP158:OD2	:THR214:HG1	H-Donor	A:ASP158:OD2	1,8638	Hydrogen Bond	Conventional Hydrogen Bond
	:ASN215:HD21 - :THR214:O	:ASN215:HD21	H-Donor	:THR214:O	2,23071	Hydrogen Bond	Conventional Hydrogen Bond
	:ALA216:HN - A:ASP158:OD1	:ALA216:HN	H-Donor	A:ASP158:OD1	2,08248	Hydrogen Bond	Conventional Hydrogen Bond
	:ILE217:HN - :ASN215:O	:ILE217:HN	H-Donor	:ASN215:O	2,20475	Hydrogen Bond	Conventional Hydrogen Bond
	:VAL220:HN - A:ARG63:O	:VAL220:HN	H-Donor	A:ARG63:O	2,15819	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG221:HN - A:TYR215:OH	:ARG221:HN	H-Donor	A:TYR215:OH	2,15695	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG221:HE - A:GLU96:O	:ARG221:HE	H-Donor	A:GLU96:O	2,15288	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG221:HH11 - A:TYR214:OH	:ARG221:HH11	H-Donor	A:TYR214:OH	1,73135	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG221:HH12 - A:THR65:OG1	:ARG221:HH12	H-Donor	A:THR65:OG1	2,26264	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG221:HH21 - A:TYR214:OH	:ARG221:HH21	H-Donor	A:TYR214:OH	2,85179	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG221:HH22 - A:GLU96:O	:ARG221:HH22	H-Donor	A:GLU96:O	1,74933	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG221:HH22 - A:HIS218:NE2	:ARG221:HH22	H-Donor	A:HIS218:NE2	2,52432	Hydrogen Bond	Conventional Hydrogen Bond

Table 3. Analysis of Bioactive peptide interaction with Glucokinase on its active conformation

	Name	From Chemistry	To	To Chemistry	Distance	Category	Type Of
41-NMAIHPR-47	:ASN41:HT1 - A:GLU51:OE1	:ASN41:HT1	H-Donor;Positive	A:GLU51:OE1	2,63	Hydrogen Bond;Electrostatic	Salt Bridge;Attractive Charge
	:ARG47:HH12 - A:ASP205:OD2	:ARG47:HH12	H-Donor;Positive	A:ASP205:OD2	2,23	Hydrogen Bond;Electrostatic	Salt Bridge;Attractive Charge
	:ARG47:NH1 - A:GLU256:OE2	:ARG47:NH1	Positive	A:GLU256:OE2	4,71	Electrostatic	Attractive Charge
	:ARG47:NH2 - A:GLU256:OE1	:ARG47:NH2	Positive	A:GLU256:OE1	2,71	Electrostatic	Attractive Charge
	A:ARG186:HH11 - :HIS45:NE2	A:ARG186:HH11	H-Donor	:HIS45:NE2	2,30	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG186:HH22 - :ILE44:O	A:ARG186:HH22	H-Donor	:ILE44:O	1,87	Hydrogen Bond	Conventional Hydrogen Bond
	A:ASN204:HN - :PRO46:O	A:ASN204:HN	H-Donor	:PRO46:O	2,43	Hydrogen Bond	Conventional Hydrogen Bond
	:ASN41:HT1 - A:GLU51:O	:ASN41:HT1	H-Donor	A:GLU51:O	2,64	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG47:HE - A:GLU256:OE2	:ARG47:HE	H-Donor	A:GLU256:OE2	1,98	Hydrogen Bond	Conventional Hydrogen Bond
	A:PRO59:CD - :ALA43:O	A:PRO59:CD	H-Donor	:ALA43:O	3,49	Hydrogen Bond	Carbon Hydrogen Bond
	:ARG47:C - A:GLU256:OE2	:ARG47:C	H-Donor	A:GLU256:OE2	2,90	Hydrogen Bond	Carbon Hydrogen Bond
	A:ARG186:NH2 - :HIS45	A:ARG186:NH2	Positive	:HIS45	3,52	Electrostatic	Pi-Cation
	A:PRO59 - :MET42	A:PRO59	Alkyl	:MET42	4,69	Hydrophobic	Alkyl
	A:LEU243 - :MET42	A:LEU243	Alkyl	:MET42	4,60	Hydrophobic	Alkyl
	:ALA43 - A:LEU58	:ALA43	Alkyl	A:LEU58	5,11	Hydrophobic	Alkyl
	:PRO46 - A:MET202	:PRO46	Alkyl	A:MET202	5,43	Hydrophobic	Alkyl
	A:HIS50 - :MET42	A:HIS50	Pi-Orbitals	:MET42	5,06	Hydrophobic	Pi-Alkyl
	:HIS45 - A:VAL182	:HIS45	Pi-Orbitals	A:VAL182	4,94	Hydrophobic	Pi-Alkyl
	:HIS45 - A:ALA201	:HIS45	Pi-Orbitals	A:ALA201	4,72	Hydrophobic	Pi-Alkyl
214-TNAIPYVR-221	:ARG221:HH11 - A:GLU196:OE2	:ARG221:HH11	H-Donor;Positive	A:GLU196:OE2	1,82	Hydrogen Bond;Electrostatic	Salt Bridge;Attractive Charge
	:ARG221:HH21 - A:GLU196:OE2	:ARG221:HH21	H-Donor;Positive	A:GLU196:OE2	1,88	Hydrogen Bond;Electrostatic	Salt Bridge;Attractive Charge
	:ARG221:NH2 - A:GLU196:OE1	:ARG221:NH2	Positive	A:GLU196:OE1	4,57	Electrostatic	Attractive Charge
	A:ARG186:HH12 - :VAL220:O	A:ARG186:HH12	H-Donor	:VAL220:O	1,81	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG447:HH11 - :ASN215:O	A:ARG447:HH11	H-Donor	:ASN215:O	2,04	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG447:HH11 - :ASN215:OD1	A:ARG447:HH11	H-Donor	:ASN215:OD1	1,79	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG447:HH21 - :ASN215:OD1	A:ARG447:HH21	H-Donor	:ASN215:OD1	2,79	Hydrogen Bond	Conventional Hydrogen Bond
	:TYR219:HH - A:ALA201:O	:TYR219:HH	H-Donor	A:ALA201:O	2,43	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG221:HN - A:VAL182:O	:ARG221:HN	H-Donor	A:VAL182:O	2,33	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG221:HE - A:VAL199:O	:ARG221:HE	H-Donor	A:VAL199:O	2,55	Hydrogen Bond	Conventional Hydrogen Bond
	:ARG221:HH22 - A:GLU196:O	:ARG221:HH22	H-Donor	A:GLU196:O	2,35	Hydrogen Bond	Conventional Hydrogen Bond
	A:ARG186:CD - :VAL220:O	A:ARG186:CD	H-Donor	:VAL220:O	3,46	Hydrogen Bond	Carbon Hydrogen Bond
	A:VAL182 - :PRO218	A:VAL182	Alkyl	:PRO218	5,09	Hydrophobic	Alkyl
	A:VAL182 - :VAL220	A:VAL182	Alkyl	:VAL220	4,61	Hydrophobic	Alkyl
	A:ARG186 - :VAL220	A:ARG186	Alkyl	:VAL220	5,11	Hydrophobic	Alkyl
	A:ARG186 - :ARG221	A:ARG186	Alkyl	:ARG221	4,27	Hydrophobic	Alkyl
	A:VAL203 - :ILE217	A:VAL203	Alkyl	:ILE217	4,40	Hydrophobic	Alkyl
	:ARG221 - A:ILE189	:ARG221	Alkyl	A:ILE189	5,47	Hydrophobic	Alkyl
	:ARG221 - A:VAL199	:ARG221	Alkyl	A:VAL199	5,43	Hydrophobic	Alkyl
	:TYR219 - A:ALA201	:TYR219	Pi-Orbitals	A:ALA201	4,34	Hydrophobic	Pi-Alkyl

Table 4. Analysis of Bioactive peptide interaction with Glucokinase on its inactive conformation

of 214-TNAIPYVR-221 to GCK displays the properties ofa good interactions.

Since the bioactive peptides appeared to be able to bind to the allosteric activator site of GCK, it is plausible that they have an activator role by improving interaction of these complexes to glucose. After comparison between interaction of glucose and GCK in it's original form and after docking with the peptides, slight differences were observed. Differences can be seen on glucose shape and distances between glucose and GCK. When the distances between glucose and GCK were measured, shorter distances were observed in the glucose and GCK interaction after docking with the peptides. The bonds between glucose before treatment showed longer distances and more limited glucose-GCK residue interactions. As mentioned before, the hydrogen bonds that occured after treatment are more plentiful compared to those in the original glucose-GCK complex, which also supports the effectiveness of bioactive peptides. Based on (14), hydrogen bonds in ligand-protein complexes are a major contributor to the stability of the binding between these molecules. Hydrogen bonds also reported to promote ligand binding affinities (13, 15). Strong hydrogen bonds were also proven to play roles in protein receptor binding affinities in the study by Chen (14). Therefore, we may say that the glucose binding in the complexes docked with peptides appeared to be much more stable. Therefore, thebioactive peptides may promote binding affinities and make GCK effectively bind to glucose.

Glucokinase can phosphorylate glucose at its sixth carbon hydroxyland turn glucose into glucose-6-phosphate. From the structural model (7,11) of glucokinase and glucose interaction, it can be seen that all the model has its sixth carbon open. The sixth carbon chain need to receive aphosphatefrom ATP to change the substance to glucose-6-phosphate. The docking result from this study also shown the sixth carbon in the complexes with bioactive peptides from CSN1S2 is exposed and not bound with glucokinase. In a previous study (16), upon the addition of glucokinase's activator on body, glucokinase found to be converting glucose in a more active way. Glucose plasma level during the addition of GCK's activator was clearly decreased in value. An activator can also

	Name	From Chemistry	To	To Chemistry	Distance	Category	Types Of
Glucose with Glucokinase	A:LYS56:HZ1 - :GLC0:O	A:LYS56:HZ1	H-Donor	: GLC 0:O	2,40152	Hydrogen Bond	Conventional Hydrogen Bond
	A:GLY258:HN - :GLC0:O	A:GLY258:HN	H-Donor	: GLC 0:O	2,45654	Hydrogen Bond	Conventional Hydrogen Bond
	A:GLY258:HN - :GLC0:O	A:GLY258:HN	H-Donor	: GLC 0:O	2,64244	Hydrogen Bond	Conventional Hydrogen Bond
	: GLC 0:H - A:CYS230:O	: GLC 0:H	H-Donor	A:CYS230:O	2,34632	Hydrogen Bond	Conventional Hydrogen Bond
	: GLC 0:H - A:THR168:OG1	: GLC 0:H	H-Donor	A:THR168:OG1	2,48077	Hydrogen Bond	Conventional Hydrogen Bond
	A:GLU256:HA - : GLC 0:O	A:GLU256:HA	H-Donor	: GLC 0:O	2,93965	Hydrogen Bond	Carbon Hydrogen Bond
	: GLC 0:H - A:GLU256:OE1	: GLC 0:H	H-Donor	A:GLU256:OE1	2,82791	Hydrogen Bond	Carbon Hydrogen Bond
Glucose with Glucokinase-41-NMAIHPR-47	N: GLC 0:H - A:GLU256:OE1	N: GLC 0:H	H-Donor	A:GLU256:OE1	2,33459	Hydrogen Bond	Conventional Hydrogen Bond
	A:LYS169:HZ2 - N: GLC 0:O	A:LYS169:HZ2	H-Donor	N: GLC 0:O	2,26521	Hydrogen Bond	Conventional Hydrogen Bond
	A:PRO153:CD - N: GLC 0:O	A:PRO153:CD	H-Donor	N: GLC 0:O	3,2173	Hydrogen Bond	Carbon Hydrogen Bond
	N: GLC 0:H - A:ASP205:OD1	N: GLC 0:H	H-Donor	A:ASP205:OD1	2,17136	Hydrogen Bond	Conventional Hydrogen Bond
Glucose with Glucokinase-214-TNAIPYVR-221	N: GLC 0:H - A:ASN204:OD1	N: GLC 0:H	H-Donor	A:ASN204:OD1	2,55448	Hydrogen Bond	Conventional Hydrogen Bond
	N: GLC 0:H - A:ASP205:OD2	N: GLC 0:H	H-Donor	A:ASP205:OD2	1,88892	Hydrogen Bond	Conventional Hydrogen Bond
	N: GLC 0:H - A:GLU256:OE2	N: GLC 0:H	H-Donor	A:GLU256:OE2	1,75546	Hydrogen Bond	Conventional Hydrogen Bond
	N: GLC 0:H - A:GLU290:OE1	N: GLC 0:H	H-Donor	A:GLU290:OE1	2,30815	Hydrogen Bond	Conventional Hydrogen Bond
	A:LYS169:HZ2 - N: GLC 0:O	A:LYS169:HZ2	H-Donor	N: GLC 0:O	2,49307	Hydrogen Bond	Conventional Hydrogen Bond
	A:LYS169:HZ2 - N: GLC 0:O	A:LYS169:HZ2	H-Donor	N: GLC 0:O	2,58229	Hydrogen Bond	Conventional Hydrogen Bond
	A:ASN231:HD21 - N: GLC 0:O	A:ASN231:HD21	H-Donor	N: GLC 0:O	2,43198	Hydrogen Bond	Conventional Hydrogen Bond
	A:PRO153:CD - N: GLC 0:O	A:PRO153:CD	H-Donor	N: GLC 0:O	3,2175	Hydrogen Bond	Carbon Hydrogen Bond

Table 5. Analysis of Glucose interaction with Bioactive peptide and Glucokinase complex.

increase the affinity of GCK and also changed it's structure.

This study indicates a new mechanism that can be seen on Figure 1(C). Bioactive peptides from CSN1S2 virtually can attach to glucokinase on its active conformation or even on its inactive conformation. Meanwhile, bioactive peptides can enter the cell and interact with glucokinase either using passive diffusion or via calcium channel (17). Bioactive peptides from CSN1S2 are also predicted to play a significant role on inhibiting the AGE-RAGE interaction that help inhibiting more cellular signal cascade reactions (18)"type" : "article-journal", "volume" : "23" }, "uris" : ["http://www.mendeley.com/documents/?uuid=da468d85-bca4-475d-b5af-e0d2c7580e45"] }], "mendeley" : { "formattedCitation" : "(18. Another role found for this compound is as an inhibitor of calmodulin-enzyme interactions (10). The CSN1S2-derivedpeptides may help all these functions and regulating GCK. The amino acids that help to regulate this function are Asn41, Ala43, His45 and Arg221. These amino acidsserve as hydrogen bond donors during their binding to glucose, GCK or even the complexes. Not only making hydrogen bonds, but also contribute salt-bridges at Asn41 in its complexes. While amino acid number 221 (Arginine) bound to complexes with four hydrogen bonds. A similar result was also seen during calmodulin-CSN1S2 bioactive peptide interactions, in which Arg221 made electrostatic pi-cation and hydrophobic pi-sigma type interactions from His45(10). During its Jak-STAT3 interaction, Asn41 bound to PepT1 and made four hydrogen bonds (4). Peptide chain 41-NMAIHPR-47 and 214-TNAIPYVR-221 also contributed in the inhibition of the AGE-RAGE interaction (18)"type" : "article-journal", "volume" : "23" }, "uris" : ["http://www.mendeley.com/documents/?uuid=da468d85-bca4-475d-b5af-e0d2c7580e45"] }], "mendeley" : { "formattedCitation" : "(18.

5. CONCLUSION

This study predicted that bioactive peptides derived from CSN1S2 from Ethawah goat milk may have a role on activating GCK. This activation may occur by binding to residues at GCK's allosteric site and may affect GCK binding to glucose after binding with the bioactive peptide.

- Supplementary Materials: Glucokinase 3D structures can be accessed on RCSB PDB with ID number 1v4s (active conformation) and 1v4t (inactive conformation).
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- Author Contributions: Fatchiyah, F and Rahasta, A: contribute equally to this study. Rahasta, A : Performed the experiment such as docking preparing molecules, and also editing for visualizing molecules to a reader-friendly mode. Fatchiyah, F : Performed analysis of the data and manuscript writing. Cairns: Editing manuscript. Co-author have read and approved the final version of the manuscript.
- Disclosures of Potential Conflict Interest: The authors declares that there are no conflict of interest.

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