In Silico Study Chitosan Snail Shell as Antioxidant Through Interesting NRF2-KEAP1 in Hypercholesterolemia

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Abstract

Hypercholesterolemia is one of the leading causes of endothelial dysfunction. Hypercholesterolemia can result in oxidative stress that exceeds antioxidant defenses. One of the antioxidants is snail shell chitosan. The research aimed to analyze the study of *in silico* chitosan snail shell as an antioxidant through inhibition of nrf2-keap1 in hypercholesterolemia. This research method is the 3D structure of Keap1 and Nrf2 (ID: 5CGJ) proteins. Canonical chitosan smiles ($C_{56}H_{103}N_9O_{39}$) (ID: 71853) were modelled with Corina software to obtain 3D structures. Chitosan was analyzed by Lipinski test. Molecular docking was analyzed by interacting chitosan, Nrf2 and Keap1 with HEX 8.0 software and visualized by discovery studio version 4.1. Data analysis was used descriptively. The results of the study of chitosan with Keap1 and Nrf2 had 43 amino acid residues (GLU444, ARG447, ASN387, ASP448, GLU79, LEU84, ALA407, TRP450, LEU76, ALA407, GLU447, LEU452, ASN495, ASN387, GLN73, GLU72, ASP79. ASP538, ASP589, GLU82, PHE70, LEU75, ASP77, GLY80, GLY81, LEU84, GLU540, ASP589, PRO549, ASN469, ASN517, ARG536, VAL536, THR545, ASP589, LEU84, GLU540, ASP538, ASP58979) and hydrogen, electrostatic, and hydrophobic bonds. Snail shell chitosan has potential activity as hypercholesterolemia because it has an affinity with nrf2 and keap1 proteins which can prevent the switch on gene process in the formation of antioxidants.

Keywords: *Hypercholesterolemia, oxidative stress, snail shell chitosan, NRF1, KEAP1.*

Introduction

The cholesterol diet can change the picture of lipoproteins to be more atherogenic, namely increasing LDL, lowering HDL levels, and increasing plasma cholesterol. Increasing LDL levels in the body will increase the risk of LDL oxidation caused by various factors such as free radicals. Oxidized LDL will be recognized by the scavenger receptor in macrophages and will become foam cells. The more LDL levels in the plasma, the more it will be oxidized and captured by macrophage cells, causing accumulation of macrophage cells. This accumulation causes reverse cholesterol transport to become unbalanced, which results in hypercholesterolemia[1,2].

Oxidative stress occurs due to an imbalance between increased free radical production and decreased antioxidant capacity. The decline in antioxidants is mainly due to impaired activation of nuclear factor-erythroid-2 related factor 2 (Nrf2) and keap1, the transcription factor that regulates genes encoding antioxidants and detoxification enzymes[3-5].

In the presence of oxidative stress, electrophiles and reactive oxygen species (ROS) can react with KEAP1

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sensor cysteines, including cysteine 151 (C151), C273, and C288, possibly releasing NRF2 from KEAP1-mediated degradation[6-8], so that newly synthesized NRF2 occurs in the nucleus and activates the expression of scattered Nrf2 and degradation occurs in the cytoplasm[9]. KEAP1-NRF2 activates NRF2. Activated NF2 accumulates in the nucleus, resulting in interactions with transcription factors and other cofactors to regulate the transcription of the target gene, which encodes a protein whose role is antioxidants[10].

Exogenous antioxidants are needed to compensate for this is snail shell chitosan. So far, snail shell chitosan has not been studied scientifically as an antioxidant *in silico*. In this study, using in silico with computational modelling which is closely related to in vitro and in vivo experiments. This modelling plays a role in the field of medicinal chemistry to find bioactive compounds that have drug candidates[10-13] in silico test using docking molecules that are predicted as target cells to be determined. This research aims to analyze preliminary studies of molluscs as chitosan producers and to predict activity as antioxidants.

Method

The 3D structures of Keap1 and Nrf2 proteins (ID: 5CGJ) were downloaded from the PDB database. Canonical smiles chitosan ($C_{56}H_{103}N_9O_{39}$) (ID: 71853) from the PubChem database modelled with Corina software to obtain 3D structures. Chitosan was analyzed by Lipinski test. Molecular docking was analyzed by interacting chitosan, Nrf2 and Keap1 with HEX 8.0 software and visualized by discovery studio version 4.1. The analysis includes H-bond, SAS, Hydrophobicity, Aromatic, Interpolated Charge, and Ionizability.

Results

Table 1 shows that the conditions that must be met by a compound based on Lipinski are a molecular weight of the compound <500 Da, there are no compounds with more than 5 hydrogen bond donors, the number of hydrogen acceptors <10, while for molar refractivity ranges from 40-130 and the value Log P <5, the ligand tested has met the requirements so that it is confirmed to be able to pass through the cell membrane.

Table 1. Lipinski Test Results

Ligand	Molecular Weight (Da)	Number of Hydrogen Bonds	Amount of Acceptor Hydrogen Bonds	Molar Refractivity	Log p	Term
Chitosan	312 Da	5	6	77.146	-0.053	Meets specifications

Discussion

In this study, the molecules studied had a higher number of rotatable bonds and had a good ability to bind Nrf2 and Keap1 because the molecules used were polymers. It is seen that the number of hydrogen bond acceptors is directly related to the binding properties of the amino acid residues; It can be seen that the results of docking chitosan→keap1, chitosan + Keap1→Nrf2, chitosan→Nrf2, chitosan + Nrf2→Keap1. The results of chitosan docking to 1 amino acid residue were SER391, SER408, THR388, ARG447, GLU444, ASP389, GLU444, ARG447, ASN387, and ASP448 with the hydrogen bond category. Docking, chitosan + Keap1→Nrf2 amino acid residues produced are SER391, SER408, THR388, ARG447, GLU444, ASP389, GLU79, LEU84, ALA407, TRP450, LEU76, ALA407, GLU447, LEU452, ASN495, GLN387, AL73A69, ASP77, THR80, LEU84, PHE83, GLU79 with the hydrogen bond category, the resulting docking results of chitosan→Nrf2 amino acid residues are LEU84, ALA72, GLN75, GLU72, ASP77, GLU79, ASP79, ASP77, LEU84, and GLU79 The bonds obtained are electrostatic and hydrogen. In contrast, the results of chitosan + Nrf2→Keap1 docking are ARG536, ASP538, LEU84, ALA72, GLN75, ASP589, GLU82, PHE70, LEU75, ASP77, GLU79, THR80, GLY80, GLY81, LEU84, GLU540, ASP589, PRO549, ALA69, PHE83 ASN517, ARG536, VAL536, THR545, ASP589, LEU84, ASP77, GLU540, ASP538, ASP589, ALA548, TRP591 and GLU79, electrostatic, hydrogen, and hydrophobic bonds.

The docking results showed that the different amino acid residues were chitosan →keap1: GLU444, ARG447, ASN387, and ASP448. Chitosan + Keap1→Nrf2 are GLU79, LEU84, ALA407, TRP450, LEU76, ALA407, GLU447, LEU452, ASN495, ASN387,

GLN73. Chitosan→Nrf2: GLU72, ASP79. Chitosan + Nrf2→Keap1: ARG536, ASP538, ASP589, GLU82, PHE70, LEU75, ASP77, GLY80, GLY81, LEU84, GLU540, ASP589, PRO549, ASN469, ASN517, ARG536, VAL536, THR545, ASP538, LEU ASP589, ALA548, TRP591 and GLU79.

Hydrophobic interactions play a role in determining the stability of the ligands against androgen receptors. Hydrophobic interactions avoid a liquid environment and tend to cluster within the globular structure of proteins. The results of this study are by several previous studies which stated that drug candidates generally have the number of hydrogen bonding bonds[14-16], because the average number of hydrophobic atoms in drugs is generally[16], with one to two donors and three to four acceptors[17], so that hydrophobic interactions play an important role in drug candidates because they can increase the binding affinity between target interfaces. The in silico test proved that the binding affinity and drug efficacy associated with hydrophobic bonds could be optimized by combining them at the hydrogen bond site[18,19]. However, this approach is not the only primary method for designing a drug. The formation of hydrophobic bonds minimizes the interaction of nonpolar residues with water. Chitosan + Keap→Nrf2 obtained 3 hydrophobic bonds, namely ALA407, TRP450, and LEU76. Chitosan + Nrf2→Keap1 obtained 3 hydrophobic bonds, namely ALA69, PHE70, and PHE83. The residue on these amino acids is hydrophobic and nonpolar. Nonpolar (hydrophobic) amino acid residues tend to form groups in the interior of the protein[20,21].

Based on the value of the bond energy obtained, it shows that chitosan compounds have potential activity as hypercholesterolemia because they have an affinity and form hydrogen-protein bonds between chitosan and nrf2 and keap1 proteins, so that chitosan can block receptors (keap1) and ligands (nrf2) so that they can be inhibited.

Conclusion

In conclusion, Snail shell chitosan has potential activity as hypercholesterolemia because it has affinity by forming hydrogen bonds with nrf2 and keap1 proteins and can inhibit hypercholesterolemia in silico.

Conflict of Interest: The author declare that they have no conflict of interest.

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