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In Silico Analysis of the Potential of the Active Compounds Fucoidan and Alginate Derived from Sargassum Sp. as Inhibitors of COX-1 and COX-2

Lestari Dewi

Department of Pharmacology, Medical Faculty, Hang Tuah University, Surabaya, East Java, Indonesia. Address: Jl. Gadung No. 1, Surabaya, East Java, Indonesia

Corresponding author. Lestari Dewi, Email: dr_lestari@yahoo.co.id

ABSTRACT

Introduction: The enzyme cyclooxygenase (COX) is an enzyme that catalyzes the formation of one of the mediators of inflammation, the prostaglandins. Inhibition of COX allegedly can improve inflammation-induced pathological conditions. **Aim:** The purpose of the present study was to evaluate the potential of *Sargassum* sp. components, Fucoidan and alginate, as COX inhibitors. **Material and methods:** The study was conducted by means of a computational *(in silico)* method. It was performed in two main stages, the docking between COX-1 and COX-2 with Fucoidan, alginate and aspirin (for comparison) and the analysis of the amount of interactions formed and the residues directly involved in the process of interaction. **Results:** Our results showed that both Fucoidan and alginate had an excellent potential as inhibitors of COX-1 and COX-2. Fucoidan had a better potential as an inhibitor of COX than alginate. COX inhibition was expected to provide a more favorable effect on inflammation-related pathological conditions. **Conclusion:** The active compounds Fucoidan and alginate derived from *Sargassum* sp. were suspected to possess a good potential as inhibitors of COX-1 and COX-2.

Key words: alginate; COX-1; COX-2; Fucoidan; *Sargassum* **sp.**

1. INTRODUCTION

Seaweeds are classified into red algae (Rhodophyta), brown algae (Ochrophyta, Phaephyceae) and green algae (Chlorophyta) (1, 2). It constitutes natural, renewable resources distributed throughout the Pacific oceans. Seaweeds have been used primarily for human consumption either as food or medicine to cope with stomach diseases, eczema, cancer, kidney disorders, asthma, arteriosclerosis, heart disease, and lung disease (3-5). It is also being studied in biomedicine due to containing abundant bioactive components, including sulfated polysaccharides, carotenoids, proteins, essential fatty acids, vitamins, minerals, terpenoids, phlorotannin, oxylipins and steroids (6, 7). For example, alginates derived from brown algae are often used as an additive to improve food textures (5).

Non-steroidal anti-inflammatory drugs (NSAIDs) are drugs most commonly used to reduce inflammation (8). Many studies reported that the therapeutic effects and the side effects of NSAIDs were targeted at inhibition of cyclooxygenase (COX) (9). An isoform of COX, the COX-1, is used to catalyze the formation of prostaglandins (PG) on platelets, vascular endothelium, mucosa of the stomach, kidney, pancreas, islets of Langerhans, seminal vesicles and brain (10, 11). The isoform COX-2 can be induced by various growth factors, proinflammatory agents, endotoxins, mitogens and agents of the tumor, indicating that the isoform has a role in pathological processes (12). The product of COX-

1, prostaglandins (PGI2 and PGE2), maintains the integrity of the gastrointestinal tract by reducing gastric acid secretion, increasing the thickness of the mucous layer, stimulating bicarbonate secretion and increasing blood flow in the mucosa (11, 13, 14).

In addition to preventing the synthesis of COX products, another mechanism of NSAID compounds is through inhibition of leukotriene, prevention of the release of the compound of oxygen radicals and lysosomal enzymes and prevention of aggregation, adhesion and chemotaxis of neutrophils (15, 16). In addition, the stimulation of peroxisome proliferator-activated receptor (PPAR) and inhibition of nuclear factor-kappa B (NF-κB) and other transcription factors are also involved in the action of NSAIDs (17).

Aspirin is one of the COX inhibitory compounds. Administration of aspirin in low doses (£100 mg/day) was reported to inhibit the activity of COX-1 by acetylating SER529 residue, leading to inhibition of the production of thromboxane A_2 (TXA_2) and inhibiting TXA_2-mediated platelet aggregation. Aspirin is also found to inhibit COX-1 on gastric and duodenal mucosa, causing a reduction in PGE_2-mediated cytoprotection against acidic environments (18). Studies of NSAIDs-induced gastric damage gave rise to a notion that inhibition of both COX-1 and COX-2 may occur, given that COX-2 can replace COX-1 in producing prostaglandins (19). The purpose of the present study was to investigate the potential of the active compounds Fucoidan and alginate derived from *Sargassum* sp. as COX inhibitors.

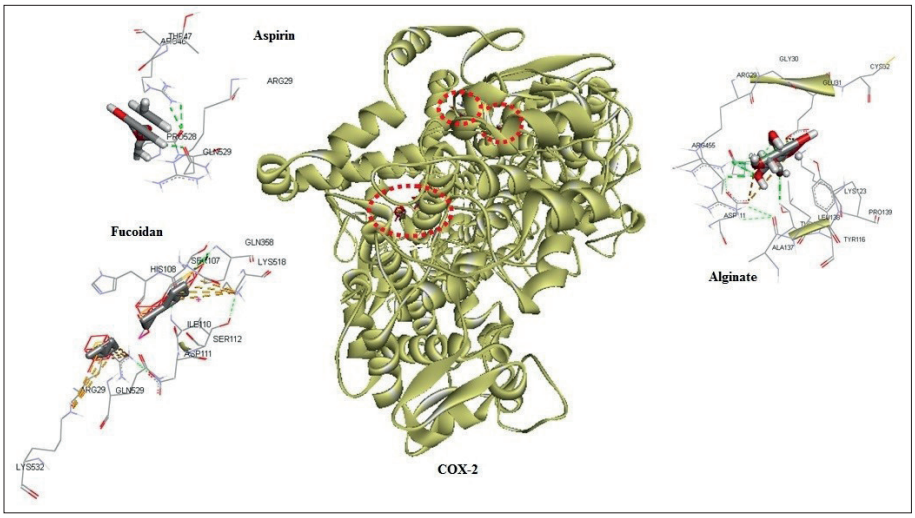

Figure 1. Possible interactions of aspirin, fucoidan and alginate with COX-1.

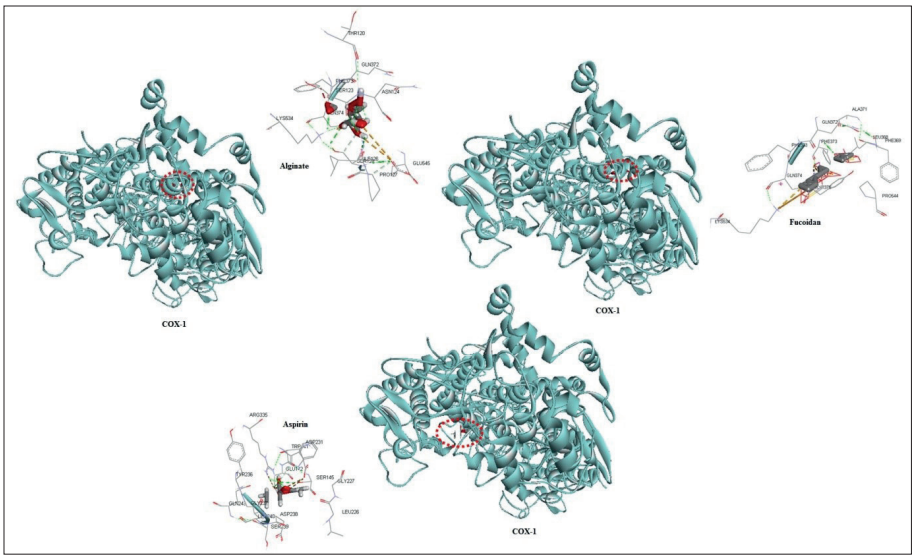

Figure 2. Possible interactions of aspirin, fucoidan and alginate with COX-2.

2. MATERIAL AND METHODS

2.1. Searching for Protein Sequences

The component structure of aspirin (CID: 2244), alginate (CID: 91666324) and Fucoidan (CID: 10452) was obtained from PubChem Open Chemistry Database, whereas the protein sequence of COX-1 (GI: 3914292) and COX-2 (GI: 2970564) was obtained from sequence database of the National Center for Biotechnology Information (NCBI), the United States National Library of Medicine (NLM) and the National Institute of Health (NIH) (http://www.ncbi.nlm.nih.gov).

2.2. 3D- Structure Modeling of DNA, Proteins, and Bioactive Components

The 3D-structure model of COX-1 and COX-2 was predicted using the SWISS-MODEL web-server (20, 21) by the homology modeling method. The 3D structure of proteins was then validated using the Ramachandran plot analysis. Conversion *.sdf files into *.pdb files of aspirin, Fucoidan and alginate was performed using the software OpenBabel (22).

2.3. Docking Computation

Docking simulation among aspirin, Fucoidan and alginate on COX-1 and COX-2 was performed using the software HEX 8.0 (23). The docking protocol consists of three stages of visualization: rigid-body energy minimization, semi-flexible repair and finishing refinement in explicit solvents. Upon completion of each stage, docking conformations were then scored and sorted based the scoring function to facilitate the selection of the best conformation to be used at later stages.

2.4. Analysis of Protein-Protein Interactions

Docking analysis results were subsequently visualized using the software Discovery Studio 4.1, LigPlot+ (24) and Chimera 1.6.2. Analysis of protein-protein inter-

actions was carried out to determine the formed bonds, including hydrogen bonding, hydrophobic bonding and van der Waals bonding. Additionally, Pharmacophore analysis was also conducted to determine residues directly involved in the process of interaction, as well as the analysis of energy minimization to improve molecular structure and shape during the interaction.

3. RESULTS

3.1. Aspirin had a higher effectiveness as an inhibitor of COX-1

Aspirin, being one of anti-inflammatory non-steroidal drugs (NSAIDs), was used as the standard in the present study. Aspirin had a higher effectiveness as ab inhibitor for COX-1 than for COX-2, as shown by the binding energy and the types of bond formed from the interaction. Aspirin was known to require a lower binding energy to COX-1 (159.68 kJ/mol) than to COX-2 (-155.37 kJ/mol). In addition, the number of the bond formed also supported these results. Aspirin interaction with COX-1 formed two electrostatic interactions (GLU142, ASP231) and three hydrogen bonds (SER145, ARG335, GLU142, TRP141), while its interaction with COX-2 formed only one hydrogen bond (GLN529).

3.2. Active compounds of *Sargassum* sp. had potential as inhibitors of COX-1 and COX-2

The active compounds of *Sargassum* sp. analyzed in this study were alginate and Fucoidan. Analysis showed that both alginate and Fucoidan could act as inhibitors of COX-1 and COX-2. Relative to aspirin, alginate and Fucoidan were thought to have a better potential as inhibitors of COX-1 and COX-2. It was shown by the fact that the energy required for the interaction between the two compounds on COX was smaller than that required by aspirin to bind to COX (Table 1).

Molecule	Point interaction	Category	From	To	Binding energy
COX 1 – Aspirin	Aspirin:O – GLU142:OE1	Electrostatic	Aspirin:O (Positive)	GLU142:OE1 (Negative)	**-159.68 kJ/mol**
	Aspirin:O – ASP231:OD2	Electrostatic	Aspirin:O (Positive)	ASP231:OD2 (Negative)	
	SER145:HG – Aspirin:O	Hydrogen bond	SER145:HG (H-Donor)	Aspirin:O (H-Acceptor)	
	ARG335:HH21 – Aspirin:O	Hydrogen bond	ARG335:HH21 (H-Donor)	Aspirin:O (H-Acceptor)	
	Aspirin:O – GLU142:OE1	Hydrogen bond	Aspirin:O (H-Donor)	GLU142:OE1 (H-Acceptor)	
	TRP141:CD1 – Aspirin:O	Hydrogen bond	TRP141:CD1 (H-Donor)	Aspirin:O (H-Acceptor)	
COX 2 – Aspirin	Aspirin:H – GLN529:OE1	Hydrogen bond	Aspirin:H (H-Donor)	GLN529:OE1 (H-Acceptor)	**-155.37 kJ/mol**
COX 1– Alginate	Alginate:O – GLU545:OE1	Electrostatic	Alginate:O (Positive)	GLU545:OE2 (Negative)	**-171.93 kJ/mol**
	SER128:HG – Alginate:O	Hydrogen bond	SER128:HG (H-Donor)	Alginate:O (H-Acceptor)	
	GLN374:H – Alginate:O	Hydrogen bond	GLN374:H (H-Donor)	Alginate:O (H-Acceptor)	
	LYS534:HZ3 – Alginate:O	Hydrogen bond	LYS534:HZ3 (H-Donor)	Alginate:O (H-Acceptor)	
	Alginate:H – ILE126:O	Hydrogen bond	Alginate:H (H-Donor)	ILE126:O (H-Acceptor)	
COX 2– Alginate	Alginate:O – GLU31:OE2	Electrostatic	Alginate:O (Positive)	GLU31:OE2 (Negative)	**-179.19 kJ/mol**
	Alginate:O – ASP111:OD2	Electrostatic	Alginate:O (Positive)	ASP111:OD2 (Negative)	
	ARG29:HH11 – Alginate:O	Hydrogen bond	ARG29:HH11 (H-Donor)	Alginate:O (H-Acceptor)	
	GLN529:HE22 – Alginate:O	Hydrogen bond	GLN529:HE22 (H-Donor)	Alginate:O (H-Acceptor)	
	THR115:HG1 – Alginate:O	Hydrogen bond	THR115:HG1 (H-Donor)	Alginate:O (H-Acceptor)	
COX 1 – Fucoidan	LYS534:NZ – Fucoidan:O	Electrostatic	LYS534:NZ (Positive)	Fucoidan:O (Negative)	-287.96 kJ/mol
	LYS534:NZ – Fucoidan:C	Electrostatic	LYS534:NZ (Positive)	Fucoidan:C (Negative)	
	PHE373:H – Fucoidan:O	Hydrogen bond	PHE373:H (H-Donor)	Fucoidan:O (H-Acceptor)	
COX 2 – Fucoidan	ARG29:NH2 – Fucoidan:O	Electrostatic	ARG29:NH2 (Positive)	Fucoidan:O (Negative)	-272.51 kJ/mol
	LYS518:NZ – Fucoidan:C	Electrostatic	LYS518:NZ (Positive)	Fucoidan:C (Negative)	
	LYS532:NZ – Fucoidan:O	Electrostatic	LYS532:NZ (Positive)	Fucoidan:O (Negative)	
	GLN358:H – Fucoidan:S	Hydrogen bond	GLN358:H (H-Donor)	Fucoidan:S (H-Acceptor)	
	GLN358:H – Fucoidan:O	Hydrogen bond	GLN358:H (H-Donor)	Fucoidan:O (H-Acceptor)	

Table 1. Comparison of possible interactions of the active compounds of *Sargassum sp.* with aspirin on COX-1 and COX-2

3.3. Fucoidan was thought to have a better potential as an inhibitor of COX-1 and COX-2 than alginate and aspirin

Analysis of bonding energy indicated that aspirin required energy of -159 kJ/mol and -155.37 kJ/mol to bind to COX-1 and COX-2, respectively. Alginate required less bonding energy of -171.93 kJ/mol and -179.19 kJ/mol to bind to COX-1 and COX-2, respectively. Fucoidan required much smaller energy to interact with COX-1 and COX-2 (-287.96 kJ/mol and -272.51 kJ/mol) than aspirin or alginate. The bonding energy required by Fucoidan was almost half of that required by aspirin to bind to COX-1 and COX-2; Fucoidan was thought to have a good potential as an inhibitor of COX-1 and COX-2.

3.4. Alginate had a better potential as an inhibitor of COX-2 than aspirin

Analysis of residues directly involved in the process of interaction showed that aspirin and alginate competitively bound COX-2, in which the two compounds would bind to the amino acid residue GLN529 in their

interactions. In the process of competitive binding it was thought that alginate more easily bound than aspirin to COX-2 due to its smaller binding energy; thus, alginate had an excellent potential as an inhibitor of COX-2. In addition, on the basis of binding energy, the interaction of COX-2/aspirin only formed one hydrogen bond (GLN529), while the interaction of COX-2/alginate formed two electrostatic bonds (GLU31, ASP111) and three hydrogen bonds (ARG29, GLN529, THR115). The number of alginate-COX-2 bonds formed showed that the bonds between them were strong and stable.

4. DISCUSSION

Of various inflammatory mediators, prostaglandins (PG) are among the most important mediators. Prostaglandins are released due to various chemical and mechanical stimuli. A key enzyme of the synthesis of prostaglandin is prostaglandin endoperoxide synthase (PGHS) or cyclooxygenase (COX), which has two catalytic sites. The first is the active site of cyclooxygenase that serves to convert arachidonic acid into endoperoxide PGG_2. The other was the active site of peroxidase that serves to convert PGG_2 into another endoperoxide, PGH_2. Furthermore, PGH_2 will be processed by a specific enzyme to form PG, prostacyclin and thromboxane A_2. Of all types of PG, PGE_2 and prostacyclin are major mediators of inflammation (25).

The active components of algae have been known to have pharmacological actions as antiviral compounds to treat a variety of diseases, including eczema, cancer, kidney disorders, asthma, arteriosclerosis, heart disease and lung disease (3-5, 26). But, the present study was the first to report the potential of the components (alginate and Fucoidan) of *Sargassum* sp. as COX-inhibiting compounds. Results of our study showed that alginate and Fucoidan were potential inhibitory compounds, either to COX-1 or COX-2, in which alginate was a more potent inhibitor of COX-2 than aspirin. COX-2 is expressed in normal endothelial cells in response to shear stress, in which inhibition of COX-2 is significantly associated with suppression of the synthesis of prostacyclin (27, 28).

Both COX-1 and COX-2 have been detected in atherosclerotic lesions in humans (29); however, the specific effects of COX inhibition in the progression of the lesion remain a matter of controversy. Administration of aspirin in low doses and COX-2 inhibitors has been known to improve or otherwise worsen endothelial dysfunction, hypercholesterolemia and hypertension (30, 31). COX-2 has been implicated in plaque destabilization via its increased expression and co-localization with microsomal PGE synthase-1 and metalloproteinase-2 (MMP-2) and MMP-9 (32).

Aspirin is among the COX-inhibiting compounds acting by acetylating the COX binding site, thus preventing the formation of prostaglandins. Aspirin bonding to COX-1 can inhibit the production of prostaglandins that are responsible for the formation of platelets, preventing blood from clotting. Its bonding to COX-2 has been known to reduce the inflammatory response. The present study found that Fucoidan and alginate were highly potential as COX-2 inhibitors; thus, consumption of *Sargassum* sp. is thought to provide an aspirin-like effect.

The 3-dimensional structure of COX-1 consists of 3 independent folding units, namely an epidermal growth factor-like domain, a membrane-bound motif and an enzymatic domain. The activity sites of peroxidase and COX is adjacent, but spatially different. The conformation of the membrane-bound motif strongly suggested the enzyme was integrated to only a layer of the lipid bilayer, thus belonging to the monotopic membrane proteins. The S(-) stereoisomer of flurbiprofen interacts by means of its carboxylic group with ARG120, thus putting the second phenyl ring in the Van der Waal's interaction of TYR385 (25). It was thought that there were other sub-sites for drug compounds to bind to the slanting channel. Results of our study indicated that alginate interacted with COX-1 at residues GLU545, SER128, GLN374, LYS534 and ILE126, while Fucoidan interacted via LYS534 and PHE373.

5. CONCLUSION

The active compounds of *Sargassum* sp., including Fucoidan and alginate, had good potential as inhibitors of COX-1 and COX-2.

• Conflict of interest: none declared.

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