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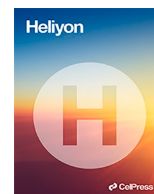

Research article

Molecular investigation of antimicrobial peptides against *Helicobacter pylori* proteins using a peptide-protein docking approach

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A R T I C L E I N F O

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A B S T R A C T

The impact of *H. pylori* resistance on patient's treatment failure is a major concern. Therefore, the development of novel or alternative therapies for *H. pylori* is urgently needed. The purpose of this study was to investigate the molecular interactions of various antimicrobial peptides (AMPs) to *H. pylori* proteins. We performed the peptide-protein molecular docking using HADDOCK 2.4 webserver. Fourteen AMPs were tested for their binding efficacy against four *H. pylori* proteins. Simulation of the peptide-protein complex was performed using molecular dynamic software package AMBER20. From molecular docking analysis, five peptides (LL-37, Tilapia piscidin 4, napin, snakin-1 and EcAMP1) showed strong binding interactions against *H. pylori* proteins. The strongest binding affinity was observed in the interactions between Snakin-1 and PBP2, TP4 and type I HopQ and EcAMP1 and type I HopQ with −11.1, −13.6 and −13.8 kcal/mol, respectively. The dynamic simulation was performed for two complexes (snakin1-PBP2 and EcAMP1-HopQ). Results of the dynamics simulation showed that EcAMP1 had stable interaction and binding to type I HopQ protein without significant structural changes. In conclusion, both results of docking and simulation showed that EcAMP1 might be useful as a potential therapeutic agent for *H. pylori* treatment. This molecular approach provides deep understanding of the interaction insights between AMPs and *H. pylori* proteins. It paves the way for the development of novel anti-*H. pylori* using antimicrobial peptides.

1. Introduction

H. pylori is a gram-negative, flagellated and microaerophilic bacteria that colonize the stomach of more than 50% of the world's population. This bacterial infection has been associated with gastric diseases such as chronic gastritis, peptic ulcer gastric cancer. and gastric MALT lymphoma. *H. pylori* infection also have been shown to be associated with a number of extragastric manifestations [1].

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The trend of *H. pylori* prevalence is declining in many developed countries; however, it remains an important and endemic public health issue in developing countries. In East Asian countries, *H. pylori* infection is associated with approximately 65–80% of gastric cancer cases [2,3]. Eradication of *H. pylori* consist of various regimens including triple, sequential, quadruple and bismuth-containing therapies. The choice of antibiotics is depending on the resistance profiles of the strains and the resistance rates in the region [4]. However, *H. pylori* infection has been hindered by the development of multidrug-resistance *H. pylori* strains. Antibiotic resistance is now the main reason for treatment failure. The prevalence of multidrug-resistance *H. pylori* strains has reached an alarming rate in many regions, rendering a therapeutic challenge for effective eradication of *H. pylori* infection. Clarithromycin-resistant *H. pylori* strain has been declared by the World Health Organization (WHO) as a high priority in the research and development of novel antimicrobial discovery [5]. The concern of increasing rate of antibiotic resistance requires urgency for the discovery of novel or alternative therapies for *H. pylori*.

Antimicrobial peptides (AMPs) are essential components of innate immunity and naturally found in many organisms of both invertebrates and vertebrates. AMPs involves in the first line of defense against infections [6]. Generally, AMPs are short peptides consisting of 10–60 amino acids with an overall positive charge (generally +2 to +9) and have a substantial proportion of hydrophobic residues (>30%). These characteristics of AMPs (amino acid sequences, net-positive charge, amphipathicity, and very small size) allow APMs to bind to and disrupt membranes of microorganims. Researches have shown that AMPs can also inhibit cell wall, nucleic acid and protein biosynthesis [7]. The broad range mechanism of actions of AMPs makes them suitable for targeting bacterial infection including the multidrug-resistant strains [8]. The advantages of AMPs over conventional antibiotics including broad spectrum activity against pathogens (bacteria, fungi, viruses and parasites) and low development of resistance among microorganisms due to short killing contact time by AMPs [9]. Thus, AMPs may provide a novel alternative approach in the treatment of *H. pylori* infection. The structural and functional diversity exhibited by AMPs upholds their antimicrobial activity, microbial cell selectivity and immunomodulatory properties that make them potential drug candidates for the development of new therapies [10].

The advent of in silico methods of bioinformatics, molecular docking, and molecular dynamics simulation provide an extraordinary advancement in the field of drug discovery [11]. These molecular approaches enhance our knowledge regarding biological pathways of protein-peptide, protein-protein, or protein-ligand complexes which provide insights into the mechanisms of novel interactions [12]. Molecular docking helps scientists to predict the best binding patterns and interactions between compound complexes [13]. Different docking techniques which involve interactions of various compounds such as ligand-based docking, protein-protein docking, protein-peptide docking, and induced fit docking have a great influence in the field of drug discovery and development. Novel and potential drug candidates can be rapidly screened and identified using molecular docking and molecular dynamics simulation approaches that predict drug-receptor interactions [14]. Recently, Pandey et al. [15] has studied the molecular dynamics aspects of moxifloxacin-induced resistance in *M. tuberculosis* DNA gyrase A and C using computational modelling. Similarly, Bera et al. [16] also used molecular docking and simulation approaches to investigate interactions of Echinocandin B with ATP-binding transporter protein. Currently, only one study investigated the interaction of plant AMPs against *H. pylori* protein using a peptide-protein docking approach. They found that snakin-1 AMP exhibited strong interaction with oxygen-insensitive NADPH nitroreductase of *H. pylori* [17]. With lacking information of other AMPs interaction against *H. pylori* proteins, we propose this study with the aim to explore the binding interactions between various AMPs and *H. pylori* receptor proteins. This study will help for further exploration and validation of new potential anti-*H. pylori* agents.

Table 1
List of antimicrobial peptides, their sources and peptide sequences used in this study.

No.	AMP	PDB ID	Source	Peptide sequence
1.	Cathelidicin, LL-37	5NNM	Homo sapiens	LLG DFF RKS KEK IGK EFK RIV QRI KDF LRN LVP RTE S
2.	Human neutrophil defensin 1 (HNP-1)	3HJD	Homo sapiens	ACYCRIPACIAGERRYGTCIYQGRLWAFCC
3.	Human β-defensin 3 (hBD3)	1KJ6	Homo sapiens	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK
4.	Tilapia piscidin 4 (TP4)	5H2S	*Oreochromis niloticus*	FIHHIIGGLFSAGKAIHRLIRRRRR
5.	Napin	1PNB	*Brassica napus*	QPQKCQREFQQEQHLRACQQWIRQQLAGSPF
6.	Snakin-1	5E5Q	*Solanum tuberosum*	GSNFCDSKCKLRCSKAGLADRCLKYCGICCEECKCVPSGTYGNKHECPCYRDKKNSKGKSKCP
7.	Knot1 domain-containing protein	7C31	*Vitis vinifera*	RVCESQSHKFEGACMGDHNCALVCRNEGFSGGKCKGLRRRCFCTKLC
8.	Amaranthus caudatus-AMP2	1MMC	*Amaranthus caudatus*	VGECVRGRCPSGMCCSQFGYCGKGPKYCGR
9.	Antimicrobial peptide EcAMP1	2L2R	*Echinochloa crus-galli*	GSGRGSCRSQCMRRHEDEPWRVQECVSQCRRRRGGGD
10.	Nigellin-1.1	2NB2	*Nigella sativa*	DRYQDCLSECNSRCTYIPDYAGMRACIGLCAPACLTSR
11.	Plant defensin NsD7	5KK4	*Nicotiana suaveolens x Nicotiana tabacum*	AKDCKRESNTFPGICITKPPCRKACIREKFTDGHCSKILRRCLCTKPC
12.	Flower-specific gamma-thionin	6DMZ	*Zea mays*	RTCQSQSHRFRGPCLRRSNCANVCRTEGFPGGRCRGFRRRCFCTTHC
13.	Acyclotide ribe 31	7KPD	*Rinorea bengalensis*	AIPCGESCVYIPCISVVIGCSCRNKVCYR
14.	Antimicrobial peptide 1a	2LB7	*Triticum kiharae*	AQRCGDQARGAKCPNCLCCGKYGFCGSGDAYCGAGSCQSQCRGC

2. Methods

2.1. Selection and retrieval of antimicrobial peptides

The list of AMPs used in this study were recovered from antimicrobial peptide databases (Table 1). The 3D structures of selected peptides were downloaded from Protein Data Bank (PDB) (https://www.rcsb.org/) in. pdb format. These AMPs were selected based on the following criteria; experimentally approved AMPs tested on other bacterial pathogens and availability of the peptide secondary structure in PDB.

2.2. Retrieval of H. pylori receptor proteins

The 3D structure of *H. pylori* receptor proteins was downloaded from PDB in. pdb format. The selected *H. pylori* proteins used in this study were as follows: transcription factor HP1043 (PDB ID: 2PLN), oxygen-insensitive NADPH nitroreductase; RdxA (PDB ID: 3QDL), penicillin binding protein-2; PBP2 (PDB ID: 5LP4), and type I HopQ (PDB ID: 5LP2).

2.3. Peptide-protein docking

Docking analysis was performed using HADDOCK 2.4 webserver to observe the binding patterns and interactions between AMPs and *H. pylori* receptor proteins [18,19]. CPORT is used for the prediction of protein-protein interface residues and the predictions are designated as active and passive residues in HADDOCK [20]. Analysis of the HADDOCK created the binding score of the peptide-protein complexes. The HADDOCK scoring consists of a linear combination of various energies and buried surface area (http://bonvinlab.org/software/haddock2.4/scoring/). The scoring is performed according to the weighted sum (HADDOCK score) of various energies including van der Waals energy, electrostatic energy, desolvation energy and restraints violation energy. PyMOL Molecular Graphics System Educational version is used to visualize and draw interactions between AMPs and receptor proteins and determine the hydrogen bonding between amino acid residues. The distance of less than 3.5 Å was considered significant (https://proteopedia.org/wiki/index.php/Hydrogen_bonds) [21]. Chain A of each receptor protein is used in docking studies. Binding affinity of the protein complexes was determined using PRODIGY webserver (https://bianca.science.uu.nl/prodigy/) [22,23]. PRODIGY (PROtein binding enerGY prediction) is a collection of web services focused on the prediction of binding affinity (strength) in biological complexed as well as the identification of biological interfaces from crystallographic interfaces. The results are automatically generated by inserting the IDs of protein and peptide structures. This tool predicts the binding affinity between the protein-peptide complexes, offering a quantitative estimation of the strength of the interaction.

2.4. Molecular dynamics (MD) simulation

In this study, MD simulation was performed to evaluate the motions and fluctuations of the two protein complexes (Snakin1-PBP2 and EcAMP1-HopQ). The initial stages of protein-peptide complexes for molecular dynamics simulation were obtained from docking studies. These protein complexes were chosen based on the lowest binding affinity scores of AMPs to different *H. pylori* proteins. Molecular docking and simulations studies provide a prediction of binding status in static condition and in physiological environment, respectively. All simulations were performed using the MD software package AMBER20 and all the proteins were simulated using the Amber ff14SB forcefield together with the TIP3P water model (http://pubs.acs.org/doi/10.1021/acs.jctc.5b00255). First, each system

Table 2
Binding scores (in kcal/mol) of the AMPs docked against selected receptor proteins of *H. pylori*.

No.	AMP	Transcription factor, HP1043	oxygen-insensitive NADPH nitroreductase, RdxA	Penicillin binding protein-2, PBP2	Type I HopQ
1.	Cathelidicin, LL-37	−72.3 ± 8.3	**−94.2 ± 1.8**	**−102.4 ± 4.4**	−78.3 ± 2.0
2.	Human neutrophil defensin 1 (HNP-1)	−60.8 ± 5.5	−51.4 ± 14.5	−51.2 ± 2.6	−75.4 ± 4.1
3.	Human β-defensin 3 (hBD3)	−67.7 ± 11.2	−71.0 ± 3.8	−56.2 ± 6.0	−84.3 ± 8.7
4.	Tilapia piscidin 4 (TP4)	**−93.3 ± 10.5**	**−93.5 ± 13.6**	−83.5 ± 4.2	**−106.0 ± 5.0**
5.	Napin	−71.4 ± 6.1	−88.6 ± 21.3	**−101.7 ± 3.8**	−84.9 ± 3.1
6.	Snakin-1	−87.4 ± 2.7	−90.4 ± 13.4	**−103.8 ± 2.4**	−94.4 ± 6.9
7.	Knot1 domain-containing protein	−45.6 ± 4.0	−79.4 ± 18.4	−56.8 ± 4.8	−87.9 ± 6.3
8.	Amaranthus caudatus-AMP2	−59.0 ± 17.2	−73.4 ± 12.6	−63.0 ± 7.0	−71.0 ± 4.7
9.	Antimicrobial peptide EcAMP1	−58.0 ± 7.4	−63.0 ± 3.9	−91.0 ± 3.7	**−113.2 ± 11.6**
10.	Nigellin-1.1	−57.2 ± 4.9	−54.0 ± 2.5	−84.6 ± 2.8	−88.3 ± 5.2
11.	Plant defensin NsD7	−78.1 ± 9.2	−53.3 ± 8.9	−65.0 ± 10.1	−64.4 ± 4.7
12.	Flower-specific gamma-thionin	−68.5 ± 7.6	−42.6 ± 1.6	−66.5 ± 16.3	−98.7 ± 6.6
13.	Acyclotide ribe 31	−48.4 ± 3.1	−68.6 ± 6.9	−44.4 ± 3.0	−62.4 ± 10.7
14.	Antimicrobial peptide 1a	−62.4 ± 4.3	−52.9 ± 1.9	−73.8 ± 3.3	−94.0 ± 5.4

underwent a restrained minimization (force constant of 300 kcal mol−1 Å−2) using 500 steps of the steepest descent followed by conjugate gradient descent each. After minimization, the temperature of each solvated system was gradually increased from 0K to 300 K over 50 ps with using a restraint force constant of 100 kcal mol−1 Å−2 (https://pubs.acs.org/doi/full/10.1021/acs.jcim.3c01252). The system temperature was kept at 300K and controlled by the Langevin thermostat. The system pressure was monitored using the Berendsen barostat and kept at 1.0 bar. All bond lengths involving hydrogen atoms were constrained by the SHAKE algorithm using a time-step of 2.0 fs for every step. The particle mesh Ewald summation (PME) approach was used to calculate the long-range electrostatic interactions. In all cases, the non-bonded cut-off was fixed at 10.0 Å. Each system was simulated for a duration of 250 ns.

3. Results

The docking interaction analysis of the AMPs revealed that all AMPs bound to the receptor proteins with a high binding capacity. Among the tested AMPs, five AMPs (i.e., cathelicidin, Tilapia piscidin 4, napin, snakin-1, and antimicrobial peptide EcAMP1) showed the best HADDOCK scores against *H. pylori* proteins (Table 2). The peptide-protein complexes with the lowest binding energy were considered to be the most stable ones.

3.1. Interactions between AMPs and H. pylori transcription factor HP1043

H. pylori transcription factors, HP1043, plays a fundamental role in regulating essential cellular processes and this protein does not display a eukaryote homolog [24]. In this study, Tilapia piscidin 4 (TP4) with a HADDOCK score of −93.3 kcal/mol showed strong interactions with active amino acids of *H. pylori* transcription factor HP1043 compared with other AMPs. The binding affinity of this complex is −8.6 kcal/mol. The interaction of protein amino acids residues showed twelve hydrogen bonds with HP1043 of *H. pylori* (Table S1 and Fig. S1).

3.2. Interactions between AMPs with oxygen-insensitive NADPH nitroreductase (RdxA) of H. pylori

RdxA protein of *H. pylori* catalyses the reduction of metronidazole to form hydroxylamine which is a potent mutagen that is toxic to *H. pylori*. Mutation in *rdxA* leads to the development of metronidazole resistance [25]. Cathelicidin (LL-37) and Tilapia piscidin (TP4) showed similar HADDOCK score of −94.2 kcal/mol and −93.5 kcal/mol, respectively to RdxA protein. Thirteen and 19 hydrogen

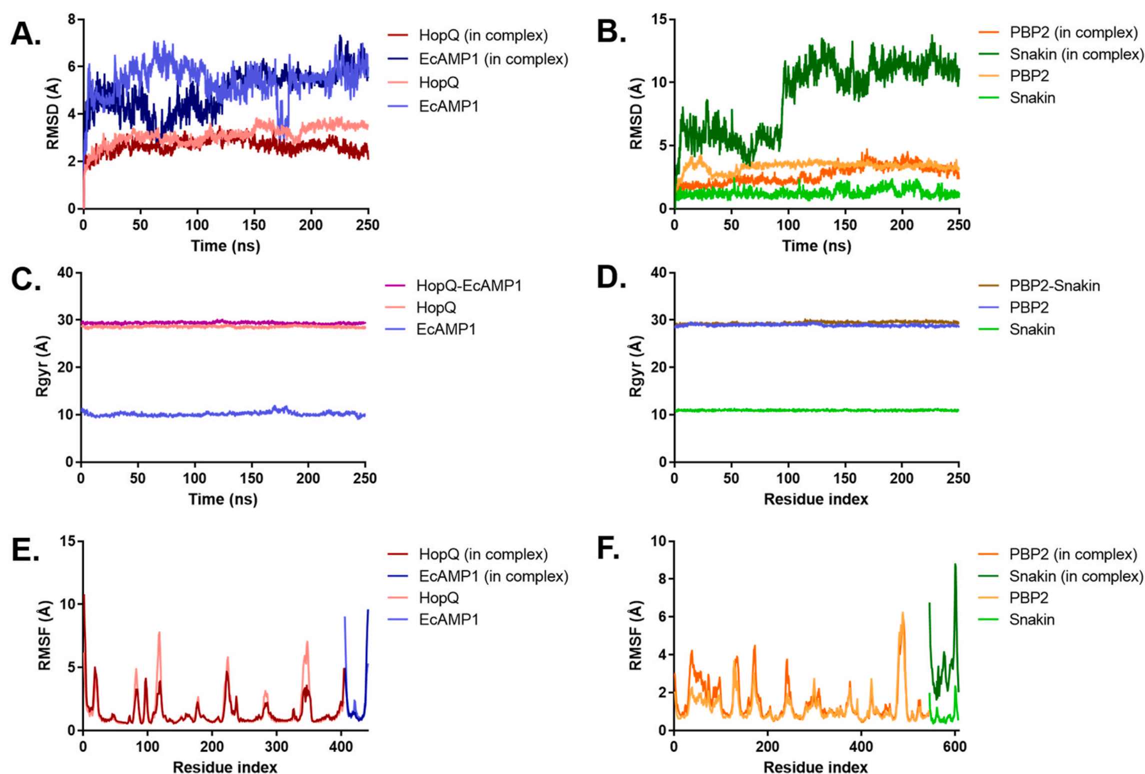

Fig. 1. Plots showing the RMSDs, Rgyr and RMSF. Plots showing the RMSDs of the components in the case of (A) EcAMP1-HopQ and (B) Snakin1-PBP2. Plots showing the Rgyr of the components in the case of (C) EcAMP1-HopQ and (D) Snakin1-PBP2. Plots showing the RMSF of the components in the case of (C) EcAMP1-HopQ and (D) Snakin1-PBP2.

bonds were formed between LL-37 and TP4, respectively to RdxA protein amino acids residues (Table S1, Figs. S2 and S3). Each LL-37-RdxA and TP4-RdxA complexes showed binding affinity of −9.9 kcal/mol.

3.3. Interactions between AMPs with penicillin-binding protein 2 (PBP2) of H. pylori

PBPs involve in bacterial cell wall formation by activating the glycosyltransferase and transpeptidase activities that lead to cross-linking of D-alanine and D-aspartic acid. Three AMPs, LL-37, napin and snakin-1 showed strong interactions with PBP2 of *H. pylori*. LL-37 and napin with a HADDOCK score of −102.4 kcal/mol and −101.7 kcal/mol showed six and seven hydrogen bonds with PBP2, respectively. Snakin-1 had the strongest binding score with a HADDOCK score of −103.8 kcal/mol showed 19 hydrogen bonds with PBP2 of *H. pylori* (Table S1, Fig. S4, S5 and S6). Binding affinity of these complexes were −10.6 kcal/mol, −9.1 kcal/mol and −11.1 kcal/mol for LL37-PBP2, napin-PBP2 and snakin1-PBP2, respectively.

3.4. Interaction between AMPs with type I HopQ of H. pylori

The outer membrane protein of *H. pylori* involves in bacterial adherence to gastric epithelial cells. *H. pylori* strains with *cag* pathogenicity island (PAI) usually carry type I HopQ alleles [26]. Among the AMPs, EcAMP1 showed the best binding score with type I HopQ of *H. pylori* with a HADDOCK score of −113.2 kcal/mol. EcAMP1 also showed the highest number of hydrogen bonds (n = 28) with amino acid residues of the receptor protein. TP4 had strong interactions with a score of −106.0 kcal/mol showed 21 hydrogen bonds with type 1 HopQ. Binding affinity of these complexes were −13.6 kcal/mol and −13.8 kcal/mol for TP4-HopQ and ECAMP1-HopQ, respectively (Table S1, Figs. S7 and S8).

3.5. Molecular dynamics simulation

Based on the best HADDOCK scores, two complexes (i.e., snakin-1 with PBP2 and EcAMP1 with type I HopQ) were selected for MD simulation analysis.

In the case of EcAMP1 with type I HopQ, the Root-Mean-Square-Deviation (RMSD) plots with respect to the starting conformation suggested that both the apo- and complexed-HopQ did not exhibit much structure fluctuation throughout the 250ns simulation (Fig. 1A). However, the EcAMP1 peptide exhibited high fluctuations between the RMSD values of 3-7Å before stabilizing after the 100ns mark (Fig. 1A). Meanwhile, in the case of Snakin-1 with PBP2, the apo-PBP2, complexed Snakin-1 and the apo-Snakin-1 maintained a constant RMSD fluctuation (Fig. 1B). This was in stark contrast with the complexed-Snakin-1 where the Snakin-1 experienced a large fluctuation after the 100ns mark (Fig. 1B). The abrupt change in the RMSD was due to the change in binding conformation of the Snakin-1 peptide to PBP2 (Fig. 2 and Fig. S9.). Next, we also analyzed the Radius of Gyration (Rgyr) for both complexes. In both EcAMP1-HopQ and Snakin1-PBP2 cases, the protein-peptide complexes, the apo-protein and the apo-peptide did not show any meaningful fluctuations. This suggested that all of them maintained their overall shape and did not undergo significant

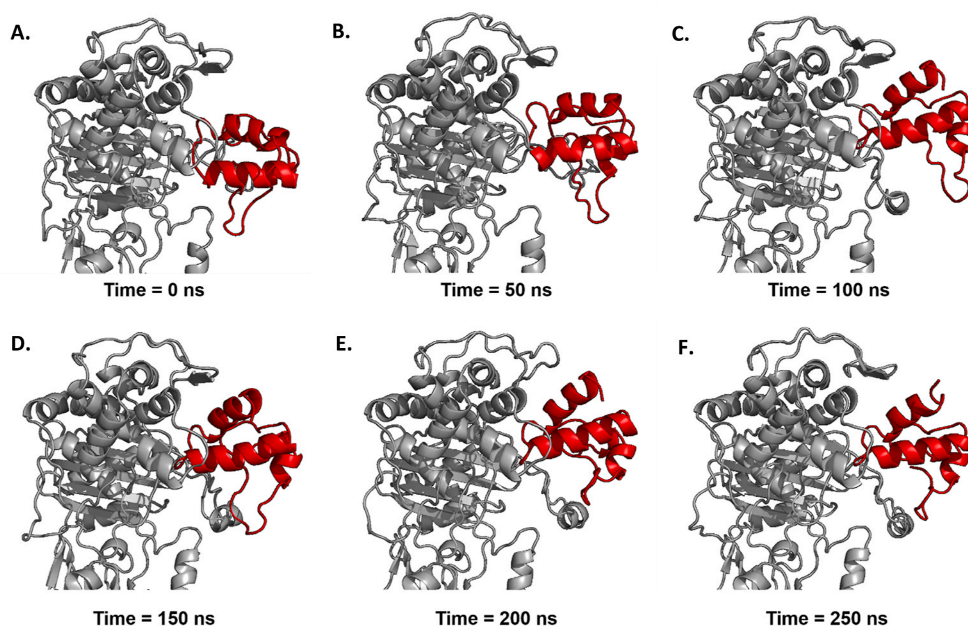

Fig. 2. Time-resolved conformations of Snakin-1 (red) bound to PBP2 (grey) at different time points starting from (A) 0 ns, (B) 50 ns, (C) 100 ns, (D) 150 ns, (E) 200 ns, and (D) 250 ns.

structural changes. (Fig. 1C and D). Finally, we analyzed the Root-Mean-Square-Fluctuation (RMSF) plot of EcAMP1-HopQ and Snakin1-PBP2. We compared the RMSF of the complexes with their respective apo-protein and apo-peptide. In the case of EcAMP1-HopQ, we noted that two regions on the HopQ protein, namely Ser128-Asp131 and Asp356-Met359 were stabilized (Fig. 1E). These two regions flank the binding site of the EcAMP1 peptide (Fig. 3A and C). At the same time, we also observed that the head domain (Gly 78 to Lys 93) of the PBP2 that was stabilized by Snakin-1 (Fig. 1F). This was surprising given that the Snakin-1 peptide was predicted to bind onto the transpeptidase domain (from Leu 233 to Leu 588; Fig. 3B and D). The protein-peptide interaction diagrams for EcAMP1-HopQ and Snakin1-PBP2 are shown in Fig. S10 and Fig. S11, respectively.

4. Discussion

Multidrug-resistant bacteria has emerged due to extensive use of antibiotics and increasing trends of the prevalence have been reported in many parts of the world. The existence of resistant strains causes traditional antibiotics to be ineffective or of limited use. Antibiotic resistance *H. pylori* strains is the major cause for the failure of standard triple therapy of *H. pylori* eradication regimen [27]. The increasing *H. pylori* resistance to clarithromycin, metronidazole and levofloxacin were found in many regions of the world. Therefore, developing novel and new anti-*H. pylori* agents to overcome the antibiotics resistant issue is urgently needed. In the present study, docking study was performed to explore molecular interactions and bindings affinity between antimicrobial peptides and *H. pylori* target proteins. Molecular dynamics simulation was then carried out with protein-peptide complexes to understand the conformational changes and the stability of their interaction in dynamic conditions. Understanding the protein-peptide interaction is important for structural-based drug designing.

Molecular docking study was used to understand the binding interactions of the peptides with four *H. pylori* proteins, i.e., transcription factor HP1043, oxygen-insensitive NADPH nitroreductase (RdxA), penicillin-binding protein-2 (PBP2) and outer membrane protein (type I HopQ). These proteins play an important role in survival and pathogenicity of *H. pylori* and seem to be the best candidates for drug or vaccine development. Our aim was to investigate the ability of the antimicrobial peptides to inhibit the protein targets as part of their mechanism of action. Our docking results showed the negative binding energy which indicate the favorable binding of peptides with all four receptor proteins. Among the tested AMPs, five peptides (LL-37, Tilapia piscidin 4, napin, snakin-1 and EcAMP1) showed the best binding interactions and HADDOCK scores (below −100 kcal/mol) against selected *H. pylori* proteins. The strongest binding affinity was observed in the interactions between Snakin-1 and PBP2, TP4 and HopQ and EcAMP1 and HopQ with −11.1, −13.6 and −13.8 kcal/mol, respectively.

The first analysis of this docking study was to determine which AMPs has good binding interaction with *H. pylori* proteins. Docking study of the AMPs against *H. pylori* transcription factor HP1043 shows that TP4 had strong binding score compared with other AMPs. For RdxA, two AMPs (LL-37 and TP4) show high binding scores towards the protein. Three AMPs (LL-37, napin and snakin-1) were docked with strong binding interaction to PBP2. Two AMPs (TP4 and EcAMP1) show high binding scores with type I HopQ, with the strongest binding was observed between EcAMPs and type I HopQ. EcAMP also shows the lowest binding affinity against type I HopQ due to the greater number of amino acids in binding interaction by hydrogen bonds. Results of this study showed snakin-1, TP4 and EcAMP1 as the best candidate with antimicrobial potential against H. pylori. These AMPs interact with PBP2 and type I HopQ proteins of *H. pylori* with strong binding affinity. The more negative and lower the value of binding affinity, the stronger the bonds between receptor and peptides. Research on the interaction of peptides against *H. pylori* proteins using molecular docking approach are scarce. Mustafa et al. [17] investigated the interaction of plant AMPs towards *H. pylori* RdxA and found that snakin-1 had the strongest binding scores compared with other AMPs. However, AMPs from other sources and other protein targets were not tested in their study. Our study is the first to investigate the binding interaction between AMPs from various sources and several protein receptors of *H. pylori*.

In this study, we didn't evaluate these properties for the selected peptides in respect to Lipinski rules. The Lipinski Rule of Five [28],

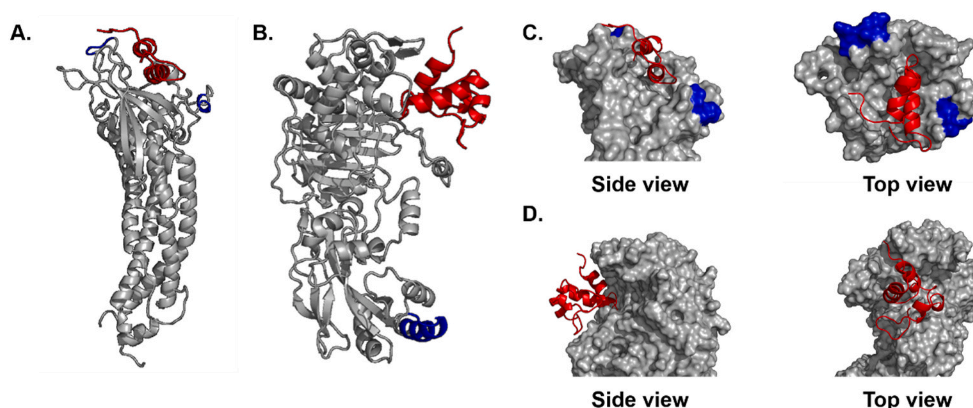

Fig. 3. Binding conformations of (A) EcAMP1-HopQ and (B) Snakin-PBP2. A zoom-up snapshot of the side and top views of (C) EcAMP1-HopQ and (D) Snakin-PBP2. The peptide is coloured in red, the protein in grey and the stabilized region coloured in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

frequently misapplied and misconstrued, was initially formulated to assist in the creation of drugs with oral bioavailability. It was not intended to provide directives for the medicinal chemistry development of all small-molecule drugs. When selecting compounds for screening, traditional "druglike" property cut-off values (e.g., Lipinski's "rule of five": MW < 500, LogP<5, hydrogen acceptor <10, and hydrogen donor <5) [28,29] might cause problems later during the lead development stage [29].

Since adherence to the Lipinski guidelines is such an important aspect of drug development, caution should be applied when investigating these peptides as potential therapeutic possibilities. To overcome this limitation, future study should include Lipinski rule analysis to provide a more detailed examination of the drug-like properties of the recommended peptides if the peptides can fulfil the rules. However, most the time the Lipinski rules are applied to chemical or small compound analysis. Peptides violate each and every one of these rules and hence, the need to improve their pharmaceutical properties.

In this study, two MD simulation were performed for snakin1-PBP2 and EcAMP1-HopQ complexes to compare proteins interaction in terms of structure and dynamic behaviour. The stability of the molecules was examined based on the RMSD, RMSF and Rg values. Comparing the RMSD values of both complexes, it shows that EcAMP1-HopQ complex is more stable than snakin1-PBP2 complex throughout the 250 ns simulation. Rg values show that both complexes are stable throughout the simulation. As shown by RMSF plot, the flexibility of residues in EcAMP1-HopQ complex is more stable than residues in snakin1-PBP2 complex. From this MD simulation analysis, EcAMP1 shows stable interaction and binding to type I HopQ of H. pylori protein without significant structural changes in different conditions. This indicates the highly potential of EcAMP1 as a new and novel antimicrobial agent that may inhibit *H. pylori* strains. This shows the potential application of antimicrobial peptide EcAMP1 as standalone or combination therapeutic agents to support the course of conventional antibiotics treatment.

In conclusion, the structural and interaction insights of the AMPs against *H. pylori* proteins in this study may provide a deep understanding for designing and developing anti-*H. pylori* agent. The knowledge obtained from this in silico analysis an able us to identify the interactive sites and interactions between EcAMP1 and active amino acids of type I HopQ protein of *H. pylori* in order to inhibit and target them directly. However, the results are preliminary and certainly need experimental validation using in vitro and in vivo studies. Further investigations are needed to understand the mechanism of action of the AMPs. Results of this study will help for further validation and exploration of new drugs to manage *H. pylori* infections and prevent the development of antibiotic resistance.

Data availability statement

All data were included in the article and supplements.

CRediT authorship contribution statement

Alfizah Hanafiah: Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Conceptualization. **Siti Nur Arifah Abd Aziz:** Writing – original draft, Methodology, Investigation, Formal analysis. **Zarith Nameyrra Md Nesran:** Writing – original draft, Methodology, Investigation, Formal analysis. **Xavier Chee Wezen:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Mohd Fadzli Ahmad:** Writing – review & editing, Validation, Supervision, Investigation, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Alfizah Hanafiah reports financial support was provided by Ministry of Higher Education (MOHE) of Malaysia. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28128.

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