Analysis of Sea Cucumber Metabolites as Phytate Inhibitor in Human ZiP Transporter: Molecular Docking Study

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Abstract. One of the causes of zinc deficiency is decreased zinc intake or impaired absorption of zinc which causes zinc bioavailability to decrease in the blood. Phytate is one of the compounds found in food that can interfere with zinc absorption in the human digestive system. The formation of a zinc complex with phytate affects zinc absorption through the human ZiP transporter (hZiP). Sea cucumber has also long been used as a dietary supplement in the East Asian region which helps maintain body health. However, there has not been much research on the potential of sea cucumber metabolites as phytate inhibitors that inhibit zinc absorption in the digestive system. The result obtained from molecular docking is that sea cucumber metabolite has the potential to inhibit the binding of phytates in ZiP2 and ZiP4 membrane proteins that act as zinc transporters from extracellular to intracellular. The metabolites in sea cucumber that have the potential as phytate inhibitors in the ZiP2 membrane protein are Holothurin, Arguside, Miliariside, Nobiliside, and Virescenoside. In the smoldering protein ZiP4, the six compounds also have the potential to inhibit phytate. Based on the molecular docking analysis, holothurin has potency as a phytate inhibitor, evidenced by binding energy and conformation in the binding site. Visualization analysis described that the holothurin precisely binds in phytate binding site in protein receptor. In addition, literature reported, that holothurin has potency as antiviral, antibacterial, and anticancer. The sea cucumber with active metabolites is one of the important marine commodities for maintaining human health and supplementation nutrition.

Keywords: sea cucumber · inhibitor · Phytate · ZiP transporter · molecular docking

I. Nurlaila et al. (Eds.): ICHR 2022, AHSR 56, pp. 65–74, 2023.
https://doi.org/10.2991/978-94-6463-112-8_7

1 Introduction

Human growth, cognitive development, and maintaining the immune system are some of the roles of the mineral zinc, one of the micronutrients needed by the body. The importance of zinc in the human health system; therefore, the bioavailability of zinc in the body is needed in the right amount, especially through the absorption of zinc and its transport to cells. On the other hand, zinc deficiency occurs due to inadequate intake or reduced zinc absorption from food sources. As a result, there is a disturbance in the immune system, which is the leading cause of susceptibility to infection and other diseases. Therefore, efforts have been made to maintain intake and increase zinc bioavailability in the body, such as through supplementation, fortification, and food modification [1, 2].

The distribution of zinc in the body is 60% zinc found in muscle, about 30% found in bone, while the concentration of zinc in human plasma is only 12–16 μmol. While in serum, zinc was mostly found to bond to albumin at about 60%, α-macroglobulin at 30%, and transferrin protein at 10%. Meanwhile, the recommended daily consumption of zinc is 2–3 mg for infants, 4–8 mg for children, and 7–10 mg for adolescents and adults, while the needs of pregnant and lactating women are 10 mg. Zinc intake comes from various food sources such as nuts, seeds, cereals, wheat, red meat, liver, and seafood. Another report stated that vegetables do not provide many sources of zinc, but phytate, a compound that can bind zinc, inhibits the absorption and distribution of zinc in the body.

Phytate (Myo-inositol hexakisphosphate) is an inhibitor of zinc absorption, a component matrix that can bind zinc from food intake. Zinc that bonds to phytate form insoluble complexes leading to a decrease in its bioavailability so that it is not available for intestinal absorption, causing zinc deficiency. The ability of phytate to bind divalent minerals such as Zn^{2+}, Fe^{2+}, Cu^{2+}, and Ca^{2+} can prevent the utilization of the minerals in the body. Peptides derived from sea cucumbers have been known to have various health benefits, one of the bioactivity is the ability to form a zinc-chelating complex that can help bind zinc in the body or as a phytate inhibitor [2]. However, research on metabolites derived from sea cucumbers as phytate inhibitors has not been widely carried out, so the aim of this study was to analyze the metabolites derived from sea cucumbers as phytate inhibitors in human ZiP transporter with molecular docking approach.

2 Materials and Methods

2.1 Database Construction and ADME Screening of Sea Cucumber Ingredients

All components of sea cucumbers were obtained from a database of natural products in Chinese herbal medicine: Traditional Chinese Medicine System Pharmacology (TCMSP) database (TCMSP - Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (tcmsp-e.com) [3]. In addition, TCMSP is also used to screen for oral bioavailability (OB) and drug-likeness (DL). One of the parameters widely used in pharmacokinetics is OB which can predict the process of absorption, distribution, metabolism, and excretion (ADME). The DL parameter determines the character of the "drug-like" compound and its effect on ADME. Screening of metabolites on OB

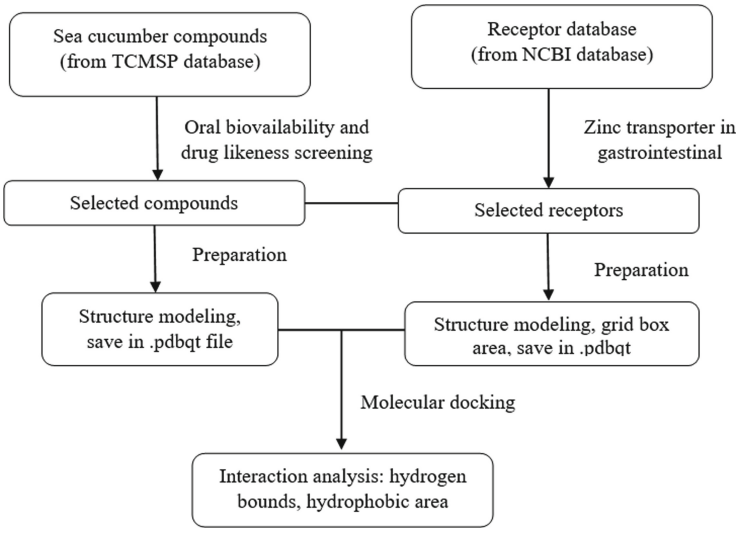


Fig. 1. The process of obtaining the interaction of compounds derived from sea cucumber with the receptor protein.

and DL parameters based on recommendations from the TCMSP database, OB $\geq$ 20 and DL $\geq$ 0.1. Chemical structure modelling, both 2D and 3D structures obtained from NCBI PubChem (www.pubchem.ncbi.nlm.nih.gov) and then the structure visualization using Discovery Studio (BIOVIA Discovery Studio) - BIOVIA - Dassault Systèmes® (3ds.com) (Fig. 1).

2.2 Receptor Protein Modeling

The receptor proteins used in this study were ZiP 4 and ZiP 2 obtained from NCBI, for ZiP 4 (www.ncbi.nlm.nih.gov/protein/np_570901.3) and ZiP 2 (www.ncbi.nlm.nih.gov/protein/Q9NP94.2). The two receptor proteins are proteins located on cell membranes that function as transport gaps in the exchange of zinc ions from extracellular to intracellular and to specific cell organelles. The fasta format on ZiP 4 and ZiP 2 obtained through NCBI was used as an amino acid sequence template in modelling the two receptor proteins using Phyre2 (PHYRE2 Protein Fold Recognition Server (ic.ac.uk)). The Ramachandran plot then validates the obtained model.

2.3 Molecular Docking Preparation

Prior to molecular docking, the receptor and ligand were prepared. Based on the analysis of ADME and Lipinski, five metabolites of the sea cucumber were obtained, namely holothurin, arguside, miliariside, nobiliside, and virescenoside, all of them will be used as ligands. The receptors are membrane proteins ZiP 4 and ZiP 2. The membrane proteins were prepared using AutoDock Tools software (AutoDock Vina (scripps.edu)) to add

polar hydrogen and remove water molecules. This research is a blind docking, where the 3D Grid Box used on the x, y, and z-axis for ZiP 2 is 56, 48, and 72, respectively. While the 3D Grid Box used on the x, y, and z-axis for ZiP 4 is 56, 72, and 100, respectively. The ligands were optimized for 3D structure and determined the axis of rotation flexibility using the same software. All ligands and receptors were saved in. pdbqt file format for further molecular docking.

2.4 Molecular Docking and Analysis

Molecular docking of ZiP 2 and ZiP 4 receptor protein was carried out separately, and each receptor protein was used to tether five test ligands (holothurin, arguside, miliariside, nobiliside, and virescenoside) and one comparison ligand, phytate. Molecular docking was carried out using AutoDock Vina. Molecular docking analysis such as determining hydrogen bond interactions, hydrogen bond distances, hydrophobic regions, and amino acids interacting with ligands was carried out using Discovery Studio in both 2D and 3D.

3 Results

3.1 Identification of Active Compounds in Sea Cucumber

The purpose of identifying the active compounds in sea cucumbers is to determine the pharmacokinetic characteristics of all compounds that are potential drug candidates. The total compounds contained in sea cucumbers are 586 compounds. Based on the screening of oral bioavailability and drug-like parameters using the Lipinski Rules, five compounds were obtained: holothurin, arguside, miliariside, nobiliside, and virescenoside. These compounds have been reported to have efficacy in the clinical field, such as holothurin was reported to have activity as an inhibitor of androgen receptors in prostate cancer [4], as an antischistosomal [5], and as an antifungal [6]. In contrast, Nobiliside was reported as a neuraminidase inhibitor from *Clostridium perfingens* bacteria [7]. The molecular structure of compounds in sea cucumber and phytate as control was presented in Table 1.

Oral Bioavailability (OB) describes the percentage of unchanged drug dose reaching the systemic circulation in the ADME process. A high OB value indicates changes in the systemic circulation in determining drug-like bioactive molecules as therapeutic agents. For example, the OB indicator was said to be good if the OB value is $\geq$20% whereas Virescenoside has an OB value of 58.71%. The value of Drug Likeness (DL) is a concept used in drug design to predict and optimize the pharmacokinetics and pharmaceutical properties of the drug, such as solubility and stability of chemical compounds. The criteria for the DL value with a good ability is $\geq$0.18. The compounds in sea cucumbers have these criteria, except for holothurin and arguside.

Based on Lipinski's rule, it was known that all the test compounds have the appropriate character in these rules with a molecular weight of around 480–488.78, with arguside as the compound with the heaviest weight. Thus, all test compounds can be continued as ligands in molecular docking to determine their interactions with several receptors. The results of the analysis of ZiP 2 and ZiP receptor protein modeling using Phyre2 are given

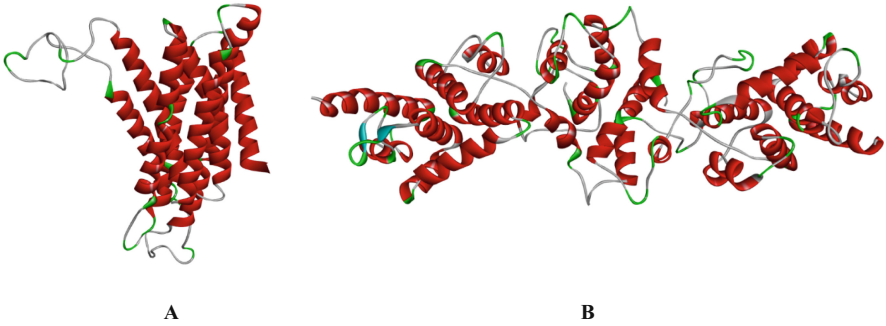


Fig. 2. The protein structure of ZiP2 (A) and ZiP4 (B)

in Fig. 2. In addition, homology modeling was performed to determine the structure of ZiP 2 and ZiP 4 proteins with the highest coverage of amino acid sequences from NCBI database.

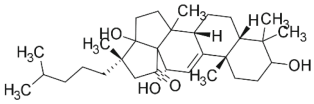
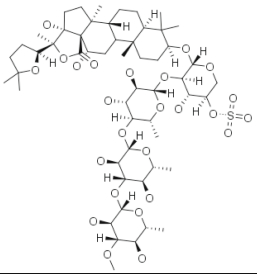
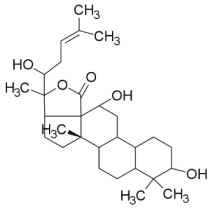
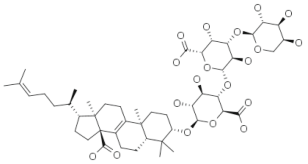
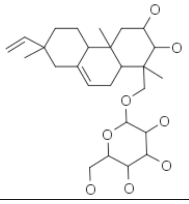
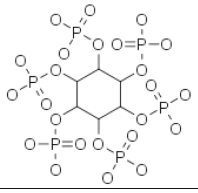
3.2 Interaction of Sea Cucumber Active Compounds on ZiP Transporter Receptor Protein

Molecular docking was used to determine the interaction of active compounds in sea cucumber with ZiP transporter receptor protein. The receptor proteins used are ZiP 2 and ZiP 4. The resulting Gibbs energy values were given in Table 1 (ordered by lowest energy). Based on the data in Table 1, it was known that holothurin and nobiliside are compounds in sea cucumber which have the most significant potential as a phytate inhibitor at the ZiP 2 receptor. It was known from the Gibbs energy value of both compounds is -9.3 kcal/mol, compared to the Gibbs free energy of phytate is -6.0 kcal/mol.

Furthermore, the active compound in sea cucumber with the highest inhibitory ability against phytate at the ZiP 4 receptor was holothurin, with a Gibbs energy value of -9.9 kcal/mol. However, all five test compounds also displayed promising energy values in their ability to inhibit phytate at the ZiP 4 receptor, with a Gibbs phytate energy value of -6.7 kcal/mol. The lower Gibbs free energy value, the easier it is for the ligand and receptor to form a stable conformation.

Gibbs free energy used in the interaction between the active compounds of sea cucumber with ZiP 2 and ZiP 4 receptor proteins causes intermolecular interactions between them. The interactions between the ligands and ZiP 2 and ZiP 4 receptor proteins formed, such as hydrogen bonds and hydrophobic regions around the ligands, were described in Table 2 and Table 3**.**

Table 1. Screening of phytate inhibitor candidate on sea cucumber metabolites

No	Compound	Structure	MW	logP	Hdon	Hacc	OB (%)	DL
1	Arguside		488.78	4.84	3	5	7.58	0.01
2	Holothurin		486.76	4.17	2	5	7.57	0.01
3	Miliariside		486.76	4.50	3	5	7.90	0.22
4	Nobiliside		466.72	5	2	4	7.51	0.30
5	Virescenodee		480	0.88	5	7	58.71	0.28
6	Phytate		660.06	-3.13	12	24	4.78	0,50

*MW: molecular weight, H-donor: , H-acceptor: ,DL: drug-likeness, OB: oral bioavailability

Table 2. Hydrogen bonding interaction of ZiP 2 receptor protein on the active compound of sea cucumber

No	Compound	Gibbs Energy	Hydrogen bond interaction	Hydrogen bond distance (A)
1	Arguside	-9.1	-	-
2	Holothurin	-9.3	Gly133-O Cys126-O Arg164-H Arg213-O Arg280-O Glu276-O	3.24 3.13 2.49 2.87 3.00 2.67
3	Miliariside	-9.0	Gln 137-O	2.98
4	Nobiliside	-9.3	Ser173-O Ser173-O Gln137-O Gln137-O Ser151-H Ser 157-H	3.19 2.84 3.19 2.80 3.03 2.31
5	Virescenoside	-9.1	Gln137-O Ser173-O Cys126-O Ser134-O Ser158-H	2.85 2.97 2.94 2.97 2.75
6	Phytate	-6.0	Ser134-O Ser134-H Ser134-O Gln137-O Thr135-H Ser151-H	2.85 2.86 3.36 2.96 2.54 2.13

Table 2 shows that holothurin and Nobiliside have six hydrogen bonds. The hydrogen bond between holothurin and the ZiP 2 receptor is between 2.49 and 3.24. The hydrogen bond between holothurin and the ZiP 2 receptor is between 2.49 and 3.24 Å. Based on the conformation of the docking results on the ZiP 2 structure, it was founded that there was an interaction in the hydrophobic region and the binding pocket protein of the ZiP receptor. A binding pocket is a place for anchoring ligands such as phytate and compounds in the sea cucumber. In this case, the compounds in sea cucumber were said to be potential if they were anchored to the same binding pocket as phytate. The compounds in sea cucumber that were successfully anchored to the binding pocket region of the ZiP protein receptor, the phytate anchorage site, have potential phytate inhibitors. Another study showed that holothurin is a potential compound as a phytate inhibitor in the ZiP 4 binding pocket. Holothurin interacts with the ZiP binding pocket with three hydrogen bonds that formed at 2.10–2.80 Å.

Table 3. Hydrogen bonding interaction of ZiP 4 receptor protein on the active compound of sea cucumber

No	Compound	Gibbs energy	Hydrogen bond interactions	Hydrogen bond distance (A)
1	Arguside	-9.7	Gly247-O Cys309-O Arg235-O	3.06 3.78 3.34
2	Holothurin	-9.9	Arg150-O Gly247-H Ser249-H	2.80 2.10 2.23
3	Miliariside	-9.4	Thr268-O Cys270-O Gln145-O	2.98 3.06 3.37
4	Nobiliside	-8.2	Ser249-H Gln148-H Lys157-O Gln154-O Asp275-O His241-O His241-O	2.76 2.52 3.62 2.80 2.23 3.14 3.15
5	Virescenoside	-9.7	Gln145-O Asp275-H His197-O Ala198-O Gln148-O	3.38 2.09 3.17 3.40 3.20
6	Phytate	-6.7	His245-O His245-O Ala248-H Ala248-H Ala308-H Glu236-H Cys270-H	2.85 2.86 3.36 2.96 2.54 2.13 2.24

4 Discussion

Seafood is a rich source of minerals that are essential nutrients for humans. However, utilization of mineral sources derived from marine is less than mineral sources from terrestrial [8]. Zinc is one of the minerals needed for the body to maintain the immune system, skin regeneration, hair and nail growth, and secretion of the sebaceous glands [8, 9]. The content of zinc minerals in several species of sea cucumbers and marine fish is presented in Table 4. Based on the data in Table 4, the zinc content in sea cucumbers and marine fish is potentially a source of zinc minerals. Furthermore, zinc sourced from sea cucumbers is expected to interact with their metabolites, such as arguside, holothurin,

Table 4. The content of zinc in the body wall of sea cucumbers and marine fish

No.	Species	Zn (mg/kg)	References
	Sea Cucumbers		
1	*Stichopus horrens*	0.96	[8]
2	*Holothuria arenicola*	0.4	[8]
3	*Parastichopus californicus*	40.4 ± 4.3	[10]
4	*Parastichopus regalis*	23.2	[11]
5	*Eupentacta fraudatrix*	13.0	[9]
	Marine Fish		
1	*Kyphosus vaigiensis*	43.39 ± 3.30	[12]
2	*Stegastes rectifraenum*	23.88 ± 0.77	[12]
3	*Balistes polylepis*	36.16 ± 1.60	[12]

miliariside, nobiliside, and virescenoside they can act as phytate inhibitors and prevent zinc deficiency in the body.

Secondary metabolite compounds in sea cucumber have not been widely investigated as phytate inhibitors in human metabolism. However, based on the results of molecular docking, it is known that all metabolite compounds have the potential to be inhibitors of phytate. This can be seen from the Gibbs-free energy produced, the conformation, and orientation of the interactions on the ZiP2 and ZiP4 receptor proteins. The holothurin conformation is in the binding pocket where the phytate was tethered, so it can be said that the Gibbs energy formed and the holothurin conformation have the potential as a phytate inhibitor. The molecular anchoring conformation between holothurin as the most potent compound and phytate as a comparison compound. Holothurin is a compound belonging to the saponin group, which was reported to have bioactive properties as antifungal [6], anticancer [4], and antibacterial [13]. This research hoped can be used as a scientific basis for further investigations on a laboratory scale. In addition, the results obtained in this study can increase the value to sea cucumber as a marine resource widely produced in Indonesia.

Acknowledgements. I would like to thank to Research Organization for Life Science and Environmental, National Research and Innovation Republic of Indonesia for grant supported fiscal year 2022.

References

1. E.LC, Mirian V, Yvonne G. Zinc bioavailability in rats fed a plant-based diet: a study of fermentation and zinc supplementation. Food Nutr Res. 2015; 59.
2. 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.

3. Ru J, Li P, Wang J, Zhou W, Li B, Huang C, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform. 2014; 6: 13.
4. Pranweerapaiboon K, Garon A, Seide lT, Janta S, Plubrukarn A, Chaithirayanon K, et al. In vitro and in silico studies of holothurin A on androgen receptor in prostate cancer. J Biomol Struct Dyn. 2021 Sep; 1–9.
5. Mona MH, Omran NEE, Mansoor MA, El-Fakharany ZM. Antischistosomal effect of holothurin extracted from some Egyptian sea cucumbers. Pharm Biol. 2012 Sep; 50(9): 1144–50.
6. Han H, Yi Y-H, Li L, Liu B-S, La M-P, Zhang H-W. Antifungal active triterpene glycosides from sea cucumber Holothuriascabra. Yao Xue Xue Bao. 2009 Jun; 44(6): 620–4.
7. Takada K, Nakao Y, Matsunaga S, VanSoest RWM, Fusetani N. Nobiloside, a new neuraminidase inhibitory triterpenoidal saponin from the marine sponge Erylus nobilis. J Nat Prod. 2002; 65(3): 411–3.
8. Barzkar N, Attaran Fariman G, Taheri A. Proximate composition and mineral contents in the body wall of two species of sea cucumber from Oman Sea. Environ Sci. Pollut. Res. 2017; 24(23): 18907–11.
9. Zaksas NP, Timofeeva AM, Dmitrenok PS, Soboleva SE, Nevins kyG. Cucumber Eupentacta fraudatrix and Its High-Molecular-Mass Multiprotein Complex. Molecules. 2022; 27(1958): 1–12.
10. Bechtel PJ, Oliveira ACM, Demir N, Smiley S. Chemical composition of the giant red sea cucumber, P arastichopus californicus, commercially harvested in Alaska. Food Sci Nutr. 2013; 1(1): 63–73.
11. Roggatz CC, González-Wangüemert M, Pereira H, Vizetto-Duarte C, Rodrigues MJ, Barreira L, et al. A first glance into the nutritional properties of the sea cucumber Parastichopus regalis from the Mediterranean Sea (SE Spain). Nat Prod Res. 2018; 32(1): 116–20.
12. Serviere-Zaragoza E, Lluch-Cota SE, Mazariegos-Villarreal A, Balart EF, Valencia-Valdez H, Méndez-Rodríguez LC. Cadmium, lead, copper, zinc, and iron concentration patterns in three marine fish species from two different mining sites inside the Gulf of California, Mexico. Int J Environ Res Public Health. 2021; 18(2): 1–18.
13. ShiS, Feng W, Hu S, Liang S, An N, Mao Y. Bioactive compounds of sea cucumbers and their therapeutic effects. Chinese J Oceanol Limnol 2016 343. 2015 Nov; 34(3): 549–58.