

Evaluating the Vaccine Potential of a Tetravalent Fusion Protein against Coronavirus (COVID-19)

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ABSTRACT

Coronaviruses are a type of viruses which cause illness ranging from the common cold to other diseases. SARS-CoV-2 is one coronavirus family that cause respiratory syndrome. The virus first isolated from three people in Wuhan. This virus became known as COVID-19. Common signs of infection comprising of fever, respiratory symptoms, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. There is not any vaccine for COVID-19. This study was aimed to design and analysis of recombinant vaccine against COVID-19.

In this research the completely sequence of Envelope and Nucleocapsid protein was fused to multi epitopes (B and MHC I epitopes) obtained from Spike protein and RNA-dependent RNA polymerase and constructed a fusion vaccine.

The vaccine has 621 amino acids which 51 negatively charged residues and 118 positive charged amino acids with 71.906 kDa. The estimated half-life of peptide was found to be greater than 30 hours in mammalian reticulocytes, greater than 20 hours in yeast cells, and greater than 10 hours in *E.coli*. The instability index II is computed to be 34.81. So, this classifies the protein as stable. The aliphatic index of COVID-19 is found to be 66.86, so the vaccine is probable to be thermostable. The results obtained from protparam and pepcalc analysis revealed that the recombinant antigen is soluble in water. Ramachandran analysis of recombinant antigen showed that 84.3% of amino acids are in most favored regions; this result supported the high-quality structure of the refined model of recombinant vaccine. The result of docking analysis proved that the vaccine has most affinity to HLA B2705-KK10, HLAB3508, HLA-A0201, and HLA B5701. The result of this research revealed that the vaccine has antigenic property and stable structure. The vaccine could be produced by Recombinant DNA technology and expressed in host cells and need to experiment on laboratory animals.

Keywords: COVID-19; Coronavirus; Recombinant vaccine; B-cell

INTRODUCTION

Coronaviruses are a type of viruses which cause illness ranging from the common cold to other diseases, for example MERS (Middle East Respiratory Syndrome) and SARS (Severe Acute Respiratory Syndrome). SARS-CoV-2 is one coronavirus family that cause respiratory syndrome. The virus first isolated from three people in Wuhan. This virus became known as COVID-19 (WHO, 2019).

The COVID-19 is the cause of the 2019-2020 coronavirus epidemic [1]. The coronavirus appeared in Wuhan and is believed to have jumped to humans at seafood and animal market where many of the first people to become infected worked (CDC, 2019). The main mode of transmission of COVID-19 is from human to human by respiratory droplets that people respire during sneezing and coughing [2]. According to the Centers for Disease Control and Prevention (CDC), the

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Received date: March 5, 2020; **Accepted date:** March 21, 2020; **Published date:** March 28, 2020

Citation: Tazehkand MN, Hajipour O (2020) Evaluating the Vaccine Potential of a Tetravalent Fusion Protein against Coronavirus. J Vaccines Vaccin. 11:411 DOI: 10.35248/2157-7560.20.11.411

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coronavirus' incubation period is believed to be 2 to 14 days. However, one case is reported as having an incubation period of 27 days [3].

Common signs of infection comprising of fever, respiratory symptoms, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death [1]. No drug has yet been discovered to treat COVID-19 infections. Antivirals being tested include chloroquine, darunavir, galidesivir, interferon beta, the lopinavir/ritonavir combination, the RNA polymerase inhibitor remdesivir, and triazavirin [4,5]. Remdesivir and chloroquine effectively inhibit the coronavirus *in vitro* [6]. There is not any vaccine for COVID-19. The United States NIH (National Institutes of Health) is collaborating with Moderna to generate an RNA vaccine from spike protein of the coronavirus. In another research, Inovio Pharmaceuticals is developing a DNA vaccine [7]. The University of Queensland (Australia) is studying the potential of a molecular clamp vaccine that would genetically modify viral proteins in order to stimulate an immune reaction [8]. Another research is doing by Canadian scientist In Canada (University of Saskatchewan), the scientists are working on a vaccine aiming to start animal testing in March 2020 and human testing in 2021 [9]. Preliminary results from a metacentric trial, announced in a press conference and described by Gao, Tian, and Yang, suggested that chloroquine is effective and safe in treating COVID-19 associated pneumonia, improving lung imaging findings, promoting a virus-negative conversion, and shortening the disease course [2].

Envelope protein, Spike glycoprotein, RNA-dependent RNA polymerase, and Nucleocapsid Protein have significant roles in pathogenesis of COVID-19. So, in this study, the epitopes of Spike protein and RNA-dependent RNA polymerase fused to Envelope protein and Nucleocapsid Protein to design a peptide vaccine against COVID-19. Then, the vaccine was analyzed with different bioinformatics analysis and software's. After that molecular docking analysis was used to recognize the affinity of vaccine to different MHC-I.

MATERIALS AND METHODS

In this research the completely sequence of Envelope and Nucleocapsid protein was fused to multi epitopes obtained from Spike protein and RNA-dependent RNA polymerase and constructed a vaccine.

Firstly, the antigenicity, allergen city and toxicity selected proteins were analyzed by vaxijen, Algpred and Toxinpred [10,11]. The result of those analysis showed that the proteins have high antigenic score and are not toxic. B-cell epitopes were predicted by Immune Epitope Database server which is a free webserver for designing of bacterial and virus vaccines. In our study the epitopes higher than 0.35 thresholds were selected to B-cell epitopes [12].

IEDB and PropredI were used to prediction of MHC class I epitopes. In this research the epitopes were evaluated for their binding affinity with different HLA alleles (p values<0.05 were considered significant) [13].

In this study we used from KK (Lysine-Lysine) linker for linking of B-cell and T-cell epitopes. The antigenicity, allergen city, and toxicity of fusion protein were tested by vaxijen, Algpred, and Toxinpred software's [10]. The toxic sequence of designed protein was removed from vaccine structure. Afterward, molecular weight, half-life, aliphatic index, isoelectric point instability index, and stability of COVID-19 vaccine were tested by Papcolc and Protparam webserver [14].

We used from Parabi webserver for prediction of Trans membrane helices of vaccine. 3D structure of protein was drowning by SWISS-MODEL. The SWISS-MODEL webserver is a computerized modeling program which develops a protein 3D structure model of an unknown structure protein based on the sequence similarity with the known structured protein [15]. For docking analysis, we need a refine model of proteins, for this reason the 3D structure of vaccine was refined by 3D refine analyzing software. 3Drefine is software for computationally efficient protein structure refinement with the ability to accomplish web based visual and statistical investigation. The 3Drefine software uses iterative optimization of hydrogen bonding network combined with atomic-level energy minimization on the optimized model using a composite physics and knowledge-based force fields for efficient protein structure refinement. The software exposed five refined model to our vaccine. The refined models were patterned for 3D refine score, RMSD score, GTD-TS, GDT-HA score and MolProbity score. The best model was taken and the chosen model was examined via Procheck Ramachandran plot analysis [16].

Docking analysis of recombinant vaccine

Protein-protein docking of recombinant vaccine was done by HEX protein protein docking analysis by considering HLA B2705-KK10, HLAB3508, HLA-A*02, and HLA B5701(the types of MCH class I) as a receptor and designed vaccine as a ligand. For this reason, molecular structure of different MCH-I was taken in PDB format from Protein Data Bank (PDB). Hex (<http://hexserver.loria.fr/>) is the first Fourier transforms based protein docking software to be powered using graphics processors. The software requires the structure of proteins in PDB format to be uploaded and start it produces a ranked list of up to 1000 docking predictions. In our research 3D structure of HLA B2705-KK10, HLAB3508, HLA-A0201, and HLA B5701 are used as receptors and our designed vaccine structure was used as a ligand were uploaded to Hex protein webserver [17].

RESULTS AND DISCUSSION

The protein sequence of Spike protein and RNA-dependent RNA polymerase fused to Envelope protein and Nucleocapsid Protein were obtained from NCBI. The completely sequence of Envelope and Nucleocapsid protein was fused to multi epitopes obtained from Spike protein and RNA-dependent RNA polymerase and constructed a vaccine.

```

10      20      30      40      50      60
MHHHHHMG TKKSAAEASK KPRQKRTATK QYNVTQAFGR RGPQQTQGNF GDQDLIRQGT

70      80      90      100     110     120
DYKHWPIAQ FAPSASAFFG MSRIGMEVTF SGTWLTLYHGA IKLDDKDPQF KDNVILLNKH

130     140     150     160     170     180
IDAYKTFPKK QEKDEDDNLK KSHVDTDLTK KKYVFKPGTS SGDATTAYKK DDYVYLPYFD

190     200     210     220     230     240
PSRIKKPLTK HPNQEYKQFV NEFYAYLKKI LHCANFNVKK YAYLRKHFSM KKQEYADVHF

250     260     270     280     290     300
LYKKRLYDSS MSYKKEVVDK YFDCYKKSSEM VMCGGSLKKS AGFPFNKWKK FVVSTGYHFK

310     320     330     340     350     360
KLTPGDSSSG WTAGKKVRQI APGQTKGIAD KKNLDSKVGK GKKILPDPFK PSKRSKKVYD

370     380     390     400     410     420
FLQPELDSFK KKNHTSPDVF LGKKWTAGAA AYYVKKFPNI TNLCPFKKWT AGAAAYYKVV

430     440     450     460     470     480
YSSANNCTFK KNVYADSFVI RKKEVFAQVK QIYKKKWPYI IWLGFKKLLM PILTLTKKSA

490     500     510     520     530     540
GFPFNKWKKK LYECLYRNRK KVVSTGYHF KKVENPHLMG WKKFVVEVVD KYKQYEVADV

550     560     570     580     590     600
FHLKQKYSF VSEETGTLV NSVLLFLAFV VFLVTLAIL TAHRLCAYCC NIVNVSIVKPV

610     620
SFYVYSRVKN LNSRVPDLL V
    
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Figure 1: The sequence of recombinant COVID-19 vaccine.

Model selected: virus

Threshold for this model: 0.4

Your Sequence:

```

MHHHHHMGTKKSAAEASKKPRQKRTATKQ
YYNVTQAFGRRGPEQQTQGNFQDQDLIRQGGDY
KHWPQIAQFAPSASAFFGMSRIGMEVTFPGT
WLTLYHGAIKLDDKDPQFKDNVILLNKHIDAY
KTFPKKQEKDEDDNLKSHVDTDLTKKYLK
PGGTS SGGDATTAYKKDDYVYLPYFDPSRIKK
FLTKHPNQEYKQFVNEFYAYLKKILHCANFN
VKKYAYLRKHFSMKKQEYADVFLHLYKRLY
DMSYKKEVVDKYFDCYKKSSEMVMCGGSLK
SAGFPFNKWKKFFVVSTGYHFKKILTPGDSSSG
WTAGKKVRQIAPGQTKGIADKKNLDSKVGK
KILPDPFKPKRSKKVYDPLQPELDSFKKK
NHTSPDVLGKKWTAGAAAYYVKKFPNINL
CPFKWTAGAAAYYKVVYSANNCTFKKNVY
ADS FVIRKKEVFAQVKQIYKKKWPYIWLGF
KKLLM P I L T L T K K S A G F P F N K W K K R L Y E C L Y
R N R K K F V V S T G Y H F K K V E N P H L M G W K K F V V E
V V D K Y K K Q E Y A D V F H L Y K K M Y S F V S E E T G T L
I V N S V L L F L A F V V F L L V T L A I L T A H R L C A Y C
C N I V N V S I V K P V S F Y V Y S R V K N L N S R V P D L L
V
    
```

Overall Prediction for the Protective Antigen = 0.4964 (Probable ANTIGEN).

Figure 2: The antigenic property of designed vaccine (Antigenic score=0.496).

The antigenicity of these sequences was tested by Vaxij server. The vixen score of these sequences were 0.6298 (Envelope protein), 0.4646 (Spike protein), 0.4064 (RNA-dependent RNA polymerase), and 0.5522 (Nucleocapsid Protein) respectively. The score higher than threshold (0.4) have good antigenicity; therefore all proteins were suitable for our research. B cell epitopes from Spike protein and RNA-dependent RNA polymerase were predicted by Immune Epitope Database and

Analysis Resource server. The B cell epitopes that having higher than 0.35 were chosen to further analysis. The MHC class I epitopes from Spike protein and RNA-dependent RNA polymerase were predicted by vaxign server. The epitopes were assessed for their binding affinity with predominant HLA I alleles (P-values < 0.05). The selected epitopes fused to Envelop and Nucleocapsid protein by KK linkage. The sequence of our designed vaccine with 621 amino acids is shown in Figure 1.

The antigenicity, allergenicity, and toxicity of fusion vaccine by analyze different software's. The result of analysis showed that the vaccine has high antigenic score and does not allergic or toxically effect on human cells (Figures 2-4).

Home Data sets Method description Contact

AllerTOP v. 2.0

Bioinformatics tool for allergenicity prediction

Your sequence is:

PROBABLE NON-ALLERGEN

The nearest protein is:

[UniProtKB accession number Q9H799](#)

defined as non-allergen

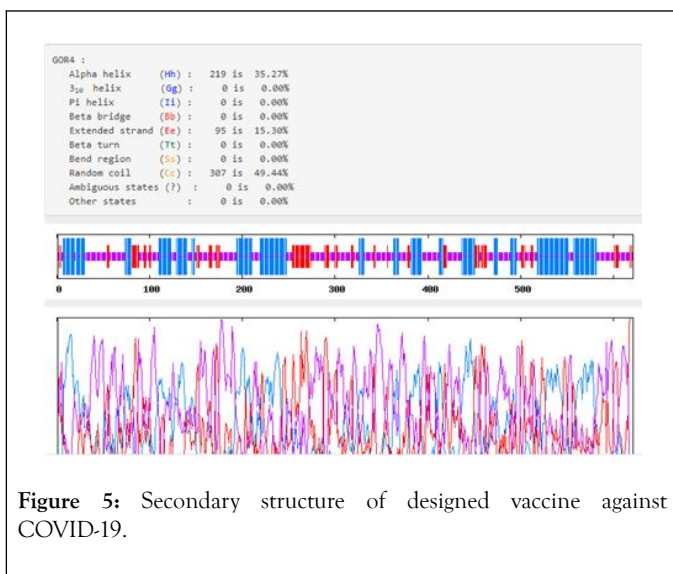
Figure 3: The allergenicity of designed vaccine (the vaccine is non allergen).

Peptide Sequence	SVM score	Prediction	Hydrophobicity	Hydropathicity	Hydrophilicity	Charge	Mol wt
MHHHHHMG	-0.96	Non-Toxin	-0.15	-1.40	-0.61	3.00	1231.54
HHHHHMGTK	-0.96	Non-Toxin	-0.19	-1.66	-0.52	3.00	1201.45
HHHHHMGTKK	-1.04	Non-Toxin	-0.26	-1.73	-0.17	3.50	1192.48
HHHHHMGTKKS	-1.06	Non-Toxin	-0.33	-1.80	0.18	4.00	1183.51
HHHHHMGTKKSA	-1.09	Non-Toxin	-0.32	-1.56	0.26	3.50	1133.44
HHHHHMGTKKSAE	-1.24	Non-Toxin	-0.25	-1.06	0.26	3.00	1067.37
HHHHHMGTKKSAE	-1.36	Non-Toxin	-0.19	-0.56	0.26	2.50	1001.30
AMGTKKSAE	-1.23	Non-Toxin	-0.21	-0.59	0.61	1.00	993.27
MGTKKSAE	-1.44	Non-Toxin	-0.21	-0.59	0.61	1.00	993.27
GTKKSAEAS	-1.42	Non-Toxin	-0.26	-0.86	0.77	1.00	949.15
TKKSAEASK	-1.50	Non-Toxin	-0.39	-1.21	1.07	2.00	1020.27
KKSAEASK	-1.25	Non-Toxin	-0.48	-1.53	1.41	3.00	1047.34
KSAAEASKP	-1.21	Non-Toxin	-0.38	-1.30	1.11	2.00	1016.28
SAAEASKPR	-1.40	Non-Toxin	-0.44	-1.36	1.11	2.00	1044.29
AAEASKPRQ	-1.34	Non-Toxin	-0.48	-1.63	1.10	2.00	1085.35
AEASKPRQK	-1.14	Non-Toxin	-0.62	-2.20	1.45	3.00	1142.45
EASKPRQKR	-1.17	Non-Toxin	-0.82	-2.83	1.80	4.00	1227.56
ASKPRQKRT	-1.08	Non-Toxin	-0.78	-2.55	1.46	5.00	1199.55
SKPRQKRTA	-1.05	Non-Toxin	-0.78	-2.55	1.46	5.00	1199.55
KPRQKRTAT	-0.92	Non-Toxin	-0.77	-2.54	1.39	5.00	1213.58
KPRQKRTATK	-1.17	Non-Toxin	-0.77	-2.54	1.39	5.00	1213.58
PRQKRTATKQ	-1.08	Non-Toxin	-0.73	-2.50	1.11	4.00	1213.54
RQKRTATKQY	-0.86	Non-Toxin	-0.72	-2.47	0.88	4.00	1279.60
QKRTATKQYN	-1.01	Non-Toxin	-0.61	-2.37	0.60	3.00	1237.52
KRTATKQYNV	-1.10	Non-Toxin	-0.48	-1.60	0.43	3.00	1208.52
RTATKQYNVT	-1.32	Non-Toxin	-0.39	-1.28	0.09	2.00	1181.45
TATKQYNVTQ	-1.54	Non-Toxin	-0.29	-1.18	-0.19	1.00	1153.40

Figure 4: The toxicity of designed vaccine (the vaccine is nontoxic).

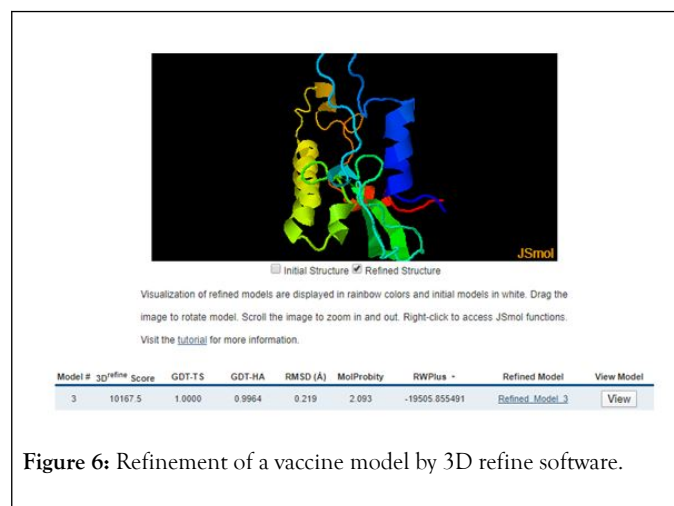
The vaccine has 621 amino acids which 51 negatively charged residues and 118 positive charged amino acids. The physicochemical property of COVID-19 vaccine was analyzed via protparam and the result showed that molecular weight of

candidate vaccine is 71.906 kDa. The estimated half-life of peptide was found to be greater than 30 hours in mammalian reticulocytes, greater than 20 hours in yeast cells, and greater than 10 hours in *E.coli*. The chemical formula of recombinant vaccine is C3315H5113N871O881S20 with 10200 atoms.

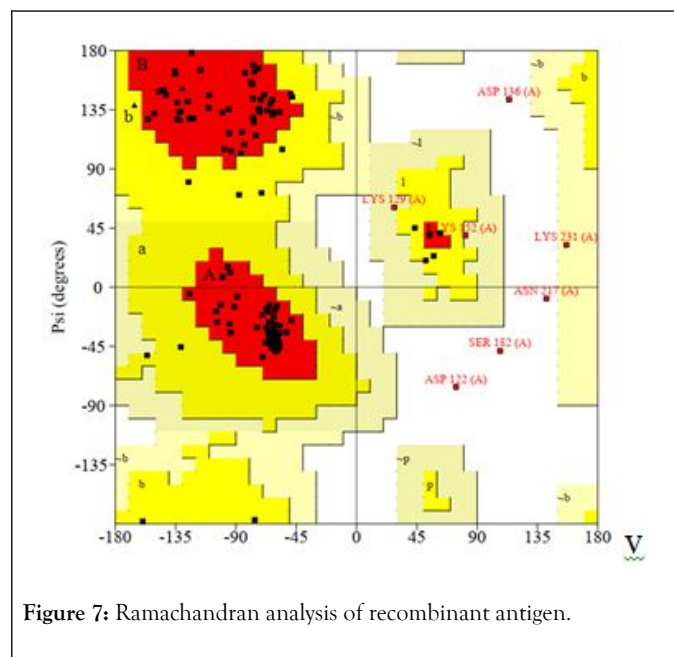


The instability index II is computed to be 34.81. So, this classifies the protein as stable. The grand average of hydropathicity of designed antigen is -0.637, thus the vaccine is a hydrophilic protein and likely interact with water. The aliphatic index of COVID-19 is found to be 66.86, so the vaccine is probable to be thermostable. The results obtained from protparam and pepcalc analysis revealed that the recombinant antigen is soluble in water. The result from Parabi showed that the membrane helices value of vaccine was 35.27% (Figure 5). The vaccine does not have any Tran's membrane helix, so the vaccine simply produced and no expression difficulties are predicted in the expression of antigen.

In this study we used from SWISS NODEL software to drawing of 3D structure of Coronavirus vaccine and then, the vaccine structure was refined by 3Drefine. The result of 3D refine analysis showed that 3Drefine score is 10167, GTD-TS score is 1.000, GTD-HA score is 0.9964, RMSD score is 0.219, MolProbity score is 2.093, and RW Plus score is -19505 (Figure 6). The selected model from 3drefine was examined by Ramachandran plot analysis using Procheck webserver.



Ramachandran analysis of recombinant antigen showed that 84.3% of amino acids are in most favored regions, 10.2% of residues are additional allowed regions, 2.4% of amino acids in generously allowed regions, and just 3.1% of amino acids are in disallowed regions (Figure 7). This result supported the high-quality structure of the refined model of recombinant vaccine.



The Pepcalc analysis showed that the vaccine has the net charge of vaccine at pH 7 is 68/3 and the isoelectric point of vaccine is 10.329 (Figure 8).

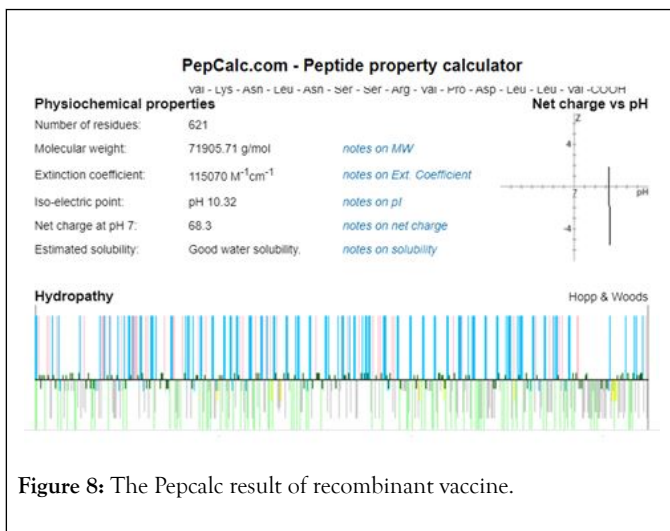


Figure 8: The Pepcalc result of recombinant vaccine.

Docking analysis of recombinant vaccine

Protein-protein docking of recombinant vaccine was done in HEX protein docking analysis by considering HLA B2705-KK10, HLAB3508, HLA-A0201, and HLA B5701 (the types of MCH class I) as a receptor and designed vaccine as a ligand. The result of protein docking analysis showed that maximum affinity of recombinant vaccine to HLA B2705-KK10, HLAB3508, HLA-A*02, and HLA B5701 with the score of -716/75, -691.28, -693.60, and -557.87 respectively (Figure 9).

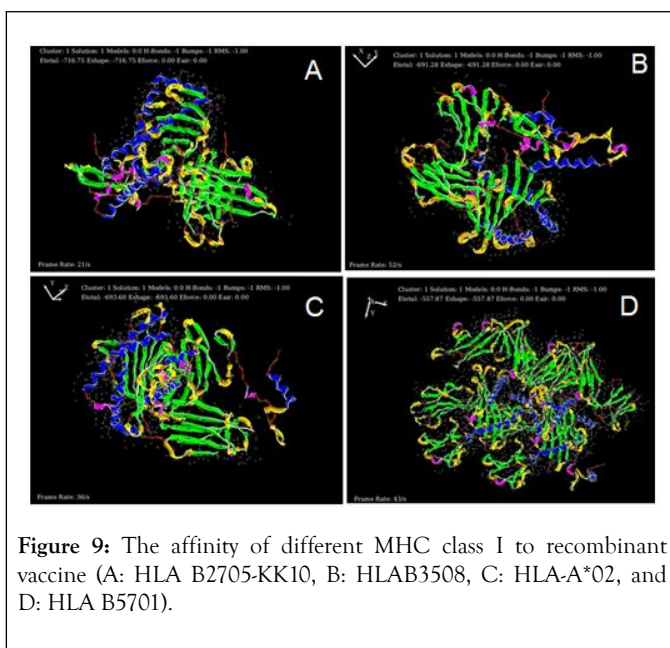


Figure 9: The affinity of different MHC class I to recombinant vaccine (A: HLA B2705-KK10, B: HLAB3508, C: HLA-A*02, and D: HLA B5701).

In the present study the selected proteins have the antigenic score higher than threshold (0.4) which can be candidate to effective vaccine against COVID-19. Having above threshold scores mean that the vaccine can be recognized by MHC-1 and B-cells. Physicochemical property of designed vaccine against COVID-19 revealed that the antigen had a molecular weight of 71 kDa. The researchers showed that the proteins with high molecular weight are more stable than low molecular weight proteins [18]. The half-life estimated of recombinant vaccine is greater than 30 hours in mammalian cells, higher than 20 hours

in yeast cells, and higher than 10 hours in *E.coli*. The result revealed that the recombinant vaccine can be overexpress in *E.coli* or other host cells. Beside that the produced vaccine can be used in human, because the vaccine can be stable 30h in human cells [19-21]. The Instability index score of recombinant vaccine is 34.81 which lower than 40, the score revealed that the vaccine is considered as stable. Our designed vaccine does not have allergenicity and toxicity effect on human and animal cells. The stability and water solubility of peptide vaccine is important and our designed vaccine is stable and soluble in water. Thus the recombinant vaccine is not estimated to drop harmful allergic responses in humans. The result of docking analysis proved that the vaccine has most affinity to HLA B2705-KK10, HLAB3508, HLA-A0201, and HLA B5701.

CONCLUSION

The result of our research showed that the vaccine can activate cellular and humoral immune responses against COVID-19. The vaccine had suitable structural, physicochemical, and immunological properties. Nevertheless, the vaccine could be produced by Recombinant DNA technology and expressed in host cells and need to experiment on laboratory animals.

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