Int J Pharm Bio Sci 2015 April;; 6(2): (B) 707 - 718

Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

VIRTUAL SELECTIVITY PEPTIDES OF CSN1S2 PROTEIN OF LOCAL GOAT ETHAWAH BREEDS MILK MODULATE BIOLOGICAL MECHANISM OF CALMODULIN

FATCHIYAH FATCHIYAH*[1], SENTOT J RAHARJO[2] AND FIRLI RP DEWI[1]

[1]Department of Biology, Faculty of Sciences, Brawijaya University, Jl. Veteran, Malang, 65145, Indonesia.
[2]Academic of Pharmacy and Food Analysis, Putra Indonesia Malang,,Jl.Barito No.5, Malang, Indonesia.

ABSTRACT

The purpose of our study is to observe virtual selectivity peptides of caprine alpha-S2 casein (CSN1S2) protein of goat Ethawah breeds milk modulate biological mechanism of Calmodulin virtual prediction of biological function of peptides of goat fresh milk of local Ethawah breed. The caprine alpha-S2 casein protein was isolated and purified from fresh goat milk local Malang, and then caprine alpha-S2 casein protein was sequenced by MALDI-TOFF analysis. The function of specific peptides were docking with Calmodulin by *in silico*.The result of this study is the caprine alpha-S2 casein protein of Ethawah breed goat milk has eight peptide fragments: CSN1S2 f41-47, f87-96, f97-107, f131-141, f182-189, f205-213, 214-221 and f214-223. The most of alpha-S2 casein peptide fragment can interacted properly with Calmodulin, except CSN1S2 fragment 97-107 peptide. The caprine alpha-S2 casein protein peptides have ability to bind Calmodulin on specific site and may enhance sites for interactions with other cellular molecules.

KEY WORD: Bioactive peptides, CSN1S2, Calmodulin, Goat milk

.

***Corresponding author**

FATCHIYAH FATCHIYAH
Department of Biology, Faculty of Sciences, Brawijaya University,
Jl. Veteran, Malang, 65145, Indonesia.

INTRODUCTION

Biology system is a source of inter/intra cellular communication encompasses the mechanism regulation on fields of genomics, post-translation modification, proteomics and metabolomics. Most of biological signaling pathway was orchestrated at the level of proteins and biological active compounds, that both molecules recognition have ability to interact and modulate molecular mechanisms underlying an organism's physiological functions. A Calmodulin molecule plays a central role as a Ca^{2+}-sensitive target in signal transduction. Calmodulin actions are as mediator protein and regulator of numerous intracellular processes for senses calcium levels to delivers the signals to various calcium-sensitive enzymes, ion channels and activates other kinases and phosphatases that play a roles in cell motility, growth, ion transport, proliferation, cell signaling and cell apoptosis [1]. Calmodulin is a small (148 amino acids, MW 17kDa) Ca^{2+}-binding protein and a ubiquitous cytoplasmic protein that exists in all eukaryotic cells for sensing calcium molecules [1, 2]. Characterization of Calmodulin structure contains loop flanked by two alpha-helices of calcium-binding motif with four closely identical high-affinity calcium binding sites and the most target enzymes binding regions include wide range fraction of basic and hydrophobic residues. These segments can modulate linear peptides bind to Calmodulin in a-helical form to preserve the affinity [3, 4]. Several biological processes are carried out by proteins interacting with each other.Activation onCa^{2+}-binding Calmodulin has to stimulated the conformational Calmodulin structure that visualized by hydrophobic amino acid residues on the surface of both lobes to creating two hydrophobic pockets, the exposure of hydrophobic sites that bind to and activate a large number of target proteins [2, 4]. Calmodulin binds to a large number of target proteins through interactions with specific Calmodulin binding domains [5, 4] including myosin light chain kinases and various Calmodulin-dependent kinases and phosphorylase kinase [2, 6]. Increasing activity of intracellular Ca^{2+} and stimulation of Ca^{2+}/Calmodulin-dependent protein kinase-β induced by activation of AMPK on the formation of pathological autophagy vacuoles in mouse cortical cells [7]. Some peptide fragments of casein protein of bovine and ovine were predicted able to prevent Calmodulin-activation of cyclic nucleotide phosphodiesterase, as major regulatory component of a variety of metabolic pathways. The peptides of casein protein suggest interact with the regulatory protein Calmodulin interfering with its binding to the enzyme [3, 5, 8]. Casein protein of milk is involved α-S1-, α-S2-, β- and κ-casein which each type has different properties and bioactive function. Differentiation of structural and physicochemical properties is important factor to determine the biological function [7, 10, 11]. Several dietary proteins produced bioactive peptide during gastrointestinal digestion and fermentation [11]. Milk casein protein is a rich source of bioactive peptide compounds that may contribute to regulate immune system of digestion, nervous, rheumatoid arthritis or cardiovascular system [12, 3.6]. Most of bovine and ovine milk bioactive peptides compounds of casein protein function have been identified as well. In contrast to bovine caseins, content of CSN1S2 on caprine caseins protein are vary. The differences content of CSN1S2 perhaps have the unique physicochemical characteristics of caprine caseins protein for each strain [9, 13]. However the caprine milk casein protein, especially α-S2 casein, CSN1S2, protein is not clearly characterized yet the properties and function of their bioactive peptides. Recently our study, we had characterized the Indonesian local Ethawah breed milk and dairy product have high amount of caprine CSN1S2 (MW: 36kDa), whether this molecular weight is not detected on bovine casein milk [14, 15]. This study focus on sequences and structures exploration and characterization of bioactive peptides from fresh milk of Indonesian local Ethawah goat breed *Capra hircus* and observe the modulation ability between the each caprine CSN1S2 peptide and Calmodulin by docking analysis. We predict that some of bioactive peptides have ability to bind the Calmodulin binding domain which is ~~be~~ able to modulate the biological activities on cellular mechanism.

MATERIALS AND METHODS

Peptide preparation

Protein Purification and Sequencing Analysis by MALDI-TOF Ethawah breed goat milk was taken from UPTD Goat Singosari at East Java. Goat milk put 1 ml on 50-ml tube and added 5 ml PBS-Tween-PMSF solution (5 x vol.). Milk protein was disrupted by high-frequency sound waves sonication with 20% amplitude sonication during 10 minute. After that solution was centrifuged at 6000 rpm at 15 minute. Supernatants added absolute alcohol-cooled (ratio volume is 1:1), incubated on 4 ºC for 12 hours, and centrifuged at 6000 rpm for 15 minute. Pellet of protein was dried-up on room temperature and added Tris-Cl buffer (pH 6.8 with ratio volume 1:1). The purified-protein of goat milk samples were separated by 15%-sodium dodecyl sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) without staining and cut the 36kDa band from gel as a protein sample[16, 17]. Protein samples were trypsin digested and peptides extracted according to standard techniques [18, 19]. Peptides were analyzed by electrospray ionization mass spectrometry using the Ultimate 3000 Nano HPLC system [Dionex] coupled to a 4000 Q TRAP mass spectrometer [Applied Biosystems].

In silico Analysis

a. Sequences Database

Bioactive peptide sequences of α-s2 Casein (CSN1S2S) of Ethawah goat milk identified by MALDI-TOF analysis, as above. The amino acid sequence of Calmodulin with number of ID CAA36839 was obtained from database The National center of Biotechnology Information, National Library of Medicine, National Institute of Health [http://www.ncbi.nlm.nih.gov/]. Calmodulin protein receptor, 3D model from PDB ID: 1CLN was obtained from RCSB protein data bank.

b. *Docking of Ligand–Protein and Visualization*

Docking of Calmodulin and bioactive peptide CSN1S2 milk protein was analyzed by using HEX software [20]. We used rigid docking the Hex 8.0 software (http://hex.loria.fr) to compute possible interaction Calmodulin with bioactive peptides of Ethawah breed goat milk on its interaction site. Output of the docking was refined using Discovery Studio Client 3.5 software. All interaction analysis are using Discovery studio 3.5. Illustration 3D structure of 3D fragment bioactive peptides on CSN1S2 protein identified by virtual screening of LigandScout[21] and Chimera software[22]. Molecular graphics[23] and analyses were performed with the UCSF Chimera package. Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311).

Ethical consideration

The study was approved by ethical review committee of Brawijaya University Research Ethics Committee as National Research Ethics Committee of Republic Indonesia Member, No. Certificate is 90-KEP-UB.

RESULTS

A. Structure of CSN1S2 Protein Indonesian Local Ethawah Breed Goat Milk (Capra hircus)

The amino acid sequence of caprine CSN1S2 protein Ethawah breed goat milk from MALDI-TOF result was examined by in silico analysis. This caprine CSN1S2 protein sequence has similarity 98% with the caprine CSN1S2 protein of *Capra hircus* sequence with Gene ID: 100861229 from NCBI. The amino acid sequence of Caprine CSN1S2 protein shows on Figure 1A and describes the sequences of each peptide of CSN1S2 protein on Figure 1B. The peptides sequences marked on red letters (Figure 1A) and the position of each peptide is appeared on orange head arrow of 3D-green structure as eight bioactive peptides sequence (Figure 1C).

A. Alpha-S2 casein (CSN1S2) Protein Sequence

```
  1 MKFFIFTCLL AVALAKHKME HVSSSEEPIN IFQEIYKQEK NMAIHPRKEK
 51 LCTTSCEEVV RNANEEEYSI RSSSEESAEV APEEIKITVD DKHYQKALNE
101 INQFYQKFPQ YLQYPYQGPI VLNPWDQVKR NAGPFTPTVN REQLSTSEEN
151 SKKTIDMEST EVFTKKTKLT EEEKNRLNFL KKISQYYQKF AWPQYLKTVD
201 QHQKAMKPWT QPKTNAIPYV RYL
```

B. Amino Acids Sequence CSN1S2 Peptide Fragment

CSN1S2 Peptide Fragment	Amino Acid Sequences of CSN1S2 Peptide
41-NMAIHPR-47	41-Asn,Met,Ala,Ile,His,Pro,Arg-47
87-ITVDDKHYQK-96	87-Ile,Thr,Val,Asp,Asp,Lys,His,Tyr,Gln,Lys-96
97-ALNEINQFYQK-107	97-Ala,Leu,Asn,Glu,Ile,Asn,Gln,Phe,Tyr,Gln,Lys-107
131-NAGPFTPTVNR-141	131-Asn,Ala,Gly,Pro,Phe,Thr,Pro,Thr,Val,Asn,Arg-141
182-KISQYYQK-189	182-Lys,Ile,Ser,Gln,Tyr,Tyr,Gln,Lys-189
205-AMKPWTQPK-213	205-Ala,Met,Lys,Pro,Trp,Thr,GlnPro,Lys-213
214-TNAIPYVR-221	214-Thr,Asn,Ala,Ile,Pro,Tyr,Val,Arg-221
214-TNAIPYVRYL-223	214-Thr,Asn,Ala,Ile,Pro,Tyr,Val,Arg,Tyr,Leu-223

C. Three-dimension Structure of CSN1S2 Protein

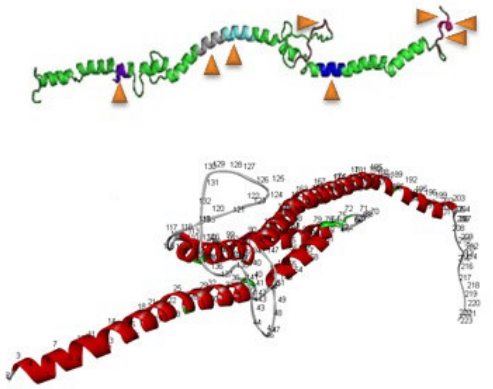

Figure1

The sequence of Goat Ethawah Breed Milk CSN1S2 protein was identified by MALDI-TOF and two types of three-dimensional structures of CSN1S2 protein. A. Goat Ethawah Breed Milk Alpha-S2 Casein Protein Sequence. B. Amino acids sequence peptide of Goat Ethawah Breed Milk Alpha-S2 Casein Protein and name of peptide. C. Two types of three-dimension of Goat Ethawah Breed Milk Alpha-S2 Casein Protein. Note: Amino acids sequence of eight of bioactive peptide fragments show on red letters (1A) and orange head arrows (1C).

The structure of eight peptide fragments of caprine CSN1S2 protein goat milk (Figure 2) has varied molecular weight on each peptide fragment. The molecular weight of each peptide fragment are CN f41-47 (MW: 577.992 Dalton), CN f87-96 (MW: 885.289 Dalton), CN f97-107 (MW: 1082.59Dalton), CN f131-141(MW: 810.266 Dalton), CN f182-189 (MW: 809.234 Dalton), CN f205-213(MW: 824.382 Dalton), CN f214-221 (MW: 699.122Dalton) and CN f214-223(MW: 873.409 Dalton). The structure of eight peptide fragments and three dimension structure of CSN1S2 protein Ethawah breed goat milk contents coil, helix and strand structures with high confidence prediction.

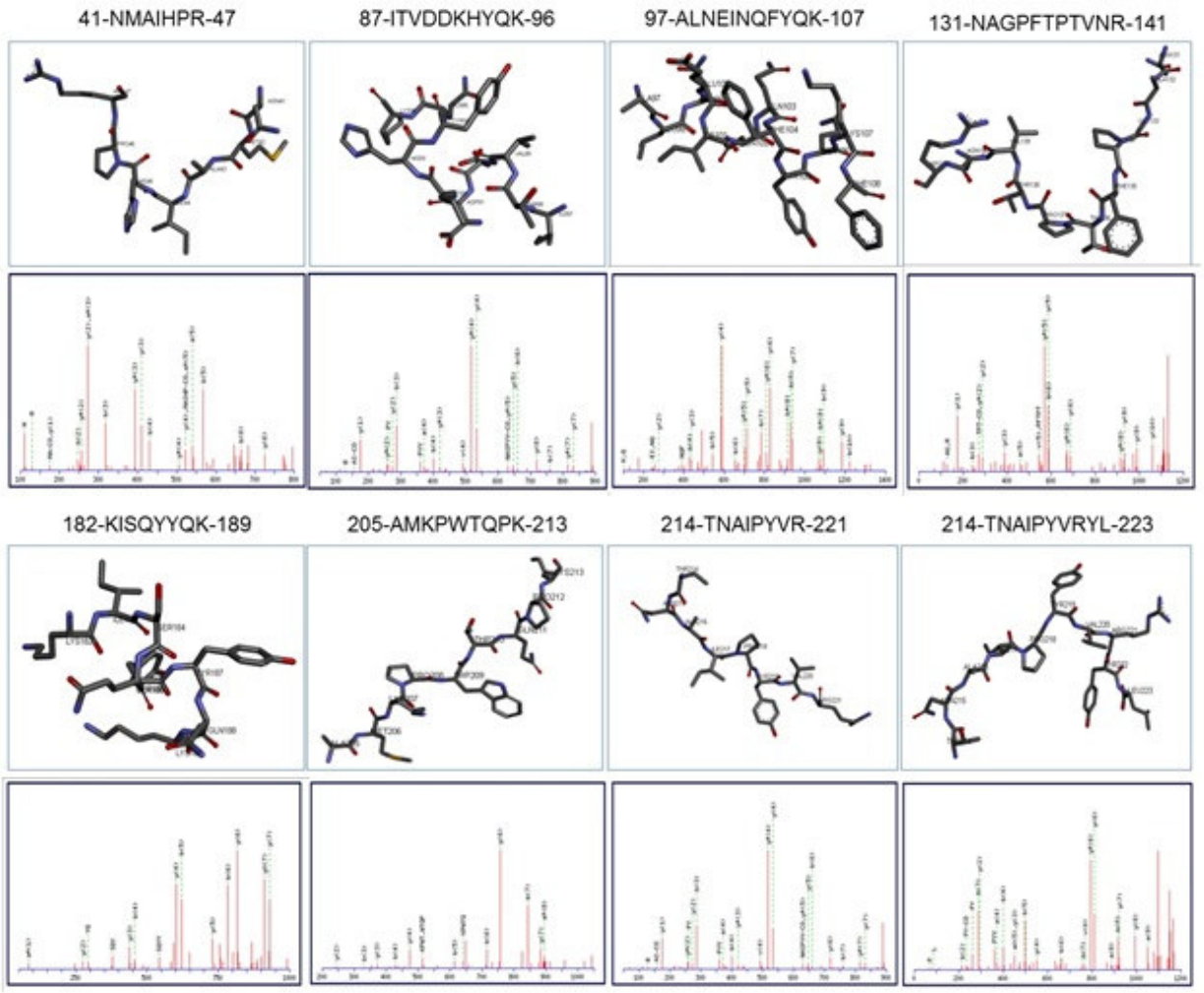

Figure2

Three-dimension of eight of bioactive peptides on CSN1S2 protein of Ethawah breeds goat milk. This result was analyzed by Chimera software and profile of monoisotopic mass of neutral Peptideof MALDI-TOF analysis.

B. Docking Analysis among bioactive peptides on CSN1S2 protein of Ethawah breed goat milk with Calmodulin

We performed the docking between Calmodulin with each of bioactive peptides of goat milk CSN1S2 protein. Seven of eight peptide fragments of caprine CSN1S2 protein Ethawah goat milk were able to interact with Calmodulin as properly with vary chemical and physical properties, except caprine CSN1S2 protein fragment 97-107 could not binding to Calmodulin, Figure 3. The energy binding of Calmodulin with each peptide are with CN f41-47 (E_{total}: -350.64 kJ/mol), CN f87-96 (E_{total}: -427.52 kJ/mol), CN f131-141(E_{total}: -407.95 kJ/mol), CN f182-189 (E_{total}: -420.14 kJ/mol), CN f205-213(E_{total}:-400.73 kJ/mol), CN f214-221 (E_{total}: -376.01 kJ/mol) and CN f214-223 (E_{total}: -413.17 kJ/mol). These results show that energy binding ability between Calmodulin and each bioactive peptide caprine CSN1S2 fragments is varying.

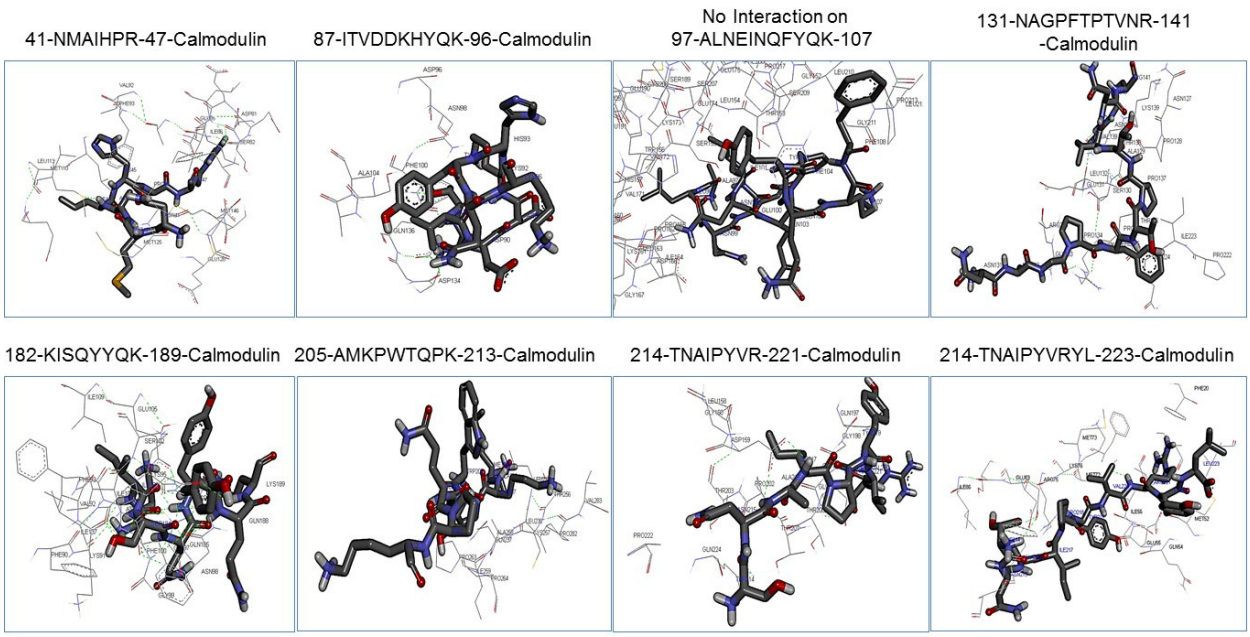

Figure 3

Docking Visualization of Interaction between Calmodulin Bioactive Peptides of CSN1S2 Protein of Ethawah breeds goat milk by Discovery Studio 3.5 software. There are seven of Bioactive Peptides of CSN1S2 Protein of Ethawah breed goat milk interacted with Calmodulin properly; meanwhile the 97-ALNEINQFYQK-107 peptide was negatively combining with Calmodulin.

According to the docking analysis on Figure 3, the general category of docking interaction among amino acid residues of seven bioactive peptides caprine CSN1S2 protein and amino acid residues of Calmodulin are containing hydrogen bond with conventional H-bond, carbon-H-bond or salt-bridge types, unfavorable with unfavorable bump or unfavorable bump: salt, hydrophobic with alkyl or Pi-alkyl types, and electrostatic with attractive charge (Table S1 to Table S7). The non-general category interactions are show specific bond such as hydrophobic p-sigma type at Leu113 of Calmodulin into His45 of CN f41-47 peptide, and X-sulfur type at Met110 of Calmodulin into Ile44of CN f41-47 peptide (Table S1) and Met125-Asn215 of CN f214-221 peptide (Table S6), electrostatic Pi-anion type at Asp96 of Calmodulin into Tyr186 of CN f182-189 peptide (Table 4S) and electrostatic Pi-cation type at Arg221 of CN f214-221 peptide into Phe20 of Calmodulin (Table 7S).

Table S 1
Analysis of Interaction Ligand 41-NMAIHPR-47 with Calmodulin receptor

Name	From	FromChemistry	To	To Chemistry	Distance	Category	Type
ARG47-GLU85	ARG47	H-Donor; Positive	GLU85	H-Acceptor; Negative	1.82755	Hydrogen Bond, Electrostatic charge	Salt bridge, Attractive charge
ASN41-GLU128	ASN41	Positive	GLU128	Negative	4.34903	Electrostatic	Attractive charge
ASN41-MET125	ASN41	H-donor	MET125	H-Acceptor	3.01308	Hydrogen bond	Conventional H-Bond
ILE44-MET110	ILE44	H-donor	MET110	H-Acceptor	2.36348	Hydrogen bond	Conventional H-Bond
ARG47-ASP81	ARG47	H-donor	ASP81	H-Acceptor	2.19726	Hydrogen bond	Conventional H-Bond
ARG47-GLU85	ARG47	H-donor	GLU85	H-Acceptor	3.60561	Hydrogen bond	Carbon Hydrogen Bond
MET110-**ILE44**	MET110	Sulfur	ILE44	O, N, S	3.26185	Other	Sulfur-X
LEU113-**HIS45**	LEU113	C-H	HIS45	Pi-Orbital	3.2707	Hydrophobic	Pi-Sigma
MET110-**ILE44**	MET110	Alkyl	ILE44	Alkyl	5.02132	Hydrophobic	Alkyl
LEU113-**ILE44**	LEU113	Alkyl	ILE44	Alkyl	4.49681	Hydrophobic	Alkyl
MET125-**MET42**	MET125	Alkyl	MET42	Alkyl	3.92876	Hydrophobic	Alkyl
ALA43-MET110	ALA43	Alkyl	MET110	Alkyl	5.2298	Hydrophobic	Alkyl
ALA43-MET146	ALA43	Alkyl	MET146	Alkyl	5.31997	Hydrophobic	Alkyl
ARG47-ILE86	ARG47	Alkyl	ILE86	Alkyl	4.48343	Hydrophobic	Alkyl
PHE93-**ALA43**	PHE93	Pi-Orbital	ALA43	Alkyl	3.28638	Hydrophobic	Pi-Alkyl

Table S 2
Analysis of Interaction Ligand 87-ITVDDKHYQK-96 with Calmodulin

Name	From	From Chemistry	To	To Chemistry	Distance	Category	Type
ILE87-ASP132	ILE87	Positive	ASP132	Negative	5.46572	Electrostatic	Attractive Charge
ILE87-ASP134	ILE87	Positive	ASP134	Negative	2.53613	Electrostatic	Attractive Charge
LYS92-ASP96	LYS92	Positive	ASP96	Negative	5.175	Electrostatic	Attractive Charge
ILE87-ASP134	ILE87	H-Donor	ASP134	H-Acceptor	1.86556	Hydrogen Bond	Conventional H-bond
ASP91-ILE87	ASP91	H-Donor	ILE87	H-Acceptor	2.799	Hydrogen Bond	Conventional H-bond
LYS92-THR88	LYS92	H-Donor	THR88	H-Acceptor	1.77129	Hydrogen Bond	Conventional H-Bond
GLN95-VAL89	GLN95	H-Donor	VAL89	H-Acceptor	2.488808	Hydrogen Bond	Conventional H-Bond
GLN95-LYS92	GLN95	H-Donor	LYS92	H-Acceptor	2.91125	Hydrogen Bond	Conventional H-Bond
LYS96-HIS93	LYS96	H-Donor	HIS93	H-Acceptor	2.85303	Hydrogen Bond	Conventional H-Bond
TYR94-VAL89	TYR94	Pi-Orbitals	VAL89	Alkyl	4.62988	Hydrophobic	Pi-Alkyl

Table S 3
Analysis of Interaction Ligand 131-NAGPFTPTVNR-141 with Calmodulin

Name	From	From Chemistry	To	To Chemistry	Distance	Category	Type
SER130-PRO137	SER130	H-Donor	PRO-137	H-Acceptor	2.38391	Hydrogen Bond	Conventional H-Bond
ARG226-**GLY133**	ARG226	H-Donor	GLY133	H-Acceptor	2.88212	Hydrogen Bond	Conventional H-Bond
ARG228-**GLY133**	ARG228	H-Donor	GLY133	H-Acceptor	3.07755	Hydrogen Bond	Conventional H-Bond
ASN140-ASN140	ASN140	H-Donor	ASN140	H-Acceptor	2.70683	Hydrogen Bond	Conventional H-Bond
ASN141-**VAL139**	ARG141	H-Donor	VAL139	H-Acceptor	2.1017	Hydrogen Bond	Conventional H-Bond
ASN141-**ASN138**	ARG141	H-Donor	ASN138	H-Acceptor	2.24069	Hydrogen Bond	Conventional H-Bond
ASN141-**GLU131**	ARG141	H-Donor	GLU131	H-Acceptor	2.24755	Hydrogen Bond	Conventional H-Bond
ASN141-**VAL139**	ARG141	H-Donor	VAL139	H-Acceptor	2.08549	Hydrogen Bond	Conventional H-Bond
PRO128-**THR138**	PRO128	H-Donor	THR138	H-Acceptor	3.79413	HydrogenBond	Carbon Hydrogen Bond
ALA129-**THR138**	ALA129	H-Donor	THR138	H-Acceptor	2.26885	HydrogenBond	Carbon Hydrogen Bond
SER130-**PHE135**	SER130	H-Donor	PHE135	H-Acceptor	2.8432	Hydrogen Bond	Carbon Hydrogen Bond
ARG141-LYS139	ARG141	H-Donor	LYS139	H-Acceptor	2.57583	Hydrogen Bond	Carbon Hydrogen Bond
GLN224-**PHE135**	GLN224	H-Donor	PHE135	Pi-Orbital	1.93418	Hydrogen Bond	Pi-Donor Hydrogen Bond
PRO128-**PRO137**	PRO128	Alkyl	PRO137	Alkyl	4.15343	Hydrophobic	Alkyl
PRO137-ILE223	PRO137	Alkyl	ILE223	Alkyl	4.32702	Hydrophobic	Alkyl
ARG141-LYS139	ARG141	Alkyl	LYS139	Alkyl	4.06459	Hydrophobic	Alkyl
PHE135-PRO137	PHE135	Pi-Orbital	PRO137	Alkyl	5.30323	Hydrophobic	Pi-Alkyl

Table S 4
Analysis of Interaction Ligand 182-KISQYYQK-189 with Calmodulin

Name	From	From Chemistry	To	To Chemistry	Distance	Category	Type
LYS182-ASP94	LYS182	Positive	ASP94	Negative	4.22913	Electrostatic	Attractive Charge
ASP-96**-ILE183**	ASP96	H-Donor	ILE183	H-Acceptor	2.36207	Hydrogen Bond	Conventional H-Bond
ILE183-LYS91	ILE183	H-Donor	LYS91	H-Acceptor	2.28196	Hydrogen Bond	Conventional H-Bond
GLN185-ASP94	GLN185	H-Donor	ASP94	H-Acceptor	2.62642	Hydrogen Bond	Conventional H-Bond
GLN185-**LYS182**	GLN185	H-Donor	LYS182	H-Acceptor	2.65789	Hydrogen Bond	Conventional H-Bond
TYR186-ASP94	TYR186	H-Donor	ASP94	H-Acceptor	2.75933	Hydrogen Bond	Conventional H-Bond
TYR186-ASP94	TYR186	H-Donor	ASP94	H-Acceptor	2.6522	Hydrogen Bond	Conventional H-Bond
TYR186-**LYS182**	TYR186	H-Donor	LYS182	H-Acceptor	1.81327	Hydrogen Bond	Conventional H-Bond
TYR187-ASN98	TYR187	H-Donor	ASN98	H-Acceptor	2.813104	Hydrogen Bond	Conventional H-Bond
TYR187-**ILE183**	TYR187	H-Donor	ILE183	H-Acceptor	1.98737	Hydrogen Bond	Conventional H-Bond
TYR187-**SER184**	TYR187	H-Donor	SER184	H-Acceptor	2.01181	Hydrogen Bond	Conventional H-Bond
GLN188-ASP96	GLN188	H-Donor	ASP96	H-Acceptor	2.03709	Hydrogen Bond	Conventional H-Bond
GLN188-SER184	GLN188	H-Donor	SER184	H-Acceptor	2.5917	Hydrogen Bond	Conventional H-Bond
GLN188-GLN185	GLN188	H-Donor	GLN185	H-Acceptor	2.39027	Hydrogen Bond	Conventional H-Bond
LYS189-GLN185	LYS189	H-Donor	GLN185	H-Acceptor	2.60604	Hydrogen Bond	Conventional H-Bond
LYS189-TYR186	LYS189	H-Donor	TYR186	H-Acceptor	2.1908	Hydrogen Bond	Conventional H-Bond
ASP96-**TYR186**	ASP96	Negative	TYR186	Pi-Orbital	3.52044	Electrostatic	P –Anion
LYS95-**LYS182**	LYS95	Alkyl	LYS182	Alkyl	4.91529	Hydrophobic	Alkyl
LYS95-**ILE183**	LYS95	Alkyl	ILE183	Alkyl	4.337775	Hydrophobic	Alkyl
ILE101**-ILE183**	ILE101	Alkyl	ILE183	Alkyl	5.29329	Hydrophobic	Alkyl
TYR186-LYS95	TYR186	Pi-Orbital	LYS95	Alkyl	4.14656	Hydrophobic	Pi-Alkyl
TYR186-LYS189	TYR186	Pi-Orbital	LYS189	Alkyl	4.95687	Hydrophobic	Pi-Alkyl

Table S 5
Analysis of Interaction Ligand 205-AMKPWTQPK-213 with Calmodulin

Name	From	From Chemistry	To	To Chemistry	Distance	Category	Type
TRP209-THR210	TRP209	H-Donor	THR-210	H-Acceptor	3.03154	Hydrogen Bond	Conventional H-Bond
GLN211-THR210	GLN211	H-Donor	THR210	H-Acceptor	2.55489	Hydrogen Bond	Conventional H-Bond
ALA258**-ALA205**	ALA258	Alkyl	ALA205	Alkyl	3.78098	Hydrophobic	Alkyl
ALA258**-PRO208**	ALA258	Alkyl	PRO208	Alkyl	5.4706	Hydrophobic	Alkyl
PRO263-**PRO208**	PRO263	Alkyl	PRO208	Alkyl	4.34457	Hydrophobic	Alkyl
PRO282-**LYS207**	PRO282	Alkyl	LYS207	Alkyl	4.92028	Hydrophobic	Alkyl
ALA205-LEU239	ALA205	Alkyl	LEU239	Alkyl	3.70167	Hydrophobic	Alkyl

Table S 6
Analysis of Interaction Ligand 214-TNAIPYVRYL-221 with Calmodulin

Name	From	FromChemistry	To	To Chemistry	Distance	Category	Type
ARG221- ASP81	ARG221	H-Donor, Positive	ASP81	H-Acceptor, Negative	2.84227	Hydrogen Bond, Electrostatic	Salt bridge, Attractive charge
ARG221- ASP81	ARG221	H-Donor, Positive	ASP81	H-Acceptor, Negative	2.41173	Hydrogen Bond, Electrostatic	Salt bridge, Attractive charge
ARG221- ASP81	ARG221	Positive	ASP81	Negative	5.2566	Electrostatic	Attractive charge
ASP221- GLU85	ARG221	Positive	GLU85	Negative	5.49382	Electrostatic	Attractive charge
THR214- MET125	THR214	H-Donor	MET125	H-Acceptor	2.90515	Hydrogen Bond	Conventional H-Bond
ASN215-THR214	ASN215	H-Donor	THR214	H-Acceptor	2.81587	Hydrogen Bond	Conventional H-Bond
VAL220-PRO218	VAL220	H-Donor	PRO218	H-Acceptor	3.09902	Hydrogen Bond	Conventional H-Bond
VAL221- GLU85	ARG221	H-Donor	GLU85	H-Acceptor	2.78536	Hydrogen Bond	Conventional H-Bond
MET125**-ASN215**	MET125	Sulfur	ASN215	O,N,S	2.70674	Other	Sulfur-X
ALA89-**PRO218**	ALA89	Alkyl	PRO218	Alkyl	4.46878	Hydrophobic	Alkyl
ALA216- MET146	ALA216	Alkyl	MET146	Alkyl	4.84868	Hydrophobic	Alkyl
PRO218- MET146	PRO218	Alkyl	MET146	Alkyl	3.36586	Hydrophobic	Alkyl
VAL220- ILE86	VAL220	Alkyl	ILE86	Alkyl	3.65492	Hydrophobic	Alkyl
PHE93**-ALA216**	PHE93	Pi-Orbitals	ALA216	Alkyl	4.48671	Hydrophobic	Pi-Alkyl
PHE142-**PRO218**	PHE142	Pi-Orbitals	PRO218	Alkyl	4.84591	Hydrophobic	Pi-Alkyl

Table S 7
Analysis of Interaction Ligand 214-TNAIPYVRYL-223 with Calmodulin

Name	From	FromChemistry	To	ToChemistry	Distance	Category	Type
THR214-GLU83	THR214	H-Donor, Positive	GLU83	H-Acceptor	2.2333	Hydrogen Bond, Electrostatic	Salt bridge, Attractive charge
ARG75-TYR219	ARG75	H-Donor	TYR219	H-Acceptor	2.81865	Hydrogen Bond	Conventional H-Bond
ASN215-THR214	ASN215	H-Donor	THR214	H-Acceptor	2.81575	Hydrogen Bond	Conventional H-Bond
TYR219-GLN54	TYR219	H-Donor	GLN54	H-Acceptor	2.82567	Hydrogen Bond	Conventional H-Bond
VAL220-PRO218	TYR220	H-Donor	PRO218	H-Acceptor	3.0987	Hydrogen Bond	Conventional H-Bond
TYR222-GLU55	TYR222	H-Donor	GLU55	H-Acceptor	2.11723	Hydrogen Bond	Conventional H-Bond
ARG221-GLU55	ARG221	H-Donor	GLU55	H-Acceptor	2.85048	Hydrogen Bond	Carbon Hydrogen Bond
ARG221-PHE20	ARG221	Positive	PHE20	Pi-Orbitals	3.18969	Electrostatic	Pi-Cation
ARG75-PRO218	ARG75	Alkyl	PRO218	Alkyl	4.95579	Hydrophobic	Alkyl
LYS76-PRO218	LYS76	Alkyl	PRO218	Alkyl	5.23921	Hydrophobic	Alkyl
VAL220-MET73	VAL220	Alkyl	MET73	Alkyl	5.16688	Hydrophobic	Alkyl
ARG221-MET52	ARG221	Alkyl	MET52	Alkyl	5.04628	Hydrophobic	Alkyl
ARG221-ILE56	ARG221	Alkyl	ILE-56	Alkyl	5.0158	Hydrophobic	Alkyl
TYR219-ILE56	TYR219	Alkyl	ILE-56	Alkyl	5.16152	Hydrophobic	Pi-Alkyl

DISCUSSION

Some protein of raw food material has physiological ability based on the composition of amino acid sequences. One of the mostimportant sources of bioactive peptides is milk protein from animal and plant.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. We also identified that the goat Ethawah breed milk CSN1S2 protein sequence is aligned with ovine alpha-S2 casein protein (data not show), thought that caprine and ovine CSN1S2 protein has carried out similar function. *In silico* study of CSN1S2 revealed that the chemical properties between caprine and ovine protein identically, however there is dissimilarity with bovine CSN1S2 proteins [16, 25, 26].The biologically active peptides have been shown to display a wide range of physiological functions including antimicrobial, anti-inflammation, antihypertensive, anti-oxidative, anti-allergy and as immunomodulatory on specific tissues [3, 2, 27, 6]. Our result 3D-structure of caprine CSN1S2 protein has similar model with the ovine and bovine alpha-S2 casein containing helixes,coils and strand structures.The three dimensional molecular model for alpha-s2-casein was produced by threading the backbone sequence of the protein onto a homologous protein is chloride intracellular channel protein-4 [28]. Thorn et al. was demonstrated by transmission electron microscopy, dye binding assays, and X-ray fiber diffraction of 3D-structure milk CSN1S2protein. The spherical particles typical of CSN1S2 at neutral pH and 37ºC incubation was rapidly converted to twisted, ribbon-like fibrils similar to 12 nm in diameter, which occasionally formed loop structures. CSN1S2 at pH 6.5-6.7 and higher incubation temperatures is particularly susceptible to fibril formation under physiological conditions [16, 17]. Peptides of caprine milk alpha-S2-casein protein may use in healthy value that have specific biological function as regulatory protein Calmodulin to prevent its binding to the enzyme. That peptide of αlpha-s2-casein is sensitive to Ca^{2+} concentrations and will be easy to precipitate it in the ion Ca^{2+} (2mM) presence [3, 11].Our study had investigated virtually that the peptides of caprine CSN1S2 protein can bind to Calmodulin as properly. 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. Recently study reported that the C-terminal of calcium-binding protein Calmodulin of fragment TR2 of E. coli Calmodulin of is cover residues 78-148. Meanwhile, the helix E N-terminus of is closer to the helix H C-terminus in TR2C than in the intact protein and that the loop connecting the EF-hands shows different conformations in the two structures [24, 17]. We found in CN f87-96-Calmodulin bound in Ca^{2+}-binding domain at one residue, ILE87-ASP132(Table S2).It seem the peptide fragment 87-96 of milk caprine CSN1S2 protein tight binding on calcium-binding domain of EF2-hand of N-terminal Calmodulin that probably the function of this peptide fragment as inhibitor protein to control cellular signaling pathway. The CN f 214-221 and CN f214-223 has differed only in residues Tyr222 and Leu223 showed the interaction with Calmodulin is completely difference. Though perhaps both peptides are at variant on biological function in molecular mechanism inside cells. Recent study showed antioxidant peptides have ability to deliver electron and bind metal cation which is to suppress the free-radical toxic effect and to prevent lipid peroxide formation. Moreover, bioactive peptides also have function as anti-inflammatory and immunomodulatory to exhibit by enhancing the protective cytokines cell production or may be able to inhibit cancer development. The other study showed that bio-peptides bind to Calmodulin could reduce Calmodulin-dependent enzymes activation which is implicated with chronic diseases [2, 5, 8]. We predict these interactions indicate peptides of caprine CSN1S2 protein have ability to modulate molecular and/or cellular pathway regulation. This study identified that the energy binding of goat milk CSN1S2 protein peptides and Calmodulin has energy total between -350.64 kJ/mol and -427.52 kJ/mol. This energy binding may due to the synergistic activity of naturally occurring among Calmodulin with specific

peptides on different binding sites. Nevalainen group reported that the Calmodulin ability to accommodate different target sequences may to provide the flexibility of central helix and local rearrangement of side chains in the binding surface[1]. The Calmodulin–peptide interactions in the complex are very strong and are dominated by hydrophobic effects[24, 5].

CONCLUSION

In summary, our finding shows that caprine CSN1S2 protein has containing eight bioactive peptides compounds. This result note that the seventh of peptide fragments caprine CSN1S2 protein are able to bind with Calmodulin has a good example of ability for multifunctional peptide. We assume that bioactive peptides of caprine CSN1S2 protein may enhance sites for interactions with negatively and/or positively charged molecules.

ACKNOWLEDGMENT

This research is supported in part by grant of the RUPT-BOPTN UB-Decentralization of Directorate of General Higher Education, Ministry of Education and Culture of Republic Indonesia. We thank to Biosains Laboratory Brawijaya University and Proteomics International Pty Ltd, Australia for providing the laboratory equipment for research analysis.

Conflict of Interests

The authors declare that they have no conflict of interests related to this paper.

REFERENCES

1. Nevalainen LT, Aoyama T, Ikura M, Crivici A, Yan, H, Chua NH and Nairn AC. Characterization of Novel Calmodulin-Binding Peptides With Distinct Inhibitory Effects on Calmodulin-Dependent Enzymes. Biochem. J., 321: 107-15, (1997)
2. Andre I, Kesvatera T, Jonsson B, Akerfeldt KS and Linse S. The Role of Electrostatic Interactions in Calmodulin-Peptide Complex Formation. Biophysical Journal, 87: 1929–1938, (2004)
3. Farrell HM, Malin EL, Brown EM and Mora-Gutierrez A. The Chemistry of Alphas2-Casein and the Generation of a Homologous Molecular Model to Explain Its Properties. Review. J. Dairy Science, 92: 1338-53, (2009)
4. Van Petegem F and Minor DL. The structural biology of voltage-gated calcium channel function and regulation. Biochemical Society transactions, 34 (pt5): 887-893, (2006)
5. Haeseleer F, Imanishi Y, Sokal I, Filipek S and Palczewski K. Breakthroughs and Views: Calcium-Binding Proteins: Intracellular Sensors from the Calmodulin Superfamily. Biochemical and Biophysical Research Communications, 290: 615–623, (2002)
6. Ryan JT, Ross RP, Bolton D, Fitzgerald GF and Stanton C. Bioactive Peptides from Muscle Sources: Meat and Fish. Review. Nutrients, 3: 765-791, (2011)
7. Xie J, Méndez JD, Valenzuela VM and Hernández MMA. Cellular signaling of the receptor for advanced glycation end products (RAGE). Review. Cellular Signaling, 25: 2185–2197, (2013)
8. Shepherd CM and Vogel HJ. A Molecular Dynamics Study of Ca21-Calmodulin: Evidence of Inter domain Coupling and Structural Collapse on the Nanosecond Timescale. Biophysical Journal, 87: 780–791, (2004)
9. Tidona, F, Criscione A, Guastella AM, Zuccaro A, Bordonaro S and Marletta D. Bioactive Peptide in Dairy Product. Ital. J. Anim. Sci., 8: 315-340, (2009)
10. Hajirostamloo B. Bioactive Component in Milk and Dairy Product. World Academy of Science, Eng. and Tech., 4:12-22, (2010)
11. Dziuba B and Dziuba M. Milk Proteins-Derived Bioactive Peptides in Dairy Products: Molecular, Biological and Methodological Aspects. Acta Sci. Pol., Technol. Aliment., 13(1): 5-25 (2014).
12. Fadaei V. Milk Proteins-derived antibacterial peptides as novel functional

food ingredients. Annals of Biological Research, 3 (5): 2520-2526, (2012)
13. Sharma S, Singh R and Rana S. Bioactive Peptides: A Review. Int. J. Bioautomation, 15(4): 223-250 (2011)
14. Budiarti IK, Padaga MC and Fatchiyah F. Nutritional Composition and Protein Profile of Goat Yogurt PE with Double Culture between Streptococcus thermophilus and Lactobacilus species. Cukurova Med. J., 38 (4): 681-686, (2013)
15. Khoiriyah LK and Fatchiyah F. Characterization of Biochemistry & Protein Profile of Goat Milk Fermented by BAL. JELS 2 (1): 1-6, (2013)
16. Lopez-Exposito I, Gomez-Ruiz JA, Amigo L and Recio I. Identification of Antibacterial Peptides from Ovine Alpha S2-Casein.Int. Dairy J., 16: 1072-1080 (2006)
17. Thorn DC, Ecroyd H, Sunde M, Poon S and Carver JA. Amyloid Fibril Formation by Bovine Milk Alpha S2-Casein Occurs Under Physiological Conditions Yet is Prevented by Its Natural Counterpart, Alpha S1-Casein. Biochemistry, 47, 3926-36, (2008)
18. Webster J and Oxley D. Peptide Mass Fingerprinting: Protein Identification Using MALDI-TOF Mass Spectrometry. Methods Mol. Biol., 310: 227-240. (2005)
19. Bringans S, Hane J.K, Casey T, Tan KC, Lipscombe R, Solomon PS and Oliver PR. Deep Proteogenomics: High Throughput Gene Validation by Multidimensional Liquid Chromatography and Mass Spectrometry of Proteins from The Fungal Wheat Pathogen Stagonospora nodorum. BMC Bioinformatics, 10. (2009)
20. Macindoe G, Mavridis L, Venkatraman V, Devignes MD and Ritchie DW. Hex server: an FFT-Based Protein Docking Server Powered by Graphics Processors. Nucleic Acids Res., 38: W445-449, (2010)
21. Wolber G and Langer T. Ligandscout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use As Virtual Screening Filters.J. Chem. Inf. Model, 45: 160-169 (2005)
22. Pettersen EF, Goddard TD, Huang CC, Couch GS, Breenblatt DM, Meng EC and Ferrin TE. UCSF Chimera--A Visualization System for Exploratory Research and Analysis. J. Comput. Chem., 25: 1605-1612, (2004)
23. DeLano, W. The Pymol: Molecular Graphics System. DeLano Scientific, USA, accessed on January 2013 (2002). http://www.pymol.org
24. Yang C, Jas GS and Kuczera K. Structure, Dynamics and Interaction With Kinase Targets: Computer Simulations of Calmodulin.Biochim. Biophys. Acta, 1697: 289-300, (2004)
25. Park YW, Ju´arez M, Ramosc M and Haenlein GFW. Physico-chemical characteristics of goat and sheep milk. Small Ruminant Research, 68: 88–113, (2007)
26. Masoodi TA and Shafi G. Analysis of Casein Alpha S1 & S2 Proteins from Different Mammalian Species. Bioinformation, 4: 430-5, (2010)
27. McCann KB, Shiell BJ, Michalski WP, Lee A, Wan J, Roginsk H and Coventry J. Isolation and Characterisation of Antibacterial Peptides Derived from The F (164-207) Region of Bovine as2-Casein. Int. Dairy J., 15: 133-143, (2005)
28. Kitts DD and Weiler K. Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. Curr. Pharm., 9 (16): 1309-23, (2003).