In Silico Study of Bioactive Peptides from Nile Tilapia (*Oreochromis niloticus*) Keratin as Antibiofilm Against *Staphylococcus aureus*

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ABSTRACT

Nile tilapia (Oreochromis niloticus) keratin can be a source of bioactive peptides. Bioactive peptides are molecules consisting of 2-20 amino acids and have biological activity as an anti-biofilm agent. The search for antibiofilm agents is important because of the emergence of biofilm layer resistance to antimicrobial compounds. Therefore, bioactive peptides from tilapia keratin have the potential to be developed as antibiofilms. This study aimed to predict bioactive peptides from tilapia keratin and determine the interaction between bioactive peptides as ligands with the Penicillin Binding Protein 3 (PBP3) receptor of S. aureus in silico. Tilapia keratin sequences were obtained from the UniProtKB database. Peptide screening using Expasy Peptide Cutter, Innovagen AB, CSM-toxin, and AntiBFP. Molecular docking was performed with PyRx 0.8 and visualization with BIOVIA Discovery Studio. The screening results obtained 10 bioactive peptides from tilapia keratin that have met the criteria of good water solubility, non-toxic, and possible antibiofilm based on the physicochemical property model. The molecular docking results showed that the LESELRNMQGLVEDFK peptide as a ligand interacted strongly with the PBP3 protein with an estimated ΔG value higher than the native ligand and other bioactive peptides, which was -12.5 kcal/mol. The amino acid residues that interact with the ligand consist of Asn-434, Glu-623, Asp-519, and Asn-432 which have hydrogen bonds, and Pro-661, Tyr-430, and Phe-625 which have hydrophobic interactions. The same amino acid residues between the LESELRNMQGLVEDFK peptide and cefotaxime are Glu-623 and Tyr-430. Peptide LESELRNMQGLVEDFK from tilapia keratin in silico has the potential as an anti-biofilm agent by inhibiting the PBP3 protein of S. aureus.

Keywords: *Keratin, Oreochromis niloticus, bioactive peptides, antibiofilm, molecular docking*

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is a type of cultivated fish that has become the third largest increase in production growth in Indonesia with a growth volume of 43.71% (1). Keratin from fish scales is an animal byproduct that is reported to have antimicrobial potential and contributes to defense against pathogens in water (2). Therefore, to reduce waste from tilapia processing, keratin could be used as a source of bioactive peptides that help suppress bacterial growth.

Bioactive peptides are short molecules made up of 2-20 amino acids with a specific sequence and have biological activity that is not present in the main protein structure (3). This peptide is produced by hydrolysis with enzymes and has certain physiological functions, such as anti-microbial, anti-inflammatory, and antioxidant (4). Bioactive peptides are also widely used because of their cheap production costs and abundant quantities (3).

Biofilm formations have become a source of contamination in the food industry and pose a serious risk to human health (5,6,7). The formation of this biofilm layer begins with the attachment of bacteria to a surface, then proliferation occurs until a microbial community is formed which is encased in extracellular polymeric substances (EPSs) resulting in resistance to

antimicrobial compounds (7,8). *Staphylococcus aureus*, a bacterium responsible for food poisoning, can form biofilm layers (9,10). These bacteria can accumulate on various surfaces that come into contact with food, thereby inducing cross-contamination and making them challenging to eliminate in the food industry (11).

So far, biofilm problems have been handled physically with UV light and steam heating and chemically with disinfectants (12). However, both methods are still unable to remove biofilm effectively. Many biofilm-producing bacteria have also been found that are resistant to antimicrobial fluids such as sanitizers. Therefore, it is important to look for alternatives to prevent biofilm formation in the food industry (5,6,13). One alternative antibiofilm agent that has potential is bioactive peptides (5,14,15).

The use of bioactive peptides has been carried out by Madrazo and Segura (16) who showed that peptides from the chia plant (*Salvia hispanica* L.) have antibiofilm capabilities against *S. aureus* both in silico and in vitro. Other peptide sources that have been studied to have anti-biofilm capabilities against *S. aureus* are milk protein (17), chicken feather keratin (14), ovomucin (9), *Odorrana graham* (18) and yellow catfish mucus (19).

The bioactive potential of a peptide can be predicted through in silico studies with molecular docking (15). Molecular docking is a bioinformatics method for predicting the interaction between a molecule and a target protein receptor to obtain binding affinity values and the stability of the bond conformation between the molecule and the receptor (20). The advantage of this method is that it saves time and costs in screening various molecules that have biological activity (21).

Until now, bioactive peptides from tilapia keratin have not been researched as antibiofilm against *S. aureus*. As a result, this study conducted an in silico analysis as a preliminary test to screen types of bioactive peptides that have the potential to act as anti-biofilms by predicting the binding interactions between the bioactive peptides and the target protein Penicillin Binding Protein 3 (PBP3) from *S. aureus.*

MATERIAL AND METHODS

The hardware used was a Windows 10 Home N computer with Intel(R) Core(TM) i3-3217U CPU @ 1.80GHz specifications, 8.00 GB RAM, 64-bit operating system, and x64-based processor. The software used were Chimera 1.18, AutoDockTools 1.5.7, PyRx 0.8, and BIOVIA Discovery Studio.

The web servers used were UniProtKB (https://www.uniprot.org/), RCSB PDB (https://www.rcsb.org/), PeptideCutter(https://web.expasy.org/peptide_cutter/),Innovagen(https://www.innovagen.com/proteomics-tools), CSM-Toxin (https://biosig.lab.uq.edu.au/csm_toxin/), AntiBioFilm-Peptide Screening (https://antibfp-antibiofilm-peptide-screening.onrender.com/).

The materials used were keratin sequences in *Oreochromis niloticus* (Nile tilapia) with code A0A669D957 and Penicillin-binding protein 3 (PBP3) with code 3VSL which were saved in FASTA format.

Protein Sequence Selection

The UniProtKB Web server was accessed for the selection of protein sequences. The *Oreochromis niloticus* (Nile tilapia) keratin protein was obtained with the code A0A669D957. The protein sequence was saved in FASTA format in Notepad.

Protein Cutting with Peptide Cutter

Cutting protein sequences using the Peptide Cutter Web server. The protein sequence in FASTA format from UniProtKB is copied into the column provided, mark the trypsin enzyme box, then press perform. The information that will be obtained is the number of cuts, the position of the cut, cutting enzyme, peptide sequence resulting from the cut, peptide length, and peptide mass (22).

Prediction of peptide solubility

The solubility of the cut peptide was predicted using the Web server. Enter the peptide sequence into the Peptide Property Calculator column, then press calculate. The estimated results of solubility in water will appear in the physiochemical properties section (23).

Prediction of peptide toxic properties

The toxin properties of peptides can be predicted using CSM-Toxin. On the web front page, press run prediction, then the peptide sequence is entered in the column provided in FASTA format (24).

Antibiofilm prediction

Peptides that have antibiofilm capabilities are predicted with AntiBFP: AntiBioFilm-Peptide Screening on the web server. The peptide sequence is entered in the peptide sequence column, then select Best Model and press Predict (25).

Preparation of proteins and ligands

Protein preparation was begun by downloading the receptor protein from the RCSB PDB Web server. The receptor protein chosen was Penicillin-binding protein 3 (PBP3) from *methicillin-resistant Staphylococcus aureus* with code 3VSL, then the file was saved in PDB format. Next, water molecules and native ligands were removed from the downloaded proteins using the USCF Chimera 1.18 program (26).

The protein was then optimized by adding hydrogen and adding Kollman charges using the AutoDockTools 1.5.7 (27). Protein optimization results are saved in pdbqt format. Ligand preparation was carried out using the USCF Chimera 1.18 program.

Molecular docking process and data visualization

The molecular docking process used PyRx 0.8 software with the Autodock Vina system (28). The proteins and ligands that had been prepared were then entered into the software and then the energy minimization of the ligands and the grid box determination were carried out. The docking results would display the binding affinity value and docking model between the ligand and the target protein. The data that had been obtained were then displayed using the BIOVIA Discovery Studio program (29).

RESULT

The screening results based on the solubility, toxicity, and antibiofilm prediction can be seen in Table 1.

Table 1. Peptide sequence screening results

Peptide Sequence	Peptide Length (Amino Acid)	Solubility Prediction	Toxicity Prediction	Antibiofilm Prediction (based on *physicochemical properties model*)
RQLDGLGNEK	10	*Good water solubility*	*Non-Toxic*	*Probable antibiofilm*
LESELRNMQGLVEDFK	16	*Good water solubility*	*Non-Toxic*	*Probable antibiofilm*
YEDEINKR	8	*Good water solubility*	*Non-Toxic*	*Probable antibiofilm*
VDALQDEINFLR	12	*Good water solubility*	*Non-Toxic*	*Probable antibiofilm*
NLDMDAIVAEVR	12	*Good water solubility*	*Non-Toxic*	*Probable antibiofilm*
AQYEDIANR	9	*Good water solubility*	*Non-Toxic*	*Probable antibiofilm*
YEEMQTSAGQYGDDLR	16	*Good water solubility*	*Non-Toxic*	*Probable antibiofilm*
LQNEIESVKGQR	12	*Good water solubility*	*Non-Toxic*	*Probable antibiofilm*
ANLESQIAEAEER	13	*Good water solubility*	*Non-Toxic*	*Probable antibiofilm*
EYQELMNVK	9	*Good water solubility*	*Non-Toxic*	*Probable antibiofilm*

Table 1 shows 10 peptides with lengths varying from 9 to 16 amino acids. The ten peptides resulting from screening are referred to as test ligands for molecular docking. Molecular docking can be used to predict the mechanism of action of molecules such as proteins, peptides or chemical compounds on a molecular scale so that it can be used to make a drug based on structure (30).

Table 2. Molecular docking results

Target Protein	Ligand	Estimated ΔG (kcal/mol)
Penicillin-binding protein 3 (code: 3VSL)	Cefotaxime	-7,5
	RQLDGLGNEK	-9,2
	LESELRNMQGLVEDFK	-12,5
	YEDEINKR	-9,8
	VDALQDEINFLR	-10,8
	NLDMDAIVAEVR	-9,9
	AQYEDIANR	-7,9
	YEEMQTSAGQYGDDLR	-12,4
	LQNEIESVKGQR	-10,2
	ANLESQIAEAEER	-11,5
	EYQELMNVK	-10,4

The molecular docking results of the ten test ligands and the native ligand, namely cefotaxime, against PBP3 from *S. aureus* can be seen in Table 2. Each docked ligand has a ΔG value (kcal/mol) which is the binding free energy or binding affinity which measures the extent to which the ligand is able to binds to the target protein (31).

The molecular docking results were then visualized with BIOVIA Discovery Studio to obtain the amino acid residues involved in the bond between the ligand and the target protein (32). The results of molecular docking that have been visualized with BIOVIA Discovery Studio can be seen in the Table 3.

DISCUSSION

Ten bioactive peptides showed good water solubility, non-toxic, and possibly had anti-biofilm activity. Peptides that have antibiofilm capabilities and are water soluble are good candidates as solutions for implementing green technologies and bio-sanitizing formulations (12).

Table 2 shows that the ten test ligands have lower ΔG values compared to native ligands, so these ten test ligands show potential for further development as antibiofilm products against *S. aureus*. A more negative ΔG value indicates a stronger binding free energy between the ligand and the target protein (33). Ligands with lower ΔG values suggest a more stable

Table 3. Visualization results with BIOVIA Discovery Studio

Target Protein	Ligand	Amino Acid Residues
Penicillin-binding protein 3 (code: 3VSL)	Cefotaxime	Hydrogen bond (Ser-392, Thr-603, Gln-524, Thr-621, Glu-623), Hydrophobic interaction (His-447, Tyr-430).
	RQLDGLGNEK	Hydrogen bond (Arg-428, Glu-508, **Gln-524**, Gly-515, **Glu-623**, Asp-519)
	LESELRNMQGLVEDFK	Hydrogen bond (Asn-434, **Glu-623**, Asp-519, Asn-432), Hydrophobic interaction (Pro-661, **Tyr-430**, Phe-625).
	YEDEINKR	Hydrogen bond (Asn-513, Gln-626, Glu-508, Tyr-525, **Gln-524**, Asp-519, **Glu-623**)
	VDALQDEINFLR	Hydrogen bond (Arg-428, **Glu-623**, Asp-519, Pro-514, Asn-513, Gly-515)
	NLDMDAIVAEVR	Hydrogen bond (Lys-427, Arg-428, Ser-429, Asn-450, **Gln-524**, Thr-621, **Glu-623**)
	AQYEDIANR	Hydrogen bond (Arg-428, Asn-516, Lys-427)
	YEEMQTSAGQYGDDLR	Hydrogen bond (Gly-515, Glu-623, **Gln-524**, **Gly-423**, Arg-428, Asn-513)
	LQNEIESVKGQR	Hydrogen bond (**Glu-623**, Asn-513), Hydrophobic interaction (Phe-421, Phe-625)
	ANLESQIAEAEER	Hydrogen bond (Arg-428, Asn-513, Asn-516, Asn-432, **Glu-623**, Asp-519, Gly-515), Hydrophobic interaction (**Tyr-430**).
	EYQELMNVK	Hydrogen bond (Lys-427, Arg-428, Thr-621, Ala-622, **Glu-623**)

bond with the target protein, enhancing the ligand's ability to inhibit the target's activity(34).The LESELRNMQGLVEDFK ligand has the lowest ΔG value among the other tested ligands, namely -12.5 kcal/mol.

Amino acid residues that have the same interactions as cefotaxime for ligands RQLDGLGNEK is Gln-524 and Glu-623, for the LESELRNMQGLVEDFK ligand, it is Glu-623 and Tyr-430, for the YEDEINKR ligand, it is Gln-524 and Glu-623, for the VDALQDEINFLR ligand, it is Glu-623, for the NLDMDAIVAEVR ligand, it is Gln-524, Thr-621, and Glu-623, for the YEEMQTSAGQYGDDLR ligand, it is Glu-623 and Gln-524, for the LQNEIESVKGQR ligand, it is Glu-623, for the ANLESQIAEAEER ligand, it is Glu-623 and Tyr-430, and for the EYQELMNVK ligand, it is Thr-621 and Glu-623.

The amino acid residues in each ligand are bound by the same active site as cefotaxime. The active site is a specific protein part where the substrate is bound and undergoes a chemical reaction (35). Amino acid residues that have hydrophobic interactions are amino acid residues that are non-polar, stay away from water and form groups on the inside of the protein, while hydrogen bonds play a role in increasing the affinity of the test ligand with the target protein (36).

CONCLUSION

The bioactive peptide obtained from *O. niloticus* keratin is the LESELRNMQGLVEDFK peptide which produces a bond energy (ΔG) of -12.5 kcal/mol. The amino acid residues with hydrogen bonds are Asn-434, Glu-623, Asp-519, and Asn-432, while those with

hydrophobic interactions are Pro-661, Tyr-430, and Phe-625. The LESELRNMQGLVEDFK peptide has the potential to be developed as an antibiofilm agent by inhibiting the PBP3 protein from *S. aureus*.

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