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Research Paper

Peptide Based Hepatitis C Vaccine Design From RNA-dependent RNA polymerase (RdRp) NS5B: Immunoinformatics Approach

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ABSTRACT: *Hepatitis is an inflammatory disease of the liver that can be caused by various factors. A common cause of hepatitis is a viral infection. Until now, there is no effective vaccine for Hepatitis C Virus infection so it is still a health problem in the world. This study aims to obtain a peptide-based vaccine design that acts as an antigen, does not cause an allergen, is not toxic, and is not homologous to human cells. This study used NS5B RNA-dependent RNA polymerase (RdRp) samples taken from PDB. All stages of the analysis are carried out using the appropriate web server and software. Sequence analysis obtained selected T-cell epitopes, namely LSAFSLHSY, VLDDHYRDV, YLFNWAVKT, TLTCYLKASAACRAA, NTLTCYLKASAACRA, and LTCYLKASAACRAAK. Meanwhile, the selected B-cell epitopes were FCVQPEKGGRK and DSTVTENDIRV. Which was given the connectors EAAAK, AAY, GPGPG, and KK on each of the corresponding epitope connectors. Then, the vaccine candidate is docked and visualized in 2D and 3D. The research shows that the design of the hepatitis C vaccine using the NS5B protein is stable and not homologous to human cells so it can be used as a vaccine candidate.*

KEYWORDS: *Epitope, Hepatitis C, Immunoinfomatics, Vaccine Design*

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I. INTRODUCTION

Hepatitis was a liver inflammation disease that could be caused by various factors such as genetic factors, viral infections, alcohol, and drugs. The common cause of hepatitis was a viral infection. The Hepatitis C virus (HCV) was one of the viruses that caused hepatitis and were considered the most dangerous among other hepatitis viruses. Most patients who were infected with the hepatitis C virus did not show any symptoms. Thus, many were unaware that they had been infected with the hepatitis C virus until liver damage appeared (Alhawaris 2019).

According to data from the World Health Organization (WHO), an estimated 58 million people worldwide have been infected with chronic hepatitis C, with about 15.2 million people being infected with chronic hepatitis C each year. There are also 3.2 million adolescents and children with chronic hepatitis C infection. In Indonesia, it is estimated that 6.6-7 million people have been infected with hepatitis C, with a prevalence that varies greatly from 0.5% to 3.37%. In 2019, WHO estimated that about 290,000 people died from hepatitis C, mainly due to cirrhosis and hepatocellular carcinoma (Prasetya, Nugroho, and Triloka 2022).

Until now, there has been no effective vaccine to prevent the spread of Hepatitis C virus infection. Therefore, it is important to design new vaccines that can be used as vaccine candidates to control the spread of hepatitis C virus infection (Pradana et al. 2021).

Technology has developed in line with the genomic era so that genetic information can be used for vaccine development. Currently, the discovery of vaccine candidates using the immunoinformatics approach has been developed. Immunoinformatics is a branch of bioinformatics that deals with the computational analysis of immunology data. The immunoinformatics approach has been used to design vaccines against several infectious diseases. By predicting the appropriate antigen, epitope, carrier, and adjuvant for vaccine candidates, the immunoinformatics can efficiently save time and costs in vaccine development (Ahammad and Sultana 2020).

The principle of immunoinformatics is to predict peptide binding with MHC (Major Histocompatibility Complex). Peptide-based vaccines do not use whole microbes, making them safe to use. The vaccine that can be developed using the immunoinformatics approach is the peptide vaccine. The peptide vaccine is a vaccine that

consists of several amino acid residues in the form of epitopes, and proteins are the main compounds of peptide vaccine candidates. The immunoinformatics approach can be used to design peptide vaccines against the hepatitis C virus using the virus peptide sequence (Rezaldi et al. 2021).

The RNA genome of hepatitis C virus encodes a polyprotein that is processed to generate at least 10 viral proteins, including structural and non-structural proteins. NS5B protein is an RNA-dependent RNA polymerase and plays a key role in the replication of hepatitis C virus, making NS5B an attractive target for the hepatitis C vaccine discovery (Polamreddy, Vishwakarma, and Saxena 2018).

This research aims to discover a peptide-based hepatitis C vaccine candidate from NS5B protein using an immunoinformatics approach. This study is expected to assist in the process of discovering more effective and efficient hepatitis C vaccine candidates, which could be beneficial for vaccine development in Indonesia, especially for hepatitis C disease.

II. MATERIALS AND METHODS

2.1 NS5B Sequence Data Retrieval

The virus sequence data was obtained from the Protein Data Bank (PDB) web server: (www.rcsb.org). The sequence of the NS5B protein was extracted from the FASTA format and further analyzed (Shin and Cho 2005)

2.2 Antigen analysis and allergen analysis

Antigen analysis was performed on the web server: (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) with the target organism: Virus and Threshold: 0.4. Allergen analysis was conducted online using the web server: (https://www.ddgpharmfac.net/AllerTOP/) (Alom et al. 2021).

2.3 MHC-1 Epitope Analysis

Analysis MHC was performed on the IEDB web server (http://ledb.org). MHC-I using identification from Net MHC pan EL 4.1 and allele codes HLA-A01:01 and HLA-A02:01 with a 9-amino acid length. The analysis results were obtained with a percentile rank value of less than 1.00 (Alom et al. 2021; Fadaka et al. 2021; Sharma et al., 2021).

2.4 MHC-II Epitope Analysis

MHC-II epitope analysis was performed by analyzing the sequence using the IEDB recommended 2.22 method and allele code DRB1*01:01, with a 15-amino acid length. The analysis results were obtained with a percentile rank value of less than 10.00 (Alizadeh et al. 2022; Alom et al. 2021; Fadaka et al. 2021).

2.5 B-Cell Epitope Analysis

B-cell epitope analysis was also performed on the IEDB web server: (http://ledb.org). The analysis that B-cells were epitope-specific with a peptide length ranging from 10-30 amino acids (Alizadeh et al. 2022).

2.6 Selected Toxin & Homolog Epitope Analysis

Toxin analysis was performed on the web server: (http://webs.iiitd.edu.in/raghava/toxinpred/design.php) using the SVM-based method (support vector method). Homology analysis was conducted on the NCBI BLAST on the web server: (http://blast.ncbi.nlm.nih.gov), and if the E-value is greater than 0.05, the epitope is considered non-homolog (Alom et al. 2021).

2.7 Final Vaccine Candidate Design

The method used to design the vaccine candidate was the design with linker and adjuvant. The vaccine candidate was formulated as follows: Adjuvant-EAAAK-(MHC-I)-AAY-(MHC-I)- GPGPG-(MHC-II)-KK-(B-cell epitope) with the appropriate linkers for each component (Alom et al. 2021).

2.8 Physicochemical analysis

Physicochemical analysis, including molecular weight, aliphatic index, amino acid composition, GRAVY, and instability index were performed using the Expasy web server (https://web.expasy.org/protparam/). Solubility analysis using the Protein-sol web server (https://protein-sol.manchester.ac.uk/) (Alom et al., 2021).

2.8 Secondary Structure Prediction of Final Vaccine

The next is to visualize the vaccine design to see the vaccine's secondary structure using the PSIPRED v4.0 web server (PSI-blast based secondary structure prediction): (http://bioinf.cs.ucl.ac.uk/psipred/) (Alom et al., 2021).

2.9 Vaccine Candidate 3D Visualization

The results of the vaccine candidate designs are visualized on the webserver page: (http://galaxy.seoklab.org/) → galaxy TBM (Alom et al., 2021). Further refinement of the predicted 3D structure with galaxy refine on the webserver: (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE) (Alom et al., 2021;Ikram et al., 2018).

3.0 *Molecular Docking*

Docking of vaccine candidates was performed using ligands and TLR4 receptors extracted from PDB data (www.rcsb.org). Haddock web server (https://wenmr.science.uu.nl/haddock2.4/) (Alom et al., 2021).

3.1 Visualization 2D & 3D Vaccine Candidates

The results of docking the vaccine candidates were then visualized using the Ligplot software to observe 2D visualization as well as the interactions and bonds between the vaccine and the TLR4 receptor. Meanwhile, the 3D visualization of the vaccine was examined using the Yasara software (Balupuri and Cho 2013).

III. RESULT AND DISCUSSION

The adaptive immune, whether humoral or cellular, was very important in fighting virus infections that entered the human body. Humoral immunity, which was mediated by antibodies produced by B-cells, bound to the virus and could prevent the virus from entering cells. Cellular immunity, which was mediated by T-cells, killed virus-infected cells, thus preventing the spread of the infection to other cells (Gustiananda et al. 2021).

With the advancement of bioinformatics technology, it became easy to find data and information about the genomics of various infectious pathogens. Bioinformatics played a crucial role in predicting potential vaccine candidates, accelerating vaccine development, and cost-saving simultaneously. This immunoinformatics technique has been used to design epitope-based vaccines for various viral, bacterial, fungal, and parasitic infections (Alom et al., 2021).

3.1 The NS5B sequence data

The sequence data used for this study was the RNA-dependent RNA polymerase (RdRp) protein NS5B from Hepatitis C virus, which was obtained from the protein data bank with the code (PDB ID: 2GIQ) (Le Pogam et al., 2006). The sequence analysis showed that the predicted antigenicity of the sequence had a score of 0.4028 using Vaxijen v2.0, with a threshold score of 0.4. This value was determined to evaluate the accuracy and sensitivity of the test. Additionally, the sequence was predicted not to cause any allergies, according to the results of the AllerTOP prediction described in Table 1 (Alom et al., 2021).

Table 1. Results of Protein Sequence Analysis

Protein	Sequence	Antigen	Allergen
RNA-dependent RNA polymerase (RdRp) NS5B	MHHHHHHMSYTWTGALITPCAAEESKLPINALSNSLLRHHNMVYATTSRSAG LRQKKVTFDRLQVLDDHYRDVLKEMKAKASTVKAKLLSVEEACKLTPPHSA KSKFGYGAKDVRNLSSKAVNHIHSVWKDLLEDTVTPIDTTIMAKNEVFCVQPE KGGRKPARLIVFPDLGVRVCEKMALYDVVSTLPQVVMGSSYGFQYSPGQRVE FLVNTWKSKKNPMGFSYDTRCFDSTVTENDIRVEESIYQCCDLAPEARQAIKSL TERLYIGGPLTNSKGQNCGYRRCRASGVLTTSCGNTLTCYLKASAACRAAKL QDCTMLVNGDDLVVICESAGTQEDAASLRVFTEAMTRYSAPPGDPPQPEYDL ELITSCSSNVSVAHDASGKRVYYLTRDPTTPLARAAWETARHTPVNSWLGNII MYAPTLWARMILMTHFFSILLAQEQLEKALDCQIYGACYSIEPLDLPQIIERLH GLSAFSLHSYSPGEINRVASCLRKLGVPPLRVWRHRARSVRARLLSQGGRAAT CGKYLFNWAVKTKLKLTPIPAASRLDLSGWFVAGYSGGDIYH	0.4028	Non Allergen
Webserver	PDB	VaxiJen v2.0	AllerTOP v2.0

3.2 T-Cell Epitopes Analysis

T-Cell is an important agent of cell-mediated immunity. T-Cell helper (Th) recognizes virus peptides associated with MHC-II proteins, while Sel-T cytotoxic (Tc) recognizes virus peptides associated with MHC-I proteins (Bhatnager et al. 2021).

Table 2. T-Cell Sequence Analysis Results (MHC-I and MHC-II)

	Epitope	*Allele*	Antigen	Allergen	Toxin	Homolog	*Percentile Rank*
MHC-I	LSAFSLHSY	HLA-A*01:01	Yes	Non	Non	Non	0.07
	ALYDVVSTL	HLA-A*02:01	Non	Yes	Non	Non	0.01
	KLQDCTMLV	HLA-A*02:01	Non	Yes	Non	Non	0.06
	VLDDHYRDV	HLA-A*02:01	Yes	Non	Non	Non	0.15
	YLFNWAVKT	HLA-A*02:01	Yes	Non	Non	Non	0.17
	HSAKSKFGY	HLA-A*01:01	Yes	Yes	Non	Non	0.17
MHC-II	TLTCYLKASAACRAA	Alel DRB1*01:01	Yes	Non	Non	Non	2.30
	NTLTCYLKASAACRA	Alel DRB1*01:01	Yes	Non	Non	Non	2.50
	AKDVRNLSSKAVNHI	Alel DRB1*01:01	Yes	Yes	Non	Non	3.30
	LTCYLKASAACRAAK	Alel DRB1*01:01	Yes	Non	Non	Non	3.30
	TCYLKASAACRAAKL	Alel DRB1*01:01	Yes	Yes	Non	Non	3.30
	KDVRNLSSKAVNHIH	Alel DRB1*01:01	Yes	Yes	Non	Non	3.90

The MHC-I analysis was performed using the allele codes HLA-A01:01 and HLA-A02:01 with a 9-amino acid length and a percentile rank of less than 1.00, resulting in several selected epitopes. Meanwhile, the MHC-II epitope analysis using Alel DRB1*01:01 code resulted in several selected epitopes with a 15-amino acid length and a percentile rank of less than 10.00, as shown in Table 2 (Alom et al., 2021; Fadaka et al., 2021; Sharma et al., 2021).

All selected Sel-T epitopes (MHC-I and MHC-II) have undergone further analysis, some of which meet the epitope requirements, where the epitope must be antigenic, non-allergenic, non-toxic, and non-homologous to humans, thus making them suitable candidates for vaccine development (Sanami et al. 2022).

3.3 B-Cell Epitopes Analysis

B-Cell plays a crucial role in vaccine development as it is the main component of humoral immunity. Antibodies produced by B-cell play a vital role in preventing the spread of viral infections. Linear B-cell epitopes are responsible for producing specific antigenic antibodies (Alizadeh et al., 2022). The B-cell analysis was performed using the Bepipred 2.0 method, which resulted in several epitopes that have been described in Table 3.

Table 3. B-Cell Sequence Analysis Results

Epitope	Antigen	Allergen	Toxin	Homolog
FCVQPEKGGRK	Yes	Non	Non	Non
PCAAEESKLPINALSNSLLR	Non	Yes	Non	Non
STLPQVVMGSSYGFQYSPGQ	Non	Non	Non	Non
DSTVTENDIRV	Yes	Non	Non	Non

The accuracy of the BepiPred method for predicting B cell epitopes reaches 80%. The BepiPred method predicts the location of B-cell linear epitopes (Simarmata et al. 2022). Figure 1 shows that the yellow area with a score above the threshold of 0.50 is the B cell epitope with a positive prediction in yellow, while the negative prediction is in the green area (Sinha, Grewal, and Roy 2020).

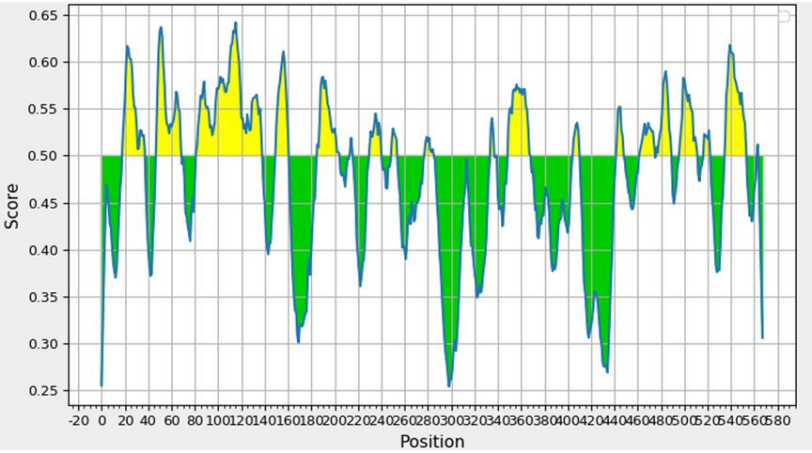

Figure 1. Graph of B Cell Epitope Prediction From NS5B Protein

3.4 Analysis of Selected Epitopes

All of the analyzed epitopes above (MHC-I, MHC-II & Sel B) have met the requirements as final vaccine candidates. After analyzing antigens, allergens, toxins, and homologs, several epitopes were obtained as vaccine candidates from MHC-I, MHC-II, and Sel B analyses, as shown in Table 4.

Table 4. Selected Epitopes

	Epitop	Antigen	Alergen	Toksin	Homolog
MHC-I	LSAFSLHSY	Yes	Non	Non	Non
	VLDDHYRDV	Yes	Non	Non	Non
	YLFNWAVKT	Yes	Non	Non	Non
MHC-II	TLTCYLKASAACRAA	Yes	Non	Non	Non
	NTLTCYLKASAACRA	Yes	Non	Non	Non
	LTCYLKASAACRAAK	Yes	Non	Non	Non
Sel-B	FCVQPEKGGRK	Yes	Non	Non	Non
	DSTVTENDIRV	Yes	Non	Non	Non

All selected epitopes were used as final vaccine candidates. Antigenicity is the ability of an antigen to stimulate the formation of specific antibodies. Meanwhile, allergenicity is the ability of a substance to cause an allergic reaction. And not homologous with humans means that the peptide vaccine protein does not show similarity to the human genome, so the vaccine does not elicit an autoimmune response when used as a component of a peptide vaccine (Rezaldi et al. 2021).

3.5 Final Vaccine Candidate Design

In the past, Human β-defensin HBD3 was chosen as an adjuvant to enhance the effectiveness of the vaccine by stimulating a strong immune response. EAAAK (Glu-Ala-Ala-Ala-Lys), AAY (Ala-Ala-Tyr), GPGPG (Gly-Pro-Gly-Pro-Gly), and KK (bi-lysine) were used as linkers to connect the vaccine sequences (Alizadeh et al., 2022; Alom et al., 2021). As described in Figure 2. Linkers that have been designed as in Table 5 are used as the final vaccine candidate sequence.

Linkers play an important role in minimizing functional immunogenicity and also in maintaining the identity of each epitope during the processing of the vaccine in cells thereby ensuring the immunogenicity of each epitope. The EAAAK linker is a rigid α-helix peptide linker that provides efficient separation of functional domains in fusion proteins, thus enhancing antigenicity and immunogenicity. Epitopes fused using the AAY linker are effectively separated within the cell, thereby reducing junctional immunogenicity and increasing epitope presentation to enhance protein stability (Ayyagari et al. 2022).

The GPGPG linker has been proven to be able to induce a highly important Th lymphocyte response for vaccines. The GPGPG linker can stimulate MHC-II response and enhance conformation-dependent immunogenicity as well as antibody epitopes. The KK linker also played an important role in reducing junctional immunogenicity by avoiding the induction of antibodies to peptide sequences that can be formed by each epitope when combined linearly (Ayyagari et al. 2022).

Tabel 5. ***Design Vaccine with Adjuvant and Linkers***

Design Vaccine with Adjuvant and Linkers	**Jumlah Asam Amino**
GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRK K**EAAAK**LSAFSLHSY**AAY**VLDDHYRDV**AAY**YLFNWAVKT**GP** **GPG**TLTCYLKASAACRAA**GPGPG**NTLTCYLKASAACRA**GPGP** **G**LTCYLKASAACRAAK**KK**FCVQPEKGGRK**KK**DSTVTENDIRV	169

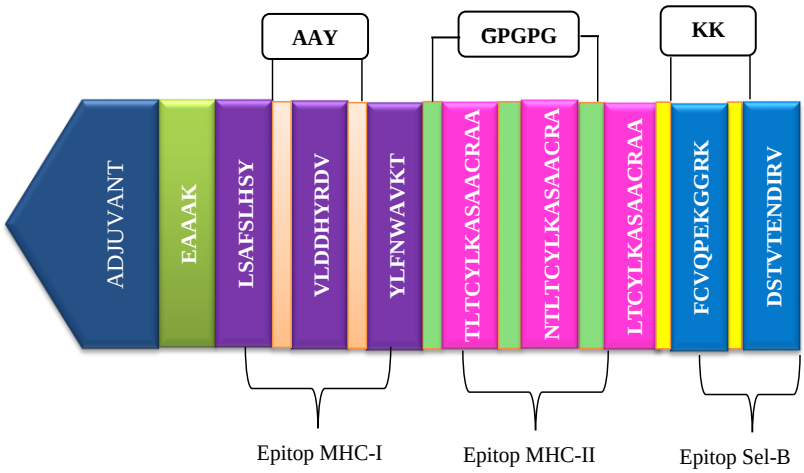

Figure 2. Graphic of Final Vaccine Candidate Designs (From Left to Right)

In this study, β-defensin was used as an adjuvant because β-defensin is known to be an effective adjuvant when conjugated with an antigen. Vaccines containing β-defensin as an adjuvant have been studied both in vivo and in vitro to activate the primary innate antiviral immune response and mediate other immunomodulatory activities against various viruses (Behmard et al., 2020). Based on this literature, the vaccine adjuvant design in this study used Human β-defensin HBD3 with the adjuvant code: GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK (Ikram et al. 2018).

3.6 Physicochemical analysis

The final result of the vaccine candidate design was re-analyzed and it was found that the designed vaccine candidate had an antigenic score of 0.6408, consistent with what was predicted by VaxiJen v2.0.

Additionally, the vaccine candidate was predicted to not cause allergies when used, according to AllerTOP v2.0. as shown in Table 5 (Alom et al., 2021).

The physicochemical properties analysis presented in Table 6 indicated that the vaccine's molecular weight is 18199.18 g/mol, with a molecular formula of $C_{794}H_{1286}N_{238}O_{226}S_{13}$. Its theoretical pI is 9.69, identifying it as a basic property. Furthermore, the vaccine's stability index is 31.05, indicating that the vaccine protein complex is stable. The aliphatic index is 67.16, indicating that the peptide vaccine is thermally stable. The total number of negatively charged residues (Asp+Glu) is 10, and the total number of positively charged residues (Arg+Lys) is 31(Ahmad and Komari 2022; Panda and Chandra 2012).

The average hydropathicity score (GRAVY) is -0.336. A positive GRAVY value indicates hydrophobic behavior, while a negative value indicates hydrophilic behavior. This average hydropathicity score indicates that overall, the protein is hydrophilic and can interact better with water molecules in its surroundings (Ahmad & Komari, 2022). And for the solubility analysis, with a score of 0.773 according to the protein-sol prediction. The scaled solubility value (QuerySol) is the solubility predicted with the population average for the experimental dataset (PopAvrSol), which is 0.45.

Table 6. Physicochemical Analysis, Antigen, Allergen and Vaccine Candidate Solubility

Parameter	Database	Value
Number of amino acids	ProtParam	169
Molecular weight	ProtParam	18199.18
Molecular formula	ProtParam	$C_{794}H_{1286}N_{238}O_{226}S_{13}$
Theoretical pI	ProtParam	9.69
Number of negatively charged residues (Asp+Glu)	ProtParam	10
Number of positively charged residues (Arg+Lys)	ProtParam	31
Stability index	ProtParam	31.05
Aliphatic index	ProtParam	67.16
Grand average of hydropathicity (GRAVY)	ProtParam	-0.336
Antigenicity	VaxiJen v.2.0	0.6408
Allergenicity	AllerTOP v2.0	Non-Allergen
Solubility	Protein-sol	0.773

3.7 Secondary Structure Prediction of Final Vaccine

The next step involved predicting the secondary structure of the vaccine candidate using the web server for PRISPRED v4.0 analysis of the vaccine construct sequence. The results of the analysis are shown in Figure 3, indicating the presence of helix, strand, and coil structures (Akter et al. 2022).

The helix structure generally describes the characteristics of transmembrane proteins because the hydrogen bonds of the helix structure form the backbone of the molecule. The coil structure functions in flexibility and conformational changes, where the peptide bonds in the coil are not involved in the hydrogen bonding of the protein (Ruslin, Putri, and Arba 2019).

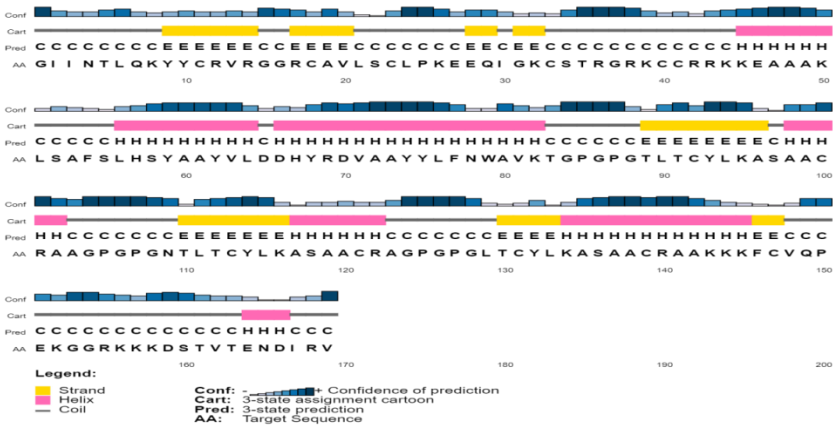

Figure 3. Secondary Structure Prediction of Final Vaccine

3.8 Vaccine Candidate 3D Visualization

The 3D structure visualization of the vaccine was modeled through GalaxyWEB. The 3D prediction visualization was used for evaluation, molecular docking with receptors, and further refinement. The refine model was used for evaluation and further refinement (Alom et al., 2021).

The GalaxyRefine server produced a refined vaccine candidate model, and the result of the GalaxyRefine visualization is shown in Figure 4. Next, the 3D visualization results were used for molecular docking, which will be combined with the receptor to become the final vaccine (Ikram *et al*., 2018).

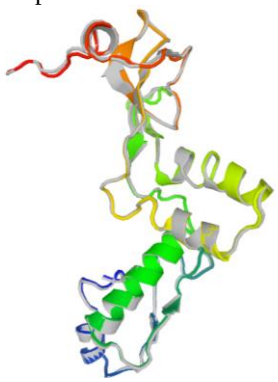

Figure 4. Vaccine 3D Structure Enhanced By Galaxyrefine

3.9 Molecular Docking

Molecular docking of the vaccine candidate was performed to combine the TLR4 receptor and ligand from the 3D visualization results of GalaxyRefine. The receptor used was TLR4, which was taken from the PDB database (PDBID: 4G8A). TLR4 is a pathogen recognition receptor on the surface of immune cells that plays an important role in the maturation process of each cell. TLR4 is used to enhance the immune response in the body (Alom et al., 2021).

The receptor was prepared first using Yasara software. The docking was performed on the Haddock web server, which combines the TLR4 receptor and the vaccine protein from GalaxyRefine. Ten best docking models were obtained in Table 7, and the docking result with the lowest score was chosen. Cluster 1 was chosen because it had the smallest score of -83.0 +/- 1.8 (Ikram et al., 2018).

Table 6. Skor Haddock Result

Model	Skor Haddock
Cluster 1	-83.0 +/- 1.8
Cluster 7	-74.8 +/- 7.6
Cluster 10	-64.2 +/- 12.8
Cluster 4	-58.5 +/- 7.0
Cluster 11	-55.0 +/- 12.0

The best docking result is the one with the lowest score among the other docking results. The negative sign in the docking score indicates a strong binding affinity between the ligand and the enzyme, which suggests the formation of a stable complex (Tambunan and Alamudi 2010). The molecular docking results were visualized using Yasara software, as shown in Figure 5. The vaccine binding is indicated in red, while the TLR4 receptor structure is shown in green.

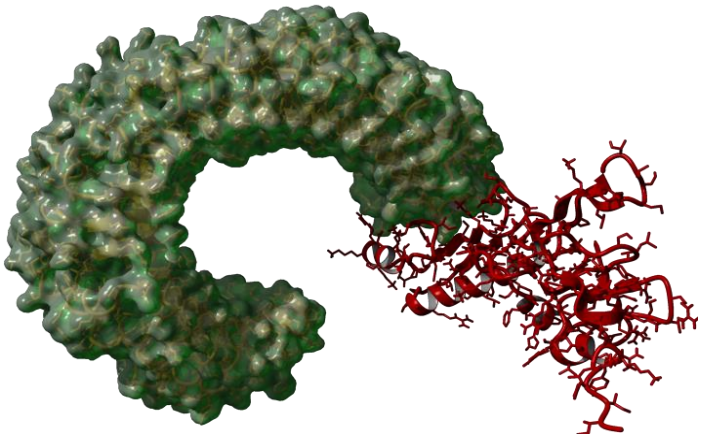

Figure 5. TLR4 (Green) and Vaccine (Red) Molecular Docking Results, 3D Visualization

The amino acids that interact between the TLR4 receptor and the vaccine in the docking results were visualized in 2D using DIMPLOT in the LigPlot application, as shown in Figure 6. There are hydrogen bonds and hydrophobic interactions between the TLR4 receptor and the vaccine. The interactions that are formed

indicate the formation of ten hydrogen bonds, between nine residues of the TLR4 receptor and six residues of the vaccine (Bouzari and Savar 2014).

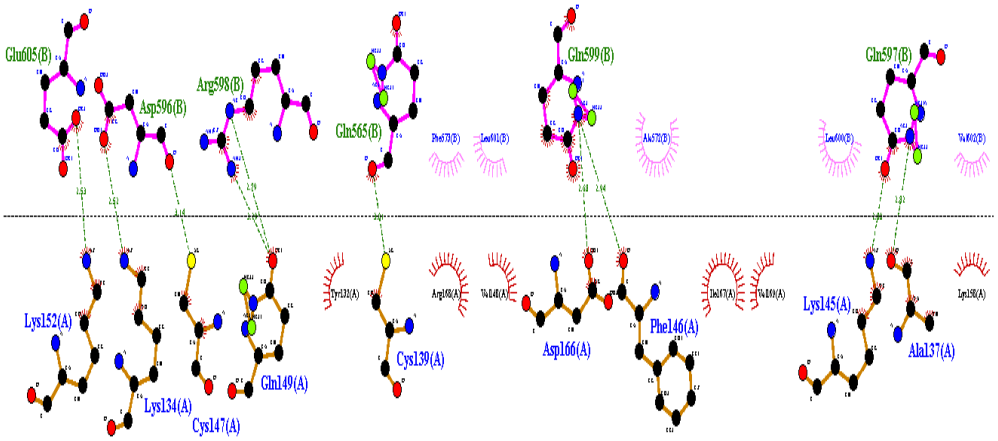

Figure 6. Interaction Between Amino Acid Chain A (TLR4) And Chain B (Vaccines), 2D Visualization

The dotted green lines indicate the hydrogen bonds between the amino acid residues of the receptor and the amino acid residues of the vaccine. Meanwhile, drawing circular, curved red lines, easy-to-curve red lines, and pink circular lines indicate the amino acid residues that form hydrophobic interactions around the amino acid residues (Rezaldi et al., 2021).

From the results and discussion above, it can be concluded that the hepatitis C vaccine candidate from the RNA-dependent RNA polymerase (RdRp) NS5B protein with TLR4 receptor binding is antigenic, non-allergenic, non-toxic, and non-homologous to humans. The vaccine candidate is predicted to have suitable physicochemical characteristics such as solubility and stability. The 2D and 3D structure of the vaccine candidate shows good binding and interactions with the receptor to enhance immune response. Therefore, this study suggests that the vaccine candidate can be used as a vaccine to combat hepatitis C infection. However, the results of this study need to be validated by further research both in vitro and in vivo.

IV. CONCLUSION

Based on the research results, the peptide sequence that has the potential as a hepatitis C vaccine candidate is GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKKEAAAKLSAFSLHSY AAYVLDDHYRDVAAYYLFNWAVKTGPGPGTLTCYLKASAACRAAGPGPGNTLTCYLKASAACRAGP GPGLTCYLKASAACRAAKKKFCVQPEKGGRKKKDSTVTENDIRV. It also has physicochemical properties that indicate the hepatitis C vaccine candidate in this study has stable stability index, antigenic properties and does not cause allergic reactions. Homologous vaccine design analysis also showed that the vaccine candidate is non-homologous to human cells, making the vaccine design a potential Hepatitis C vaccine candidate.

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