Archives of Razi Institute, Vol. 77, No. 1 (2022) 45-56

DOI: 10.22092/ARI.2021.353377.1601

Original Article

Development of a Novel Multi-Epitope Vaccine Candidate against *Streptococcus Iniae* Infection in Fish: An Immunoinformatics Study

Forouharmehr, A[1 *], Banan, A[2], Mousavi, S. M[1], Jaydari, A[3]

1. Department of Animal Science, Faculty of Agriculture, Lorestan University, Khorramabad, Iran
2. Department of Marine Science, Faculty of Agriculture, Lorestan University, Khorramabad, Iran
3. Department of Pathobiology, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran

Received 26 January 2021; Accepted 3 April 2021
Corresponding Author: forouharmehr.a@lu.ac.ir

Abstract

Streptococcus iniae infection is recognized as a disease with substantial economic losses, infecting a wide range of fish species. The limitations of current vaccines and strategies have led to the identification of new methods to control this disease. Multi-epitope vaccines which employ various immunogenic proteins can be promising. The current research project aimed to design an efficient multi-epitope vaccine against *Streptococcus iniae* infection in fish. To this end, six immunogenic proteins of *Streptococcus iniae*, including FBA, ENO, Sip11, GAPDH, MtsB, and SCPI proteins, were applied for epitope prediction. The best B cell, T cell, and IFNγ epitopes of the immunogenic proteins, as well as interleukin-8, were used to construct a multi-epitope vaccine. Thereafter, different parameters of the designed vaccine, including physicochemical features, antigenicity, secondary structure, and tertiary structure, were evaluated. Moreover, the interaction of the interleukin-8 domain of the designed vaccine and its receptor was investigated by molecular docking strategy. Finally, nucleotide sequence of the vaccine was adapted to express in *Escherichia coli.* The results of the present study pointed out that the designed vaccine was a stable vaccine with molecular weight and antigenicity score of 45 kDa and 0.936, respectively. Furthermore, the structure analysis results revealed that the designed vaccine contained 23.49% alpha helix, with 90.5% residues in favored region. Finally, it was demonstrated that the interleukin-8 domain of the designed vaccine could be successfully docked to its receptor with the lowest energy of -1020.9. Based on the obtained results, it seems that the designed vaccine can be an efficient candidate to prevent *Streptococcus iniae* infection in fish.

Keywords: Multi-epitope scaffold, *Streptococcus iniae*, Immunoinformatics, Immunogenic proteins

1. Introduction

Streptococcus iniae (*S. iniae*) is a pathogenic and gram-positive bacterium belonging to the *Streptococcaceae* family (1). Multiple reports have demonstrated that this bacterium is able to infect both freshwater and saltwater fish (2-4). Initially, *S. iniae* infection was observed in Japan in 1958; nonetheless, this infection was gradually reported in other countries (5). Moreover, *S. iniae* can infect people who touch raw fish; therefore, this infection is known as a zoonotic disease (5, 6). The *S. iniae* infection in fish has symptoms with a species-dependent pattern, and it is classified into two clinical forms, including sub-acute and acute.

The most important symptoms of this infection include exophthalmia, erratic swimming, dark ended coloration, corneal opacity, and death (7-9). Although numerous projects have been conducted to fight against

fish diseases, this issue is still known as one of the most important economic losses of the aquaculture industry (10). In general, antibiotics therapy and chemical drugs have been able to control some fish diseases; nonetheless, drug resistance and safety issues have limited the application of these strategies (11). Nowadays, vaccination is considered one of the most effective strategies used for the prevention of bacterial and viral diseases (12). The first vaccination in fish was reported in 1940. Currently killed, inactivated, and attenuated vaccines are applied in the aquaculture industry (13, 14).

New-generation vaccines which take advantage of recombinant DNA technology, compared to traditional vaccines, are more protective and less pathogenic (15, 16). Therefore, owing to the advantages of recombinant vaccines, they can be introduced as a proper alternative to traditional vaccines. The multi-epitope vaccine is one of the most famous classes of recombinant vaccines designed to control a wide variety of diseases. In general, this kind of vaccine, using different epitopes (e.g. B cell, T cell, and IFNγ) of immunogenic proteins, can trigger a robust immune response against a particular disease (17). It is worthy to note that the epitopes are short peptides of immunogenic proteins recognized by the immune system (18). In a multi-epitope vaccine, to take advantage of antigenic properties of different immunogenic proteins, epitopes of proteins are used instead of their whole amino acid sequences (19, 20).

During designing the construct of a multi-epitope vaccine, a molecular adjuvant is usually conjugated to the vaccine for increasing immune responses (21). Furthermore, multi-epitope vaccines have flexible construction to design; consequently, they can be easily manipulated by designers for different aims (22, 23). Bioinformatics is a potent science which merges computer and biology sciences to predict affordable and reliable data. Immunoinformatics is a major branch of bioinformatics which provides accurate tools for researchers working in vaccine designing projects (24). In the current study, an immunoinformatics approach was applied to design an efficient multi-epitope vaccine against *S. iniae* infection in fish. To this end, six immunogenic proteins of *Streptococcus iniae*, including FBA, ENO, Sip11, GAPDH, MtsB, and SCPI proteins, were applied for epitope prediction. It is worth mentioning that immunogenic proteins were selected since their protection abilities have been confirmed using immunoprotective analysis (1).

The best B cell, T cell, and IFNγ epitopes of the immunogenic proteins were isolated using the most accurate tools. The predicted epitopes were applied to design a multi-epitope vaccine; moreover, Interleukin-8 (IL-8) was linked to the designed vaccine as a molecular adjuvant to reinforce immune responses. In general, cytokines, such as IL-8, are secretory and low weight proteins which have crucial roles against different pathogenic agents; therefore, they are suggested to be applied as a molecular adjuvant in recombinant vaccines (25).

2. Materials and Methods

2.1. Amino Acid Sequences Collection

To conduct this project, amino acid sequences of the immunogenic proteins, including FBA (A0A1J0MXH9), ENO (A0A1J0MZJ1), Sip11 (D5I7F8), GAPDH (Q7BB80), MtsB (G4W7K3, and SCPI (B4YVA3), were collected from The Universal Protein Recourse(UniProtKB) database (26).

2.2. B Cell Epitope Prediction

To predict B cell epitopes of FBA, ENO, Sip11, GAPDH, MtsB, and SCPI proteins, two accurate servers, including IEBD (27) and ABCpred (28), were applied. It is noteworthy that primary epitopes were selected among common results of all servers. Finally, the best epitopes were screened based on antigenicity score which was determined by the VaxiJen server (29). The prediction accuracies of IEBD, ABCpred, and VaxiJen servers are estimated at 78%, 65.93%, and 80%, respectively (27-29).

2.3. T Cell Epitope Prediction

To identify T cell epitopes of FBA, ENO, Sip11, GAPDH, MtsB, and SCPI proteins, the IEBD server

(27) was employed. In this case, the NetMHCpan El4.0 method was selected for prediction. At the same time, the amino acid sequence of fish MHCI protein was pasted in the specified MHC alleles sequence window. To find the best T cell epitopes, the predicted epitopes were filtered based on their antigenicity scores.

2.4. IFNγ Epitope Prediction

In the current project, IFNγ epitopes of FBA, ENO, Sip11, GAPDH, MtsB, and SCPI proteins were predicted by the INFepitope server (30). In this case, the best epitopes were isolated based on their score and probable positivity. The accuracy of the INFepitope server is estimated at 82.2% (30).

2.5. Designing Multi-Epitope Vaccine

To design an efficient multi-epitope vaccine against *S. iniae* infection in fish, the best B cell, T cell, and IFNγ epitopes of the immunogenic proteins were applied. In fact, the epitopes were employed to construct different fragments which were assembled by proper linkers. Moreover, an appropriate molecular adjuvant was linked to the designed vaccine.

2.6. Physicochemical Features and Antigenicity Analysis of the Designed Vaccine

In the current study, ProtParam server (31) was applied to compute different physicochemical features of the designed vaccine, including molecular weight, theoretical pI, estimated half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY). In this case, an amino acid sequence of the designed vaccine was submitted to the server, and the achieved outputs were used to make a decision. Finally, the antigenicity of the designed vaccine was evaluated by VaxiJen and Predicting Antigenic Peptides tool (32).

2.7 Secondary and Tertiary Structures of the Designed Vaccine

In order to evaluate secondary structure parameters of the designed vaccine, including alpha helix, extended strand, and random coil, the GOR4 server (33) was applied. Moreover, the initial tertiary structure of the designed vaccine was modeled by the I-TASSER server (34). To use both servers, an amino acid sequence of the designed vaccine was employed as input.

2.8 Refinement and Validation of the Tertiary Structure

To refine the initial tertiary structure of the designed vaccine, GalaxyRefine server (35) was applied. Moreover, the best-refined model was screened based on Ramachandran plots which were drawn by the MolProbity server (36). Finally, ProSA (37) and Verify3D (38) tools were employed to validate the tertiary structure of the designed vaccine.

2.9. Molecular Docking Codon Adaptation

In the current study, to investigate the interaction between IL-8 which was conjugated to the designed vaccine and CXCR1 receptor, a protein-protein docking strategy was conducted by the ClusPro server (39). In this case, PDB files of both proteins (ligand and receptor) were submitted to the ClusPro server, and the best docking model was selected based on the lowest energy. Moreover, JCat server (40) was applied to optimize the nucleotide codons of the designed vaccine in *Escherichia coli (E. coli*

3. Results

3.1. Epitope Prediction

The best B cell, T cell, and IFNγ epitopes of the FBA, ENO, Sip11, GAPDH, MtsB, and SCPI proteins are presented in tables 1, 2, and 3, respectively. The results demonstrated that the length of the best B cell epitopes of all immunogenic proteins was 16 residues. Moreover, the highest antigenicity score (1.34) of B cell epitopes belonged to GAPDH protein, whereas the lowest antigenicity score (1.03) of these epitopes belonged to SCPI protein (Table 1). Based on the results, the lengths of the best T cell epitopes were estimated at 9-10 residues; moreover, the results suggested that the ENO protein had the highest antigenicity scores (1.44) of the best T cell epitopes, and MtsB protein had the lowest score (1.02) (Table 2). It should be noted that antigenicity is defined as a score to determine antigen and non-antigen peptides. Once

the antigenicity score of a peptide is less than 0.5, it is considered a non-antigen. As displayed in Table 3, the results disclosed that the length of all the best IFNγ epitopes of the immunogenic proteins was 15 residues; moreover, the positive and negative probabilities of these epitopes were positive (Table 3).

Table 1. B cell epitopes of the immunogenic proteins

Antigen	Rank	Sequence	Length	Start Position	End Position	Antigenicity score
FBA	1	IGGEEDGIVGKGELAP	16	140	150	1.27
ENO	1	GETEDSTIADIAVATN	16	375	390	1.25
SCPI	1	SLDGTFYYTPEEGQDK	16	714	729	1.14
MtsB	1	YVEQRSMIDFHFPITV	16	74	89	1.03
Sip11	1	TETTNRPVRKDAKVSK	16	290	305	1.27
GAPHD	1	TETTNRPVRKDAKVSK	16	189	204	1.34

Table 2. T cell epitopes of the immunogenic proteins

Antigen	Rank	Sequence	Length	Start position	End position	Antigenicity Scores
FBA	1	ESMGITVPV	9	72	80	1.24
ENO	1	YLGGFNAKV	9	133	141	1.44
SCPI	1	LSAPGYELY	9	478	486	1.02
MtsB	1	SLNNVNISI	9	17	25	1.06
Sip11	1	KYGVKVELI	9	58	66	1.65
GAPDH	1	VSSDIVGISY	10	279	288	1.06

Table 3. IFNγ epitopes of the immunogenic proteins

Antigen	Sequence	Length	Start position	End position	Positivity/Negativity
FBA	IKLGVAKVNVNTESQ	15	222	236	Positive
ENO	PTLEVEVYTESGAFG	15	19	33	Positive
SCPI	VQEHVKKKYPQYSPQ	15	514	528	Positive
MtsB	VFTVESLADTYGNDL	15	220	234	Positive
Sip11	VSKEAQDALGTETTN	15	280	294	Positive
GAPDH	VKVSAEREPANIDWA	15	72	86	Positive

3.2. Designing Multi-Epitope Vaccine

The best B cell, T cell, and IFNγ epitopes of immunogenic proteins, which are presented in tables 1-3, were employed to design a novel multi-epitope vaccine against *S. iniae* infection in fish. In fact, B cell, T cell, and IFNγ domains of the designed vaccine were constructed using the best B cell, T cell, and IFNγ epitopes of the immunogenic proteins, respectively. Moreover, amino acid sequence of fish IL-8 was intercalated at N terminal of the designed vaccine as a molecular adjuvant. It is worth noting that each domain was built by the KPKP linker, whereas the EAAAK linker was applied to link various domains of the vaccine (Figure 1).

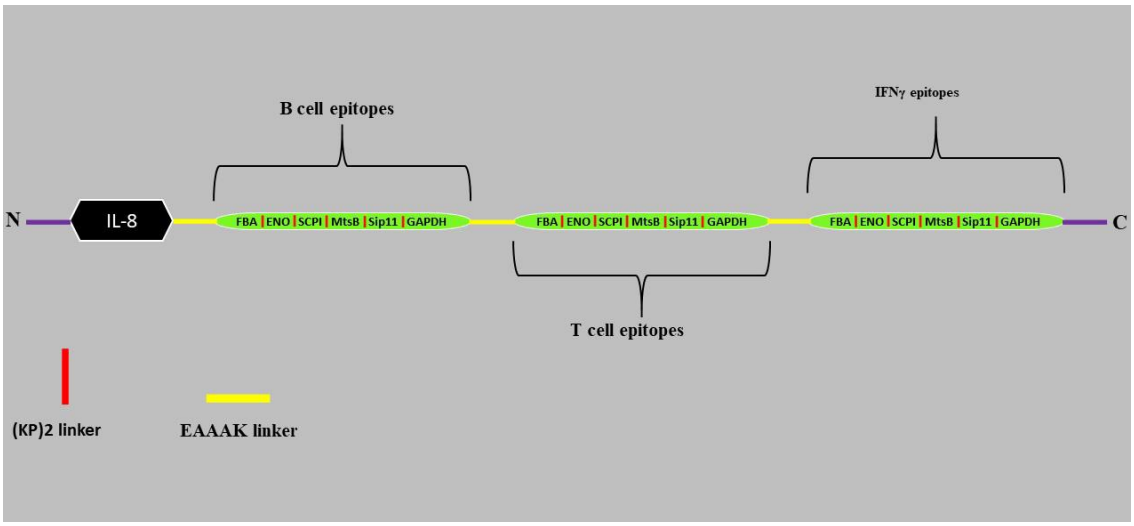

Figure 1. Schematic presentation of the designed vaccine with 413 amino acids in length. As illustrated, the designed vaccine from N terminal to C terminal contained IL-8 domain (as an adjuvant), B cell domain, T cell domain, and IFNγ domain, respectively

3.3. Physicochemical Features and Antigenicity Analysis of the Designed Vaccine

According to the results of the ProtParam server, molecular weight, theoretical PI, instability index, aliphatic index, and GRAVY of the designed vaccine were 45kDa, 9.5, 36, 75.79, and -0.58, respectively. Furthermore, the estimated half-life of the designed vaccine in different hosts, including mammalian, yeast, and *E. coli*, were 30 h, >20 h, and >10 h, respectively. The antigenicity analysis results demonstrated that the designed vaccine was an antigen with an antigenicity score of 0.936; moreover, the average antigenic propensity of the whole vaccine was 1.02 (Figure 2).

3.4. Secondary and tertiary structures of the designed vaccine

The results of the GOR4 server revealed that the secondary structure of the designed vaccine contained 23.49%, 17.93,% and 58.60 of alpha-helix, extended strand, and random coil, respectively (Figure 3). The initial tertiary structure of the designed vaccine was modeled by the I-TASSER server (Figure 4). The results pointed out that the C-score of the initial model was -2.39.

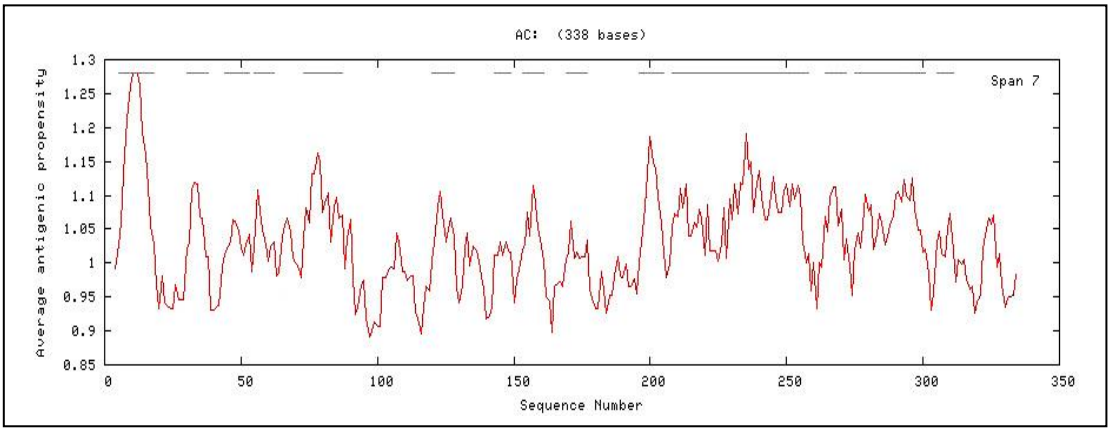

Figure 2. Antigenic plot of the designed vaccine, the average antigenic propensity of the protein was 1.02

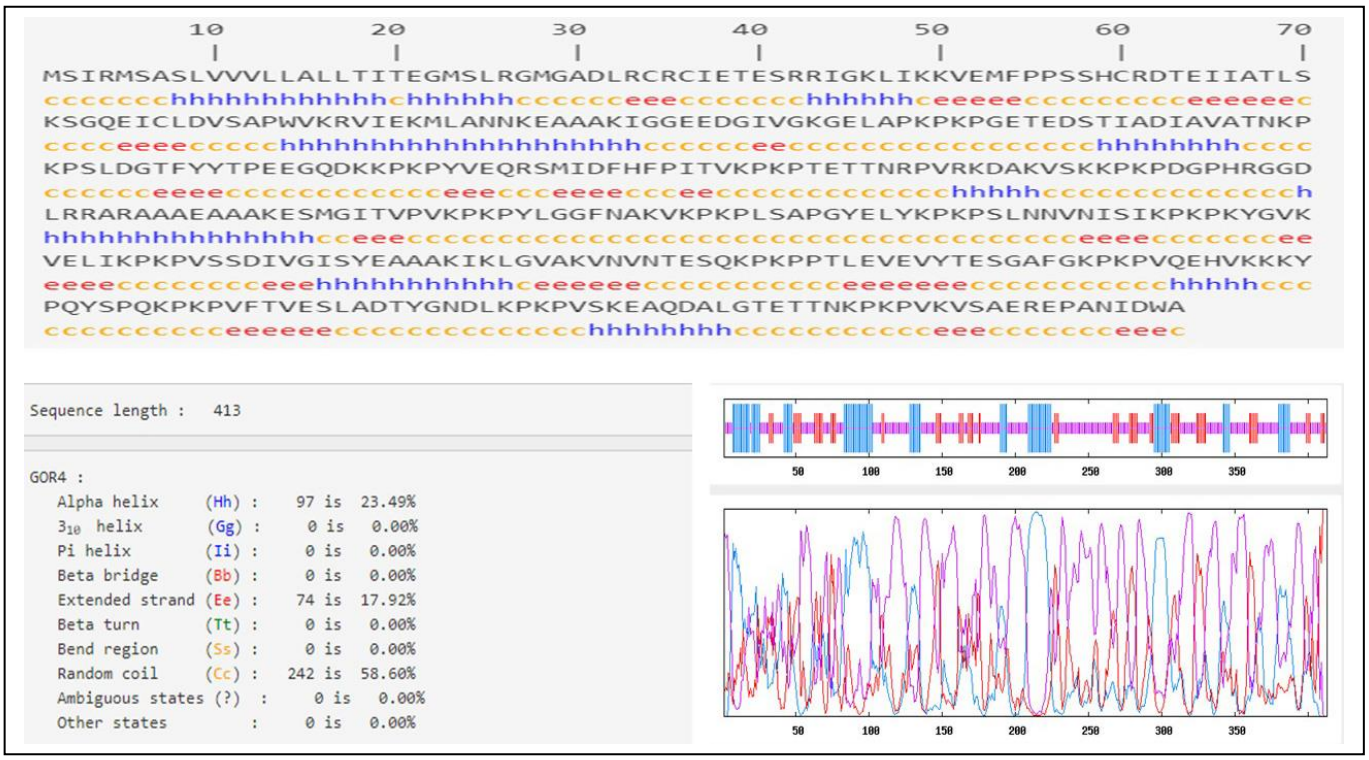

Figure 3. Secondary structure of the designed vaccine predicted by GOR4 server. As illustrated, 97, 74, and 242 of the residues were located in alpha-helix (blue), extended strand (red), and random coil (brown), respectively

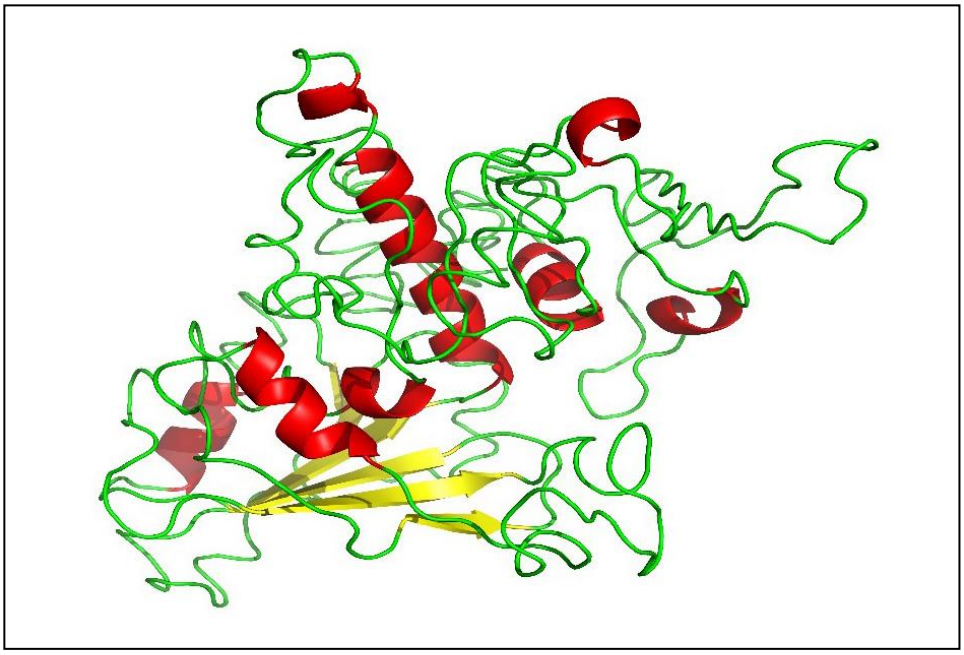

Figure 4. Tertiary structure of the designed vaccine modeled by I-TASSAR software, different regions including helix, sheet, and loop have been colored by red, yellow, and green, respectively. PyMol software (41) was applied for visualization.

3.5. Refinement and Validation of the Tertiary Structure

As noted, to refine the initial tertiary structure of the designed vaccine, the GalaxyRefine server was employed. The results demonstrated that in the initial model, 56% of residues were in the favored region, whereas in the refined model, 90.5% of residues were in the favored region (Figure 5). The validation of the tertiary structure was carried out by Verify3D and ProSA servers. The results of validation demonstrated that 61.74% of residues of the refined model had an average 3D-1D score >=0.2 (Figure 6A). Moreover, the results showed that the Z-score of the refined tertiary model was -3.08 (Figure 6B).

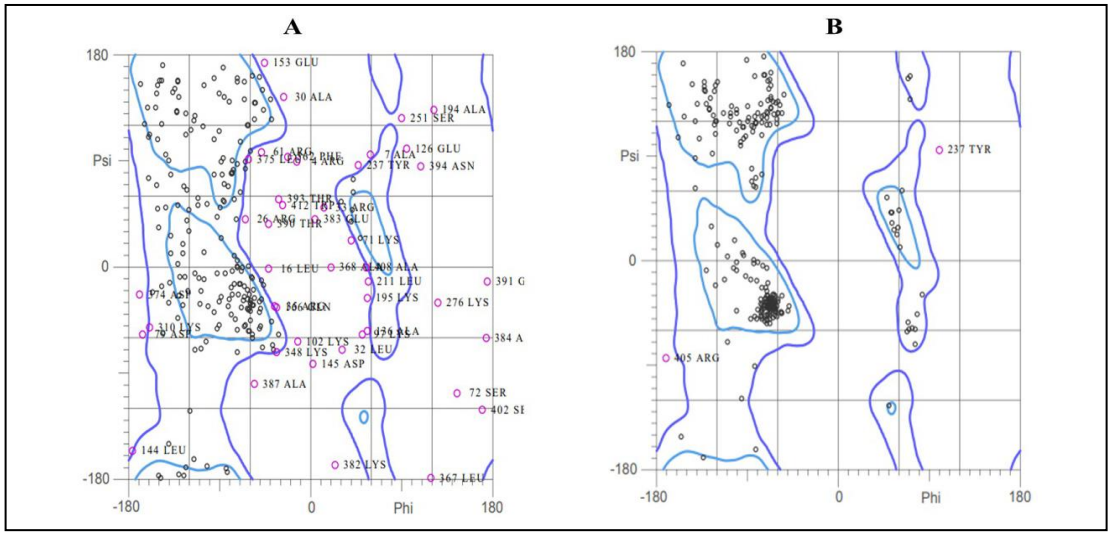

Figure 5. Ramachandran plots of the designed vaccine before (**A**) and after (**B**) refinement. Before refinement, 230 (56%) residues were located in the favored region; nonetheless, after refinement, 372 (90.5%) of residues were in the favored region

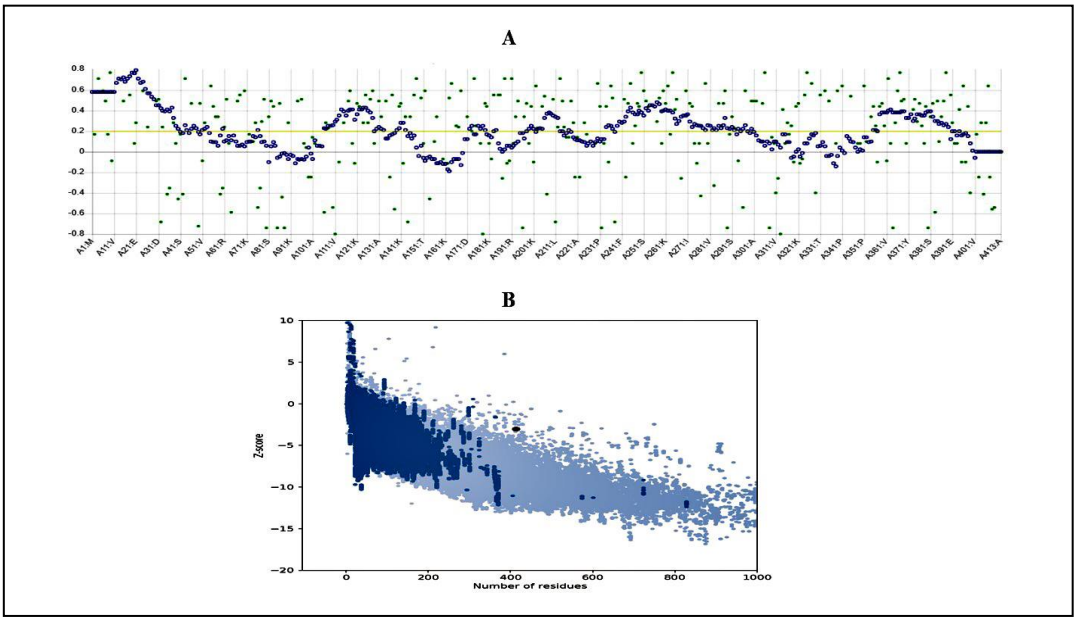

Figure 6. (A): Verify3D plot of the refined model**,** blue points represent averaged scores and green points show raw scores. **(B)**: Z-score plot of the refined model, X-ray and NMR regions have been demonstrated by light and dark blue, respectively. The Z- scores of the refined model are displayed with a highlighted dot

3.6. Molecular Docking and Codon Adaptation

ClusPro server was applied to investigate molecular docking between the IL-8 domain of the designed vaccine and the CXCR1 receptor. The results of docking revealed that the IL-8 domain was successfully docked to its receptor with the lowest energy of -1020.9 (Figure 7). Based on the results of codon adaptation in *E. coli*, the codon adaptation index (CAI) of the raw sequence was 0.2, while after adaptation, this index was improved to 0.98 (Figure 8).

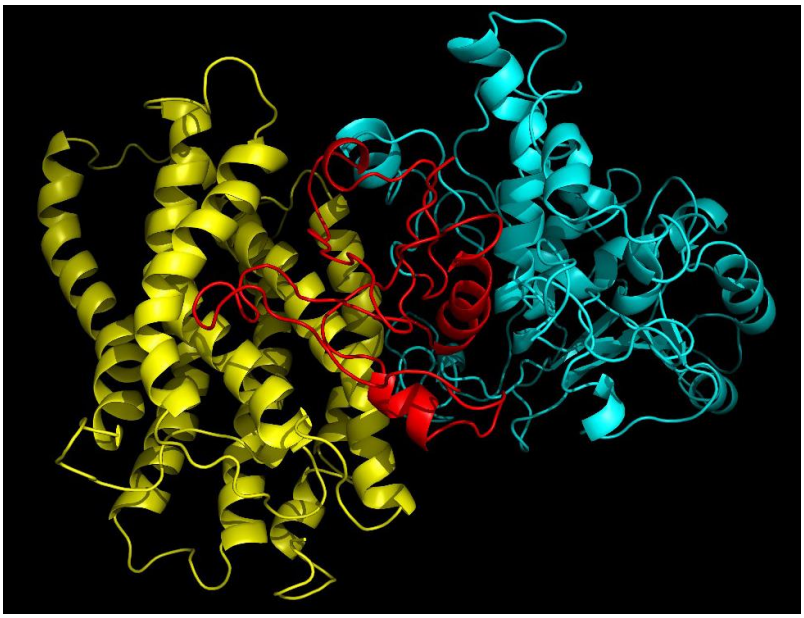

Figure 7. Schematic presentation of interaction between the CXCR1 receptor (yellow) and the designed vaccine (blue). The adjuvant domain (red) has been successfully docked to its receptor

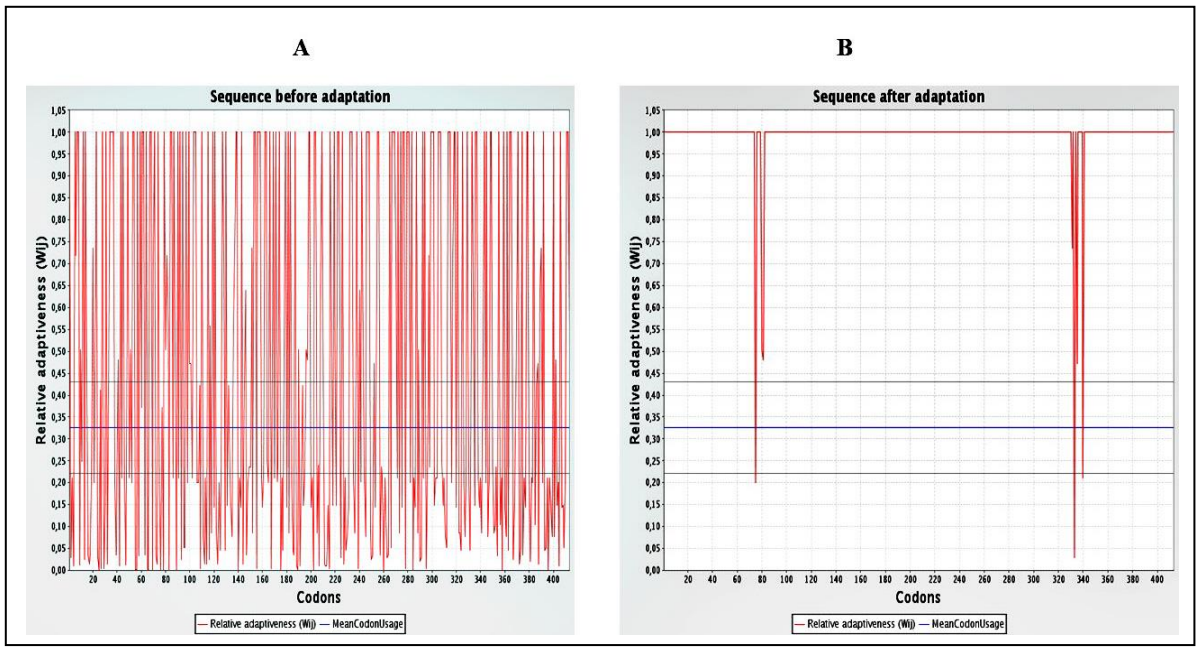

Figure 8. Codon adaptation plots of raw **(A)** and adapted **(B)** sequences. The CAI value of the codon adapted sequence was 0.98, the maximum CAI value which is considered for a codon-adapted sequence is 1.

4. Discussion

Nowadays, the aquaculture industry is considered one of the biggest food resources which guarantee human life (42); therefore, it is necessary to identify the dangers which menace this industry. Fish diseases (e.g. *S. iniae)* are recognized as a serious problem which can extremely decrease aquaculture products. Antibiotics are widely applied for fighting fish diseases; nonetheless, antibiotic resistance of this drug has limited its application (43). Consequently, new strategies, such as the multi-epitope vaccine, must be applied to prevent fish diseases. Multi-epitope vaccines are safe, stable, and easy synthetic products which can stimulate both humoral and cellular immunities (44). Regarding the advantages of multi-epitope vaccines and the limitations of traditional methods, the current study was conducted to design an efficient multi-epitope vaccine against *S. iniae* infection in fish.

Firstly, to stimulate humoral immunity, the best B cell epitopes of the FBA, ENO, Sip11, GAPDH, MtsB, and SCPI proteins were screened (Table 1) and applied to construct a B cell fragment (Figure 1). Thereafter, to apply cellular immunity, the best T cell epitopes of immunogenic proteins were predicted and used to build a T cell fragment (Figure 1). Moreover, an IFNγ fragment was constructed by the best IFNγ epitopes of the immunogenic proteins and linked to the designed vaccine. In general, IFNγ can intensify macrophage activities, as well as B cell proliferation and antibody class switching; consequently, it can be claimed that IFNγ plays a major role in the stimulation of both innate and adaptive immunities (45). Therefore, the presence of epitopes which can increase the level of IFNγ in the body is crucial in vaccine designing.

Despite the advantages of multi-epitope vaccines, low antigenicity is recognized as a major drawback of these vaccines (44). To solve this problem, a molecular adjuvant is mostly linked to the multi-epitope vaccine. In the present study, to increase the antigenicity of the designed vaccine, two strategies were considered. Firstly, the best B cell and T cell epitopes were selected based on their antigenicity score; moreover, the antigenicity of the whole vaccine was confirmed by its antigenicity score (0.936) and average antigenic propensity (1.02). Secondly, IL-8 was put in the N terminal of the designed vaccine (Figure 1). More recently, it has been demonstrated that IL-8 as a molecular adjuvant can improve immunoprotection and period of immune responses (42).

It is worth noting that to assemble the designed vaccine, two types of rigid linkers, including, EAAAK and KPKP, were applied (Figure 1). In general, linkers are known as short peptides which can separate different proteins domains (46). It has been reported that constructing a multi-epitope vaccine without a proper linker can intensify the problems of protein folding (44). The EAAAK and KPKP linkers can not only keep proper distances among epitopes and fragments of the designed vaccine but also eliminate unwanted interactions (47). In the next step, the most important physicochemical features of the designed vaccine were investigated. The results revealed that the molecular weight of the designed vaccine was 45kDa.

In general, a protein with a molecular weight of less than 10kDa can be easily cleared from the body using the renal system (24, 46). Consequently, our designed vaccine with a molecular weight of 45kDa can circumvent the renal system. Moreover, our designed vaccine was recognized as a stable protein since its instability index was less than 40 (48, 49). As illustrated by the obtained results, the instability index of the designed vaccine was 36. As noted earlier, the tertiary structure of the designed vaccine was modeled and refined by reliable tools.

The results of tertiary structure refinement indicated that most of the residues (90.5%) were located in the favored region; therefore, it can be concluded that the designed vaccine was well modeled. The results of molecular docking confirmed that the IL-8 domain of the designed vaccine could successfully dock to the CXCR1 receptor. Accordingly, it can be claimed that the IL-8 domain can successfully play its role as a

molecular adjuvant. Eventually, the nucleotide sequence of the designed vaccine was appropriately optimized for expression in *E. coli* as a prokaryotic expression system. As a matter of fact, compared to other systems, the prokaryotic system is a cheaper and more utilitarian host which can be applied for the expression of simple proteins (50).

5. Conclusion

Multi-epitope vaccines are a new generation of vaccines suggested as an appropriate alternative for traditional methods, such as antibiotic therapy. The current study was designed to introduce a novel multi-epitope vaccine candidate against *S. iniae* infection in fish. To design the vaccine, six immunogenic proteins, along with IL-8 (molecular adjuvant), were applied. The major parameters of the designed vaccine were assessed by the most reliable tools. The results of this research project confirmed that the designed vaccine can be an appropriate candidate for the control of *S. iniae* in fish. It is worth mentioning that the results of this project were theoretically obtained; therefore, these results should be experimentally confirmed.

Authors' Contribution

Study concept and design: A. F.
Acquisition of data: A. F.
Analysis and interpretation of data: A. F.
Drafting of the manuscript: A. F.
Critical revision of the manuscript for important intellectual content: A. F.
Statistical analysis: A. B., S. M. M., and A. J.
Administrative, technical, and material support: A. F.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

The current project was performed without funding support.

Acknowledgment

This project was conducted in the Department of Animal Science, Faculty of Agriculture, Lorestan University. The authors would like to deeply thank all people who supported this project.

References

1. Sheng X, Liu M, Liu H, Tang X, Xing J, Zhan W. Identification of immunogenic proteins and evaluation of recombinant PDHA1 and GAPDH as potential vaccine candidates against Streptococcus iniae infection in flounder (Paralichthys olivaceus). PloS One. 2018;13(5):0195450.
2. El Aamri F, Padilla D, Acosta F, Caballero M, Roo J, Bravo J, et al. First report of Streptococcus iniae in red porgy (Pagrus pagrus, L.). J Fish Dis. 2010;33(11):901-5.
3. Eldar A, Frelier PF, Assenta L, Varner PW, Lawhon S, Bercovier H, et al. Streptococcus shiloi, the name for an agent causing septicemic infection in fish, is a junior synonym of Streptococcus iniae. Int J Syst Evol Microbiol. 1995;45(4):840-2.
4. Facklam R, Elliott J, Shewmaker L, Reingold A. Identification and characterization of sporadic isolates of Streptococcus iniae isolated from humans. J Clin Microbiol. 2005;43(2):933-7.
5. Agnew W, Barnes AC. Streptococcus iniae: an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. Vet Microbiol. 2007;122(1-2):1-15.
6. Goh SH, Driedger D, Gillett S, Low DE, Hemmingsen SM, Amos M, et al. Streptococcus iniae, a human and animal pathogen: specific identification by the chaperonin 60 gene identification method. J Clin Microbiol. 1998;36(7):2164-6.
7. Bromage Ea, Owens L. Infection of barramundi Lates calcarifer with Streptococcus iniae: effects of different routes of exposure. Dis Aquat Organ. 2002;52(3):199-205.
8. Evans JJ, Shoemaker CA, Klesius PH. Experimental Streptococcus iniae infection of hybrid striped bass (Morone chrysops× Morone saxatilis) and tilapia (Oreochromis niloticus) by nares inoculation. Aquaculture. 2000;189(3-4):197-210.
9. Perera RP, Fiske RA, Johnson SK. Histopathology of Hybrid Tilapias Infected witha Biotype of Streptococcus iniae. J Aquat Anim Health. 1998;10(3):294-9.
10. Ma J, Bruce TJ, Jones EM, Cain KD. A review of fish vaccine development strategies: Conventional methods

and modern biotechnological approaches. Microorganisms. 2019;7(11):569.
11. Sneeringer S, Bowman M, Clancy M. The US and EU Animal Pharmaceutical Industries in the Age of Antibiotic Resistance. 2019.
12. Horzinek M, Schijns V, Denis M, Desmettre P, Babiuk L. General description of vaccines. Vet Vaccine. 1997:131-52.
13. Gudding R, Goodrich T. The history of fish vaccination. Fish Vaccine. 2014:1-11.
14. Snieszko S, Friddle S. Prophylaxis of furunculosis in brook trout (Salvelinus fontinalis) by oral immunization and sulfamerazine. Progress Fish Cult. 1949;11(3):161-8.
15. Hansson M, Nygren PAk, Sta˚ hl S, biochemistry a. Design and production of recombinant subunit vaccines. Biotechnol Appl Biochem. 2000;32(2):95-107.
16. Shams N, Gandabeh ZS, Nazifi N, Forouharmehr A, Jaydari A, Rashidian E, et al. Computational Design of Different Epitope-Based Vaccines Against Salmonella typhi. Int J Pept Res Ther. 2019:1-13.
17. Slingluff Jr CL. The present and future of peptide vaccines for cancer: single or multiple, long or short, alone or in combination? J Cancer. 2011;17(5):343.
18. Nazifi N, Mousavi SM, Moradi S, Jaydari A, Jahandar MH, Forouharmehr A. In silico B cell and T cell epitopes evaluation of lipL32 and OmpL1 proteins for designing a recombinant multi-epitope vaccine against leptospirosis. Int J Infect. 2018;5(2).
19. Jebastin T, Narayanan S, Chemistry. In silico epitope identification of unique multidrug resistance proteins from Salmonella Typhi for vaccine development. Comput Biol Chem. 2019;78:74-80.
20. Saadi M, Karkhah A, Nouri HR, Genetics, Evolution. Development of a multi-epitope peptide vaccine inducing robust T cell responses against brucellosis using immunoinformatics based approaches. Infect Genet Evol. 2017;51:227-34.
21. Rashidian E, Gandabeh ZS, Forouharmehr A, Nazifi N, Shams N, Jaydari A, et al. Immunoinformatics Approach to Engineer a Potent Poly-epitope Fusion Protein Vaccine Against Coxiella burnetii. Int J Pept Res. 2020:1-11.
22. Hajissa K, Zakaria R, Suppian R, Mohamed Z. Immunogenicity of Multiepitope Vaccine Candidate against Toxoplasma gondii Infection in BALB/c Mice. Iran J Parasitol. 2018;13(2):215.
23. Zhang L, immunology m. Multi-epitope vaccines: a promising strategy against tumors and viral infections. Cell Mol Immunol. 2018;15(2):182-4.
24. Jaydari A, Forouharmehr A, Nazifi N. Determination of immunodominant scaffolds of Com1 and OmpH antigens of Coxiella burnetii. Microb Pathog. 2019;126:298-309.
25. Secombes C, Zou J, Bird S. Fish cytokines: discovery, activities and potential applications. Fish Defenses. 2009;1:1-36.
26. Consortium U. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 2019;47(1):506-15.
27. Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, et al. The immune epitope database (IEDB): 2018 update. Nucleic Acids Res. 2019;47(1): 339-43.
28. Saha S, Raghava GPS. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins: Struct, Funct, Bioinf. 2006;65(1):40-8.
29. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC Bioinf. 2007;8(1):1-7.
30. Dhanda SK, Vir P, Raghava GP. Designing of interferon-gamma inducing MHC class-II binders. Biol Direct. 2013;8(1):1-15.
31. Gasteiger E, Hoogland C, Gattiker A, Wilkins MR, Appel RD, Bairoch A. Protein identification and analysis tools on the ExPASy server. The proteomics protocols handbook. 2005:571-607.
32. Molero-Abraham M, Lafuente EM, Flower DR, Reche PA. Selection of conserved epitopes from hepatitis C virus for pan-populational stimulation of T-cell responses. Clin Dev Immunol. 2013;2013.
33. Sen TZ, Jernigan RL, Garnier J, Kloczkowski A. GOR V server for protein secondary structure prediction. Bioinformatics. 2005;21(11):2787-8.
34. Yang J, Zhang Y. I-TASSER server: new development for protein structure and function predictions. Nucleic Acids Res. 2015;43(1):174-81.
35. Heo L, Park H, Seok C. GalaxyRefine: Protein structure refinement driven by side-chain repacking. Nucleic Acids Res. 2013;41(1):384-8.
36. Williams CJ, Headd JJ, Moriarty NW, Prisant MG, Videau LL, Deis LN, et al. MolProbity: More and better reference data for improved all-atom structure validation. Protein Sci. 2018;27(1):293-315.

37. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res. 2007;35(2):407-10.
38. Eisenberg D, Lüthy R, Bowie J. VERIFY3D: assessment of protein models with three-dimensional profiles. InMethods in enzymology 1997 Jan 1 (Vol. 277, pp. 396-404). Academic Press DOI.10:s0076-6879.
39. Kozakov D, Hall DR, Xia B, Porter KA, Padhorny D, Yueh C, et al. The ClusPro web server for protein–protein docking. Nat Protoc. 2017;12(2):255.
40. Grote A, Hiller K, Scheer M, Münch R, Nörtemann B, Hempel DC, et al. JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. Nucleic Acids Res. 2005;33(2):526-31.
41. Schrodinger L. The PyMOL molecular graphics system. Version. 2010;1(5).
42. Wang E, Long B, Wang K, Wang J, He Y, Wang X, et al. Interleukin-8 holds promise to serve as a molecular adjuvant in DNA vaccination model against Streptococcus iniae infection in fish. Oncotarget. 2016;7(51):83938.
43. Evensen Ø, Leong J-AC, immunology s. DNA vaccines against viral diseases of farmed fish. Fish Shellfish Immunol. 2013;35(6):1751-8.
44. Goumari MM, Farhani I, Nezafat N, Mahmoodi S. Multi-Epitope Vaccines (MEVs), as a Novel Strategy Against Infectious Diseases. Curr Proteomics. 2020;17(5):354-64.
45. Alspach E, Lussier DM, Schreiber RD. Interferon γ and its important roles in promoting and inhibiting spontaneous and therapeutic cancer immunity. Cold Spring Harb Perspect Biol. 2019;11(3):028480.
46. Jaydari A, Nazifi N, Forouharmehr A. Computational design of a novel multi-epitope vaccine against Coxiella burnetii. Hum Immunol. 2020.
47. Validi M, Karkhah A, Prajapati VK, Nouri HR. Immuno-informatics based approaches to design a novel multi epitope-based vaccine for immune response reinforcement against Leptospirosis. Mol Immunol. 2018;104:128-38.
48. Gamage DG, Gunaratne A, Periyannan GR, Russell TG. Applicability of instability index for in vitro protein stability prediction. Protein Pept Lett. 2019;26(5):339-47.
49. Guruprasad K, Reddy BB, Pandit MW. Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. Protein Eng. Des. Sel. 1990;4(2):155-61.
50. Forouharmehr A, Nassiri M, Ghovvati S, Javadmanesh A. Evaluation of different signal peptides for secretory production of recombinant bovine pancreatic ribonuclease A in gram negative bacterial system: an in silico study. Curr Proteomics. 2018;15(1):24-33.