Computational Study Enlightens the Structural Role and Molecular Mechanism of Marine Algal Compound Fucoidan against Hepatocellular Carcinoma Markers

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Abstract: Hepatocellular carcinoma (HCC) is reported to be the major death causing cancers in the world. In the market, several drugs and synthetic compound are able to block HCC in humans. Fucoidan is a marine sponge derived compound with less or no side effects has been increasingly considered as a potential inhibitor of CDK2/CDK4 dependent kinase. In our study, an attempt has been made to effectively design new drugs without any significant side effects against aflatoxin induced HCC. Therefore, we have docked with cancer inducing five proteins into fascaplysin. Our findings showed that among the five proteins, the best docking score of ProstaglandinH2 synthase is 57.973, Crystal structure of human cytochrome P450 3A4 is 54.32 and Crystal Structure of Human Microsomal P450 1A2 is 45.91. This binding affinity of fucoidan might be capable of inhibiting the aflatoxin induced hepatocellular carcinoma. Hydrogen bonding, electrostatic and Vanderwaals stabilizes the fucoidan and target protein interactions as predicted by Discover Studio. Our results strongly prove that fucoidan act as potential inhibitor against aflatoxin induced hepatocellular carcinoma. Further, this fucoidan compound will be tested for biochemical assays *in vitro* and *in vivo*.

Key words: Sea weed, marine sponges, fucoidan, hepatocellular carcinoma, molecular docking.

1. Introduction

Today's life causing diseases are mainly due to the most important factors of diet and life style [1]. Some addicting habitats such as drug abuse, tobacco smoking, and alcohol drinking may increase the risk of developing certain diseases, especially later in age. These life style related diseases are commonly cancer, osteoporosis and atherosclerosis *et al.* It has been reported that high of cancer incidence in connection with the life style [1]. Osteoporosis is a bone disease, which leads to a high risk of bone fracture. Osteoporosis is estimated to affect bones for 200 million women worldwide [2]. In addition to cancer and osteoporosis, life style is the cause of atherosclerosis. It has been reported atherosclerosis is the leading cause of mortality in industrialized countries. The major manifestations of atherosclerosis are coronary artery disease (myocardial infarction, angina, sudden death), cerebrovascular disease (ischemic stroke), and peripheral vascular disease (ischemic limbs), all of these diseases are referred as cardiovascular diseases (CVDs) [3]. So far, the situation is still serious. According to the report from American Heart Association, more than 81 million Americans suffer from some form of cardiovascular disease, making it the leading cause of death in

the country. As of 2006, cardiovascular disease is responsible for one in every 2.9 deaths in the United States. Preventing of these diseases is an important task for the society to lead a healthy life. Many human epidemiological studies and animal studies also showed that high intake of fruits and vegetables over a prolonged period of time will reduce the cancer disease [4]-[6]. Thus, cancer preventive plant has been attracted a great attention. Various factors in plant food, such as antioxidative vitamin, carotenoids, phenolic, terpenoid and fibers, have been considered as responsible for reducing the life style related diseases.

Recent studies in the cancer research have revealed promising compounds, doscovered from natural sources, with proven anticancer activity [7]. Seaweeds have great potential as a supplement in functional food. Seaweeds are known for their richness in polysaccharides, minerals and certain vitamins, also they contain bioactive substances like polysaccharides, proteins, lipids and polyphenols, with antibacterial, antifungal, antiviral properties, etc. [8] and used in the development of novel pharmaceutical agents [9]. Fucoidan, a sulfated polysaccharide found in cell walls of various species of brown and green seaweeds, is known to be used as food and medicine for over a thousand years in Asia and in parts of northern Europe [10]. Recently, the antitumor effect of fucoidan has been intensively studied. It has been reported that the intake of seaweed is associated with a lower incidence of breast cancer [11]. In vivo studies conducted using mouse xenograft models have suggested that fucoidan suppresses the growth of 4T1-derived breast cancer [12], A20-derived lymphoma [13], and Ehrlich ascites carcinoma [14], inhibits metastasis of Lewis lung adenocarcinoma [15] and 13762 MAT rat mammary adenocarcinoma, [16] and has antiangiogenesis activity against B16 melanoma [17]. The lines of evidence of in vitro studies have demonstrated that fucoidan inhibits the growth of non- small cell bronchopulmonary carcinoma NSCLCN6 cells [18] and also induces apoptosis in cells derived from human lymphoma [19], including the prevention of angiogenesis by suppressing expression and secretion of the angiogenesis factor, vascular endothelial growth factor (VEGF) [20], [21]. However, the mechanism involved in the anticancer action of fucoidan is not completely understood, and drug interactions of fucoidan-anticancer targeted therapy have not been studied.

Hence, in our current study, is to designing new drugs against funcoidan induced Hepato cellular carcinoma. Molecular Docking Simulation [22]-[24] was carried out to reveal the binding mechanism of Cancer inducing targeting enzymes with Fucoidan.

2. Materials and Methods

2.1. Ligand Preparation

Fucoidan was retrieved from the CHEMBANK database (http://chembank.broadinstitute.org/) in 2D-strucuture data file (SDF) format were converted into 3D-MOL2 file with the program OpenBABEL2.3.1 [25] (Figure 1). The Fucoidan were further energy minimized for 100 steps with Swiss-PDB viewer with steepest descent and conjugated gradient algorithms [26].

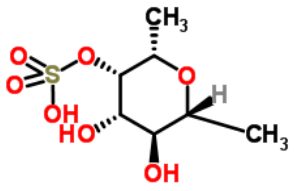

Fig. 1. A chemical structure of brown seaweed sulfated polysaccharide.

2.2. Target Protein Selection

Cancer inducing three different proteins was selected as targets for gold docking studies with Fucoidan.

The proteins selected for the analysis were, 1W0E - Crystal structure of human cytochrome P450 3A4, 2HI4 - Crystal Structure of Human Microsomal P450 1A2 and 1PRH – ProstaglandinH2 synthase. These proteins were significant in fucoidan induced hepatocellular carcinogenesis. The 3D structures of all target proteins were extracted from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/).

2.3. GOLD Docking

GOLD uses a genetic algorithm to explore the full range of ligand conformational flexibility with partial flexibility of the protein [27]. The Docking was performed using GOLD Software (Genetic Optimization Ligand Docking). GOLD uses a genetic algorithm to explore the full range of ligand conformational flexibility with partial flexibility of the protein. Docking procedure consisted of three interrelated components; a) identification of binding site b) a search algorithm to effectively sample the search space (the set of possible ligand positions and conformations on the protein surface) and c) a scoring function. The GOLD fitness function consisted of four components: a) protein-ligand hydrogen bond energy (external H- bond); b) protein-ligand vanderwals (vdw) energy (external vdw); c) ligand internal vdw energy (internal vdw); d) ligand torsional strain energy (internal torsion). Default settings for GOLD docking were adopted. Ten poses were kept for each ligand, and the one yielding the best score was used for further analysis.

3. Results and Discussion

3.1. GOLD Docking

In order to confirm the binding site of Cancer inducing 3 different proteins with Fucoidan one of the most appropriate methods to explore its predicted structural features is through docking studies with the known inhibitor Fucoidan. For such prediction we considered cavity 10 Å around PHE108 because it has been reported to be the key residue for human cytochrome P450 3A4 (1WOE). For Crystal Structure of Human Microsomal P450 1A2 (2HI4), we considered cavity 10 Å around Asn257.

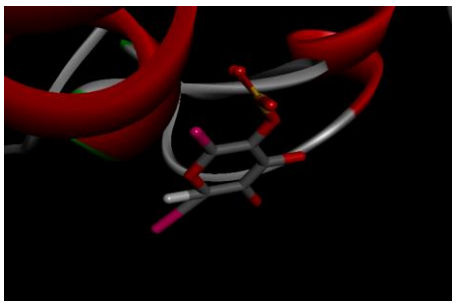

Fig. 2. Docking pose of Fucoidan with Prostaglandin H2 Synthase at a larger view.

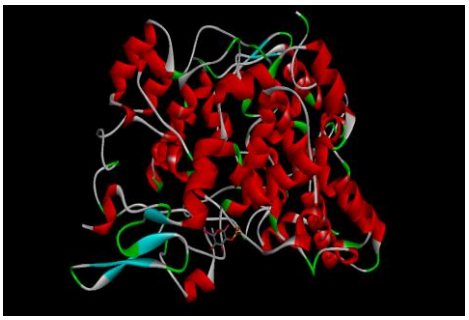

Fig. 3. Docking pose of Fucoidan with Prostaglandin H2 Synthase in cartoon representation.

For ProstaglandinH2 synthase (1PRH), Thr212 around 10 Å considered for the cavity. Further, we considered the active site residues used for docking with the respective receptor (Cancer inducing 3

different proteins) binding to Fucoidan with excellent binding affinity. The best docking scores are shown in the Table 1. Based on our results, among the five proteins, the best GOLD docking score of ProstaglandinH2 synthase is 45.71, Crystal structure of human cytochrome P450 3A4 is 57.37 and Crystal Structure of Human Microsomal P450 1A2 is 54.32. The docking result shows that the Fucoidan is located in the main hydrophobic pocket of the protein and also showed that Fucoidan mainly interacted with target proteins through H-bonding, electrostatic and vanderwaals interactions stabilizes the Fucoidan and target protein interactions as predicted by Discover Studio. This binding allowed us to predict that this Fucoidan might be capable of inhibiting the hepatocellular carcinoma. Docking pose and interacting amino acids within 5 Å distances were shown in Fig. 2 and Fig. 3.

Table 1. The Best Docking Conformations of GOLD and the Calculated Scores Using GOLDscore, Chemscore and ASPscore for Fucoidan and the Cancer Inducing 3 Different Proteins

Proteins	Ligand	GOLD score	Chemscore	ASPscore
1PRH	Fucoidan	45.91	27.25	43.46
1WOE	**Fucoidan**	**57.97**	**36.12**	**55.93**
2HI4	Fucoidan	54.32	32.78	53.79

3.2. Interactions of Prostaglandin H2 Synthase with Fucoidan

Hydrogen bonds are indicated by dashed lines between the atoms involved, while hydrophobic contacts are represented by an arc with spokes radiating towards the ligand atoms they contact. The contacted atoms are shown with spokes radiating back. The following interactions were observed between Fucoidan and ProstaglandinH2 synthase (Fig. 4). His207, Phe210 and Thr212 residues make electrostatic contacts with the inhibitor. Thr206, Lys211, His274, Gln289, Glu290, Val291, Asn382 and His386 form Vanderwaals interactions with Fucoidan. His207 and Lys211 residues involved in forming strong π- π interactions with Fucoidan.

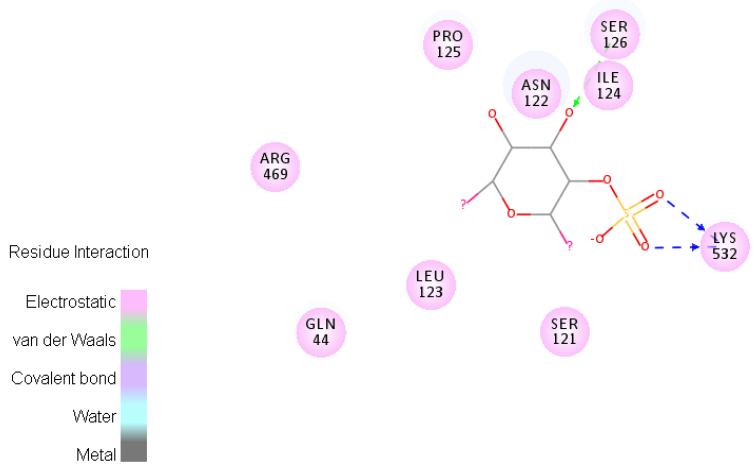

Fig. 4. Hydrogen bond interactions of Fucoidan with Prostaglandin H2 synthase were predicted by LIGPLOT.

3.3. Interactions of Crystal Structure of Human Microsomal P450 1A2 with Fucoidan

Phe226, Val227, Gly316, Ala317 and Asp320 residues make electrostatic contacts with the inhibitor. Ile117, Thr118, Thr124, Phe125, Phe256, Asn257, Phe260, Asp313, Thr321, Leu 382, Ile386, Leu497 and Thr498 form Vanderwaals interactions with Fucoidan. Phe 226 residue involved in forming strong π- π

interaction with Fucoidan (Fig. 5-Fig. 7).

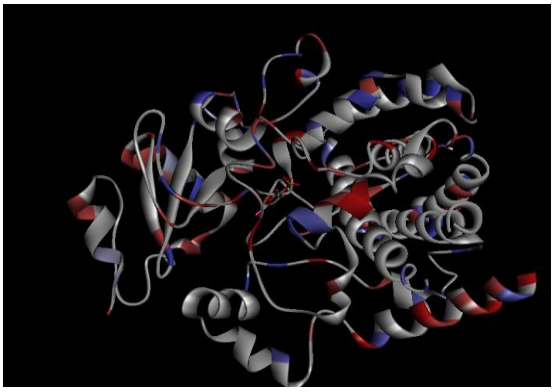

Fig. 5. Docking pose of Fucoidan with Human Microsomal P450 1A2 in cartoon.

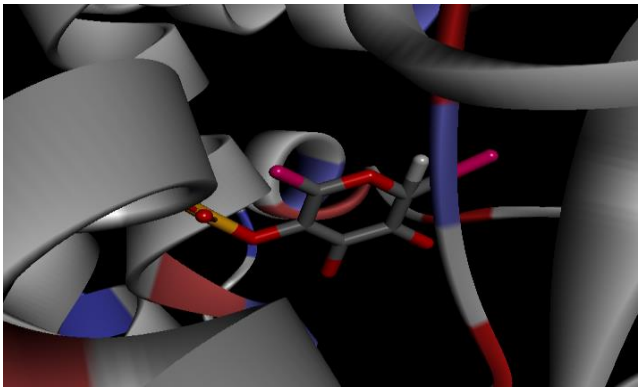

Fig. 6. Docking pose of Fucoidan with Human Microsomal P450 1A2 at a larger view.

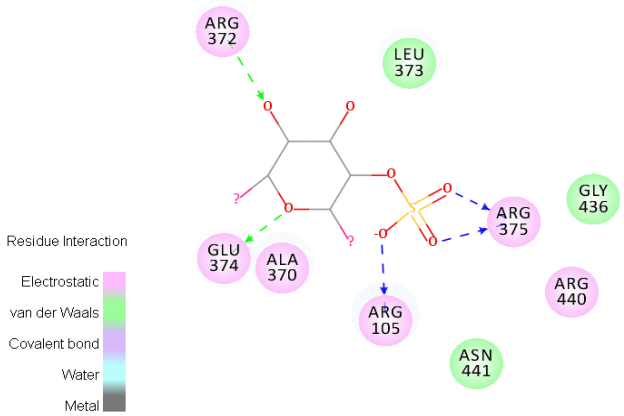

Fig. 7. Hydrogen bond interactions of Fucoidan with Human Microsomal P450 1A2 were predicted by LIGPLOT.

3.4. Interactions of Crystal Structure of Human Cytochrome P450 3A4 with Fucoidan

Phe108 make two hydrogen bonds with N and H of Fucoidan. Phe215 and Thr224 form hydrogen bonds with O atom of Fucoidan. Phe57, Arg106, Phe108, Phe215 and Thr224, residues make electrostatic contacts with the inhibitor. Tyr53, Asp76, Arg105, Ser119, Ile120, Leu216, Phe220, Leu221, Arg372 and Glu374 forms Vanderwaals interaction with Fucoidan. Phe215 residue involved in forming strong π- π interaction with Fucoidan (Fig. 7-Fig. 10).

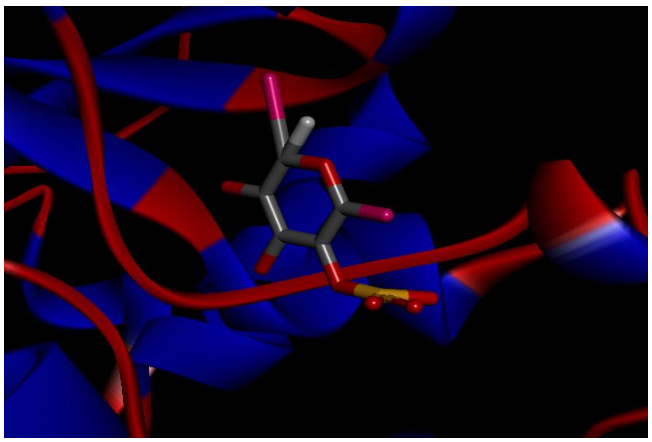

Fig. 8. Docking pose of Fucoidan with human cytochrome P450 3A4 at bigger view.

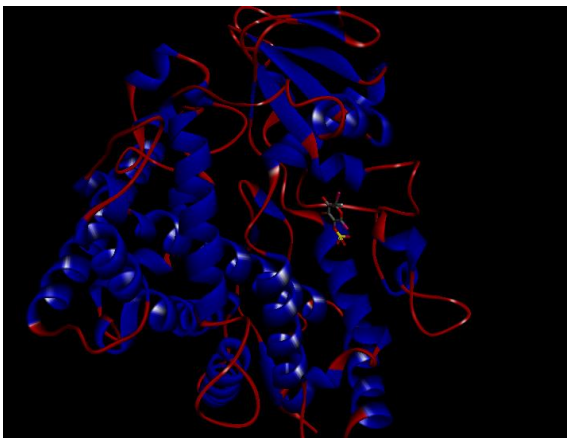

Fig. 9. Docking pose of Fucoidan with human cytochrome P450 3A4 in cartoon.

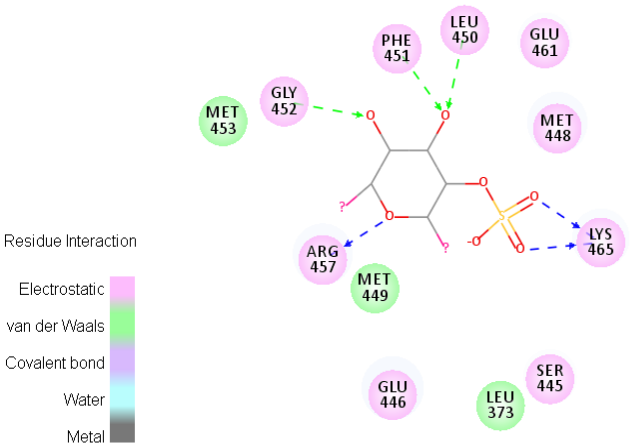

Fig. 10. Hydrogen bond interactions of Fucoidan with human cytochrome P450 3A4 were predicted by LIGPLOT.

4. Conclusion

Life style related diseases emerged in middle age after long exposure to usage of tobacco, a lack of regular physical activity, and consumption of highly rich diets include saturated fats, sugars, and salt, and then it develops to chronic diseases. Diet plays a role as important risk factor for chronic diseases. Diet and nutrition are important factors in the promotion and maintenance of good health throughout the entire life. Their role as determinants of chronic disease is well established and they therefore maintain a prominent position in prevention activities. Traditional, largely plant-based diets have been rapidly replaced by high-fat, energy- dense diets with a substantial content of animal-based foods. The change in diet causes the increase of chronic diseases. It has been projected that, by 2020, chronic diseases will account for almost three-quarters of all deaths worldwide, and that 71% of deaths are due to ischaemic heart disease, 75% of deaths due to stroke, and 70% of deaths are due to diabetes will occur in developing countries(2). Indeed, people are affected by cardiovascular diseases are more numerous in India and China than in all the economically developed countries in the world put together (3). Changing to a healthy diet containing function food such as kelp is a good approach to prevent life style related disease.

Seaweed is an important source of functional and potentially valuable bioactive polysaccharides. Among the polysaccharides from brown seaweeds, fucoidans have turned out to exert potentially significant pharmaceutical effects, at the same time to be a family of differently structured polysaccharides comprising structural units having distinct traits. Despite intensive research, the exact correlations between the various bioactivities and the molecular and structural features of fucoidans still have to be clarified. In this study, the molecular docking was applied to explore the binding mechanism and to correlate its docking score with Fucoidan with targeting cancer proteins. Fucoidan showed interactions and strong binding with aflatoxin induced HCC proteins of Prostaglandin H2 synthase, Crystal Structure of Human Microsomal P450 1A2, and Crystal structure of human cytochrome P450 3A4. The results of our present study can be useful for the design and developing new drugs against aflatoxin induced HCC. Based on the prediction of our work the efficacy of Fucoidan as an anticancer agent can be further confirmed by *in vitro* and *in vivo* studies.

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