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Selective Inhibition on RAGE-binding AGEs Required by Bioactive Peptide Alpha-S2 Case in Protein from Goat Ethawah Breed Milk: Study of Biological Modeling

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ABSTRACT

Background: Advanced Glycation End Products (AGE) play a pivotal role in the development various degenerative diseases such as diabetes, cardiovascular disease, stroke, neuropathy, and nephropathy. Different studies have been done to employ AGEs as drug targets for the diseases therapy. In previous study, we have found bioactive peptide from Ethawah goat milk for anti-diabetic that may work through inhibition of AGE receptor function. However, the mechanism of bioactive peptides inhibits AGE- AGE receptor (RAGE) bonding still not clear yet. Therefore we investigated the inhibition mechanism by calculate the potential energy binding among the peptides, AGEs and RAGE using molecular docking system. **Methods:** Modeling 3D-structure was predicted by SWISS-MODEL web server. The virtual interaction was analyzed by docking system using HEX 8.0, Pymol and Discovery Studio 4.0 software. **Results:** this study showed that AGEs (Argyrimidine, Imidazole, Pentosidine and Pyrraline) bind to C-domain of RAGE. The total energy binding of RAGE with Argyrimidine, Imidazole, Pentosidine and Pyrraline were 378.35kJ/mol, -74.57kJ/mol, -301.25kJ/mol and -400.72kJ/mol, respectively. We have found three peptides among eight peptides from Ethawah goat milk, which are able bind to C-domain of RAGE, there are CSN1S2 f41-47, CSN1S2 f182-189, and CSN1S2 f214-221. The CSN1S22 f41-47 at arginine residue 47 interacts with proline162, leusine163 and leusine158 of RAGE. The total binding energy between CSN1S2 f41-47, CSN1S2 f182-189, and CSN1S2 f214-221 with RAGE were -378.35 kJ/mol, -359.97kJ/mol, -356.78 kJ/mol, respectively. Total binding energy and binding pattern indicated that RAGE more prefer bind with peptide and block AGE bind to functional site of RAGE. Further analysis showed that complex peptide-RAGE shifted binding site of AGE on function domain RAGE. **Conclusion:** This study suggested that the peptides from Ethawah goat milk may act as an inhibitor of AGEs-RAGE interaction that impaired signal transduction cascade at the cellular level.

Key words: Argyrimidine; bioactive peptide; glycation; goat milk; imidazole; receptor for glycation end products.

1. INTRODUCTION

Advanced Glycation End Products (AGEs) play a pivotal role in the cause and development of diabetic complications cardiovascular disease, stroke, and microvascular diseases, including retinopathy, neuropathy, nephropathy and other complications (1, 2). AGEs also provide new possible targets for the treatment of both type I and type II diabetes. Hyperglycemia is an abnormally high blood glucose (blood sugar) level. Among the irreversible changes that occur as a result of hyperglycemia is the formation of AGE through a reaction between sugars and the free amino groups on proteins, lipids, and nucleic acids. Glucose is a reactive aldehyde can react spontaneously, although slow, with protein. Through a process called non-enzymatic glycosylation, protein experienced modification. Aldehyde group of glucose reacts with the amino group contained on a protein, forming products glycosylation which is reversible. This product underwent a series of reactions with group NH₂ of protein and intercalated with AGE (2,3, 4).

Non-enzymatic glycation product and advanced glycation

end products (AGEs) acquire gradually in a wide diversity of environments. Maillard reaction firstly characterized by the formation of brown-colored substances resulted from non-enzymatic reaction come off between reducing sugars and amino acids of protein (5, 6). This reaction is develop from chemical linkage between the amino group of protein and the carbonyl groups of sugar to form Schiff bases and Amadori compounds, resulting in heterogeneous derivatives termed AGEs (7, 8). The AGEs compound consist three categories: Argyrimidine and Imidazole as molecules adduct of arginine product, Pentosidine as crosslink product of lysine-arginine residues and last, Pyrraline as amadori product degradation (9, 10).

Receptor for AGE (RAGE) is multi-ligand cell surface receptors belong to the immunoglobulin superfamily. AGEs interact with Receptor for AGEs (RAGE) leading to oxidative stress and activation of pro-inflammatory pathways mechanisms dependent on RAGE signal transduction. The level of AGEs elevate deeply in diabetes due to chronic hyperglycemic condition (2, 6, 11).

Goat milk derived bioactive peptides play vital roles in having better digestibility, buffering capacity, alkalinity and certain therapeutic values in medicine and human nutrition than cow milk. The protein in goat milk is more digested and their constituent amino acids efficiently absorbed more than those of cow milk. The bioactivities of peptides encrypted in major milk protein are latent until released and activated by enzymatic proteolysis, e.g. during gastrointestinal digestion or food processing (12, 13). Bioactive peptides of milk protein are synthesized in the small intestines in the form of large prepropeptides, which are cleaved and modified to give active products. As signaling molecules, the bioactive peptides play important roles in physiological functions and pathogenesis (14, 15). Recent study, we found the goat Ethawah breed milk CSN1S2 protein as caprine protein has eight peptides residues contain seven to twelve amino acid residues which are suggest to reveal multifunctional properties (16). We predict that some of bioactive peptides have a function as agent of reducing RAGE signaling in diabetic development or progression. Structural information is critical to understand the molecular constituents and inhibition mechanisms of goat milk bioactive peptides prevent RAGE signaling and to design modeling structures and optimize the affectivity of bioactive peptide as therapeutic agents against RAGE cause the diabetic pathologies.

2. MATERIALS AND METHODS

RAGE Protein, AGEs compound and Protein Peptide Sequence Retrieval

The protein sequences of RAGE (GI: 259089426) and AGE's compound such as Argypirimidine (ID: 17750123), Imidazole (ID: 795), Pyrraline (ID: 1222228), and Pentosidine (ID: 119593) was retrieved from the sequence database of NCBI (*National Center of Biotechnology Information*). The peptide sequence fragments of caprine milk CSN1S2 protein was isolated and identified by MALDI-TOF (16).

Receptor and Ligand Modeling 3D-structure Preparation

Modeling 3D-structure of RAGE and peptide sequence fragments of caprine milk CSN1S2 protein were predicted by SWISS-MODEL web server by homology modeling

method. SDF file format of Argypirimidine, Imidazole, Pyrraline, and Pentosidine were converted to be as PDB format using Open Babel (17).

Docking of Ligand-Protein and Visualization

To analyze the virtual interaction among RAGE, AGEs, and peptide sequence fragments of caprine milk CSN1S2 protein, we used HEX 8.0, Pymol and Discovery Studio 4.0 software to docking for possibility interaction. The docking between RAGE & AGE, RAGE & peptide sequence fragments of caprine milk CSN1S2 protein, and among RAGE-peptide sequence fragments of caprine milk CSN1S2 protein-AGE were elucidated by Hex 8.0. Interaction visualization among them was showed off by Pymol and Discovery Studio 4.0 as proper.

Analysis for Binding interaction and Binding Energy

The type of binding among receptor, protein peptide and other ligand was identified using HEX 8.0 such as amino acids residue; atoms belong to the protein and ligand and also type of hydrogen bonds, van der Waals contacts and covalent bonds. The binding energy of their interaction was calculated by HEX 8.0.

Ethical consideration

The study was approved by ethical review committee of Brawijaya University Research Ethics Committee.

3. RESULTS

The protein sequences of RAGE and AGE's compound was obtained from the sequence database of NCBI and PDB. Structure 3D modeling of RAGE and peptide fragments of Caprine alpha-S2 casein protein was provided by SWISS-SPROT.

Virtual docking RAGE and AGEs interaction

The possibility interaction of AGEs (Argypirimidine, Imidazole, Pentosidine and Pyrraline) and RAGE presented in Figure 1, Argypirimidine interacts on upstream of C-domain type 1 (Figure 1A, yellow arrow), Imidazole and Pentosidine connecting between C-domain type 1 and V-domain (Figure 1B and 1C, yellow arrow), and Pyrraline bind at downstream C-domain type 1 of RAGE (Figure 1D, yellow arrow). Amino acids residues of RAGE interacted with each AGE appear explicitly, and has various total binding energy (Table 1). Ar-

No	Interaction	Point Interaction	Donor Atom	Acceptor Atom	Type	Chemistry Bond	Energy binding
A	RAGE- Argypirimidine	ARG 214- Argypirimidine	ARG214:H	:Argypirimidine: N	Hydrogen Bond	Hydrogen Bond	-378.35 kJ/mol
		ARG 215- Argypirimidine	ARG215:H	:Argypirimidine: O	Hydrogen Bond	Hydrogen Bond	
		ARG 216- Argypirimidine	ARG216:H	:Argypirimidine: N	Hydrogen Bond	Hydrogen Bond	
B	RAGE-Imidazole	GLU 93- Imidazole	GLU93:H	Imidazole :N	Hydrogen Bond	Hydrogen Bond	-74.57 kJ/mol
		ARG 115- Imidazole	ARG115:H	Imidazole:N	Hydrogen Bond	Hydrogen Bond	
		VAL116- Imidazole	VAL116:H	Imidazole :N	Hydrogen Bond	Hydrogen Bond	
		TYR149- Imidazole	TYR149:H	Imidazole :N	Hydrogen Bond	Hydrogen Bond	
		PRO150- Imidazole	PRO150:H	Imidazole :H	Hydrogen Bond	Hydrogen Bond	
C	RAGE- Pentosidine	LEU212- Imidazole	LEU212:H	Imidazole :N	Hydrogen Bond	Hydrogen Bond	-301.25 kJ/mol
		ASN25- Pentosidine	ASN25: H	Pentosidine: H	Hydrogen Bond	Hydrogen Bond	
		ARG 155- Pentosidine	ARG 155: H	Pentosidine: N	Hydrogen Bond	Hydrogen Bond	
		GLY 221- Pentosidine	GLY 221- :H	Pentosidine: O	Hydrogen Bond	Hydrogen Bond	
D	RAGE- Pyrraline	LEU212- Pentosidine	LEU212: H	Pentosidine: H	Hydrogen Bond	Hydrogen Bond	-400.72 kJ/mol
		PRO213- Pentosidine	PRO213: H	Pentosidine: O	Hydrogen Bond	Hydrogen Bond	
		GLU131- Pyrraline	GLU131: H	Pyrraline: H	Hydrogen Bond	Hydrogen Bond	
		THR 133- Pyrraline	THR 133 : H	Pyrraline: N	Hydrogen Bond	Hydrogen Bond	
		ARG 228- Pyrraline	ARG 228 :H	Pyrraline: O	Hydrogen Bond	Hydrogen Bond	

Table 1. Ligand Interaction and Total Energy Binding of RAGE and AGEs. Note: Bold letter and number is Donor

No	Interaction	Point Interaction	Donor Atom	Acceptor Atom	Type	Chemistry Bond	Energy binding
A	RAGE- CSN1S22 f 41-47	PRO 137- ASN 41	PRO 137:	ASN 41:	Unfavorable	H-Donor	-378.35 kJ/mol
		VAL 136- MET 42	VAL 136:	MET 42:	Alkyl	Alkyl	
		PRO 162- ARG 47	PRO 162:	ARG 47:	Unfavorable	Steric	
		LEU 163- ARG 47	LEU 163:	ARG 47:	Unfavorable	Steric	
B	RAGE- CSN1S22 f 182-189	LEU 158- ARG 47	LEU 158:	ARG 47:	Unfavorable	Steric	-356.78 kJ/mol
		ILE 119- GLN 188	ILE 119:	GLN 188:O	Hydrogen Bond	Hydrogen Bond	
		GLY 121- GLU 146	GLY 121:	GLU 146:O	Hydrogen Bond	Hydrogen Bond	
		GLU 124- VAL 144	GLU 124:	VAL 144:O	Hydrogen Bond	Hydrogen Bond	
		TYR 149- GLN 118	TYR 149:	GLN 118:O	Hydrogen Bond	Hydrogen Bond	
		LYS 182- GLU146	LYS 182:	GLU 146: O	Hydrogen Bond	Hydrogen Bond	
		LYS 182- GLU146	LYS 182:	GLU 146: O	Hydrogen Bond	Hydrogen Bond	
		SER 184- VAL144	SER 184:	VAL 144: O	Hydrogen Bond	Hydrogen Bond	
		TYR 186- ILE 183	TYR 186:	ILE 183: O	Hydrogen Bond	Hydrogen Bond	
		TYR 187- ILE 183	TYR 187:	ILE 183: O	Hydrogen Bond	Hydrogen Bond	
		TYR 188- GLN 185	TYR 188:	GLN 185: O	Hydrogen Bond	Hydrogen Bond	
C	RAGE- CSN1S22 f 214-221	GLN 188- ILE119	GLN 188:	ILE 119: O	Hydrogen Bond	Hydrogen Bond	-359.97 kJ/mol
		LYS 189- TYR 186	LYS 189:	TYR 186: O	Hydrogen Bond	Hydrogen Bond	
		GLY199- VAL220	GLY199:	VAL220:O	Hydrogen Bond	Hydrogen Bond	
		GLN224- THR214	GLN224:	THR214:O	Hydrogen Bond	Hydrogen Bond	
		GLN224- THR214	GLN224:	THR214:O	Hydrogen Bond	Hydrogen Bond	
		ASN215- THR203	ASN215 :	THR203:H	Hydrogen Bond	Hydrogen Bond	
		ALA216- ASP159	ALA216: O	ASP159:H	Hydrogen Bond	Hydrogen Bond	
ILE217- ASP159	ILE217:O	ASP159:H	Hydrogen Bond	Hydrogen Bond			

Table 2. Ligand Interaction and Total Energy Binding of RAGE and Peptide fragment of Caprine Alpha-S2 Casein Protein. Note: Bold letter and number is Donor

No	Interaction	Point Interaction	Donor Atom	Acceptor Atom	Type	Chemistry Bond	Energy binding
A	RAGE- CSN1S22 f 41-47- Argyrimidine	THR 176- ARG 47	THR 176:	ARG 47:O	Unfavorable	Steric	-208,60 kJ/ mole
		THR 153- ALA 43	THR 153:	ALA 43:H	Unfavorable	H-Donor	
		SER 155- ASN 41	SER 155:	ASN 41:H	Unfavorable	H-Donor	
		THR 176- ARG 45	THR 176:	ARG 45:H	Unfavorable	H-Donor	
		SER 209- ASN 41	SER 209:	ASN 41:H	Unfavorable	H-Donor	
		GLY 152- ALA 43	GLY 152:	ALA 43:H	Carbon Hydrogen	H-Donor	
		PRO 150- ILE 44	PRO 150:	ILE 44	Alkyl	Alkyl	
		ALA 151- PRO 46	ALA 151:	PRO 46	Alkyl	Alkyl	
		LEU 212- ILE 44	LEU 212:	ILE 44	Alkyl	Alkyl	
		PRO 213- MET 42	PRO 213:	MET 42	Alkyl	Alkyl	
PRO 217- MET 42	PRO 217:	MET 42	Alkyl	Alkyl			
B	RAGE- CSN1S22 f 214- 221- Imidazole	Argyrimidine- ILE 44	Argyrimidine :C	ILE 44: H	Alkyl	Alkyl	-72,66 kJ/ mole
		GLY199- VAL220	GLY199 :	VAL220:O	Hydrogen Bond	Hydrogen Bond	
		GLN224- THR214	GLN224:H1	THR214:O	Hydrogen Bond	Hydrogen Bond	
		GLN224- ARG 221	GLN224:H2	ARG 221:O	Hydrogen Bond	Hydrogen Bond	
		ASN215- THR203	ASN215:O	THR203:H	Hydrogen Bond	Hydrogen Bond	
		ALA216- ASP159	ALA216: H	ASP159:O	Hydrogen Bond	Hydrogen Bond	
		ILE217- ASP159	ILE217:H	ASP159:O1	Hydrogen Bond	Hydrogen Bond	
		ILE217- ASP159	ILE217:H	ASP159:O2	Hydrogen Bond	Hydrogen Bond	
		ARG 221- Imidazole	ARG 221: H	Imidazole: O	Hydrogen Bond	Hydrogen Bond	
		GLY199- VAL220	GLY199 :	VAL220:O	Hydrogen Bond	Hydrogen Bond	
GLN224- THR214	GLN224:H1	THR214:O	Hydrogen Bond	Hydrogen Bond			
GLN224- ARG 221	GLN224:H2	ARG 221:O	Hydrogen Bond	Hydrogen Bond			

Table 3. Ligand Interaction and Total Energy Binding between RAGE, Peptide fragment of Caprine Alpha-S2 Casein Protein and AGEs complex. Note: Bold letter and number is Donor

gypiridine bind at three of arginine residues 214-215-216 of RAGE and total energy binding Σ : -378.35kJ/mol (Table 1A). Imidazole binds at six of amino acid residues 63-212 of RAGE with total binding energy Σ : -74.57kJ/mol (Table 1B). Pentosidine Imidazole binds at five of amino acid 25-213 residues of RAGE and total binding energy is -301.25kJ/mol (Table 1C). Pyrraline bind to three of amino acid 131-133-228

residues of RAGE with total binding energy is -400.72kJ/mol (Table 1D).

Possibility Interaction of RAGE and Peptide Fragments of Caprine alpha-S2 casein protein

We have performed the docking of interaction between RAGE and peptide fragments of Caprine alpha-S2 casein protein. We have identified the eight sequences of peptide

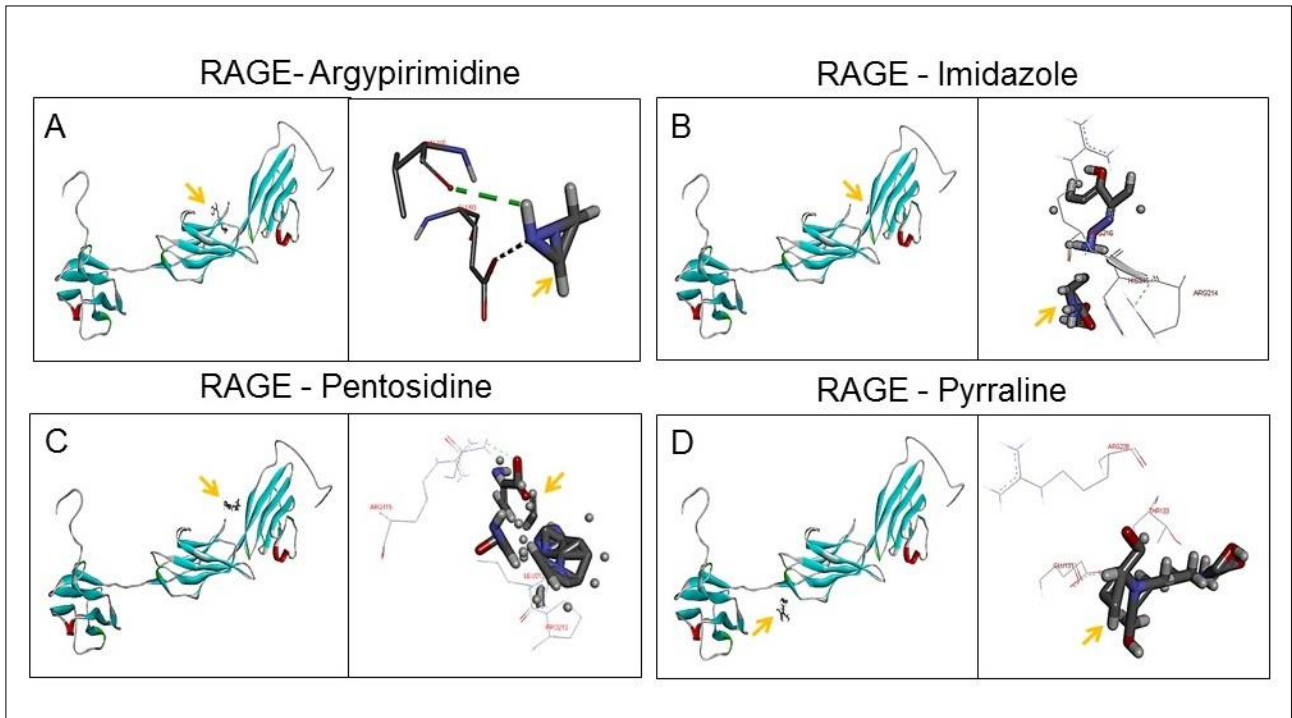


Figure 1. Possibility Interaction between RAGE and four types of AGEs. A. RAGE- Argypirimidine. B. RAGE-Imidazole. C. RAGE-Pentosidine. D. RAGE- Pyrraline. Yellow Arrow is AGEs.

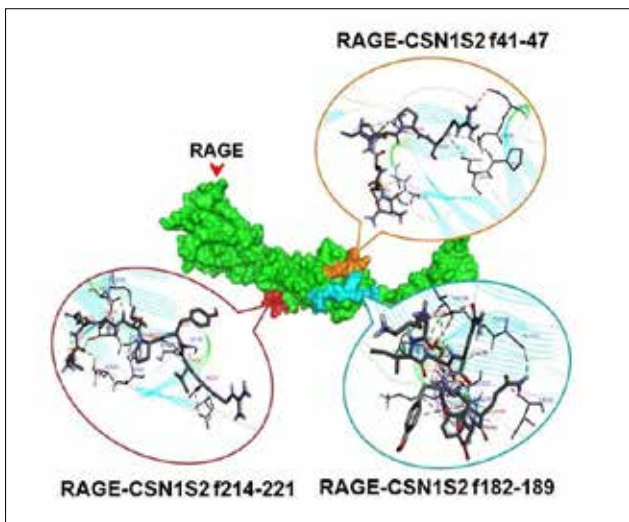


Figure 2. Possibility Interaction between RAGE and three of peptide fragments of Caprine alpha-S2 casein protein. The green molecule is RAGE. The orange molecule is a peptide fragment of Caprine alpha-S2 casein protein at amino acid 41-47 residues. The light blue is molecule peptide fragment of Caprine alpha-S2 casein protein at amino acid 182-189 residues. The red molecule is a peptide fragment of Caprine alpha-S2 casein protein at amino acid 214-221 residues.

fragments that belong to milk Caprine alpha-S2 casein protein (16). We detected that only three of peptide fragments of Caprine alpha-S2 casein protein able to interact with RAGE in a different area of C-domain type 1 of RAGE (Figure 2). These three of peptide fragments of Caprine alpha-S2 casein protein, there are CSN1S22 f41-47 (⁴¹Asn,⁴²Met,⁴³Ala,⁴⁴Ile,⁴⁵His,⁴⁶Pro,⁴⁷Arg), CSN1S2 f182-189 (¹⁸²Lys,¹⁸³Ile,¹⁸⁴Ser,¹⁸⁵Gln,¹⁸⁶Tyr,¹⁸⁷Tyr,¹⁸⁸Gln,¹⁸⁹Lys), and CSN1S2 f214-221 (²¹⁴Thr,²¹⁵Asn,²¹⁶Ala,²¹⁷Ile,²¹⁸Pro,²¹⁹Tyr,²²⁰Val,²²¹Arg) (16). Molecular binding between peptide fragment 41-47 of Caprine alpha-S2 casein protein interact at downstream C-

domain type 1 of RAGE (Figure 1) which is five amino acid residues of RAGE as donor. Whereas three of CSN1S2 peptide were recipient which is arginine 47 residues of the pep-

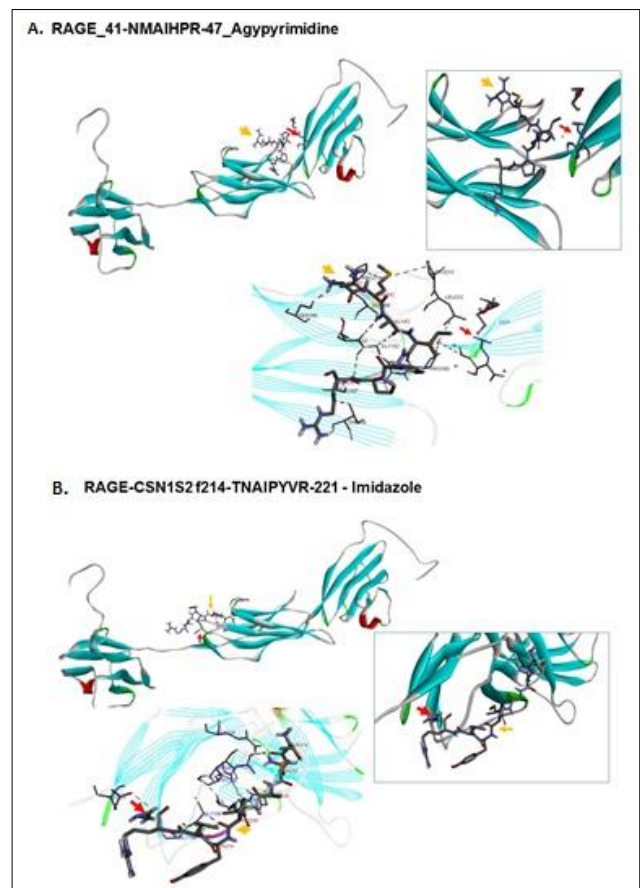


Figure 3. Possibility Interaction of RAGE, Peptide fragment of Caprine Alpha-S2 Casein Protein and AGEs. Yellow arrow is Peptide fragment. Red arrow is AGE.

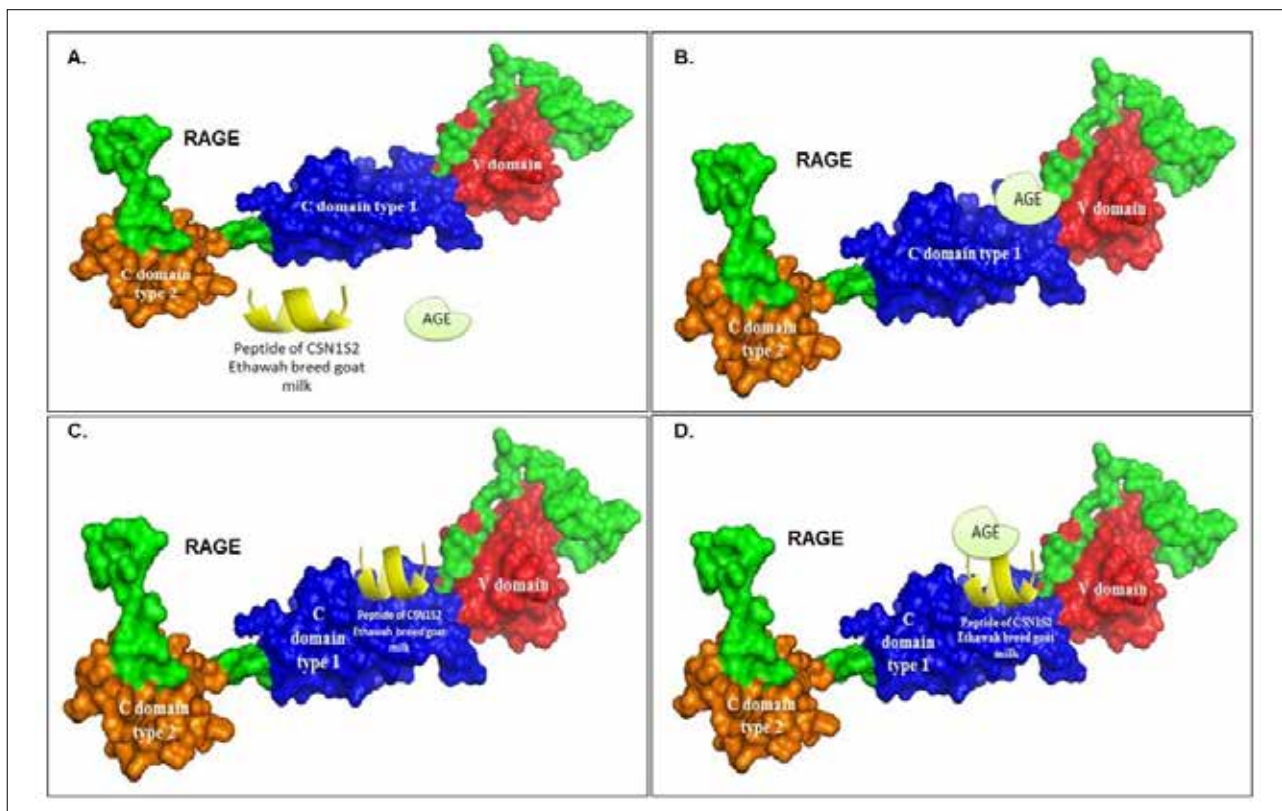


Figure 4. Highlight Abstract of 3D-Structure of RAGE-AGEs-Bioactive Peptides Interaction. A. RAGE-AGEs-Bioactive Peptides Molecules. B. RAGE-AGEs Interaction. C. RAGE- Bioactive Peptides. D. RAGE- Bioactive Peptides-AGEs Interaction.

ptide made three branches with proline162, leusine163 and leusine 158 of amino acids residue of RAGE. Moreover, total binding energy between CSN1S2 and RAGE was -378.35 kJ/mol (Table 2A). Further investigation also suggested that CSN1S2 f214 is required as a recipient for amino acids residue of RAGE and acted tightly up on one another by hydrogen bond of upstream C-Domain type 1 with total binding energy Σ : -359.97 kJ/mol (Table 1C). Different profile appeared in interaction between amino acid 182-189 residues peptide fragment of Caprine alpha-S2 casein protein is as donor for amino acids residue of RAGE and need total binding energy around Σ : -356.78 kJ/mol (Table 2B). Though the peptide fragment 41-47 and 213-221 residues of caprine alpha-S2 casein protein may have the biological function totally unlike with the peptide fragment 182-189 residues.

The highlight abstracting of all possibility interaction of RAGE-AGEs, RAGE-bioactive peptides and RAGE-bioactive peptides-AGEs is providing at Figure 4. AGEs usually interacted in C-domain Type 1 of RAGE-binding domain. Some peptides protein also interact into the C-domain Type 1 of RAGE-binding domain. However, when AGEs compound and peptides protein appear around the cell, both will compete to connect into RAGE-binding domain. In this study show that the bioactive peptide of caprine CSN1S2 protein succeed properly interacted into RAGE-binding C-type-1 domain and AGEs compounds bonding in one amino acid residue of peptide of caprine CSN1S2 protein.

Virtual Molecular Dynamic and Binding Energy Calculation of RAGE, Peptide fragment of Caprine Alpha-S2 Casein Protein and AGEs Interaction

Virtual dynamic of molecular interaction among RAGE, peptide fragment of caprine alpha-S2 Casein protein and

AGEs was identified and provided by Discovery 4.0. The results are only two of the peptide fragment of Caprine alpha-S2 casein protein compete with two AGEs compounds to interact with RAGE as properly (Figure 3 and Table 3). The Caprine alpha-S2 casein protein peptide fragment 41-47 residues was bond in C-domain type 1 and the Argypirimidine held on to isoleucine 44 residue of the peptide fragment (Figure 3A), as Argypirimidine-ILE44. The total binding energy of RAGE, peptide fragment of caprine alpha-S2 casein protein and Argypirimidine complex is $-208,60$ kJ/mol is higher than RAGE-Argypirimidine (Σ : $-378,35$ kJ/mole, Table 3A). Meanwhile the fragment 214-221 of Caprine alpha-S2 casein protein bind to RAGE C-domain type 1 region and was brought forward Imidazole compound (Figure 3B) connected to arginin221 residue of peptide fragment as Imidazole-ARG221 (Table 3B). RAGE bind to complex of casein protein (peptide fragment 214-221) with Imidazole has high binding energy (Σ : $-72,66$ kJ/mol).

4. DISCUSSION

Full-length structure of RAGE contains extracellular (exRAGE) and cytosolic signal region (ctRAGE). AGE-RAGE complexes mostly due to the huge heterogeneousness of AGEs generated by glycation reactions: Glycation reactions are not largely dependent on sequence specificity, and lysine and arginine residues, which are particularly susceptible to glycation, are very common in proteins (18,19). The extracellular regions are signal recognition domain as N-terminal signaling, the V-type domain which functions for ligand binding, and two C-type domains, a transmembrane spinning helix. Cytosolic region is a domain of C-terminal which is required for signal transduction of cell (19). According to

the UNIPROT analysis, the amino acid sequences of RAGE domains include the upstream are 22 amino acid signal recognition sequence followed V-domain (amino acid 23-109 residues), the middle region is C-domain type 1 (amino acid 123-219 residues), C-domain Type2 (amino acid 233-315 residues), and transmembrane domain (amino acid 345-363 residues) and cytosolic signal domain is 363-440 amino acids residues.

In this study report that Argypirimidine, Imidazole, Pentosidine and Pyrraline connected by hydrogen bonds. The electrostatic attraction between polar molecules of hydrogen bond occurs when a hydrogen (H) atom binds to a highly electronegative atom such as nitrogen (N) or oxygen (O). The bonding of them need low energy except RAGE-Imidazole (Σ : -74.57kJ/mole) seem this ligand-binding stronger than others. A hydrogen bond (5 to 30kJ/mole) is tighter than van der Waals interaction (20, 21). Oligomer forms of RAGE interface localized at the link between the C1 and C2 domains that are may play the mediators role of the signal transduction cascade of an extracellular event and intracellular downstream signaling (10). Specific of AGEs compound such as Argypirimidine and Imidazole due to their interactions with the Receptor for AGEs (RAGE), may play pivotal role increasing to complications of diabetes evident and chronic inflammation. These ligands are binding to RAGE induce dysfunction of cellular signal transduction through up-regulation oxidative stress, synthesis and secretion of pro-inflammatory cytokine (18, 22)

During the last two decades, it has clear that milk proteins have been widely used as source of biologically active peptides. The alpha2-casein family has complex structure and function that accounts for up to 20% of all casein fractions in goat milk. Bioactive peptides of food proteins can lower blood pressure and inhibit the activity of proline specific endopeptidase stimulate the immune system (23). Moreover, the arginine in the N- or C-terminal region of goat milk peptide is important structural entity recognized by specific membrane-bind receptors. The present of arginine has structural activity in relationship and mechanism of immunomodulatory effects that may stimulate the proliferation and maturation of T cells and natural killer cells for defense of newborn against a large number of bacteria, particularly enteric bacteria (14). An in vitro cell system digested dietary peptide with serum albumin show that this system may play a role in the regulation of RAGE and downstream inflammatory pathways (24). This study show there is three kinds of caprine CSN1S2 peptide fragments may play a role to prevent RAGE activities in interact with ligand. These bioactive peptides fragments are bind in C-domain type 1 and localized in the similar with AGEs-binding region. RAGE and caprine CSN1S22 peptide fragment on amino acid 41-47 and 214-221 residues ligand were succeed moving the Argypirimidine in upstream C domain type 1 to downstream C domain type 1 and Imidazole from V domain into C domain type 1 that seem it may act as regulatory compounds on physiological cellular mechanism. Therapeutic strategies to block RAGE may represent high therapeutic potentials. There is a growing interest in unraveling the intracellular signaling pathways by RAGE controls these disease-related processes (19). In vitro study showed high dose of CSN1S2 protein of

goat milk (0.100 mg/L) in high methyl glyoxal environment inhibits precisely decreasing the mitogenic activity due to increasing the proliferation of MC3T3E1 pre-osteoblast cell (25). It seems caprine alpha-S2 casein protein have potency for bone loss therapeutic agent and prevent inflammation.

The interaction between RAGE and CSN1S2 fragment 182-189 shown caprine CSN1S2 protein peptide as donor binding despite the other bonding as recipient. Thought this caprine CSN1S2 fragment has a specific function differ with other peptide. The amino acids residues 165-203 of milk alpha-S2 casein protein are proposed as casocidin-I or related peptides of milk that influence the human small intestinal flora, particularly of the suckling which perform a protective activities (15).

The changes of amino acids on protein can affect the formation of the 3-D structure of proteins. Interaction RAGE-Argypirimidine shows that arginine residue at position 214, 215 and 216 of RAGE bind with Argypirimidine using hydrogen bonding. Therefore the binding was shifted into Alkyl-Alkyl bonding between isoleucine number 44 of peptide and Argypirimidine after intervened by CSN1S2 fragment 41-47. Whereas after their binding are given bioactive peptides of CSN1S2 fragment 41-47 induced new binding changing of Argypirimidine position caused by a number of amino acid residues thus reducing the stability of the conformation. Alkyl bond of isoleucine residue belongs to Van der Wall bond. Alkyl bond may cause a conformational 3-D structure of Argypirimidine with RAGE and peptide fragment. Alkyl bond is stronger than hydrogen bonds. Polar nature, charge and hydrophobicity of the alkyl bond resulting in this interaction are not easy to dissociation (19). The difference in the active region resulted in amino acid changes in a protein seem that affects to the formation of three-dimensional structure of the protein. These changes may abrogate functional interaction between AGE and RAGE. The result indicated that fragment 41-47 of CSN1S2 has inhibitor activity of Argypirimidine-RAGE interaction. In other hand, CSN1S2 fragment 214-221 can also require the imidazole bind to arginine residue 221 of the peptide. These results indicated the possibility of caprine CSN1S2 peptide able to take place biological function as a competitive inhibitor of AGEs and RAGE interaction that may intervenes its cellular mechanism and signal transduction. Previous study reported the most of peptides fragments of caprine CSN1S2 protein were bound closer to N-terminal and loop of Calmodulin than to C-terminal of Calmodulin that probably this peptide function as inhibitor protein to regulate cellular signaling pathway (16).

Energy is required for ligand and receptor interactions, the lower negative energy indicate the tight of receptor-ligand bond, but the high energy may cause instability or difficulty binding between receptor and ligand. In this study showed that Argypirimidine-RAGE binding energy is $\Sigma = -221.28$ kJ/mol, lower than the caprine CSN1S2 peptides 41-47 and RAGE interaction at $\Sigma = -374.35$ kJ/mol. Interestingly after RAGE-CSN1S2 peptide Argypirimidine binding together increasing total energy into $\Sigma = -208.60$ kJ/mol. The ligand binding energy RAGE & Imidazole or RAGE, caprine CSN1S2 peptide fragment 214-221 & Imidazole was high, there are $\Sigma = -74.54$ and 72.66 kJ/mol, respectively. Meanwhile the binding energy of caprine CSN1S2 fragment 214-

221 and RAGE was declined in $\Sigma = -374.35$ kJ/mol. This binding energy indicates that influence by the position and the interaction of the ligand and receptor and after a given caprine CSN1S2 peptide provided different affinity of binding energy in both AGEs into RAGE interaction.

5. CONCLUSION

This study predicted that the bioactive peptides of Ethawah goat milk may act as an inhibitor of AGEs-RAGE interaction that impaired signaling transduction cascade at the cellular level.

List of abbreviations: AGE: *Advanced Glycation End Products*; CSN1S2: *Alpha-S2 Casein Protein*; RAGE: *Receptor for Glycation End Products*

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CONFLICT OF INTEREST: NONE DECLARED.

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