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Original Article

STRUCTURAL PREDICTION OF HUMAN ZIP 2 AND ZIP4 BASED ON HOMOLOGY MODELLING AND MOLECULAR SIMULATION

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ABSTRACT

Objective: This study aimed to analyze the structural proteins of zinc transporters as the target for drug actions and their molecular interactions.

Methods: The present study is about the homology modelling and analysis of the zinc transporter function using the *in silico* molecular modelling method. Homology modelling predicts the 3D structure of a protein based on the sequence alignment with one or more template proteins of known structure. This study using *in silico* molecular modelling method, explains the 3D structure of human ZIP 2 and ZIP4 with Ramachandran Plot analysis, physical and chemical characteristics, transmembrane prediction with structural biology, and binding site prediction through molecular docking simulation.

Results: Based on the physicochemical properties of the 3D structure of the ZIP2 and ZIP4 proteins, each comprises 309 amino acids and 582 amino acids with pI values of 5.85 and 5.24. The amino acid composition analysis showed that both proteins contain many Leucine amino acids. The Ramachandran diagram concludes that both proteins are stable in the stereochemical conformation forming a secondary structure. The binding amino acids on ZIP2 include Glu281, His216, Ser284, and Arg46. The binding amino acids in ZIP4 include Gln148, Gln154, Thr155, His197, Ala138, and Lys157.

Conclusion: Establishment of the structure and function of human ZIP2 and ZIP4 as zinc transporters in cell membranes and prediction of ZIP2 and ZIP4 binding sites through molecular dcoking.

Keywords: 3D structure, Zinc transporter, Molecular modelling, Molecular docking, Structural biology

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INTRODUCTION

Zinc bioavailability is influenced by the concentration and amount of zinc that can successfully transmit through the small intestine membrane [1]. The gastrointestinal tract's zinc absorption factors include solubility, binding with other compounds, pH, and enzymatic reactions. The factors affect zinc absorption through the small intestine membrane e [2, 3]. Studies related to zinc bioavailability have not been studied, especially the effect of pancreatic secretion on zinc absorption and homeostasis.

The Zinc and Iron Regulated Transport Protein (ZIP) family of proteins is central to the transition homeostasis of metals. Their function is to increase the cytosolic concentrations of zinc and iron. There are 14 mammals ZIP family members, namely ZIP 1–ZIP 14 [4]. The ZIP family functions to facilitate cellular uptake or efflux from intracellular compartments. Studies show that ZIP2 is one of the key zinc transporters associated with zinc requirements during pregnancy. At the same time, ZIP4 and ZIP2 are transporter proteins that play a role in zinc absorption in the small intestine (duodenum) [5]. A mechanism that supports the description of ZIP transporters is the ZN2+/[HCO3-ion] [6, 7]. The transport mechanism of the ZIP family is not precisely known, so it is necessary to determine the model and function of ZIP2 and ZIP4 proteins.

Zinc transporters were also reported to be synthetic intracellular transducers that regulate cellular functions [8, 9], and various cytosol proteins can associate with zinc [10]. Conditions of physiological responses, such as developmental, immune, and neurological functions and the onset of several human diseases result from changes in zinc mineral concentration and intracellular distribution. The many reports related to zinc control in cells and its regulation make it important to know the biological function of the role of zinc transporters more deeply [11].

Determination of the protein model of zinc transporters ZIP2 and ZIP4 in the body's cellular function must be understood through the protein structural modelling. However, the protein database (PDB) used to find the protein structure is sometimes only partially complete. Therefore, a homology modelling method is needed to determine the comparative protein structure model from the query protein. The molecular docking method used in this study is determining the predicted binding sites of ZIP2 and ZIP4 while interacting with zinc carnosine as a third-generation zinc supplement [12, 13]. This research aimed to obtain the 3D structure of the ZIP2 and ZIP4 proteins that can be applied to further studies, especially molecular simulations.

MATERIALS AND METHODS

This research is *in silico* with a homology and molecular modelling approach. The databases used are National Center for Biotechnology Information (NCBI) (National Center for Biotechnology Information (nih.gov)), (Swiss Model (SWISS-MODEL (expasy.org)), and UniProt (UniProt). Validation and protein function using several sites such as Verify3D (Verify3D–UCLA-DOE Institute), ERRAT (ERRAT: An Empirical Atom-Based Method for Validating Protein Structures (1993-2015) | (ucla.edu), Procheck (PROCHECK. bio. tools), TMHMM 2.0 (TMHMM-2.0-Services-DTU Health Tech).

Nucleotide sequence determination for amino acid sequences of human ZIP2 and ZIP4

Based on the NCBI database, the DNA sequences of the ZIP2 and ZIP4 proteins were searched. The search results were obtained successively from the "gene" search with the official full name SLC39A2 solute carrier family 39 member 2 (Homo sapiens) and SLC39A4 solute carrier family 39 member 4 (Homo sapiens). Then, an Open Reading Frames (ORFs) analysis was performed on all DNA sequences from SLC39A2 and SLC39A4. Amino acid sequences translated from peptide sequence results from homology sequence tracing on NCBI. The results of ORFs with the highest similarity DNA sequences were used for BLASTp analysis from NCBI to determine the amino acid sequences of target proteins with high similarity [13].

Amino acid sequence tracing and homology modelling of human ZIP 2 and ZIP 4

After tracing the homology of the protein amino acid sequences of ZIP2 and ZIP4 with BLASTp on NCBI, the ZIP2 sequence with Reference Sequence: NP_055394.2 (Zinc transporter ZIP2 isoform a (Homo sapiens) and ZIP4 sequence with Reference Sequence: NP_001361768.1 (Zinc transporter ZIP4 isoform 4 precursors (Homo sapiens). Furthermore, the amino acid sequences of the two target proteins are stored in fasta format for protein modeling-homology modelling using Swiss Model on target proteins ZIP2 and ZIP4 on each reference sequence obtained. The model's homology results are from the highest critical scoring structure. The protein model is saved in a PDB file, which will be used for further structural analysis. The visualization used is Biovia Discovery Studio [14].

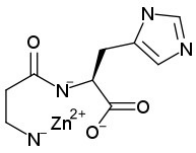


Fig. 1: The structure of Zn-carnosince

Analysis of geometrical conformation, stereochemical quality, and physicochemical properties of proteins

This study used the PROCHECK tool, ProtParam, and TMHMM database applications to determine the conformational geometry and stereochemical quality. The analysis carried out on the application is to visualize the Ramachandran diagram and physicochemical properties. In addition, this application is also used to determine the amino acid composition of the target protein, as well as other physicochemical parameters, protein expression location, topology, and transmembrane segment of the target protein.

Analysis of binding site with molecular docking

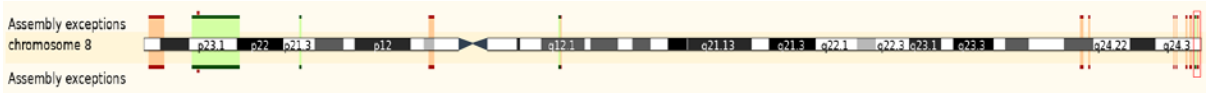
This study was done through a blind docking technique with the grid box size for ZIP2 being x, y, and z sizes of 52, 54, and 80, respectively. The grid box sizes for ZIP4 are x,y, and z sizes of 56, 72, and 100, respectively. Molecular docking used is flexible docking to determine the most stable conformation and orientation with minimal energy between Zn-carnosine and ZIP2 or ZIP4 proteins. Zn-carnosine and target protein structures were saved in. pdb and. pdbqt formats [15]. Molecular docking was performed using AutoDock Vina software with AutoDock Tools 1.5.6 (www.vina.scripps.edu). The peptide structure was visualized and prepared using MarvinSketch 20.12 (www.chemaxon.com), while the target protein structure was visualized and prepared using Biovia Discovery Studio 19.1.0 (www.3ds.com) [17]. The 2D structure of Zn-carnosine is as follows fig. 1.

RESULTS AND DISCUSSION

Searching nucleotide sequences of ZIP2 and ZIP4 using the NCBI database found that the ZIP2 target protein was in the SLC39A2 gene, and for ZIP4, the target protein was in the SLC39A4 gene. Based on the analysis of the Ensembl database, the SLC39A2 gene sequence is located on chromosome 14 in the q11.2 region (20.999.225–21.001.887). Fig. 2 shows the presence of the SLC39A2 gene. The SLC39A4 gene is located on chromosome 8 region 24.3 (144,409,742–144,416,844).

The SLC39A2 sequence had 2511 bp of nucleotides, and the SLC39A4 sequence had 4431 bp of nucleotides. They were analyzed by Open Reading Frame Finder (ORF Finder) to obtain protein translation segments using BLAST. The ORF will find several amino acid sequences translated into all nucleotide sequences SLC39A2 and SLC39A4. The longest translated amino acid values from each nucleotide sequence SLC39A2 and SLC39A4 will be used as templates for the ZIP2 and ZIP4 target proteins previously carried out by BLASTp. Table 1 shows the ORF results of the nucleotide sequences SLC39A2 and SLC39A4 with the+strand.

(a)



(b)

Fig. 2: The location of the SLC39A2 gene is on chromosome 14 (a), while SLC39A4 is on chromosome 8 (b)

Each ORF results in a nucleotide sequence; the longest amino acid is determined and followed by BLASTp analysis to determine the ZIP2 and ZIP4 target protein templates. For example, ORF finder analysis on the nucleotide SLC39A2 found that the ORF 8 region at nucleotides 1655–2287 has the longest amino acid sequence, 210 amino acids translated from 633 nucleotides. Furthermore, ORF 8 was carried out by BLASTp on NCBI to determine the highest similarity of the longest amino acid sequence in table 2. In addition, an ORF finder analysis was performed on the SLC39A4 nucleotide and translated from 678 nucleotides. Therefore, to find out the protein designated by ORF 14, it is necessary to carry out homology similarity from the NCBI database using the BLASTp tool, resulting in phylogenetic data in table 3.

Table 1: Five ORF finder results of the nucleotide sequences SLC39A2 and SLC39A4 with the longest amino acids

	Label	Start	Stop	Length	
				Nucleotide	Amino acid
SLC39A2	ORF 8	1655	2287	633	210
	ORF 11	1503	1901	399	132
	ORF 2	1603	1893	291	96
	ORF 7	1460	1654	195	64
	ORF 4	155	304	150	49
SLC39A4	ORF 14	2628	3305	678	225
	ORF 2	637	1236	600	199
	ORF 7	536	970	435	144
	ORF 12	1233	1622	390	129
	ORF 11	3476	3823	348	115

Table 2: Five genes with the highest query cover value in ORF 8-SLC39A2

Description	Max score	Total score	Query cover (%)	E value	Identn (%)	Accession
Zinc transporter ZIP2 isoform a [Homo sapiens]	409	409	100	4e-145	100	NP_055394.2
Zinc transporter ZIP2 [Mus musculus]	311	311	100	2e-106	80.95	NP_001034765.2
Zinc transporter ZIP1 [Danio rerio]	160	160	96	3e-47	41.63	NP_997748.2
Zrt (ZRT), Irt-(IRT-) like Protein Transporter [Caenorhabditis elegans]	102	102	97	1e-24	33.80	NP_001122755.1
Zinc/iron-regulated transporter-related protein 42C.1 [Drosophila melanogaster]	92.8	92.8	83	4e-21	36.75	NP_525107.1

Table 3: Five genes with the highest query cover value at ORF 14-SLC39A4

Description	Max score	Total score	Query cover (%)	E value	Identn (%)	Accession
Zinc transporter ZIP4 isoform 4 precusor [Homo sapiens]	64.7	64.7	28	7e-11	76.81	NP_001361768.1
Zinc transporter ZIP4 precusor [Mus musculus]	61.6	61.6	20	8e-10	68.75	NP_0832340.1
Zinc transporter ZIP4 isoform 2 precusor [Homo sapiens]	64.7	64.7	28	8e-11	76.81	NP_570901.3
Zinc transporter ZIP4 isoform 1 [Homo sapiens]	64.7	64.7	28	9e-11	76.81	NP_060237.3
Zinc transporter ZIP4 isoform X1 [Homo sapiens]	63.5	63.5	20	2e-10	95.74	XP_024302957.1

The NCBI access number was obtained based on ORF and BLASTp analysis, which was used as an amino acid sequence template for molecular modelling. Based on these two analyses, the NCBI access number found that the target protein for ZIP2 was NP_055394.2 while ZIP4 was NP_001361768.1. Also, the results of modelling ZIP2 and ZIP4 on the SwissModel, are shown in table 4 and 5, respectively.

The modelling of 6pgi.1. A as the ZIP2 model and 4x82.1 as the ZIP4 model, respectively, is depicted in fig. 3 and fig. 4. Meanwhile, determining the physicochemical properties of the 3D model of the ZIP2 and ZIP4 proteins is in table 6 and table 7. The conformations of the 3D models of the ZIP2 and ZIP4 proteins are depicted in the Ramachandran diagrams in fig. 4a and 4b, respectively. ZIP2 and ZIP4 proteins are proteins located on the cell membrane. Therefore, table 8 describes the amino acid residues transmembrane in ZIP2 and ZIP4. The transmembrane residues ZIP2 and ZIP4 are also depicted in each amino acid residue's hydropathy and amphipathy profiles, forming the transmembrane properties in fig. 5 and 6.

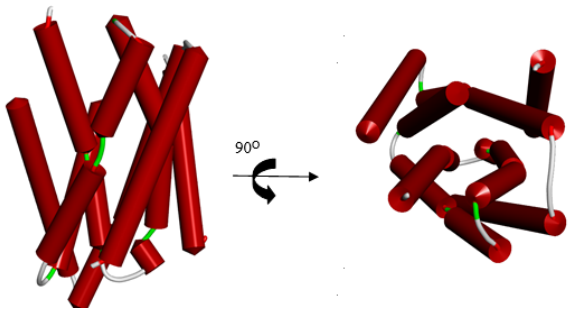


Fig. 3: ZIP2 3D protein modelling on the 6phi.1. A. model template

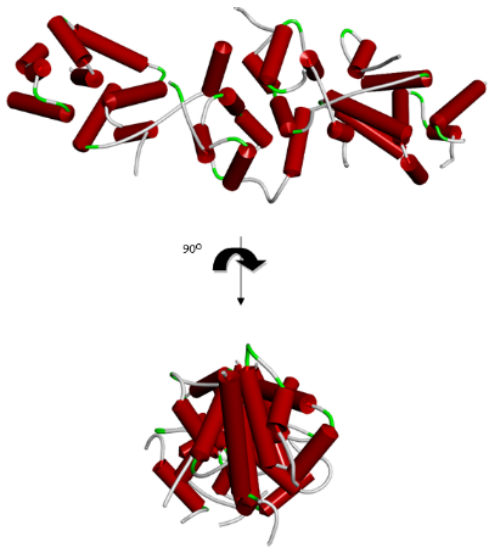


Fig. 4: 3D ZIP 4 protein modelling on 4 x 8 2.1. A and 4x82.1. B. model templates

Table 4: The top five protein modelling with the swiss model on ZIP2

Template	Sequence identity	Sequence similarity	Range	Coverage	QMEAN
6pgi.1. A	21.116	0.298	3-308	0.812	0.50
5tsa.1. A	20.717	0.295	3-308	0.812	0.50
6pgi.1. A	17.073	0.272	221-307	0.265	0.08
5tsa.1. A	17.500	0.276	223-307	0.259	0.08
5tsa.1. A	15.385	0.283	43-122	0.168	0.04

Table 5: The top five protein modelling with the swiss model on ZIP4

Template	Sequence identity	Sequence similarity	Range	Coverage	QMEAN
4x82.1. A	69.39	0.51	34-229	0.35	0.22
4x82.1. B	69.39	0.51	34-229	0.35	0.21
6pgi.1. A	18.90	0.29	229-547	0.46	0.29
5tsa.1. A	18.97	0.30	230-547	0.46	0.29
4x82.1. A	68.82	0.51	63-232	0.31	0.16

Table 6: Physicochemical properties of the 3D ZIP2 model on the 6pgi.1. A. model template

Physicochemical identities	3D ZIP2 model	Amino acid composition	No.	Composition (%)
Number of amino acids	309	Ala (A)	27	12.3
Molecular weight	32742.38	Arg (R)	19	3.2
Theoretical pI	5.85	Asn (N)	16	0.6
No. of negatively charged (Asp+Glu)	23	Asp (D)	36	1.6
No. of positively charged (Arg+Lys)	16	Cys (C)	10	2.6
Atomic composition		Gln (Q)	24	3.2
Carbon (C)	1501	Glu (E)	35	5.8
Hydorgen (H)	2340	Gly (G)	21	11.3
Nitrogen (N)	384	His (H)	14	3.6
Oxygen (O)	404	Ile (I)	35	3.9
Sulfur (S)	15	Leu (L)	52	16.5
Formula	$C_{1501}H_{2340}N_{384}O_{404}S_{16}$	Lys (K)	39	1.9
Total number of atoms	4645	Met (M)	17	2.6
Aliphatic index	112.46	Phe (F)	27	6.8
Grand average of hydropathicity	0.683	Pro (P)	25	3.6
Instability index	41.17	Ser (S)	39	7.4
		Thr (T)	26	3.2
		Trp (W)	1	1.6
		Tyr (Y)	18	1.0
		Val (V)	24	7.1

Human zinc transporters ZIP2 and ZIP4 are two of the 14 types of the ZIP family, both of which are types of transport proteins responsible for the transport of mineral zinc from extracellular to intracellular. Therefore, ZIP2 and ZIP4 protein modelling are needed to determine the 3D structure of the protein, its function, and the protein's physicochemical properties. Furthermore, protein modelling can be used in determining the interaction of receptors to ligands in determining drug candidates and their role in molecular dynamics simulations [16].

The 3D structures deposited in the Protein Data Bank (PDB) database are not always complete. Therefore, it is necessary to approach protein modelling with homology modelling to determine the comparative model of the protein we need. The modelling in this research uses the Swiss model to determine the protein homology of human ZIP2 and ZIP4. Therefore, several databases are needed from the nucleotide sequences that will be translated into amino acid sequences to determine the tertiary structures of the protein to obtain the 3D structure of human ZIP2 and ZIP4.

Table 7: Physicochemical properties of the 3D ZIP4 model on the 4x82.1. A and 4x82.1. B. model templates

Physicochemical identities	3D ZIP4 Model	Amino acid composition	No.	Composition (%)
Number of amino acids	582	Ala (A)	72	12.4
Molecular weight	61642.95	Arg (R)	21	3.6
Theoretical pI	5.24	Asn (N)	9	1.5
No. of negatively charged (Asp+Glu)	52	Asp (D)	22	3.8
No. of positively charged (Arg+Lys)	27	Cys (C)	14	2.4
Atomic composition		Gln (Q)	21	3.6
Carbon (C)	2776	Glu (E)	30	5.2
Hydrogen (H)	4356	Gly (G)	48	8.2
Nitrogen (N)	736	His (H)	23	4.0
Oxygen (O)	804	Ile (I)	8	1.4
Sulfur (S)	23	Leu (L)	107	18.4
Formula	$C_{2776}H_{4356}N_{736}O80_4S23$	Lys (K)	6	1.0
Total number of atoms	8695	Met (M)	9	1.5
Aliphatic index	109.36	Phe (F)	21	3.6
Grand average of hydropathicity	0.373	Pro (P)	35	6.0
Instability index	46.8	Ser (S)	53	9.1
		Thr (T)	24	4.1
		Trp (W)	9	1.5
		Tyr (Y)	10	1.7
		Val (V)	40	6.9

Starting from the search for the nucleotide level database, the nucleotide sequences known that the chromosomes in the SLC39A2 and SLC39A4 genes are genes encoding the protein ZIP2 and ZIP4. Furthermore, based on the ORF analysis of the nucleotide sequences, it is known that the ZIP2 protein has homology in the "zinc transporter ZIP2 isoform a [Homo sapiens]" protein database in NCBI with a 3D protein structure template homology in PDB "6pgi.1. A". On the other hand, an ORF analysis of nucleotide sequences shows that the ZIP4 protein is like "zinc transporter ZIP4 isoform 4 precursors [Homo sapiens]" in the NCBI database, which has a 3D structure called "4x82.1. A" that is like the structure of the homology template.

Fig. 3 and fig. 4 are the 3D protein structures of ZIP2 and ZIP4 with physicochemical properties in table 6 and table 7, and it is known that in ZIP2 protein, there are 309 amino acids, the theoretical pI is 5.85 with leucine as the largest constituent amino acid, which is 16.5%, followed by Alanine 12.5% and the third highest was Glysin at 11.3%. In the 3D structure of the ZIP4 protein, it is known that there are 582 amino acids, with a theoretical pI value of 5.24. Like protein ZIP2, Protein ZIP4 contains much leucine with a composition of 18.4%, then Alanine at 12.4%, and Serine at 9.1%. The Ramachandran diagram (fig. 5) shows a 3D model of the ZIP2 and ZIP4 proteins, which have more than 85% of the amino acid residues distributed in the quadrant 1 conformation, where the stereochemistry formed in the secondary structure of the protein is antiparallel-sheet and parallel-sheet. Meanwhile, in fig. 5a and 5b, other amino acid residues are in quadrants 2 and 3 as amino acids, forming the secondary structures of the left-handed and right-handed helix. Less than 1% of ZIP2 and ZIP4 proteins are in quadrant 4, where the smaller the percentage of amino acid residues in quadrant 4, the more stable the protein model is formed. The stability of protein models is important in preparing proteins for molecular simulations in bioinformatics applications such as molecular docking and molecular dynamics.

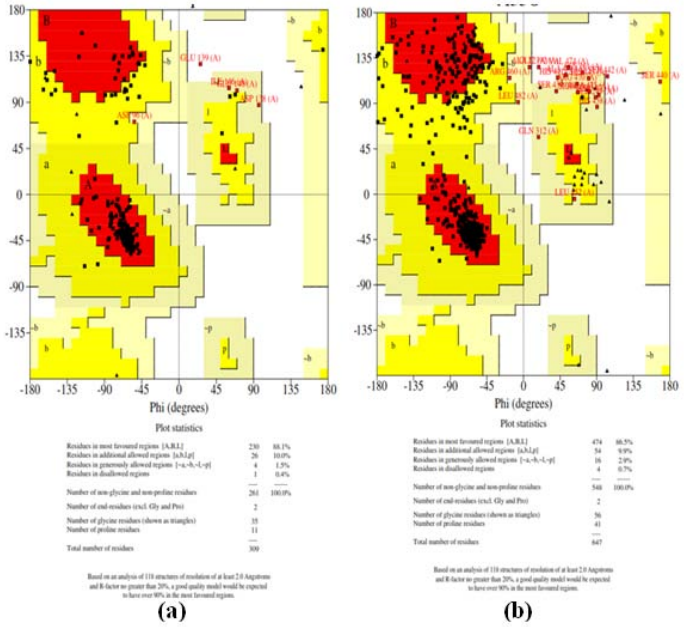


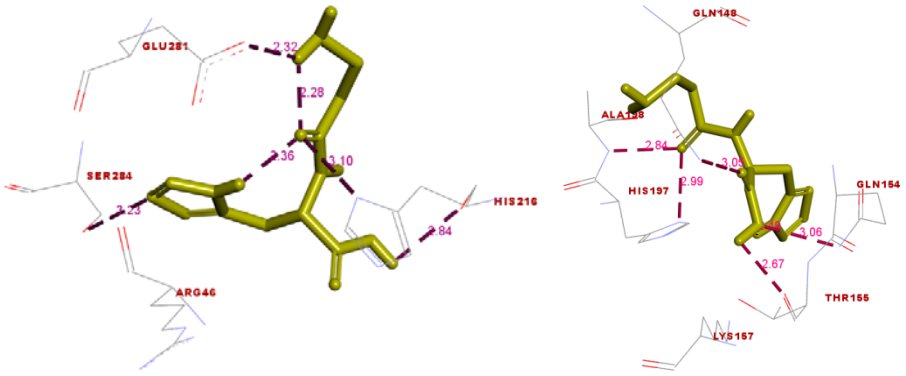
Fig. 5: Ramachandran diagram on 3D models ZIP2 (a) and ZIP4 (b)

Table 8: Topological locations of transmembrane sequences on ZIP2 and ZIP4

ZIP2 protein	Transmembrane location	ZIP4 protein	Transmembrane location
TMS 1	9-29	TMS 1	327-249
TMS 2	47-67	TMS 2	355-375
TMS 3	107-127	TMS 3	559-582
TMS 4	165-185	TMS 4	588-611
TMS 5	193-213	TMS 5	619-641
TMS 6	225-246		
TMS 7	256-276		
TMS 8	288-308		

The ZIP2 and ZIP4 proteins are also distributed in the cell membrane as zinc mineral transporter receptors, so analyzing the secondary structure of amino acids at the transmembrane position is necessary. Based on the hydropathy and amphipathy analysis of amino acid residues in the ZIP2 and ZIP4 proteins, there are 8 secondary structures and 5 secondary structures at the transmembrane position, respectively, described in table 8. Analysis of transmembrane proteins is important in determining zinc minerals' binding and transport mechanisms in the extracellular transport process. to intracellular and vice versa. More than half of the ZIP transporters have His-rich sequences found in the cytoplasmic part of the intracellular loop between TMDs III and IV [17]. The presence of this loop has its role in the ZIP transporter family. In ZIP 1, this loop plays a role in the di-leucine sorting signal as a mediator of endocytosis.

On the other hand, this loop in ZIP 4 plays a role in mediating ubiquitination and degradation [18]. In mammals, in the LIV-1 subfamily, there is a metalloprotease motif (HEXPHEXGD) on TMD V and a CPALLY motif located just before TMD 1, except for ZIP 7 and 13 [19]. The location of ZIP transporters is mostly on the plasma membrane. However, ZIP and ZnT transporters also change with zinc stimulation and availability in the subcellular. The diverse localization and transport of zinc are important in homeostasis and biological processes [20-22].



(a) **(b)**

Fig. 6: Interaction Zn-carnosine with ZIP2 (a) and ZIP4 (b) through molecular docking simulations

Zn-carnosine ($C_9H_{13}N_4O_3Zn$) is a zinc-binding peptide, a third-generation zinc supplement. Carnosine, a peptide binding with zinc, enhances cell bioabsorption and bioavailability [23-25]. To determine the binding activity of ZIP2 and ZIP4 with zinc supplements, Zn-carnosine is used as a ligand to determine the binding site on ZIP2 and ZIP4. Fig. 6a and 6b are the conformation and orientation of Zn-carnosine binding with ZIP2 and ZIP4. The results obtained several amino acids on ZIP2 formed hydrogen bonds with Zn-carnosine, such as Glu281 formed hydrogen bonds along 2.32 A, His216 along 2.84A, and Ser284 along 3.23. Another amino acid in ZIP2 that forms van der Waals Zn-carnosine bonds is Arg46. Meanwhile, fig. 6b shows the hydrogen bond that is the interaction of amino acids in ZIP4 with Zn-Carnosine, while the hydrogen bond occurs at Ala138 along 2.84 A, His197 along 2.99, Gln154 along 3.06, Thr155 along 2.67, and Gln148 along 3.05. Lys157 on ZIP4 forms van der Waals bond with Zn-Carnosine.

Molecular docking simulation shows the binding side of ZIP2 and ZIP4 on amino acids that interact with hydrogen and van der Waals bonds. The binding amino acids on ZIP2 include Glu281, His216, Ser284, and Arg46. The binding amino acids in ZIP4 include Gln148, Gln154, Thr155, His197, Ala138, and Lys157. Based on the analysis of transmembrane proteins (TMS) in table 8, in ZIP2, the amino acids Arg46, His216, Glus281, and Ser 284 are not in the transmembrane region, which is in the loop part of the ZIP2 protein both in the interior and exterior of the protein. The amino acids in ZIP4 are not in the TMS region but in the exterior and interior loops of the protein. The factor of the region causes a potential interaction on Zn-carnosine.

CONCLUSION

The 3D structures of the ZIP2 and ZIP4 proteins have been obtained by tracing the nucleotide sequences encoding the genes SLC39A2 and SLC39A4. These two genes are translated into amino acid-forming proteins in ZIP2 and ZIP4. Based on the physicochemical properties of the 3D structure of the ZIP2 and ZIP4 proteins, each comprises 309 amino acids and 582 amino acids with pI values of 5.85 and 5.24. The amino acid composition analysis showed that both proteins contain many Leucine amino acids. The Ramachandran diagram concludes that both proteins are stable in the stereochemical conformation forming a secondary structure. Therefore, they suit molecular simulation applications, such as molecular docking and molecular dynamics. Its function is to move zinc minerals across cell membranes. ZIP2 protein comprises 8 secondary structures of a transmembrane protein, while ZIP4 protein comprises 5 secondary structures. Molecular docking simulation shows the binding side of ZIP2 and ZIP4 on amino acids that interact with hydrogen and van der Waals bonds with Zn-carnosine. The binding amino acids on ZIP2 include Glu281, His216, Ser284, and Arg46. The binding amino acids in ZIP4 include Gln148, Gln154, Thr155, His197, Ala138, and Lys157.

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Nil

AUTHORS CONTRIBUTIONS

GS worked all experiments and technical design, ML and FF the main conceptual ideas and proofreader, MYP and FF designed the experiments and proofreader; NG edited the manuscript and proofreader. GS proposed the experiment in discussions with ML, MYP, and FF. GS wrote the manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest in this article.

REFERENCES

1. Whitehead MW, Thompson RPH, Powell JJ. Regulation of metal absorption in the gastrointestinal tract. Gut. 1996;39(5):625-8. doi: 10.1136/gut.39.5.625, PMID 9026473.
2. Lonnerdal B, Schneeman BO, Keen CL, Hurley LS. Molecular distribution of zinc in biliary and pancreatic secretions. Biol Trace Elem Res. 1980;2(3):149-58. doi: 10.1007/BF02785351, PMID 24271265.
3. Finley JW, Johnson PE, Reeves PG, Vanderpool RA, Briske Anderson M. Effect of bile/pancreatic secretions on absorption of radioactive or stable zinc. *In vivo* and *in vitro* studies. Biol Trace Elem Res. 1994;42(2):81-96. doi: 10.1007/BF02785381, PMID 7981007.
4. Kambe T, Taylor KM, Fu D. Zinc transporters and their functional integration in mammalian cells. J Biol Chem. 2021;296:(100320). doi: 10.1016/j.jbc.2021.100320, PMID 33485965.
5. Jeong J, Eide DJ. The SLC39 family of zinc transporters. Mol Aspects Med. 2013;34(2-3):612-9. doi: 10.1016/j.mam.2012.05.011, PMID 23506894.
6. Gaither LA, Eide DJ. The human ZIP1 transporter mediates zinc uptake in human K562 erythroleukemia cells. J Biol Chem. 2001;276(25):22258-64. doi: 10.1074/jbc.M101772200, PMID 11301334.
7. Gaither LA, Eide DJ. Functional expression of the human hZIP2 zinc transporter. J Biol Chem. 2000;275(8):5560-4. doi: 10.1074/jbc.275.8.5560, PMID 10681536.
8. Hojyo S, Fukada T. Roles of zinc signaling in the immune system. J Immunol Res. 2016;2016:6762343. doi: 10.1155/2016/6762343, PMID 27872866.
9. Maret W. Zinc coordination environments in proteins as redox sensors and signal transducers. Antioxid Redox Signal. 2006 Sep-Oct;8(9-10):1419-41. doi: 10.1089/ars.2006.8.1419, PMID 16987000.
10. Andreini C, Banci L, Bertini I, Rosato A. Counting the zinc-proteins encoded in the human genome. J Proteome Res. 2006;5(1):196-201. doi: 10.1021/pr050361j, PMID 16396512.
11. Kimura T, Kambe T. The functions of metallothionein and ZIP and ZnT transporters: an overview and perspective. Int J Mol Sci. 2016;17(3):336. doi: 10.3390/ijms17030336, PMID 26959009.

12. Guo H, Yu Y, Hong Z, Zhang Y, Xie Q, Chen H. Effect of collagen peptide-chelated zinc nanoparticles from pufferfish skin on zinc bioavailability in rats. J Med Food. 2021;24(9):987-96. doi: 10.1089/jmf.2021.K.0038, PMID 34448624.
13. Kumar B, Praveen D, Chowdary PR, Aanandhi MV. A prospective single-blinded study on safety and efficacy of cholecalciferol supplementation in pulmonary tuberculosis. Drug Invent Today. 2019;12(11).
14. Kibenge F, McKibbon A, Kibenge M, Wang Y. Bioinformatics analysis identifies a small ORF in the genome of fish nidoviruses of genus oncotshavirus predicted to encode a novel integral protein. Microbiology Research. 2021;12(4):753-64. doi: 10.3390/microbiolres12040055.
15. Sanchez R, Sali A. Large-scale protein structure modeling of the *Saccharomyces cerevisiae* genome. Proc Natl Acad Sci USA. 1998;95(23):13597-602. doi: 10.1073/pnas.95.23.13597, PMID 9811845.
16. Arwansyah A, Arif AR, Syahputra G, Sukarti S, Kurniawan I. Theoretical studies of thiazolyl-pyrazoline derivatives as promising drugs against malaria by QSAR modelling combined with molecular docking and molecular dynamics simulation. Mol Simul. 2021;47(12):988-1001. doi: 10.1080/08927022.2021.1935926.
17. Nurhasanah N, Fadilah F, Bahtiar A. Prediction of active compounds of Muntingia calabura as a potential treatment for chronic obstructive pulmonary diseases by network pharmacology integrated with molecular docking. Int J Appl Pharm. 2023;15(1):274-9.
18. Sala D, Giachetti A, Rosato A. Insights into the dynamics of the human zinc transporter ZnT8 by MD simulations. J Chem Inf Model. 2021;61(2):901-12. doi: 10.1021/acs.jcim.0c01139, PMID 33508935.
19. Haraguichi H. Springrer. Metallom Integr Biometal Sci; 2017.
20. Mao X, Kim BE, Wang F, Eide DJ, Petris MJ. A histidine-rich cluster mediates the ubiquitination and degradation of the human zinc transporter, hZIP4, and protects against zinc cytotoxicity. J Biol Chem. 2007;282(10):6992-7000. doi: 10.1074/jbc.M610552200, PMID 17202136.
21. Hogstrand C, Kille P, Nicholson RI, Taylor KM. Zinc transporters and cancer: a potential role for ZIP7 as a hub for tyrosine kinase activation. Trends Mol Med. 2009;15(3):101-11. doi: 10.1016/j.molmed.2009.01.004, PMID 19246244.
22. Kambe T, Weaver BP, Andrews GK. The genetics of essential metal homeostasis during development. Genesis. 2008;46(4):214-28. doi: 10.1002/dvg.20382, PMID 18395838.
23. Schmitt Ulms G, Ehsani S, Watts JC, Westaway D, Wille H. Evolutionary descent of prion genes from the ZIP family of metal Ion transporters. Plos One. 2009;4(9):e7208. doi: 10.1371/journal.pone.0007208, PMID 19784368.
24. Maywald M, Rink L. Zinc in human health and infectious diseases. Biomolecules. 2022;12(12):1-29. doi: 10.3390/biom12121748, PMID 36551176.
25. Udechukwu MC, Collins SA, Udenigwe CC. Prospects of enhancing dietary zinc bioavailability with food-derived zinc-chelating peptides. Food Funct. 2016 Oct;7(10):4137-44. doi: 10.1039/c6fo00706f, PMID 27713952.